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The Interindividual Variation in Femoral Neck Width Is Associated With the Acquisition of Predictable Sets of Morphological and Tissue-Quality Traits and Differential Bone Loss Patterns

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ABSTRACT

A better understanding of femoral neck structure and age-related bone loss will benefit research aimed at reducing fracture risk. We used the natural variation in robustness (bone width relative to length) to analyze how adaptive processes covary traits in association with robustness, and whether the variation in robustness affects age-related bone loss patterns. Femoral necks from 49 female cadavers (29–93 years of age) were evaluated for morphological and tissue-level traits using radiography, peripheral quantitative computed tomography, micro–computed tomography, and ash-content analysis. Femoral neck robustness was normally distributed and varied widely with a coefficient of variation of 14.9%. Age-adjusted partial regression analysis revealed significant negative correlations ($p < 0.05$) between robustness and relative cortical area, cortical tissue-mineral density (Ct.TMD), and trabecular bone mineral density (Ma.BMD). Path analysis confirmed these results showing that a one standard deviation (SD) increase in robustness was associated with a 0.70 SD decrease in RCA, 0.47 SD decrease in Ct.TMD, and 0.43 SD decrease in Ma.BMD. Significantly different bone loss patterns were observed when comparing the most slender and most robust tertiles. Robust femora showed significant negative correlations with age for cortical area ($R^2 = 0.29$, $p < 0.03$), Ma.BMD ($R^2 = 0.34$, $p < 0.01$), and Ct.TMD ($R^2 = 0.4$, $p < 0.003$). However, slender femora did not show these age-related changes ($R^2 < 0.09$, $p > 0.2$). The results indicated that slender femora were constructed with a different set of traits compared to robust femora, and that the natural variation in robustness was a determinant of age-related bone loss patterns. Clinical diagnoses and treatments may benefit from a better understanding of these robustness-specific structural and aging patterns. © 2012 American Society for Bone and Mineral Research.

KEY WORDS: BIOMECHANICS; FEMUR; AGING; ROBUSTNESS; BONE LOSS; pQCT; PATH ANALYSIS; STRENGTH; FUNCTIONAL INTERACTIONS

Introduction

Identifying skeletal trait variants that increase the risk of hip fractures is critical for reducing the associated morbidity, mortality, and cost of these fractures.[1] These variants are typically identified by comparing the average traits measured for fracture cases to those of age- and sex-matched nonfracture controls. This approach allows for a dichotomous outcome, such that physical traits contributing to fracture risk can be either greater or less than the population average. Not surprisingly, studies using this approach found fracture cases to have either narrow[2–4] or wide[3,5–7] femoral necks compared to controls. Despite finding small but significant differences (2%–3%) in bone width between fracture and nonfracture groups, these studies also showed that fracture cases expressed the full range in bone size. This means that, like bone mineral density (BMD), there is sufficient overlap in bone width between fracture and nonfracture groups to reduce confidence in using a single trait as a general clinical diagnostic measure. Thus, it is unclear whether we are missing opportunities to identify structural and tissue-quality traits contributing to fracture risk using this traditional approach.

We propose that developing a better understanding of how a complex system like bone establishes and maintains mechanical

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function will benefit efforts to identify traits contributing to fracture risk. Complex systems like bone have strong adaptive processes in place to compensate for common variants; this results in function being defined by the coordination of multiple traits.[8] Consequently, evaluating an individual's skeletal strength may come down to assessing the set of interrelated traits they acquired during growth and maintained during aging. Translating this concept into a clinically useful technology would require finding a pattern in the way trait sets are acquired among individuals, and then determining if this pattern is biomechanically and biologically meaningful.

We have used the interindividual variation in robustness, a measure of bone cross-sectional size relative to length, as a model to systematically evaluate how morphological and tissue-level mechanical properties are coordinated to establish whole-bone mechanical function.[9,10] All morphological traits are expected to be adaptive to mechanical loads, including external size.[11] Some individuals are prone to having narrow bones, independent of height and weight, and this should not be viewed as a failure to adapt mechanically to applied loads. Rather, because external size is nonlinearly related to whole bone stiffness, the interindividual variation in external size should be associated with large functional changes in other morphological traits and tissue-level mechanical properties in order for all individuals in a population to have bones that are adapted to support physiological loads.

Prior work in mouse[9] and human[12] long bone identified functional interactions among morphological traits and tissue-level mechanical properties that accompany the natural variation in robustness. Functional interactions among traits have also been identified in corticocancellous structures like the vertebral body[13] and the proximal femur.[14] However, the adaptation process is more complex in structures like the proximal femur because it involves functional interactions among morphological traits and tissue-level mechanical properties for both cortical and trabecular tissue types. Recent work showed that femoral neck volume correlated negatively with volumetric BMD (vBMD), but was uncorrelated with bone mineral content (BMC), suggesting that bone traits are adapted so that similar amounts of material are distributed over varying volumes.[14] To advance the idea that this pattern of trait interactions has biological and biomechanical meaning requires further analyses using invasive methods to identify the morphological and tissue-quality traits that are being coordinated to produce functionally adapted structures.

The idea that individuals acquire sets of traits specific to external size is important, because it may complicate our understanding of the aging process. First, although slender and robust femora are expected to acquire different sets of traits by adulthood, it remains to be determined whether age-related bone loss patterns vary with robustness. Second, the bone-width specific trait sets acquired during growth and maintained during aging are expected to be adapted to compensate for loads incurred during daily activities. How these trait sets differentially affect bone strength during a fall to the side is unknown. In this study, we conducted a biomechanical analysis to test the hypotheses that variation in external size of the femoral neck is associated with predictable functional changes in morphology and tissue-quality, and that slender and robust femora age differently.

Materials and Methods

Sample population

The sample population included 49 female cadaveric femora, which were acquired through the Ohio Valley Tissue and Skin Center (Cincinnati, OH, USA). The population ranged from 29 to 93 years of age with a mean and standard deviation (SD) of 68.2 ± 14.6 years. Race/ethnicity, weight, and height were available for a small subset of the cohort and thus could not be included in the analysis. Medical history was unavailable and consequently femora could not be segregated for prior use of prophylactic treatments. Cause of death was wide ranging, but no individuals died subsequent to a hip fracture.

Bone morphology

Gross morphological traits of the proximal femur (Fig. 1) were measured from plain film radiographs using published methods.[15] Radiographs were acquired (Faxitron Bioptics, LLC, Linconshire, IL, USA) with an aluminum step wedge (Gammex, Inc., Middleton, WI, USA) included for exposure calibration. Radiographic exposure was set at 1 mA and 50 kV for a duration of 0.7 minutes on direct exposure film (Biomax MS film; Eastman Kodak Company; Rochester, NY, USA). The femoral shaft was held in an anteroposterior position with a clamp, so the neck axis was visually perpendicular to the X-ray beam. The direct exposure film was digitized using a high resolution scanner (10000 XL; Epson, Inc., Long Beach, CA, USA) and analyzed with ImageJ (version 1.44f; U.S. National Institutes of Health, Bethesda, MD, USA). Neck axis length (NAL) was measured as the linear distance from the lateral aspect of the greater trochanter to the apex of the femoral head, passing through the center of the femoral head and neck. Head diameter was obtained by fitting a circle to the outline of the femoral head. Neck shaft angle (NSA) was measured as the angle formed between the neck axis and the shaft axis.

Cross-sectional morphology and tissue-mineral density

Morphological traits were quantified using peripheral quantitative computed tomography (pQCT) (XCT2000L; Stratec Medizintechnik, Gmbh., Pforzheim, Germany). Cross-sectional images were acquired at 2-mm increments between the base of the head and the base of the trochanter with an in-plane pixel size of $0.16\,\text{mm} \times 0.16\,\text{mm}$. Measurement quality was assured by conducting a calibration scan daily using a standard phantom with known densities. Scans were obtained for the full length of the femoral neck, from the greater trochanter to the femoral head. The femoral neck was aligned perpendicular to the beam by holding the femoral shaft at a complementary angle to the neck shaft angle. The scanned region was fully submerged in saline solution.

The cortex was manually segregated from trabecular bone. Femoral neck traits were analyzed using ImageJ, and these included total cross-sectional area (Tt.Ar), marrow area (Ma.Ar), cortical area (Ct.Ar), cortical tissue mineral density (Ct.TMD),

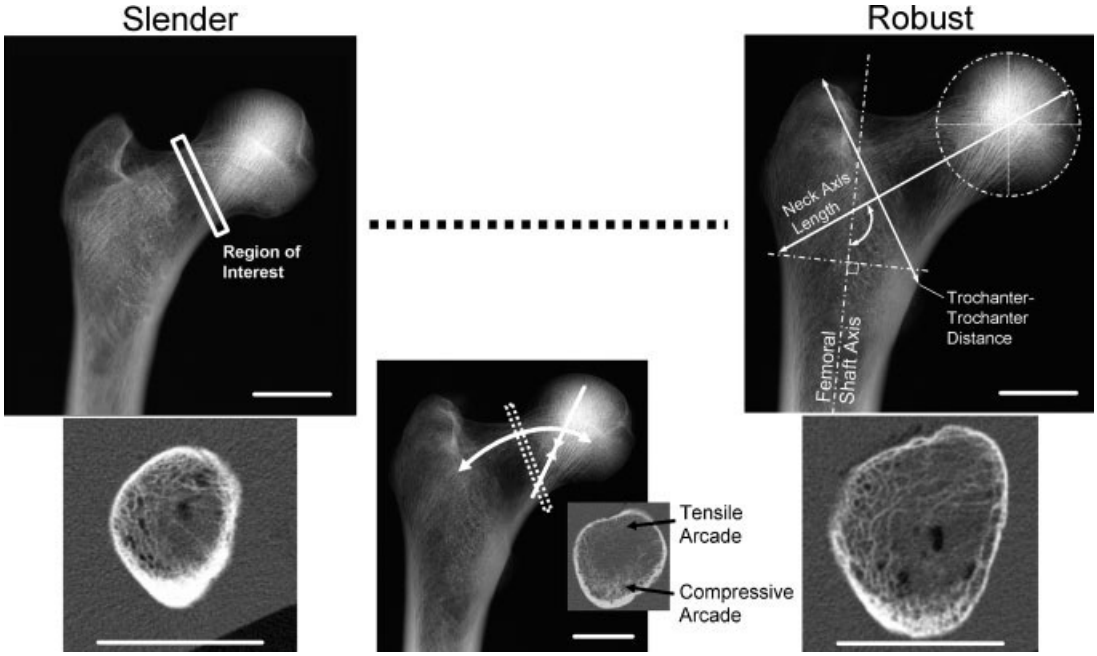

Fig. 1. Radiographic images of slender and robust femora and the associated cross-sections derived from pQCT depict the natural variation in bone size that exists among individuals. The region of interest (narrow neck region) is indicated on the slender image, and the manner in which gross morphological measures were made are indicated on the robust image. All white bars are 25 mm in length.

marrow-bone mineral density (Ma.BMD), robustness (Tt.Ar/NAL), and relative cortical area (RCA = Ct.Ar/Tt.Ar). Grayscale values of the cortical and trabecular regions were converted to Ct.TMD and Ma.BMD, respectively, using calibration constants derived from the phantom. The grayscale value of cortical bone included bone voxels only, and consequently we referred to the mineral density as tissue-mineral density. The marrow space included both bone and non-bone voxels, and consequently we referred to the mineral density as bone mineral density. For this study, femoral neck traits were reported for the narrow neck region, which was defined as the site with the smallest total area (Tt.Ar). Because cortical and trabecular traits vary along the femoral neck,[14] we chose the narrow neck region to standardize the analysis site among samples. Intrarater repeatability was assessed by measuring traits at two distinct times using the same hardware and software. No significant difference, based on a *t* test, was found between repeat measures for Tt.Ar ($p < 0.81$), Ma.Ar ($p < 0.48$), Ct.Ar ($p < 0.19$), RCA ($p < 0.88$), NAL ($p < 0.77$), Ct.TMD ($p < 0.96$), and Ma.BMD ($p < 0.61$). A validation study was conducted using a subset of the femora ($n = 26$) to relate the Ma.BMD measured by pQCT to indices of trabecular architecture measured using microCT (Supporting Information, Appendix 1). We further tested whether the Ct.TMD measured by pQCT could also be used as an indicator of tissue-mineralization.

Biological constraints affecting compensation

If a common biological strategy was used to establish function during growth for a population expressing the normal range in robustness, then functionally related traits would be expected to correlate across an adult population.[9,12,16] We postulated that the femoral neck would show functional trait interactions among robustness, cortical area, and tissue-mineral density similar to that reported for mouse femora[9] and human tibias.[12] However, because the femoral neck also includes trabecular bone, we postulated that trabecular architectural traits, particularly those related to tissue-stiffness (eg, BV/TV), will correlate negatively with robustness. We tested for these functional interactions by conducting a series of univariate regression analyses to identify significant correlations among cortical and trabecular traits. We then conducted a multivariate analysis to test whether the cohort exhibited a pattern in the way these traits covaried. Multiple regression and principal components analysis test whether traits are related, but make no specific assumption about the underlying biology. We used path analysis, because this multivariate analysis allowed us to not only test whether select traits are related, but to also test the hypothesis that traits are related in a particular way that reflects a common biological strategy to build functional structures that maximize stiffness while minimizing mass.[17]

For the path analysis, we arranged the traits and specified the direction of the arrows among the traits to test whether variation in robustness was accompanied by coordinated changes in the amount of cortical bone (Ct.Ar, RCA) and tissue-level stiffness. Tissue-level stiffness, although not directly measured, is proportional to the mineral content of the cortical shell (Ct.TMD) and trabecular mass (Ma.BMD). Path coefficients, which represent the magnitude of the direct and indirect relationships among traits, were calculated using standardized (*Z*-transformed) data (LISREL v.8.8; Scientific Software International, Lincolnwood, IL, USA). Structural equations were constructed using the path coefficients to specify the interconnected relationships. For traits with direct and indirect connections, the structural equations were rederived in terms of

the independent traits (robustness, age, Ct.Ar) and these are reported as the reduced form equations. Observed and model-implied covariance matrices were compared using maximum likelihood estimation. Chi-squared values with an associated p value greater than 0.05 indicate that the model adequately fits the data. The root mean square error of approximation (RMSEA), which is a measure of fit that is adjusted for population size, was also reported as an additional fit index. For RMSEA, the p value represents the significance of fit with $p < 0.05$ indicating a close fit.

Mechanical testing

To assess how the functional interactions among traits affect bone strength, 38 of the proximal femora were subjected to a conventional load condition that simulated a fall to the side.[18] Femora were sectioned 4.5 inches inferior to the lesser trochanter to isolate the proximal femur. The shaft was embedded in a square aluminum channel using orthodontic acrylic (Dentsply International, Milford, DE, USA) with the femoral neck in 15 degrees of internal rotation. Impressions of the greater trochanter were made in a Petri dish filled with Bondo (3M, Maplewood, MN, USA), which was necessary to distribute the load across the greater trochanter during testing. Femora were placed in a simulated fall configuration with 10 degrees of rotation in the coronal plane, and held in a custom-designed fixture with the greater trochanter sitting in the preformed impression. A compressive force was applied to the femoral head through stainless steel hemispherical cups to simulate acetabular contact. Each femoral head was matched to the best fitting cup size, which ranged from 20 to 29 mm radii in 1-mm increments. A 100-N preload was applied with an Instron 8511 materials test machine (Instron, Inc., Norwood, MA, USA) to assure proper sample contact. Femora were loaded to failure at 100 mm/s. Stiffness and maximum load were calculated from load-deflection curves.

Robustness-specific bone loss patterns

Linear regression analysis was used to test whether age-related bone-loss patterns varied with robustness. Femora were grouped by robustness values into robust ($n = 17$) and slender tertiles ($n = 17$). Cortical and trabecular traits were compared between the two tertiles (Student's t test) and differences in how each trait changed with age were evaluated by analysis of covariance (ANCOVA).

Results

Interindividual variation in femoral neck robustness

We normalized total area (Tt.Ar) by neck axis length (NAL) as a measure of femoral neck robustness. Total cross-sectional area increased with neck axis length ($R^2 = 0.34$, $p < 0.0001$), as expected (Fig. 2*A*). Residuals calculated from this regression correlated significantly with the ratio, Tt.Ar/NAL ($R^2 = 0.94$, $p < 0.0001$) (Fig. 2*B*), such that individuals with a negative Tt.Ar-NAL residual were characterized as slender, whereas those with a positive Tt.Ar-NAL residual were characterized as robust. This regression indicated that the ratio, Tt.Ar/NAL, could be used as a measure of robustness even though the y-intercept was not taken into consideration. Tt.Ar/NAL correlated positively with alternative measures of robustness such as femoral head diameter/NAL ($R^2 = 0.24$, $p < 0.0005$) and Tt.Ar/neck length ($R^2 = 0.70$, $p < 0.001$). Neck length was measured from the base of the femoral head to a line connecting the greater and lesser trochanters. We chose to use Tt.Ar/NAL as a measure of femoral neck robustness, because the lack of distinct anatomic markers delineating the beginning and end of the neck region made neck length a less reliable measure compared to NAL. Robustness

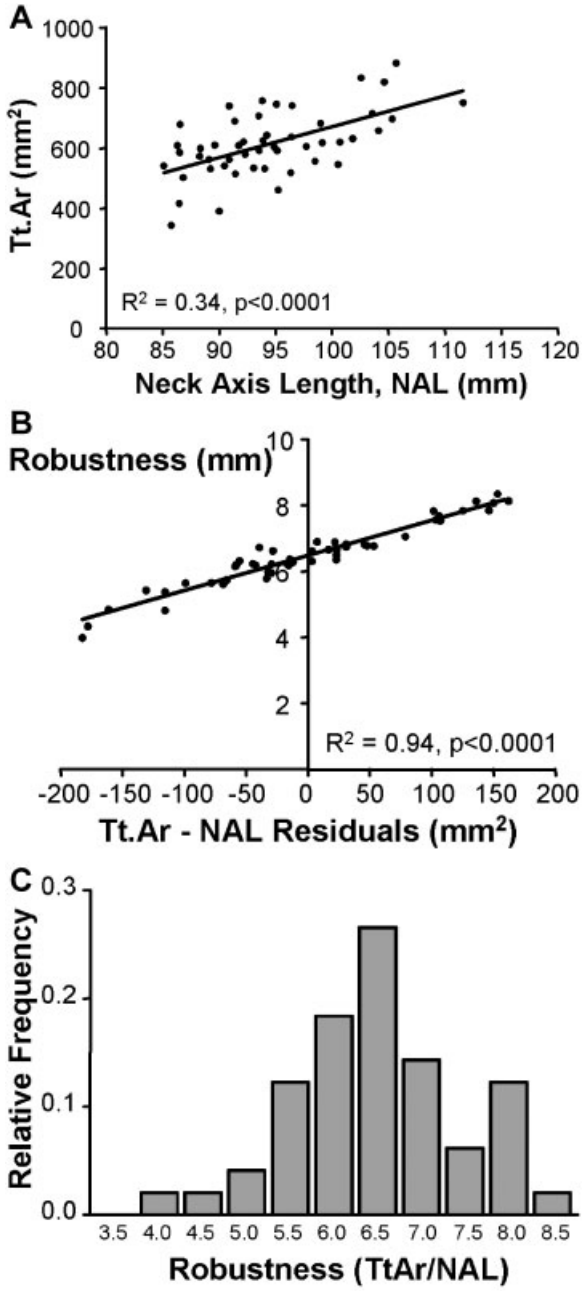

Fig. 2. (*A*) Variation in total cross-sectional area as a function of neck axis length. (*B*) Robustness (Tt.Ar/Le) was plotted against residuals calculated from the Tt.Ar-Le regression to determine if the ratio differentiated slender from robust femora. (*C*) Robustness was normally distributed across the female cadaveric cohort ($p > 0.10$, Kolmogorov-Smirnov test).

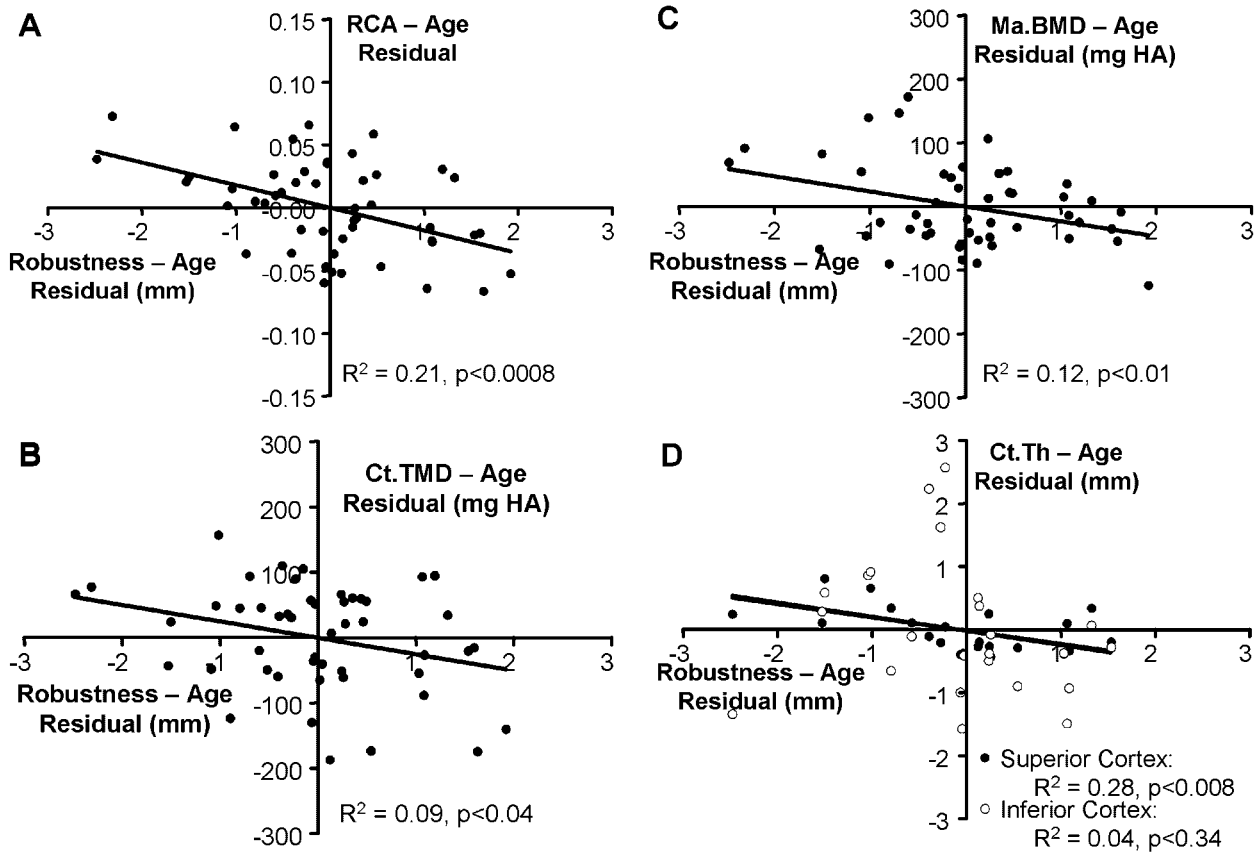

Fig. 3. Partial regression analyses were conducted to take the effects of age into consideration, showing that (*A*) relative cortical area (RCA = Ct.Ar/Tt.Ar), (*B*) Ct.TMD, (*C*) Ma.BMD, and (*D*) Ct.Th all correlate negatively with robustness.

(Tt.Ar/NAL) was normally distributed ($p > 0.10$, Kolmogorov-Smirnov test) and varied widely among women (Fig. 2*C*), showing a coefficient of variation of 14.9%. Robustness correlated weakly with age ($R^2 = 0.04$, $p > 0.20$) and neck shaft angle ($R^2 = 0.025$, $p > 0.27$).

Correlations between bone morphology and tissue quality

Linear regression analysis was conducted to identify traits that co-varied with robustness. Significant negative correlations were observed between RCA and robustness (Fig. 3*A*), Ct.TMD and robustness (Fig. 3*B*), and Ma.BMD and robustness (Fig. 3*C*) when the effects of age were taken into consideration by partial regression analysis. Similar negative correlations were observed for the unadjusted data, but the significance was improved for most regressions when accounting for age-effects, as expected (data not shown). MicroCT images were used to quantify cortical thickness in the superior and inferior aspects of the narrow neck region for a subset of the femora ($n = 24$). Ct.Th correlated negatively with robustness for both the superior and inferior regions (Fig. 3*D*), and the regression was significant for the superior ($R^2 = 0.28$, $p < 0.008$) but not the inferior neck region ($R^2 = 0.04$, $p < 0.34$). Further analysis of the apical Ct.Th showed no significant correlation between superior Ct.Th and age ($R^2 = 0.06$, $p < 0.24$) or inferior Ct.Th and age ($R^2 = 0.005$, $p < 0.73$).

Path analysis

Path analysis was conducted to assess the functional interactions among morphological and tissue-quality traits. The path model included age, robustness, Ct.Ar, RCA, Ct.TMD, and Ma.BMD. The χ^2 and RMSEA values both indicated there was an excellent fit between the data and the model (Fig. 4). The path coefficients, which were calculated based on *Z*-transformed data, reflect the number of SD changes in a trait arising from a 1 SD change in robustness. When all direct and indirect paths were considered, a 1 SD increase in robustness was associated with a 0.70-SD decrease in RCA, a 0.47-SD decrease in Ct.TMD, and a 0.43-SD decrease in Ma.BMD. The path model confirmed the linear regression results, indicating that the variation in femoral neck robustness was negatively associated with the relative amount of space occupied by the cortex, the degree of cortical mineralization, and trabecular bone mineral density, as hypothesized. The validation study confirmed that Ma.BMD and Ct.TMD provided measures that could be related to tissue-level mechanical properties (Supporting Information, Appendix 1). Ma.BMD correlated positively with BV/TV ($R^2 = 0.66$, $p < 0.0001$), Tb.Th ($R^2 = 0.66$, $p < 0.0001$), Tb.N ($R^2 = 0.42$, $p < 0.001$), and Tb.TMD

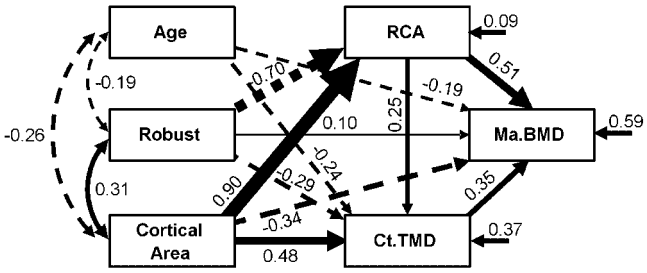

Fig. 4. The results of the path analysis show a significant goodness of fit for the hypothesized interactions among cortical and trabecular traits. Solid arrows indicate positive associations, whereas dashed arrows indicate negative associations. The reduced form structural equations are shown, which are written in terms of the independent variables. The path coefficients for the reduced form structural equations take both the direct and indirect paths into consideration. The path coefficients for dependent variables with only direct paths (eg, RCA) will be similar to those indicated in the path model. However, the path coefficients necessarily change for dependent variables having both direct and indirect paths (eg, Ct.TMD, Ma.BMD).

($R^2 = 0.48$, $p < 0.001$). Ct.TMD measured by pQCT was indirectly correlated with ash content.

Age-related bone loss

Individuals were stratified into robustness tertiles to evaluate whether robust femora aged differently compared to slender femora. There was no difference in age between robust (64.0 ± 8.6 years) and slender (69.9 ± 17.2 years) tertiles ($p < 0.25$, t test). The robust and slender tertiles differed with respect to Tt.Ar ($p < 0.0001$, t test), but not NAL ($p < 0.19$, t test), suggesting the variation in robustness resulted largely from differences in transverse expansion rather than axial growth of the neck. The slender tertile showed a significantly greater RCA compared to the robust tertile ($p < 0.01$, t test), which was expected based on the results of the bivariate and multivariate analyses. Although the cortex occupied proportionally greater space for the slender tertile, the slender tertile had a lower Ct.Ar compared to the robust tertile ($p < 0.018$, t test), indicating that slender femoral necks were constructed with a lower absolute amount of cortical bone. Linear regression analysis of the entire cohort (Table 1) revealed that Ct.Ar ($R^2 = 0.06$, $p < 0.08$), Ct.TMD ($R^2 = 0.20$, $p < 0.11$), and Ma.BMD ($R^2 = 0.10$, $p < 0.05$) decreased with age, as expected. However, stratifying the data into robustness tertiles revealed that slender femora aged differently compared to robust femora. Ct.Ar decreased significantly with age for the robust tertile ($R^2 = 0.29$, $p < 0.05$), but not for the slender tertile ($R^2 = 0.02$, $p > 0.58$). The slopes of the Ct.Ar - age regressions were significantly different between robust and slender tertiles ($p < 0.012$, ANCOVA), suggesting that slender and robust femoral necks exhibited differential rates of cortical bone loss with aging. Likewise, Ct.TMD ($R^2 = 0.48$, $p < 0.01$) and Ma.BMD ($R^2 = 0.34$, $p < 0.05$) decreased significantly with age for the robust tertile, but not for the slender tertile ($R^2 = 0.0005$–0.09, $p < 0.25$). The slopes of the mineral density - age regressions were significantly different between robust and slender tertiles for Ct.TMD ($p < 0.005$, ANCOVA), but not Ma.BMD. This suggested that slender and robust femoral necks exhibited differential rates of loss in Ct.Ar and Ct.TMD with aging, but showed fairly similar losses in Ma.BMD.

Table 1. Age-Related Changes in Femoral Neck Traits

	Entire dataset		Slender tertile		Robust tertile		ANCOVA[a]	
Trait	R^2	p	R^2	p	R^2	p	Slope	Intercept
Ct.Ar	0.06	0.08	0.02	0.60	0.29	0.03	0.01	N/A
Ct.TMD	0.12	0.01	0.001	0.90	0.44	0.004	0.003	N/A
Ma.BMD	0.10	0.03	0.09	0.24	0.34	0.01	0.28	0.08

ANCOVA = analysis of covariance; Ct.Ar = cortical area; Ct.TMD = cortical tissue mineral density; Ma.BMD = marrow-bone mineral density.
[a]The ANCOVA compared the slopes and intercepts for the slender and robust tertiles. N/A indicates that p values for y-intercepts were not calculated when a difference in slope was found.

Bone mechanics

Whole-bone mechanical tests were conducted to test how acquisition of robustness-specific trait sets affected bone stiffness and maximum load during a simulated fall to the side. Linear regression analysis applied to the entire cohort showed that maximum load ($R^2 = 0.19$, $p < 0.004$) but not stiffness ($R^2 = 0.05$, $p < 0.18$) decreased with age. A multiple regression analysis (Table 2), also applied to the entire cohort, indicated that 63.1% of the variation in maximum load was explained by robustness, Ct.Ar, Ct.TMD, Ma.BMD, and age ($R^2 = 0.631$, $p < 0.0001$). Of these traits, only Ct.Ar and Ma.BMD were significant contributors, accounting for the majority of the variation in maximum load. When segregating the data into robustness tertiles, femora in the robust tertile showed a 31% greater stiffness ($p < 0.05$, t test) and 13% greater maximum load ($p < 0.3$, t test) compared to slender femora (Table 3). Maximum load decreased with age for both tertiles and the slope of this regression was approximately twofold greater (not significant) for the robust tertile compared to the slender tertile.

Discussion

We used the natural variation in robustness as a model to define the functional interactions that exist among adult bone traits, to determine how these trait interactions contribute to femoral neck strength, and to test for differential aging effects. Assessing variation in robustness for a metaphyseal region like the proximal femur is more complicated than long bone,[19] because this site is shorter and has a more variable cross-sectional size. After comparing different ways of normalizing external size, we found that Tt.Ar/NAL measured at the narrow neck region could be quantified reliably. Other formulations of robustness are certainly possible, but these would likely also reflect a measure of

Table 2. Multiple Regression Analysis

Equation[a]	R^2 - adj	p
Max Load = –3175–20.9 Age + 212 Robustness + **23.6 Ct.Ar** + 3.39 Ct.TMD + **6.41 Ma.BMD**	63.1%	0.0001
Max Load = –1161 + **34.9 Ct.Ar** + **8.05 Ma.BMD**	58.9%	0.0001

Ct.Ar = cortical area; Ct.TMD = cortical tissue mineral density; Ma.BMD = marrow-bone mineral density.
[a]Bold font indicates traits making significant ($p < 0.05$) contributions to the variation in Max Load.

cross-sectional size normalized by a measure of length since variation in external size affects the resistance to deformation under all loading conditions. Beginning these integrative studies with Tt.Ar/NAL is appropriate, because it is simple and makes no assumptions about the loading conditions.

The natural variation in robustness allowed us to systematically evaluate femoral neck traits in a manner that provided new insight into how the functional adaptation process works across an adult population. Research by others showed how individual traits in the femoral neck such as Ct.Th, matrix mineralization, and Ct.Ar contribute to fracture risk.[20–23] Our study of 49 female cadaveric femora contributed to this literature by showing that the functional adaptation process results in a predictable network of functional interactions among these morphological and tissue-quality traits. This means that there is a pattern in the way traits covary in the proximal femur when viewed across our cohort, and that function is defined by a set of acquired traits, rather than a single trait or even a set of unrelated traits. The bivariate correlation analysis and the multivariate path analysis demonstrated that robustness correlated negatively with RCA, Ct.TMD, and Ma.BMD. Furthermore, after validating the pQCT information using microCT, our data indicated that, at one extreme, robust femoral necks had a thinner and less mineralized cortical shell and reduced trabecular bone mineral density, whereas, at the other extreme, slender femoral necks had a thicker and more highly mineralized cortical shell and greater trabecular bone mineral density. Thus, slender femoral necks were constructed using a different set of traits compared to robust femoral necks. These results are consistent with Zebaze and colleagues,[14] as well as our work examining trait networks in long-bone diaphyses[8,9,12] and mouse vertebrae.[13] Acquisition of robustness-specific trait sets makes sense from an engineering perspective, particularly when considering that the skeletal system must adjust traits in substantial ways to compensate for the nonlinear relationship between width and bending/torsional stiffness. At one extreme, slender femoral necks need to maximize bone mass and increase tissue-stiffness to compensate for the small external width. At the other extreme, robust femoral necks can easily achieve physiologically relevant stiffness and strength values, but must strategically minimize mass without compromising structural integrity (eg, developing a thin-walled tube without internal support that would increase the risk of buckling failure[24]).

The range of trait sets acquired by individuals in our study cohort was consistent with the idea that bone cells adjusted traits during growth to maximize stiffness while minimizing mass.[17] Work by others suggested that the amount of bone acquired was independent of external size.[14] However, the age regressions suggested that robust and slender tertiles may have different Ct.Ar values in young adulthood (ie, 30–40 years of age), but then differential resorption rates resulted in the two tertiles showing similar Ct.Ar values in older age (ie, >65 years of age). Ma.BMD appeared to be similar in young adulthood for the robust and slender tertiles, but then declined significantly for the robust tertile but not the slender tertile. These results suggested that individuals do not acquire the same amount of bone during growth, but an amount that is specific to their particular femoral neck robustness. This is consistent with prior work in long-bone diaphyses,[8,25] and is an important observation because the impact of these naturally varying robustness-specific trait sets on clinical measures like BMD have yet to be established.

The predictable pattern in the way traits covaried relative to robustness, as indicated by the path model, suggested that individuals used a common biological strategy to adjust traits like Ct.Ar and trabecular BMD relative to robustness, despite having diverse genetic backgrounds and life histories. This is consistent with the idea that the traits which are adjusted during growth relative to a common stimulus will emerge as a set of correlated traits when viewed across an adult population.[16] When translating the systems-based concepts learned in long bones[8,9,12] to the femoral neck, we left open the possibility that there would be far greater ways to adjust cortical and trabecular traits to compensate for robustness, and that finding a pattern would be more difficult. Further, the trabecular bone traits measured at the narrow neck region represent only a part of the tensile and compressive arcades (Fig. 1) and thus may not necessarily be locally adapted to satisfy the biomechanical functionality of a single anatomical site. Incorporating these

Table 3. Age-Related Changes in Mechanical Properties

	Slender tertile			Robust tertile			ANCOVA	
Trait	Slope	R^2	p	Slope	R^2	p	Slope	Intercept
Stiffness	1.05 (Nmm/year)	0.002	0.9	–15.7 (Nmm/year)	0.5	0.4	0.4	0.07
Max load	–30.2 (N/year)	0.16	0.13	–74.3 (N/year)	0.15	0.14	0.3	0.5

ANCOVA = analysis of covariance.

arcades into a three-dimensional (3D) model of functionality may be needed to better understand the pattern of functional trait interactions observed in this study. Nevertheless, seeing a relationship between cortical and trabecular traits in the two-dimensional (2D) model presented here thus suggested that trabecular architecture within the tensile and compressive arcades is modified during growth in association with the natural variation in femoral neck width. Because traits measured in the current study were at the higher end of the structural hierarchy (eg, neck axis length, total area, marrow area, cortical thickness, Ma.BMD), we suspect there is a limited range in the way bone cells can adjust these traits within constraints imposed by cellular dynamics (eg, number of cells per surface area, rate of cellular replacement, rate of formation and resorption).

The path model identified important trait interactions, but a limitation of this model is that we could not include a measure of body weight and whole-bone mechanical function (ie, stiffness relevant to the way bone is loaded during daily activities) that together serve as a stimulus for functional adaptation during growth.[26] Although femoral neck strength during a simulated fall-to-the-side configuration correlates well with the strength under a single-leg stance configuration,[27] the lack of precision in how these strength values predict each other precluded us from using the current strength values in the path model. Future studies could include stiffness measured by loading femora in a single-leg stance position or from finite element analyses. Nevertheless, our biomechanical analysis successfully evaluated how the robustness-specific trait sets affected bone strength during a fall-to-the-side configuration, which is clinically meaningful. The tertile analysis indicated that robust femora were only 13% stronger compared to slender femora, suggesting that the coordination of morphological and tissue-quality traits within the proximal femur was successful in achieving strength equivalence across our study cohort. Additional studies with more data on young adults are needed to confirm these findings and to rule out the effects of differential bone-loss patterns.

The strength of engineered structures subjected to bending and torsional loads is generally proportional to measures of robustness and the cross-sectional moment of inertia (CSMI). However, we found that nearly 60% of the variation in fracture strength of the proximal femur was explained by Ct.Ar and Ma.BMD, rather than an external size related trait like robustness (Table 2). This is consistent with the results of others showing that cross-sectional area of the narrow neck region was similar to BMD in its ability to discriminate incident hip fracture cases from noncases.[6] We attribute this phenomenon to the fact that bone is a complex system with strong adaptive processes; theoretically, biological processes should work so that slender femora are as stiff and strong as robust femora, otherwise there is functional inequivalence across a population.[8] Our data suggested that variation in bone strength results from variation in the degree to which morphological traits and tissue-level mechanical properties are adjusted relative to robustness. Norms for these acquired trait sets would need to be established to evaluate the magnitude of impairment in adaptation that would lead to increased fracture risk. Because this adaptive response involves multiple traits, this approach opens the possibility that individuals may be at increased risk of fracturing for different biomechanical and biological reasons,[28] making it tricky to identify a single trait that is useful clinically as a prognosticator of fracture risk. The pattern in the way traits covary could be used clinically to better assess bone strength and fracture risk, by inviting the option of not only comparing individual traits to the population mean, which is the traditional approach, but also comparing an individual's trait set relative to their peers with similar robustness values.

The interindividual variation in robustness was accompanied by predictable differences in morphology and tissue-quality, and the results showed for the first time that age-related bone loss patterns differed significantly between robust and slender femora, as hypothesized. In particular, the robust tertile showed a significant, negative correlation with age for Ct.Ar, Ma.BMD, and Ct.TMD. In contrast, femora in the slender tertile did not show these age related changes. Bone loss in the cortical shell was affected to a greater extent than trabecular bone, but how loss in each compartment differentially affected the strength of robust and slender femora has yet to be determined. The pattern of bone loss in the femoral neck may place robust femora at increased risk of fracturing during a fall to the side, since robust femora have a thinner superior cortex, a structural feature implicated in fracture risk.[22] This is consistent with studies showing that having a wide femoral neck combined with a thin cortical shell is a risk factor for fractures.[5] Slender femora appeared to retain cortical area, whereas robust femora lost significant amounts of cortical area with age. This suggested that resorption rates differ based on bone size. This is consistent with prior work showing that cortical porosity associated with internal remodeling correlated with robustness of adult human tibiae.[8] A number of factors contributing to age related bone loss have been identified, but the natural variation in external size has not been previously studied as a determinant of bone loss. Our data suggested that the variation in bone robustness may be a global factor influencing bone remodeling. Although the biological mechanism explaining the robustness-specific bone loss patterns is unclear, knowledge of a person's robustness may be an important factor to consider when personalizing prophylactic treatments to the biological needs of the individual.

Our conclusions are based on a cross-sectional study design utilizing 49 female cadaveric femora, and thus are limited to a single sex. These analyses will benefit from a longitudinal study design that can better elucidate the association between morphology and bone loss while ruling out secular changes. Further, a longitudinal study design will also provide insight into how body size information can be incorporated into the path models. Bone size is sensitive to body size, but how the set of bone traits change in response to changes in body size after young adulthood remains to be determined. Variables that may affect bone loss such as lifestyle, diet, and medications were not available for a majority of our samples and could not be controlled for in our analyses. This research analysis was limited to the narrow neck region, and further work is needed to confirm that similar associations are observed at other sites along the femoral neck.[14] The 49 femora provided ample power to test for functional interactions among morphological and tissue-quality traits, and although the tertile analysis consisted of a relatively small sample size, we observed significant differential bone-loss

patterns. Future studies employing a larger cadaveric cohort combined with a prospective clinical study are needed to confirm these differential age-related changes in bone mass and strength.

In conclusion, this study provided new insight into how the functional adaptation process works across an adult population. The natural variation in robustness was accompanied by specific and predictable changes in cortical and trabecular traits in the femoral neck. Robust femoral necks had a thinner and less mineralized cortical shell and reduced trabecular bone mineral density, whereas slender femoral necks had a proportionally thicker and more highly mineralized cortical shell and greater trabecular bone mineral density. We suspect that a common biological strategy is driving a patterned adaptation across our study cohort. Understanding this variation and its implications on bone strength will be critical for advancing our ability to identify individuals at increased risk of fracturing. Further, we also demonstrated that the amount of bone lost with aging was inversely related to robustness. Thus, the natural variation in femoral neck robustness was associated with predictable differences in the acquisition and maintenance of trait sets that have important biomechanical and biological meaning.

Disclosures

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