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Mapping the natural variation in whole bone stiffness and strength across skeletal sites

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Abstract

Traits of the skeletal system are coordinately adjusted to establish mechanical homeostasis in response to genetic and environmental factors. Prior work demonstrated that this 'complex adaptive' process is not perfect, revealing a two-fold difference in whole bone stiffness of the tibia across a population. Robustness (specifically, total cross-sectional area relative to length) varies widely across skeletal sites and between sexes. However, it is unknown whether the natural variation in whole bone stiffness and strength also varies across skeletal sites and between men and women. We tested the hypotheses that: 1) all major long bones of the appendicular skeleton demonstrate inherent, systemic constraints in the degree to which morphological and compositional traits can be adjusted for a given robustness; and 2) these traits covary in a predictable manner independent of body size and robustness. We assessed the functional relationships among robustness, cortical area (Ct.Ar), cortical tissue mineral density (Ct.TMD), and bone strength index (BSI) across the long bones of the upper and lower limbs of 115 adult men and women. All bones showed a significant ($p < 0.001$) positive regression between BSI and robustness after adjusting for body size, with slender bones being 1.7–2.3 times less stiff and strong in men and 1.3–2.8 times less stiff and strong in women compared to robust bones. Our findings are the first to document the natural inter-individual variation in whole bone stiffness and strength that exist within populations and that is predictable based on skeletal robustness for all major long bones. Documenting and further understanding this natural variation in strength may be critical for differentially diagnosing and treating skeletal fragility.

Keywords

Functional adaptation; Bone strength index (BSI); Robustness; Cortical area; Mineralization; pQCT

Introduction

The natural variation in skeletal robustness (specifically, total cross-sectional area relative to length) is a mechanically and clinically important trait. The broad range in bone robustness, as defined by Martin and Saller [1], is well tolerated within and between populations. Bone

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is a 'complex adaptive system,' which is a term used to describe systems that coordinately adjust multiple traits in response to genetic and environmental perturbations in order to establish system-level homeostasis [2–7]. For bone, the homeostasis of clinical interest refers to the biological processes that are involved in establishing and maintaining mechanical function. However, the flexibility in how bone establishes mechanical function, or stiffness [8], comes at a clinical cost, with individuals acquiring reduced fracture resistance through various biomechanical and biological pathways [9]. This phenomenon raises two primary issues that should be considered to better define and expand our ability to identify individuals with increased fracture risk. First, the adaptive process is not perfect [10]. Biological constraints in cellular activity (e.g. osteoclastic/osteoblastic driven modeling and remodeling) limit the degree to which traits can be adjusted to mechanically offset the natural variation in bone robustness. This in part explains why slender bones, those that are narrow relative to length, are less stiff and strong in relation to body size compared to more robust bones that are wide relative to length [10]. This natural variation in stiffness and strength, or functional inequivalence, has only been quantified for the tibia and has not been explicitly incorporated into clinical studies. Fully defining the magnitude of how bone stiffness and strength naturally vary is important. Both slender and robust bones perform adequately well under routine loading conditions [9,10]. However, slender bones are more at risk of fracturing when subjected to extreme loading conditions, such as military training and falls in the elderly [11–14]. Therefore, a segment of the population (i.e. individuals with a skeleton comprised of slender bones) is at risk of fracturing despite their bones being as well adapted as biologically possible to maximize stiffness while minimizing mass [15]. Second, cortical area (Ct.Ar) and cortical tissue mineral density (Ct.TMD) naturally vary relative to robustness [9,10,16], resulting in a circumstance wherein variations in Ct.Ar and Ct.TMD are superimposed on the natural variation in robustness. Understanding this variation is important for determining when the covariation between traits is impaired, resulting in reduced fracture resistance. Furthermore, how this covariation between morphological and compositional traits impacts clinical bone mineral density (BMD) assessments has yet to be defined.

Previously we found that the slender or robust phenotype of an individual is consistently represented throughout the appendicular skeleton. This suggests that the covariation among robustness, Ct.Ar, and Ct.TMD is system wide (Fig. 1) [17]. Moreover, slender bones, after adjusting for body size, demonstrated a 25–50% lower Ct.Ar and a 5–8% greater Ct.TMD, relative to robust bones, depending on the long bone considered [17]. However, this prior work did not establish whether the covariation of these skeletal traits resulted in similar strength differences across skeletal sites. Our previous work found that the slender tibiae of young adult men and women were two to three times less stiff compared to those with robust tibiae [10]. To put this 100–200% natural variation in bone stiffness into a clinical context, work by others reported 6.5–40.8% reductions in bone strength between fracture and non-fracture groups, depending on the skeletal site considered [13,18,19]. Therefore, the natural variation in stiffness we previously documented overshadows the mean differences others have reported in bone strength within and between men and women, and has yet to be clinically defined or acknowledged. How this variation presents itself across the major long bones of the appendicular skeleton is unknown. Taking our previous findings into

consideration, the goals of the current study are to quantify the natural variation in whole bone stiffness and strength across the major long bones and to systematically evaluate how this natural variation in strength can be attributed to the degree to which skeletal robustness, Ct.Ar, and Ct.TMD covary. We further tested whether a person showing less Ct.Ar or Ct.TMD for robustness at one skeletal site demonstrates this same deficit across all major long bones. This would provide insight in the degree to which impairments in the adaptive process are systemically versus locally influenced. Though diaphyseal fractures are much less frequent than those of metaphyses, the diaphysis provides us with a relatively simple model from which to establish basic principles of how the skeletal system coordinately adjusts multiple traits to establish mechanical homeostasis. These principles can then be translated to the metaphysis in future work, since these cortico-cancellous regions pose their own unique challenges [20,21].

Materials and methods

Sample

The sample used in this study consisted of 63 men and 52 women of African-American ethnicity. These individuals died in the Greater Cleveland area of Northern Ohio between 1910 and 1940, and their skeletal remains are presently curated by the Cleveland Museum of Natural History within the Hamann–Todd Osteological Collection. Individuals comprising this collection are predominately indigent and of low socioeconomic standing for this young 20th century region of early urban industrialism. This sample was specifically chosen due to its scientific value. It is rare to acquire substantial data from multiple human skeletal sites from which to estimate whole bone stiffness and strength based on engineering theory. These volumes of data, at such a high scan resolution (e.g. 100 μm voxel size), are not obtainable from most clinical and/or research databases given the expense and well-documented risks of radiation exposure. Moreover, anatomical collections are readily available for analysis, opposed to the time and expense required to collect multiple skeletal elements of substantial number from modern donors. Furthermore, this sample was potentially subject to a large amount of environmental noise, as they demonstrate a high propensity for acute infections and degenerative diseases [22–26]. Therefore, these individuals are expected to demonstrate a wide range of variation in how well skeletal traits were functionally adapted, increasing our ability to test to what degree variation among covariant traits is systemically affected. This complements our prior work focused on healthy, modern men and women of predominately European ancestry [9,10].

Individuals selected for this study were from 20 to 30 years of age, and consisted of no observable skeletal pathology that potentially impacted bone morphology and/or tissue level mechanical properties. Their cortices demonstrated no endocortical or intracortical resorption uncharacteristic for their age. This adult age range was chosen because the skeletons of these individuals represent the end product of the functional adaptation process during growth, wherein bone loss should be minimal. Skeletal elements in this study consisted of the left humeri, radii, second and third metacarpi, femora, and tibiae. Body height and weight were also documented for each individual at the time of autopsy; however, there may be inaccuracies in the data. Deceased individuals appropriated for this

osteological collection were not necessarily received immediately following death, leaving some individuals to have measured weights below their actual weight due to variable fluid loss and decomposition [27]. Moreover, documented weights were comprised of both direct measurement and estimates [28]. Notwithstanding the potential inaccuracies in the documented data, we chose to use reported body weight, along with a femoral head breadth measure obtained at the time of our analysis to serve as a complementary proxy for body size [29]. We previously reported that body weight minimally influenced the associations among covarying traits, and that using other proxies to estimate body weight (e.g. height and femoral head breadth) was suitable [17]. Comparisons between individuals included in this study and those of known body weight from our previous study [10] confirm that the effects of body weight minimally influence the expected associations among traits. Moreover, regression-based estimations of body weight, which are useful only for the population from which they are derived, would potentially introduce additional statistical error when using these values to adjust traits for body size.

Data acquisition and statistical analyses

Quantification of skeletal traits for each long bone was conducted using a pQCT, or peripheral quantitative computed tomography system (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany), as described previously [17]. Briefly, a single axial scan was taken at the 50% midshaft of each bone, as defined by longitudinal length (Le). Bone length was quantified in accordance with Ruff [30]. Images were acquired at 0.10mm×0.10mm in-plane pixel size, and the quality and consistency of scanned images were maintained via a daily calibration scan of a standard phantom with known densities.

Anatomical orientation was maintained for each scan using custom adjustable holders and a line level, allowing each bone to be centered within the gantry with the anterior surface up. For each image slice, total cross-sectional area (Tt.Ar), cortical area (Ct.Ar), mean gray value, and the rectangular area moments of inertia about the anteroposterior (I_{AP}) and mediolateral (I_{ML}) axes were quantified using ImageJ freeware (NIH), along with a custom macro (Momentmacro.J; www.hopkinsmedicine.org/fac/mmacro.htm) that calculates cross-sectional morphological traits that are correlated with whole bone mechanical properties. From these data, robustness was calculated by dividing Tt.Ar by Le (Tt.Ar / Le), which was validated in prior work [17], creating an index related to the biological relationship between longitudinal and transverse bone growth. Cortical tissue mineral density (Ct.TMD) was derived from the mean gray value using calibration constants. In a prior study, we validated that variation in Ct.TMD correlated with ash content and porosity for fresh human cadavers [10]. We further validated that Ct.TMD correlated with the tissue-modulus of bone samples machined from tibial diaphyses and subjected to 4-point bending tests. To obtain a proxy for whole bone stiffness and strength, since it cannot be directly measured from this osteological museum collection, Ct.TMD was multiplied by I_{ML} to generate a bone strength index (BSI). A validation study was conducted to confirm that BSI accurately estimated whole-bone bending stiffness (EI) and the maximum bending moment from pQCT derived images. Fifteen long bones readily available to us from three cadaveric individuals under the age of 50 years (2 females, 1 male) were scanned at the 50% midshaft site using pQCT. After each bone was scanned, the cortical diaphyses were loaded to failure in four-point

bending at 0.05mm/s using a servohydraulic material testing system (Instron 8872, Instron Corp., Canton, MA), as described previously [31]. These long bones consisted of the left femora, tibiae, humeri, radii, and ulnae of each individual. For each bone, the distance between the two lower supports (L) was adjusted so that they contacted the bone at the 25% and 75% anatomical sites. The upper two supports were then placed at the one-third and two-third expanse of the lower supports. Data derived from the load–deflection graphs included stiffness, failure load, maximum load, postyield deflection, and work-to-fracture, with deflection being corrected for system compliance. To obtain EI, load and deflection for the geometry of the loading setup relative to each bone were corrected using the following equation:

$$EI = (P/y) \left(a^3/3 - a^2L/4 \right)$$

where, P/y = stiffness derived from the load–deflection curve, L = the span of the lower two supports, and a = the span of the upper loading points (one-third L). To obtain the maximum bending moment, a measure of bone strength in whole bone mechanical terms, the following equation was used:

$$\text{Maximum Bending Moment} = (\text{maximum load} \times b/2),$$

where b = the span between the upper and lower supports (i.e. $(L-a)/2$) [32]. Linear regression analysis was used to determine whether BSI derived from pQCT images accurately estimated EI and the maximum bending moment directly measured from four-point bending tests. Our current sample cohort is from a museum collection and cannot be directly validated using destructive techniques. However, we expect that our prior study correlating Ct.TMD with ash content and porosity [10], and our current study correlating BSI with the actual bending stiffness and strength of fresh cadaveric bone, provide reasonable validation for the use of pQCT in assessing morphology, Ct.TMD, and whole bone mechanical properties.

Minitab 16.1.1 (Minitab Inc., State College, PA) software was used for all statistical analyses. The data were normally distributed. To adjust for body size differences between individuals, we performed partial linear regressions among all three variables and body mass times bone length (BM–Le), which is proportional to the bending moment applied to each bone [33]. Residuals resulting from these regressions were used in all subsequent analyses. They represent variation within each trait that is not explained by the weight of each individual or the moment arm length of each bone; both of which significantly influence the phenotypic plasticity of bone [30]. For each bone, linear regression analyses were performed to test the hypotheses that 1) the degree of variation in stiffness and strength among slender and robust bones is relatively consistent across bones of a similar phenotype; and 2) Ct.Ar and Ct.TMD, independent of body size and diaphyseal robustness, intraskeletally covary in a consistent manner wherein these skeletal traits for a given bone predict that of another.

Natural variation in stiffness and strength

Previously it was reported that individuals within this population showed covariation among robustness, Ct.Ar, and Ct.TMD throughout the appendicular skeleton [17]. After adjusting for body size, Ct.Ar was found to be positively associated with robustness ($r^2 = 0.12\text{--}0.65$, $p < 0.006$) for all skeletal elements. Therefore, for our current analyses partial linear regressions were performed on data from each bone, comparing whole bone stiffness and strength (BSI) to robustness after adjusting for body size (BM–Le). Additionally, we performed regressions to calculate the degree to which whole bone stiffness and strength varied between slender and robust skeletal elements. To do so in a standardized manner, robustness was regressed against BM–Le. The residuals were then plotted to identify cohorts within the upper 90th and lower 10th percentiles that demonstrated greater and lesser robustness, respectively, for their body size (Fig. 2). Once the cohorts were defined, the BSI of all individuals within each percentile group was averaged, providing an estimate of the variation present within the population. Following this, the average of the upper 90th percentile (i.e. individuals robust for their body size) was divided by the average of the lower 10th percentile (i.e. individuals slender for their body size) to calculate the magnitude to which slender bones for body size were less stiff and strong compared to bones that are robust relative to body size. To ensure the accuracy of the defined 90th and 10th percentiles, body size was also compared to confirm that this standardized approach resulted in cohorts with similar body sizes but different bone sizes. Pearson correlations were performed to assess whether BSI residuals significantly correlate across skeletal sites.

Intraskkeletal covariation of cortical area and mineral density

Since we previously reported that robustness, Ct.Ar, and Ct.TMD systemically covary, irrespective of body size [17], we assessed the degree of covariation between Ct.Ar and Ct.TMD when also accounting for robustness. Thus, we tested whether individuals having a given bone that is slender with less Ct.Ar than expected for their slenderness and body size also demonstrate this deficit at all skeletal sites. Likewise, we tested whether individuals that have a robust bone with greater Ct.Ar than expected for their robustness and body size also demonstrate greater Ct.Ar at all skeletal sites. To investigate these questions, both robustness and body size were taken into account since Ct.Ar significantly varies with both traits. Body size adjusted residual values for Ct.Ar and Ct.TMD were regressed against robustness. The residual values represent variation in these traits that is not explained by body size or robustness. These residuals were then used to identify which long bones and skeletal traits correlate significantly. Additionally, Pearson correlations were performed to assess the significance of associations among skeletal elements for Ct.Ar and Ct.TMD.

Results

Natural variation in stiffness and strength

The relationship between whole bone stiffness, strength, and robustness was assessed to map the degree to which these bone traits naturally vary across the major long bones. Our validation study to determine whether BSI derived from pQCT images accurately estimated EI and maximum bending moment directly measured from four-point bending tests found all long bones to have a significant association between BSI and EI ($r^2 = 0.98$, $p < 0.001$) (Fig.

3a) and BSI and maximum bending moment ($r^2 = 0.92$, $p < 0.001$) (Fig. 3b). This confirms that BSI accurately estimates whole-bone stiffness and strength as directly measured by EI and maximum bending moment, respectively. Confident in our use of BSI as a proxy for whole bone stiffness and strength, all bones showed a significant ($p < 0.001$) positive regression between BSI and robustness after adjusting for body size (Figs. 4a–b), indicating that slender bones for body size were less stiff and strong than robust bones for body size (Table 1). Slender bones for body size were 1.7–2.3 times less stiff and strong in men and 1.3–2.8 times less stiff and strong in women compared to robust bones for body size (Fig. 5). On average, both men and women demonstrated slightly less variation in stiffness and strength in the lower limbs compared to the upper limbs. In terms of the major long bones of the upper and lower limbs of men and women, the femora showed the least amount of inter-individual variation in stiffness and strength with slender femora being 1.7 times less stiff and strong in men and 1.3 times less stiff and strong in women, compared to robust femora. The greatest degree of variation in stiffness and strength in men and women was within the radius with slender radii demonstrating 2.3 times less stiffness and strength in men and 2.8 times less stiffness and strength in women compared to more robust radii.

With a significant degree of natural variation in stiffness and strength present within each skeletal element examined, we then compared these traits across skeletal sites. Not surprisingly, significant correlations in the variation of stiffness and strength were observed throughout the major long bones (Table 2) of both men and women, suggesting that an individual with a bone less stiff and strong relative to the population also demonstrated less stiffness and strength in other bones of the appendicular skeleton. The variation in stiffness and strength of all bones was highly correlated in men. The 3rd metacarpal were the best correlate of body size adjusted BSI in all long bones, followed by the humeri, tibiae, 2nd metacarpal, femora, and radii, respectively. Similarly, in women, all bones demonstrated significant correlations. However, the humeri were generally the best correlate of body size adjusted BSI in this cohort, followed by the radii, tibiae, femora, 2nd metacarpal and 3rd metacarpal, respectively. As expected, the most significant correlation was between the metacarpal in both men ($r = 0.81$, $p < 0.001$) and women ($r = 0.73$, $p < 0.001$).

Intraskkeletal covariation of Ct.TMD adjusted for body size and robustness

The degree to which Ct.TMD relates to one another across skeletal elements, after adjusting for body size and robustness, was assessed. Significant correlations were prevalent when comparing adjusted Ct.TMD values across bones (Table 3) (Fig. 6a). In men, the radii were consistently the best correlate of Ct.TMD across skeletal sites. Mineral density of the radii correlated best with the metacarpal ($r = 0.66$, $p < 0.001$), followed by the femora ($r = 0.56$, $p < 0.001$), tibiae ($r = 0.54$, $p < 0.001$), and humeri ($r = 0.54$, $p < 0.001$). To a lesser degree, the humeri, femora, tibiae, and metacarpal were also strong correlates of Ct.TMD throughout the appendicular skeleton. In women, the upper and lower long bones significantly correlated with one another in regard to Ct.TMD, with the tibiae being the best correlate in this sexual cohort ($r = 0.66$ – 0.49 , $p < 0.001$) across skeletal sites. The 2nd and 3rd metacarpal did not significantly correlate with the femora ($r = 0.17$ – 0.03 , $p > 0.05$). As expected, the most significant correlation was between the metacarpal for both men ($r = 0.88$, $p < 0.001$) and women ($r = 0.84$, $p < 0.001$).

Intraskkeletal covariation of Ct.Ar adjusted for body size and robustness

For adjusted Ct.Ar, the skeletal elements of men significantly correlated with one another (Table 3) (Fig. 6b). Similar to the Ct.TMD results, the radii were consistently the best correlate of Ct.Ar across all skeletal sites. The radii correlated best with the 2nd metacarp (r= 0.76, p < 0.001), followed by the 3rd metacarp (r= 0.73, p < 0.001), humeri (r= 0.67, p < 0.001), femora (r= 0.68, p < 0.001), and tibiae (r= 0.63, p < 0.01). The outcome in women was similar to that for men with all bones significantly correlating. However, the femora were the best correlate of adjusted Ct.Ar across all skeletal elements (r= 0.71–0.54, p < 0.001). Overall, the metacarp were the weakest correlates in this cohort, but as expected demonstrated the most significant correlation with one another in both men (r = 0.80, p < 0.001) and women (r = 0.78, p < 0.001).

Discussion

We estimated the magnitude of the inter-individual variation in strength and mapped this across the major long bones to establish how strength naturally varies among individuals and across long bones. Our findings revealed a 30–180% difference in whole bone stiffness and strength between slender and robust bones. The magnitude of this inter-individual variation in whole bone stiffness and strength overshadows the differences in stiffness and strength reported between fracture and non-fracture groups in clinical studies [13,18,19]. Thus, this natural variation in bone strength warrants clinical consideration when assessing fracture risk. Another important outcome of this study is that the skeletal traits of the metacarp significantly correlate with the major long bones of the appendicular skeleton. Confirming this association between skeletal elements advocates for the use of the many longitudinal studies, both historic and contemporary, that are consisted of a plethora of hand radiograph data. These databases are ideal for investigating the underlying biology [16,34] as longitudinal studies would define how these interactions develop during growth and how they change with aging.

The findings of our study demonstrate that all major long bones of both African-American men and women comprising our study sample have a high degree of natural variation in stiffness and strength, after accounting for body size, which is predictable based on the natural variation in robustness. This systemic phenomenon of variation in stiffness and strength is not an artifact of the population sample chosen. These findings are statistically consistent with previous reports of stiffness and strength variation in the tibiae of young adults of predominately European ancestry that enjoyed a moderately healthier lifestyle [10]. When comparing men and women derived from the Hamann–Todd Osteological Collection to those of healthy, live men and women of the same age [10], an analysis of covariance (ANCOVA) demonstrated similar tibial robustness (p < 0.309 and p < 0.783, respectively) and Ct.Ar (p < 0.926 and p < 0.751, respectively) traits for body size. Moreover, when both robustness and Ct.Ar were adjusted for body size and regressed against one another, the two samples demonstrated a similar relationship between traits as expected, with more slender individuals having less Ct.Ar. Although this skeletal collection may be criticized for its selective mortality [35–37], as these individuals represent members of the population that died prematurely, this comparison suggests that the greater proclivity for acute infectious

and degenerative diseases within this collection did not negatively impact skeletal traits. Therefore, these individuals were suitable for the systematic assessment of robustness and associated skeletal traits within a population wherein longitudinal and transverse diaphyseal growth was potentially impacted. Further research is required to confirm this assumption.

The results of this study and our previous research [10] are contrary to earlier proposals that suggest biological compensation among traits restrains phenotypic variance, and thus leads to functional equivalence [6]. All bones exhibit a similar range of impairment in the degree to which Ct.Ar and Ct.TMD can be adjusted to compensate for the natural variance in robustness. This indicates how it is possible for the skeletal system to tolerate a rather large range of morphological variation while retaining function. A slender bone may be susceptible to fracturing throughout life under extreme loading conditions, and a robust bone may be at risk of fracturing later in life as endocortical resorption and basic multicellular unit (BMU)-based remodeling increasingly accumulate [21]. However, both phenotypic extremes appear to perform well under routine loading, as there are large safety factors in place to curtail fractures [38]. The variance in skeletal structure appears not to hamper a given skeletal element's ability to withstand routine loads. However, we assert that the biomechanical trade-off, wherein a modest degree of variation in stiffness and strength in the skeletal system is tolerated in modern populations, may increase the risk of fracturing under extreme loading that often occurs during intense exercise and falls. When stiffness is reduced relative to body size, bone is also proportionally weaker. Greater tissue strains generated through extreme loading would result in considerable damage to the cortical matrix, increasing the probability of fracturing [39]. This natural variation in stiffness and strength apparent in slender bones may in part explain the high prevalence of stress fractures among military recruits [9,10,12,13] and athletes [14]. Moreover, biological compensatory deficits may also help explain why slenderness is often a predictive indicator of fracture risk among the elderly [40,41], though we are careful not to generalize our diaphyseal findings to the cortico-cancellous structure of the hip.

That an individual exhibiting reduced Ct.Ar and/or Ct.TMD at one skeletal site can be expected to have a similar deficit at other sites is clinically important. This would suggest that a reduction in tissue strength may be diagnosed and treated using a global methodology. This raises the question of how one might clinically diagnose and treat individuals in relation to the natural variation in bone stiffness and strength apparent within the population. Also, how this natural variation in stiffness and strength would be revealed in cortico-cancellous sites such as the distal radius and proximal femur has yet to be fully understood [20,42]. Assuming this variation could be accurately diagnosed in the clinic, it remains unclear how a physician would effectively treat impairments in trait covariation. Presumably, differential prophylactic treatment strategies would be needed, since what might be best for a slender-boned individual demonstrating reduced Ct.Ar and increased Ct.TMD may differ for a more robust-boned individual demonstrating increased Ct.Ar and reduced Ct.TMD. Based on engineering principles, individuals with slender bones would need to be treated with anabolic therapies that increase periosteal expansion [43,44], particularly since we would expect these individuals to arrive at a given fracture-risk threshold sooner in life as they already demonstrate reduced Ct.Ar relative to body size as young adults, prior to age-related bone loss. In contrast, individuals with robust bones

already have the benefit of a large external size. Thus, treatments for these patients may be geared toward optimizing cortical thickness [21,45]. We recognize that the personalization of these treatment strategies is currently not a clinical reality [46]. Nevertheless, our data provide important insight into specific inter-individual differences in the set of traits acquired by individuals to establish function. Defining the variation in stiffness and strength within a population may allow clinicians to take better advantage of the growing arsenal of prophylactic treatments to optimize bone strength on a personalized basis [46].

This study provides further insight into how the skeletal system maintains mechanical function when faced with genetic and environmental perturbations. Our experimental approach has been to use the variation in robustness as a natural perturbation and to study how the system coordinately adjusts other traits to maximize stiffness while minimizing mass. Other research has often characterized a population based on group means [13,18,19]. However, our research, showing there are patterns in the way individuals coordinate traits, allows us to examine a population in a novel way that is not limited to group means but that now compares all individuals in a population simultaneously (Fig. 7). This allows us to systematically evaluate these trait interactions individually and then, by regression analysis, to identify whether two populations show similar trait–trait interactions uniformly across the entire population (e.g. difference in y-intercept) or show similar bone traits at one morphological extreme but different at the other (e.g. difference in slope). This pattern in the way the skeletal system coordinately adjusts for robustness, Ct.Ar, and Ct.TMD also provides a novel way to evaluate how well a skeleton is adapted to body size. For example, a poorly adapted skeleton would be one that demonstrates reduced Ct.Ar and/or Ct.TMD in relation to the population mean for a given robustness. Given differences in the set of traits acquired by slender versus robust bones, it would be more accurate to assess whether a genetic or environmental perturbation affected Ct.Ar and/or Ct.TMD by first adjusting for an individual's robustness. However, whether the natural variation in stiffness and strength systemically detected in the appendicular skeleton of an individual is attributable to the same biological compensatory impairment in all bones has yet to be elucidated. Determining this is important toward identifying whether a global or local treatment is required to effectively treat individuals demonstrating significant degrees of variation in stiffness and strength that may threaten their mechanical homeostasis.

We observed a general trend that weight-bearing bones show less variation among traits compared to nonweight-bearing bones. Moreover, bones that are nearer to each other tend to show higher correlations. Although we ranked these correlations (highest to lowest), we recognize that our sample cohort had the power to test for significant correlations but not to rigorously evaluate whether one bone correlated better with another bone. A much larger dataset would be needed to draw meaningful conclusions about the biological and functional significance of the difference in correlation coefficients across skeletal sites.

Currently, a change in strength on the level of 20–30% is of clinical concern. Our findings report a much greater magnitude of 30–180% when the natural variance in robustness is considered. This disparity in strength is present prior to the aging process, placing some individuals at greater risk of fracturing. Recognizing and acknowledging this natural variation in whole bone stiffness and strength may help us identify individuals at risk of

fracturing earlier in life, so that they may be treated more strategically based on their biomechanical demands. However, to benefit clinicians, future work must translate these findings of stiffness and strength variance in the diaphysis to the cortico-cancellous region of the metaphysis (i.e. distal radii, lumbar vertebrae, and proximal femora). Moreover, the impact this variation has on other fracture resistance properties (e.g. ductility, toughness, porosity) will need to be determined.

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Fig. 1. Example of systemic intraskeletal covariance of traits for four individuals within the study population. Bone length and body mass are similar between each set of men and women.

Fig. 2.

Example of how the natural variation in whole bone stiffness and strength was calculated to determine the magnitude by which slender bones were less stiff and strong for body size compared to robust bones. Since there is a small but significant correlation between robustness and body size ($r^2 = 0.21$, $p < 0.0007$), the residuals as indicated by the vertical dashed arrows indicate the natural variation in robustness that we are studying. This figure demonstrates that individuals of similar body size can express wide variation in robustness.

Fig. 3.

Linear regression results of bone strength index (BSI) validation study. a) Regression of directly measured whole bone stiffness (EI) against BSI, and b) regression of directly measured maximum bending moment against BSI.

Fig. 4.

Example of linear regressions derived from comparisons between bone strength index (BSI) and robustness after accounting for body size via partial linear regression analysis. a) Humeri, and b) femora.

Fig. 5. Schematic mapping of the degree of variation in strength and stiffness among all bones examined in both men and women. Fold values reported represent the degree to which slender bones, relative to body size, were less stiff and strong compared to robust bones, relative to body size.

Fig. 6.
Example of linear regressions between Ct.TMD and Ct.Ar values of femora and humeri after accounting for body size and robustness. a) Ct.TMD, and b) Ct.Ar.

Fig. 7.

The schematic illustrates the differences between previous research approaches that emphasize population differences focused on a 'typical' phenotype derived from an analysis of group means. Our current approach highlights the natural variation that exists within and across populations, and shows how individuals acquire unique sets of traits by adulthood. By acknowledging the pattern by which the system coordinates multiple traits, we now view this continuum of trait sets as the 'typical' bone phenotype.

Table 1

Results of partial linear regression analyses between BSI and robustness, while accounting for body size ($BM \times Le$).

Bone	Males				Females			
	r ²	Slope	y-Int	p	r ²	Slope	y-Int	p
Femur	0.66	45420	0.0	0.0001	0.74	36103	0.0	0.0001
Tibia	0.56	25679	0.0	0.0001	0.66	20874	0.0	0.0001
Humerus	0.79	18398	0.0	0.0001	0.81	11261	0.0	0.0001
Radius	0.73	8902	0.0	0.0001	0.80	6866	0.0	0.0001
2nd MC	0.52	656.1	0.0	0.0001	0.69	559	0.0	0.0001
3rd MC	0.52	683.2	0.0	0.0001	0.71	521	0.0	0.0001

Table 2

Pearson correlations (r) of whole bone stiffness (BSI) values between skeletal elements, while accounting for body size ($BM \times Le$). Bold values denote significance at $p < 0.05$.

Bone	Tibia		Humerus		Radius		2nd MC		3rd MC	
	M	F	M	F	M	F	M	F	M	F
Sex										
Femur	0.59	0.51	0.60	0.50	0.38	0.46	0.48	0.32	0.51	0.17
Tibia			0.49	0.51	0.43	0.53	0.62	0.44	0.72	0.42
Humerus					0.39	0.56	0.58	0.51	0.53	0.53
Radius							0.48	0.38	0.54	0.36
2nd MC									0.81	0.73

Correlations (r) of intraskeletal comparisons for skeletal elements, after accounting for body size and robustness. Bold values denote significance at $p < 0.05$.

Table 3

Bone	Tibia		Humerus		Radius		2nd MC		3rd MC	
	M	F	M	F	M	F	M	F	M	F
Ct.TMD										
Femur	0.52	0.51	0.79	0.53	0.56	0.38	0.44	0.17	0.39	0.03
Tibia			0.62	0.64	0.54	0.66	0.44	0.56	0.34	0.49
Humerus					0.54	0.48	0.46	0.38	0.50	0.25
Radius							0.66	0.67	0.66	0.61
2nd MC									0.88	0.84
Ct.Ar										
Femur	0.68	0.71	0.65	0.69	0.68	0.60	0.49	0.54	0.58	0.56
Tibia			0.62	0.55	0.63	0.56	0.39	0.44	0.44	0.38
Humerus					0.69	0.67	0.66	0.48	0.69	0.49
Radius							0.76	0.53	0.73	0.51
2nd MC									0.80	0.78