CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Aging and Bone

ABSTRACT

Bones provide mechanical and protective function, while also serving as housing for marrow and a site for regulation of calcium ion homeostasis. The properties of bones do not remain constant with age; rather, they change throughout life, in some cases improving in function, but in others, function deteriorates. Here we review the modifications in the mechanical function and shape of bones, the bone cells, the matrix they produce, and the mineral that is deposited on this matrix, while presenting recent theories about the factors leading to these changes.

KEY WORDS: bone properties, aging, senescence, bone cells, bone mineral, review.

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INTRODUCTION

The image of bone as a static "skeleton in the closet" has created the false impression that, once formed, bones do not change. The purpose of this review is to demonstrate how bones change with development and aging, from the whole bone level down to the specific components, and to suggest some mechanisms for the age-dependent modifications in bone structure, composition, and function.

Bone serves mechanical and homeostatic functions, protecting the internal organs, allowing for locomotion and load-bearing, and serving as a home for marrow, and as a reservoir for calcium homeostasis. With aging, these functions become impaired, bone becomes more fragile and less able to perform its mechanical functions, and the calcium stores are often depleted (Chan and Duque, 2002). To understand why this does not occur in all individuals and how the factors that control these processes change with age is a major area of investigation.

Bone is a composite structure, consisting of inorganic mineral crystals, an extracellular organic matrix, cells, lipids, and water. The mineral crystals are analogous to the geologic mineral, hydroxylapatite. Since bone mineral is made of OH-deficient nano-particles, we will refer to it here as hydroxyapatite (HA) (Boskey, 2007). Most of the mineral crystals contain impurities, mainly carbonate, magnesium, citrate, and other trace elements whose content depends on what the animal has ingested (Grynpas, 1993b). The organic matrix is mainly type I collagen, but other types of collagen and several non-collagenous proteins, reviewed elsewhere, are also present (Zhu *et al*., 2008). The cells, which produce, nurture, and remodel the mineralized extracellular matrix, also respond to mechanical and other signals, which determine the properties (morphology and function) of the bone. The relative composition of bone varies with health and disease, tissue site, and animal and tissue age. It is the variation with age that is the focus of this review.

Independent of age, bone has a hierarchical structure: from the level of whole tissue, where there are different types of bones—long and short, flat and tubular—to the tissue level, where bone is arranged into cortical (compact) and trabecular (woven and lamellar) structures, to the microscopic level, where bone consists of cells, matrix, and mineral, and to the nanometer level, where the individual crystals and collagen fibrils can be seen (Fig. 1).

BONE GROWTH AND DEVELOPMENT

Bone development begins in the embryo with the formation of a cartilage anlagen that gradually becomes replaced with bone in a process known as endochondral ossification (Provot *et al*., 2008) (Fig. 2). As reviewed in detail elsewhere (Zuscik *et al*., 2008), during this process, developmental signals within the "growth plate", cartilaginous tissue found at the distal and proximal ends of the developing long bone, regulate the differentiation and maturation of the resident cells (chondrocytes). Chondrocytes proliferate, deposit a matrix, and then become surrounded by a mineralized matrix, and subsequently

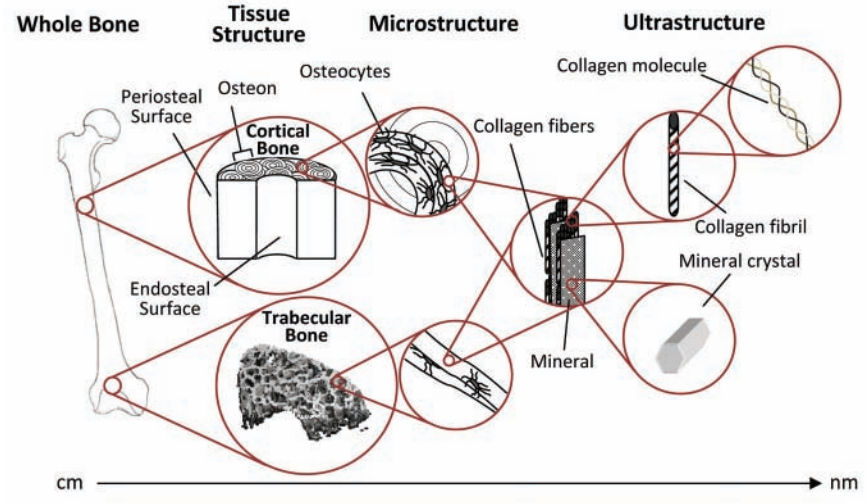

Figure 1. The structural levels of bone. Cortical bone is made up of longitudinally oriented osteons, and the trabecular bone within the metaphyses is made up of connected struts and plates. In both bone types, the bone is laid down in layers (lamellae). Both tissue types contain identical components, and their properties are dependent on the amount, morphology, and interaction of these components at each level.

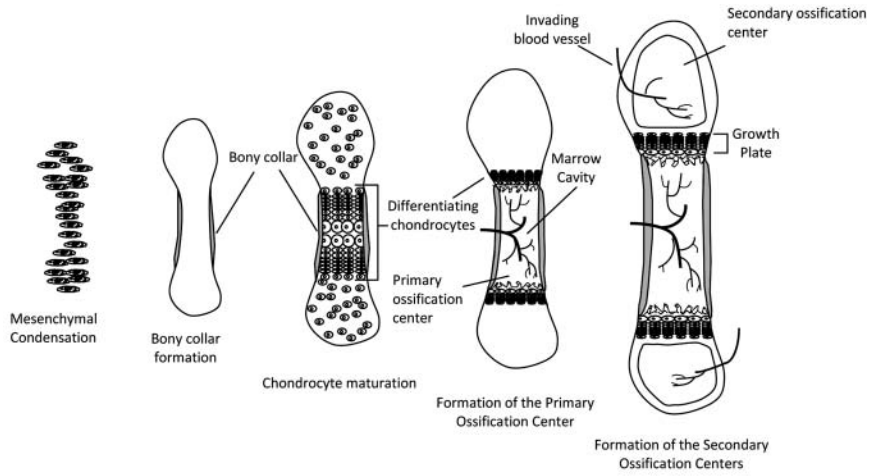

Figure 2. Endochondral ossification of long bones. Condensation of mesenchymal cells forms the general shape of the long bone. Differentiation of these cells into chondrocytes begins the process of bone formation in the primary ossification center. In late differentiation, chondrocytes undergo apoptosis, leaving behind a mineralized scaffold onto which osteoblasts brought in by the invading vasculature lay down bone, lengthening the bone while forming the marrow cavity. As development continues, secondary ossification centers form in the epiphyses. Bone increases in width through deposition of bone on the periosteal side and, through endochondral ossification, continues to increase in length until the growth plates fuse.

undergo apoptosis (programmed cell death). Blood vessels then invade the tissue, forming a marrow cavity, and the calcified cartilage is replaced by bone, hence the bone grows in length. Bone grows in width (appositional bone growth) by periosteal expansion, with new bone forming on already existing surfaces. In most species, the growth plates close at puberty, and long bone growth essentially stops. Appositional bone growth continues, and this accounts for the changes in bone shape, and consequent mechanical changes (Rauch, 2005).

The endochondral ossification process is recapitulated during fracture healing (Ferguson *et al*., 1999); thus, it is interesting to ask whether there are changes in fracture healing with age, although anecdotally one knows that, in general, children heal their fractured bones more rapidly than do adults. In rabbits, studied throughout their lifespan, the chondrogenic potential of the periosteum decreased with age (O'Driscoll *et al*., 2001). Age also affects the rate at which rats regain their mechanical competence after fracture (Meyer *et al*., 2001). In mice, the rate of cellular differentiation, and hence the rate of repair, decreases with age (Naik *et al*., 2009). The rate of revascularization also decreases with increasing age (Lu *et al*., 2008). No such comparative analysis of the rate of fracture healing in humans has been reported. These observations may explain some of the observed shifts in bone morphological and mechanical properties with age, with decreases in the rates of multiple processes such as cell proliferation and differentiation, as well as tissue revascularization, all being affected.

AGE-DEPENDENT CHANGES IN BONE MECHANICAL BEHAVIOR

The components of bone are maintained in a balance to resist fracture while optimizing the weight of the skeleton. Stiffness (resistance to deformation) and strength (maximum stress to failure) are required to carry large loads, while toughness, or ductility, is required to absorb the energy from impact loads. A shift in the balance to a higher tissue mineral content will generally yield stiffer but more brittle bones. It is important to recognize that changes in collagen structure may also contribute to increased brittleness due to the shift in its cross-linking profile, which not only stiffens the organic matrix, but also affects the morphology of the mineral component, as will be discussed below. It is also important to realize that, although bone mineral density (BMD) decreases in some fragility diseases such as osteoporosis (Manolagas, 2010), it is increased in others such as osteopetrosis (Kaste *et al*., 2007). Thus, it is the tissue-level properties in combination with the bone geometry that determine fracture risk.

There are reports that older individuals may have a 10-fold-increased 10-year fracture risk compared with younger individuals with the same BMD (Kanis, 2002). Since many investigators and clinicians believe that BMD is a marker of fracture susceptibility, because it declines with age in both women and men, it is important to examine why there is an age-dependent increase in bone fragility or in brittleness of bone. Several studies have reported that cortical bone becomes more brittle and weaker with age (Currey and Butler, 1975; Burstein *et al*., 1976; McCalden *et al*., 1993; Tommasini *et al*., 2007). Similarly, the trabecular struts (see Fig. 1) also weaken with age (Nagaraja *et al*., 2007). In studies that extend to extremely old age (> 85 yrs), the incidence of fractures is reported to be 10-15 times as likely as in persons aged 60-65 yrs (Melton, 1996; Yates *et al*., 2007).

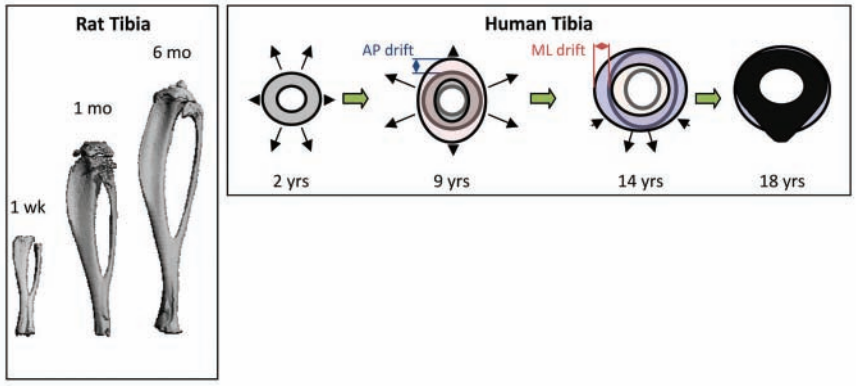

Figure 3. Long bone geometrical changes with age. **Left**: micro-CT 3D renderings of rat tibias with increasing age. **Right**: Cortical drift in the tibias of human bones with age. Changes in periosteal and endosteal remodeling around the bone are non-uniform throughout life. Note that the AP drift is larger from 2 to 9 yrs, and that the ML drift is greater from 9 to 14 yrs. Right panel adapted from Goldman *et al.* (2009). AP: anterior-posterior direction. ML: medial-lateral direction.

The strength of bone as a tissue is determined by the amount of mineral that is there (usually provided clinically as a two-dimensional BMD and a T- or Z-score comparing the value with that of healthy sex-matched 25-year-olds or with healthy age-matched control individuals, respectively), and the way that mineral is distributed relative to the forces applied to the bone (Ammann and Rizzoli, 2003). With aging, sex-related differences (not discussed here) in the distribution (geometry and morphology) become more pronounced, and these differences are believed to contribute to increased fracture incidence in the extremely elderly population (Yates *et al*., 2007). In a study of mice ranging in age from 3-77 wks, where the mice were grouped into age ranges of fewer than 12 wks, 13-50 wks, and 50-77 wks, bone density and elasticity decreased with age (Kavukcuoglu *et al*., 2007). Testing cortical bones of old and elderly specimens in tension, Nyman *et al*. (2007) also found that increasing age affected all parameters (including strength) except for the elastic stiffness. Age-dependent changes were associated with increases in cortical porosity, non-enzymatic collagen cross-links, and absolute collagen content, as will be discussed below.

Bone that is repetitively loaded (by normal activities of daily life, by extreme exercise, or in *ex vivo* experimental situations) develops cracks, initially at the sub-micron level, but eventually these cracks become visible, and if they are not repaired by the bone remodeling process, they can lead to failure (O'Brien *et al*., 2005). The cause of the initial damage is hypothesized (Mohsin *et al*., 2006) to be either disruption of bone mineral crystallites, debonding at the mineral organic interface, or disruption of collagen fibrils, or some combination of all three. Disruption of the structure of the bone cells that are embedded in mineral, the osteocytes, also can contribute (Verborgt *et al*., 2000). For example, in an animal model of early premature aging, the klotho mouse, osteocytes die and their interaction with the matrix is altered (Suzuki *et al*., 2005). The extent of this micro-damage increases exponentially with age in humans (Schaffler *et al.*, 1995), as the micro-crack densities and lengths also increase (Vashishth, 2007). It is likely that both the inability to repair the cracks (Hirano *et al*., 2000) and their increasing propagation with age, contribute to the reduced toughness of both cortical and trabecular bone (Currey *et al*., 1996).

CHANGES IN BONE MORPHOLOGY WITH AGE

Morphology describes the shapes (geometry) of bones, in terms of whether they are long bones (such as the femur and tibia), short bones (such as the bones of the feet and hands), or flat bones (such as the calvaria or breast bones). The morphological traits that determine strength are the sizes and the shapes of the bones (Jepsen, 2009). There are compact areas (cortices) and spongy areas (trabecular) found in the ends of all long bones and in the central region of other bones (see Fig. 1). Bones change in shape to facilitate their mechanical functions—being strong enough to withstand large forces and streamlined enough to minimize energy demands (Seeman, 2003; Wang and Seeman, 2008; Seeman, 2009, Jepsen, 2009) (Fig. 3).

During development and aging, bone shape changes in response to load, as postulated in Wolff's Law and demonstrated in exercise experiments in various animals (Woo *et al*., 1981; Wallace *et al*., 2007; Chen *et al*., 2009), as well as hormonal and growth factor signals. As the shape changes (lengthening, narrowing walls, shifting the center of gravity), the bone's functional ability may also be modified. This can be seen in animal models, as well as in humans. In mice, for example, cortical bone size, trabecular bone volume, and bone strength decline with age after 3 mos corresponding to achievement of peak bone mass in this species (Ramanadham *et al*., 2008), as the bones become more slender (Kawashima *et al*., 2009). In mature rats (from 8-36 mos of age), the only change reported in bone structure is an increase in the cross-sectional moment of inertia (distribution of the bone around the central axis), due to the expansion of the outer diameter (periosteal deposition) of their bones, with a thinning of the cortical walls (endosteal resorption) (LaMothe *et al*., 2003;

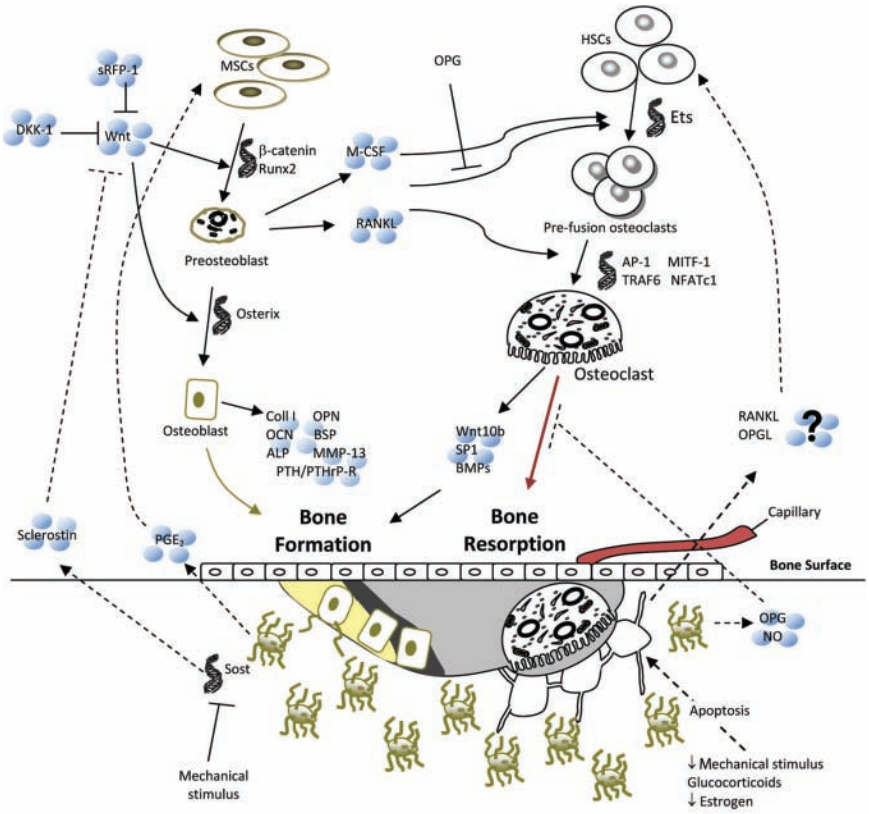

Figure 4. Bone remodeling pathways. Several factors can initiate bone remodeling, which, in a balanced system, begins with resorption by osteoclasts and ends with formation by osteoblasts. Signals from external sources (Wnts) and from within the bone (osteocyte apoptosis) contribute to osteoblast and osteoclast differentiation and activity, a selection of which is shown in this figure. Light gray = resorption pit, dark gray = osteoid, yellow = newly mineralized osteoid.

Westerbeek *et al*., 2008). However, in a more recent study, when the trabecular bones in proximal tibias of 23-month-old and 5-month-old rats were compared, mineral density, bone volume fraction, and trabecular number were significantly reduced in the aged rats compared with the younger rats (Pietschmann *et al*., 2007). Serum markers of bone formation were also reduced in the older rats. In bovine samples (Nagaraja *et al*., 2007), the trabecular struts decreased in thickness with age, and the degree of anisotropy was decreased in the older specimens, as shown by micro-computed tomography.

Human samples, although more difficult to assess in such numbers, show similar patterns of morphological changes with age. In necropsied tibias from males ranging in age from 17 to 46 yrs, significant changes in mechanical properties were correlated with the size (slenderness) of the tibia in an age-independent fashion, with increased mechanical weakening, and more brittle behavior observed with increasing age (Tommasini *et al*., 2005, 2007).

The shape of long bones changes during development (Fig. 3). Beginning early in life, the cross-sectional geometry of long bones undergoes modification, beginning with a more uniform outer wall thickness and progressing to a more ellipsoidal shape through a process known as "cortical drift" (Goldman *et al*., 2009). Cortical drift occurs when formation is decreased and resorption increased on the endosteal surface, while bone is deposited on the periosteal surface. This leads to an increase in bone diameter and, in the case where endosteal resorption is greater than formation, cortical thinning. Cortical drift occurs rapidly during pre-pubertal growth, levels off after closure of the epiphyseal plate, and increases again in the elderly, often resulting in weaker bone with a wider diameter and significantly thinner cortices. Cortical drift does not occur uniformly around the bone diameter. Like many of the transformations we will discuss, it is the response to mechanical demands and biological signaling that results in the alteration in bone geometry throughout life (Goldman *et al*., 2009).

In humans, there is some debate (Seeman, 2003) as to whether the patterns of resorption and formation are similar in men and women, but this is beyond the scope of this review. It must be noted, however, that within trabecular bone, the age-related loss in men is predominantly due to thinning of the individual struts, while in women the loss is due to a decrease in connectivity (Aaron *et al*., 1987). Thus, in most animals studied, as well as in humans, bone gets stiffer with age, and the cross-sectional area (trabecular surface over which mechanical load is distributed) decreases. The impact of this is that stress (load/unit area) increases and bone deformation increases, and in fragile bone, this may lead to fracture.

In the healthy individual, bone formation and resorption are in a state of balance. The variations in bone morphology are related to the changes in this balance between bone formation and bone remodeling. While these changes do not affect all bones equally, the general trends are similar. For example, in terms of hip structure, men and women older than 85 yrs of age have been reported to have the most "unfavorable" hip geometry, narrower cortices, and decreased resistance to bending/buckling (Yates *et al*., 2007). Similar "unfavorable" properties also exist in tibias (Tommasini *et al*., 2005), and perhaps in other bones, but this might not be detected in bones that are loaded to a lesser extent than tibias and femurs. The reason for these morphological changes is related to genetics, the loading of the bones, and the activity of the cells.

THE BONE CELLS AND HOW THEY AGE

There are several types of cells in bone (Fig. 4) (for review, see Blair *et al*., 2007; Bonewald, 2007; Bonewald and Johnson, 2008; Lian and Stein, 2008; Boyce *et al*., 2009; Chen *et al*., 2009). The majority of the bone cells are of mesenchymal cell origin: chondrocytes, which are responsible for the deposition of the growth plate and its subsequent remodeling (Fig. 2); osteoblasts,

which synthesize the bone matrix and facilitate the mineralization process; and osteocytes, which respond to load and regulate bone resorption and formation (Adachi *et al*., 2009).

Osteoblastogenesis is regulated by numerous pathways, some of which are illustrated in Fig. 4. From 60 to 80% of osteoblasts recruited to a resorption pit die by apoptosis within 200 days (Jilka *et al*., 2007; Manolagas and Parfitt, 2010). Some of the osteoblasts become small, quiescent bone-lining cells along inactive surfaces. The remaining osteoblasts are surrounded by mineral and extend long processes (dendrites), which allow signaling and nutrition to pass from cell to cell through channels in the bone called canaliculi. These mineral-surrounded cells are known as osteocytes, and they make up approximately 90% of the cells in bone. They have a lifespan of 1 to 50 yrs (Manolagas and Parfitt, 2010), and they are the cells that are most responsive to mechanical signals (Matsuo, 2009; Rochefort *et al*., 2010). These cells maintain contact with other osteocytes and the surface through gap junctions. In fact, the most abundant gap junction protein in bone, connexin 43, is required for bone modeling and remodeling (Matsuo, 2009; Rochefort *et al*., 2010). Theoretically, mechanical loading results in fluid flow within the canalicular-lacunar network, resulting in cell-level shear forces, membrane deformation, and tension in elements connecting osteocytes with the canalicular walls, which results in the release of anti-apoptotic factors. Osteocyte apoptosis due to disruption of the canalicular network or lack of mechanical stimulus is hypothesized to regulate osteoclast and osteoblast function to induce the remodeling of the local bone tissue (Rochefort *et al*., 2010).

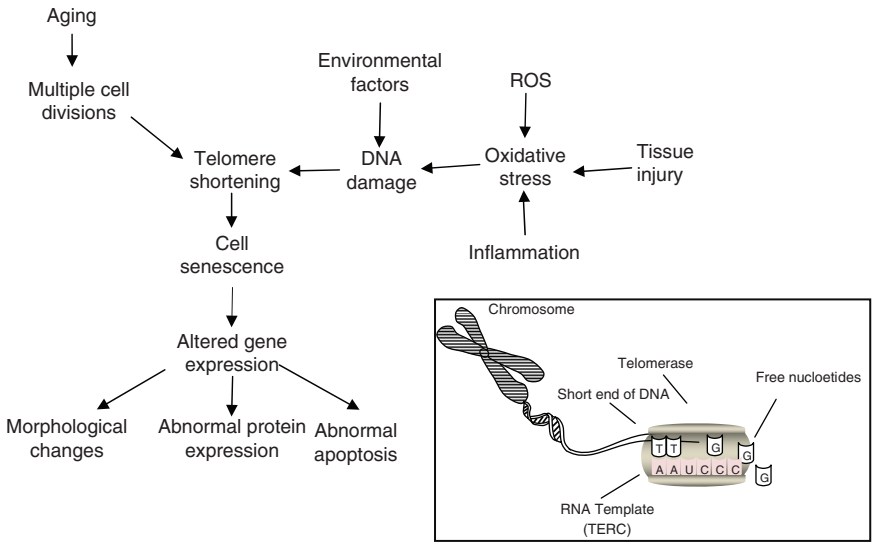

Figure 5. Factors affecting cell senescence. Cells reach senescence when regions at the ends of chromosomes, telomeres, reach a critical length due to the cells' inability to replicate them properly. The inset shows the activity of an enzyme, telomerase, that allows stem cells to extend the length of the telomeres and therefore maintain an almost indefinite capacity for self-renewal. There are many factors that also contribute to telomere shortening, which may lead to abnormal cell behavior. The accumulation of damaged cells and the inability of a depleting stem cell population to replace these cells with aging may affect tissue function. Adapted from Muller (2009).

The other major bone cells, the osteoclasts, are of hematopoietic origin, and they are multinucleated giant cells responsible for removing bone (resorption) following signals from osteoblasts and osteocytes. In male rats and in all mice studied to date, osteoclast differentiation is impaired in old as compared with younger animals (Cao *et al*., 2005, 2007; Pietschmann *et al*., 2007), and the relative rates of bone removal far exceed the rates of new bone formation. Another interesting finding is that osteoclasts develop to a greater extent when they encounter older bone *in vitro*, so that older bone may be resorbed preferentially (Henriksen *et al*., 2007). Some of the factors involved in the differentiation of these cells are shown in Fig. 4. These factors and the way they control the development of the skeleton have been reviewed by Provot *et al*. (2008).

All normal cells, including osteoblasts, osteoclasts, and osteocytes, have a limited lifespan, which is controlled by the number of replication cycles and external factors (Fig. 5). The Hayflick limit of cell division indicates that cells can undergo only a limited number of divisions, and that the number of such divisions declines with age (Martin *et al*., 1970). In the cell nucleus, the lengths of the telomeres at the ends of genes are presumably the determinant of that number. A telomere is a repetitive length of DNA and associated proteins that provide stability to the ends of chromosomes. With each cell division, telomeres decrease in length due to the inability of the cell to fully replicate this region, and once the telomere length reaches some critical level, cell senescence and apoptosis are initiated (Calado, 2009). Self-renewing hematopoietic and mesenchymal stem cells have low levels of an enzyme, telomerase, which extends their life cycle significantly compared with that of mature cells. However, the activity of this enzyme is not indefinite and decreases with age (Calado, 2009). Damage to telomeres by UV light and oxidative stress may also accelerate telomere shortening, thereby curtailing the lifespan of cells (Muller, 2009).

It has been noted in mice null for telomerase-specific genes that the first generation of the knock-out animals had no advanced aging phenotype, presumably due to telomere reserves that retained their chromosome stabilizing functions (Blasco *et al*., 1997). However, mice from the third generation and beyond exhibited systemic impaired organ function, tissue atrophy, and classic age-related disorders such as osteoporosis (Lee *et al*., 1998). Werner syndrome in humans results in the premature onset of age-associated diseases with accelerated telomere attrition after puberty (Martin, 2005). However, *Wrn*-null mice do not demonstrate any of the classic symptoms of the disease until the knock-out is developed in mice with telomerase-deficient backgrounds, further highlighting the role telomere maintenance in the onset of the premature aging phenotype (Chang *et al*., 2004). Studies in humans have found that telomere length was associated with

decreased development of age-associated diseases; the connection between telomere length and longevity is still in question (Sahin and DePinho, 2010).

Cells often show changes in gene expression and activity with age, perhaps due to altered gene (mRNA) translation. A study by Kennedy and Kaeberlein (2009) found that treatments that decrease mRNA translation in animals increase their lifespan. Of particular importance to the cells in bone, although this appears to be true for all cells, aging is associated with the development of an inability to respond to forces ["mechanotransduction" (Chen *et al*., 2009)]. As recently reviewed (Wu *et al*., 2010), the ability of cells, in general, to sense, process, and respond to mechanical stimuli appears to be altered with aging, and these changes are associated with increased susceptibility to mechanical damage, increased apoptosis, alterations in intracellular signaling, and an impaired regulation of gene expression.

Among the key cell-based changes that occur with age, which in turn affect bone's decline in mechanical function are modifications in the amount and rate of bone remodeling, the process by which osteoclasts remove existing bone (resorption), and osteoblasts then replace that bone (formation). With age, the amount of bone deposited with each cycle of remodeling decreases (Szulc and Seeman, 2009), possibly due to a reduction in the number of cell precursors of osteoblasts, a reduction in the number of stem cells from which these precursors are derived, or a reduction in the lifespan of osteoblasts. The signals that lead to differentiation of osteoblast precursors decrease with age (Lee *et al*., 2005), which may also contribute to the loss in osteoblast numbers. The number of hematopoietic cells, which are osteoclast precursors, declines with age in non-human primates (Lee *et al*., 2005), and there may also be a decrease in the amount of surface available for resorption. The net result is a decrease in the amount of bone with age, starting fairly early in life (Szulc and Seeman, 2009).

Few studies have examined whether human bone cell differentiation is age-dependent. In one study of 80 patients aged 14-79 yrs, bone marrow stromal cell gene expression was characterized for several markers of differentiation: Runt-related transcription factor 2 (Runx2) for osteoblasts, Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) for adipocytes, SRY-box containing gene 9 (sox9) for chondrocytes, and Receptor Activator for Nuclear Factor κB Ligand (RANKL) for osteoclasts were measured (Jiang *et al*., 2008). Markers of apoptosis were elevated with increasing age, and RANKL and PPAR-γ levels were positively correlated with age in female patients, but not in males. There was also a slight (not significant) decrease with increasing age in the osteoblast marker, Runx2. In another study (Zhou *et al*., 2008), the patients ranged in age from 17 to 90 yrs, and the markers examined were senescence-associated β-galactosidase, proliferation, apoptosis, p53 pathway genes, and osteoblast differentiation by alkaline phosphatase activity and osteoblast gene expression analysis. There were 4 times as many marrow stromal cells that were positive for senescence, and the doubling times for the older subjects' samples were correlated with age. There was also an age-dependent decrease in marrow stromal cell proliferation and osteoblast differentiation. All of these indicate that cell differentiation and proliferation in human bone is age-dependent.

Apoptosis is a regulatory mechanism in most tissues and plays a role in normal tissue maintenance (Carrington, 2005). The dysregulation of apoptosis contributes to the imbalance between bone resorption and formation, as well as changes in local tissue mechanical properties (Carrington, 2005). It has been noted that osteocyte apoptosis increases with tissue age, which contributes to bone weakening independent of BMD through at least two mechanisms: (i) the formation of areas of micropetrosis due to mineralization of empty lacunae; and (ii) disruption of the canalicular system, thereby decreasing repair of microcracks (Jilka *et al*., 2007; Manolagas and Parfitt, 2010). Studies have also shown that many of the factors that regulate cell function in bone remodeling, such as steroids and hormones (glucocorticoids and estrogen), local autocrine and paracrine factors (inflammatory cytokines, Wnts, and the TGF-β superfamily), and mechanical stimuli also regulate apoptosis of osteocytes and osteoblasts (Almeida *et al*., 2007b). Cell senescence may alter the responses of cells to apoptotic signals (see Muller, 2009, for review), although whether this is the case in bone cells has yet to be investigated.

GENETIC, MOLECULAR, AND OTHER CHANGES IN BONE CELL RESPONSES WITH AGING

There are multiple pathways that control differentiation and metabolism by the bone cells. These were recently reviewed in detail elsewhere (Provot *et al.,* 2008; Chau *et al*., 2009), and here we will consider only those that are known both to be affected by age and to result in age-dependent changes in bone properties.

Signaling by a family of genes called wingless-type MMTV integration site (Wnt) (Williams and Insogna, 2009) is believed to regulate cellular aging in all cells, although the mechanism through which this occurs has not been completely accepted (Brack *et al*., 2007; DeCarolis *et al*., 2008) (Fig. 6). A study comparing the expression levels of all genes in the Wnt pathway in bones of different ages, and in cells isolated from young and old bones, found that all Wnt-associated genes were decreased in adult and old mice compared with younger mice, with many being significantly decreased (Rauner *et al*., 2008). Of note, transgenic overexpression of one of the Wnt genes (Wnt 10b) prevented bone mass loss in aged mice, again demonstrating the importance of the Wnt pathway in aging osteoblasts (Bennett *et al*., 2005). Studies in an animal model of early senescence, the senescence-accelerated mouse strain P6 (SAMP6), reported that a soluble frizzled related protein, sFRP-4, a protein that increases osteoblast differentiation, is negatively associated with peak bone mass (Nakanishi *et al*., 2006). In the canonical Wnt pathway, Wnt proteins bind to a Frizzled family member transmembrane receptor to initiate the signaling cascade by activating the Disheveled protein (Dsh) by excessive phosphorylation. This in turn prevents phosphorylation of β-catenin by the complex (GSK-3, APC, and Axin) (Fig. 6). If not proteolytically degraded, β-catenin accumulates exterior to the cell nucleus, where stable β-catenin interacts with lymphoid enhancer factor/T-cell factor (Lef/Tcf) and is translocated into the nucleus as a complex of β-catenin/Lef/Tcf to stimulate target gene transcription (Novak and Dedhar, 1999). When Wnt signals are not present, β-catenin is kept in low concentrations *via* the degradation

complex that consists of 4 proteins (GSK-3, Axin, APC, and β-TrCP/Slimb) and by the proteolytic pathway. In the degradation complex, Axin and APC are the scaffolds that bind to both β-catenin and GSK-3 to facilitate the phosphorylation of β-catenin's amino terminus by GSK-3, facilitating its ubiquitination and degradation.

Recently, oxidative stress has been suggested to be an additional factor contributing to bone cell aging. This again occurs through the Wnt pathway, albeit indirectly, as reactive oxygen species (ROS) activates FoxOs [a series of transcription factors reviewed elsewhere (Salih and Brunet, 2008), which in turn ties up β-catenin, minimizing its effective concentration in the cells and decreasing the formation of osteoblasts (Almeida *et al*., 2007a). A decrease in Wnt signaling by the activation of PPAR-γ *via* ligands generated from lipid oxidation also contributes to the age-dependent decrease in osteoblast formation (Manolagas, 2010).

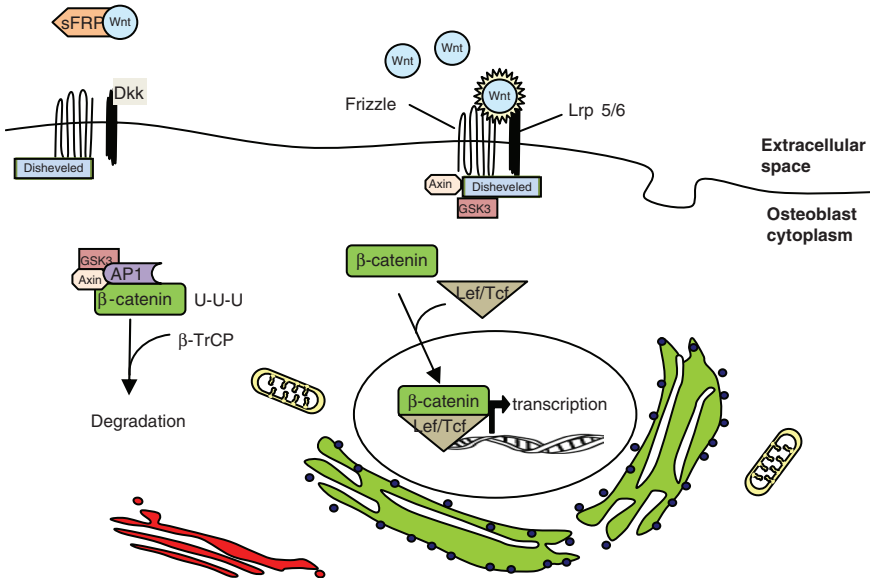

Figure 6. Wnt/β-catenin pathway of osteoblast differentiation. The translation of genes that stimulate osteoblast differentiation require the translocation of β-catenin from the cytoplasm to the nucleus of mesenchymal stem cells. β-catenin is targeted for degradation by the GSK3/AP1/Axin complex. Wnt binding to frizzled and Lrp 5/6 interrupts this process, allowing β-catenin to complex with Lef/Tcf. Dkk inhibits Wnt binding by interacting with Lrp 5/6. sFRP also inhibits this pathway by sequestering Wnt.

BONE PROTEIN CHANGES WITH AGE

The organic matrix of bone consists of collagen (mainly type I) and approximately 5% (by weight) non-collagenous proteins. The collagen provides the flexibility (toughness) to the bone structure, which provides resistance to impact loading, and serves as a template for the oriented deposition of mineral crystals. Collagen is secreted from the cell as triple-helical fibrils which self-associate to form larger fibrils and then fibers. Extensive post-translational modifications (hydroxylation, glycation) occur before the fibrils associate within the cell. Once extruded from the cell, globular domains that help keep the fibrils soluble in the cell are cleaved. These fibrils are then stabilized and modified extracellularly by the formation of cross-links, based both on reduction of Schiff-bases and aldol condensation products within and between the fibrils, and by the addition of sugars to the collagen fibrils (advanced glycation end-products) [for review, see Saito and Marumo (2010). It is the cross-linking of the collagen fibrils that has the greatest impact on the strength of collagen fibrils.

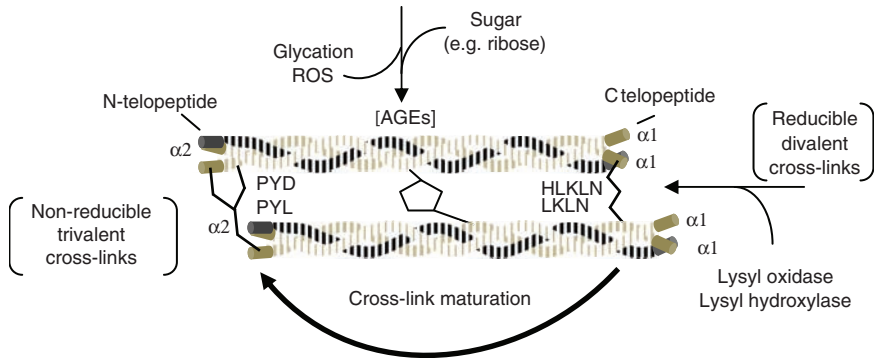

Figure 7. Chronological formation of collagen cross-links. As collagen matures, reducible cross-links become non-reducible. Advanced glycation end-products (AGEs) also accumulate between the helical parts of the molecules as the collagen persists in the tissue. Both contribute to the stiffening of the collagen matrix with age, which may contribute to bone tissue properties. Adapted from Leeming et al. (2009) and Saito and Marumo (2010).

There are two different categories of collagen cross-links, and they vary differently with age (Fig. 7). The cross-links that are formed enzymatically by lysyl hydroxylase and lysyloxidase (enzymatic cross-links) connect the N- or C-terminus of one collagen molecule to the helical region of another. They then mature, with age, to trivalent pyridinoline (PYD) and pyrrole (PYL) cross-links, which connect two terminal regions and a helical region, thereby increasing the stiffness of the collagen (Vashishth *et al*., 2001). Those formed by glycation- or oxidation-induced non-enzymatic processes, advanced glycation end-products (AGEs), such as glucosepane and pentosidine, increase in formation as the collagen persists for longer times in the tissue. Limited numbers of non-enzymatic cross-links were found to be structurally related to the morphology of the trabecular

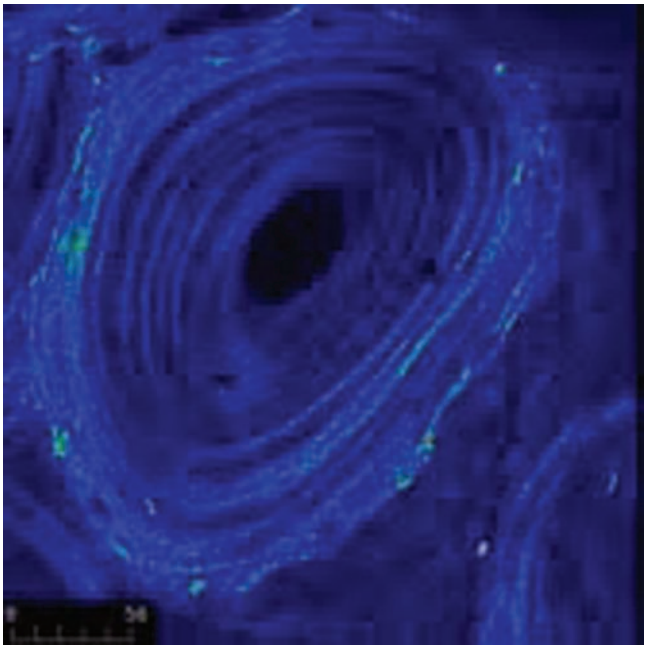

Figure 8. Collagen orientation of osteons obtained by second-harmonic generation microscopy, where the intensity is proportional to the orientation of the collagen. The orientation increases with tissue age, as is apparent in the outer, older rings of the osteon, which have a brighter intensity. Image from a 6-year-old baboon courtesy of Jayme Burket.

bone (Banse *et al*., 2002). In a study of autopsy samples from individuals 54-95 yrs old, Viguet-Carrin *et al*. (2010) reported that the enzymatic cross-links were not correlated with micro-architecture, while there was a strong correlation with the non-enzymatic glycation cross-link, pentosidine, in vertebral trabecular bone. The study also reported an increase in the amount of pyridinoline but not the other cross-links, a finding noted earlier in iliac crest trabecular bone (Bailey *et al*., 1999). Age-dependent increases in both pentosidine and lysyl pyridinoline cross-links, but not in hydroxylysyl-pyridinoline cross-links, were found in cortical bone osteons (Nyman *et al*., 2006). This finding led the authors to speculate that the rate of non-enzymatic cross-linking increases with age, while formation of mature enzymatic cross-links may decrease. Such changes would result in decreased strength and toughness of the bone and, ultimately, decreased resistance to crack propagation. Similar findings were also reported for bovine bone (Tang *et al*., 2007). Thus, cross-links based on non-enzymatic glycation occur to a greater extent with age than do enzymatic cross-links.

Formation of cross-links affects both the way the collagen mineralizes and the way micro-damage is propagated. There is also evidence to suggest that the accumulation of AGEs within bone tissue can be removed only by bone resorption, and their presence increases osteoclast activity while decreasing formation by osteoblasts, thereby contributing to the fragility of bone with age (Viguet-Carrin *et al*., 2010).

Important features of the bone collagen network include the orientation of the collagen fibrils and the co-alignment of mineral crystals with the fiber axis of the collagen. Collagen orientation increases with tissue age, as is seen in images of osteons obtained by second-harmonic generation microscopy (Fig. 8), where the intensity is proportional to the orientation (Campagnola and Loew, 2003). Similar to the accumulation of non-enzymatic cross-links, orientation is an age-dependent feature.

There are also age-dependent changes in the expression and relative presence of the non-collagenous proteins (Ikeda *et al*., 1995). Such proteins, reviewed in detail elsewhere (Zhu *et al*., 2008), are for the most part multifunctional proteins important for regulating cell-matrix and mineral-matrix interactions, as regulators of mineralization, and for playing a role in signaling. Much of their multifunctionality is related to the extensive post-translations they undergo (fragmentation, glycosylation/de-glycosylation, phosphorylation/dephosphorylation). Thus, it is important to note that not only do their distributions change with age, but also the extent of their post-translational modification decreases with increasing age (Plantalech *et al*., 1991; Grzesik *et al*., 2002).

Decreased protein production with age was reported by Grynpas *et al*. (1994), where a comparison of trabecular bone from human femoral necks showed that younger individuals (ages 18-37 yrs) had more extracellular bone matrix proteins than individuals aged 51-79 yrs, and that there were increased bone matrix protein fragments in the older group. This was also shown in cultures of undifferentiated osteoblasts (pre-osteoblasts) obtained from human trabecular bone from the embryo to age 60 yrs (Fedarko *et al*., 1992). Cell proliferation was greatest in the 16-18th wks of gestation, declining after birth and then continuously with donor age, so that the rate of proliferation in the 30-year-old age group was ¼ that of the fetal samples; the rates of total protein synthesis mirrored the age-dependent changes in proliferation. The relative distribution of the proteins expressed in culture was not constant, however, indicating that synthesis rates do vary. Of the proteins studied, small proteoglycan content followed total protein content, but osteonectin, a cell-binding protein, increased in the teen years until puberty, and then steadily decreased thereafter. Similarly, in the klotho knockout mouse, a model of early senescence discussed later in this review, comparison of the distribution of proteins in 6-month-old mutant and wild-type mice of the same sex, background, and age showed increased histochemical staining for dentin matrix protein 1 (DMP1) and osteopontin in the mutant mice. DMP1 is a protein relatively specific for osteocytes; osteopontin is made by a multitude of cells (Suzuki *et al*., 2005) and functions in cell signaling and control of mineralization, among other things. Analysis of the data stresses the alteration in protein expression with age in the klotho mice, although the effect of genetic manipulation on the expression of these proteins is not known.

MINERAL CHANGES WITH AGE

The mineral content of bone (also referred to as "mineralization" or "ash content") increases with age, and classic studies have shown that the breaking stress of bone increases exponentially with ash content, while the toughness of bone (resistance

to fracture, or the inverse of brittleness) declines as the ash content reaches a maximum (Currey, 1969). This is seen not only in ash weight determination but also in the areal BMD, which is more frequently measured. BMD measurements have been compared by a variety of techniques for a variety of species, demonstrating the increase in mineral content during growth and development, and the decline with later aging (Fig. 9).

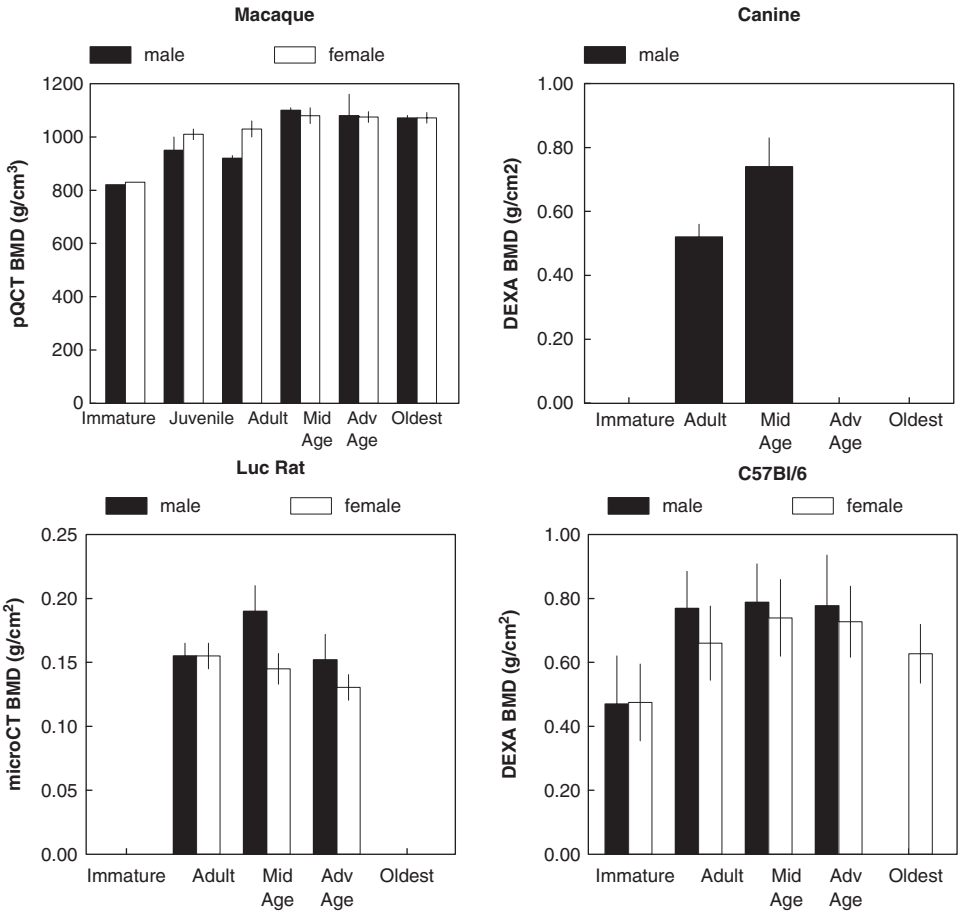

Figure 9. Change in BMD of various species with age. All of the plotted species show an increase in BMD with age. Male increases faster and female decreases earlier, but both sexes decline with age. Adapted from the following: macaque – Cerroni *et al.* (2000); canine – Schneider *et al.* (2004); Luc rat – Duque *et al.* (2009); C57Bl/6 – Somerville *et al.* (2004).

The mineral found in bone is an analogue of the natural occurring mineral, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Bone mineral crystals are nano-crystals of approximate dimensions (1-1.5 nm thick x 5-25 nm wide x 8-40 nm long) (Eppell *et al*., 2001; Tong *et al*., 2003; Burger *et al*., 2008). They also contain a variety of inclusions and substitutions that also vary with age. Prevalent among these substituents is carbonate, which substitutes for hydroxyl and phosphate within the apatite surface and the crystal lattice (LeGeros, 2002). The most comprehensive report describing how normal human bone mineral changes in composition and crystal size as a function of age was based on x-ray diffraction analyses by Hanschin and Stern (1995), who examined 117 homogenized iliac crest biopsies from patients aged 0-95 yrs. They found that the bone mineral crystal size and perfection increased during the first 25-30 yrs and then decreased thereafter, slightly increasing in the oldest individuals. Maturation of the mineral, as shown by the line-broadening of the x-ray diffraction data, was later also noted in human embryonic vertebrae as a function of age (Meneghini *et al*., 2003). While in some of these cases cortical and trabecular bone tissues were examined separately, there was no attempt to distinguish locations in the bone (surface *vs*. internal) or male and female differences, which are known to exist (Tosi *et al*., 2005).

The same 117 homogenized biopsy samples were analyzed by wavelength-dispersive x-ray fluorescence to quantify the carbonate substitution in the hydroxyapatite mineral as a function of age. Although the changes observed in carbonate substitution were relatively slight (at most 10%), there was a general increase from 0-90 yrs that is distinct from the absence of a change in crystallinity after age 30 in these samples. The increase in carbonate content with age has also been reported based on Raman (Tarnowski *et al*., 2002; Yerramshetty *et al*., 2006; Donnelly *et al*., 2009) and infrared spectroscopy (Miller *et al*., 2007; Kuhn *et al*., 2008; Gourion-Arsiquaud *et al*., 2009b), as well as x-ray diffraction and chemical analyses (Pellegrino and Biltz, 1972; Burnell *et al*., 1980; Rey *et al*., 1991a,b) of bones from a variety of animal species.

Combining x-ray diffraction, Fourier transform infrared spectroscopy (FTIR), and chemical analysis, Kuhn and coworkers analyzed mineral crystals in bovine bones at two different ages, and reported that the crystals mature with age with greater increases of crystal size and Ca/P ratio for the cortical as compared with the trabecular bone of younger (1- to 3-month-old calves) *vs.* 4- to 5-year-old animals. The Ca/[P + CO_3] was relatively constant within a given bone type and in both bone types in the older animals. Analysis of the FTIR data showed that the labile ions decreased with age, to a greater extent in cortical in contrast to trabecular bone, but in general, similar patterns of maturation were seen for both bone types when old and young bones were compared (Kuhn *et al*., 2008). It is important to note that all the x-ray diffraction data and most of the spectroscopic data came from homogenized bone.

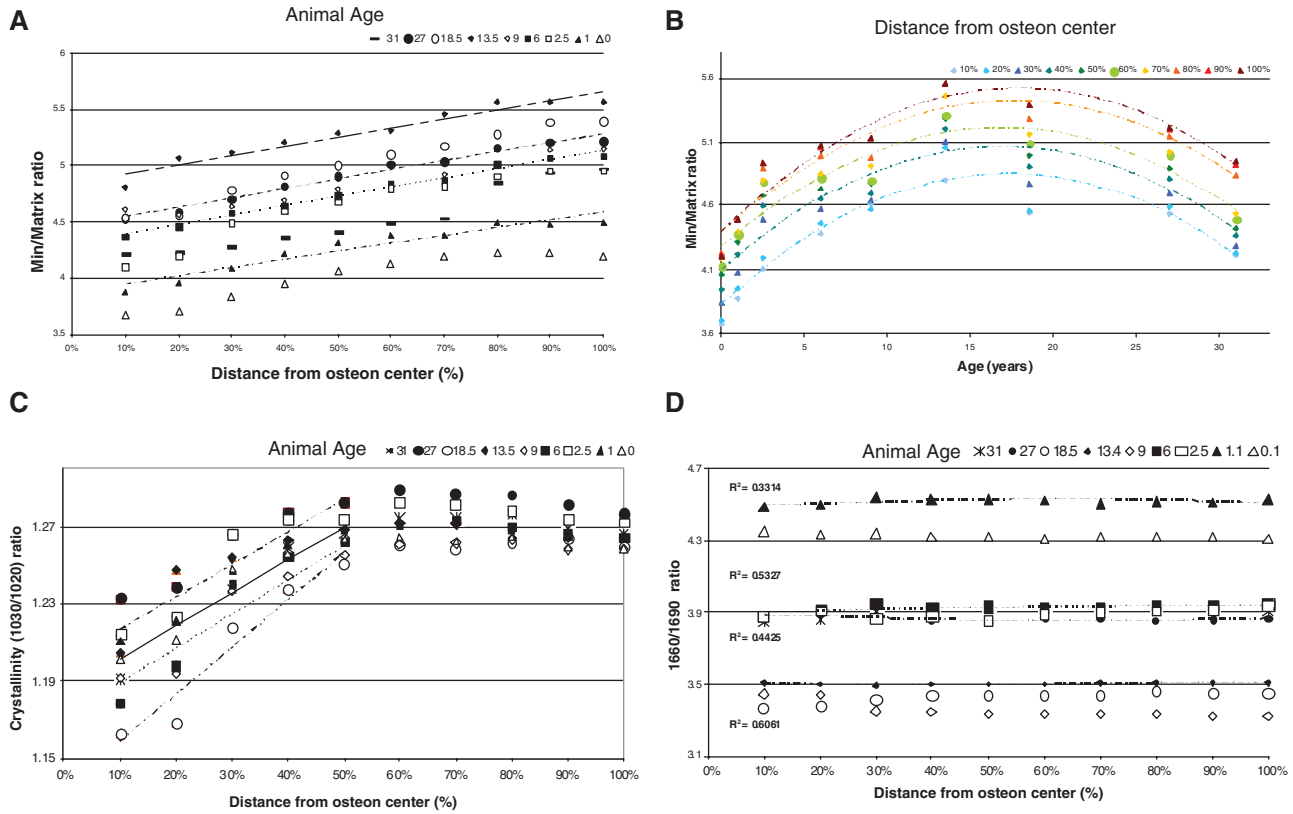

Figure 10. Heterogeneity of FTIR parameters in baboon osteonal bone. **(A)** Mineral/matrix ratio as a function of tissue age (distance from osteon center) in baboons of ages ranging from 0 to 37 yrs (R^2 ~ 0.92-0.99). **(B)** Mineral/matrix ratio plotted as a function of baboon age for each section along the line from the center of the osteon (R^2 ~ 0.83-0.89). **(C)** Crystallinity for each baboon sample as a function of tissue age (distance from the osteon center) ($0.84 < R^2 < 0.94$). **(D)** Collagen cross-link ratio (maturity) for each baboon sample ($0.1 < R^2 < 0.5$) as a function of tissue age (distance from the osteon center). Modified from Gourion-Arsiquaud *et al.* (2009b).

In the homogenized bulk bone samples of all species examined, parallel to the age-dependent increase in carbonate content, there is a decrease in acid phosphate content (Rey *et al.*, 1991a,b) and an increase in the hydroxyl content (Rey *et al.*, 1995a,b; Loong *et al.*, 2000; Wu *et al.*, 2002). Bone apatite is non-stoichiometric, and is both hydroxyl- and calcium-deficient (Cho *et al.*, 2003), but these deficiencies decrease with age. Whether this is because the crystals are actually perfecting with time (which argues against their incorporating carbonate ions) or whether, as suggested by *in vitro* studies showing that osteoclasts preferentially resorb older bone (Henriksen *et al.*, 2007), the accelerated remodeling of bone leaves behind only the least soluble, more perfect crystals, has not been fully established.

To measure actual crystal size, rather than estimating the sizes of particles causing the broadening of the x-ray diffraction pattern of powdered bone mineral, investigators have used transmission electron microscopy and atomic force microscopy. These studies confirm the sizes suggested by x-ray diffraction, but, to date, perhaps because of the amount of work required for each sample, and the site-to-site variation that occurs within the bony tissues (Weiner and Traub, 1989), there are few reports of the age-dependent variation in actual mineral crystal size. However, Eppell's group studied immature and mature bovine bone, from which the changes in crystal size suggested by the x-ray diffraction discussed above were confirmed (Eppell *et al.*, 2001; Tong *et al.*, 2003). There is a TEM study in which crystal sizes in lamellar bone of "mature" and "senior" individuals were compared by TEM, and, similar to the findings above, the length and the width of crystal aggregates were significantly higher in senile age as compared with the younger "mature" group (Denisov-Nikolskii *et al.*, 2002)

A half-century ago, backscattered electron imaging suggested that there was a gradient of mineral density around osteons (Jowsey, 1966; Barer and Jowsey, 1967), with the youngest bone, closest to the blood vessels (Haversian canals), being the least dense. Within cortical bone, a series of almost concentric circles surrounds the blood vessel, cutting a cone through the bone (see Fig. 1). With the application of infrared microspectroscopy and microspectroscopic imaging to bone (for review, see Carden and Morris, 2000; Boskey and Pleshko Camacho, 2007), this pattern was extended to mineral crystallinity, carbonate content, and acid phosphate content, as well as to the maturity of the collagen in osteons (Fig. 10) (Paschalis *et al.*, 1996; Fuchs *et al.*, 2008; Gourion-Arsiquaud *et al.*, 2009b). Other studies scanning across cortical bone, or going from the formative surface to the resorptive surface of cortical or trabecular bone in rodents (Boskey *et al.*, 2003, 2005b; Ling *et al.*, 2005), canines

(Gourion-Arsiquaud *et al*., 2009a), and humans (Paschalis *et al*., 1997, 2003; Boskey *et al*., 2005a), demonstrated that the changes noted as a function of animal age also changed as a function of tissue age. The wide range of values (and large coefficients of variation) in the whole bone samples noted above could thus be partly explained by the relative contributions of younger and older bone, or by the relative numbers of new and old osteons examined. Similar spatial variations were noted by Raman microspectroscopy (Akkus *et al*., 2003). Thus, as summarized in the Table, there are parallel tissue-age- and animal-age-dependent changes in bone mineral composition.

Table. Age-dependent Changes in Bone Mineral Composition with Increasing Animal or Tissue Age

Increasing mineral content
Increasing carbonate substitution
Decreasing acid phosphate substitution
Increasing hydroxyl content
Increasing Ca/P molar ratio
Increasing crystal size and perfection

ANIMAL MODELS OF AGING BONE

Much of the information available on age-dependent changes in bone has come from animal studies and in particular from models where there is early senescence. The SAMP6 mouse (Jilka *et al*., 1996), along with the 11 related senescence-accelerated mouse strains and the Klotho mouse (Kuro-o *et al*., 1997; Kuro-o, 2009), are examples of such premature aging models. A mutation in the klotho gene (co-receptor for FGF-23) leads the "klotho" mice to show decreased bone formation and increased bone turnover, associated with kidney abnormalities, at an early age (Negri, 2005). Knockout of the klotho gene, which is predominantly expressed in the kidney, leads to premature aging starting at about 4 wks of age, while overexpression extends the mouse lifetime (Masuda *et al*., 2005). Overexpression can rescue the premature aging changes noted in bones and teeth; hence this is an excellent model for studying age-related changes over a short time frame. There are also other rodents that undergo premature aging, such as the Louvain rat (Luc/c) (Duque *et al*., 2009). In this model, rats avoid serious systemic disorders while demonstrating low-turnover bone loss, which may allow investigators to study bone fragility independent of the effects of decreased estrogen levels of OVX rats.

Almeida *et al*. suggested a potential mechanism for the aging effects seen in bone. In a study where female and male C57BL/6 mice were compared as a function of age, the authors found that the expected age-related loss of strength in the spine and hind limbs was detectable 9 mos earlier than the loss of BMD (Almeida *et al*., 2007b). Suggestive of the mechanism was the finding that aging in C57BL/6 mice was associated with an increase in adrenal production of glucocorticoids, as well as bone expression of 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1, the enzyme that activates glucocorticoids. Aging also decreased the volume of the bone vasculature and bone fluid transport. These changes could be reproduced by pharmacologic hyperglucocorticoidism or by preventing cell-specific transgenic expression of the enzyme that inactivates glucocorticoids (11β-HSD 2). Glucocorticoids suppressed angiogenesis in fetal metatarsals and hypoxia-inducible factor 1α transcription and VEGF production in osteoblasts and osteocytes. The mechanism suggested for increased skeletal fragility in old age is *via* similar effects associated with the increases in endogenous glucocorticoids that are known to occur with aging in humans (Van Cauter *et al*., 1996; Dennison *et al*., 1999; Purnell *et al*., 2004; Reynolds *et al*., 2005). Identical to the way excess glucocorticoids affected the mouse, it is suggested that the age-associated increase in endogenous glucocorticoids decreases the lifespan of osteoblasts and osteocytes, the size of the vascular bed in bone, and solute transport through the lacunar-canalicular network. These results revealed that endogenous glucocorticoids increase skeletal fragility in old age as a result of cell-autonomous effects on osteoblasts and osteocytes, leading to interconnected decrements in bone angiogenesis, vasculature volume, and osteocyte-fluid flow.

There are also other mechanisms that may account for the age-dependent changes in bone properties, since aging mice show reduced cell proliferation, apoptosis, and loss of IGF-I-induced signaling at the receptor level and at key regulatory sites along the MAPK (ERK1/2) and PI3K (AKT) pathways (Cao *et al*., 2007). These pathways have been reviewed elsewhere (Provot *et al*., 2008).

Non-human primates have been studied as aging models, since they closely resemble changes in humans (Grynpas, 1993a; Jayo *et al*., 1994; Colman *et al*., 1999; Cerroni *et al*., 2000; Black and Lane, 2002). As discussed earlier, the standard clinical measurement for the assessment of human bone mineral changes is dual-photon absorptiometry (DEXA). While this method is not sensitive to drug-induced changes, it does show developmental changes in the two-dimensional bone mineral densities measured. For example, BMD in male and female rhesus macaques differs with age (Cerroni *et al*., 2000) (Fig. 9). Females show an initial increase with age, with peak bone density occurring around age 9.5 yrs, and remaining constant until 17.2 yrs, after which there is a steady decline in BMD. Males acquire bone mass at a faster rate, showing a higher peak BMD at an earlier age than females, and BMD remains constant between ages 7 and 19 yrs. Older males were not included in that study.

Based on analyses in changes in osteonal mineral and matrix properties in baboons of different ages (Havill, 2004), our laboratory (Gourion-Arsiquaud *et al*., 2009b) characterized the mineral and matrix changes in these osteons as a function of animal age and tissue age from newborn to 33 yrs of age in female animals. We found that there was an increase in mineral content, based on FTIR analyses of mineral/matrix ratio from age 0 to age 13 yrs, in agreement with the data reported by Cerroni *et al*. (2000), and then there was a decrease in mineral content, although the slope of the change as a proportion of the osteonal radius (tissue age) was similar (Fig. 10). Crystal size and perfection and collagen maturity, assessed by FTIR imaging, showed similar patterns, with an age-dependent increase up to age 13 yrs, a decrease in the global average after that, but similar increases in the carbonate/phosphate ratio in all bones analyzed.

IS OSTEOPOROSIS A DISEASE OF AGING?

Osteoporosis, also known as the "silent disease", occurs in women and men, and is associated with a loss of bone mass and increased risk of fracture. As has been pointed out throughout this review, with aging there is a loss of bone mass and bone strength. But does that mean that osteoporosis is a disease characteristic of aging, or that its incidence increases in old age? It is our contention that these are not the same, and for reasons discussed below, osteoporosis is not necessarily a disease of aging (Carrington, 2005; Blair and Carrington, 2006).

Osteoporotic bone, by definition, is bone that fractures easily. Both trabeculae and cortices are often thinner, the mineral content *per* area of tissue is often increased, and the mean crystal size, collagen maturity, and carbonate contents are increased relative to those of age-matched controls (Gourion-Arsiquaud *et al*., 2010). Further, there is a less broad distribution of the pixel histograms describing each of these properties (Boskey *et al*., 2009). The general characteristics are also true, as discussed above, of bones in older animals. However, although the incidence of fragility fractures and decreased bone strength (Banks *et al*., 2009) increases exponentially with age in males and females (Nieves *et al*., 2009), we suggest that the answer to the question above is a definite "no": Osteoporosis is not a disease of aging; it is only age-associated. There are several reasons for this conclusion. First, and most obvious, is that not all the elderly, here referring to people over age 85 yrs, show signs of skeletal fragility or have fractures (Mellibovsky *et al*., 2007). Second, while there are specific genes associated with bone loss in rodents and humans (Richards *et al*., 2009), their presence or absence does not offer specific protection to any age group, and finally, because osteoporosis does occur in the very young—for example, in idiopathic juvenile osteoporosis, a disease of skeletal fragility in teenagers (Rauch *et al*., 2002; Płudowski *et al*., 2006). To argue against osteoporosis being a disease of aging, as opposed to a disease which has a greater prevalence in older individuals, we point to the case of two centenarians and their relatives (one being 113 years old) who had no fractures and no genetic abnormalities that could be linked to their younger-appearing bone properties (Mellibovsky *et al*., 2007).

OSTEOPOROSIS AND ORAL BONE LOSS

As demonstrated by the expression of the intramembranous bone-specific marker periostin (Kashima *et al*., 2009), alveolar bone forms through the process of intramembranous ossification, *i.e*., without forming a cartilage model, it too undergoes age-dependent bone loss. Interestingly, this loss occurs primarily in the mandibular bone and not in the maxilla (Sarajlic *et al*., 2009), although changes in the maxilla have also been reported.

In human studies, significant, moderate correlations were found between hip or vertebral BMD and some measure of alveolar bone density or quality in most studies (Von Wowern and Stoltze, 1978; Kribbs *et al*., 1989; Krall *et al*., 1996; Taguchi *et al*., 1999; White and Rudolph, 1999; Jonasson *et al*., 2006). However, no other association between osteoporosis and oral bone loss in humans has been reported in previous investigations (Daniell, 1983; Mohajery and Brooks, 1992; Mohammad *et al*., 1997; Earnshaw *et al*., 1998; Southard *et al*., 2000). These variable findings can be attributed to small sample sizes, use of different methods of analysis, analysis of different sites within the jaw, and failure to account for mechanical factors such as missing teeth. Oral hygiene, often not reported, also appeared to be a critical factor in predicting oral bone loss (Streckfus *et al*., 1999). Additionally, Sanfilippo and Bianchi (2003) reported that oral bone loss was due more to alterations in maxillary function, which in turn changed the mechanical environment.

Rodent experiments, such as those in ovariectomized rats, have similarly reported both positive (Tanaka *et al*., 2002, 2003) and negative (Moriya *et al*., 1998) associations between osteoporosis in long bones and spine and effects in the jawbone. Recently, in a study of male mice of different ages, not specifically treated with any pathogens, there was significant oral bone loss from 9 mos to 18 mos, with markers of periodontitis significantly elevated in the gingivae but not the spleen of these animals, suggesting that mice are subject to age-related bone loss (Liang *et al*., 2010). It is difficult, however, to separate which came first, periodontal disease or bone loss. Periodontitis is characterized by inflammation of the tissues that support the teeth, and it causes resorption of the alveolar bone and the loss of the soft tissue attachment to the tooth, hence causing tooth and bone loss. The effect of inflammation on oral bone loss, and on osteoporosis, has been reviewed previously (Chung *et al*., 2009).

A recent study that compared lumbar and mandibular bone in ovariectomized monkeys also reported a direct correlation between bone BMD and jaw BMD. The monkeys were more than 9 yrs old, adults but not elderly, and were examined 76 wks after ovariectomy, relative to sham-operated controls of the same age (Binte Anwar *et al*., 2007). Periodontal disease and jaw mechanics *per se* were not noted.

As in the case of osteoporosis, oral bone loss may be age-associated because of poorer nutrition (Ritchie *et al*., 2002), alterations in dental hygiene, and hormonal changes. However, it is thought that aging alone does not cause the loss of periodontal attachment in the healthy elderly (Huttner *et al*., 2009). Thus, because there is only an association, rather than causality, we maintain that while oral bone loss may be accelerated in aging, in both animals and humans, the loss of bone is not directly attributable to aging.

CONCLUSIONS

The composition of bone and its mechanical properties vary as a function of age. New understanding of the factors controlling the aging of the cell in general, and the aging of osteoblasts, osteocytes, and osteoclasts in particular, is pinpointing the pathways that could be targeted to delay these age-dependent changes. But since not all individuals' bones age similarly, whom to target and which specific pathway should be selected may become the greatest challenge.

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