Clin Orthop Relat Res (2011) 469:2150–2159
DOI 10.1007/s11999-010-1706-9

SYMPOSIUM: BONE QUALITY: FROM BENCH TO BEDSIDE

Functional Interactions Among Morphologic and Tissue Quality Traits Define Bone Quality

Karl J. Jepsen PhD

Published online: 2 December 2010

Abstract

Background Advances in diagnostic and treatment regimens that aim to reduce fracture incidence will benefit from a better understanding of how bone morphology and tissue quality define whole-bone mechanical properties.

Questions/purposes The goal of this article was to review what is known about the interactions among morphologic and tissue quality traits and how these interactions contribute to bone quality (ie, whole-bone mechanical function). Several questions were addressed. First, how do interactions among morphology and tissue quality traits relate to functional adaptation? Second, what are the emergent patterns of functionally adapted trait sets in long bones? Third, how effective is phenotypic integration at establishing function across a population? Fourth, what are the emergent patterns of functionally adapted trait sets in corticocancellous structures? Fifth, how do functional interactions change with aging?

Methods A literature review was conducted with papers identified primarily through citations listed in reference sections as well as general searches using Google Scholar and PubMed.

Results The interactions among adult traits or phenotypic integration are an emergent property of the compensatory mechanisms complex systems used to establish function or homeostasis. Traits are not regulated independently but vary simultaneously (ie, covary) in specific ways to establish function. This covariation results in individuals acquiring unique sets of traits to establish bone quality.

Conclusions and Clinical Relevance Biologic constraints imposed on the skeletal system result in a population showing a pattern of trait sets that is predictable based on external bone size and that can be used to identify individuals with reduced bone quality relative to their bone size and body size.

Introduction

Bone quality can be simply defined in engineering terms as the mechanical behavior of a skeletal structure at the whole-bone level (Fig. 1). This behavior includes common, more predictable properties, such as stiffness and failure load (also known as whole-bone strength), as well as more complex, less predictable properties, such as toughness, creep, and fatigability. A major challenge to reducing the worldwide incidence of fractures and the associated mortality, morbidity, and cost [50] is that the repertoire of mechanical properties defining bone quality can only be accurately measured ex vivo by destructive mechanical tests. According to engineering principles, the mechanical behavior of any structure, whether it is a bridge, building, or bone, depends on the morphology of the structure and the mechanical properties of the materials used in construction (Fig. 1). In situ values for bone quality can be approximated from noninvasively derived measures of morphology and tissue quality. This poses an enormous barrier to accurately identifying individuals who are at risk of fracturing and for determining how to treat them to prevent fractures. Consequently, we must rely on physical

This work was supported by grants from the National Institutes of Health (AR44927, AR056639), the Department of Defense (DAMD17-01-1-0806), and the USA Medical Research and Material Command (USAMRAA08072006).

K. J. Jepsen (✉)
Leni and Peter W. May Department of Orthopaedics, Mount Sinai School of Medicine, Box 1188, One Gustave Levy Place, New York, NY 10029, USA
e-mail: karl.jepsen@mssm.edu

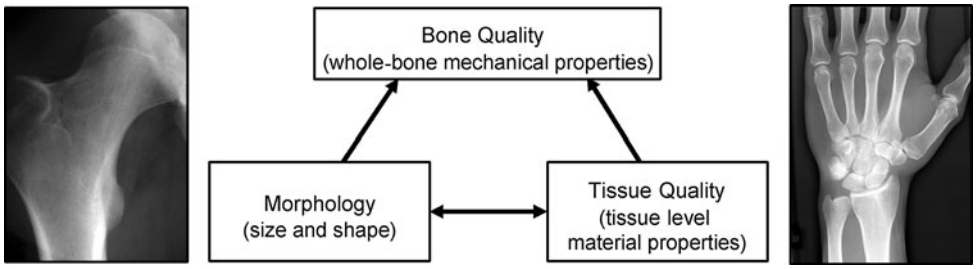

Fig. 1 Bone quality is a generic term describing whole-bone mechanical properties. The repertoire of whole-bone mechanical properties is determined from morphologic traits, tissue quality traits, and their interactions.

traits that are related to whole-bone mechanical properties or that can be used in analytical models to predict whole-bone strength [13].

Morphology is defined here as any physical trait that quantifies the size and shape of bone. Tissue quality encompasses all tissue-level material properties, such as modulus, strength, ductility, toughness, fatigability, and creep. For cortical bone, "tissue" is comprised of the mineralized matrix, including pores. For trabecular bone, "tissue" is defined on the same length scale as that used to define cortical bone and thus includes traits describing the mineralized matrix and trabecular architecture (eg, bone volume/tissue volume [BV/TV], anisotropy, trabecular thickness).

Identifying the physical traits that predict bone quality and fracture risk should seem like a straightforward problem to solve. However, bone is anything but simple, and a major problem is that adaptive processes allow variation in one trait to be compensated by changes in other traits [53]. Furthermore, the construction of bone, like buildings and bridges, faces a common design constraint in that function must be achieved using limited resources. Finally, the process must be sufficiently flexible to account for variations in the system. Bone structure varies widely among individuals, largely independent of height and weight, and this variation results from genetic and environmental factors [56]. Because the functional adaptation process must work for all individuals, bone must be artfully constructed using a clever combination of traits. Given the wide variation in bone size relative to body size, each person arrives at bone strength in very different ways by acquiring a particular combination of traits during growth. Morphology can be modified depending on the tissue-level material properties [5]. Likewise, tissue quality can be modified depending on the morphologic constraints [41, 80]. Thus, not only are morphology and tissue quality important, but the interaction among these traits is also particularly important for bone quality.

Although much is known about bone morphology and tissue quality, little is known about how bone integrates morphologic and tissue quality traits in the context of skeletal function. The goal of this article is to review what is known about the interactions among morphologic and tissue quality traits and how these interactions contribute to whole-bone mechanical properties.

This review covers phenomenologic data showing that interactions among morphologic and tissue quality traits are central to establishing skeletal function, and experimental data showing these interactions are part of the biologic machinery central to functional adaptation and the development of bone strength. Several questions are addressed. First, how do interactions among morphology and tissue quality traits relate to functional adaptation? Second, what are the emergent patterns of functionally adapted trait sets in long bones? Third, how effective is phenotypic integration at establishing function across a population? Fourth, what are the emergent patterns of functionally adapted trait sets in corticocancellous structures? Fifth, how do functional interactions change with aging?

Search Strategy and Criteria

The number of papers and book chapters that deal with the interaction between morphology and tissue quality are quite small in comparison to the number of papers that address morphology and tissue quality separately. In fact, these papers are rare and difficult to find using traditional PubMed and Google Scholar searches. The papers listed in this review were primarily found through citations listed in the reference sections of several papers. Searches were generally more successful in Google Scholar than in PubMed, and keywords included "bone morphology tissue quality," "bone phenotypic integration," "bone functional adaptation," "bone compensatory interactions biomechanics," "bone compensatory interactions biomechanics aging."

How Do Interactions Among Morphologic and Tissue Quality Traits Relate to Functional Adaptation?

Traits that covary or are coordinated in a way to satisfy a common function are considered functionally related or functionally integrated [11]. Functional interactions have

been well studied during the past century primarily in the context of how morphologic integration impacts evolutionary changes in skeletal structure [12, 53, 81]. More recently, these interactions have also been studied in the context of skeletal stiffness and strength [34, 75]. Functional interactions are not limited to morphologic traits but also involve interactions with tissue quality traits. When viewing whole-bone mechanical properties across species and for structures with very diverse functions, skeletal stiffness and strength are facilitated by the particular combination of morphologic and tissue quality traits [16]. For example, the reduced mineralization of the deer antler results in very high toughness, a property critical for sparring during the rutting season. In contrast, the increased mineralization of the fin whale's tympanic bulla results in very high tissue stiffness (modulus), a property critical for transmitting sound waves with high fidelity.

Experimental evidence indicating morphologic and tissue quality traits interact to establish function is not limited to a comparison of bones from different species with diverse functions, but these interactions have also been observed for the same bone within a single species. Functional interactions have been reported for phalangeal segments from the brown bat [55], for long bones during growth [7, 9, 31, 33], for adult human long bones [74, 76], and for adult mouse long bones [34] and vertebral bodies [73]. Although tissue quality is often assumed to be the same for all individuals [63], it is well known tissue quality and matrix organization vary widely along the length of human long bones [39] and around the cortex [30, 42, 68]. Much of this variation has been attributed to site-specific loading conditions. These studies clearly show bone is capable of exhibiting a wide range of adaptive responses to establish function, from gross modulation of cross-sectional morphology to more refined modulation of matrix deposition, organization, and mineralization. However, the biologic mechanisms responsible for coordinating these traits during growth are not well understood.

What Are the Emergent Patterns of Functionally Adapted Trait Sets in Long Bones?

The interindividual variation in diaphyseal bone width provides a straightforward model to illustrate the interactions among morphologic and tissue quality traits play a central role in establishing bone quality. Long-bone robustness, a measure of cross-sectional size (eg, width, total area, moment of inertia) relative to length (Fig. 2), is a heritable trait [35, 40, 71] resulting from variation in the biologic processes regulating the relationship between growth in width (subperiosteal expansion) and growth in length [56]. This relationship is established early in life, by 2 years of age in humans [38, 54] and by 2 weeks in mice [57]. Ural and Vashishth [76], who examined male tibiae over a large age range, showed a negative correlation between external bone size and the area fraction of unremodeled tissue. This indicated slender tibiae contain

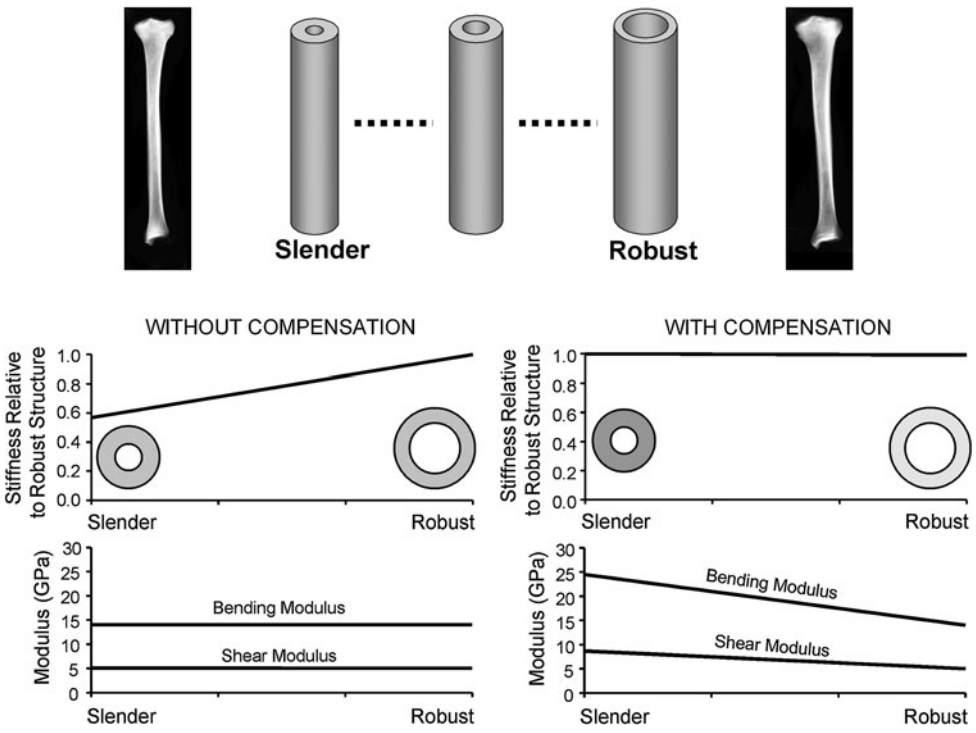

Fig. 2 A schematic shows how bone morphology varies widely among individuals, from slender (narrow relative to length) to robust (wide relative to length). If the skeletal system had no biologic mechanism to compensate for the variation in robustness, then slender bones would have a lower whole-bone stiffness and strength relative to robust bones. However, if the skeletal system has a biologic mechanism to compensate for the variation in robustness, then whole-bone stiffness and strength will be similar for slender and robust bones.

a greater amount of interstitial tissue compared with robust tibiae. Variation in robustness is particularly important clinically because variants leading to a slender phenotype could result in reduced whole-bone stiffness and strength relative to a robust phenotype if the system were not able to compensate by modifying tissue modulus [77]. For example, a 15% reduction in the width of a hollow cylinder, with no change in cortical area or tissue modulus, is associated with a 35% reduction in global bending stiffness relative to a robust cylinder. However, a proportional increase in tissue modulus could rescue this global phenotype, resulting in similar stiffness values for slender and robust structures. Thus, tissue modulus should theoretically correlate negatively with bone robustness so all individuals achieve functionally adapted bones (Fig. 2). A recent study by Tommasini et al. [74] showed ash content was negatively related to bone robustness, measured as total area/bone length (Tt.Ar/Le), when the effects of body size were taken into consideration. Reanalysis of the data presented in Tommasini et al. [74] confirmed tissue modulus correlated negatively with measures of bone robustness such as Tt.Ar/Le or the polar section modulus (J/r) normalized by bone length (Fig. 3). Similar functional interactions have been observed in adult mouse long bone [34, 45].

These studies show the skeletal system is capable of compensating for variants affecting external bone size. The process of functional adaptation within the skeletal system thus results in individuals acquiring particular sets of traits that are predictable based on bone robustness. Individuals with slender bones will tend to have a small external width combined with increased relative cortical area (cortical area/total area) and increased mineralization. At the other extreme, individuals with robust bones will tend to have a large external width combined with reduced relative cortical area and reduced mineralization. Importantly, this pattern was consistent among individuals because cortical area was similar for robust and slender diaphyses, consistent with the idea that bone maximizes stiffness using minimum mass [18, 26]. This pattern is important because it may be used to identify individuals that acquire "suboptimal" sets of traits relative to their bone size and body size and thus have reduced bone quality. Importantly, small increases in mineralization, on the order of 2% to 4%, are associated with substantial (20%–30%) increases in tissue modulus and strength [17]. This small but important variation in mineralization poses a challenge for accurately measuring tissue quality noninvasively. The downside of this functional adaptation process is that increasing mineralization to compensate for slender phenotypes may occur at the expense of reduced tissue toughness [17]. Having higher matrix mineralization would generally not be a problem under normal daily activities, but the reduced tissue toughness may be problematic under extreme conditions, such as intense physical exercise (eg, military training) or during a fall [24]. The compensatory nature of the skeletal system may thus put individuals with slender bones at risk of fracturing [1, 2, 10, 14, 20, 21, 27, 28, 37, 43, 51], even if they are functionally adapted to support daily loading conditions.

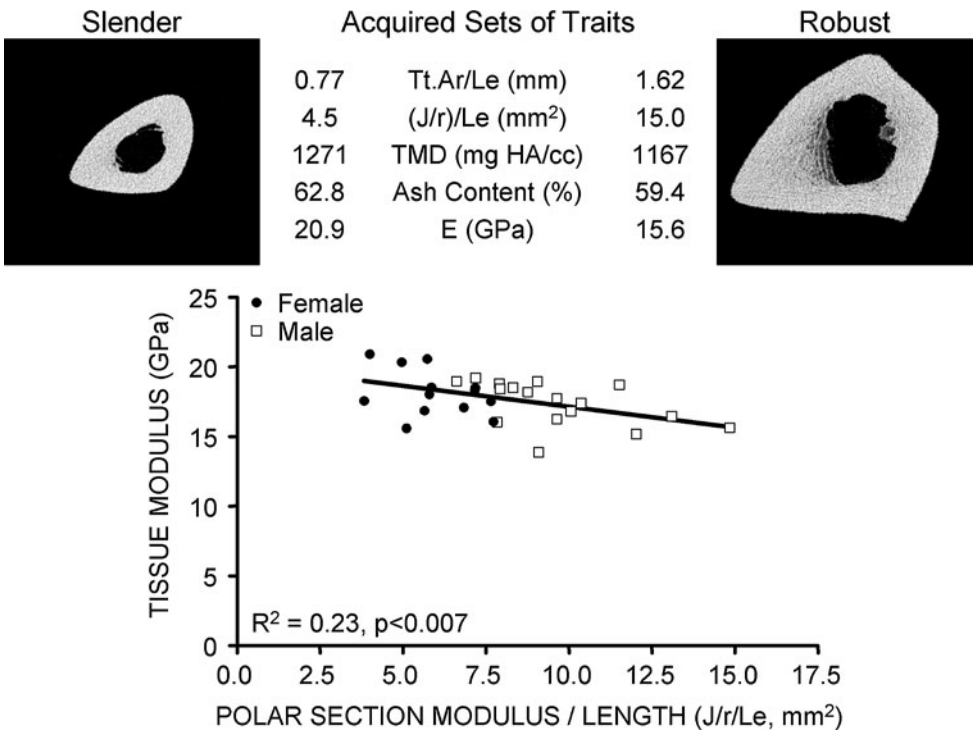

Fig. 3 Data from Tommasini et al. [74] were reanalyzed to confirm tissue modulus, measured from samples machined from the diaphyseal cortex of tibiae from young adult men and women, correlates negatively with a measure of bone robustness (polar section modulus/bone length). Acquired sets of traits, including measures of tissue mineral density (TMD) from peripheral quantitative CT (pQCT) (Stratec 2000L; Stratec Medizintechnik GmbH, Pforzheim, Germany) and ash content (% ash weight/hydrated weight), are shown for two representative pQCT images taken from a diaphyseal site located 38% from the distal articulating surface. pQCT images were courtesy of G. Felipe Duarte. E = Young's modulus; HA = hydroxyapatite.

How Effective Is Phenotypic Integration at Establishing Function?

The effectiveness of the interactions among morphologic and tissue quality traits to equalize bone quality across a population exhibiting a wide range in robustness has not been established. Solving this problem has both academic value for advancing our understanding of how bone works and clinical value for understanding how physical bone traits contribute to bone quality and fracture risk. To address this problem, a functional end point for bones needs to be defined to provide context to the word "effective." Bone is believed to use a closed-loop feedback process to model or remodel tissue to achieve a particular peak tissue level strain [26, 44, 49, 61]. Using the idea that bone adapts to achieve a common peak strain, we asked whether individuals with slender bones are adapted to body size in the same way as individuals with robust bones. Data from Tommasini et al. [74] were reanalyzed to determine whether the morphology–tissue quality interactions were sufficient to generate similar strains relative to body size for slender and robust tibiae of young adult men and women. Tissue strains were estimated using beam theory. The tibiae were assumed to be subjected to a combination of bending and compressive loads. The applied bending moment was assumed to be proportional to body weight times tibial length and the compressive force was assumed to be proportional to body weight [62, 66]. Cross-sectional morphology and tissue modulus were measured at multiple locations along the length of the tibiae and averaged to generate a diaphyseal structure with homogeneous tissue quality. The tibiae were divided into tertiles based on robustness (Tt.Ar/Le). For males, the peak compressive strain calculated for tibiae in the slender tertile was similar ($p < 0.28$, t test) to the peak strain for tibiae in the robust tertile (Fig. 4). However, for women, the peak compressive strain for tibiae in the slender tertile was 41% greater compared with the strain for tibiae in the robust tertile ($p < 0.02$, t test). The estimated peak strains were on average 43% greater for women compared with men ($p < 0.001$, t test). This analysis suggested the functional adaptation process works well across the range of robustness values for men but not women. Although women have more slender bones compared with men ($p < 0.00002$, t test), the functional adaptation process appears to result in greater tissue strains and thus reduced stiffness relative to body size for women, particularly those with slender tibiae. Although this analysis must be confirmed using ex vivo whole-bone mechanical tests and a larger cohort of individuals, the results emphasize the importance of understanding how the interactions among morphologic and tissue quality traits contribute to bone quality and fracture risk on an individualized basis.

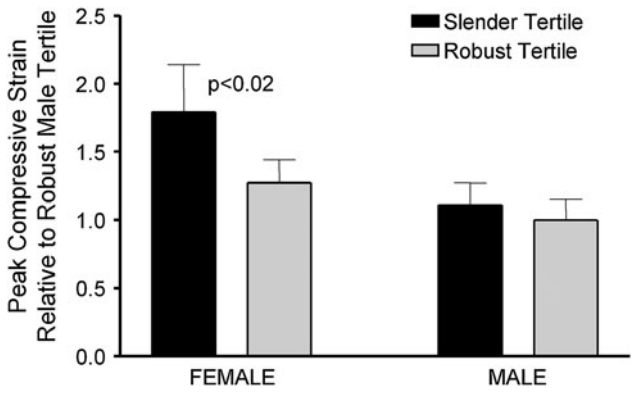

Fig. 4 Data from Tommasini et al. [74] were reanalyzed to estimate tissue level strains. Peak compressive strain was estimated for the tibiae of young adult men (n = 17) and women (n = 14) and expressed relative to strains for robust male tibiae. Tibiae were assumed to be subjected to bending and compressive loads. No differences in peak strain were found for men with slender and robust tibiae. However, peak strains were higher for tibiae in the slender tertile compared with the robust tertile for women, suggesting the combination of traits for slender tibiae resulted in reduced stiffness relative to body weight. Error bars are SDs.

What Are the Emergent Patterns of Functionally Adapted Trait Sets in Corticocancellous Structures?

Functional interactions among morphologic and compositional traits have also been observed in corticocancellous structures such as the femoral neck and the vertebral body. The number of traits involved in establishing function, and thus the complexity of the trait interactions, is dramatically higher for corticocancellous structures compared with diaphyseal structures. These traits include the morphologic and compositional traits of the cortical shell and the morphologic and compositional traits of the trabecular tissue. Functional interactions among corticocancellous traits have been reported largely in regard to the relative proportion of cortical and trabecular tissues, which is associated with fracture risk [3, 4, 19, 59, 60, 70, 78]. This should not be surprising because both tissue types contribute to skeletal strength and fracture resistance [6, 23, 46, 47, 69]. For example, despite the fact that the average thickness of the cortical shell of the vertebral body is only approximately 0.38 mm, the mass fraction of the cortical shell ranged from 21% to 39% and the maximum load fraction supported by the shell ranged from 38% to 54% [23].

Despite the large number of potential trait interactions in corticocancellous structures, there is a pattern to the way traits covary among individuals. A recent study examining the femoral neck of nearly 700 healthy women showed the relative proportion of cortical and trabecular tissues acquired by a person during growth correlated with femoral neck width and body weight [82]. Women with wide

femoral necks had proportionally less cortical bone and lower trabecular density, whereas women with narrow femoral necks had proportionally greater cortical bone and higher trabecular density (Fig. 5). Further, the amount of bone was independent of external bone size, consistent with the notion that bone adapts to maximize stiffness using minimum mass [18, 26]. Thus, healthy individuals with functionally adapted structures show different relative proportions of cortical and cancellous tissues that are predictable based on bone size. A similar pattern of trait interactions was observed for the vertebral body of inbred mouse strains [73]. Multivariate analyses showed inbred mouse strains, which tended to have smaller vertebrae relative to body size, achieved mechanical functionality by increasing mineralization and the relative amounts of cortical and trabecular bone. The interdependence among corticocancellous traits in the vertebral body indicated variation in trabecular bone traits was determined in part by the adaptive response to variation in traits describing the cortical shell. Given that physiologic forces are important for the normal maturation of trabecular tissue into a highly adapted, anisotropic structure [25, 72], the amount of load imparted to the trabecular tissue during growth may be a critical determinant of adult trabecular bone mass and architecture. Thus, the degree of load sharing between the cortical and trabecular components during growth may provide a biomechanical mechanism explaining functional interactions among corticocancellous traits.

The functional adaptation process in corticocancellous structures, such as long bone diaphyses, compensates for variants affecting external bone size, resulting in each person acquiring a specific set of cortical and trabecular traits [73, 82]. These studies clearly showed variation in the relative proportion of cortical and trabecular traits in relation to bone size is a normal trait variant. This outcome may help to interpret recent work showing fracture risk is not simply related to bone mass or bone diameter but to specific sets of trait. Women with fractures had wider femoral neck widths combined with reduced cortical thickness, whereas men with fractures had narrower femoral neck widths combined with reduced cortical thickness [19]. Similar results were observed in the Rotterdam study for 2740 women [60] and the Osteoporotic Fractures in Men study for 3347 men [4]. However, the Rotterdam study showed men with hip fractures had wider bones with thinner cortices [60]. Further research is required to better understand why these structural determinants vary with gender and among study populations and whether the individuals with fractures exhibit trait sets outside the normal range for their bone size and body size.

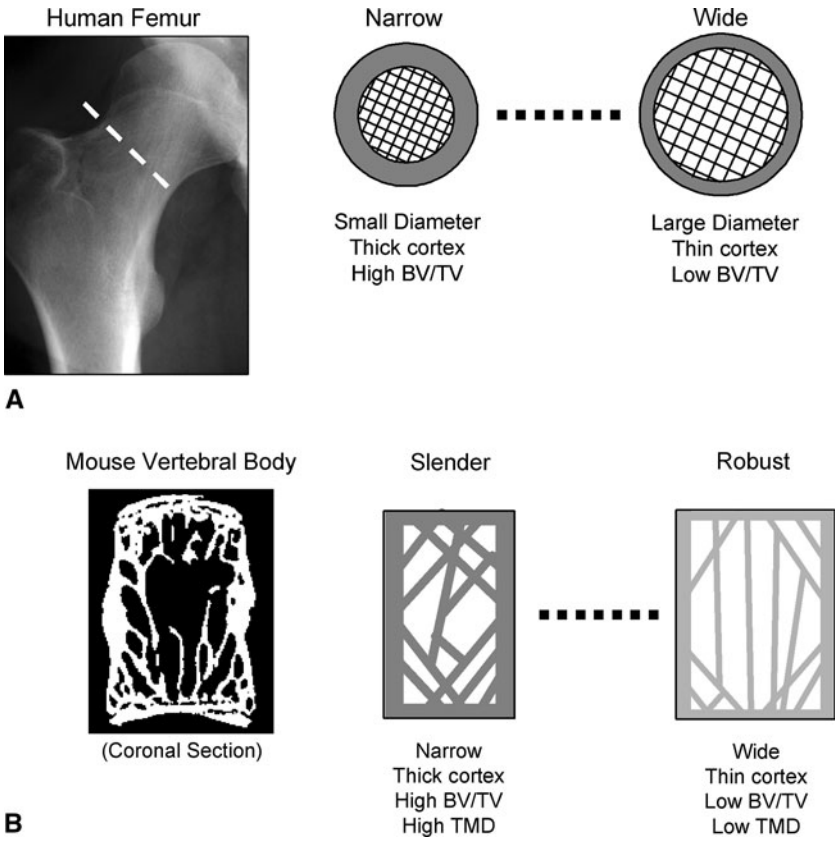

Fig. 5A–B Studies investigating functional interactions among morphologic and tissue quality traits in corticocancellous structures such as (**A**) the human femoral neck [82] and (**B**) the mouse vertebral body [73] reveal an emergent pattern of trait sets expressed by individuals within these populations that were predictable based on external bone size.

How Do Functional Interactions Change With Aging?

Recent studies using high-resolution imaging methods revealed aging of corticocancellous structures cannot be characterized simply in terms of a net loss of bone mass. Bone loss occurs in a gender-specific manner [36], and the reduction in trabecular bone volume fraction (BV/TV) in men and women begins earlier than expected [36, 58]. Although men have greater trabecular BV/TV than women, both genders show similar age-related decreases in trabecular BV/TV. However, women lose bone mass through loss of trabecular number, whereas men lose bone mass through loss of trabecular thickness [36]. This gender-specific bone loss pattern has important mechanical consequences because the loss of trabecular number has a three to four times worse effect on bone strength compared with loss of BV/TV through trabecular thinning [67]. Longitudinal studies using in vivo micro-CT revealed age- and ovariectomy-related bone loss is accompanied by a tremendous amount of remodeling in the remaining trabecular elements [79]. This remodeling may represent an adaptive response to maintain function during a time of net bone loss through compensatory changes that occur among trabecular elements. Waarsing et al. [79] suggested the state of bone loss during estrogen depletion is better described as "accelerated bone metabolism that resulted in fast, possibly mechanically driven, bone adaptation." The adaptive response is not limited to trabecular tissue but has also been observed for the surrounding cortical tissue in human [22, 58] and mouse [29] bone. Age-related decreases in trabecular bone mass are often accompanied by age-related increases in periosteal expansion, depending on race and gender [22].

Discussion

Advances in diagnostic and treatment regimens that aim to reduce fracture incidence will benefit from a better understanding of how bone morphology and tissue quality define whole-bone mechanical properties. The goal of this review was to report what is known about how the interactions between morphologic and tissue quality traits contribute to whole-bone mechanical properties (ie, bone quality).

A primary limitation of this review is the difficulty in accessing papers dealing with the interactions among traits. Many papers discuss the interactions among morphologic and tissue quality traits as a way to explain observations in their data, but few studies have actually studied these interactions on a scientific basis. Consequently, these integrative studies are just in their infancy and much work needs to be done to understand the biologic control mechanisms coordinating relative trait values. Many unresolved issues remain. First, functional interactions are often described as compensatory and this implies a degree of causality in that variation in one trait elicits adaptive changes in other traits. Although the temporal sequence in which traits mature during growth may provide a sense of causality [33], there is no scientific basis for causality yet. Second, the coordination among traits is facilitated by osteoblasts and osteoclasts, and yet what controls their relative activities to coordinate traits is not known. Third, how the components of negative feedback control (eg, set point, sensors, etc), which are responsible for coordinating morphologic and tissue quality traits, relate to biologic processes remains unclear. Fourth, although this review touched on whether this covariation process is effective at establishing function across a human population, very little is understood about this topic. Are slender bones as stiff and strong relative to body size as robust bones? This has yet to be answered in a large cohort. Finally, osteoblasts and osteoclasts are expected to have constraints imposed on their formative and resorptive activities, and it is unclear whether these constraints limit the degree to which traits can be compensated. We know clinically many single gene mutations (eg, COL1A1 mutations) cannot be fully compensated, but we do not know the range of genetic and environmental variants that can be fully compensated in the general population. Do these constraints, and thus limitations intrinsic to the biologic processes regulating the interactions among traits, contribute to fracture susceptibility?

Although this review focused primarily on interactions among adult traits, there are several studies examining the development of these trait interactions during growth [7–9, 11, 12, 33, 54], which can be the topic of future review papers. The interactions among traits provide a systematic way to study the functional adaptation process across a population. Genomic studies found interacting traits were regulated largely independently, suggesting these traits may be regulated in a nonpleiotropic manner [32]. This is a critical outcome for prophylactic interventions that aim to increase bone mass to reduce fracture risk because it tells us some traits can be modulated independently of others, allowing for selective treatments tailored to the biologic needs of the individual. Much work needs to be done to further understand the genetic regulation of this process. Importantly, genetic studies will need to focus not only on the traits themselves, which is most often done, but also on the relationships among traits [45].

This review showed the adaptive response of bone involves interactions among morphologic and tissue quality traits and these interactions define bone quality. These compensatory interactions are critical for health and disease because they buffer genetic and environmental

variants that would otherwise lead to dysfunction [48, 64]. Although each person inherits approximately 300 mutations with deleterious effects [15], most individuals are healthy. This health has been attributed in large part to the buffering mechanisms compensating for deleterious genetic variants [52]. These compensatory interactions complicate the search for genetic and environmental perturbations leading to disease because an unbuffered perturbation may increase disease risk in one person, whereas the same perturbation, if buffered, may have no effect in another person [52]. Genetic or environmental variants that alter compensatory relationships or that cannot be fully compensated as a result of biologic constraints can tip a system from health to disease [52] or expose cryptic genetic variants [65]. Although much work still needs to be done, the data thus far suggest bone traits covary in predictable ways and patterns are emerging when looking at acquired trait sets across a population. Because all whole-bone mechanical properties depend on the particular combination of morphologic and tissue quality traits acquired during growth, the pattern of trait sets across the population is expected to reveal biologic control mechanisms used by all individuals to establish function. This pattern of trait sets may also provide a novel approach to identifying individuals with trait combinations that are underdesigned (ie, less stiff, less strong) and at increased risk of fracturing. Efforts to reduce fracture risk on an individualized basis will benefit from further investigation of how the skeletal system uses these compensatory interactions to establish bone quality and how genetic and environmental factors impair this process, leading to reduced strength, toughness, and fracture resistance.

Acknowledgments I thank Philip Nasser and G. Felipe Duarte for their help in the preparation of the manuscript.

References

1. Albright F, Smith PH, Richardson AM. Post-menopausal osteoporosis: its clinical features. *JAMA*. 1941;116:2465–2474.
2. Beck TJ, Ruff CB, Shaffer RA, Betsinger K, Trone DW, Brodine SK. Stress fracture in military recruits: gender differences in muscle and bone susceptibility factors. *Bone*. 2000;27:437–444.
3. Bell KL, Loveridge N, Power J, Garrahan N, Stanton M, Lunt M, Meggitt BF, Reeve J. Structure of the femoral neck in hip fracture: cortical bone loss in the inferoanterior to superoposterior axis. *J Bone Miner Res*. 1999;14:111–119.
4. Black DM, Bouxsein ML, Marshall LM, Cummings SR, Lang TF, Cauley JA, Ensrud KE, Nielson CM, Orwoll ES. Proximal femoral structure and the prediction of hip fracture in men: a large prospective study using QCT. *J Bone Miner Res*. 2008;23:1326–1333.
5. Bonadio J, Jepsen KJ, Mansoura MK, Jaenisch R, Kuhn JL, Goldstein SA. A murine skeletal adaptation that significantly increases cortical bone mechanical properties: implications for human skeletal fragility. *J Clin Invest*. 1993;92:1697–1705.
6. Bousson V, Le Bras A, Roqueplan F, Kang Y, Mitton D, Kolta S, Bergot C, Skalli W, Vicaut E, Kalender W, Engelke K, Laredo JD. Volumetric quantitative computed tomography of the proximal femur: relationships linking geometric and densitometric variables to bone strength: role for compact bone. *Osteoporos Int*. 2006;17:855–864.
7. Brear K, Currey JD, Pond CM. Ontogenetic changes in the mechanical properties of the femur of the polar bear Ursus maritimus. *J Zool (Lond)*. 1990;222:49–58.
8. Carrier D, Leon LR. Skeletal growth and function in the California gull (Larus californicus). *J Zool (Lond)*. 1990;222:375–389.
9. Carrier DR. Postnatal ontogeny of the musculo-skeletal system in the black-tailed jack rabbit. *J Zool (Lond)*. 1983;201:27–55.
10. Chan GM, Hess M, Hollis J, Book LS. Bone mineral status in childhood accidental fractures. *Am J Dis Child*. 1984;138:569–570.
11. Cheverud JM. Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution*. 1982;36:499–516.
12. Cheverud JM. Developmental integration and the evolution of pleiotropy. *Am Zool*. 1996;36:44–50.
13. Crawford RP, Cann CE, Keaveny TM. Finite element models predict in vitro vertebral body compressive strength better than quantitative computed tomography. *Bone*. 2003;33:744–750.
14. Crossley K, Bennell KL, Wrigley T, Oakes BW. Ground reaction forces, bone characteristics, and tibial stress fracture in male runners. *Med Sci Sports Exerc*. 1999;31:1088–1093.
15. Crow JF. The origins, patterns and implications of human spontaneous mutation. *Nat Rev Genet*. 2000;1:40–47.
16. Currey JD. Mechanical properties of bone tissues with greatly differing functions. *J Biomech*. 1979;12:313–319.
17. Currey JD. Effects of differences in mineralization on the mechanical properties of bone. *Philos Trans R Soc Lond B Biol Sci*. 1984;304:509–518.
18. Currey JD, Alexander RM. The thickness of the walls of tubular bones. *J Zool (Lond)*. 1985;206:453–468.
19. Duan Y, Beck TJ, Wang XF, Seeman E. Structural and biomechanical basis of sexual dimorphism in femoral neck fragility has its origins in growth and aging. *J Bone Miner Res*. 2003;18:1766–1774.
20. Duan Y, Parfitt A, Seeman E. Vertebral bone mass, size, and volumetric density in women with spinal fractures. *J Bone Miner Res*. 1999;14:1796–1802.
21. Duan Y, Seeman E, Turner CH. The biomechanical basis of vertebral body fragility in men and women. *J Bone Miner Res*. 2001;16:2276–2283.
22. Duan Y, Wang XF, Evans A, Seeman E. Structural and biomechanical basis of racial and sex differences in vertebral fragility in Chinese and Caucasians. *Bone*. 2005;36:987–998.
23. Eswaran SK, Gupta A, Adams MF, Keaveny TM. Cortical and trabecular load sharing in the human vertebral body. *J Bone Miner Res*. 2006;21:307–314.
24. Evans RK, Negus C, Antczak AJ, Yanovich R, Israeli E, Moran DS. Sex differences in parameters of bone strength in new recruits: beyond bone density. *Med Sci Sports Exerc*. 2008;40:S645–S653.
25. Fazzalari NL, Moore AJ, Byers S, Byard RW. Quantitative analysis of trabecular morphogenesis in the human costochondral junction during the postnatal period in normal subjects. *Anat Rec*. 1997;248:1–12.
26. Frost HM. Bone 'mass' and the 'mechanostat': a proposal. *Anat Rec*. 1987;219:1–9.
27. Giladi M, Milgrom C, Simkin A, Stein M, Kashtan H, Margulies J, Rand N, Chisin R, Steinberg R, Aharonson Z, Kedem R, Frankel VH. Stress fractures and tibial bone width: a risk factor. *J Bone Joint Surg Br*. 1987;69:326–329.

28. Gilsanz V, Loro ML, Roe TF, Sayre J, Gilsanz R, Schulz EE. Vertebral size in elderly women with osteoporosis. Mechanical implications and relationship to fractures. *J Clin Invest*. 1995;95: 2332–2337.
29. Glatt V, Canalis E, Stadmeyer L, Bouxsein ML. Age-related changes in trabecular architecture differ in female and male C57BL/6 J mice. *J Bone Miner Res*. 2007;22:1197–1207.
30. Goldman HM, Bromage TG, Thomas CD, Clement JG. Preferred collagen fiber orientation in the human mid-shaft femur. *Anat Rec A Discov Mol Cell Evol Biol.* 2003;272:434–445.
31. Heinrich R. Ontogenetic changes in mineralization and bone geometry in the femur of muskoxen (Ovibos moschatus). *J Zool (Lond)*. 1999;247:215–223.
32. Jepsen KJ, Courtland HW, Nadeau JH. Genetically-determined phenotype covariation networks control bone strength. *J Bone Miner Res*. 2010;25:1581–1593.
33. Jepsen KJ, Hu B, Tommasini SM, Courtland HW, Price C, Cordova M, Nadeau JH. Phenotypic integration of skeletal traits during growth buffers genetic variants affecting the slenderness of femora in inbred mouse strains. *Mamm Genome*. 2009;20: 21–33.
34. Jepsen KJ, Hu B, Tommasini SM, Courtland HW, Price C, Terranova CJ, Nadeau JH. Genetic randomization reveals functional relationships among morphologic and tissue-quality traits that contribute to bone strength and fragility. *Mamm Genome*. 2007;18:492–507.
35. Karasik D, Dupuis J, Cupples LA, Beck TJ, Mahaney MC, Havill LM, Kiel DP, Demissie S. Bivariate linkage study of proximal hip geometry and body size indices: the Framingham study. *Calcif Tissue Int*. 2007;81:162–173.
36. Khosla S, Riggs BL, Atkinson EJ, Oberg AL, McDaniel LJ, Holets M, Peterson JM, Melton LJ 3rd. Effects of sex and age on bone microstructure at the ultradistal radius: a population-based noninvasive in vivo assessment. *J Bone Miner Res*. 2006;21: 124–131.
37. Kiel DP, Hannan MT, Broe KE, Felson DT, Cupples LA. Can metacarpal cortical area predict the occurrence of hip fracture in women and men over 3 decades of follow-up? Results from the Framingham Osteoporosis Study. *J Bone Miner Res*. 2001;16: 2260–2266.
38. Kimura K. Growth of the second metacarpal according to chronological age and skeletal maturation. *Anat Rec*. 1976;184: 147–157.
39. Kimura T, Amtmann E. Distribution of mechanical robustness in the human femoral shaft. *J Biomech*. 1984;17:41–46.
40. Koller DL, Liu G, Econs MJ, Hui SL, Morin PA, Joslyn G, Rodriguez LA, Conneally PM, Christian JC, Johnston CC Jr, Foroud T, Peacock M. Genome screen for quantitative trait loci underlying normal variation in femoral structure. *J Bone Miner Res*. 2001;16:985–991.
41. Kozloff KM, Carden A, Bergwitz C, Forlino A, Uveges TE, Morris MD, Marini JC, Goldstein SA. Brittle IV mouse model for osteogenesis imperfecta IV demonstrates postpubertal adaptations to improve whole bone strength. *J Bone Miner Res*. 2004; 19:614–622.
42. Lai YM, Qin L, Hung VW, Chan KM. Regional differences in cortical bone mineral density in the weight-bearing long bone shaft—a pQCT study. *Bone*. 2005;36:465–471.
43. Landin L, Nilsson BE. Bone mineral content in children with fractures. *Clin Orthop Relat Res*. 1983;178:292–296.
44. Lanyon L, Skerry T. Postmenopausal osteoporosis as a failure of bone's adaptation to functional loading: a hypothesis. *J Bone Miner Res*. 2001;16:1937–1947.
45. Li R, Tsaih SW, Shockley K, Stylianou IM, Wergedal J, Paigen B, Churchill GA. Structural model analysis of multiple quantitative traits. *PLoS Genetics*. 2006;2:e114.
46. Lotz JC, Cheal EJ, Hayes WC. Stress distributions within the proximal femur during gait and falls: implications for osteoporotic fracture. *Osteoporos Int*. 1995;5:252–261.
47. Manske SL, Liu-Ambrose T, Cooper DM, Kontulainen S, Guy P, Forster BB, McKay HA. Cortical and trabecular bone in the femoral neck both contribute to proximal femur failure load prediction. *Osteoporos Int*. 2009;20:445–453.
48. Marder E, Goaillard JM. Variability, compensation and homeostasis in neuron and network function. *Nat Rev Neurosci*. 2006;7: 563–574.
49. McMahon T. Size and shape in biology. *Science*. 1973;179: 1201–1204.
50. Melton LJ 3rd, Wahner HW, Richelson LS, O'Fallon WM, Riggs BL. Osteoporosis and the risk of hip fracture. *Am J Epidemiol*. 1986;124:254–261.
51. Milgrom C, Giladi M, Simkin A, Rand N, Kedem R, Kashtan H, Stein M, Gomori M. The area moment of inertia of the tibia: a risk factor for stress fractures. *J Biomech*. 1989;22:1243–1248.
52. Nadeau JH, Topol EJ. The genetics of health. *Nat Genet*. 2006; 38:1095–1098.
53. Olson EC, Miller RL *Morphological Integration.* Chicago, IL: The University of Chicago Press, Ltd; 1958.
54. Pandey N, Bhola S, Goldstone A, Chen F, Chrzanowski J, Terranova CJ, Ghillani R, Jepsen KJ. Inter-individual variation in functionally adapted trait sets is established during post-natal growth and predictable based on bone robusticity. *J Bone Miner Res*. 2009;24:1969–1980.
55. Papadimitriou HM, Swartz SM, Kunz TH. Ontogenetic and anatomic variation in mineralization of the wing skeleton of the Mexican free-tailed bat, Tadarida brasiliensis. *J Zool (Lond)*. 1996;240:411–426.
56. Pearson OM. Activity, climate, and postcranial robusticity: implications for modern human origins and scenarios of adaptive change. *Curr Anthropol*. 2000;41:569–607.
57. Price CP, Herman BC, Lufkin T, Goldman HM, Jepsen KJ. Genetic variation in bone growth patterns defines adult mouse bone fragility. *J Bone Miner Res*. 2005;20:1983–1991.
58. Riggs BL, Melton LJ 3rd, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, Rouleau PA, McCollough CH, Bouxsein ML, Khosla S. Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. *J Bone Miner Res*. 2004;19:1945–1954.
59. Rivadeneira F, Houwing-Duistermaat JJ, Vaessen N, Vergeer-Drop JM, Hofman A, Pols HA, Van Duijn CM, Uitterlinden AG. Association between an insulin-like growth factor I gene promoter polymorphism and bone mineral density in the elderly: the Rotterdam Study. *J Clin Endocrinol Metab*. 2003;88:3878–3884.
60. Rivadeneira F, Zillikens MC, De Laet CE, Hofman A, Uitterlinden AG, Beck TJ, Pols HA. Femoral neck BMD is a strong predictor of hip fracture susceptibility in elderly men and women because it detects cortical bone instability: the Rotterdam Study. *J Bone Miner Res*. 2007;22:1781–1790.
61. Rubin CT, Lanyon LE. Dynamic strain similarity in vertebrates; an alternative to allometric limb bone scaling. *J Theor Biol*. 1984;107:321–327.
62. Ruff C, Holt B, Trinkaus E. Who's afraid of the big bad Wolff? 'Wolff's law' and bone functional adaptation. *Am J Phys Anthropol*. 2006;129:484–498.
63. Ruff CB. Body size, body shape, and long bone strength in modern humans. *J Hum Evol*. 2000;38:269–290.
64. Rutherford SL. From genotype to phenotype: buffering mechanisms and the storage of genetic information. *Bioessays*. 2000;22: 1095–1105.
65. Rutherford SL, Lindquist S. Hsp90 as a capacitor for morphological evolution. *Nature*. 1998;396:336–342.

66. Selker F, Carter DR. Scaling of long bone fracture strength with animal mass. *J Biomech*. 1989;22:1175–1183.
67. Silva MJ, Gibson LJ. Modeling the mechanical behavior of vertebral trabecular bone: effects of age-related changes in microstructure. *Bone*. 1997;21:19119–9.
68. Skedros JG, Mendenhall SD, Kiser CJ, Winet H. Interpreting cortical bone adaptation and load history by quantifying osteon morphotypes in circularly polarized light images. *Bone*. 2009; 44:392–403.
69. Spadaro JA, Werner FW, Brenner RA, Fortino MD, Fay LA, Edwards WT. Cortical and trabecular bone contribute strength to the osteopenic distal radius. *J Orthop Res*. 1994;12: 211–218.
70. Szulc P, Seeman E, Duboeuf F, Sornay-Rendu E, Delmas PD. Bone fragility: failure of periosteal apposition to compensate for increased endocortical resorption in postmenopausal women. *J Bone Miner Res*. 2006;21:1856–1863.
71. Tabensky A, Duan Y, Edmonds J, Seeman E. The contribution of reduced peak accrual of bone and age-related bone loss to osteoporosis at the spine and hip: insights from the daughters of women with vertebral or hip fractures. *J Bone Miner Res*. 2001; 16:1101–1107.
72. Tanck E, Homminga J, van Lenthe GH, Huiskes R. Increase in bone volume fraction precedes architectural adaptation in growing bone. *Bone*. 2001;28:650–654.
73. Tommasini SM, Hu B, Nadeau JH, Jepsen KJ. Phenotypic integration among trabecular and cortical bone traits establishes mechanical functionality of inbred mouse vertebrae. *J Bone Miner Res*. 2009;24:606–620.
74. Tommasini SM, Nasser P, Hu B, Jepsen KJ. Biological co-adaptation of morphological and composition traits contributes to mechanical functionality and skeletal fragility. *J Bone Miner Res*. 2008;23:236–246.
75. Tommasini SM, Nasser P, Schaffler MB, Jepsen KJ. Relationship between bone morphology and bone quality in male tibias: implications for stress fracture risk. *J Bone Miner Res*. 2005; 20:1372–1380.
76. Ural A, Vashishth D. Interactions between microstructural and geometrical adaptation in human cortical bone. *J Orthop Res*. 2006;24:1489–1498.
77. van der Meulen MC, Jepsen KJ, Mikic B. Understanding bone strength: size isn't everything. *Bone*. 2001;29:101–104.
78. Vega E, Ghiringhelli G, Mautalen C, Rey Valzacchi G, Scaglia H, Zylberstein C. Bone mineral density and bone size in men with primary osteoporosis and vertebral fractures. *Calcif Tissue Int*. 1998;62:465–469.
79. Waarsing JH, Day JS, Verhaar JA, Ederveen AG, Weinans H. Bone loss dynamics result in trabecular alignment in aging and ovariectomized rats. *J Orthop Res*. 2006;24:926–935.
80. Wallace JM, Rajachar RM, Allen MR, Bloomfield SA, Robey PG, Young MF, Kohn DH. Exercise-induced changes in the cortical bone of growing mice are bone- and gender-specific. *Bone*. 2007;40:1120–1127.
81. Wright S. On the nature of size factors. *Genetics*. 1918;3: 367–374.
82. Zebaze RM, Jones A, Knackstedt M, Maalouf G, Seeman E. Construction of the femoral neck during growth determines its strength in old age. *J Bone Miner Res*. 2007;22:1055–1061.