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Abstract

The biomechanics literature contains many well-understood mechanisms behind typical fracture types that have important roles in treatment planning. The recent association of "atypical" fractures with long-term use of drugs designed to prevent osteoporosis has renewed interest in the effects of agents on bone tissue-level quality. While this class of fracture was recognized prior to the introduction of the anti-resorptive bisphosphonate drugs and recently likened to stress fractures, the mechanism(s) that lead to atypical fractures have not been definitively identified. Thus, a causal relationship between these drugs and atypical fracture has not been established. Physicians, bioengineers and others interested in the biomechanics of bone are working to improve fracture-prevention diagnostics, and the design of treatments to avoid this serious side-effect in the future. This review examines the mechanisms behind the bone tissue damage that may produce the atypical fracture pattern observed increasingly with long-term bisphosphonate use. Our recent findings and those of others reviewed support that the mechanisms behind normal, healthy excavation and tunnel filling by bone remodeling units within cortical tissue strengthen mechanical integrity. The ability of cortical bone to resist the damage induced during cyclic loading may be altered by the reduced remodeling and increased tissue age resulting from long-term bisphosphonate treatment. Development of assessments for such potential fractures would

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restore confidence in pharmaceutical treatments that have the potential to spare millions in our aging population from the morbidity and death that often follow bone fracture.

Keywords

bone remodeling; osteocytes; osteoblasts; osteoclasts; antiresorptives; osteoporosis

1. Introduction

Relationships between many typical bone fracture types and their mechanisms are well understood, are found in the biomechanics literature, and play an important role in treatment planning. For example, osteoporotic fracture has been associated with decreased bone density at skeletal sites composed predominately of trabecular tissue. Over the past two decades bisphosphonate (BP) therapy has been the gold standard used to reduce osteoporotic fracture risk by suppressing osteoclast-mediated bone resorption. However, increased reports of rare but serious "atypical" femur fracture (AFF) associated with long-term BP therapy have intensified examination by the Food and Drug Administration (FDA) and the orthopaedic research community (Figure 1) (e.g., Lenart et al., 2008; National Guideline Clearinghouse, 2013). This review summarizes work that may hint at the potential underlying mechanisms behind the bone tissue damage that produces this atypical fracture pattern.

2. Fracture classification

Fractures are classified based on location, the estimated energy that produced them and the resulting breakage patterns. Classification criteria inform a great deal about the biomechanical environment prior to fracture. Fractures due to pathologic conditions such as osteoporosis, Paget's disease, osteogenesis imperfecta, rickets or bone cancer are generally closed, have intact overlying skin, and result from low-energy events. Conversely, high-energy impacts often result in open trauma fractures and are classified by the AO Trauma system (Müller, 1980). These typical fractures include those that have at least one large crack that completely traverses all cortices, including the entire width of the bone. The simple fractures are spiral, oblique and transverse. More complex, higher-energy fractures include burst, comminuted with many small bone fragments and/or impacted.

2.1 Osteoporotic fractures and their prevention

Osteoporotic fractures present with typical patterns. They are the result of age-related metabolic bone wasting characterized by highly porous, low density bone ends with reduced bone strength where trabecular structure is predominantly found (Atkinson, 1965). The wasting is due to rates of osteoclastic bone resorption outpacing osteoblastic bone formation, resulting in a highly porous structure. Hip fractures usually result from falls that would not otherwise produce fractures in non-osteoporotic individuals (Sanders et al., 1998). Collapsing crush fractures of the principally cancellous spinal vertebrae are also common with osteoporosis (Kleerekoper et al., 1985).

While osteoporotic fractures normally occur in the predominantly trabecular ends (metaphyseal region), cortical bone also plays a role in the propensity to fracture. Cortical cross-sectional geometry (i.e., bone structure) includes bone width, cortex thickness and distribution of tissue matrix. Bone width and cortex thickness are important to resisting failure by local buckling, as structures buckle when they have a slender aspect ratio (small width to length) (Beck et al., 2001; Giladi et al., 1987). The distribution of bone about the centroidal axis is important because a smaller periosteal versus endosteal adaptive expansion of the cortex offsets a propensity to fragility due to the effect on structural cross-sectional moment of inertia (Ruff and Hayes, 1982; Smith and Walker, 1964). Thus, bone normally adapts to meet biomechanical needs and some of these abilities, such as cortical thickening, may be observed in the pathogenesis of AFF (section 3), possibly with imaging techniques (Section 4).

2.1.1 Bisphosphonate mechanisms of action—BPs are the most commonly prescribed drug for the prevention of osteoporotic fracture (FDA, 2011). By suppressing resorption, BPs and other anti-resorptive agents slow the loss of bone mass at the hip and spine (Rodan and Fleisch, 1996). Consequently, fracture risk is reduced (Seeman and Delmas, 2006). Non-nitrogen containing, first-generation BPs have fallen out of favor, especially in the U.S. because they may affect mineralization, although a few are still used clinically including clodronate (Bayer), etidronate (Warner Chilcott) and tiludronate (Sanofi) (Russell, 2011). Nitrogen-containing BPs administered orally and intravenously (i.v.) have captured the majority of the clinical market. The orals most commonly prescribed are alendronate (Merck, Sharp & Dohme), ibandronate (Genentech) and risedronate (Sanofi), and the most commonly prescribed i.v. administered BPs are pamidronate (Novartis) and zoledronate (Novartis Pharma AG) (Rogers et al., 2011). Many are now becoming available in generic form.

Each BP ultimately acts to slow resorption by osteoclasts (OC). However, osteocytes, the terminally differentiated osteoblasts (OB) embedded in the bone matrix during formation (Figure 2), are now believed to be the primary activators of resorption (section 3.1) (Bonewald, 2011; Schaffler, 2003). Damage may trigger osteocyte apoptosis to signal and target for resorption (Bentolila et al., 1998; Cardoso et al., 2009; Herman et al., 2010; Verborgt et al., 2002). In order for resorption to occur, pre-OCs are recruited to a site at which proliferation and differentiation cues are present to drive the OCogenesis, and the resulting OCs have to attach onto exposed mineral and begin normal bone resorption (Burr, 2002; Parfitt, 2002). BPs exploit the mechanism by which OC membrane rigidity is regulated through cholesterol synthesis for attachment of the OC to bone and push OCs toward apoptosis, thus altering osteoclast activity (Rogers et al., 2011), so less bone is removed and the normal, coupled-action of OBs to form new bone and fill the OC pits is lower (Parfitt, 1982). Thus, long-term BP treatment may affect bone quality through many bone multi-cellular unit (BMU)-related cortical tissue features, including a flux in osteocyte numbers (section 3.1.1).

Suppression of bone resorption and remodeling initially alters tissue-level micro-architecture and composition positively, most notably by slowing the loss of trabecular micro-architecture, quantified as number, thickness and interconnectedness of plate-like and rod-

like elements (Boivin et al., 2000). Suppression of resorption may also have added biomechanical effects by allowing OB to more completely fill existing resorption cavities, in addition to the prevention of new stress risers that resorption cavities may eventually become, especially in cancellous bone (Dempster, 2003; Easley et al., 2012; McNamara et al., 2006). Regardless of mechanism(s), the short-term benefit of BPs is clear as large randomized trials have demonstrated that BPs greatly reduce osteoporotic fracture risk (Black et al., 1996).

2.2 Stress fractures

Since some retrospective assessments of atypical fractures propose similarities with common stress fractures, research in that field may yield potential mechanisms (Lappe et al., 2005; Schilcher and Aspenberg, 2009). While some similarities exist, stress fractures are different from pathologic and traumatic fractures. Stress fractures typically occur as a result of excessive loading cycles ("fatigue") within healthy bone tissue and may be exacerbated in lesser quality ("insufficient") bone (Giladi et al., 1987; McKenna et al., 2014; Shane et al., 2014). While insufficient-type stress fractures have been observed in patients with osteomalacia and hypophosphatasia, fatigue-type stress fractures are solely observed in high load-bearing sites like the femur, tibial shaft (most frequent), metatarsals and calcaneus. Fatigue fractures are a result of repetitive activity in high-performance athletes, including runners and race horses, and elevated activity for military recruits when their training begins (Giladi et al., 1987).

Stress fractures normally present oblique to the diaphysis when incomplete, affecting a single cortex within the shaft of long bones (Shane et al., 2014). They are associated with a painful, periosteal healing response with cortex thickening acting to attempt a stabilization of the fracture gap. A similar generalized cortical thickening has been observed prior to some AFFs and is classified as a minor feature that may be due to a localized periosteal reaction of the lateral cortex (Shane et al., 2014). In the case of a stress fracture such a callus formation is usually followed by normal bone remodeling and healing if the repetitive activity that is the root cause of the fracture is eliminated (McKenna et al., 2014). Likewise, unless normal remodeling is restored after an initial AFF indicator, such as cortical thickening, the deficit may allow progression to fracture.

2.3 Atypical fractures

Recently, a subset of mainly female osteoporotic patients on long-term anti-resorptive therapy, principally with BPs, has sustained fractures with a pattern that is atypical of osteoporotic, traumatic or stress fractures. Differences from stress fractures are fewer and include location, angle of progression across the cortex and activity level of the presenting patient (Shane et al., 2014). These atypical fractures may start as an incomplete transverse fracture (Figure 3) that sometimes advances to a complete fracture through all cortices of the subtrocanteric or diaphyseal femur. The ulna has been recently proposed as another site of atypical fracture in isolated clinical case studies (Ang et al., 2013; Bjorgul and Reigstad, 2011; Tang and Kumar, 2011). Additionally, in some retrospective reviews, approximately 20% of patients exhibiting an atypical fracture have a similar bi-lateral finding, usually at the same location on the contralateral limb at first clinical presentation or within a short

window of < 6 months. One possible explanation for this type of fracture is that over many years of treatment, bone tissue treated with BPs may age differently, with lower tissue turnover than in the drug-naïve condition. Eventually, the rate of damage initiation and accumulation may outpace the suppressed remodeling process, and mechanical integrity becomes compromised.

The uncommon features of these fractures have raised concern for long-term effects on bone health due to the use of BPs. Many clinical reports, meta-analyses and reviews of BP-associated atypical fracture cases have been published since the first recognition of this problem in 2008 (Gedmintas et al., 2013; Lenart et al., 2008; Lenart et al., 2009; Neviaser et al., 2008). In response, an American Society for Bone and Mineral Research (ASBMR) task force recently reviewed all literature pertaining to reports of AFF, demonstrating the most common presentation location along the lateral cortex of the femoral diaphysis, outside of the neck and/or intertrochanteric regions with a transverse orientation or "short oblique" (<30 degrees from a transverse plane) trajectory (Figure 3) (Shane et al., 2014; Shane et al., 2010). The radiographic features also demonstrate localized periosteal or endosteal thickening of the lateral cortex at the site of initial fracture (cortical beaking or flaring) and non- or minimal comminution, i.e., few fragments. A minor feature of AFF, groin or thigh pain, is a symptom causing patients to seek additional medical attention prior to experiencing a complete fracture. While the ASBMR report concluded an association with BP use, osteonecrosis of the jaw (ONJ) was only briefly addressed as a problem related to BPs, principally associated with high-dose cancer treatments (Allen and Burr, 2009; Compston, 2011; Subramanian et al., 2013). High-dose BPs are effective treatments to prevent cancer metastases to bone and have had similar incidences of ONJ in cancer trials (1–2% of patients) that appear to be a consequence of marked inhibition of bone resorption (Clezardin et al., 2011; Gartrell et al., 2013; Saad et al., 2012; Woo et al., 2006). Though they exist, there are few reports of AFF in the cancer population on high-dose BPs (Chang et al., 2012; Grasko et al., 2009; Hayashi et al., 2014; Iwata et al., 2014; Puhaindran et al., 2011; Waterman et al., 2011; Wernecke et al., 2008). One plausible reason may be the relatively short amount of time that cancer patients have been maintained on BPs due principally to the morbidity and guarded life expectancy for patients with metastatic bone disease (Saad et al., 2012). Meanwhile, the incidence of BP-associated AFF increases with long-term use (>3–5 years); dose time may have a large influence on this side effect (Abrahamsen et al., 2009; Dell et al., 2012; FDA, 2011; Lenart et al., 2009; Shane et al., 2014). Thus, whether the advent of new therapeutic options that allow cancer patients longer life will lead to greater reports of AFF remains to be seen. Mechanisms behind AFF and ONJ may also be un-related and cancer patients likely maintain a lower level of physical activity than that expected to lead to any type of long-bone fracture.

3. Possible mechanisms for AFF: Biological, biochemical and biomechanical damage to bone

There is a clear lack of understanding with regards to the mechanism(s) behind atypical fracture (Shane et al., 2014). However, the functions of bone are well recognized: to provide mechanical stability and mineral homeostasis, allowing for locomotion and ion exchange.

Bone's properties thus rely on tight regulation of biological and biochemical processes. If man-made, bone would be considered one of the most technologically-advanced, tough composite materials, designed to withstand cyclic loading and dissipate energy. The main advantage of bone tissue over artificial materials is that damage accumulated is repaired via intrinsic mechanisms during remodeling (Figure 2) (Currey, 1999; Martin et al., 1998; Recker, 1983; Schaffler and Burr, 1988).

In the case of the adult taking a BP, the primary effect is on bone tissue turnover. As recently demonstrated, the tissue renewal process is dependent on damage cues from the local cells (osteocytes), and there are several direct influences of the BPs (Aguirre et al., 2006; Manolagas and Parfitt, 2013; Nakashima et al., 2011; Schaffler et al., 2014). While this review focuses on these cell- and tissue-level bone quality effects, cells and tissue will be subject to differing stress distributions based on whole-bone structure, including femoral-shaft curvature and offsets from lines of force application (i.e., femoral-neck length and angle). The cortices of many long bones, including the femoral shaft, have a natural curvature. Thus, some have argued that patients of Asian ancestry with a larger natural curving bow to their femoral shaft are at greater risk of atypical fracture (Sasaki et al., 2012; Shane et al., 2014).

3.1 Osteocyte damage

Osteocytes and their cellular processes in the lacunar-canalicular pores establish a dense communication network that is vital for nutrient and gas exchange. This network also establishes a highly sensitive mechanosensory system (Cowin et al., 1991; Wang et al., 2000). Upon incorporation into the matrix, osteocytes form cellular protrusions that function as connections between the cytoplasm of neighboring osteocytes. Each osteocyte has at least 50 processes with the potential to directly contact a neighbor (for reviews, see (Bonewald, 2011; Manolagas and Parfitt, 2013; Schaffler et al., 2014)) (Beno et al., 2006; Kerschnitzki et al., 2013). Osteoctye processes connect directly by multi-pass transmembrane proteins, primarily connexin 43 (Cx43), located at the ends of the processes, that tightly regulate active transport of nutrients, metabolites, signaling ions (including Ca^{2+}), small molecules and possibly RNA from one cell to another (Doty, 1981; Harris, 2007). Gap junction intercellular communication in response to anabolic agents (PTH) appears to be decreased as the age of the animal from which cells are isolated increases, independent of the ability of cells to produce Cx43 (Genetos et al., 2012). Paracrine and endocrine communication also occurs outside the osteocyte through the fluid-filled lacunar-canalicular network; exported from the cell are signaling molecules such as receptor activator of nuclear factor – κB ligand (RANKL) and Ca^{2+}, released through un-paired Cx43 hemi-channels and other Ca^{2+}-specific channels (Ke et al., 2013). Thus, mechanisms of cell signaling, both direct and indirect, occur in continuous passageways and are vital to the maintenance of osteocytes and good bone tissue quality.

Damage to cells constantly occurs within bone and also may result from disruption of the processes, affecting cell-to-cell signaling. As in most cells, osteocytes accumulate damage to their intra-cellular machinery due to the normal aging processes of oxidation and telomere shortening that eventually leads to death (Almeida et al., 2007; Nojiri et al., 2011). Rarely,

osteocyte death is by overwhelming rupture of the cell body. Normally, osteocyte death occurs in a regulated fashion, by apoptosis (Bonewald, 2011; Manolagas and Parfitt, 2013; Schaffler et al., 2014). Estrogen withdrawal, loss of normal mechanical loading, accumulation of advanced glycation end (AGE) products or direct, mechanically-induced tissue damage accelerate this apoptosis (Aguirre et al., 2006; Burr et al., 1985; Follet et al., 2007; Schaffler and Kennedy, 2012; Tomkinson et al., 1998).

3.1.1 Anti-apoptotic effects of bisphosphonates: Is a long life best?—BPs may have both short-term and long-term effects on osteocytes. Certainly, in the short-term BPs are anti-apoptotic for osteocytes (Loiselle et al., 2013; Plotkin et al., 2008; Plotkin et al., 1999). Possibly, this action involves the Cx43 hemi-channels that are independent of gap junctions (Bellido and Plotkin, 2011; Plotkin et al., 2005; Plotkin et al., 2002).

For any anti-resorptive therapy a long-term consequence of reducing osteocyte apoptosis and OC resorption may be that the average age of the osteocyte population increases as there is a lower demand for new cells. After the initial closure of the remodeling space with the OB more completely filling existing resorption pits, a smaller amount of matrix is laid down for mineralization upon each remodeling activation cycle (Allen et al., 2010; Bajaj et al., 2014). Thus, one possible consequence to the sparing of osteocytes by reduced resorption is increased cell aging. Yet, even this long-lived (decades) cell has a limited lifespan so that eventually death ensues (Dallas et al., 2013). If this death occurs without replacement, the logical eventual outcome is decreased osteocyte density. This less cellular tissue would have decreased sensitivity to mechanical loading and damage, raising the possibility that osteocyte density helps determine bone tissue properties (Vashishth et al., 2002; Vashishth et al., 2000). Indeed, a decline in osteocyte and lacunar density has been associated with aging, increased risk of osteoporotic fracture, and estrogen withdrawal (Frost, 1960a; Mullender et al., 1996; Qiu et al., 2002; Qiu et al., 2003; Tomkinson et al., 1997; Wong et al., 1985).

We recently reported osteocyte lacunae density, tissue mechanical properties under fatigue loading, and tissue micro-structure (section 3.2) of cortical rib bone from female beagles treated for 3 years with two different doses of alendronate or saline control (Bajaj et al., 2014). The alendronate doses tested were 0.2 and 1.0 mg/kg/day, chosen to correspond to those used clinically for the treatment of postmenopausal osteoporosis (low-dose) and Paget's disease (high-dose), respectively. Tissue was bulk stained in 1% basic fuchsin, embedded in polymethyl-methacrylate, cut transverse to the bone long axis and imaged under bright-field microscopy. We found the density of osteocyte lacunae was reduced (~20%) by the same degree for both doses of alendronate (Bajaj et al., 2014). Our findings were similar in both the osteonal and interstitial space of the rib. However, our histological technique did not discriminate between possibly empty versus "missing" lacunae, due to either poor tissue construction or infilling after osteocyte death. Utilizing high-resolution (1 μm) 3D synchrotron μCT to separate porosity fractions, (Tommasini et al., 2012) recently found a similar (~16%) reduction in the density of osteocyte lacunae with 6-month alendronate treatment, equivalent to our low-dose group, 1 month after the ovariectomy (OVX) of rat. Their comparisons were to the non-treated, young, OVX, while ours were to non-treated, age-matched adult controls. So, in addition to species and cortical bone-type

differences, these similar results may be due to many factors other than the drug treatment. There are no other osteocyte evaluations of models on long-term BP treatment for comparison, and while we attempted to relate our findings to tissue mechanical properties (section 3.2), others have not done that to date.

3.2 Cortical bone tissue damage

Functioning as one mechanism for energy dissipation, micro-cracks initiate, accumulate and effectively distribute the applied daily cyclic loads such that one single loading event does not cause fracture (Frost, 1960b; Mori et al., 1997; Schaffler et al., 1995; Wenzel et al., 1996). Fatigue-induced micro-cracks compromise mechanical integrity, tend to be restricted to more densely-mineralized older-bone tissue and are capable of signaling the remodeling process (Burr et al., 1998; Carter et al., 1976; Hoshaw et al., 1997; Parfitt, 2002; Pattin et al., 1996; Qiu et al., 2005; Schaffler et al., 1990). To prevent fracture, cracks are removed through the process of bone remodeling (Figure 2). In the same beagle model (section 3.1.1) in which osteocyte lacunae were reduced, increases in the average length (25%) of micro-cracks with BP treatment were evident within cortical bone of the rib after one and three years (Allen et al., 2008b; Mashiba et al., 2000). This aspect of micro-damage, and no other, including the density of cracks (#/mm^2), has been found consistently altered in cortical bone with BP treatment in this model.

Obviously, not all aspects of the human condition are recapitulated in the beagle model and any extrapolations should be made in a cautionary light. However, iliac biopsies of osteoporotic women on BP treatment have demonstrated differences in cortical matrix composition (greater mineral crystal homogeneity) (Donnelly et al., 2012a) and lower toughness, or the amount of energy absorption to fracture (Tjhia et al., 2012). Greater homogeneity at many levels of the hierarchical structure of bone tissue are associated with bone tissue aging, greater damage and lower toughness (Akkus et al., 2003; Zimmermann et al., 2011).

Estimates from simple, mono-tonic, quasi-static bending of whole beagle rib suggest that the increased crack length in alendronate-treated cortical bone is associated with a reduction in tissue-level toughness (Allen et al., 2008b). These and other studies with BP-treated beagles have demonstrated increased stiffness, or initial resistance to a mechanical load, as expected due to the beneficial effects of BPs. However, the BP treatment also decreased toughness, as estimated from whole-bone mechanical testing, more significantly so for the higher doses and longer durations tested (Allen and Burr, 2007; Allen et al., 2006; Komatsubara et al., 2003; Mashiba et al., 2001). More direct, tissue-level tests on machined specimens from bone tissues of BP-treated animals and patients have also suggested lower toughness (Tang et al., 2009; Tjhia et al., 2012). Furthermore, the modulus determined at the calcaneus with ultrasound was significantly lower among women on long-term (>3 years) versus shorter BP therapy and both treatment groups had lower modulus (normalized to density) versus non-treated osteoporotics (Richer et al., 2005).

Since loading conditions *in vivo* are cyclic, we sought to determine the mechanical properties of long-term, BP-treated adult female beagle bone subjected to fatigue (Bajaj et al., 2014). After treatment for 3 years (section 3.1.1), we machined prismatic beams of

rectangular cross-sectional geometry (1.5 mm × 0.5 mm) and 10–12 mm length from rib cortices. The long-axis of osteons was oriented parallel to the beam length. Our cyclically loaded beams demonstrated an increasing dose-response decline in number of loading cycles to failure under 4-point bending. Furthermore, a positive relationship was established between osteocyte lacunar density and the initial elastic modulus (E_i) measured within the first few loading cycles of the fatigue test.

The possible mechanisms accounting for lower osteocyte density affecting material properties includes an impaired detection of damage by osteocytes at the cement line (section 3.2.1) or within the rest of the tissue, and a loss of structural (lacunae and canaliculae) discontinuities in the matrix (Skedros et al., 2011). We hypothesize that degradation in damage detection at the cement line could occur through loss of the canalicular supply chain since the osteocytes near the cement line are the furthest away from a nutrient blood vessel of the Haversian system. The damage regulation role of the osteocyte lacunar-canalicular system has been hypothesized to be due to the structural discontinuities in mineralized tissue that serve a toughening role. Toughness is also attributed to the altered mineralization around the cement line and alternating lamellae of the osteon that provide ductile interfaces to slow crack growth (Burr et al., 1988; Lakes and Saha, 1979; Schaffler et al., 1987; Skedros et al., 2005).

3.2.1 Bone tissue as a viscoelastic, damaging material—Osteons, representing the youngest and least mineralized of the heterogenous bone tissue, provide an attractive sink for cracks that initiate in the interstitial regions oriented toward the cement line (Carter and Hayes, 1976; Schaffler et al., 1989). Differences in tissue modulus are responsible for this property; as the osteons age, and become more mineralized with greater modulus, the preference for crack directionality to cement lines is lost and cracks are repelled into the interstitial space (Lakes and Saha, 1979; Thompson, 1980). This suggests that as the overall age of osteons increases, the toughness of cortical bone tissue is both decreased and the location where cracks might be detected is changed. In addition to lower osteocyte lacunae density, E_i and fatigue cycles to failure found in 3-year, BP-treated beagle rib, we found osteonal cross-sectional area, determined by the vigor of the bone resorption phase of remodeling, to be reduced by approximately 14%. However, this was the case for the high-dose treated group only (Bajaj et al., 2014). A similar finding of reduced depth of BMU resorption cavities within trabecular bone in this beagle model, also only found with high-dose treatment, supports this cortical data (Allen et al., 2010). Therefore, both the numbers of new rib cortical osteons formed over the 3-year treatment period, estimated from the activation frequency of calcein-labeled osteons formed over the last 2 weeks of life to be approximately 60% of the total number of osteons, and the size of those newly formed osteons were reduced significantly with the high-dose alendronate treatment (Allen et al., 2006; Allen et al., 2008b; Bajaj et al., 2014; Mashiba et al., 2000).

Decreases in the resorption width dug by the osteoclasts of BMUs decrease the average osteon area and total cement line perimeter (Figure 4) and fail to decrease or replace the interstitial, oldest bone area, where most micro-cracks initiate and lengthen (Allen et al., 2010; Bajaj et al., 2014; Skedros et al., 2005). If osteon density remains unaffected, a reduced cement line perimeter around each osteon further reduces the tough, energy-

absorbing interfaces available for slowing crack growth and accumulation (Burr et al., 1988; Lakes and Saha, 1979; Schaffler et al., 1987; Skedros et al., 2005). These losses in energy-absorbing capacity may lead to a reduction in material quality. Without increased activation of BMUs, average tissue age increases. Thus, without this cell-facilitated renewal process, old and damaged tissue may persist and the mechanical quality of bone eventually degrades.

Bone matix is a composite of the collagen and non-collagen proteins, minerals, lipids and water. Of these individual components, only water content, which contributes heavily to viscoelasticity of the tissue and the fluid flow that is thought to allow osteocytes to monitor the mechanical health of the bone tissue environment (for review see Fritton and Weinbaum, 2009), has been directly examined in an animal model exposed to a BP. Collagen is primarily responsible for bone's flexibility under both compressive and tensile loading and provides a measure of toughness to resist fracture. As newly deposited organic matrix matures, calcium and phosphate ions infiltrate the collagen framework and develop calcium hydroxyapatite (HAP), plate-like crystals to provide bone with compressive strength and rigidity. Toughness is built in with the discontinuities just discussed, plus those between the matrix components, including AGEs (section 3.2.2). Many of the matrix components can be broken and rebuilt (Zimmermann et al., 2011). Water serves a structural role as demonstrated by examining the differences in mechanical properties between stiff, brittle, dry bone and tough, wet bone (Sasaki and Enyo, 1995). In pre-mineralized osteoid matrix water is bound by a network of acidic proteoglycans with long glycosaminoglycan chains. Water becomes depleted as ossification proceeds.

Utilizing ultra-short echo-time MRI, supported by NMR, µCT, and ashing, Wehrli and colleagues recently found such a decreased water (^{1}H content) and increased phosphorus (^{31}P content as indicator of mineralization) concentration after 6-week alendronate treatment, with doses equivalent to our dosing groups, 1 week after the OVX of rat. The effects were dose-dependent with effect sizes at the greatest dose of -14% (^{1}H) and $+6\%$ (^{31}P) compared to non-treated OVX (Anumula et al., 2010). Again, species and cortical bone-type differences make extrapolation to the human condition difficult, and no reports of this drying effect of a BP have yet appeared in the literature for any other species.

3.2.2 Effects of aging on collagen and mineral—There is also evidence that BP therapy increases AGE presence in bone (Allen et al., 2008a; Saito et al., 2008; Tang et al., 2009). What is not known is whether the effect is primarily due to increased tissue age resulting from suppressed bone turnover or some other factor. Evidence is lacking for an effect on OB collagen fiber production, orientation or initial cross-linking (enzymatic), factors that normally affect mechanical properties (Martin and Boardman, 1993). Fiber orientation is doubly important because HAP crystal alignment appears dependent on the collagen fibril axis and determined during the initial mineral seeding, i.e., orientation does not change during collagen and crystal maturation (Chatterji et al., 1981; Skedros et al., 2006). Proper enzymatic cross-linking provides the framework for new HAP crystal incorporation and growth. While mineral maturation occurs through crystal growth, collagen maturation is the result of enzymatic (short-term) and non-enzymatic cross-links (AGE, Figure 5) (Byers, 1990).

Tissue aging is a likely candidate for increased AGE in BP-treated bone. While enzymatic cross-linking is a tightly controlled process, non-enzymatic cross-linking is a spontaneous event between mature collagen fibers and free sugars, like glucose, known as glycation and resulting in AGE accumulation in tissue. AGE may be enhanced by oxidation, and both oxidative and AGE byproducts typically increase with tissue age (Saito and Marumo, 2010). The presence of these cross-links may be exacerbated by diabetes, likely due to reduced glucose control and glycemia (Dominguez et al., 2005).

Clearly, AGE accumulation in animal models is associated with altered bone strength (increased demineralized tensile stiffness without effect on mineralized compressive stiffness), reduced bone mechanical quality (cortical tissue-level toughness), and increased fracture risk (Gourion-Arsiquaud,et al., 2010; Karim and Vashishth, 2012; Tang et al., 2007; Vashishth, 2009; Vashishth et al., 2001). Again, the limited human data are in support of the research primarily completed in dogs. Across a wide range of alendronate and risedronate doses, Allen and colleagues demonstrated elevated AGE content in trabecular bone (Allen et al., 2008a; Tang et al., 2009). Also reported have been accumulation of AGE following treatment with high-dose incadronate, a non-clinical BP (Saito et al., 2008). Cortical sites remain to be fully evaluated.

Collagen and HAP maturity (crystallinity) demonstrate variability with aging (Akkus et al., 2003; Boskey and Pleshko Camacho, 2007; Pleshko et al., 1991) and certain drug treatments, including BPs (Gourion-Arsiquaud et al., 2010). These are characterized by Fourier transform infra-red (FTIR) and x-ray diffraction microscopy (Figure 6). Tissue composition varies with skeletal site, and an area associated with atypical fracture, the subtrochanteric cortex of the femur, exhibits a 30% narrower crystallinity distribution than other commonly examined locations (Donnelly et al., 2012b). Interestingly, the AFF subset of fractures demonstrated an 8% greater cortical mineral content compared to typical fracture sites. Similarly, mineral content, collagen maturity and crystallinity have been shown to increase with age of the tissue (Akkus et al., 2003; Boskey and Pleshko Camacho, 2007; Pleshko et al., 1991).

4. Atypical fracture risk assessment

As pointed out by the ASBMR task force, there is a need for better imaging techniques to identify atypical fracture risk as early as possible. Over the past decade, advancements in radiologic imaging technologies have led to high-resolution systems promising non-invasive imaging and the ability for "in silico" analysis based on computational mechanics techniques (Seeman and Delmas, 2006). Resolution currently limits use to evaluating bone at a structural level higher than the width (<1 μm) of micro-cracks (Sornay-Rendu et al., 2009). Conventional radiographic x-ray imaging allows characterization of larger cracks; a skilled reading provides for identifying atypical fractures (Shane et al., 2014). However, by definition, this is too late, as fracture avoidance is the objective. Once an AFF is diagnosed, a surgical intervention is often required.

Imaging for biological activity seems more promising than structural imaging techniques for identifying potential underlying problems (Figure 7). Tagging BPs with contrast agents such

as fluorescent probes or radiolabels might be a move toward accomplishing this by providing an estimate for the amount of BP taken up in bone (Coxon et al., 2008; Kozloff et al., 2010; Roelofs et al., 2010; Turek et al., 2012). These agents may also be useful for identifying AFF by bone scintigraphy (Figure 7) that identifies newly exposed mineral surfaces (Einhorn et al., 1986). Other techniques, including MRI, may be useful for determining mineral and water tissue content (section 3.2.1) (Ahovuo et al., 2004; Anumula et al., 2010; Gaeta et al., 2005). Dual-energy x-ray absorptiometry (DEXA) in combination with additional advancements in image analysis techniques is helping community physicians identify abnormalities like cortical beaking or thickening associated with atypical femoral fractures (McKenna et al., 2013). This extension of the DEXA scan, previously used only to identify patients for osteoporosis treatment, has demonstrated an increased automated identification and detection of AFF features. While possibly a more concrete method to identify AFF over conventional x-ray, this method has not yet been proven to alter the course of treatment or outcome. If indeed this thickening, considered a minor feature, is a localized periosteal reaction of the lateral cortex indicative of a symptom (periosteal irritation due to excessive motion at the cortex) rather than a cause for AFF, this finding may not occur early enough to substantially alter the course of treatment (Shane et al., 2014). Indeed, increased cortical thickness is a feature that should lower, not increase, the risk of fracture. Though again, tissue-level mechanical quality must also be considered if normal remodeling is being impeded by a drug or any other cause (section 2.2).

5. Summary and conclusions

While anti-resorptive therapy has proven very effective over the past 20 years at preventing osteoporotic fractures, the risk of side-effects, including atypical fracture, limits their use (Pazianas and Abrahamsen, 2011). The incidence of atypical fracture is quite small, yet rises with length of use (Figure 1) (Dell et al., 2012). Therefore, questions remain regarding the best use of anti-resorptives. While not specifically covered in this survey, many have suggested a drug holiday, use of lower BP doses or switching to an anabolic drug. These seem like logical quick solutions. However, the optimization of holidays off of a drug (time-dose), and actual doses while not on holiday are likely specific to an individual and their age. Anabolics, specifically PTH, tend to increase the frequency of remodeling cycles and therefore may reverse some of the changes related to tissue age; again these may need to be individualized.

Novel assessment tools for specifying drug doses will require a better understanding of the mechanism(s) behind AFF and a way to balance risk with treatment reward. These tools should be based on biomechanical measures of tissue-level quality that assess changes over the duration of treatment to dynamic loads and directly relate to each type of fracture risk, including atypical. Biological assessments related to tissue-level mechanical properties will also be important.

One of the main biological questions remaining is whether osteocyte and/or osteocyte lacunar density are affected by long-term BP use in humans as has been suggested by our data from the alendronate-treated beagle (Bajaj et al., 2014). We have hypothesized that the sparing of osteocytes in the short-term may have long-term implications. As cellular

networks get older due to delayed death of some osteocytes, signaling for replacement is impaired. This loss of signaling would thus result in more lacunae empty of the cellular machinery required for tissue maintenance than would have been the case if normal, on-time apoptosis had occurred. This tissue aging may also increase AGE content, mineralization, collagen and HAP crystal homogeneity, risk of damage in the form of increased micro-crack length and fracture. Additionally, there may be a decrease in cortical tissue water content and toughness.

While the new data for BP-treated cortical bone tissue surveyed here is not definitive for tissue-aging over long-term human use, this mechanism is in line with the differences found thus far in long-term treated animal models. Bone tissue turnover is known to be important to the overall bone health of long-lived animals, supported by a large body of literature, some of which was reviewed here. Certainly, tissue age and other possible causative factors behind AFF should be further investigated so that even more optimal treatments for prevention of fractures may be developed in the future.

Acknowledgments

Abbreviations

AFF	atypical femoral fractures
AGE	advanced glycation end-products
ASBMR	American Society for Bone and Mineral Research
BMU	basic multi-cellular unit
BP	bisphosphonate
Cx43	connexin 43
DEXA	dual energy x-ray absorptiometry
E	elastic modulus
FDA	Food and Drug Administration
FTIR	Fourier transform infra-red
HAP	hydroxyapatite
MRI	magnetic resonance imaging
NMR	nuclear magnetic resonance
OB	osteoblast

OC	osteoclast
ONJ	osteonecrosis of the jaw
OVX	ovariectomy
PET	positron emission tomography
PTH	parathyroid hormone
RANKL	receptor activator of nuclear factor –κB ligand
SAXS	small-angle x-ray scattering
WAXS	wide-angle x-ray scattering
μCT	micro-computed tomography

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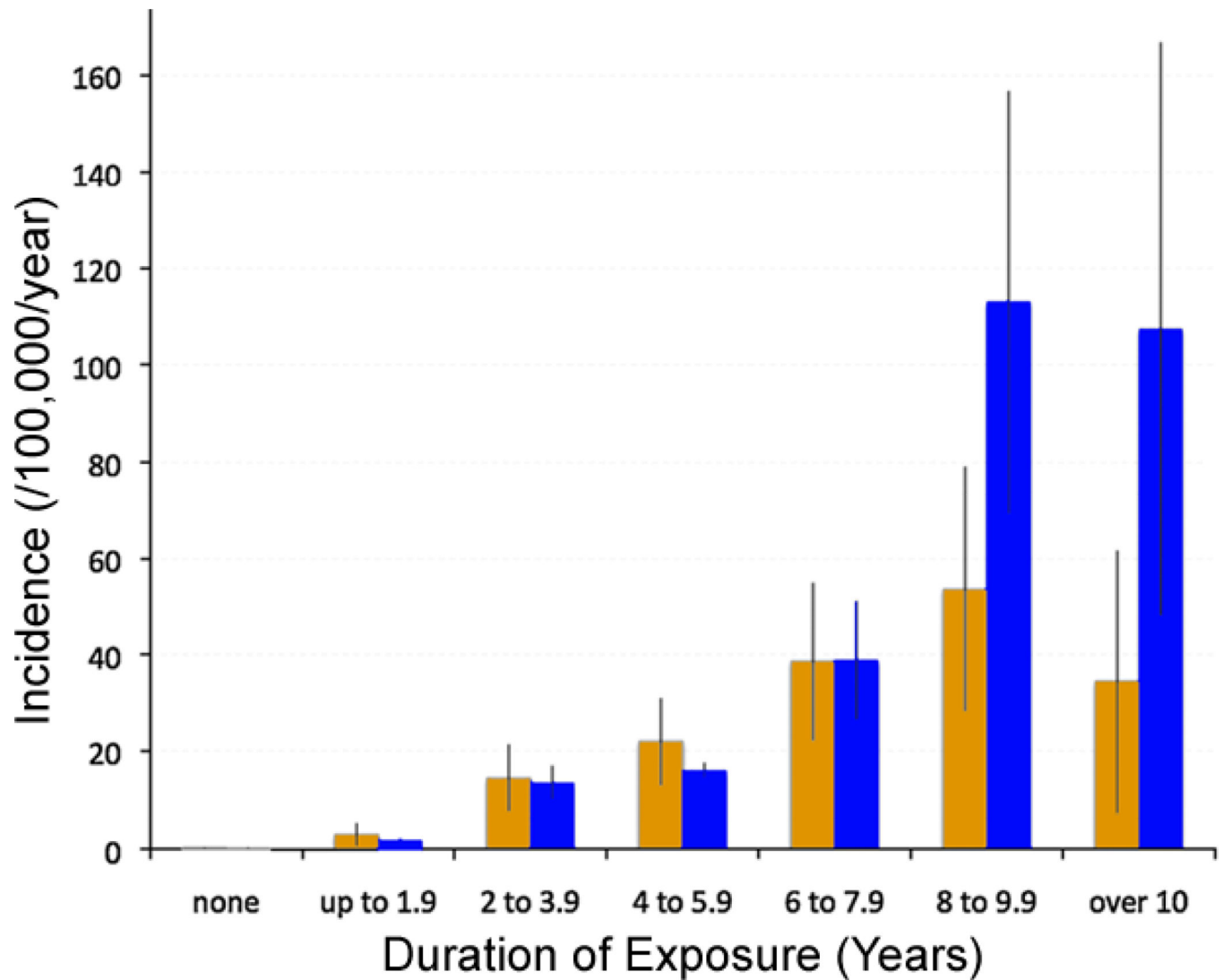
Figure 1.
In the only large, long-term prospective clinical report to date, the incidence of non-traumatic diaphyseal fractures of the femur increased with duration of bisphosphonate (BP) exposure. Reproduced with permission from Dell et al. (2012). Data demonstrate unadjusted (blue) and age-adjusted (yellow) (error bars are 95% confidence intervals) incidence of atypical femur fracture (AFF) in 188,814 patients on BPs for increasing numbers of years (x-axis). The study population was over 45 years old, and approximately half of those sustaining AFF were of Asian ancestry.
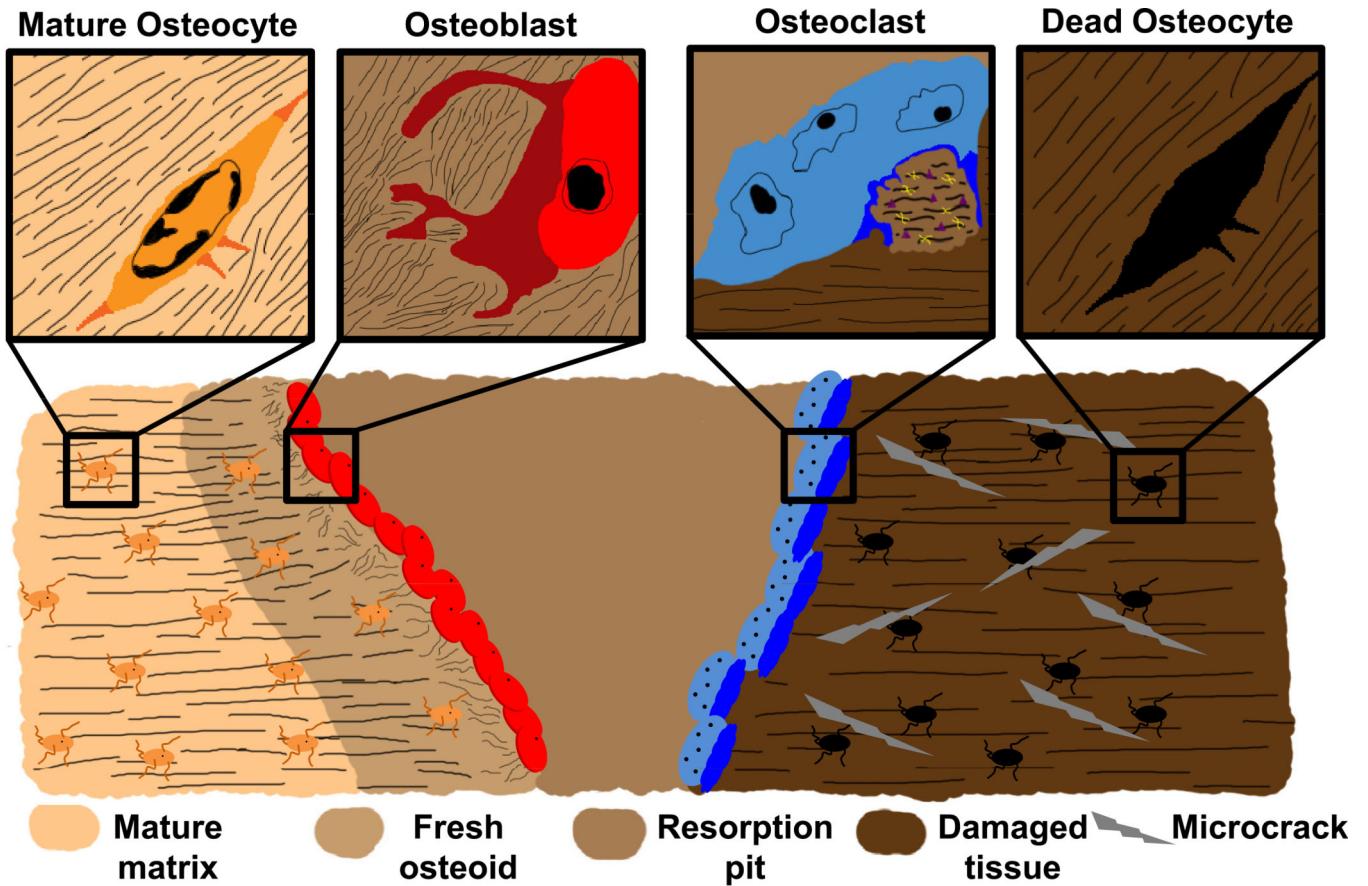
Figure 2.
The primary mechanism of bone tissue replacement in the adult occurs in the basic multi-cellular unit (BMU). The BMUs are in both trabecular and cortical bone (Frost, 1979; Parfitt, 1982). Bone resorption is completed by multi-nucleated osteoclasts (OC) that degrade both inorganic and organic phases, leaving a resorption pit (center). Following closely behind are osteoblasts (OB) that fill the pit by secreting components of the osteoid matrix. Some OBs become entombed within the mineralizing matrix, transition into terminally-differentiated osteocytes and take up residency within the lacunar space of bone.
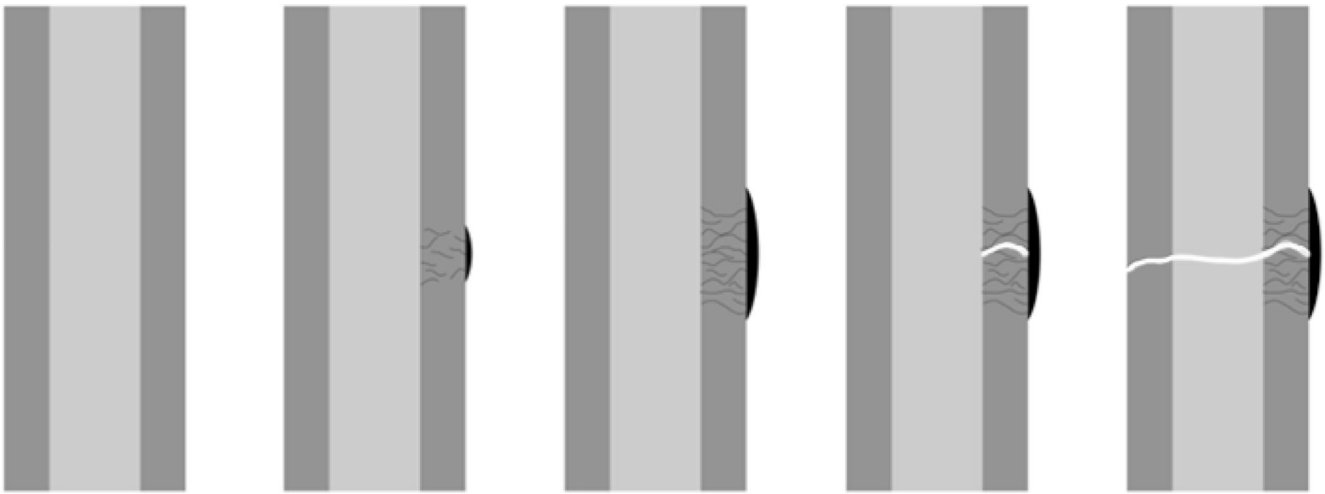
Figure 3.
Model for atypical fracture that presents with symptoms similar to that of an insufficiency-type stress fracture. A full, single cortex, transverse fracture on the tensile (lateral) side of the femur leads to irritation or disruption of the outer periosteal soft-tissue layer of bone. The reaction includes a small callus (black) of rapid bone formation attempting to stabilize the periosteum, and the patient presents with what appears to be an insufficiency-type stress fracture.
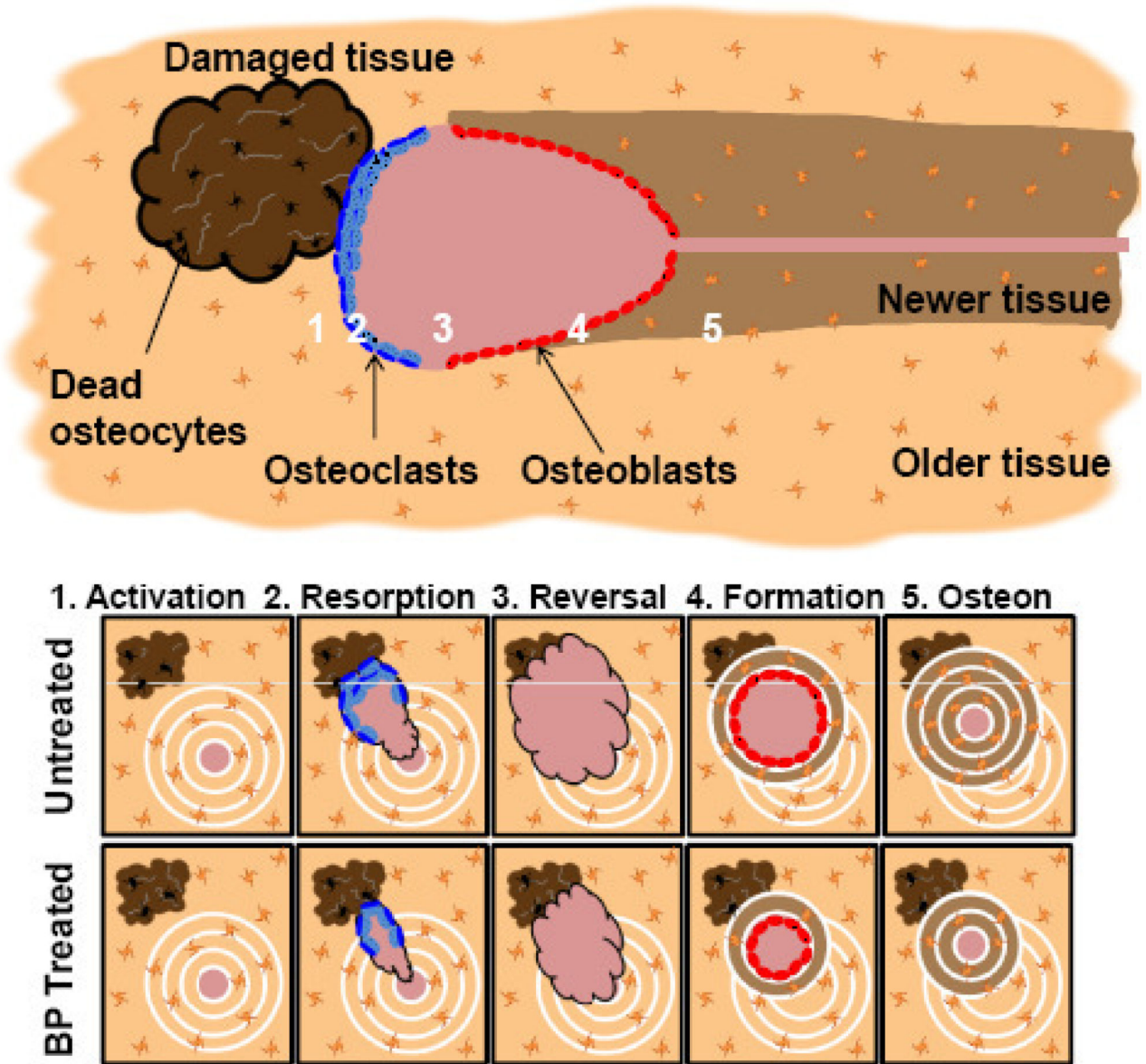
Figure 4.
Osteoclastic tunneling to remodel cortical bone tissue (top), and a model for the observations of decreased osteonal size, osteocyte lacunae density and increased micro-crack length in long-term bisphosphonate (BP) treated cortical bone tissue (bottom). Top: Osteoblasts of the BMU are shown filling in the resorption pit, leaving a central canal for the blood and nerve supply of the Haversian system (Cooper et al., 1966; Petrtyl et al., 1996; Piekarski and Munro, 1977). Bottom: Damage due to "wear and tear" accumulates in young normals, and is removed at a regulated rate that prevents coalescence (Burr, 2002; Parfitt, 2002). Long-term BP treatment may affect bone quality through many BMU-related cortical tissue features.
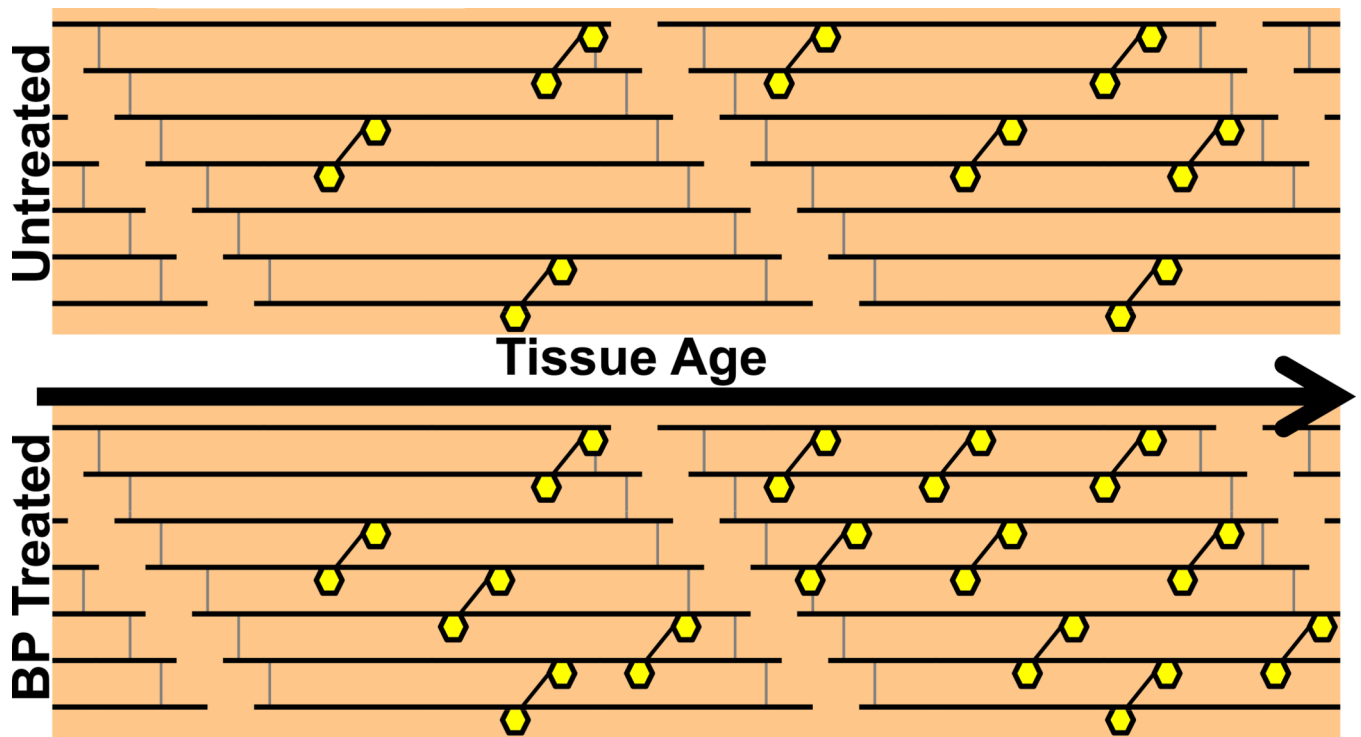
Figure 5.
Mechanical integrity of bone tissue is altered by two mechanisms of cross-linking between collagen fibers: enzymatic (gray lines) and non-enzymatic (yellow hexagons). Enzymatic occurs during collagen deposition and subsequent mineralization (Saito and Marumo, 2010). This process is tightly regulated. As tissue age increases with BP treatment, spontaneous non-enzymatic cross-linking may lead to the formation of advanced glycation end products.
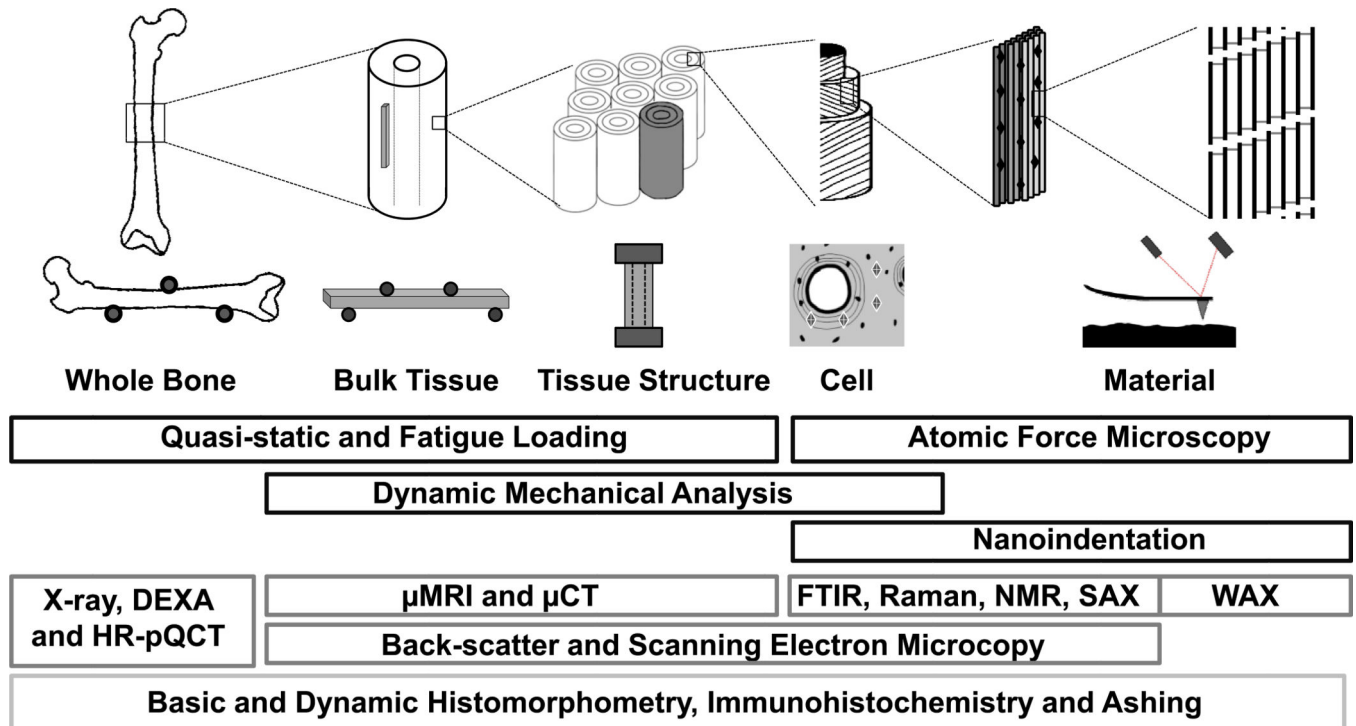
Whole Bone **Bulk Tissue** **Tissue Structure** **Cell** **Material**

Quasi-static and Fatigue Loading	Atomic Force Microscopy

Dynamic Mechanical Analysis

Nanoindentation

X-ray, DEXA and HR-pQCT	μMRI and μCT	FTIR, Raman, NMR, SAX	WAX

Back-scatter and Scanning Electron Microcopy

Basic and Dynamic Histomorphometry, Immunohistochemistry and Ashing

Figure 6.
The hierarchical structure of bone provides many length scales upon which to measure structural and biomechanical properties (after (Currey, 2005; Ruppel et al., 2008)). Each is integral to the overall function of cortical tissue. Quasi-static or fatigue loads are applied to characterize (left-to-right) whole bone, tissue-, cell-, and material-level mechanical properties. Beams or coupons are isolated to evaluate osteonal and interstitial tissue (Bajaj et al., 2014). Osteons may be further isolated for testing (Ascenzi et al., 1997). Microindentation and nanoindentation analyses characterize hardness of the composite material of bone (Anumula et al., 2010). Imaging techniques (reviewed in (Cardoso et al., 2013)), including Fourier transform infra-red (FTIR), Raman, small-angle (SAXS), and wide-angle (WAXS) x-ray diffraction microscopy, are used to characterize sub-micrometer properties.
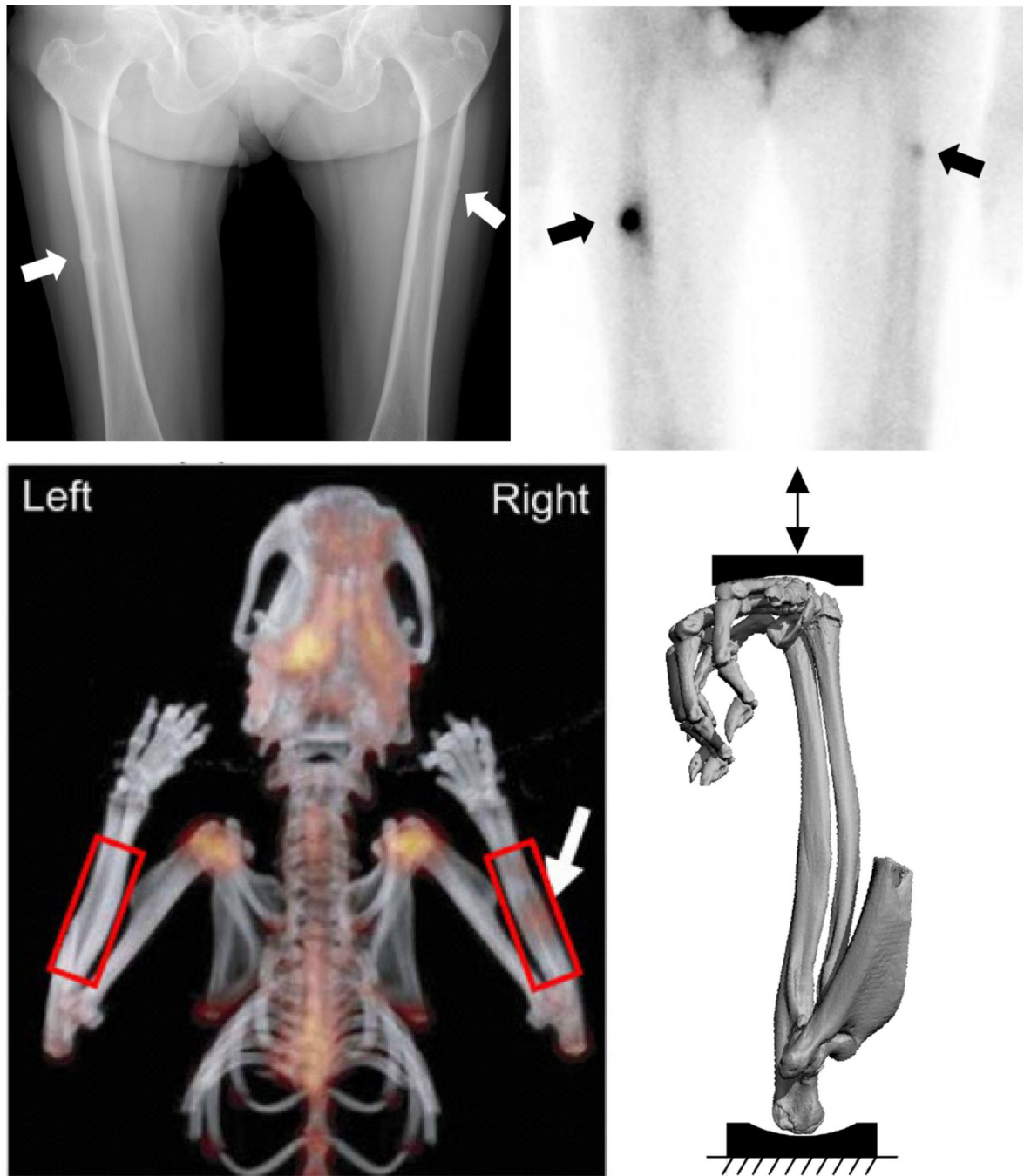
Figure 7.

(top) An x-ray combined with a Technetium-99m scan may allow a physician early identification of an atypical femur fracture (AFF). Reproduced with permission from Jo et al. (2013). The x-ray effectively identifies a radiolucent line indicative of the AFF on left (left most white arrow). However, only the Technetium-99m scan on right delineates an increased and unusual bone formation activity in the contralateral AFF (right most black arrow). (bottom) Co-registered *in vivo* positron emission (PET) and computed-tomography (CT) demonstrates increased uptake of radio-labeled fluoride (^{18}F) due to increased mineral

exposure after external mechanical loading used to create damage and stress fracture in the living rat. Reproduced with permission from Silva et al. (2006); Uthgenannt and Silva (2007).