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Bisphosphonate effects on Bone Turnover, Microdamage, and Mechanical

Properties: What we think we know and what we know that we don't know

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Abstract

The bisphosphonates (BPs) have been useful tools in our understanding of the role that bone remodeling plays in skeletal health. The purpose of this paper is to outline what we know, and what is still unknown, about the role that BPs play in modulating bone turnover, how this affects microdamage accumulation, and ultimately what the effects of these changes elicited by BPs are to the structural and the material biomechanical properties of the skeleton. We know that BPs suppress remodeling site-specifically, probably do not have a direct effect on formation, and that the individual BPs vary with respect to speed of onset, duration of effect and magnitude of suppression. However, we do not know if these differences are meaningful in a clinical sense, how much remodeling is sufficient, the optimal duration of treatment, or how long it takes to restore remodeling to pre-treatment levels. We also know that suppression is intimately tied to microdamage accumulation, which is also site-specific, that BPs impair targeted repair of damage, and that they can reduce the energy absorption capacity of bone at the tissue level. However, the BPs are clearly effective at preventing fracture, and generally increase bone mineral density and whole bone strength, so we do not know whether these changes in damage accumulation and repair, or the mechanical effects at the tissue level, are clinically meaningful. The mechanical effects of BPs to the fatigue life of bone, or BP effects on bone subject to an impact, are entirely unknown. This paper reviews the literature on these topics, and identifies gaps in knowledge that can be addressed with further research.

Introduction

The effect of bisphosphonates (BPs) to reduce bone remodeling has been appreciated since the early days of their discovery. Implicitly, reducing bone loss should result in reduced fracture risk.   But suppression of remodeling alters material properties of bone and allows microdamage accumulation, changes that interact in complex ways to affect biomechanical integrity of the skeleton. Over the past 40 years, a number of reviews have detailed the effects of BPs on various tissue-level aspects of bone. The goal of this review is not to regurgitate what already can be found in these works but rather to briefly highlight key points regarding what is known about how BPs affect bone turnover, microdamage accumulation, and mechanical properties and then discuss where gaps in knowledge exist in each of these areas.   We hope outlining some of these knowledge gaps will continue to stimulate work on BPs with the goal of optimizing BP treatment, and skeletal health in general, in the years to come.

BPs and Bone Turnover – what we know (Table 1)

In the adult skeleton, the majority of osteoblast and osteoclast activity involves coupled remodeling in which the process of bone resorption and formation are linked in space and time. The negative bone balance associated with each remodeling unit (more bone is resorbed than is formed) combined with the accelerated number of remodeling units both contribute to bone loss in postmenopausal osteoporosis. To a lesser extent the adult skeleton also undergoes modeling – where either formation or resorption occur, without the other, at a given site and time. The most prominent site of modeling in the adult is on the periosteal bone surface. Here formation modeling serves to expand the outer diameter of the bone in an attempt to biomechanically offset the loss of bone adjacent to marrow that occurs with aging and estrogen deficiency.

The most prominent and most clearly understood action of BPs is to suppress the initiation of osteoclast activity [1, 2]. By suppressing osteoclast activity, the number of active bone remodeling sites is reduced although BPs have also been shown to significantly reduce the size of the resorption cavities that form [3] **(Fig 1)**. Reductions in the number of active remodeling sites, routinely assessed histologically as the activation frequency, or reflected by reduced bone formation rate, are a consistent finding in both pre-clinical and clinical studies. This suppression of remodeling significantly reduces the loss of bone that occurs through remodeling. Early in treatment, the rapid reduction in newly initiated remodeling sites combined with the re-filling of pre-existing remodeling sites, is responsible for the early increase in bone mass that is routinely observed with BP treatment. With continued treatment, it is both fewer and smaller remodeling sites that reduce bone loss compared to untreated individuals, and maintain mass and architecture.

The effects on bone remodeling are generally similar among the BPs, yet there are differences that are thought to be directly related to the differences in binding affinity [4, 5] and potency on the farnesyl pyrophosphate synthase enzyme in osteoclasts [6, 7] along with the differences in dose and route of administration. The magnitude of remodeling suppression has been shown to differ in several separate head-to-head studies using serum and urine biomarkers [8-10]. These studies consistently show alendronate suppresses remodeling more than risedronate [8, 9] while zoledronate suppresses more than risedronate [10]. No head-to-head assessments of bone remodeling among the BPs has been conducted using histology as an outcome, yet based on iliac crest biopsy studies from the major clinical trials the percent suppression of remodeling relative to placebo-treated controls tends to be quite similar over 3 years with daily risedronate (-40%) [11], daily alendronate (-92%) [12], intermittent oral ibandronate (-50%) [13], and zoledronate (-63%) [14].

Preclinical models have shown us that the effect of BPs on bone turnover is highly site-specific. Histological analyses of remodeling show that values can differ by an order of magnitude among several bone sites. Treatment of beagle dogs for 6 months with oral alendronate at doses used to treat osteoporosis reduced initiation of new remodeling sites by 15% in the mandible, but 46% in the rib, while having no effect at all in the tibial cortex [15]. Treatment with ibandronate in ovariectomized non-human primates produced a 79% reduction in vertebral bone remodeling, a 56% reduction at the iliac crest and only a 25% reduction at the proximal femur [16]. This site-specific variability in remodeling suppression has two important implications. First, it means that assessment of bone remodeling using systemic markers will over- or underestimate remodeling activity at certain sites. It also means that caution must be used when interpreting data from iliac crest biopsies in humans – the primary site on which we have histological data concerning how BPs affect bone turnover.

In addition to site-specific effects, there is also a time-dependent effect on turnover suppression. These time-dependent effects appear greatest in cortical bone, which takes longer to experience significant remodeling suppression compared to trabecular bone. While clinical doses of alendronate minimally affect intracortical remodeling in the mandible of beagle dogs after 6 months of treatment [15], treatment for three years at these same doses reduced remodeling by 67% compared to vehicle-treated controls [17]. A similar time-dependent suppression exists in the cortex of the rib where clinical doses of alendronate had no effect after one year but reduced remodeling by more than 85% after three years of treatment [18, 19]. After 1 year of treatment, vertebral and femoral neck trabecular bone remodeling is suppressed by ~70% and is not further suppressed with continued treatment at least out to three years.

While the overall reduction in turnover is generally similar among the BPs, the kinetics of these effects – the speed of suppression following treatment initiation and the recovery of turnover following treatment withdrawal - are where the most apparent differences exist.

Risedronate produces a significantly more robust reduction in vertebral trabecular bone turnover following treatment initiation than does alendronate [20] (**Fig 2**). These differences are consistent with clinical data from separate studies suggesting that risedronate tends to reduce fracture risk more quickly than alendronate [1, 21]. There have been no head-to-head trials to directly compare fracture risk reductions among BPs.

Far more data exist regarding the recovery of turnover following treatment withdrawal. Discontinuing BP therapy results in turnover recovery although the dynamics of the recovery profiles appears to differ among the BPs and depends on the dose and duration of treatment [22-25]. Women treated with daily alendronate for 2 years still had a reduction in markers of bone resorption and formation of 10-15% seven years after discontinuation of the therapy [26]. In women treated for 5 years with alendronate, and then withdrawn from treatment for 5 years, biomarkers of bone remodeling were higher than those of patients who continued treatment, yet also remained significantly lower compared to baseline [27]. After a single dose of intravenous zoledronate, remodeling biomarkers are significantly suppressed compared to baseline for at least three years [28, 29]. Recovery of remodeling following cessation of BP may occur more quickly in cancer patients although the kinetics appear dependent on the duration of treatment prior to withdrawal [30]. In pre-clinical studies, the recovery of remodeling after treatment withdrawal is time, dose, and drug dependent [31-33]. In ovariectomized rats treated with either alendronate or risedronate for 8 weeks and then withdrawn for up to 16 weeks, trabecular bone formation rate returned to levels comparable to controls in risedronate, but not alendronate treated animals [32] (**Fig 2**).

Effects of BPs on bone modeling are less studied compared to remodeling yet the majority of evidence suggests there is no effect on formation-based modeling. The most prominent site used to study bone modeling is the periosteal surface, where the majority of activity is formation modeling. Clinical data describing bone formation activity on periosteal

surfaces show no effect of BPs on the periosteal surface of iliac crest biopsies – although the low amount of activity on the iliac crest periosteal surface produces a fair amount of variability among patients [34, 35]. Pre-clinical studies in larger animals (dogs and mini pigs) consistently show absence of a BP effect on periosteal bone formation [18, 19, 36-39]. More detailed studies have been conducted in rats and mice, although in these species conflicting results exist. Following ovariectomy, which produces a robust increase in periosteal bone formation, BP treatment (alendronate, risedronate, or zoledronate) does not alter periosteal expansion compared to vehicle [40]. Furthermore, when animals were stimulated with mechanical loading, another stimulus for periosteal bone formation, there again was no difference in BP-treated animals compared to vehicle-treatment [40]. A number of other rodent studies have also shown no effect of BPs on periosteal activity [32, 41]. Some studies in rats and mice, however, have produced contrary results – showing suppression of mineral apposition rate on periosteal surfaces with BP treatment and suggesting a direct effect on the individual activity of osteoblasts [42-44].

BPs and Bone Turnover – what we don't know (Table 1)

With the goal of reducing fracture risk to the greatest degree, the push within the field has generally been to reduce bone turnover to the greatest degree possible. Yet it remains unclear whether the basic premise of more turnover suppression equals greater fracture risk reduction is correct. In fact, it seems rather clear that it is not. Raloxifene, a selective estrogen receptor modulator, suppresses bone remodeling by only 20-40%, yet achieves nearly the same fracture risk reduction in the vertebra as do the BPs [45]. Determining how much suppression is enough to significantly reduce fracture risk is important given the growing number of suggested side effects, such as osteonecrosis of the jaw and atypical femoral fractures, postulated to result from significant suppression of remodeling. It's not clear whether either of these effects are

directly tied to the magnitude of turnover suppression, or if they could be eliminated if bone turnover suppression were less.

Pre-clinical studies using histology as an end-point have provided convincing evidence that turnover suppression with BPs is site-specific yet little information is available as to the mechanism for these differences. It is possible that the drug distributes to sites differently and that the physiological effects at a given site are related to the amount of BP that accumulates. Gaining a greater understanding of the distribution of BPs throughout the skeleton could help determine whether fracture efficacy is tied to the ability of the drug to reach certain sites.

Higher rates of basal turnover result in greater increases of BMD [46] and greater reductions in fracture risk [47], but it is not clear how baseline turnover dictates the degree of remodeling suppression for BPs. This could have important clinical implications as patients with modest increases in turnover may necessitate a different BP-treatment regimen compared to those with higher turnover prior to treatment. Pre-clinical studies suggest that trabecular sites with high and low turnover eventually plateau at similar remodeling rates [48]. The same may not be true for cortical bone as sites with very high remodeling, such as the alveolar bone of the mandible, experience near complete suppression of intracortical remodeling while sites with relatively low turnover such as the tibia midshaft, retain measurable amounts of turnover [15]. Alternatively these differences between trabecular and cortical bone could be due to site-specific differences, rather than cortical versus trabecular bone differences.

There is an increasing interest in utilizing BP drug holidays. Although some data exist on recovery of remodeling following treatment withdrawal, the kinetics of this recovery differ among the BPs and depends, in unknown ways, on both the dose and duration of treatment. The rate of remodeling prior to treatment also likely affects recovery following treatment withdrawal [30, 49]. The need to understand how remodeling recovers during a drug holiday is

important as it will likely serve as the main tool, along with BMD, to assess when patients should resume treatment. Drug holidays are most commonly advocated for individuals who necessitate oral surgery, where it has been proposed that cessation of BP-treatment for 3 months could allow recovery of remodeling and thus reduce the risk of osteonecrosis of the jaw. It is unlikely that simply stopping BP-treatment would allow any significant recovery of remodeling over this short timeframe although combining a drug holiday with an anabolic stimulus may accelerate the return to more active turnover. Although we know that pre-treatment with a BP affects subsequent response to parathyroid hormone, it is not clear whether recombinant human [PTH-1-34] (teriparatide), or other emerging anabolic agents such as anti-sclerostin antibodies, could accelerate the return to pre-treatment remodeling rates during a period of BP withdrawal.

BPs and Microdamage – what we know (Table 2)

It is now well established in animal models that the suppression of remodeling allows microdamage accumulation [36, 50-52], and that this is not necessarily a BP-specific effect [53]. Any suppression of remodeling will prevent the repair of naturally occurring damage to bone, and will allow it to accumulate. Even a 40% suppression of remodeling with a half-dose of risedronate caused a 3-fold increase in microdamage accumulation [54], and a 20% suppression with a non-BP, raloxifene, doubled the microdamage burden in vertebral bone [53]. The relation between remodeling suppression and damage accumulation in bone has been shown repeatedly in pre-clinical animal models, at different doses and over different periods of time. Although initial studies [36, 37, 52, 55] used high doses to achieve this effect, later studies using clinically-relevant doses [54] also show that suppression of remodeling even by small amounts will allow an accumulation of microdamage. The amount of damage that accumulates appears to be related in a nonlinear fashion to the severity of suppression (**Fig 3**) [56], so the BPs may allow damage to accumulate at a greater rate or to a greater extent than do less potent agents.

Even with prolonged suppression of remodeling, though, microdamage is self-limiting and does not continue to accumulate to any significant extent [57]. Three years treatment even with doses of alendronate 5x higher than clinical doses did not increase the microdamage burden in the L3 vertebra of dogs compared either to 1 yr of treatment at similar doses, or to age-matched untreated dogs. This suggests that any deleterious effect that microdamage accumulation may have on the mechanical properties of bone will be achieved early, and that continued deterioration of the properties of bone with prolonged treatment using BPs is likely not associated with the burden of microdamage in the tissue [51].

The amount of microdamage that accumulates at different skeletal sites varies, and is greater in cancellous bone than in cortical bone (**Table 3**). The only data on site-specificity that exist are derived from an intact canine model treated at various doses and durations of time, with different BPs. These data show the primary site of accumulation is in the spine, mainly in the lumbar spine. The microdamage burden in lumbar vertebrae, even using clinical doses of BPs given for only one year, is twice that from the iliac crest [58]. This observation suggests that estimate of microdamage accumulation in the spine based on the analysis of iliac crest biopsies may underestimate the true microdamage burden in the vertebrae. However, it is possible to estimate the microdamage burden in the spine based on measurements from the iliac crest using previously derived equations [58].

Microdamage is a well-known stimulus for the activation of new remodeling [59, 60], mediated through the apoptosis of ostectyes in the vicinity of the damage [61, 62]. The general suppression of remodeling certainly prevents the repair of some of this damage, but studies have also shown a more specific suppression of targeted remodeling by BPs [63]. Typically when bone is loaded to the point of creating microcracks, the probability of finding a microcrack with the resorbing bone remodeling unit (BMU) or an adjacent BMU is 3-4 times higher than one would expect if remodeling were completely random [63, 64]. However, in bone from BP

treated animals, the probability of finding cracks in association with resorption spaces is less than one. This indicates not only that cracks are not being repaired by random remodeling, but that there is a selective suppression of remodeling targeted specifically to microcracks. This allows microcracks to accumulate in bone at an even faster rate than if they were repaired incidentally as a result of stochastic processes. Thus the accumulation of microdamage in BP-treated bone does not occur just because of a generalized suppression of remodeling, but also occurs through a disruption of the normal signaling processes that would originate new remodeling sites.   The likely reason for this suppression of targeted remodeling is the well-known BP effect that prevents the apoptosis of osteocytes [65, 66].   Because it is nearly certain now that osteocyte apoptosis is the critical signaling step to initiate new targeted remodeling events [61], any delay or prevention of this apoptosis will have the singular effect of suppressing targeted remodeling.   This suggests that the suppression of bone remodeling by the BPs occurs not just through an interruption in the mevalonate pathway that prevents activation of fully differentiated osteoclasts, but also through a disruption of cell-level signaling pathways that impairs recruitment of new pre-osteoclasts to a site in need of repair.

BPs and Microdamage – what we don't know (Table 2)

It is still an unresolved question whether microdamage accumulates in postmenopausal women who have taken BPs. Stepan et al. [67], using human transilial biopsies, showed in a subsample that microcrack accumulation occurs in women treated for an average of 5 years with alendronate, but the study is inconclusive because the analysis of biopsies from the two different research centers associated with the study differed.   However, Stepan et al. were able to show definitively that microcrack accumulation is associated with age, low BMD, and prevalent fractures. Others, however, have not found an association between BP treatment and damage accumulation in the iliac crest [68].   However, this study used an aged, incompletely characterized control sample from the dissecting room.   Subject controls were neither age-

matched, nor was their clinical or pharmacologic history known. Moreover, the study was underpowered. Microdamage studies using iliac crest biopsies are inherently difficult to perform because damage is created during the process of coring the biopsy. Therefore, for the time being, we must conclude that the issue of whether microdamage accumulates in human bone following a period of treatment with BPs is unresolved.

One important question is whether the microdamage that is observed to accumulate in bone with BP treatment is only the result of the suppression of remodeling, or whether BPs alter the properties of the bone in ways that increase the likelihood that microcracks will initiate. BPs allow the formation and accumulation of advanced glycation end-products (AGEs) [69]. The increase in AGE's after one year of treatment in dogs using clinical doses of alendronate or risedronate ranges from 37-50%. It is well known that AGE accumulation causes embrittlement of bone [70, 71], and any property that reduces the bone's ability to absorb energy will increase the probability for the initiation of microcracks. It is not clear whether the large increase in AGEs found in animal models following one year of administration of BPs occurs only in the small fraction of bone that is replaced each year, or within the 98% of pre-existing bone matrix that is not replaced. If it is the latter, this suggests that BPs have a direct effect on the non-enzymatic cross-linking of collagen, through some unknown mechanism.

To address the question of increased crack initiation, vertebrae from animals treated with various doses of alendronate, and subsequently loaded in cyclic fatigue for 100,000 cycles, demonstrated 2.4-3.1 fold more microdamage than control animals treated with saline. Animals treated with dose-equivalents of risedronate generated significantly fewer microcracks than alendronate-treated animals, more equivalent to vehicle-treated controls [72]. The increased damage formation in alendronate treated bone occurred despite the fact that bone from the BP treated groups was loaded at lower maximum stress compared to controls because of their significantly higher bone volume. More recently, a study used finite element analysis

correlated to microdamage morphology to calculate von Mises stresses in trabecular bone from the distal femur of dogs treated with alendronate for three years, and compared them to untreated dogs. This study showed that one year of treatment with alendronate decreased the stress required for microcrack initiation and increased the probability that microcracks would form [73]. These separate analyses suggest that the threshold for the initiation of new cracks in bone is reduced following a period of BP treatment, at least with alendronate. If there is an increased susceptibility to cancellous bone damage with BP-treatment, the mechanism is not clear. One possibility is that aspects of the tissue matrix (increased mineralization, AGE accumulation, lamellar organization) make the trabecular struts more brittle and susceptible to crack initiation. Another possibility is that microarchitectural rearrangements increase the probability of finding microcracks in thicker trabeculae [74]. At this point, whether there is increased susceptibility to crack formation, the balance between increased crack initiation and decreased crack repair to the eventual microdamage burden, and a mechanism for increased susceptibility to crack formation if it occurs, are all unknowns and require further investigation.

Quasi-static mechanical tests using bone from dogs treated for 1-3 years with BPs show a decrease in material toughness, but typically do not demonstrate significant changes in elastic modulus, or whole bone stiffness (see following section). It is interesting, and perhaps instructive, that a 3-4 fold increase in total microdamage does not alter elastic modus, given that the engineering definition of damage within a structure is defined by a change in modulus. The weak relationship between damage and reduced modulus in BP treated bone is difficult to explain, unless even a several-fold increase in damage is insufficient to sustain a measurable loss of stiffness. Biomechanical tests of non-BP treated bone show that fatigue reduces the residual strength of the canine femur [75], and decreases the overall fatigue life of bone, as microcrack formation reduces the sites within the bone that can dissipate energy and delay the

fracture [76]. Surprisingly, we do not yet know whether microdamage accumulation to levels found in BP treated bone is clinically important, or not.

BPs and Biomechanical Properties – what we know (Table 4)

The efficacy of BPs on reducing fracture risk is clear and relatively consistent [1, 77]. New vertebral fractures are significantly reduced over three years with alendronate, risedronate, ibandronate, and zoledronate relative to placebo-treated controls. Non-vertebral fracture risk reduction is also significantly lower with all four BPs over the first three years. When hip fractures are assessed independent of other non-vertebral sites, only alendronate, risedronate, and zoledronate show significant efficacy in risk reduction. The longest duration of follow-up in any of these studies show continued fracture risk reduction after 7 years of risedronate treatment and 10 years of alendronate treatment. As has been outlined in detail elsewhere [46], issues with patient compliance, follow-up, and ethical considerations that disallow continued placebo-treated controls once efficacy is established in these studies complicates the interpretation of these long-term data. It is important to note that no head-to-head trials among the different BPs have been conducted, nor are they likely to ever occur, meaning it is unlikely we will ever have true comparative data on fracture efficacy among the various BPs.

Reductions in fracture risk in BP-treated patients are assumed to be the result of improved or maintained mechanical properties. No assessments have ever been made showing bone from patients treated with BPs has better biomechanical properties compared to untreated controls. The only human bone strength data consist of estimates of bone strength using finite element models based on QCT measures. These studies show that vertebral compressive strength estimates are significantly greater after 3 [78] and 6 months of treatment with alendronate [79] compared to placebo-treated patients. Similar results have been shown

for the femur following 24 months of BP treatment [80] and at both sites after 12 months of ibandronate [81].

More direct data on the biomechanical effects of BPs come from animal studies. Presentation of biomechanical data is not standardized and therefore it's important to pay attention to what is being discussed in the different studies. Whole bone mechanical tests, such as compression of vertebrae or bending of long bones, provide a global assessment of a bones' biomechanical integrity in for form of strength, stiffness, and energy to failure. These tests do not control for differences in bone geometry/architecture or BMD; larger bones would be expected to have enhanced structural properties. Using established methods its possible to normalize bone geometry/architecture and calculate material-level biomechanical properties from structural tests [82]. These material-level biomechanical data provide information on the strength, stiffness, and energy absorption of the tissue independent of how much material is present. Alternative methods for directly assessing material-level biomechanical properties, including machining specimens to produce similar bone geometries and nanoindentation, have also been used in BP studies.

Over the last 20 years, we and others have studied the biomechanical changes caused by BP-treatment using an intact beagle dog model. Following one year of treatment with clinical or high doses of alendronate or risedronate, ultimate load [52] and stiffness [54] were significantly higher than controls. Following 3 years, clinical doses do not produce differences in structural strength, stiffness, or energy absorption compared to controls [57] while high doses of incadronate result in significantly higher ultimate load [55]. Whole bone biomechanical tests of the rib - a predominantly cortical bone site –show no difference in ultimate load, stiffness, or energy absorption following one year of treatment with either alendronate or risedronate at clinical (data unpublished) or high doses [36]. Three years of incadronate treatment (both low and high doses) produce higher ultimate load and stiffness compared to controls while the same

duration of alendronate treatment, at either clinical or high doses, produces no effect on any of the structural biomechanical properties [19, 37].

The changes in structural biomechanical properties that occur with BPs are almost completely explained by increases in bone density/mass. Following treatment with clinical doses of BPs for 1-3 years, the relationship between vertebral ultimate load and areal BMD is nearly identical in animals treated with BP and controls [57, 83] (**Fig 4**). That is, the increased compressive strength in BP-treated animals is entirely accounted for by the higher bone density, and at a given bone density, BP and control animals have similar bone strength. A similar conclusion was drawn from the human FEM studies of BP-treated patients, where improvements in estimated vertebral strength were directly tied to improvements in BMD [79, 80]. These findings are consistent with clinical data demonstrating that those patients who experience the greatest increase in BMD with BP treatment have the greatest reduction in fracture risk [46]. This relationship between BMD and bone strength may have important clinical implications as those individuals that do not experience large increases in BMD may have reduced fracture protection.

Structural biomechanical properties are determined not only by the amount (and density) of bone but also the properties of the material. Calculation of material properties from structural tests have shown that treatment for up to three years with either clinical or high doses of BPs does not affect either ultimate stress or the modulus of vertebrae [36, 37, 54, 57]. More direct material-level tests on isolated trabecular cores show equivocal effects on modulus and stress [84, 85] while nanoindentation data show 1 year of clinical dose treatment significantly increases tissue hardness and modulus of trabecular bone [86]. Although the strength and modulus of the material appear to be relatively unaffected by BPs, toughness, the ability of the material to absorb energy, is consistently reduced, and declines progressively with longer treatment duration. After 1 year of treatment with either high or clinical doses of BPs, vertebral toughness

is 15-20% lower than controls, although this was not statistically significant in two separate studies [52, 54]. Following three years of either incadronate or alendronate treatment (both clinical and high doses) toughness was significantly lower than controls (-27-40%) [55, 57].

Similar to trabecular bone, 1 year of high dose BP does not affect ultimate stress or modulus, but significantly reduces toughness in the rib [36]. Following three years of alendronate treatment, significant reductions in toughness of the rib were noted with high dose alendronate (-33%) but not with the clinical dose (-19%) compared to controls [19]. These changes were almost completely due to reductions in post-yield toughness; Komatsubara et al. reported no change in rib toughness following three years of incadronate treatment but this measurement excluded the post-yield portion of the stress strain curve [37]. Neither of the two three year studies showed an effect of BP-treatment on rib ultimate stress or modulus [19, 37]. Direct assessment of material-level properties on cortical bone beams machined from the long bones have produced conflicting results. Following either one or three years of treatment with clinical or high doses of alendronate, four point bending of femoral shaft beams revealed no difference in stress, modulus, or toughness compared to controls [87]. Conversely, three-point bending of cortical beams from the tibia of animals treated for 1 year with high dose alendronate or risedronate resulted in lower post-yield toughness, but no change in stress or modulus [88]. The lower basal remodeling rate in long bones (1-2%/year) compared to the rib (~15-20%/year) is the likely explanation for the less dramatic effects in the long bones.

One limitation of the beagle dog model is that it is not estrogen deficient and thus does not have either the accelerated bone remodeling or the loss of bone mass that occurs in post-menopausal women. Two studies in non-human primates have investigated mechanical property changes in ovariectomized animals. Compared to untreated ovariectomized controls, treatment with high doses of alendronate (0.25 mg/kg IV every 2 weeks) significantly increased ultimate stress of isolated lumbar vertebral cores; lower doses (0.05 mg/kg IV every 2 weeks)

did not have any effect [89]. There was no effect of either dose on femoral midshaft ultimate load or femoral neck ultimate load or stiffness. In a separate study, sixteen months of ibandronate resulted in higher vertebral ultimate load, stiffness, stress and modulus in only the highest dose group (150 ug/kg IV injection every 30 days) compared to ovariectomized controls; the more clinically relevant dose had no effect on any of these parameters [16, 90]. Unfortunately, neither of these studies in ovariectomized animals reported toughness parameters and it therefore remains unclear whether the changes in toughness noted in the beagle dog studies occur in a high-turnover, low bone mass model.

A search of PubMed for studies in rats/mice treated with BPs that report biomechanical data reveals nearly 100 references. While a comprehensive review of these studies is beyond the scope of this review, in general most studies document that BPs increase structural biomechanical properties – ultimate load and stiffness - of both vertebra and the long bones. Unfortunately, the majority of these studies do not report material properties. Assessing toughness in rodent studies would provide data that could be compared to larger animal studies – where changes in toughness have been one of the few consistent changes due to BPs. Recently, an exquisite comprehensive evaluation of biomechanical properties was conducted in BP-treated aged rats [91] – yet even this detailed report does not provide calculations of toughness consistent with those previously reported. The strength of these rodent studies is they provide data on how BPs alter biomechanical properties in an estrogen-deficient model. One limitation of rodents, however, is that the absence of secondary Haversian systems makes assessment of BP effects on cortical bone of little translational value to humans.

BPs and Biomechanical Properties – what we don't know (Table 4)

In life, bone is loaded cyclically and not quasi-statically, and the properties of the bone in cyclic loading, and its residual fatigue life with additional cycles of loading, may not be estimated

very accurately from quasi-static tests of structural or material biomechanical properties. Cyclic fatigue tests of bone treated with BPs have not been performed to determine whether the reduced toughness measured quasi-statically translates into reduced residual strength and shorter fatigue life of the bone under more physiologically relevant cyclic loading conditions. From a clinical standpoint, cyclic fatigue studies of BP treated bone are critical to interpreting fracture risk.

In addition to cyclic fatigue tests, assessment of impact properties of BP-treated bone could be informative. The high strain rates imparted during a fall are not mimicked under the relatively slow quasi-static mechanical tests routinely used in the laboratory. Increasing strain rate can reveal more brittle behavior of bone, and differences in toughness could be magnified if tested at strain rates above 1 mm s^{-1} [92]. At lower strain rates cracks can initiate and propagate to dissipate energy, while at higher rates it is more difficult to stop crack growth [93]. Since BP-treated bones have higher amounts of microdamage to begin with, as well as tissue properties that are more conducive to forming cracks, the biomechanical response of the tissue at high strain rates could be considerably more dramatic than those previously described by quasi-static tests.

While changes in toughness have been consistently documented, the cause of these changes remains elusive. Studies have not been able to demonstrate any relationship between reduced toughness in BP-treated bone and changes in microdamage, mineralization, or collagen cross-linking [51]. Determining the cause of reduced toughness is becoming particularly relevant in light of the recent increase in the incidence of atypical subtrochanteric femoral fractures - which have features consistent with a bone having reduced toughness. It will also be important to determine whether changes in toughness are occurring in human bone although this will necessitate either a non-invasive method to assess bone toughness or studies on cadavers in which the treatment with BP can be confirmed.

Structural properties are the product of both the amount and the quality of the material. While BPs increase the amount of bone, the biomechanical quality (toughness) of the tissue is reduced. Although the cause of the reduced toughness is not known, it seems plausible that if these changes to the material could be reduced, the overall positive effect on the structural properties would be enhanced. Raloxifene has been shown to significantly enhance the material properties of bone, including toughness, through mechanisms other than reductions in remodeling. This suggests that co-treatment with BPs and raloxifene could produce a combination of effects (higher bone mass and improved material properties) that would result in better structural biomechanical properties beyond those achieved with BP-treatment alone. Such co-treatment approaches have been tried using a rat model [94], with some positive effects on BMD and vertebral strength. In humans, combination therapy with alendronate and raloxifene has been shown to increase femoral neck BMD more than monotherapies with these agents, but was equivalent to alendronate alone at the lumbar spine [95]. Clearly, biomechanical strength could not be assessed in this human study.

Summary

The BPs have been extremely successful in reducing fracture risk and improving patients' quality of life. Still, given how much attention has been paid to these agents, there is still much to learn. Much of this has less to do with the drugs themselves, than with the role that remodeling suppression plays in bone physiology and maintenance. For example, while we know that suppressing bone turnover is important to prevent fractures in postmenopausal women, we don't know how suppression is related to baseline bone turnover, how much suppression is necessary to have the maximal effect on fracture risk reduction, or what the effects of withdrawal are and how these interact with dose and duration of exposure. It is clear that suppression of remodeling allows microdamage accumulation, but we don't know whether this is clinically important. We have very limited understanding of the mechanical effects of BP

treatment, or the mechanical effects of long-term exposure. The most important and physiologicalloy meaningful mechanical parameters – fatigue life and impact strength – have not been measured. These are important questions to study not just from the standpoint of the mechanism of action of BPs, but to expand our understanding of the role that bone turnover plays in the health of our skeletons. From this standpoint, the BPs have revolutionized the field of bone biology not just because of their clear therapeutic value, but because they have been – and hopefully will continue to be – a valuable tool with which to understand the role of bone remodeling in skeletal health and disease.

Figure Legends

Figure 1. BP-treatment significantly reduces the number (A) and size (B) of remodeling sites. (A) Treatment of beagle dogs for 1 year with daily oral alendronate or risedronate (the middle dose of each being equivalent to the clinical dose for osteoporosis) significantly suppresses vertebral trabecular bone activation frequency - the number of newly initiated remodeling sites – compared to vehicle controls (adapted from [54]). (B) In this same experiment, the size of the newly formed remodeling sites is also significantly reduced in BP-treated animals compared to controls (adapted from [3]). * $p < 0.05$ versus vehicle controls. Numbers in the bars represent percent difference from vehicle controls.

Figure 2. Speed of onset following BP initiation (A) and speed of recovery following BP withdrawal (B) in pre-clinical studies. (A) In skeletally mature rabbits, the magnitude of bone turnover suppression in vertebral trabecular bone was significantly greater in animals treated with risedronate compared to alendronate (adapted from [20]). (B) In ovariectomized rats, the magnitude of bone formation rate suppression following treatment with alendronate and risedronate was similar yet following treatment withdrawal the return toward vehicle-treated

levels of bone formation tended to be more rapid with risedronate compared to alendronate (adapted from [32]).

Figure 3. Non-linear relationship between suppression of remodeling and increases in microdamage. (A) In the cortical bone of the rib of beagle dogs treated for 1 year, decreased activation frequency (Ac.F) was associated with a nonlinear increase in crack surface density. (B) Exponential increases in Cr.S.Dn with reductions in Ac.F in the lumbar vertebra of control and 1 year treated beagle dogs. Figure reproduced with permission from [56].

Figure 4. Higher bone strength with bisphosphonate treatment is explained by the drugs' effect on bone density. Strength-density relationships of vertebral bone from beagles treated for 1 year with vehicle or clinical doses of risedronate, or alendronate. The strength-density relationship was similar for untreated ((•), y=17264x − 1927.2) and bisphosphonate-treated animals ((○),y=16709x − 1724.8). Figure reproduced with permission from [83].

Table 1. Summary of What is Known and What is Unknown About BPs and Bone Turnover

What is Known

- BPs suppress remodeling by reducing both the number and size of resorption cavities
- The effect of BPs on bone remodeling varies by skeletal site
- BPs do not suppress formation modeling
- The BPs used clinically generally produce similar effects – yet some subtle differences exist in speed of onset, duration of effect and magnitude of remodeling suppression

What is Not Known

- Are differences among BPs in remodeling suppression, including speed of onset, duration of effect, and magnitude of suppression clinically meaningful?
- How much remodeling suppression is ideal and is more necessarily better to reduce fracture risk?
- Is the degree of remodeling suppression related to the rate of turnover prior to BP treatment?
- How long does it take to restore turnover to pre-treatment levels after treatment cessation? How do dose and duration affect recovery and can it be altered by anabolic drugs?

Table 2. Summary of What is Known and What is Unknown About BPs and Microdamage

What is Known
- Microdamage accumulates with remodeling suppression, whether by BPs or other agents
- The amount varies site-specifically, and is greater in trabecular bone than cortical bone
- BPs are associated with a decline in bone toughness measured quasi-statically
- Microdamage is probably not responsible for the decline in toughness
- BPs reduce targeted remodeling as well as stochastic remodeling

What is Not Known

- Does microdamage accumulation occur in people who have taken BPs?
- Are cracks initiated more easily in BP-treated tissue? Do cracks grow more easily?
- Is damage accumulation the result only of suppressed remodeling, or also increased initiation?
- Are the signals for targeted repair of microdamage altered with BP treatment, or is the accumulation of damage the result of a more brittle tissue combined with a generalized suppression of remodeling?
- Is the accumulation of microdamage that occurs with BPs important to fracture risk?
- Is the increase in microdamage accumulation with BPs implicated in the pathogenesis of atypical femoral fractures and/or ONJ?
- How do we apply the data from human iliac crest biopsies to those from other sites?
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Table 3. Site-Specific Fold-Increase in Microdamage Burden Compared to Controls, Pre-clinical

	RIS		ALN		INC		REF
	Clin	High	Clin	High	Low	High	
L3 (1 yr)	3.13			4.17			[52]
L3 (1 yr)	2.95	5.44	3.68	4.73			[54]
L3 (3 yr)			1.28	1.68			[57]
L3 (3 yr)					2.98	3.52	[55]
Th2 (1 yr)		1.70		2.02			[52]
Th2 (3 yr)					2.98	3.52	[55]
Iliac crest (1yr)		1.77		1.76			[52]
Rib (1 yr)		4.91		6.90			[36]
Rib (1 yr)	1.53		1.93				Unpulbished[1]
Rib (3 yr)			1.81	1.64			[19]
Rib (3 yr)					2.05	3.00	[37]
FN (2 yr)		1.43[2]					[96]
FN (1 yr)		1.28		1.92			[52]

RIS = risedronate; ALN = alendronate; INC = incadronate; Clin = clinical dose for post-menopausal osteoporosis on a mg/kg basis; L3 = third lumbar vertebra; Th2 = second thoracic vertebra, FN = femoral neck. [1]Unpublished data from Allen and Burr. [2]Cancellous bone, averaged over three different doses

Table 4. Summary of What is Known and What is Unknown About the Role of BPs and Mechanical Properties

<u>What is Known</u>
- BPs reduce fracture risk at least over 3-5 years
- Increased bone strength with BPs can be explained almost entirely by increases in BMD
- Increased mechanical properties of the whole bone are partially offset by adverse changes to the bone material
- BP treatment is associated with reduced bone toughness, but not ultimate stress or modulus.

<u>What is Not Known</u>

- What are the fatigue properties of bone that has been treated with BPs?
- How does BP-treated bone respond to testing at high strain rates more closely associated with those that occur during impact from a fall?
- What is the cause of reduced bone toughness with BP treatment? Does it occur in humans? Is it related to atypical femoral fracture?
- Is it possible to combine other treatments with BPs to allow BPs to positively affect bone structure while preventing (or reducing) the adverse affects to the material?

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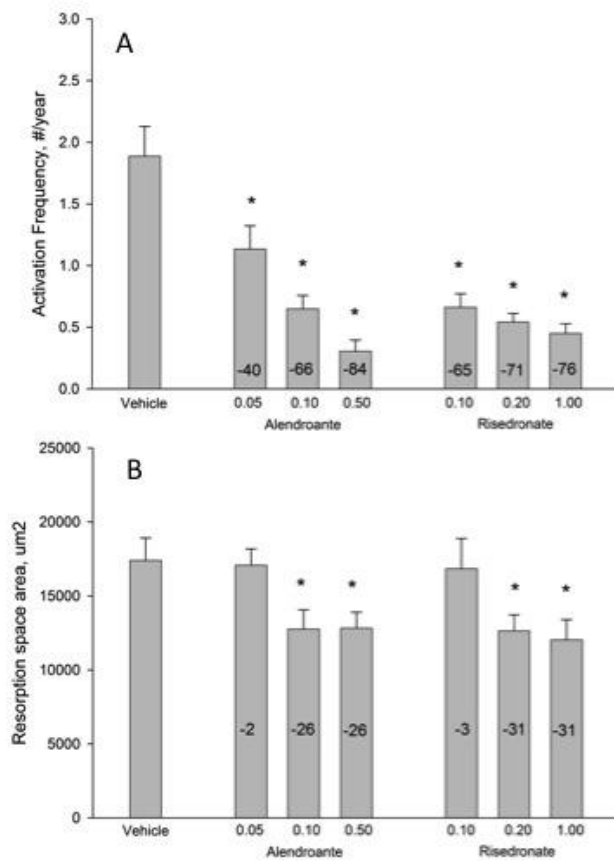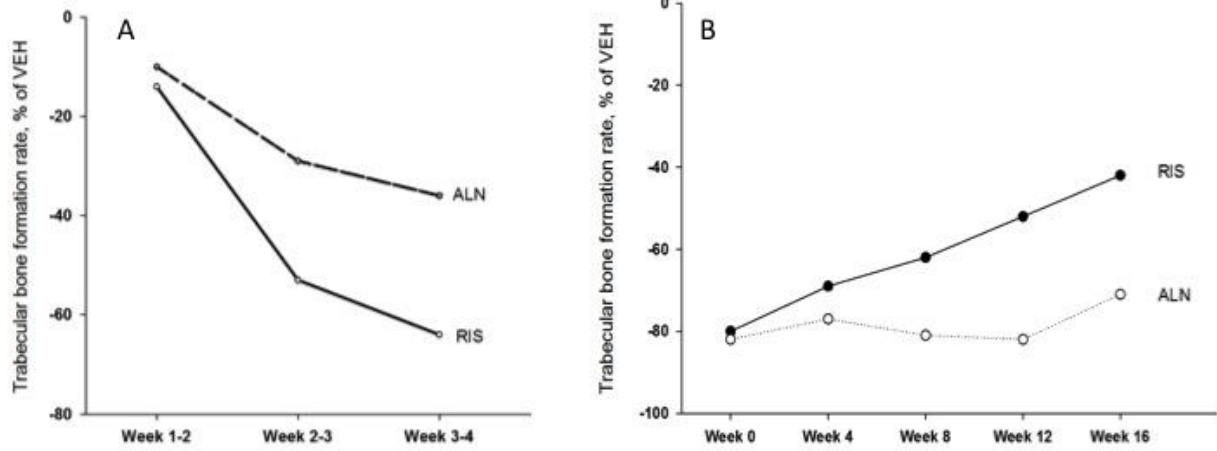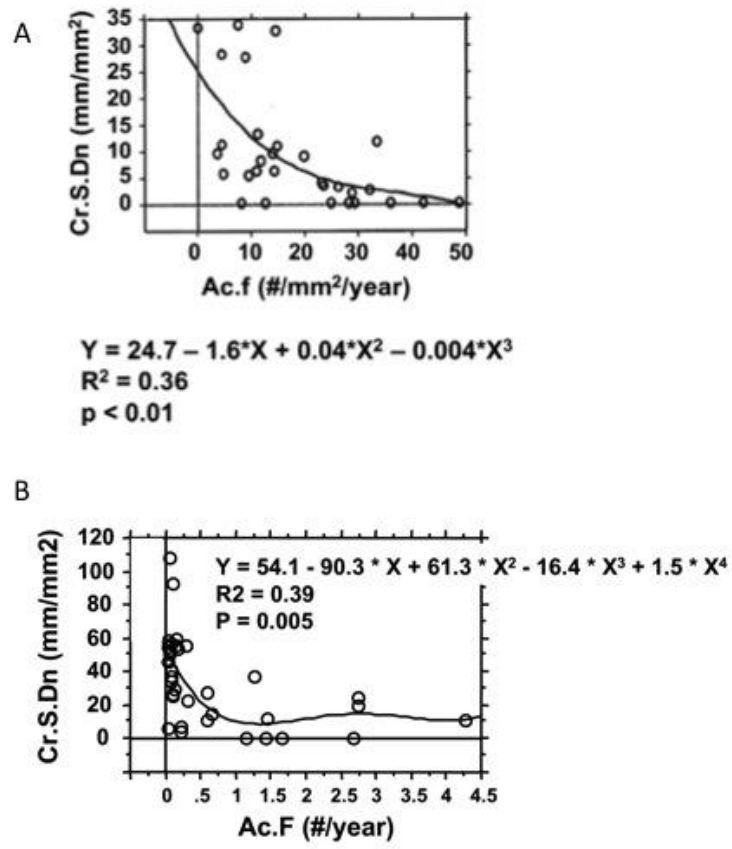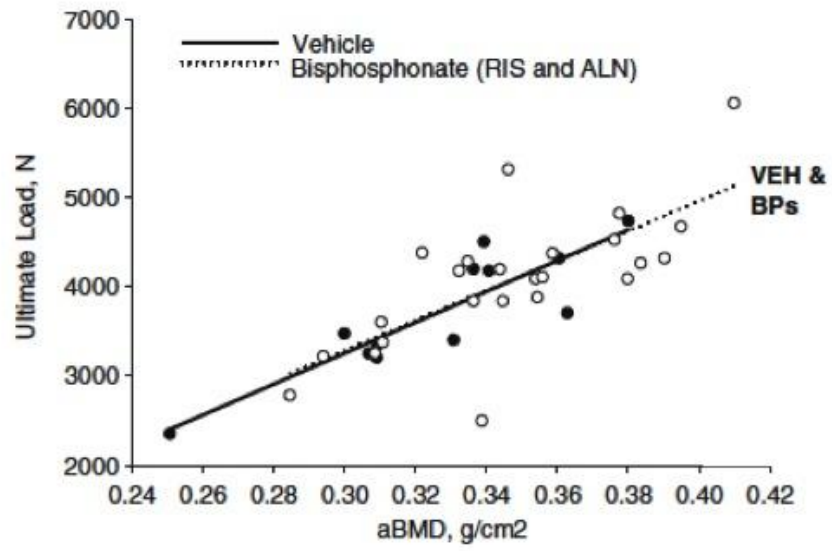
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Figure 1

Figure 2

A

$$Y = 24.7 - 1.6*X + 0.04*X^2 - 0.004*X^3$$
$$R^2 = 0.36$$
$$p < 0.01$$

B

$$Y = 54.1 - 90.3 * X + 61.3 * X^2 - 16.4 * X^3 + 1.5 * X^4$$
$$R2 = 0.39$$
$$P = 0.005$$

Figure 3

Figure 4