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Functional integration of skeletal traits: An intraskeletal assessment of bone size, mineralization, and volume covariance

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Abstract

Understanding the functional integration of skeletal traits and how they naturally vary within and across populations will benefit assessments of functional adaptation directed towards interpreting bone stiffness in contemporary and past humans. Moreover, investigating how these traits intraskeletally vary will guide us closer towards predicting fragility from a single skeletal site. Using an osteological collection of 115 young adult male and female African-Americans, we assessed the functional relationship between bone robustness (i.e. total area/length), cortical tissue mineral density (Ct.TMD), and cortical area (Ct.Ar) for the upper and lower limbs. All long bones demonstrated significant trait covariance ($p < 0.005$) independent of body size, with slender bones having 25–50% less Ct.Ar and 5–8% higher Ct.TMD compared to robust bones. Robustness statistically explained 10.2–28% of Ct.TMD and 26.6–64.6% of Ct.Ar within male and female skeletal elements. This covariance is systemic throughout the skeleton, with either the slender or robust phenotype consistently represented within all long bones for each individual. These findings suggest that each person attains a unique trait set by adulthood that is both predictable by robustness and partially independent of environmental influences. The variation in these functionally integrated traits allows for the maximization of tissue stiffness and minimization of mass so that regardless of which phenotype is present, a given bone is reasonably stiff and strong, and sufficiently adapted to perform routine, habitual loading activities. Covariation intrinsic to functional adaptation suggests that whole bone stiffness depends upon particular sets of traits acquired during growth, presumably through differing levels of cellular activity, resulting in differing tissue morphology and composition. The outcomes of this intraskeletal examination of robustness and its correlates may have significant value in our progression towards improved clinical assessments of bone strength and fragility.

Keywords

Bone; Robustness; Functional morphology; Cortical area; Mineralization

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Introduction

Variation in whole bone stiffness is directly linked to the functional adaptation process. Increasingly, this variation in stiffness appears to be established during growth and maintained throughout the aging process via the coordination of morphological and compositional traits acting through biomechanical pathways [1–7]. These traits include, but are not limited to, diaphyseal size, tissue mineralization, and cortical area. These three interacting traits account for 73–79% of whole bone bending stiffness [9], making them important determinants of whole bone strength. Additionally, these traits are clinically convenient for assessing fracture risk in that they are non-invasively measured. Previous studies demonstrated that total cross-sectional area of the diaphysis relative to length (i.e. robustness) varies along a biological continuum from slender to robust, in which the cross-sectional diameter varies from narrow to wide, irrespective of body size [1,8–10]. Slender bones, though smaller in cross-sectional diameter, have proportionally thicker cortices and greater mineralization (i.e. tissue stiffness) compared to robust bones, which demonstrate relatively thinner, less mineralized cortices [9,11,12]. This covariance between robustness, cortical area, and mineralization is present both between and within sexual cohorts [1,2,12] and populations [9,10]. Given the observed range in bone size and stiffness, one of the ongoing questions surrounding bone is how this tissue ascertains and preserves adequate stiffness throughout growth and aging to accommodate static and dynamic loading associated with body mass and physical activity. Resisting peak strains within the normal physiological range, which may differ between skeletal regions, is one primary objective. As new evidence comes to light, it appears that variation in bone size and an individual's susceptibility to fracture results from genetic and environmental perturbations [13–20]. To better understand how this variation is established and modified throughout growth and development it is important to look past population means in assessing function [21], which mask the variation within, and begin focusing on an individual's unique trait set that may lie on the slender or robust periphery of the population average. No single trait defines whole bone stiffness. Thus, understanding covariance among traits is essential towards identifying genetic and environmental factors that impair the development of stiffness, while enhancing evaluations of skeletal growth.

Bone robustness appears to be established early postnatally in both males and females [2], with this relationship between subperiosteal expansion and longitudinal growth persisting to adulthood despite fundamental sex differences in growth patterns [1]. Thus, we must consider that robustness naturally varies within a population and that geometric strength parameters based on habitual loading patterns do not fully explain this phenomenon. Given that there is a covariant pattern as to how traits establish function (i.e. a bone that is sufficiently stiff and strong to support physiological loads) during growth, variation in bone stiffness may be better defined by the specific combination of traits acquired by adulthood and maintained during aging. Having a slender phenotype does not necessitate lower habitual loading or a bone that is poorly adapted, as there may be a selective advantage of constructing a bone that minimizes mass while maximizing stiffness to perform particular functions [22].

The functional morphology of bone is complex, and compounded by genetically guided structural and tissue level mechanical properties that fluctuate over time [20]. Thus, individuals within a population are at risk of fracturing their bone for different life history, temporal, and genetic reasons. Despite this understanding, the systemic nature by which traits covary has yet to be fully investigated. Previous human populational research into bone size and tissue level trait variants, as discussed above, have been largely limited to radiographic and computed tomography scans comprised of individuals who are physically fit [9,23–25], and those of European ancestry with moderate to high socioeconomic standing [1,2,17]. The following investigation seeks to complement these studies while both confirming and enhancing our understanding of functional morphology and the systemic nature of robustness trait sets throughout the human skeleton. Specifically, are patterned trait interactions previously reported for robustness, tissue modulus, and cortical bone area, consistent among weight bearing and non-weight bearing bones? All long bones are subjected to loading, albeit in differing manners, and as a result they are functionally adapted relative to habitual loading regimes. Understanding the intraskeletal variance of complex trait sets, and determining whether robustness and its correlates behave systemically throughout the skeleton, has significant clinical value towards predicting variation in bone stiffness and strength using data obtained from a single skeletal site.

To progress towards this goal, the upper and lower limbs from a population of African-American individuals of low socioeconomic standing were analyzed. The specific aims of this study were three-fold: 1) to confirm the presence of a significant negative relationship between skeletal robustness and tissue modulus (i.e. as robustness increases for a given bone, tissue modulus decreases) relative to body mass and bone length 2) to confirm the presence of a significant positive relationship between skeletal robustness and cortical area (i.e. as robustness increases for a given bone, tissue volume increases) relative to body mass and bone length; and 3) to assess the intraskeletal relationship of all bones examined in respect to skeletal robustness, tissue modulus, and cortical area. Better understanding these patterns for different bones and an ethnic group that is not predominately of European origin, will benefit efforts aimed at understanding loading patterns across ethnic groups, which is fundamental for the continual advancement of clinically assessing fracture risk, and the behavioral interpretation of prehistoric and historic osteologic material.

Materials and Thethods

Sample

The sample used in this study was comprised of 52 women and 63 men of African-American ethnicity obtained from the Hamann-Todd Osteological Collection that was amassed between 1910 and 1940 from dissecting room cadavers in Cleveland, Ohio and is now curated by the Cleveland Museum of Natural History. This population was chosen specifically because it is predominantly comprised of indigents of low socioeconomic status that were a subjugated people during early 20th century urban industrialism. Moreover, this population was undoubtedly susceptible to health-related factors stemming from nutritional deficits and disease [26–29], presumably accounting for many of these individuals' young age-at-death. We acknowledge the limitation of the sample chosen and the 'osteological

paradox' [30] considerations associated with a sample of this nature. However, this sample was intentionally chosen to complement previous investigations into robustness trait variants in healthy Caucasians of higher socioeconomic status, determining whether the covariation of traits remains significant amid environmental considerations that may impact longitudinal and transverse skeletal growth [31,32]. Thus, this sample provides us with a starting point for looking at individual differences within populations that we hope to build upon in future work.

All individuals selected had stated ages of 20–30 years, and they were devoid of any gross or radiologically observable skeletal pathology that may have impacted bone structure and/or tissue level mechanical properties. The left upper and lower limbs of each individual were used in this study and included the humerus, radius, second metacarpal, third metacarpal, femur, and tibia. Body weight was documented at time of autopsy for each individual; however, these data may not be reliable. Todd and Lindala [33,34] noted that the condition of the bodies varied greatly at the time they were received, and that reported weights were not always directly measured but also estimated. However, regression equations given for the estimation of body weight are widely variable, occasionally reliant upon mean data and/or self-reported weights, contingent upon varying morphological indicators and quantification methods, and useful only for the population from which they were derived. Thus, rather than introduce further bias in the application of body weight estimation equations, documented body weight, along with femoral head breadth as a proxy, are used in this study. Despite the uncertainties in this variable, our analyses below will demonstrate that body weight has little influence on the significance of the relationships reported.

Data acquisition methods

Robustness and tissue moduli estimations were quantified for each bone using peripheral quantitative computed tomography, or pQCT (XCT 2000; Stratec Medizentechnik, Pforzheim, Germany). Each bone was axially scanned with an in-plane pixel size of 0.10 mm × 0.10 mm. To ensure quality and consistency of images generated, a calibration scan was performed daily using a standard phantom with known densities.

Scans were taken at the 50% midshaft of each bone according to length. Length was measured parallel to the longitudinal axis of each bone using a standard osteometric board in accordance with Ruff [35]. Femoral head breadth was measured using standard calipers, with an offsetting attachment, and was quantified along the anteroposterior axis. Each long bone was placed in two custom holders designed to hold the bone along the proximal and distal metaphyses. This facilitated the orienting of each bone along the anteroposterior and mediolateral axes in a similar manner to that described by Ruff and Hayes [36]. The design of the holders allowed each bone to be oriented in a consistent, reproducible manner that accounted for variation in bone width and curvature. Each bone was positioned with the distal end facing away from the gantry opening. To make certain each slice obtained from the midshaft was perpendicular to the longitudinal axis of the bone, a line level was placed on the anterior surface of the bone and the holders on either end were adjusted accordingly until the bone was level and centered within the gantry of the scanner. The 2nd and 3rd metacarpals were oriented in the same manner as the long bones, however due to their short

length a custom acrylic block was used to allow for the midshaft of both metacarpals from each individual to be scanned simultaneously. A validation study was conducted to ensure that the acrylic block, and the presence of two bones positioned side-by-side, did not significantly affect X-ray attenuation, and thus the resulting gray values. Both factors were found to have an insignificant effect on attenuation.

Following image acquisition the following traits were quantified: total cross-sectional area (Tt.Ar); cortical area (Ct.Ar); and mean gray value. From these data the remaining variables were derived. Robustness was calculated by dividing total cross-sectional area by bone length (Le), an index reflecting the biological relationship between longitudinal and transverse growth [37,38]. Cortical tissue mineral density (Ct.TMD) was estimated from the mean gray values. To convert attenuation into a density measure the slope and intercept values is required. These values comprise the calibration equation calculated by the scanner, which is derived from the standard phantom of known densities. For our particular scanner the slope and intercept equal 1724 and -322, respectively. Density values were then calculated by multiplying the mean gray value for each bone by the slope, dividing this by 1000 and then adding the intercept to the resulting value (i.e. $[(\text{mean gray value} \times 1724) / 1000] + -322$). Normal attenuation is expressed as a value between 0.0 and 0.1, however the image values given by the pQCT is attenuation times 1000. Therefore, the mean gray value and slope were divided by 1000 in the interest of standardizing the data. It is important to note that pQCT density values are scaled with fat at 0 mg/cm^3 , which is different from the standard Hounsfield units scaled with water at 0 mg/cm^3 . Thus, gray values obtained from the pQCT are not comparable with standard in vivo CT scanners without validated standardization. Cortical tissue mineral density was used as an estimate of tissue modulus. Previous work found that Ct.TMD is significantly associated with mechanically quantified tissue modulus, histomorphologically assessed bone porosity, and ash content in cadaveric tibiae. This work validated that Ct.TMD accurately accounts for the full range of variation in tissue modulus across the full range in robustness [9]. However, Jepsen and colleagues [9] only validated this relationship in tibiae that were scanned while submerged in phosphate buffered saline. Whether this relationship is consistent across bones that are scanned in air and of differing cross-sectional size has yet to be validated.

Statistical analysis methods

All statistical analyses were performed using Minitab 16.1.1 (Minitab Inc., State College, PA) software. All data had near normal distributions with the metacarpals of six individuals falling outside the mean range of the population. However, the remaining bones of these individuals were within the mean range. With only the metacarpals presenting as potential 'outliers', these data points were not eliminated, but were instead treated as representatives of natural variation within the population, as we make no presumption of the biology. Means and their range for all data included in this study are shown in Table 1 for each bone.

We hypothesize that robustness, mineralization (Ct.TMD), and tissue volume (Ct.Ar) functionally covaries for all bones within both males and females. Previous studies in murine models and humans suggest that early variation in robustness leads to adaptive changes in Ct.TMD and Ct.Ar. Thus, to test the current hypothesis and expand upon our

working paradigm, linear regression analyses were performed on the data in its raw form and after adjusting for body mass and bone length via partial regressions, since these dimensions are known to significantly impact bone morphology (i.e. moment arm length and diaphyseal size) [32]. Body mass and bone length values were multiplied and then the product was divided by 100 to create a composite variable (BM-Le) and then linearly regressed against robustness, Ct.TMD, and Ct.Ar for all bones. Using the residuals, which represents variation within each trait not explained by body size (BM-Le), linear regressions were systematically performed between robustness, Ct.TMD, and Ct.Ar for all bones. To determine the degree to which systemic factors may affect trait variation within the skeleton (e.g. whether an individual with a slender and relatively stiff radius also demonstrates this phenotype within other long bones), Pearson's correlations between skeletal elements for robustness, Ct.TMD, and Ct.Ar were performed both before and after adjusting the data for body size. To compare how well robustness explained Ct.TMD and Ct.Ar across bones, linear regressions between robustness, Ct.TMD, and Ct.Ar were plotted for all bones, and an ANCOVA test was used to assess the level of trait covariance between bones both before and after adjusting the data for body size.

To allay potential concerns with using bone length as a scaling factor in our robustness index, we also considered additional indices that may be potentially employed to assess robustness. These include total area divided by body mass, body mass $\times$ bone length, height, and femoral head breadth. Statistical analyses between these indices, Ct.TMD, and Ct.Ar were also conducted on the weight-bearing femur and non-weight bearing radius, determining whether our definition of robustness is appropriate for use as an indicator of bone size.

Results

Relationships between bone robustness and tissue stiffness

The relationship between robustness (Tt.Ar/Le) and tissue mineralization (Ct.TMD) for each bone site was assessed to determine the degree of covariance between these traits throughout the skeleton. Males and females were significantly ($p < 0.001$) different from one another in terms of diaphyseal robustness and Ct.TMD in a consistent manner for all bones. Male skeletal elements tended to be more robust with a lower Ct.TMD, compared to those of females, as expected. However, Gaussian distributions of each trait demonstrate considerable overlap within and between males and females, particularly within the 2nd and 3rd metacarpals (see value ranges in Table 1).

Robustness and Ct.TMD comparisons unadjusted for body size revealed a significant relationship between traits for most of the skeletal elements (Table 2a). Within males, robustness and Ct.TMD significantly negatively correlated with one another in all skeletal elements of the upper and lower limbs ($p < 0.001$ – 0.006), except for the 3rd metacarpal ($p < 0.278$). Robustness statistically explained 13.9–22.4% of Ct.TMD variation in the upper and lower limbs, with the femur and radius demonstrating the greatest relationship between traits. Females revealed a similar association between robustness and Ct.TMD, with significant negative relationships between traits within all major long bones ($p < 0.001$ – 0.013), but not within the 2nd ($p < 0.548$) and 3rd ($p < 0.712$) metacarpals. Robustness

explained 11.1–25.7% of the variation in Ct.TMD, with the lower limb elements demonstrating the greatest relationship between traits in females. The metacarpals in both females and males included outlying individuals that demonstrated very low Ct.TMD values. Removal of these individuals made no significant difference to the poor relationship between traits among these skeletal elements.

Adjusting robustness and Ct.TMD for body size generally improved these relationships (Table 2a). The degree to which robustness explained Ct.TMD increased for all male bones with a range of 16.4–28% in the major long bones of the upper and lower limbs. For females, adjusted values maintained negative significant relationships, however, only the relationship between traits for the 2nd metacarpal and tibia improved with a 0.4–3.3% shift in how well robustness explained Ct.TMD, respectively. The femur demonstrated no change, while the relationship between bone size and mineralization decreased within the upper limb bones. This suggested strong covariance between traits independent of body size. Thus, it would appear additional mechanical and systemic factors, other than those associated with body size and sex, significantly impact the functional morphology of bone. These results confirm that in both males and females, slender bones tend to have a higher Ct.TMD compared to robust bones, irrespective of body size. Robustness trait variants, in conjunction with tissue mineralization, not only significantly vary between sexes in adherence with the principles of sexual dimorphism, but also widely between individuals of each sexual cohort.

Relationships between robustness and tissue volume

Relationships between robustness and cortical area (Ct.Ar) within each bone were evaluated to determine the extent of agreement between these traits throughout the skeleton. In terms of Ct.Ar, males and females were consistently significantly ($p < 0.001$) different from one another for all bones. However, similar to Ct.TMD, Gaussian distributions of Ct.Ar for males and females demonstrate considerable overlap both within and between the sexes, particularly within the metacarpals, with more robust bones tending to have less tissue volume compared to the thicker cortices of slender bones.

Relationships between values unadjusted for body size were positively significant in both males ($p < 0.001$ –0.007) and females ($p < 0.001$ –0.002) for all bones (Table 2b). For males, the range in which robustness explained variation in Ct.Ar for all bones was 11.5–50.3%, with the radius demonstrating the greatest relationship between traits, followed by the femur. In females the range was 18.0–62.4%, with the strongest relationship between traits found in the femur, closely followed by the radius. When adjusting for body size, significant relationships between traits for all bones were maintained in males and females (Table 2b). However, the degree of association between traits slightly changed with the radius now revealing the strongest relationship between traits in females, followed by the femur. In all, these results confirm that independent of body size both male and female slender bones tend to have less tissue volume compared to that of robust bones, which widely varies not just between sexual cohorts but also within them.

Intraskkeletal variation of trait sets

Establishing that robustness, tissue mineralization and tissue volume significantly covary within each bone, we then assessed the level to which these trait sets relate to one another among bones. When comparing robustness, Ct.TMD, and Ct.Ar between individual bones, both before and after adjusting for body size, significant correlations were prevalent (see Table 3 for body size adjusted results). Interestingly, skeletal elements from both the upper and lower limbs demonstrated strong correlations between traits, suggesting in part a systemic mechanism. Covarying traits of the femur, tibia, and humerus, but not the radius, were highly correlated in males. However, in the females, the femur, tibia, humerus, and radius all demonstrated significant trait associations. Thus, male radii did not demonstrate a strong covariance of traits, which is contrary to female radii. Reasons for this disparity are unknown. The radius, which is by far the most slender long bone, may have correlated better in females since on average their bones tend to be more slender in comparison to males. Additionally, the radius, though unequally, shares loads with the ulna. Therefore, the radius and ulna may need to be considered as a unit, particularly since they potentially form a single beam with a fluctuating center of mass dependent on forearm rotation. Sexual differences were also apparent when comparing covarying traits in the metacarpals to the major long bones. Traits of neither the 2nd nor 3rd metacarpals significantly correlated with bones of the upper and lower limbs in females. Again, removal of 6 females with very low Ct.TMD values from the analysis did not affect the resulting outcome. However, in males, correlations between traits of both metacarpals and the remaining long bones demonstrated significant associations ($p < 0.001$). Interestingly and somewhat unsurprisingly, trait sets of both the 2nd and 3rd metacarpals in males and females did significantly ($p < 0.001$) correlate with one another. This suggests that the long bones of the hand may developmentally and biomechanically behave differently from the major long bones of the upper and lower limb. Similar size and tissue volume between metacarpals may reflect biological growth constraints, and similar tissue mineralization may indicate load sharing with bones of the palm cooperating, in part, as a single entity. Additionally, the threshold set points believed to direct the modeling and remodeling processes may be different for the metacarpals compared to the limb bones.

Figs. 1 and 2 are regression plots of Ct.TMD and Ct.Ar versus robustness for males and females. When considering the Ct.TMD values unadjusted for body size, the femur, tibia, and humerus were most similar, followed by the metacarpals (Fig. 1a). The radius is clearly different from all bones included in this study, emphasizing the need to revisit its association with the ulna. However, when adjusting for body size, the range of variation in which robustness explained Ct.TMD was similar for all bones. Significance of ANCOVA tests between slopes for each bone ranged between $p < 0.06$ and 0.82 for males, and $p < 0.19$ and 0.91 for females (Fig. 1b). A significant negative relationship between Ct.TMD and robustness, independent of body size, suggests that this is partially a systemic trait variant within the skeleton. Comparisons between Ct.Ar and robustness values unadjusted for body size demonstrated a similar pattern, with the femur, tibia, and humerus being the most related (Fig. 2a). Furthermore, Ct.Ar values were distributed according to whether the bones were primarily weight bearing or non-weight bearing, as expected. However, the slope and linear positioning of the metacarpals was significantly different from all remaining bones.

Interestingly, when adjusting for body size, all bones demonstrated a similar range of variation between values and their slopes were staggered in accordance with weight bearing and non-weight bearing status (Fig. 2b). Significance of ANCOVA tests between slopes for each male bone ranged between $p < 0.0001$ and 0.93 when the metacarpals were included, however, without the metacarpals significance ranged between $p < 0.12$ and 0.93 . For females, significance of ANCOVA tests between slopes for each bone ranged between $p < 0.0001$ and 0.75 when including the metacarpals, and $p < 0.0004$ and 0.75 when excluding these skeletal elements. This confirms a partial systemic mechanism between tissue volume and robustness, which is not explained by body size. However, the humeri of females were found to be significantly different from the lower limbs, demonstrating some differences between upper and lower skeletal elements that at this point are unexplained. The metacarpal results, both before and after adjusting for body size, suggests that robustness needs to be redefined when regarding the hand, or, as postulated earlier, biological constraints in size and mass are different within the hand compared to other long bones. That both male and female residuals for all bones, except for the humeri, encompass a similar range in values for Ct.TMD and Ct.Ar, suggests that sexually dimorphic differences in the skeleton may only be applicable to external size, and not tissue volume or mineralization. This is consistent with the significant overlap observed in Gaussian distributions of individual trait values between males and females.

Tt.Ar/Le as an appropriate measure of skeletal robustness

Understanding that bone length is non-linearly related to body mass and muscle strength, we employed regression analyses between four additional robustness indices, Ct.TMD, and Ct.Ar of male and female femora and radii to examine their effects, if any, on the outcome of the study. Coefficients of determination with significance values are given in Table 4 for all regressions performed. Overall, the substitution of body mass, body mass $\times$ length, height, or femoral head breadth in place of bone length as a scaling factor for total cross-sectional area, had very little effect on the results generated from this study. Though our definition of robustness (Tt.Ar/Le) performed the best for both the femora and radii of males and females, all indices demonstrated significant relationships with Ct.TMD and Ct.Ar. Indices using body mass generally performed the worst, which is probably due to the inaccuracies in documented body weight for the individuals examined. Based on the results from these analyses we are confident that use of Tt.Ar/Le as an index of robustness does not produce artifactual results, as the relationships observed when using all indices remains the same between bone size, mineralization, and tissue volume. Therefore, either index can be used; however, we prefer Tt.Ar/Le as it reflects the biological relationship between transverse expansion and longitudinal growth of the diaphysis.

Discussion

Bone is functionally and structurally complex. To circumvent this challenge we have chosen a top down approach, translating functional relationships between bone morphology and tissue stiffness observed in the murine model [20,39–45] to the human skeleton. The research herein seeks to better understand the gross variation in functional morphology within the skeleton of a variable population, with the ultimate goal of investigating the

molecular development and adaptability of bone, which is of interest to anthropologists, biologists, clinicians, and biomedical engineers. Preliminary human investigations demonstrated significant covariance between bone robustness, mineralization, and tissue volume in select skeletal elements, including the tibiae of physically fit military recruits [9,10] and small-scale cadaver studies of young adult and middle-aged tibiae [3,12]. Our study expands this research, seeking to confirm this trait covariance throughout the human skeleton and explore how these robustness trait sets, that in part establish moment of inertia, intraskeletally vary in an environmentally noisy population of African-Americans with high rates of acute and/or chronic infectious and degenerative diseases [28].

A thorough assessment of robustness trait variants in the population subsample selected for this study, confirmed that significant covariance between the gross morphology and mineralization of bone is present throughout the skeletal system in a predictable manner (Fig. 3). This relationship is consistent not only within bones but also between them, with upper and lower limbs demonstrating similar degrees of trait covariance (Fig. 4). These findings suggest strong systemic influences in the obtainment of adult skeletal function that is not fully explained by mechanical forces placed on bone via an individual's physique. These trait sets vary widely within and between individuals and sexual cohorts. Men and women of similar body size can both parallel one another in their combination of traits and be fundamentally different (as exemplified in Fig. 3), suggesting that each person attains their own unique trait set by adulthood through differing levels of osteoclastic and osteoblastic activity during growth, resulting in differing tissue morphology and composition that is both predictable based on robustness and partially independent of environmental influences. However, the covarying relationship between traits must be further validated via ash content tests to ensure Ct.TMD estimates, based on pQCT gray values, are consistent across all bones.

A challenge to bone functionality is to maximize stiffness, while simultaneously minimizing mass [22]. Variation in functionally integrated traits largely accomplishes this feat, constructing a bone that is reasonably stiff and strong, and sufficiently adapted to perform routine, habitual loading activities. Slender and robust bones maximize their stiffness, and thus strength, by packing in as much bone as possible without unduly exceeding their safety factor and metabolic cost by keeping tissue mass relatively minimal [46]. The potential risk of having a slender bone that is defined by a small cross-sectional diameter is partially negated by increasing Ct.TMD to improve tissue modulus and thus whole bone stiffness, whereas risk associated with a robust bone is improved by reducing Ct.Ar relative to Tt.Ar to minimize mass. Both trait variants, though reasonably adapted to complement daily loading demands, are not without their drawbacks. The proportionally thick, highly mineralized cortices of slender bones potentially make them more brittle [47], while the proportionally thin cortices of robust bones possibly make them less tolerant of prolonged endosteal and intracortical remodeling; all factors that do not bode well for structures under extreme loads. Thus, there appears to be a functional limit to bone that may be partially defined by robustness trait sets and the degree to which bone cells can adequately compensate for this skeletal variation, which is ordered to maximize stiffness while minimizing mass. Epelboym and colleagues [11] reported differential aging of the femoral neck, such that Ct.Ar decreased significantly with age in robust bones ($r^2 = 0.29$; $p < 0.05$)

but to a lesser degree in slender bones ($r^2 = 0.02$; $p > 0.58$), with regression slopes between morphological phenotypes being significantly different ($p < 0.012$; ANCOVA). This suggested that slender and robust bones in their sample exhibited differential rates of cortical bone loss. Furthermore, Ct.TMD of the femoral neck also decreased significantly with age among robust bones ($r^2 = 0.48$; $p < 0.01$), but not within slender bones ($r^2 = 0.09$; $p < 0.25$). Therefore, robust bones would appear to lose relatively more bone in order to decrease their tissue mass [9,11,48]. Preliminary investigations into intracortical remodeling differences between robustness phenotypes suggests slender bones adjust for size limits by suppressing intracortical remodeling in order to maintain tissue volume and increase tissue modulus. Goldman and colleagues [48] reported significant relationships between tibial robustness and the percentage of porosity ($r^2 = 0.61$, $p < 0.009$), osteon area ($r^2 = 0.52$, $p < 0.02$), osteon population density ($r^2 = 0.49$, $p < 0.02$), and the percent of secondary remodeling tissue ($r^2 = 0.68$, $p < 0.004$), suggesting slender tibiae have fewer and smaller osteons per unit of area than their robust counterparts. However, a much larger sample of bones comprising the appendicular skeleton will need to be assessed to confirm these initial findings. Despite these potential pathways in which bones of varying degrees of robustness accommodate their inherent functional limitations, neither is without risk. Slender bones may have a propensity to become more brittle as intracortical remodeling is suppressed and microdamage accumulates, which would make them susceptible to fracturing throughout life [5,6,25,49], and robust bones potentially become more brittle as porosities coalesce and cortical area decreases, making them prone to fracturing later in life [1,50,51].

Finding that robustness trait variants are systemically prevalent throughout the skeletal system contributes to our understanding of bone functionality and trait covariation. However, it is important to note that our paradigm for this study is experimental, focusing only on whole bone stiffness relative to body size as an indication of peak tissue level strains. This does not negate the importance of toughness and fatigue resistance when assessing fracture resistance, but our analysis is limited to two-dimensional data that does not permit for the quantification of these post-yield variables. Therefore, a noted difference in bone size and tissue level mechanical properties does not fully explain the variation in bone strength. Additional adaptations at the tissue level, which also adjust toughness and fatigue resistance, should be considered in future investigations, such as collagen fiber orientation, collagen cross-links, osteonal morphotypes, and remodeling rates [52,53]. Furthermore, bones comprising the appendicular skeleton may behave differently in resisting peak strains [54,55], which would alter the way in which the tissue microstructurally adjusts through remodeling. Bones such as the femora experience more torsional loads [56] compared to the radii which may be exposed to more bending loads. This may in part explain why the radii do not correlate well with the other primary long bones, as there may be fundamental differences in mechanical demands among bones.

That our study found systemically patterned trait covariance throughout the skeleton emphasizes that all long bones adapt to habitual loads in a similar fashion, predicted by robustness, and independent of weight-bearing status. Mapping covariation to understand how these trait interactions manifest intraskeletally may benefit clinical advancements geared towards developing models that predict variation in bone strength. In this study we observed a rather large systemic effect in how functionally integrated traits covary. This was

detected with only accounting for body size, and not addressing local mechanical factors attributable to the physiological loads experienced by each bone. Thus, we might expect further refinement in the systemic variation of robustness trait sets if we were to include information on differential loads among skeletal sites. The presence of systemic variation in robustness and its correlates may help identify long bones that are either more or less amendable to prophylactic treatments (e.g. bisphosphonates, PTH). Further work is needed to determine whether the higher Ct.TMD of radii, for example, results from greater mineralization and/or reduced porosity. This is clinically important because greater mineralization could lead to reduced toughness, and reduced porosity would mean that remodeling is further suppressed at the radius compared to other skeletal elements. Mapping differential control of intracortical remodeling among skeletal elements may help explain why several clinical trials have found the radius to be less responsive to bisphosphonate treatments for bone loss compared to other sites [57–60]. Additionally, determining how traits covary systemically may identify particular skeletal sites that are well suited for estimating the structural and compositional state of the entire appendicular skeleton. Moreover, mapping the systemic covariation of traits may provide important clues as to whether genetic and environmental factors responsible for a decrease in fracture prevalence among particular individuals act locally, systemically, or both. This knowledge may benefit our ability to best treat individuals by maximizing strength throughout the skeleton.

Mapping the covariation of traits may enhance current clinical practices concerned with assessing activity levels and fracture risk between individuals and populations through the quantification of bone mineral density, cortical thickness, and section-moduli. Bone mineral density (BMD) measures have become the gold standard in the clinical assessment of an individual's susceptibility to fracture, and are useful in predicting strength relative to the population average. However, in its current form clinical BMD analyses do not account for the variation in robustness, mineralization, and tissue volume that this study has found to significantly covary within a population in a systemic fashion. Understanding how these traits are functionally integrated, and that there are subtle variations in function that are not considered when comparing individuals to the population mean, may enhance future BMD analyses. Similarly, findings from this study may improve investigations into individual and populational differences in cortical thickness and section-moduli. Slender bones appear to have thicker cortices, however, slender bones in our sample were found to have approximately 25–50% less Ct.Ar, once adjusted for body size, in comparison to their robust counterpart. This estimate was based on the linear regression plots of body size adjusted data, and the range of deviation from the population mean. Considering these differences, estimations of cortical thickness based on periosteal and endosteal diameter averages about the cross-section may not accurately reflect function, since slender bones have less tissue volume than those that are more robust, relative to body size. Additionally, cortical thickness and other morphological indices like section-modulus, do not account for the significant covariance these traits have with tissue level mechanical properties, which our current and previous investigations into tissue stiffness, porosity, and tissue volume demonstrates is a crucial component of the functional adaptation process [9,10,48]. For example, in the current study slender bones were found to be approximately 5–8% less stiff at the tissue level than robust bones after adjusting for body size, based on the range of deviation from the

population mean in the linear regression plots. Thus, populational interpretations based on cortical thickness and section-moduli, without accounting for both bone robustness and tissue level mechanical properties, may not fully reflect functional differences within and between populations. Moreover, robustness trait sets are unique to the individual, which is not accounted for in physical activity and fracture risk assessments based on population means.

That bone morphology and mineralization are functionally, and by extrapolation, developmentally integrated traits that potentially modularly evolved, does not minimize the role environmental factors also have in the achievement of a sufficiently functional bone. Undoubtedly, the environment in which bone develops can influence and modify skeletal robustness during growth and adolescence, and we acknowledge that though our study population presumably performed similar loading activities, we were unable to account for physical activity. Previous studies have demonstrated that bone robustness is intricately linked to environmental factors on a populational level [16,61–70]. Observations of asymmetry in the external dimensions of the dominant arm in tennis players, particularly of individuals that began training as children, indicates that periosteal apposition is associated with physical activity [61–63]. Additionally, a shift in dietary resources and the activity levels employed to acquire them, has had a significant impact on skeletal development and long bone robustness. Skeletal differences in bone size and strength between highly mobile prehistoric foraging populations and more sedentary modern agricultural populations are well established [64–67]. Temporal examinations of skeletal robustness among hominins indicate that bone strength, relative to body size, has progressively decreased over the last 2 million years by about 30% [68]. Interestingly, approximately half of this reduction has likely occurred since the emergence of the Holocene (~10,000 year ago), and thus the advent of sedentary agricultural practices [69]. Though sedentism as causality has yet to be substantially confirmed, it does appear that technological innovation increasingly deflects physical subsistence requirements placed on the body [69]. Other considerations that may reveal causative biomechanical factors attributable to this progressive decline in bone strength include the terrain in which populations subsist, division of labor ratios influencing sexual dimorphic differences, and the physical demands required for exploiting various resources. Thus, the importance of understanding biocultural evolutionary trends when assessing modern populational variation in robustness trait sets cannot be overstated.

Considering the evidence for environmental influences on skeletal size, in conjunction with the noted covariance of robustness traits, ‘functional adaptation’ may be better defined by the combination and coordination of traits obtained by skeletal maturity. Ancestral environmental conditions may have guided the functional integration and present variation in morphological traits observed in modern human populations [71]. If so, there is no reason to doubt that environmental factors continue to shape these trait interactions towards the maximization of stiffness and minimization of mass. Thus, the total amalgamation of factors influencing bone development and adaptation should be considered when assessing bone function in clinical and osteologic contexts. For example, suppose you were presented with a slender and a robust femur, what could be functionally said about these macroscopic morphological differences? Would one assume that the slender femur is significantly ill-adapted to support routine loading, representative of a physically unfit individual? Or would

the morphological differences between bones be interpreted as defined divisions of labor between individuals or sexual cohorts? Or would one interpret this variation as indicative of differing ancestral geographic origins? The short answer is yes, all of these explanations could reflect the dichotomy in physical activity and geographic domains. However, as our research suggests these conclusions cannot be easily made. Slender and robust bones appear to be sufficiently capable of sustaining routine physiological loads by maximizing stiffness while minimizing mass, as previously observed in healthy human tibiae [9,10]. However, whether both phenotypes compensate for their trait differences adequately throughout the appendicular skeleton has yet to be determined. These trait differences naturally vary within and across populations of differing ethnicity (i.e. individuals of European and African ancestry) and social standing (i.e. individuals of middle and upper class). Moreover, there is a significant overlap in bone size between males and females, wherein individuals of similar body size can and do demonstrate differing phenotypic expressions of robustness. Herein lies the primary question; is the variation in robustness within a given population also accompanied by similarly coordinated changes in Ct.TMD and Ct.Ar? Or are slender and robust bones constructed in fundamentally different ways?

Conclusion

The results of this study confirm the presence of significant covariance between the external size, volume, and stiffness of bone that is not wholly defined by one's physique and sexual characteristics. Clearly environmental factors have an influential role in skeletal trait sets obtained by adulthood, along with an individual's activity regime. However, to accurately define and assess functional adaptation, the covariance between traits related to skeletal robustness should also be considered. Future studies should concentrate on the underlying variation within a population and move past analyses limited to the population mean. Though populational comparisons based on the mean have undoubtedly contributed to our understanding of how environmental perturbations affect bone growth and development, emphasizing individual variation within populations will move us closer towards understanding the inherent phenotypic variance in skeletal morphology and function, which may improve clinical fracture risk assessments and prophylactic treatments for bone loss. In defining and attributing bone function to individuals and populations, we should also consider the total set, and covariant pattern of functionally integrated traits, which may contribute to phenotypic variances in skeletal morphology that is attributable to both genetic and environmental factors. This is paramount in our progression towards a more holistic comprehension of the evolutionary development and maintenance of variation in bone strength that has shaped the functional landscape apparent in contemporary human populations. Embracing this investigatory direction will not only improve interpretations of past activity distinctions between genders and populations, but also advance clinical research methodologies, such as BMD analyses, used to assess fracture risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Intraskkeletal regression plots between robustness and Ct.TMD. a) plotted values unadjusted for body size; b) plotted values adjusted for body size. Note the 6 females with very low Ct.TMD values in comparison to the population for both the unadjusted and adjusted values. Corresponding r^2 values for each bone, both unadjusted and adjusted for body size, are provided in Table 2a.

Fig. 2.

Intraskelletal regression plots between robustness and Ct.Ar. a) plotted values unadjusted for body size; b) plotted values adjusted for body size. Note the drastically different linear relationship of the 2nd and 3rd metacarpals in comparison to the long bones. Corresponding r^2 values for each bone, both unadjusted and adjusted for body size, are provided in Table 2b.

Fig. 3.

Example of intraskkeletal covariance of traits for four individuals within the study population. Femora and radii for two females and males demonstrating maximum slenderness and robustness are presented. For individuals within each sexual cohort, body mass and bone length are similar. Note the range of variation between individuals with similar body size, the intraskkeletal consistency in robustness within the same individuals, and the gray value differences between femora and radii, indicating higher mineralization in the radii compared to the femora.

Fig. 4.

Schematic showing trait covariance of robustness, Ct.TMD, and Ct.Ar. a) expected relationship between traits for a given robustness; b) populational variance of trait sets demonstrating a narrow range that is predictable in relation to robustness. The solid line indicates the range of males within the study population, and the dashed line indicates the range of females. The overlap of the two ellipsoids represents the population average. Gray value differences in a) and b) represent the variation in mineralization, with lighter shades reflecting lower Ct.TMD and darker shades reflecting higher Ct.TMD.

Table 1

Means with maximum and minimum value range for all variables quantified.

Bone	Trait	Males			Females		
		Mean	SD	Range	Mean	SD	Range
Femur	Bone Le (mm)	450.78	26.01	399–506	419.15	22.57	373–471
	Tt.Ar (mm ²)	591.77	65.88	464.9–786.8	485.98	66.07	369.0–642.4
	Ct.Ar (mm ²)	424.52	44.55	396.7–507.3	328.14	39.49	259.8–416.9
	Robustness	1.31	0.13	1.08–1.71	1.15	0.13	0.91–1.54
Tibia	Ct.TMD (mg/HA)	1267.1	21.2	1203–1320	1289.7	24.3	1234–1347
	Bone Le (mm)	390.75	25.17	345–438	358.25	22.38	309–416
	Tt.Ar (mm ²)	507.17	72.51	386.3–705.6	403.10	55.54	309.8–558.8
	Ct.Ar (mm ²)	338.83	44.52	252.4–473.9	267.51	37.18	200.3–342.6
Humerus	Robustness	1.29	0.15	1.04–1.80	1.12	0.13	0.87–1.64
	Ct.TMD (mg/HA)	1256.0	25.0	1183–1297	1293.1	19.7	1247–1333
	Bone Le (mm)	336.14	16.93	305–372	309.73	15.79	285–348
	Tt.Ar (mm ²)	360.15	46.41	267.4–536.2	270.39	44.55	188.5–369.2
Radius	Ct.Ar (mm ²)	254.92	27.04	197.1–319.4	172.50	22.35	120.1–219.8
	Robustness	1.07	0.13	0.78–1.50	0.87	0.13	0.60–1.28
	Ct.TMD (mg/HA)	1269.1	24.50	1209–1326	1304.4	22.3	1251–1352
	Bone Le (mm)	257.63	16.54	224–295	229.25	13.46	206–260
2nd MC	Tt.Ar (mm ²)	145.11	19.50	102.0–192.4	104.45	14.43	71.6–143.8
	Ct.Ar (mm ²)	115.86	12.66	86.0–143.8	83.93	10.97	63.3–107.4
	Robustness	0.56	0.06	0.41–0.73	0.45	0.06	0.32–0.66
	Ct.TMD (mg/HA)	1320.8	22.7	1267–1377	1360.7	15.0	1329–1388
3rd MC	Bone Le (mm)	72.69	4.71	65–83	68.13	3.87	60–79
	Tt.Ar (mm ²)	66.88	7.65	51.0–89.3	53.52	7.22	38.8–71.2
	Ct.Ar (mm ²)	50.62	5.79	38.9–72.0	40.84	4.32	28.3–49.6
	Robustness	0.92	0.09	0.72–1.16	0.78	0.09	0.60–0.99
3rd MC	Ct.TMD (mg/HA)	1287.5	29.9	1180–1338	1318.9	43.4	1174–1372
	Bone Le (mm)	71.87	4.66	60–82	66.62	3.71	64–78
	Tt.Ar (mm ²)	69.06	8.10	52.5–94.5	54.49	6.80	41.9–68.8

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Bone	Trait	Males			Females		
		Mean	SD	Range	Mean	SD	Range
	Ct.Ar (mm ²)	51.28	5.80	40.9–68.6	40.40	4.73	29.1–50.0
	Robustness	0.96	0.10	0.76–1.21	0.83	0.16	0.64–1.04
	Ct.TMD (mg/HA)	1294.5	28.9	1201–1355	1319.0	45.9	1159–1367

Table 2

Regression values for Ct.TMD and Ct.Ar versus robustness without adjusting data for body size and with body size adjustment. a) Ct.TMD; b) Ct.Ar.

Bone	Unadjusted for BM-Le						Adjusted for BM-Le					
	Males			Females			Males			Females		
	r ²	Slope	y-int	p	r ²	Slope	r ²	Slope	y-int	p	r ²	Slope
<i>a</i>												
Femur	0.22	-76.7	1368	0.001	0.26	-90.0	0.28	-89.5	0.0	0.001	0.26	-95.0
Tibia	0.14	-60.8	1335	0.003	0.22	-70.4	0.16	-70.5	0.0	0.001	0.26	-76.8
Humerus	0.15	-73.0	1347	0.002	0.11	-53.2	0.17	-77.3	0.0	0.001	0.10	-53.7
Radius	0.16	-146.0	1403	0.001	0.12	-85.5	0.18	-153.0	0.0	0.001	0.13	-88.9
2nd MC	0.12	-104.0	1383	0.006	0.007	-38.9	0.13	-106	0.0	0.004	0.01	-47.2
3rd MC	0.02	-38.9	1332	0.278	0.007	-38.3	0.02	-41.7	0.0	0.233	0.004	-30.0
<i>b</i>												
Femur	0.40	215.0	143	0.001	0.62	228.0	0.34	183.9	0.0	0.001	0.58	205
Tibia	0.34	170.0	119	0.001	0.51	200.0	0.27	144.5	0.0	0.001	0.50	180
Humerus	0.38	128.0	117	0.001	0.37	97.4	0.37	124	0.0	0.001	0.32	92.4
Radius	0.50	143.0	35.5	0.001	0.61	143.0	0.51	140	0.0	0.001	0.65	140
2nd MC	0.24	29.2	23.7	0.001	0.18	19.1	0.26	28.6	0.0	0.001	0.17	18.6
3rd MC	0.11	19.1	32.9	0.007	0.19	20.5	0.12	18.4	0.0	0.006	0.20	21.2

Table 3

Correlations of intraskeletal comparisons for males and females. Correlation coefficients (r) are given and bold values denote significance at $p < 0.05$.

	Bone	Femur	Tibia	Humerus	Radius	2 nd MC	3 rd MC
Robust	Femur	-----	0.66	0.68	0.04	0.39	0.39
	Tibia	0.78	-----	0.58	0.06	0.30	0.19
	Humerus	0.64	0.59	-----	0.09	0.64	0.57
	Radius	0.60	0.65	0.74	-----	0.13	0.14
	2 nd MC	0.04	0.05	0.10	0.00	-----	0.75
	3 rd MC	0.00	0.00	0.21	0.03	0.57	-----
	Females						
	Bone	Femur	Tibia	Humerus	Radius	2 nd MC	3 rd MC
Ct.TMD	Femur	-----	0.57	0.40	0.23	0.40	0.40
	Tibia	0.64	-----	0.64	0.22	0.39	0.33
	Humerus	0.57	0.57	-----	0.31	0.47	0.46
	Radius	0.44	0.65	0.45	-----	0.48	0.46
	2 nd MC	0.33	0.19	0.04	0.15	-----	0.91
	3 rd MC	0.33	0.27	0.09	0.22	0.93	-----
	Females						
	Bone	Femur	Tibia	Humerus	Radius	2 nd MC	3 rd MC
Ct.Ar	Femur	-----	0.66	0.58	0.18	0.36	0.47
	Tibia	0.76	-----	0.58	0.08	0.36	0.43
	Humerus	0.63	0.62	-----	0.08	0.70	0.67
	Radius	0.69	0.66	0.79	-----	0.22	0.24
	2 nd MC	0.07	0.04	0.24	0.18	-----	0.75
	3 rd MC	0.07	0.00	0.13	0.07	0.74	-----
	Females						

Table 4

Regression values for Ct.TMD and Ct.Ar versus various robustness indices, unadjusted for body size, for male and female femora and radii. BM = body mass; BM-Le = body mass $\times$ bone length; FH = femoral head breadth.

Bone	Ct.TMD vs robustness (Tt.Ar/Le)					Ct.Ar vs robustness (Tt.Ar/Le)				
	Males		Females			Males		Females		
	r ²	Slope	y-int	p		r ²	Slope	y-int	p	
Femur	0.22	-76.7	1368	0.001	0.26	-90.0	1394	0.001	0.40	214.7
Radius	0.16	-145.9	1403	0.001	0.12	-85.5	1400	0.013	0.50	142.8
Ct.TMD vs Tt.Ar/BM										
Bone	Males		Females			Males		Females		
	r ²	Slope	y-int	p		r ²	Slope	y-int	p	
Femur	0.09	-5.9	1321	0.016	0.13	-5.9	1345	0.008	0.16	16.6
Radius	0.19	-27.2	1382	0.001	0.07	-11.1	1383	0.063	0.51	54.7
Ct.TMD vs Tt.Ar/BM-Le										
Bone	Males		Females			Males		Females		
	r ²	Slope	y-int	p		r ²	Slope	y-int	p	
Femur	0.18	-38.9	1346	0.001	0.13	-25.6	1347	0.009	0.04	39.6
Radius	0.22	-81.7	1392	0.001	0.12	-31.5	1389	0.013	0.29	51.6
Ct.TMD vs Tt.Ar/Height										
Bone	Males		Females			Males		Females		
	r ²	Slope	y-int	p		r ²	Slope	y-int	p	
Femur	0.12	-226.2	1343	0.004	0.21	-335.2	1388	0.001	0.43	881.8
Radius	0.11	-711.1	1380	0.008	0.04	-415.5	1387	0.135	0.55	882.3
Ct.TMD vs Tt.Ar/FH										
Bone	Males		Females			Males		Females		
	r ²	Slope	y-int	p		r ²	Slope	y-int	p	
Femur	0.11	-5.9	1340	0.007	0.22	-8.8	1391	0.001	0.40	23.3
Radius	0.15	-22.2	1388	0.002	0.06	-12.7	1392	0.091	0.56	24.1