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PERSPECTIVES

Mineralization, Microdamage, and Matrix: How Bisphosphonates Influence Material Properties of Bone

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Abstract

Bisphosphonates provide clear anti-fracture efficacy by suppressing bone turnover. The effects of turnover suppression extend beyond slowing the rate of bone loss to changing the properties of the bone matrix. The goal of this review is to summarize the effects of bisphosphonates on material-level properties of bone, including tissue mineralization, microdamage, and the organic matrix (e.g., collagen cross-linking). The mechanical implication of these changes is also addressed. Because of a reduction in turnover that increases the mean tissue age, bisphosphonates increase the mean degree and homogeneity of mineralization, the accumulation of microdamage, and the degree of both enzymatic and non-enzymatic collagen cross-linking. These changes combine to reduce the energy absorption capacity (material-level toughness) without altering the material-level strength or modulus of the tissue. The implication of these material-level changes remains unclear, as the reduced rate of bone loss, together with the improvement in bone mineral density and the maintenance of trabecular architecture, appears sufficient to reduce fracture risk. Continued study of the changes in material-level properties with bisphosphonate treatment is warranted. With treatment duration now extending into its second decade in some patients, it remains possible that over time the changes in material-level properties could override the structural-level benefits. *BoneKEy-Osteovision*. 2007 February;4(2):49-60.

Keywords: Collagen cross-links; Turnover suppression; Anti-remodeling agents; Biomechanics; Toughness

What Are "Material Properties" of Bone?

The biomechanical properties of bone exist at two hierarchical levels (Fig. 1). Structural-level biomechanical properties, such as ultimate load, stiffness, and energy absorption to fracture, describe the bone as a composite unit. These properties are determined by factors such as bone mass, geometry/architecture, and the biomechanical properties of the material (1). Material-level biomechanical properties, such as ultimate stress, modulus, and toughness, describe the biomechanical properties of the tissue, independent of mass or geometry. The biomechanical properties of the material are determined by factors including, but not limited to, the degree and heterogeneity of mineralization, the level of microdamage, and collagen content and cross-linking. A change in one or more of these variables, as occurs with bisphosphonate treatment, can alter the material-level biomechanical properties, affecting structural biomechanical properties and presumably fracture risk (Fig. 2) (2).

Clinical trials in post-menopausal osteoporotic women have consistently shown reductions in both vertebral and non-vertebral fracture with various bisphosphonates (for review see (3;4)). These reductions are assumed to be related to an increase in the structural biomechanical properties of the bone, e.g., the bones are 'stronger'. Indeed, animal

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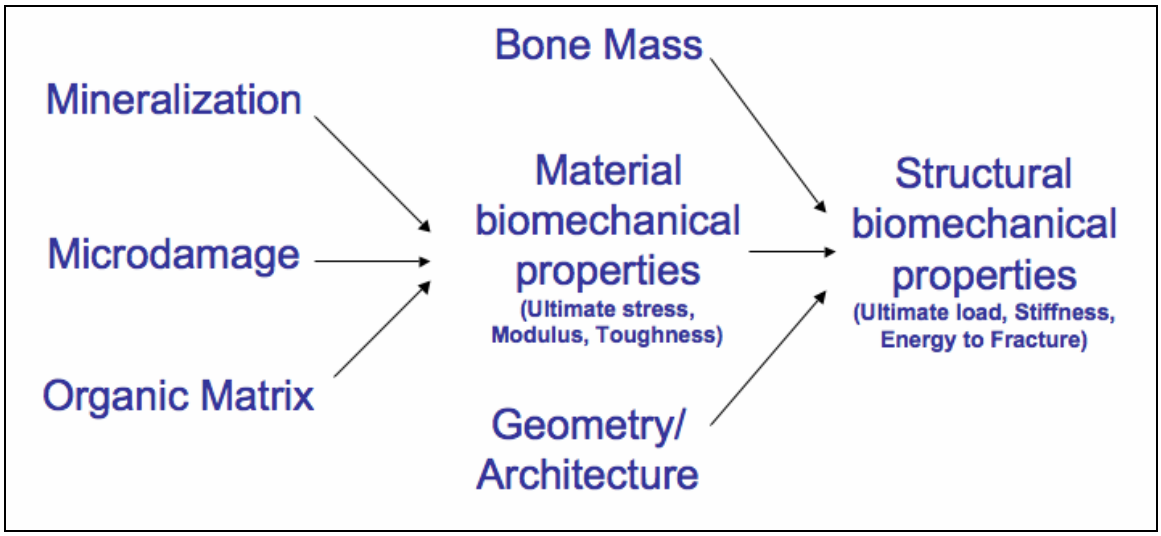

Figure 1. Hierarchical nature of bone biomechanical properties. Structural-level biomechanical properties are determined by a combination of factors including bone mass, geometry/architecture, and the biomechanical properties of the bone tissue (material properties). Material-level biomechanical properties are determined by factors such as mineralization (both degree and heterogeneity), the level of microdamage accumulation, and the organic matrix (e.g., collagen cross-linking).

experiments have consistently shown increased whole bone strength and stiffness with bisphosphonate treatment (5-11). Bisphosphonates likely enhance structural-level biomechanical properties through a combination of changes in bone mass, geometry/architecture, and material-level biomechanical properties. By suppressing bone turnover, bisphosphonates reduce the rate of bone loss, thereby maintaining bone mass relative to untreated controls (12;13). Bone geometry and architecture are improved with bisphosphonates through reductions in cortical bone porosity (14) and maintenance of trabecular number, thickness, and connectivity (12;13;15). The effects of bisphosphonates on the material-level biomechanical properties, and more specifically the components that determine them, are less prominent in the literature. The goal of this Perspective is to highlight what is known concerning the effect of bisphosphonates on material-level properties of bone. Specifically, we will summarize the effects of bisphosphonate treatment on three key material properties of bone: mineralization, microdamage, and the organic matrix, and describe how these changes affect the material-level biomechanical properties.

Mineralization: More Is Not Necessarily Better

By reducing bone turnover, bisphosphonates significantly alter the mineralization profile of bone, increasing the average tissue mineralization (often termed mean degree of mineralization (16)) and reducing the heterogeneity of mineralization across the mineralized bone matrix. These changes occur because of reduced formation of new basic multicellular units (BMUs), which transiently lowers mineralization levels because new BMUs are undermineralized, relative to mature BMUs. A reduction in new BMU formation also increases the lifespan of existing

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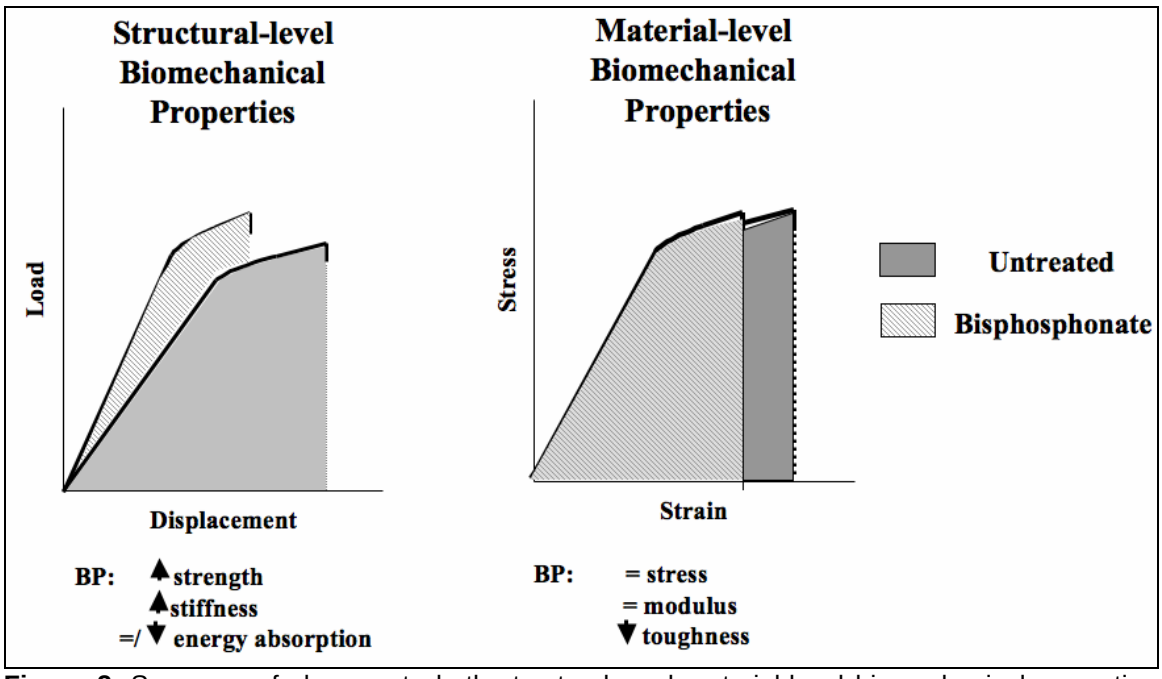

Figure 2. Summary of changes to both structural- and material-level biomechanical properties with bisphosphonate treatment. Structural properties are defined in terms of strength (the highest load the bone can withstand), stiffness (the linear slope of the load/displacement curve), and energy absorption to fracture (the energy under the curve). Analogous properties can be defined for material-level properties using a stress/strain curve (corrected for differences in bone mass and geometry).

BMUs, allowing more of the bone to become fully mineralized, although it does not lead to greater mineralization per unit of collagen, or to "hypermineralization". Using quantitative backscatter electronic imaging (qBSE), Boivin et al. (17) and Roschger et al. (14) have documented significantly higher average tissue mineralization and mineralization homogeneity in both cortical and trabecular bone of the iliac crest from alendronate-treated patients after 2-3 years, compared to untreated patients. Borah et al. (18), using microCT to assess biopsies from patients at baseline and after 3 and 5 years on risedronate, showed an increase in average tissue mineralization, and a reduction in the ratio of low/high mineralization (an indication of increased mineralization homogeneity) by three years of treatment. There was no significant difference in average mineralization or homogeneity values between years 3 and 5 of risedronate treatment, suggesting mineralization changes may be maximized early in treatment (19).

Animal studies have provided results consistent with data from humans. Increased ash fraction, a more global measure of tissue mineralization, has been reported following bisphosphonate treatment in numerous dog studies (5;6;8-11;20). Using qBSE, Roschger et al. (21) documented an increase in both mean degree of mineralization and mineralization homogeneity following 1 year of alendronate treatment in minipigs, while Mashiba et al. (22) found an increased mean degree of mineralization in dog rib following 3 years of treatment with incadronate. Although increased mineralization was not found using qBSE in ribs from dogs treated with alendronate or risedronate for 1 year, using density centrifugation, Burr et al. found a significantly greater percentage of bone with higher levels of mineralization in these same bones (23).

An unresolved question is whether bisphosphonates alter the process of mineralization. Specifically, it is unclear

whether bisphosphonates alter either the rate of mineralization, or the eventual degree of mineralization, of a specific BMU. One or both of these changes would be expected to have a significant effect on the biomechanical properties at the tissue level. Some insight can be gained from a study by Burr et al. (23), in which levels of mineralization were assessed in newly formed bone (determined by fluorochrome labeling in the tissue) using Fourier transformed infrared microspectroscopy. This study showed that the phosphate/protein ratio, a measure of mineralization, was significantly lower in newly deposited bone (< 1 month old) of bisphosphonate-treated animals compared to controls. As the phosphate/protein ratio was similar in bone that was ~5 months old, it appears that the time-course of mineralization is acutely altered but that the final level of mineralization of a newly deposited piece of bone is at least equivalent to that of untreated bone. No data exist on whether the eventual level of mineralization that a particular BMU achieves is increased with bisphosphonate treatment.

The effects of altered mineralization on biomechanical properties are well-established (24-30). Increased mineralization is positively correlated to strength and stiffness, and inversely related to energy absorption (toughness) (30). This is true both at the whole bone (structural-) and material-level. Consistent with these expected changes, bones from animals treated with bisphosphonates have been shown to have increased strength and stiffness, and reduced toughness (5;6;10;31). These changes in toughness have been shown to be closely predicted by the changes in mineralization (32). Interestingly, these animal studies showing increased mineralization did not document increased material strength (ultimate stress) or modulus, changes that would be expected to occur. The most plausible explanation for this finding is that the positive changes induced by increased mineralization are offset by other material-level changes that result from bisphosphonate treatment, including increases in microdamage accumulation (33).

Microdamage: More Is Not Necessarily Worse

Bisphosphonate treatment results in a significant accumulation of microdamage. To date, six papers have reported microdamage levels in animals treated with bisphosphonates (5;6;9-11;34). These studies have used various bisphosphonates (alendronate, risedronate, incadronate), at doses ranging from 0.5 to 6x the dose used for treatment of post-menopausal osteoporosis, and with treatment durations lasting from 1 to 3 years. Significantly higher levels of microdamage have been consistently noted in the trabecular bone of the lumbar vertebra and cortical bone of the rib with bisphosphonate treatment (Fig. 3). Although increased levels of microdamage have also been noted in the ilium, thoracic spinous process, and femoral neck, these sites appear to be less prone to significant microdamage accumulation (< 2-fold relative to untreated bone) (5;6;9-11;34). While these experiments have all been conducted using a beagle dog model, recent data from iliac crest biopsies of treatment-naive and bisphosphonate-treated women similarly show increased microdamage with bisphosphonates (35).

The mechanism of bisphosphonate-induced microdamage accumulation has not been established, although conventional wisdom suggests it is due to a combination of increased microdamage formation and reduced microdamage removal. Bisphosphonates suppress turnover, reducing both targeted and stochastic remodeling (36), thereby allowing microdamage to persist for a greater period of time compared to non-treated bone. A large suppression of turnover is not necessary to induce a significant

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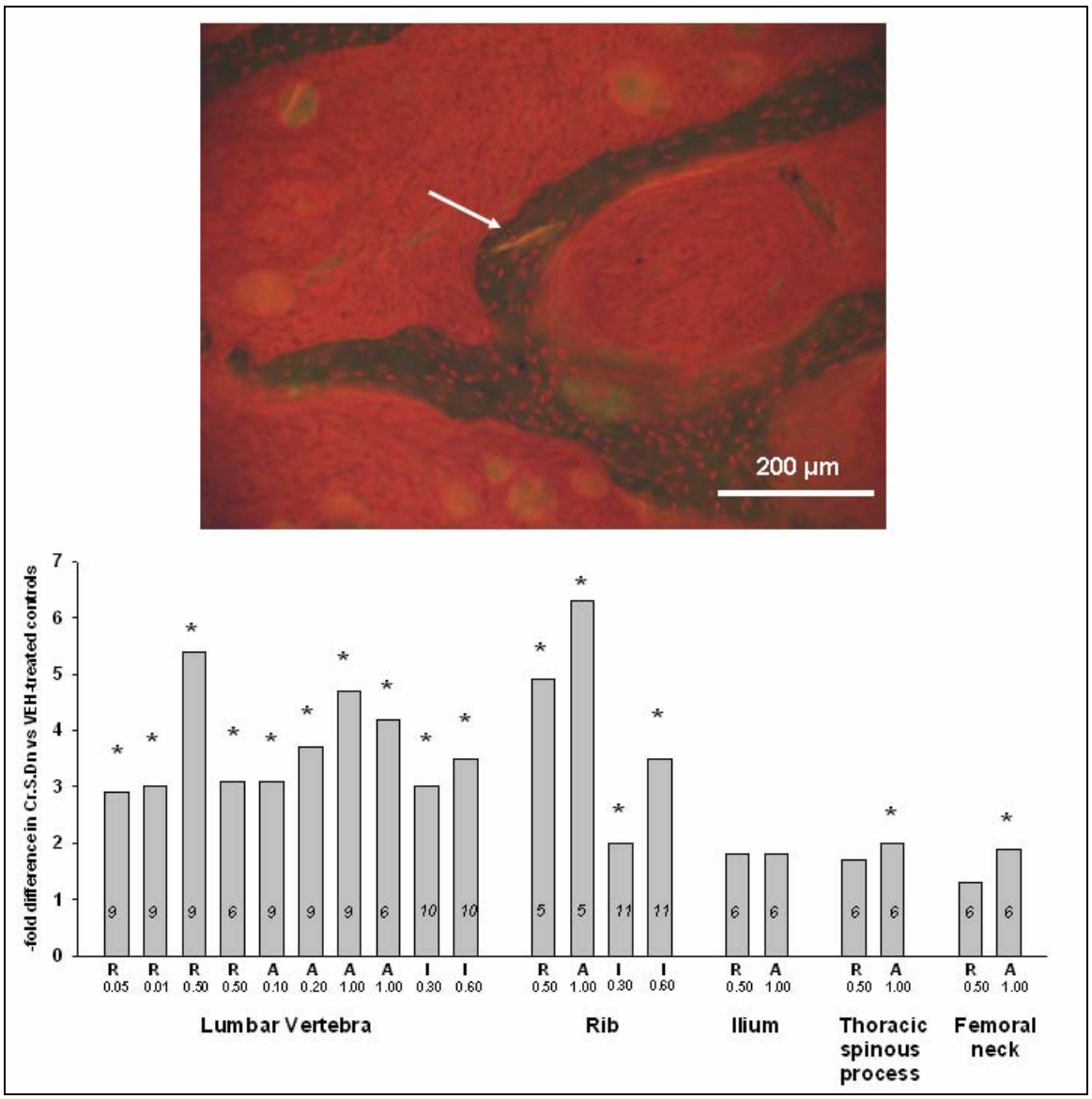

Figure 3. Summary of results from beagle dog studies examining changes in microdamage accumulation with bisphosphonate treatment. Data are expressed as –fold change in crack surface density (Cr.S.Dn.) of the treated group relative to vehicle-treated animals within the same study. Results are divided by site of analysis, and within a particular tissue each bar represents the results from a specific group of treated animals. The bisphosphonate (R, risedronate; A, alendronate; I, incadronate), as well as the dose (in mg/kg/day), are represented on the x-axis below each bar. The citation corresponding to each experiment is noted within the bar. (*) denotes that the level of microdamage was significantly higher than in vehicle-treated animals in a particular study ($p < 0.05$). The photomicrograph shows a typical linear microcrack in the trabecular bone of a beagle dog vertebra (stained with basic fuchsin and viewed under fluorescent light).

accumulation of microdamage. Suppression of trabecular bone activation frequency in the vertebra by just ~40% is associated with a 3x increase in microdamage compared to untreated controls (9). Similarly, a wide-range of microdamage accumulation (from 3x to 5x higher compared to untreated controls) occurs in treatment groups having

similar degrees of remodeling suppression (9). So, while there is a significant inverse relationship between remodeling suppression and microdamage accumulation (6;9;10), there is more to the story than a suppression of microdamage removal. One explanation is that bisphosphonates also likely alter microdamage formation. Factors such as increased mineralization and increased tissue homogeneity, both of which occur with bisphosphonate treatment, are known to be permissive to the formation and growth of microdamage (37-39). Data concerning changes in damage formation and/or propagation with bisphosphonate-treatment are limited but are consistent with the hypothesis of increased microdamage formation (40).

The main controversy concerning bisphosphonates and microdamage arises with respect to the biomechanical implications. In the majority of studies that have documented increased microdamage with bisphosphonate treatment, a concomitant decrease in bone toughness has also been quantified (5;6;9;10). However, a cause/effect relationship between these two parameters has not been documented. It is generally accepted that microdamage reduces material-level biomechanical properties, including strength, modulus, and toughness (41-46). The difficulty in teasing out the contribution of microdamage to these deteriorations in biomechanical properties is that the concomitant changes to mineralization (as outlined above) and organic matrix (see below) occur with bisphosphonates. Although not definitive, a recent study assessing microdamage and biomechanical properties in dog vertebra suggests that microdamage accumulation may not be the predominant reason for reduced toughness (9). In this study, there was minimal congruence between changes in microdamage accumulation and material-level toughness in vertebra from several groups of bisphosphonate-treated dogs (9). Although these data do not eliminate the possibility of a direct cause/effect connection, they suggest factors other than microdamage contribute significantly to the material-level biomechanical changes associated with bisphosphonate treatment. Whereas mineralization likely plays some role in these material-level biomechanical changes, emerging data suggest the non-mineral component of bone, the organic matrix, may also contribute.

Organic Matrix: Looking Beyond The Mineral in Bone

There are limited data concerning bisphosphonate effects on the organic component of bone, yet the data that do exist suggest the changes are significant. Early studies by Guenther et al. (47) showed significant increases in divalent enzymatic cross-linking of the collagen matrix of rat tibia following treatment with a bisphosphonate. These changes were associated with decreased digestibility of the tissue, suggesting a more stable collagen matrix (47). We (48) and others (49) have recently documented changes in both enzymatic and non-enzymatic cross-linking of the organic matrix in bisphosphonate-treated animals. Following one year of treatment with a wide range of bisphosphonate doses, the ratio of pyridinoline to deoxypyridinoline (PYD/DPD, an index of trivalent enzymatic cross-linking) in the trabecular bone of lumbar vertebrae was significantly increased compared to vehicle-treated animals. The level of pentosidine, an advanced glycation end-product (AGE) that forms non-enzymatically mediated collagen cross-links, was significantly increased in the trabecular bone of bisphosphonate-treated animals compared to controls (48). In these same animals, the level of AGEs in cortical bone of the tibia was also increased with bisphosphonates (50). In a separate experiment, levels of pentosidine were found to be increased in the ribs of dogs following 3 years of treatment with incadronate (49).

The increasing interest in changes in the organic matrix with bisphosphonate treatment is due to the known contributions of the organic matrix to a bone's fracture resistance (51;52). Specifically, the organic matrix constitutes the principal toughening mechanism in bone, and therefore plays a

substantial role in determining properties of energy absorption/toughness (53). This is because collagen properties are the main determinants of the post-yield properties of bone (51;54;55). Changes in the organic matrix may have some effect on tissue strength and stiffness (56;57), although these properties are determined predominantly by the mineral fraction.

While changes in the amount, structure, and organization of collagen can alter tissue biomechanical properties, the key component appears to be the extent of collagen cross-linking. Collagen cross-linking occurs via either enzymatically-mediated or non-enzymatically mediated processes. The enzymatic process, mediated by lysyl oxidase, results in the trivalent collagen cross-links PYD and DPD. The ratio of PYD/DPD has been shown to be positively associated with strength and stiffness in bone (58-62), but appears to have a minimal effect on toughness (53;57;63;64). Non-enzymatic collagen cross-linking (e.g., AGEs such as pentosidine) occurs via spontaneous condensation of arginine, lysine and free sugars (65;66). Cross-links formed through non-enzymatic processes make the tissue more brittle (67-69). Increased pentosidine concentration in bone has been shown to reduce the amount of post-yield deformation (53;70;71) and work to fracture (72). Consistent with these known effects of increased cross-linking, our dog studies, which showed increased enzymatic cross-linking and AGE accumulation in both the vertebrae and tibiae, also showed decreased toughness in both tissues (9;48;50). There was a significant inverse, non-linear relationship between levels of AGEs and post-yield work to fracture in the cortical bone (50). While this does not show a cause/effect relationship between collagen cross-linking and mechanical properties, it provides intriguing data supporting the hypothesis that changes in the organic matrix play a significant role in altering material-level biomechanical properties with bisphosphonate treatment.

Conclusions

By reducing turnover, bisphosphonates result in significant changes to three key material properties of bone, increasing the mean degree and homogeneity of mineralization, the accumulation of microdamage, and the degree of collagen cross-linking. Each of these changes in the bone material has a significant effect on material-level biomechanical properties, independent of changes in bone mass, although their specific individual contribution is difficult to assess experimentally. By all accounts, it appears that changes to mineralization and collagen cross-linking, which tend to increase material-level strength and stiffness, are offset by the increased microdamage (which tends to lower both). This results in minimal change to material-level strength (ultimate stress) and stiffness (modulus). Conversely, changes to all three parameters, mineralization, microdamage, and cross-linking, likely contribute to reducing energy absorption capacity at the material level (toughness) (Fig. 2).

What remains unclear is the importance of these changes in material-level biomechanical properties, given the dramatic changes in bone mass and geometry/architecture that occur with bisphosphonates. To date, it appears these macro-level changes drive the anti-fracture efficacy of bisphosphonates, and can adequately compensate for diminutions in material properties (Fig. 2). However, as treatment duration extends into its second decade for some patients, continued study of the changes in material-level properties with bisphosphonates is warranted. Over time, the changes in material-level properties could potentially override the structural-level benefits of bisphosphonates.

Conflict of Interest: Dr. Allen reports that he has research contracts from Eli Lilly and the Alliance for Better Bone Health. Dr. Burr reports that he has research contracts from Eli Lilly, the Alliance for Better Bone Health, and Amgen; owns stock in Amgen; has a material transfer agreement from Merck; serves on the advisory board for Eli Lilly; and is a speaker for Eli Lilly, the Alliance for Better Bone Health, and Amgen.

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