Aging and Bone

Adele L. Boskey, PhD and **Rhima Coleman, PhD**
Hospital for Special Surgery, 535 E. 70th Street, New York, NY 10021

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.

Keywords

bone properties; aging; senescence; bone cells; bone mineral; review

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 locomotion and load-bearing, serving as a home for marrow, and is a reservoir for calcium homeostasis. With aging these functions become impaired, bone becomes more fragile, 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 of 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, 1993]. The organic matrix is mainly type I collagen, but other types of collagen and a number of 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.

Adele Boskey (Corresponding author) Tel: 212-606-1453 Fax: 212-774-7877 boskeya@hss.edu Rhima Coleman colemanr@hss.edu.

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 (Figure 1).

BONE GROWTH AND DEVELOPMENT

Bone development begins in the embryo with the formation of a cartilage anlagen which gradually becomes replaced with bone in a process known as endochondral ossification [Provot *et al.*, 2008] (Figure 2). As reviewed in detail elsewhere [Zusick *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, they become surrounded by a mineralized matrix, and subsequently 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 adults. In rabbits, studied throughout their life-span, 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 wells 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, are required to absorb the energy from impact loads. This is why younger bones are more ductile and older bones are stiffer. 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 tenfold increased 10-year fracture risk compared to younger individuals with the same BMD [Kanis *et al.*, 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. A number of studies have reported that cortical bone becomes more brittle and weaker with age [Burstein *et al.*, 1976; McCalden *et al.*, 1993; Currey and Butler, 1975; Tomassini *et al.*, 2007]. Similarly, the trabecular struts (see Figure 1) also weaken with age [Nagaraja *et al.*, 2007]. In studies which extend to extremely old age (>85 years) the incidence of fractures is reported to be 10–15 times as likely as in persons aged 60–65 [Melton *et al.*, 1996; Yates *et al.*, 2007].

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 to healthy sex-matched 25 year olds or to healthy age-matched controls, respectively), and the way that mineral is distributed relative to the forces applied to the bone [Ammann & 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 the increased fracture incidence in the extremely elderly population [Yates *et al.*, 2007]. In a study of mice ranging in age from 3–77 weeks, where the mice were grouped into age ranges of less than 12 weeks, 13–50 weeks, and 50–77 weeks, 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, nonenzymatic 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 [Vasishth 2007]. It is likely that both the inability to repair the cracks [Hirano *et al.*, 2000] and their propagation increase with age, contributing to the reduced toughness of both cortical and trabecular bone [Currey *et al.*, 1996].

CHANGES IN BONE MORPHOLOGY WITH AGE

Morphology describes the shape (geometry) that bones have, both 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 size and the shape of the bones [Jepsen, 2009]. Within the bones 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 Figure 1). Bones change in shape to facilitate their mechanical functions – being strong enough to withstand large forces and streamlined enough to minimize energy demands [Jepsen 2009; Seeman, 2003; Wang and Seeman, 2009] (Figure 3).

During development and aging, bone shape (geometry) 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 months (corresponding to achievement of peak bone mass in this species [Ramandham *et al.*, 2008]), as the bones become more slender [Kawashima *et al.*, 2009]. In mature rats (from 8–36 months 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; Westerbeek *et al.*, 2008]. However, in a more recent study, when the trabecular bone in proximal tibia 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 to 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 male subjects ranging in age from 17– 46 years, 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].

As shown in Figure 3, the shape of long bones changes during development. Beginning early in life, the cross-sectional geometry of long bones undergo modification, going from having 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 by this time. 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 the bone geometry throughout life [Goldman *et al.*, 2009].

In humans there is some debate [Seeman *et al.*, 2003] as to whether the patterns of resorption and formation are similar in men and women, but this is beyond the scope of this current review. It must be noted, however, that within trabecular bone, the age related loss in men is predominately 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, with age bone gets stiffer 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, bone deformation increases, and in fragile bone, this may lead to fracture.

In the healthy individual, bone formation and resorption is 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 impact all bones equally, the general trends are similar. For example, in terms of hip structure, men and women older than 85 years of age were 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 [Tommassini *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 (Figure 4) [for review see Boyce *et al.*, 2009;Chen *et al.*, 2009;Blair *et al.*, 2008;Bonewald, 2008;Bonewald & Johnson, 2008;Lian & Stein, 2008]. The majority of the bone cells are of mesenchymal cell origin; chondrocytes, which as illustrated in Figure 2, are responsible for the deposition of the growth plate and its subsequent remodeling; 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 Figure 5. Sixty to eighty percent 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 years [Manolagas and Parfitt, 2010] and they are the cells which are most responsive to mechanical signals [Rochefort *et al.*, 2010;Matsuo, 2009]. 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 canilicular-lacunar network, resulting in cell-level shear forces, membrane deformation, and tension in elements connecting osteocytes with the canilicular walls which results in the release of anti-apoptotic factors. Osteocyte apoptosis due to disruption of the canilicular 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].

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 to younger animals [Pietschmann *et al.*, 2007; Cao *et al.*, 2005; 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 Figure 5. These factors and the way they control the development of the skeleton were reviewed by Provot *et al.* [2008].

All normal cells, including osteoblasts, osteoclasts and osteocytes, have a limited life-span, which is controlled by the number of replication cycles and external factors (Figure 5). The Hayflick limit of cell division indicates that cells can only undergo 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 end 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 that extends their life cycle significantly compared to 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 fist 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 classical symptoms of the disease until the knock-out is developed in mice with telomerase-deficient backgrounds, further highlighting the role in 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 life-span. 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 reviewed in 2009 [Wu *et al.*, 2009] 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 a impaired regulation of gene expression.

Among the key cell-based changes that occur with age which in turn impact 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 & Seeman, 2009] possibly due to reduction in 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 life span 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, decline 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 [Sczulc &Seeman, 2009].

There have been few studies examining whether human bone cell differentiation is age-dependent. In one study of 80 patients aged 14 – 79 years, 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 κ B 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 17–90 years, 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 four times as many marrow stromal cells that were positive for senescence, and the doubling times for the older subject's 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 disregulation 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 canilicular 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 regulate apoptosis of osteocytes and osteoblasts [Almeida *et al*. 2007b]. Cell senescence may alter the response of cells to apoptotic signals [see Muller 2009 for a 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 [Chau *et al*, 2009; Provot 2008], and here we will only consider those which are both known 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 & Insogna, 2009] is believed to regulate cellular aging in all cells, although the mechanism through which this occurs is not completely accepted [DeCarolis *et al*., 2008; Brack *et al*. 2007] (Figure 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 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 over-expression 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 mice 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. As seen in Figure 6, this in turn prevents phosphorylation of β-catenin by the complex (GSK-3, APC, and Axin). 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 four 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 contributing factor 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 [Almedia *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].

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 which 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 & Marumoto, 2010]. It is the cross-linking of the collagen fibrils that have the greatest impact on the strength of collagen fibrils.

There are two different categories of collagen cross-links and they vary differently with age (Figure 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 to trivalent pyridinoline (PYD) and pyrrole (PYL) cross-links with age which connects 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. A limited number of non-enzymatic cross-links were found to be structurally related to the morphology of the trabecular bone [Banse *et al.*, 2002]. In a study of autopsy samples from individuals 54–95 years old, Viguet-Carrin *et al.* [2009] reported that the enzymatic cross-links were not correlated with microarchitecture, while there was a strong correlation with the nonenzymatic 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-pridionoline 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 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 only be removed by bone resorption and their presence increases osteoclast activity while decreasing formation by osteoblasts, thereby contributing to the fragility of bone with age [Vaguette-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 using second harmonic generation microscopy (Figure 8) where the intensity is proportional to the orientation [Campagnola & 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 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 their distributions change with age, but 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 years) had more extracellular bone matrix proteins than individuals aged 51–79 years, and that there were increased bone matrix proteins 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 years [Fedarko *et al*., 1992]. Cell proliferation was greatest in the 16–18th week of gestation, declining after birth and then continuously with donor age, so that the rate of proliferation in the age 30 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 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 after that time. 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 wildtype 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. The data stress the alteration in protein expression with age in the klotho mice although the effect of the 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 *et al*., 1969]. This is seen in ash weight determination but also in the areal BMD that is more frequently measured. Figure 9 compares BMD measurements using a variety of techniques for a variety of species, and demonstrates the increase in mineral content during growth and development, and the decline with later aging.

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 × 5–25 nm wide × 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 which 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 years and found the bone mineral crystal size and perfection increased during the first 25–30 years and then decreased after that, 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 tissue 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 years 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 [Donnelly *et al.*, 2009; Tarnowsky *et al.*, 2002; Yerramashetty *et al.*, 2006] and infrared spectroscopy [Gourion-Arsiquaud *et al.*, 2009b; Miller *et al*, 2007; Kuhn *et al.*, 2008], as well as x-ray diffraction and chemical analyses [Rey *et al.*, 1991a,b; Pellegrino & Blitz, 1972; Burnell *et al.*, 1980] of bones from a variety of animal species.

Combining x-ray diffraction, FTIR, and chemical analysis, Liisa Kuhn 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 to the trabecular bone of younger (1–3 month old calves) vs. 4–5 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. The FTIR data showed that the labile ions decreased with age, to a greater extent in cortical as contrasted with 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.

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*, 1991] and an increase in the hydroxyl-content [Wu *et al.*, 2002; Rey *et al.*, 1995; Loong *et al.*, 2000]. 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 [Henrickson *et al.*, 2007], the accelerated remodeling of bone leaves behind only the least soluble, more perfect crystals, is not fully established.

To actually measure crystal size, rather than estimating the size 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 from the x-ray diffraction discussed above was confirmed [Tong *et al*., 2003; Eppell *et al*., 2001]. 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 was significantly higher in senile age as compared to the younger "mature" group [Denisov-Nikol'skiĭ IuI *et al*., 2002]

A half-century ago backscattered electron imaging suggested that there was a gradient of mineral density around osteons [Barer & Jowsey, 1967; Jowsey 1966] with the youngest bone, closest to the blood vessels (Haversian canals) being the least dense. Within cortical bone, a series of almost concentric circles surround the blood vessel, cutting a cone through the bone (see Figure 1). With the application of infrared microspectroscopy and microspectroscopic imaging to bone [for review see Boskey & Pleshko Camacho, 2007; Carden & Morris, 2000] this pattern was extended to mineral crystallinity, carbonate content, and acid phosphate content, as well as to the maturity of the collagen in osteons (Figure 10) [Gourion-Arsiquaud *et al*., 2009b; Fuchs *et al*., 2008; Paschalis *et al*., 1996]. 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; Boskey *et al*., 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 in part be explained by the relative contributions of younger and older bone, or by the relative number of new and old osteons examined. Similar spatial variations were noted by Raman microspectroscopy [Akkus *et al*., 2003]. Thus, as summarized in Table 1, there are parallel tissue age and animal age dependent changes in bone mineral composition.

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 mice strains and the Klotho mouse [Kuro-o, 1997; 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 about 4 weeks of age, while overexpression extends the mouse life-time [Masudo *et al.*, 2005]. Over-expression 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 the expected age-related loss of strength in the spine and hind limbs was detectable 9 months 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 increases in endogenous glucocorticoids which 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, as 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 are reviewed elsewhere [Provot, 2008].

Non-human primates have been studied as aging models as they closely resemble changes in humans [Grynpas 1993, Jayo *et al.*, 1994; Colman *et al.*, 1999; Cerroni *et al.*, 2000; Black & Lane, 2002]. As discussed earlier, the standard clinical measurement for assessing human bone mineral changes is dual photon absorbtiometry (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, as shown in Figure 9, BMD in male and female rhesus macaques differ with age [Cerroni *et al.*, 2000]. Females show an initial increase with age, with peak bone density occurring around age 9.5 years, and remaining constant until 17.2 years, 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 7–19 years. 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 years 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 years, in agreement with the Cerroni *et al* data [Cerroni *et al.*, 2000], and then there was a decrease in mineral content, although the slope of the change was a proportion of the osteonal radius (tissue age) was similar (Figure 10). Crystal size and perfection and collagen maturity, assessed by FTIR imaging showed similar patterns, with an age dependent increase up to age 13, 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 [Blair & Carriington, 2006; Carrington 2005].

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 age-matched controls [Gourion-Arsiquaud *et al*, 2009c]. 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 would like to suggest 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, not all the elderly, here referring to people over age 85 years, show signs of skeletal fragility, or have fractures [Melibovsky *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; Pludowski *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 centagenarians 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 [Melibovsky *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 Solte, 1978; Krall *et al.*, 1996; Taguchi *et al.*, 1999; White and Rudolph, 1999; Kribbs *et al.*, 1989; Jonasson *et al.*, 2006]. However, other no association between osteoporosis and oral bone loss in humans has been reported in previous investigations [Daniell, 1983; Mohammad *et al.*, 1997; Earnshaw *et al.*, 1998; Mohajery and Brooks, 1992; 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, Sanfillippo 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 months to 18 months, with markers of periodontitis significantly elevated

in the gingival but not the spleen of these animals, suggesting that mice are subject to age-related bone loss [Liang *et al.*, 2010]. It is hard, 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 as well as the loss of the soft tissue attachment to the tooth, hence causing tooth as well as bone loss. The effect of inflammation on oral bone loss, and on osteoporosis, is reviewed in the accompanying manuscript [Chung *et al.*, 2009].

A recent study in ovariectomized monkeys that compared lumbar and mandibular bone also reported a direct correlation between bone BMD and jaw BMD. The monkeys were greater than 9 years old, adults but not elderly, and were examined 76 weeks 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, both in 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, are 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.

Acknowledgments

REFERENCES

Aaron JE, Makins NB, Sagreiya K. The microanatomy of trabecular bone loss in normal aging men and women. Clin Orthop Relat Res 1987;215:260–271. [PubMed: 3802645]

Adachi T, Aonuma Y, Ito S, Tanaka M, Hojo M, Takano-Yamamoto T, Kamioka H. Osteocyte calcium signaling response to bone matrix deformation. J Biomech 2009;42:2507–2512. [PubMed: 19665124]

Akkus O, Polyakova-Akkus A, Adar F, Schaffler MB. Aging of microstructural compartments in human compact bone. J Bone Miner Res 2003;18:1012–1019. [PubMed: 12817753]

Almeida M, Han L, Martin-Millan M, O'Brien CA, Manolagas SC. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box O-mediated transcription. J Biol Chem 2007a;282:27298–27305. [PubMed: 17623658]

Almeida M, Han L, Martin-Millan M, Plotkin LI, Stewart SA, Roberson PK, Kousteni S, O'Brien CA, Bellido T, Parfitt AM, Weinstein RS, Jilka RL, Manolagas SC. Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. J Biol Chem 2007b; 282:27285–27297. [PubMed: 17623659]

Ammann P, Rizzoli R. Bone strength and its determinants. Osteoporos Int 2003;14(Suppl 3):S13–18. [PubMed: 12730800]

Bailey AJ, Sims TJ, Ebbesen EN, Mansell JP, Thomsen JS, Mosekilde L. Age-related changes in the biochemical properties of human cancellous bone collagen: relationship to bone strength. Calcif Tissue Int 1999;65:203–210. [PubMed: 10441651]

Banks E, Reeves GK, Beral V, Balkwill A, Liu B, Roddam A, Million Women Study Collaborators. Hip fracture incidence in relation to age, menopausal status, and age at menopause: prospective analysis. PLoS Med 2009;6:e1000181. [PubMed: 19901981]

Banse X, Devogelaer JP, Lafosse A, Sims TJ, Grynpas M, Bailey AJ. Cross-link profile of bone collagen correlates with structural organization of trabeculae. Bone 2002;31:70–76. [PubMed: 12110415]

Barer M, Jowsey J. Bone formation and resorption in normal human rib. A study of persons from 11 to 88 years of age. Clin Orthop Relat Res 1967;52:241–247. [PubMed: 6048913]

Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, MacDougald OA. Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci U S A 2005;102:3324–3329. [PubMed: 15728361]

Binte Anwar R, Tanaka M, Kohno S, Ikegame M, Watanabe N, Nowazesh Ali M, Ejiri S. Relationship between porotic changes in alveolar bone and spinal osteoporosis. J Dent Res 2007;86:52–7. [PubMed: 17189463]

Black A, Lane MA. Nonhuman primate models of skeletal and reproductive aging. Gerontology 2002;48:72–80. [PubMed: 11867928]

Blair HC, Carrington JL. Bone cell precursors and the pathophysiology of bone loss. Ann N Y Acad Sci 2006;1068:244–249. [PubMed: 16831925]

Blair, HC.; Simonet, S.; Lacey, DL.; Zaidi, M. Osteoclast Biology. In: Marcus, R.; Feldman, D.; Nelson, D.; Rosen, CJ., editors. Osteoporosis. Third Edition. Academic Press, NY; NY: 2008. p. 151-168.

Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 1997;91:25–34. [PubMed: 9335332]

Bonewald, LF. Osteocytes. In: Marcus, R.; Feldman, D.; Nelson, D.; Rosen, CJ., editors. Osteoporosis. Third Edition. Academic Press, NY; NY: 2008. p. 169-190.

Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone 2008;42:606–615. [PubMed: 18280232]

Boskey, A. Mineralization of Bones and Teeth. In: Sahai, Nita, editor. Elements Magazine. Vol. 3. 2007. p. 387-393.

Boskey AL, DiCarlo E, Paschalis E, West P, Mendelsohn R. Comparison of mineral quality and quantity in iliac crest biopsies from high- and low-turnover osteoporosis: an FT-IR microspectroscopic investigation. Osteoporos Int 2005a;16:2031–2038. [PubMed: 16088360]

Boskey AL, Moore DJ, Amling M, Canalis E, Delany AM. Infrared analysis of the mineral and matrix in bones of osteonectin-null mice and their wildtype controls. J Bone Miner Res 2003;18:1005–1011. [PubMed: 12817752]

Boskey A, Pleshko Camacho N. FT-IR imaging of native and tissue-engineered bone and cartilage. Biomaterials 2007;28:2465–2478. [PubMed: 17175021]

Boskey AL, Spevak L, Weinstein RS. Spectroscopic markers of bone quality in alendronate-treated postmenopausal women. Osteoporos Int 2009;20:793–800. [PubMed: 18769963]

Boskey AL, Young MF, Kilts T, Verdelis K. Variation in mineral properties in normal and mutant bones and teeth. Cells Tissues Organs 2005b;181:144–153. [PubMed: 16612080]

Boyce BF, Yao Z, Xing L. Osteoclasts have multiple roles in bone in addition to bone resorption. Crit Rev Eukaryot Gene Expr 2009;19:171–180. [PubMed: 19883363]

Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007;317:807–810. [PubMed: 17690295]

Burger C, Zhou HW, Wang H, Sics I, Hsiao BS, Chu B, Graham L, Glimcher MJ. Lateral packing of mineral crystals in bone collagen fibrils. Biophys J 2008;95:1985–1992. [PubMed: 18359799]

Burnell JM, Teubner EJ, Miller AG. Normal maturational changes in bone matrix, mineral, and crystal size in the rat. Calcif Tissue Int 1980;31:13–19. [PubMed: 6770970]

Burstein AH, Reilly DT, Martens M. Aging of bone tissue: Mechanical properties. J Bone Joint Surg Am 1976;58:82–86. [PubMed: 1249116]

Calado RT. Telomeres and marrow failure. Hematology Am Soc Hematol Educ Program 2009:338–343. [PubMed: 20008219]

Campagnola PJ, Loew LM. Second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms. Nat Biotechnol 2003;21:1356–1360. [PubMed: 14595363]

Cao JJ, Kurimoto P, Boudignon B, Rosen C, Lima F, Halloran BP. Aging impairs IGF-I receptor activation and induces skeletal resistance to IGF-I. J Bone Miner Res 2007;22:1271–1279. [PubMed: 17488198]

Cao JJ, Wronski TJ, Iwaniec U, Phleger L, Kurimoto P, Boudignon B, Halloran BP. Aging increases stromal/osteoblastic cell-induced osteoclastogenesis and alters the osteoclast precursor pool in the mouse. J Bone Miner Res 2005;20:1659–1668. [PubMed: 16059637]

Carden A, Morris MD. Application of vibrational spectroscopy to the study of mineralized tissues. J Biomed Opt 2000;5:259–268. [PubMed: 10958610]

Carrington JL. Aging bone and cartilage: cross-cutting issues. Biochem Biophys Res Commun 2005;328:700–708. [PubMed: 15694404]

Cerroni AM, Tomlinson GA, Turnquist JE, Grynpas MD. Bone mineral density, osteopenia, and osteoporosis in the rhesus macaques of Cayo Santiago. Am J Phys Anthropol 2000;113:389–410. [PubMed: 11042540]

Chan GK, Duque G. Age-related bone loss: old bone, new facts. Gerontology 2002;48:62–71. [PubMed: 11867927]

Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, Pathak S, Guarente L, DePinho RA. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nature Genet 2004;36:877–882. [PubMed: 15235603]

Chau JF, Leong WF, Li B. Signaling pathways governing osteoblast proliferation, differentiation and function. Histol Histopathol 2009;24:1593–1606. [PubMed: 19795357]

Chen JH, Liu C, You L, Simmons CA. Boning up on Wolff's Law: Mechanical regulation of the cells that make and maintain bone. J Biomech 2009;43:108–118. [PubMed: 19818443]

Cho G, Wu Y, Ackerman JL. Detection of hydroxyl ions in bone mineral by solid-state NMR spectroscopy. Science 2003;300:1123–1127. [PubMed: 12750514]

Chung HY, Choi YJ, Kim JM, Kim DH, Zou Y, Kim CH, Yu BP. Molecular inflammation as an underlying mechanism of the aging process and age-related diseases. Ageing Res Rev 2009;8:18–30. [PubMed: 18692159]

Colman RJ, Lane MA, Binkley N, Wegner FH, Kemnitz JW. Skeletal effects of aging in male rhesus monkeys. Bone 1999;24:17–23. [PubMed: 9916779]

Currey JD. The relationship between the stiffness and the mineral content of bone. J Biomech 1969;2:477–480. [PubMed: 16335147]

Currey JD, Butler G. The mechanical properties of bone tissue in children. J Bone Joint Surg Am 1975;57:810–814. [PubMed: 1158919]

Currey JD, Brear K, Zioupos P. The effects of ageing and changes in mineral content in degrading the toughness of human femora. J Biomech 1996;29:257–262. [PubMed: 8849821]

Daniell HW. Postmenopausal tooth loss. Contributions to edentulism by osteoporosis and cigarette smoking. Arch Intern Med 1983;143:1678–1682. [PubMed: 6615088]

DeCarolis NA, Wharton KA Jr, Eisch AJ. Which way does the Wnt blow? Exploring the duality of canonical Wnt signaling on cellular aging. Bioessays 2008;30:102–106. [PubMed: 18200563]

Denisov-Nikol'skiĭ, IuI; Zhilkin, BA.; Doktorov, AA.; Matveĭchuk, IV. Ultrastructural organization of the human lamellar bone tissue mineral component in aged and elderly. Morfologiia 2002;122:79–83. [PubMed: 12530314]

Dennison E, Hindmarsh P, Fall C, Kellingray S, Barker D, Phillips D, Cooper C. Profiles of endogenous circulating cortisol and bone mineral density in healthy elderly men. J Clin Endocrinol Metab 1999;84:3058–3063. [PubMed: 10487665]

Donnelly E, Boskey AL, Baker SP, van der Meulen MC. Effects of tissue age on bone tissue material composition and nanomechanical properties in the rat cortex. J Biomed Mater Res A 2009;92A: 1048–1056.

Duque G, Rivas D, Li W, Li A, Henderson JE, Ferland G, Gaudreau P. Age-related bone loss in the LOU/c rat model of healthy ageing. Exp Gerontol 2009;44:183–189. [PubMed: 18992316]

Earnshaw SA, Keating N, Hosking DJ, Chilvers CE, Ravn P, McClung M, et al. Tooth counts do not predict bone mineral density in early postmenopausal Caucasian women. EPIC study group. Int J Epidemiol 1998;27:479–483. [PubMed: 9698139]

Eppell SJ, Tong W, Katz JL, Kuhn L, Glimcher MJ. Shape and size of isolated bone mineralites measured using atomic force microscopy. J Orthop Res 2001;19:1027–1034. [PubMed: 11781001]

Fan W, Crawford R, Xiao Y. Structural and cellular differences between metaphyseal and diaphyseal periosteum in different aged rats. Bone 2008;42:81–9. [PubMed: 17962095]

Fedarko NS, Vetter UK, Weinstein S, Robey PG. Age-related changes in hyaluronan, proteoglycan, collagen, and osteonectin synthesis by human bone cells. J Cell Physiol 1992;151:215–227. [PubMed: 1572898]

Ferguson C, Alpern E, Miclau T, Helms JA. Does adult fracture repair recapitulate embryonic skeletal formation? Mech Dev 1999;87:57–66. [PubMed: 10495271]

Fuchs RK, Allen MR, Ruppel ME, Diab T, Phipps RJ, Miller LM, Burr DB. In situ examination of the time-course for secondary mineralization of Haversian bone using synchrotron Fourier transform infrared microspectroscopy. Matrix Biol 2008;27:34–41. [PubMed: 17884405]

Goldman HM, McFarlin SC, Cooper DM, Thomas CD, Clement JG. Ontogenetic patterning of cortical bone microstructure and geometry at the human mid-shaft femur. Anat Rec 2009;292:48–64.

Gourion-Arsiquaud S, Allen MR, Burr DB, Vashishth D, Tang SY, Boskey AL. Bisphosphonate treatment modifies canine bone mineral and matrix properties and their heterogeneity. Bone. Nov 17;2009a Epub ahead of print.

Gourion-Arsiquaud S, Burket JC, Havill LM, DiCarlo E, Doty SB, Mendelsohn R, van der Meulen MC, Boskey AL. Spatial variation in osteonal bone properties relative to tissue and animal age. J Bone Miner Res 2009b;24:1271–1281. [PubMed: 19210217]

Gourion-Arsiquaud S, Faibish D, Myers E, Spevak L, Compston J, Hodsman A, Shane E, Recker RR, Boskey ER, Boskey AL. Use of FTIR spectroscopic imaging to identify parameters associated with fragility fracture. J Bone Miner Res 2009c;24:1565–1571. [PubMed: 19419303]

Grzesik WJ, Frazier CR, Shapiro JR, Sponseller PD, Robey PG, Fedarko NS. Age-related changes in human bone proteoglycan structure. Impact of osteogenesis imperfecta. J Biol Chem 2002;277:43638–43647. [PubMed: 12221073]

Grynpas M. Age and disease-related changes in the mineral of bone. Calcif Tissue Int 1993;53(S1):S57–64. [PubMed: 8275381]

Grynpas MD, Hancock RG, Greenwood C, Turnquist J, Kessler MJ. The effects of diet, age, and sex on the mineral content of primate bones. Calcif Tissue Int 1993;52:399–405. [PubMed: 8504378]

Grynpas MD, Tupy JH, Sodek J. The distribution of soluble, mineral-bound, and matrix-bound proteins in osteoporotic and normal bones. Bone 1994;15:505–513. [PubMed: 7980961]

Hanschin RG, Stern WB. X-ray diffraction studies on the lattice perfection of human bone apatite (Crista iliaca). Bone 1995;16(4 Suppl):355S–363S. [PubMed: 7626325]

Havill LM. Osteon remodeling dynamics in Macaca mulatta: normal variation with regard to age, sex, and skeletal maturity. Calcif Tissue Int 2004;74:95–102. [PubMed: 14973638]

Henriksen K, Leeming DJ, Byrjalsen I, Nielsen RH, Sorensen MG, Dziegiel MH, Martin TJ, Christiansen C, Qvist P, Karsdal MA. Osteoclasts prefer aged bone. Osteoporos Int 2007;18:751–759. [PubMed: 17216130]

Hirano T, Turner CH, Forwood MR, Johnston CC, Burr DB. Does suppression of bone turnover impair mechanical properties by allowing microdamage accumulation? Bone 2000;27:13–20. [PubMed: 10865204]

Huttner EA, Machado DC, de Oliveira RB, Antunes AG, Hebling E. Effects of human aging on periodontal tissues. Spec Care Dentist 2009;29:149–55. [PubMed: 19573041]

Ikeda T, Nagai Y, Yamaguchi A, Yokose S, Yoshiki S. Age-related reduction in bone matrix protein mRNA expression in rat bone tissues: application of histomorphometry to in situ hybridization. Bone 1995;16:17–23. [PubMed: 7742077]

Imai Y, Youn MY, Kondoh S, Nakamura T, Kouzmenko A, Matsumoto T, Takada I, Takaoka K, Kato S. Estrogens maintain bone mass by regulating expression of genes controlling function and life span in mature osteoclasts. Ann N Y Acad Sci Sep;2009 1173(Suppl 1):E31–9. [PubMed: 19751412]

Jayo MJ, Jerome CP, Lees CJ, Rankin SE, Weaver DS. Bone mass in female cynomolgus macaques: a cross-sectional and longitudinal study by age. Calcif Tissue Int 1994;54:231–236. [PubMed: 8055372]

Jepsen KJ. Systems Analysis of Bone. Wiley Interdiscip Rev Syst Biol Med 2009;1:73–88. [PubMed: 20046860]

Jiang Y, Mishima H, Sakai S, Liu YK, Ohyabu Y, Uemura T. Gene expression analysis of major lineage-defining factors in human bone marrow cells: effect of aging, gender, and age-related disorders. J Orthop Res 2008;26:910–917. [PubMed: 18302252]

Jilka RL, Weinstein RS, Takahashi K, Parfitt AM, Manolagas SC. Linkage of decreased bone mass with impaired osteoblastogenesis in a murine model of accelerated senescence. J Clin Invest 1996;97:1732–1740. [PubMed: 8601639]

Jilka RL, Weinstein RS, Takahashi K, Parfitt AM, Manolagas SC. Quantifying osteoblast and osteocyte apoptosis: challenges and rewards. J Bone Miner Res 2007;22:1492–1501. [PubMed: 17542686]

Jonasson G, Jonasson L, Kiliaridis S. Changes in the radiographic characteristics of the mandibular alveolar process in dentate women with varying bone mineral density: A 5-year prospective study. Bone 2006;38:714–721. [PubMed: 16326156]

Jowsey J. Studies of Haversian systems in man and some animals. J Anat 1966;100:857–864. [PubMed: 4961449]

Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 2002;359:1929–1936. [PubMed: 12057569]

Kashima T, Nishiyama K, Shimazu M, Shimazaki I, Kii A, Grigoriadis M, Fukayama A, Kudo T. Periostin, a novel marker of intramembranous ossification, is expressed in fibrous dysplasia and in c-Fos-overexpressing bone lesions. Hum Path 2009;40:226–37. [PubMed: 18799196]

Kaste SC, Kasow KA, Horwitz EM. Quantitative bone mineral density assessment in malignant infantile osteopetrosis. Pediatr Blood Cancer 2007;48:181–5. [PubMed: 16421907]

Kavukcuoglu NB, Denhardt DT, Guzelsu N, Mann AB. Osteopontin deficiency and aging on nanomechanics of mouse bone. J Biomed Mater Res A 2007;83:136–144. [PubMed: 17390367]

Kawashima Y, Fritton JC, Yakar S, Epstein S, Schaffler MB, Jepsen KJ, LeRoith D. Type 2 diabetic mice demonstrate slender long bones with increased fragility secondary to increased osteoclastogenesis. Bone 2009;44:648–655. [PubMed: 19150422]

Kennedy BK, Kaeberlein M. Hot topics in aging research: protein translation, 2009. Aging Cell 2009;8:617–623. [PubMed: 19747234]

Krall EA, Garcia RI, Dawson-Hughes B. Increased risk of tooth loss is releated to bone loss at the whole body, hip and spine. Calcif Tissue Int 1996;59:433–437. [PubMed: 8939767]

Kribbs PJ, Chestnut CH III, Ott SM, Kilkoyne RJ. Relationships between mandibular and skeletal bone in an osteoporotic population. J Prosthet Dent 1989;62:703–707. [PubMed: 2585328]

Kuhn LT, Grynpas MD, Rey CC, Wu Y, Ackerman JL, Glimcher MJ. A comparison of the physical and chemical differences between cancellous and cortical bovine bone mineral at two ages. Calcif Tissue Int 2008;83:146–154. [PubMed: 18685796]

Kuro-o M. Klotho and aging. Biochim Biophys Acta 2009;1790:1049–1058. [PubMed: 19230844]

Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997;390:45–51. [PubMed: 9363890]

LaMothe JM, Hepple RT, Zernicke RF. Selected contribution: Bone adaptation with aging and long-term caloric restriction in Fischer 344 × Brown-Norway F1-hybrid rats. J Appl Physiol 2003;95:1739–1745. [PubMed: 12807893]

Lee CC, Fletcher MD, Tarantal AF. Effect of age on the frequency, cell cycle, and lineage maturation of rhesus monkey (Macaca mulatta) CD34+ and hematopoietic progenitor cells. Pediatr Res 2005;58:315–322. [PubMed: 16006431]

Lee HW, Blasco MA, Gottlieb GJ, Horner JW, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. Nature 1998;392:569–574. [PubMed: 9560153]

Leeming DJ, Henriksen K, Byrjalsen I, Qvist P, Madsen SH, Garnero MA, Karsdal MA. Is bone quality associated with collagen age? Osteoporos Int 2009;20:1461–1470. [PubMed: 19330423]

LeGeros RZ. Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthop Relat Res 2002;395:81–98. [PubMed: 11937868]

Lian, JB.; Stein, GS. Osteoblast Biology. In: Marcus, R.; Feldman, D.; Nelson, D.; Rosen, CJ., editors. Osteoporosis. Third Edition. Academic Press, NY; NY: 2008. p. 93-128.

Liang S, Hosur KB, Domon H, Hajishengallis G. Periodontal inflammation and bone loss in aged mice. J Periodont Res. 2010 e publication.

Ling Y, Rios HF, Myers ER, Lu Y, Feng JQ, Boskey AL. DMP1 depletion decreases bone mineralization *in vivo*: an FTIR imaging analysis. J Bone Miner Res 2005;20:2169–2177. [PubMed: 16294270]

Loong CK, Rey C, Kuhn LT, Combes C, Wu Y, Chen S, Glimcher MJ. Evidence of hydroxyl-ion deficiency in bone apatites: an inelastic neutron-scattering study. Bone 2000;26:599–602. [PubMed: 10831931]

Lu C, Hansen E, Sapozhnikova A, Hu D, Miclau T, Marcucio RS. Effect of age on vascularization during fracture repair. J Orthop Res 2008;26:1384–1389. [PubMed: 18464248]

Manolagas SC. From Estrogen-Centric to Aging and Oxidative Stress: A Revised Perspective of the Pathogenesis of Osteoporosis. Endocr Rev. Jan 5;2010 Epub ahead of print.

Manolagas SC, Parfitt AM. What old means to bone. Trends Endocrinol Metab. 2010 2010 [Epub ahead of print].

Martin GM, Sprague CA, Epstein CJ. Replicative life-span of cultivated human cells. Effects of donor's age, tissue, and genotype. Lab Invest 1970;23:86–92. [PubMed: 5431223]

Martin GM. Genetic modulation of senescent phenotypes in *Homo sapiens*. Cell 2005;120:523–532. [PubMed: 15734684]

Masuda H, Chikuda H, Suga T, Kawaguchi H, Kuro-o M. Regulation of multiple ageing-like phenotypes by inducible klotho gene expression in klotho mutant mice. Mech Ageing Dev 2005;126:1274–1283. [PubMed: 16144705]

Matsuo K. Cross-talk among bone cells. Curr Opin Nephrol Hypertens 2009;18:292–7. [PubMed: 19395964]

McCalden RW, McGeough JA, Barker MB, Court-Brown CM. Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure. J Bone Joint Surg Am 1993;75:11993–12005.

Mellibovsky L, Bustamante M, Lluch P, Nogues X, Grinberg D, Balcells S, Diez-Perez A. Bone mass of a 113-year-old man. J Gerontol A Biol Sci Med Sci 2007;62:794–795. [PubMed: 17634329]

Melton LJ 3rd. Epidemiology of hip fractures: implications of the exponential increase with age. Bone 1996;18(3 Suppl):121–125.

Meneghini C, Dalconi MC, Nuzzo S, Mobilio S, Wenk RH. Rietveld refinement on x-ray diffraction patterns of bioapatite in human fetal bones. Biophys J 2003;84:2021–2029. [PubMed: 12609904]

Meyer RA Jr, Tsahakis PJ, Martin DF, Banks DM, Harrow ME, Kiebzak GM. Age and ovariectomy impair both the normalization of mechanical properties and the accretion of mineral by the fracture callus in rats. J Orthop Res 2001;19:428–435. [PubMed: 11398856]

Miller LM, Little W, Schirmer A, Sheik F, Busa B, Judex S. Accretion of bone quantity and quality in the developing mouse skeleton. J Bone Miner Res 2007;22:1037–1045. [PubMed: 17402847]

Mohammad AR, Bauer RL, Yeh CK. Spinal bone density and tooth loss in a cohort of postmenopausal women. Int J Prosthodont 1997;10:381–385. [PubMed: 9484049]

Mohajery M, Brooks SL. Oral radiographs in the detection of early signs of osteoporosis. Oral Surg Oral Med Oral Pathol 1992;73:112–117. [PubMed: 1603549]

Mohsin S, O'Brien FJ, Lee TC. Microcracks in compact bone: a three-dimensional view. J Anat 2006;209:119–124. [PubMed: 16822275]

Moriya Y, Ito K, Murai S. Effects of experimental osteoporosis on alveolar bone loss in rats. J Oral Sci 1998;40:171–5. [PubMed: 10036831]

Muller M. Cellular Senescence: Molecular mechanisms, *in vivo* significance, and redox considerations. Antioxid Redox Signal 2009;11:59–99. [PubMed: 18976161]

Nagaraja S, Lin AS, Guldberg RE. Age-related changes in trabecular bone microdamage initiation. Bone 2007;40:973–980. [PubMed: 17175210]

Naik AA, Xie C, Zuscik MJ, Kingsley P, Schwarz EM, Awad H, Guldberg R, Drissi H, Puzas JE, Boyce B, Zhang X, O'Keefe RJ. Reduced COX-2 expression in aged mice is associated with impaired fracture healing. J Bone Miner Res 2009;24:251–264. [PubMed: 18847332]

Nakanishi R, Shimizu M, Mori M, Akiyama H, Okudaira S, Otsuki B, Hashimoto M, Higuchi K, Hosokawa M, Tsuboyama T, Nakamura T. Secreted frizzled-related protein 4 is a negative regulator of peak BMD in SAMP6 mice. J Bone Miner Res 2006;21:1713–1721. [PubMed: 17002585]

Negri AL. The klotho gene: a gene predominantly expressed in the kidney is a fundamental regulator of aging and calcium/phosphorus metabolism. J Nephrol 2005;18:654–658. [PubMed: 16358222]

Nieves JW, Bilezikian JP, Lane JM, Einhorn TA, Wang Y, Steinbuch M, Cosman F. Fragility fractures of the hip and femur: incidence and patient characteristics. Osteoporos Int. May 30;2009 Epub ahead of print.

Novak A, Dedhar S. Signaling through beta-catenin and Lef/Tcf. Cell Mol Life Sci 1999;56:523–537. [PubMed: 11212302]

Nyman JS, Roy A, Acuna RL, Gayle HJ, Reyes MJ, Tyler JH, Dean DD, Wang X. Age-related effect on the concentration of collagen crosslinks in human osteonal and interstitial bone tissue. Bone 2006;39:1210–1217. [PubMed: 16962838]

Nyman JS, Roy A, Tyler JH, Acuna RL, Gayle HJ, Wang X. Age-related factors affecting the postyield energy dissipation of human cortical bone. J Orthop Res 2007;25:646–655. [PubMed: 17266142]

O'Brien FJ, Brennan O, Kennedy OD, Lee TC. Microcracks in cortical bone: how do they affect bone biology? Curr Osteoporos Rep 2005;3:39–45. [PubMed: 16036100]

O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. The chondrogenic potential of periosteum decreases with age. J Orthop Res 2001;19:95–103. [PubMed: 11332626]

Paschalis EP, Betts F, DiCarlo E, Mendelsohn R, Boskey AL. FTIR microspectroscopic analysis of normal human cortical and trabecular bone. Calcif Tissue Int 1997;61:480–486. [PubMed: 9383275]

Paschalis EP, DiCarlo E, Betts F, Sherman P, Mendelsohn R, Boskey AL. FTIR microspectroscopic analysis of human osteonal bone. Calcif Tissue Int 1996;59:480–487. [PubMed: 8939775]

Pellegrino ED, Biltz RM. Mineralization in the chick embryo. I. Monohydrogen phosphate and carbonate relationships during maturation of the bone crystal complex. Calcif Tissue Res 1972;10:128–135. [PubMed: 4343465]

Pietschmann P, Skalicky M, Kneissel M, Rauner M, Hofbauer G, Stupphann D, Viidik A. Bone structure and metabolism in a rodent model of male senile osteoporosis. Exp Gerontol 2007;42:1099–1108. [PubMed: 17949933]

Plantalech L, Guillaumont M, Vergnaud P, Leclercq M, Delmas PD. Impairment of gamma carboxylation of circulating osteocalcin (bone gla protein) in elderly women. J Bone Miner Res 1991;6:1211–1216. [PubMed: 1666807]

Płudowski P, Lebiedowski M, Olszaniecka M, Marowska J, Matusik H, Lorenc RS. Idiopathic juvenile osteoporosis--an analysis of the muscle-bone relationship. Osteoporos Int 2006;17:1681–1690. [PubMed: 16951909]

Provot, S.; Schipanni, E.; Wu, J.; Kronenberg, H. Development of the Skeleton. In: Marcus, R.; Feldman, D.; Nelson, D.; Rosen, CJ., editors. Osteoporosis. Third Edition. Academic Press, NY; NY: 2008. p. 241-270.

Purnell JQ, Brandon DD, Isabelle LM, Loriaux DL, Samuels MH. Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body composition, leptin levels, and aging in adult men and women. J Clin Endocrinol Metab 2004;89:281–287. [PubMed: 14715862]

Ramanadham S, Yarasheski KE, Silva MJ, Wohltmann M, Novack DV, Christiansen B, Tu X, Zhang S, Lei X, Turk J. Age-related changes in bone morphology are accelerated in group VIA phospholipase A2 (iPLA2beta)-null mice. Am J Pathol 2008;172:868–881. [PubMed: 18349124]

Rauch F. Bone growth in length and width: the Yin and Yang of bone stability. J Musculoskelet Neuronal Interact 2005;5:194–201. [PubMed: 16172510]

Rauch F, Travers R, Norman ME, Taylor A, Parfitt AM, Glorieux FH. The bone formation defect in idiopathic juvenile osteoporosis is surfSace-specific. Bone 2002;31:85–89. [PubMed: 12110417]

Rauner M, Sipos W, Pietschmann P. Age-dependent Wnt gene expression in bone and during the course of osteoblast differentiation. Age (Dordr) 2008;30:273–282. [PubMed: 19424851]

Rey C, Hina A, Tofighi A, Glimcher MJ. Maturational of poorly crystalline apatites – Chemical and Structural aspects *in vivo* and *in vitro*. Cells Materials 1995;5:345–356.

Rey C, Miquel JL, Facchini L, Legrand AP, Glimcher MJ. Hydroxyl groups in bone mineral. Bone 1995;16:583–586. [PubMed: 7654473]

Rey C, Renugopalakrishnan V, Collins B, Glimcher MJ. Fourier transform infrared spectroscopic study of the carbonate ions in bone mineral during aging. Calcif Tissue Int 1991a;49:251–258. [PubMed: 1760769]

Rey C, Shimizu M, Collins B, Glimcher MJ. Resolution-enhanced Fourier transform infrared spectroscopy study of the environment of phosphate ion in the early deposits of a solid phase of calcium phosphate in bone and enamel and their evolution with age: 2. Investigations in the nu3PO4 domain. Calcif Tissue Int 1991b;49:383–388. [PubMed: 1818762]

Reynolds RM, Dennison EM, Walker BR, Syddall HE, Wood PJ, Andrew R, Phillips DI, Cooper C. Cortisol secretion and rate of bone loss in a population-based cohort of elderly men and women. Calcif Tissue Int 2005;77:134–138. [PubMed: 16151676]

Richards JB, Kavvoura FK, Rivadeneira F, Styrkársdóttir U, Estrada K, Halldórsson BV, Hsu YH, Zillikens MC, Wilson SG, Mullin BH, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra BA, Pols HA, Sigurdsson G, Thorsteinsdottir U, Soranzo N, Williams FM, Zhou Y, Ralston SH, Thorleifsson G, van Duijn CM, Kiel DP, Stefansson K, Uitterlinden AG, Ioannidis JP, Spector TD. In: Genetic Factors for Osteoporosis Consortium. Collaborative meta-analysis: associations of 150 candidate genes with osteoporosis and osteoporotic fracture. Ann Intern Med 2009;151:528–37. [PubMed: 19841454]

Ritchie CS, Joshipura K, Hung HC, Douglass CW. Nutrition as a mediator in the relation between oral and systemic disease:associations between specific measures of adult oral health and nutrition outcomes. Crit Rev Oral Biol Med 2002;13:291–300. [PubMed: 12090466]

Rochefort GY, Pallu S, Benhamou CL. Osteocyte: the unrecognized side of bone tissue. Osteoporos Int. 2010 Epub ahead of print.

Saito M, Marumoto K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int 2010;21:195–214. [PubMed: 19760059]

Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 2010;464:520–528. [PubMed: 20336134]

Salih DA, Brunet A. FoxO transcription factors in the maintenance of cellular homeostasis during aging. Curr Opin Cell Biol 2008;20:126–36. [PubMed: 18394876]

Sanfilippo F, Bianchi AE. Osteoporosis: the effect on maxillary bone resorption and therapeutic possibilities by means of implant prostheses--a literature review and clinical considerations. Int J Periodontics Restorative Dent 2003;23:447–57. [PubMed: 14620119]

Sarajlic N, Topic B, Brkic H, Alajbeg IZ. Aging quantification on alveolar bone loss. Coll Antrhopol 2009;33:1165–70.

Schaffler MB, Choi K, Milgrom C. Aging and matrix microdamage accumulation in human compact bone. Bone 1995;17:521–552. [PubMed: 8835305]

Schneider S, Breit SM, Grampp S, Künzel WW, Liesegang A, Mayrhofer E, Zentek J. Comparative assessment of bone mineral measurements obtained by use of dual-energy x-ray absorptiometry, peripheral quantitative computed tomography, and chemical-physical analyses in femurs of juvenile and adult dogs. Am J Vet Res 2004;65:891–900. 2004. [PubMed: 15281646]

Seeman E. The structural and biomechanical basis of the gain and loss of bone strength in women and men. Endocrinol Metab Clinic NA 2003;32:25–38.

Seeman E. Bone modeling and remodeling. Crit Rev Eukaryot Gene Expr 2009;9:219–33. [PubMed: 19883366]

Somerville JM, Aspden RM, Armour KE, Armour KJ, Reid DM. Growth of C57BL/6 mice and the material and mechanical properties of cortical bone from the tibia. Calcif Tissue Int 2004;74:469–75. [PubMed: 14961209]

Southard KA, Southard TE, Schlecthte JA, Meis PA. The relationship between the density of the alveolar process and that of post-cranial bone. J Dent Res 2000;89:964–9. [PubMed: 10831099]

Streckfus CF, Parsell DE, Streckfus JE, Pennington W, Johnson RB. Relationship between oral alveolar bone loss and aging among African-American and Caucasian individuals. Gerontology 1999;45:110–114. [PubMed: 9933734]

Suzuki H, Amizuka N, Oda K, Li M, Yoshie H, Ohshima H, Noda M, Maeda T. Histological evidence of the altered distribution of osteocytes and bone matrix synthesis in klotho-deficient mice. Arch Histol Cytol 2005;68:371–381. [PubMed: 16505583]

Szulc P, Seeman E. Thinking inside and outside the envelopes of bone: dedicated to PDD. Osteoporos Int 2009;20:1281–1288. [PubMed: 19590836]

Taguchi A, Suei Y, Ohtsuka M, Otani K, Tanimoto K, Hollender LG. Relationship between bone mineral density and tooth loss in elderly Japanese women. Dentomaxillofac Radiol 1999;28:219–23. [PubMed: 10455385]

Tanaka M, Ejiri S, Toyooka E, Kohno S, Ozawa H. Effects of ovariectomy on trabecular structures of rat alveolar bone. J Periodontal Res 2002;37:161–5. [PubMed: 12009186]

Tanaka M, Toyooka E, Kohno S, Ozawa H, Ejiri S. Long-term changes in trabecular structure of aged rat alveolar bone after ovariectomy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:495–502. [PubMed: 12686937]

Tang SY, Zeenath U, Vashishth D. Effects of non-enzymatic glycation on cancellous bone fragility. Bone 2007;40:1144–1151. [PubMed: 17257914]

Tarnowski CP, Ignelzi MA Jr, Morris MD. Mineralization of developing mouse calvaria as revealed by Raman microspectroscopy. J Bone Miner Res 2002;17:1118–1126. [PubMed: 12054168]

Tommasini SM, Nasser P, Jepsen KJ. Sexual dimorphism affects tibia size and shape but not tissue-level mechanical properties. Bone 2007;40:498–505. [PubMed: 17035111]

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. [PubMed: 16007335]

Tong W, Glimcher MJ, Katz JL, Kuhn L, Eppell SJ. Size and shape of mineralites in young bovine bone measured by atomic force microscopy. Calcif Tissue Int 2003;72:592–598. [PubMed: 12724830]

Tosi LL, Boyan BD, Boskey AL. Does sex matter in musculoskeletal health? The influence of sex and gender on musculoskeletal health. J Bone Joint Surg Am 2005;87:1631–1647. [PubMed: 15995134]

Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 1996;81:2468–2473. [PubMed: 8675562]

Vashishth D. Hierarchy of Bone Microdamage at Multiple Length Scales. Int J Fatigue 2007;29:1024–1033. [PubMed: 18516216]

Vashishth D, Gibson GJ, Khoury JI, Schaffler MB, Kimura J, Fyhrie DP. Influence of nonenzymatic glycation on biomechanical properties of cortical one. Bone 2001;28:195–201. [PubMed: 11182378]

Verborgt O, Gibson GJ, Schaffler MB. Loss of osteocyte integrity in association with microdamage and bone remodelling after fatigue *in vivo*. J Bone Miner Res 2000;15:60–67. [PubMed: 10646115]

Viguet-Carrin S, Follet H, Gineyts E, Roux JP, Munoz F, Chapurlat R, Delmas PD, Bouxsein ML. Association between collagen cross-links and trabecular microarchitecture properties of human vertebral bone. Bone 2010;46:342–347. [PubMed: 19836004]

Von Wowern N, Solte K. Histoquantitation on small jaw specimens. Scand J Dent Res 1978;86:193–9. [PubMed: 276918]

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. [PubMed: 17240210]

Wang Q, Seeman E. Skeletal growth and peak bone strength. Best Pract Res Clin Endocrinol Metab 2008;22:687–700. [PubMed: 19028352]

Weiner S, Traub W. Crystal size and organization in bone. Connect Tissue Res 1989;21:259–265. [PubMed: 2605950]

Westerbeek ZW, Hepple RT, Zernicke RF. Effects of aging and caloric restriction on bone structure and mechanical properties. J Gerontol A Biol Sci Med Sci 2008;63:1131–1136. [PubMed: 19038827]

White SC, Rudoolph DJ. Alterations of the trabecularo pattern of the jaws in patients with osteoporosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 1999;88:628–35.

Williams BO, Insogna KL. Where Wnts went: the exploding field of Lrp5 and Lrp6 signaling in bone. J Bone Miner Res 2009;24:171–178. [PubMed: 19072724]

Woo SL, Kuei SC, Amiel D, Gomez MA, Hayes WC, White FC, Akeson WH. The effect of prolonged physical training on the properties of long bone: a study of Wolff's Law. J Bone Joint Surg Am 1981;63:780–787. [PubMed: 7240300]

Wu M, Fannin J, Rice KM, Wang B, Blough ER. Effect of aging on cellular mechanotransduction. Ageing Res Rev. Nov 20;2009 Epub ahead of print.

Wu Y, Ackerman JL, Kim HM, Rey C, Barroug A, Glimcher MJ. Nuclear magnetic resonance spin-spin relaxation of the crystals of bone, dental enamel, and synthetic hydroxyapatites. J Bone Miner Res 2002;17:472–480. [PubMed: 11874238]

Yates LB, Karasik D, Beck TJ, Cupples LA, Kiel DP. Hip structural geometry in old and old-old age: similarities and differences between men and women. Bone 2007;41:722–732. [PubMed: 17662680]

Yerramshetty JS, Lind C, Akkus O. The compositional and physicochemical homogeneity of male femoral cortex increases after the sixth decade. Bone 2006;39:1236–1243. [PubMed: 16860007]

Zhang Y, Dong XL, Leung PC, Wong MS. Differential mRNA expression profiles in proximal tibia of aged rats in response to ovariectomy and low-Ca diet. Bone Jan;2009 44(1):46–52. [PubMed: 18848653]

Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, Leboff MS, Glowacki J. Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 2008;7:335–343. [PubMed: 18248663]

Zhu, W.; Robey, PG.; Boskey, AL. The regulatory role of matrix proteins in mineralization of bone. In: Marcus, R.; Feldman, D.; Nelson, D.; Rosen, CJ., editors. Osteoporosis. Third Edition. Academic Press, NY; NY: 2008. p. 191-240.

Zuscik MJ, Hilton MJ, Zhang X, Chen D, O'Keefe RJ. Regulation of chondrogenesis and chondrocyte differentiation by stress. J Clin Invest 2008;118:429–38. [PubMed: 18246193]
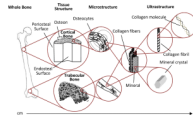
Figure 1.
The structural levels of bone. Cortical bone is made up of longitudinally oriented osteons and the trabecular bone within the metaphyses is made up of connected struts and plates. In both bone types, the bone is laid down in layers (lamellae). Both tissue types contain identical components and their properties are dependent on the amount, morphology, and interaction of these components at each level.
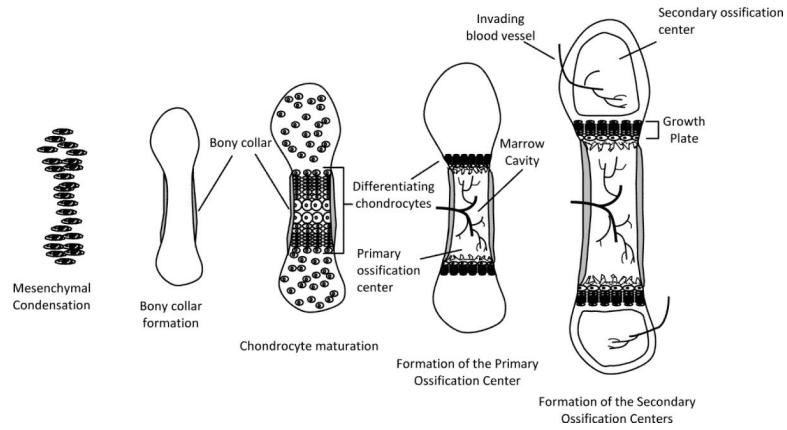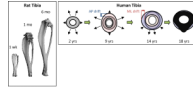
Figure 2.
Endochondral ossification of long bones. Condensation of mesenchymal cells form the general shape of the long bone. Differentiation of these cells into chondrocytes begins the process of bone formation in the primary ossification center. In late differentiation, chondrocytes undergo apoptosis leaving behind a mineralized scaffold onto which osteoblasts brought in by the invading vasculature lays down bone, lengthening the bone the 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.

Figure 3.
Long bone geometrical changes with age. Left: micro-CT 3D renderings of rat tibias with increasing age. Right: Cortical drift in the tibia 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 years. Right panel adapted from Goldman et al., 2009. AP: anterior-posterior direction, ML: medial-lateral direction.
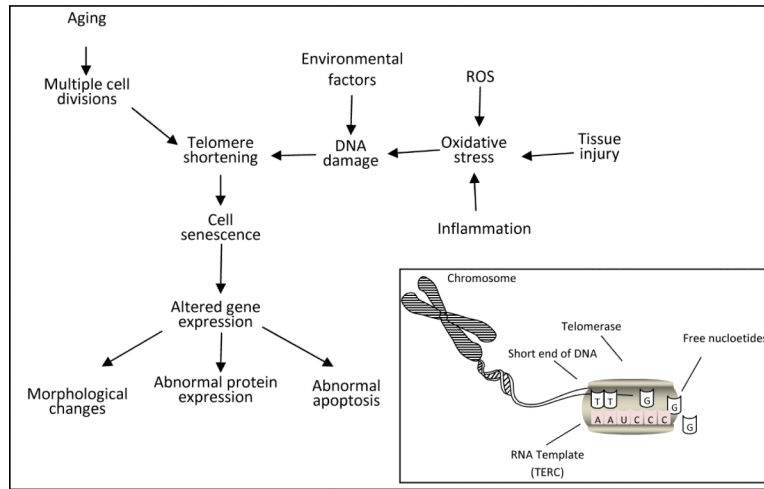
Figure 4.
Bone remodeling pathways. Several factors can initiate bone remodeling, which, in a balanced system, begins with resorption by osteoclasts and ends with formation by osteoblasts. Signals from external sources (Wnts) and from within the bone (osteocyte apoptosis) contribute to osteoblast and osteoclast differentiation and activity, a selection of which is show in the above figure. Light gray – resorption pit, dark gray – osteoid, yellow – newly mineralized osteoid

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 stems 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.
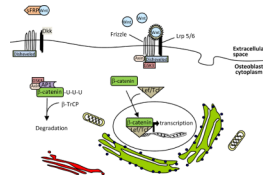
Figure 6.
Wnt/beta-catenin pathway of osteoblast differentiation. The translation of genes that stimulate osteoblast differentiation require the translocation of b-catenin from the cytoplasm to the nucleus of mesenchymal stem cells. b-catenin is targeting for degradation by the GSK3/AP1/Axin complex. Wnt binding to frizzled and Lrp 5/6 interrupts this process, allowing b-catenin to complex with Lef/Tcf. Dkk inhibits Wnt binding by interacting with Lrp 5/6. sFRP also inhibits this pathway by sequestering Wnt.
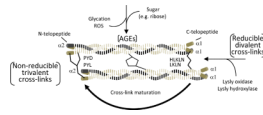
Figure 7.
Chronological formation of collagen cross-links. As collagen matures, reducible cross-links become non-reducible. Advanced glycation end products (AGEs) also accumulate between the helical part of the molecules as the collagen persists in the tissue. Both contribute to the stiffening of the collagen matrix with age, which may contribute to bone tissue properties. Adapted from Leeming et al., 2009 and Saito and Marumo, 2010.
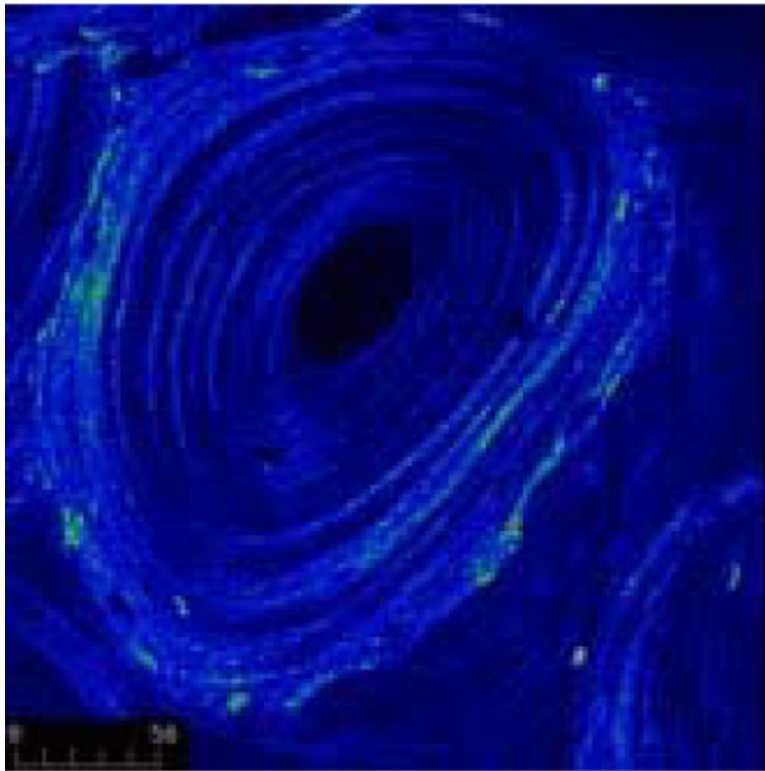
Figure 8.
Collagen orientation of osteons obtained using second harmonic generation microscopy, where the intensity is proportional to the orientation of the collagen. The orientation increases with tissue age as is apparent in the outer, older rings of the osteon, which have a brighter intensity. Image from a 6 year old baboon courtesy of Jayme Burket.
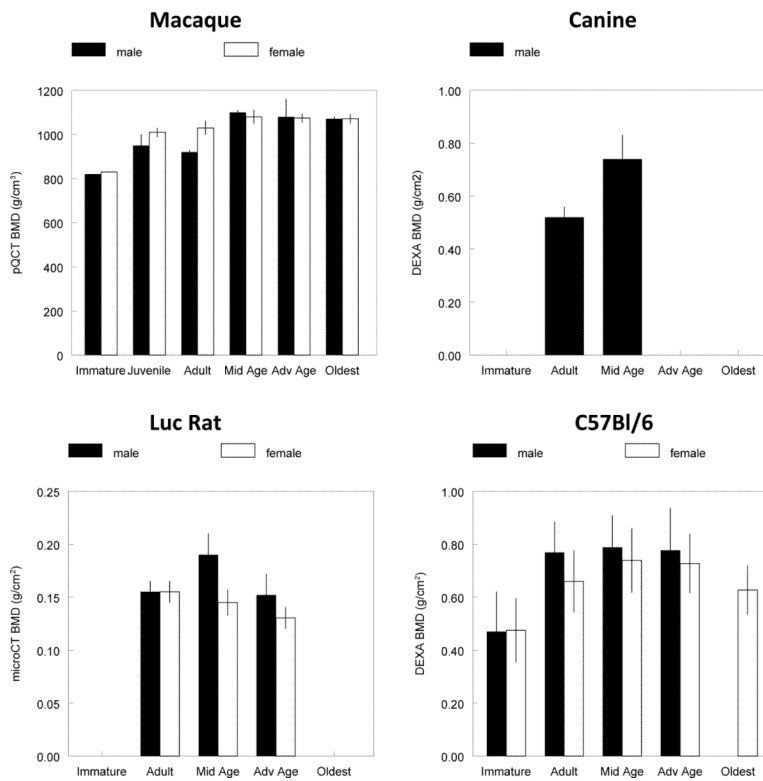
Figure 9.
Change in BMD of various species with age. All of the plotted species show an increase in BMD with age – male increases faster and female decreases earlier – but both sexes decline with age. Adapted from the following: Macaque – Cerroni, 2000; dog – Schneider et al., 2004; Luc rat – Duque et al., 2009; C57Bl/6 – Somerville and Aspden, 2004
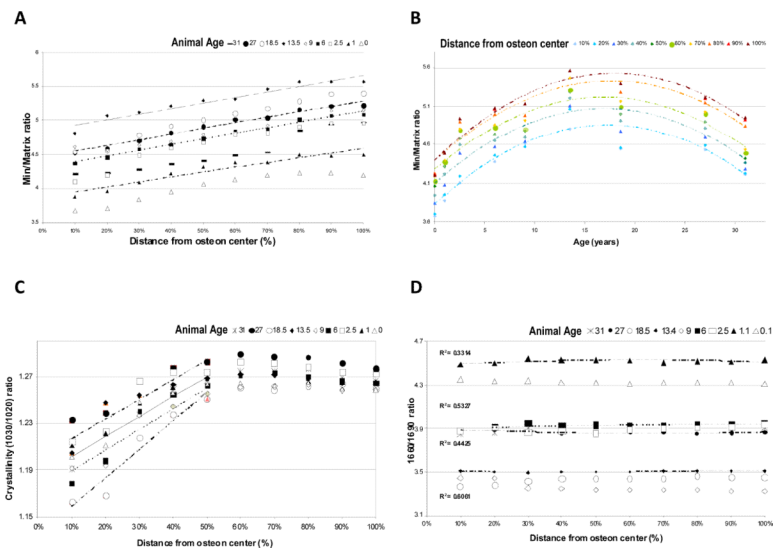
Figure 10.
Heterogeneity of FTIR parameters in baboon Osteonal bone. a) Mineral/Matrix ratio as a function of tissue age (distance from osteon center) in baboons of ages ranging from 0–37 years (R^2 ~0.92–0.99). b) Mineral/Matrix ratio plotted as a function of the baboon age for each section along the 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.

Table 1

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