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Changes in bone matrix properties with aging

David B. Burr*

Dept. of Anatomy and Cell Biology, Indiana Center for Musculoskeletal Health, Indiana University School of Medicine, United States of America
Dept. of Biomedical Engineering, Indiana University-Purdue University, Indianapolis (IUPUI), United States of America

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ABSTRACT

It is well known that bone loss accompanies aging in both men and women and contributes to skeletal fragility in the older population, but changes that occur to the bone tissue matrix itself are less well known. These changes in bone quality aggravate the skeletal fragility associated with loss of bone mass. Bone tissue quality is affected by age-related changes in bone mineral, collagen and its cross-linking profiles, water compartments and even non-collagenous proteins. It is commonly assumed that greater tissue mineralization accompanies aging as bone turnover slows down in elderly individuals, but the data for this are weak. However, there may be changes in the quality of the mineral crystals, and the substitutions found within the crystal. Both enzymatically-mediated and non-enzymatically-mediated collagen cross-links multiply with age. The former tend to make the bone stiffer and stronger, but the latter, while making the bone stiffer can also make it more brittle and more likely to fracture. Bone pore water that is not bound to collagen or mineral increases with age as bone mass is lost, but water that is bound to collagen and mineral declines with age. These changes contribute to skeletal fragility by reducing the amount that bone can deform before fracturing. Finally, non-collagenous proteins have physical properties that can alter matrix mechanical properties and can also have molecular signaling functions that regulate bone remodeling. Whether these change with age, how they change, and how this affects skeletal fragility with aging is still largely a black box, and requires much more investigation. The roles of any of these factors in skeletal fragility are difficult to assess clinically as there is no easy or economical way to evaluate them, but a picture of fragility in the aging skeleton is incomplete without them.

The risk of bone fracture increases with age, and it is commonly thought that the reason for this is the loss of bone mass and alterations to skeletal architecture, especially after the menopause in women. However, these changes contribute to only about 75% of the increased risk of fracture. Both cortical and trabecular bone strength begin to decline by ~2–5% and 5–10% respectively beginning in our 30's [see Ref. [1] for a more detailed description of this], well before there is a significant loss of bone mass or trabecular re-organization. Age itself contributes to increased skeletal fragility, independent of bone mass. For example, an osteopenic individual (with a T-score between −1.0 and −2.5 standard deviations below the young adult normal) in his or her 80's has a four-fold greater risk of fracture at either the radius or the hip than does someone in their 50's with exactly the same BMD [2,3] (Fig. 1). If increased fragility cannot be entirely explained by changes in bone mass and architecture, then it seems logical that the bone tissue and matrix properties themselves must change with age, and contribute to the risk of fracture.

The material properties of bone matrix contribute to bone's overall strength, and can exacerbate strength changes caused by a loss of bone. This may be particularly important under certain types of fracture situations. For example, energy absorption and bone toughness decline by 30–40% between the ages of 50–80 [4], and by > 80% compared to someone in their 20's [5–8]. This results from a combination of declines in crack initiation toughness (a measure of energy required to initiate a microcrack in bone, which declines by 40% between the ages of 40 and 100 years) and toughness associated with crack growth, which declines by close to 100% by the 10th decade [5,9,10]. These are much greater declines than changes to overall strength, which may fall by only about 30–35% over the same time period. Likewise, tensile ultimate strain (a measure of how much bone can deform before it breaks) also declines by 80% between the ages of 20 and 80 [11] (Fig. 2). Energy absorption and ultimate strain are much more important indicators than strength of whether the hip, for instance, will fracture from the impact of a fall [12]. It is no surprise, then, that one in three women and one in five men will sustain a hip fracture during their lifetime.

These changes in material mechanical properties are independent of bone mineral density (BMD) and architecture, and suggest that the changing properties of the tissue itself may explain the currently

* Dept. of Anatomy and Cell Biology, MS 5035, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202, United States of America.
E-mail address: dburr@iupui.edu.

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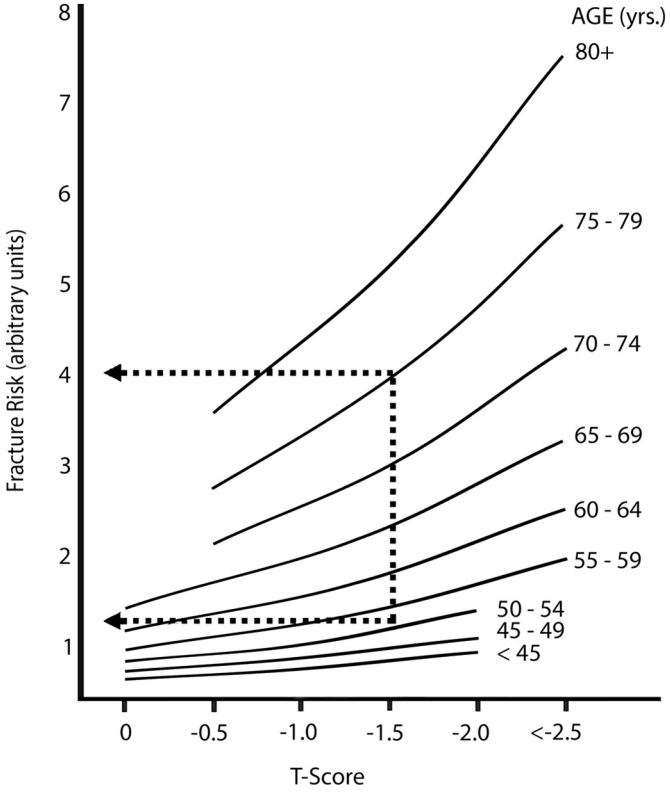

Fig. 1. Fracture risk increases in a non-linear fashion as people age. Even for the same bone density, fracture risk can increase four-fold over a 20 year period between the 6th and the 8th decades (dotted lines).
(Modified from Hui et al. [2] and Kanis et al. [3].)

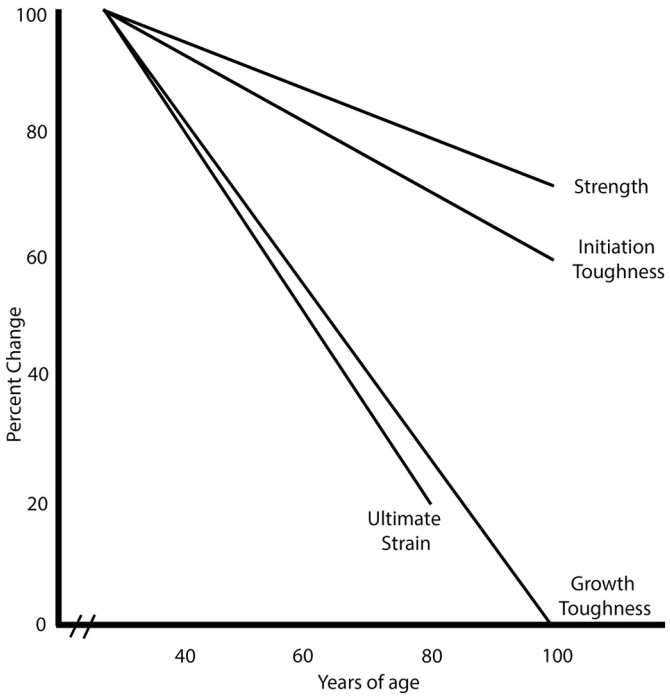

Fig. 2. There is a significant decline in mechanical properties from age 40 to age 80–100. Bone strength and crack initiation toughness decline by about 30% over this period of time, but the ultimate strain (a measure of how much deformation can occur prior to fracture) and crack growth toughness decline by 80 to nearly 100%.
(Based on data from Nalla et al. [5] and Morgan et al. [11].)

"unexplained" portion of fracture risk once BMD and architecture have been taken into account. The question is: what are these age-related changes in bone matrix properties and how do they contribute to bone's overall fragility?

Bone matrix is composed of mineral, collagen, water and a small amount of non-collagenous proteins. Changes to any of these individual components – or to the interactions among them, for example at the collagen-mineral interface – contribute to fragility. We will explore each of these matrix components, and its contribution to fracture risk.

1. Bone mineral

Bone mineral is composed of a poorly crystalline carbonated apatite that is initially deposited as an amorphous calcium phosphate between the ends of the collagen fibrils (intra-fibrillar mineral, hole zones) and along the length of the fibril (extra-fibrillar mineral, pore zones) (Fig. 3). The deposited mineral aggregates, in part regulated by non-collagenous proteins [13], into platelets about 2.5–4 nm thick (the a-axis), whose long axis aligns parallel to the collagen fibrils (the c-axis). The platelets are thought to be separated by octacalcium phosphate-citrate layers that are hydrated (see below, Bone water) [14]. As the bone ages, the mineral crystals lengthen and become more crystalline [15], so the average size of the crystals is dependent on tissue age, which in turn is dependent on turnover rate and is regulated by osteocalcin [13]. The increased crystallinity also may be regulated in part by both the water and citrate layers between the platelets to prevent them from aggregating over time into single plates [14]. This would preserve the mechanical properties of the tissue as increased crystallinity has been associated with decreased ultimate strain and toughness [15,16]. As mineral crystallinity is a property often measured using Fourier-transform Infrared (FTIR) or Raman spectroscopy, it is important to note that smaller crystals with many imperfections may appear similar to larger crystals with only a few imperfections. This can make measurements of crystallinity, and how crystallinity changes with age, difficult to interpret. In addition, carbonate substitutions occur for phosphate in the hydroxyapatite lattice during aging, reducing elastic modulus (stiffness) of the bone matrix [15].

Although it is well known that bone mineral density declines with age in both men and women, it is less clear whether tissue mineral density, i.e. bone mineral per bone volume, changes with age. Variations in tissue mineralization are highly dependent on the rate of bone remodeling, and to what extent new bone formation in remodeling units is suppressed in older individuals. If remodeling slows with age, then tissue mineralization will likely increase because older and more highly mineralized regions of bone tissue are not replaced by newer, not-yet-fully mineralized bone. On the other hand, if remodeling is accelerated with age, but resorption and formation are still coupled, then tissue mineralization might appear to decrease with age because of the mineralization lag between the deposit of osteoid and its full mineralization [17]. These variations in the rate of bone remodeling probably account for the wide range of opinions in the literature about whether mineralization of the bone tissue itself changes with age.

Although at one time it was thought that tissue mineralization increased with age [18–21] accumulated and more comprehensive data suggest that tissue mineralization remains stable over the lifetime [4,22,23]. Although bone mineral is lost as bone mass is lost, the amount of hydroxyapatite per volume of bone remains about the same, at least once growth is complete and peak bone mineral density has been reached [24].

Likewise, there is a diverse set of opinions about whether the heterogeneity in mineralization changes with age, even when the data are generated from the same laboratory [25,26]. Some have suggested that heterogeneity is reduced because there is an increased fraction of high density bone in older people [18]. Others suggest that heterogeneity is increased [27,28]. Again, the reason for the discrepancy is likely due to individual variations in remodeling rate, and this may be why some

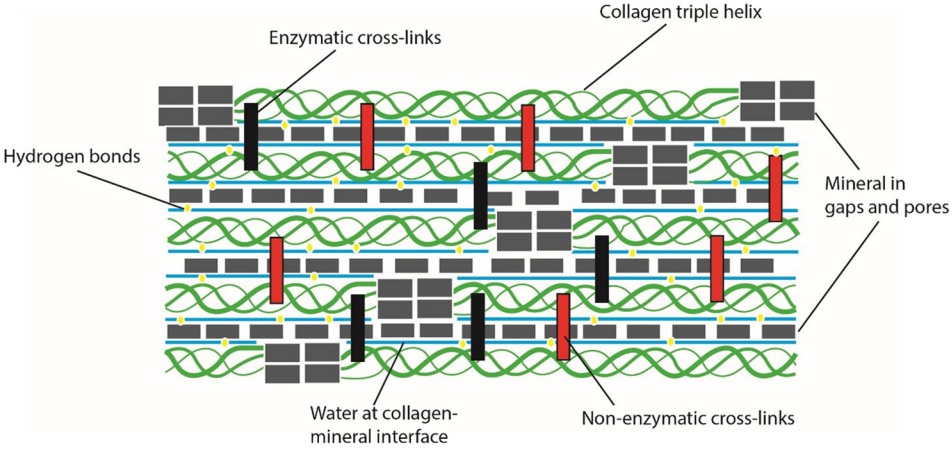

Fig. 3. Mineral platelets are deposited in the gap (between the ends of the collagen molecules) and pore (between adjacent collagen molecules) zones of the collagen-mineral composite. Loosely bound water (blue) and hydrogen bonds (yellow) are found at the interface between the collagen molecules and mineral platelets. The water at this interface allows some deformation (through sliding) and stress relief at the boundary between them. As people age, this loosely bound water will in part be transferred to the more tightly bound water compartments, causing a loss of ductility. Mature collagen cross-links formed by enzymatic processes (black) stiffen and strengthen the collagen matrix, and do not change appreciably over the aging process. Bonds between collagen molecules that are formed by non-enzymatic processes such as oxidation reactions accumulate as the bone matrix ages (mean tissue age of the bone), and naturally accumulate with age of the individual. These bonds, or advanced glycation end-products (AGEs) form bonds in the middle of adjacent collagen molecules, and reduce the toughness of the bone tissue. This is one reason that fracture risk increases with age, even for the same bone density (see Fig. 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) (From D.B. Burr and M.R. Allen, Basic and Applied Bone Biology, (2014), Amsterdam: Elsevier/Academic Press, p. 7.)

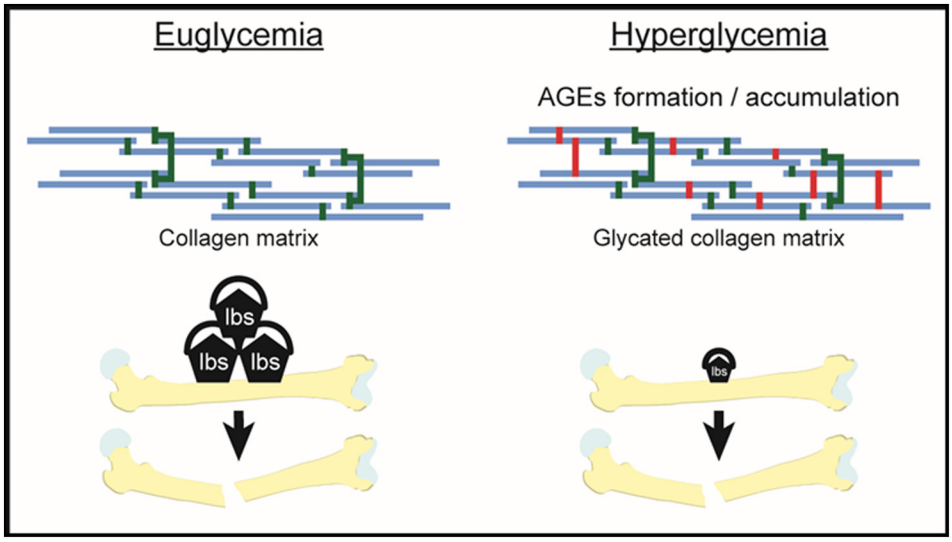

Fig. 4. Enzymatic cross links (green) such as pyridinium and deoxypyridium form at the ends of the collagen molecules, serving to strengthen the bone matrix. Advanced glycation end-products (AGEs), on the other hand, form in the middle of the collagen molecules (red) and serve to reduce the toughness of bone, making it more likely to fracture. AGEs accumulate naturally during the aging process. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

investigators have found increasing heterogeneity with age only in women, who may well be in various stages of heightened remodeling associated with menopause [29]. If bone is not removed by remodeling, so that most of the bone present in a volume of tissue is older and has achieved most of its potential for mineralization, all areas of bone will be similarly mineralized and so the heterogeneity will be decreased. On the other hand, if active remodeling continues to replace old bone with new bone which is not fully mineralized and which may undergo secondary mineralization for a year [17] or more [24], then the range of tissue mineral in a given volume of bone will be more heterogeneous.

Thus, although increased skeletal fragility has been attributed to an increased brittleness caused by greater tissue mineralization, the data in support of that are at best equivocal. A conservative interpretation of the data would conclude that there is no clear populational trend in tissue mineralization that could account for the increased fragility of bone with age.

There may, however, be age-related changes in the nature of the mineral crystal that affect bone mechanical properties [30]. Paschalis et al. [31,32] suggested that mineral becomes more crystalline with age, and that this could reduce the amount of elastic and plastic deformation [15,33] that occurs before the bone fractures. Others, however, have found that crystallinity, which is defined by both the crystal size and crystal lattice perfection, and indicates crystal maturity, decreases with age as carbonate ions substitute for the phosphate (PO_4^3) in the hydroxyapatite [34]. The substitution of carbonate with mineral maturity changes the shape of the mineral crystal [35–37] reportedly increasing the length along the c-axis [34]. This change in mineral conformation may increase Young's modulus [38], but could reduce the toughness of the bone tissue [1] as a result of the inverse relationship between stiffness and toughness [39].

2. Collagen cross-linking

2.1. Enzymatic-mediation

Both collagen content, and also the extent and nature of its interfibrillar (pyrroles) and intrafibrillar (pyridinoline, deoxypyridinoline) cross-linking, all have a significant effect on bone mechanical properties. Cross-links can be formed by either enzymatic processes or non-enzymatic processes. Immature, divalent cross-links formed by enzymatic means are post-translationally modified via an enzymatic process initiated by lysyl oxidase to more mature trivalent cross-links such as pyridinoline (PYD, or hydroxylysyl pyridinoline) and deoxypyridinoline (DPD, or lysyl pyridinoline). These cross-links link the ends of the

collagen molecules (Fig. 4). Typically, divalent cross-links are converted to trivalent cross-links within a few weeks after collagen is deposited by the osteoblasts, and so any variations in the quantity of divalent or trivalent cross-links in bone are indicative of either changes in turnover rate, or an enzymatic deficiency or other metabolic condition. The quantity of PYD and DPD trivalent cross-links in bone tends to be relatively constant by the end of puberty, unlike the pyrroles which are known to decrease with loss of bone mass [40,41].

Although the bulk of trivalent crosslinks does not change with age, and the individual amounts of PYD and DPD do not correlate with bending strength [42,43], a higher PYD to DPD ratio is indicative of a more stable collagen network (i.e., more mature cross-links) that does contribute to bone's strength and stiffness [44–47]. In contrast to the effects of non-enzymatic crosslinks (see below), enzymatic crosslinks make bone tougher. There also is some evidence that the interfibrillar pyrroles have a positive effect on bending [21] and tensile [48] strength as well, especially in cortical bone [49,50].

2.2. Non-enzymatic mediation

Intra-fibrillar (inter-molecular) collagen crosslink-like bonds can also be formed by a series of posttranslational modifications that occur through a process involving the condensation of arginine, lysine and free sugars (Fig. 4). This non-enzymatic glycation forms intra-molecular collagen bonds known as advanced glycation end-products (AGEs). These form when reducing sugars spontaneously condensate with free amino groups such as lysine and arginine. Because AGEs are formed in the presence of sugars, they accumulate in collagenous tissues of people with diabetes, and are considered to be one cause for skeletal fragility that is found in this condition. However, AGEs are also produced by oxidation reactions, and form naturally as collagen ages. Proteins with long half-lives, such as collagen, can accumulate substantial amounts of AGEs. Therefore, unlike the enzymatically-mediated crosslinks, AGEs accumulate naturally in bone with age [42,51–54], purportedly achieving levels 3–10 times those in younger bone [13,55]. Accumulation may be less evident in trabecular bone, probably because of its higher turnover rate, although one laboratory has shown increased trabecular accumulation both in vitro [54], probably because of increased trabecular surface area, and in trabecular rods (but not plates) in vivo [46]. Trabecular rods represent older bone, and this may be the reason for the difference in glycation between rods and plates. Although there are many different AGEs (vesperlysine, pentosidine, carboxymethyl-lysine, glucosepane, imidazolone, furosine), quantitative measurements in bone are based on measurements of a single AGE marker, pentosidine, which is one of only two AGEs that can be measured accurately in bone. The other, carboxymethyl-lysine (CML), is present in bone in amounts 40–100 times greater than pentosidine. Although these AGEs accumulate with age, it is not clear whether the other AGEs do, or whether they accumulate at the same rates and amounts, or have the same effects on bone matrix properties.

AGEs form in the middle of the collagen fibrils, and restrict deformation (plasticity) of the collagen fibers, reducing bone strength and toughness by making the tissue more brittle (Fig. 4). A single not very common AGE, pentosidine, has been reported to reduce trabecular ductility by 9% [56], and is associated with 23% of the variation in bone toughness [46]. Moreover, they have been shown to alter the interface between collagen and hydroxyapatite [57], in particular the spacing between them. The now well-documented age-related loss of post-yield energy absorption capacity of bone [20,58–60] and reduced plasticity [56] and toughness [1,8,42] has been attributed in large part to the natural accumulation of these collagen bonds. Although the enzymatic trivalent crosslinks may enhance bone toughness [61], they are not sufficient to offset the decline in bone toughness with age because they do not increase or decrease with age. Bone's reduced capacity to absorb energy prior to fracture makes the bone more prone to fracture, particularly fractures generated by impact (as during a fall on the hip). This may be partly because the loss of ductility prevents stress relaxation that is the result of crack initiation, or because the change in matrix properties facilitates crack growth [62–64].

The accumulation of AGEs in the extracellular matrix of bone also has cell-dependent effects that are important to aging. AGEs can impair the proliferation and differentiation of osteoblasts [65,66] through interaction with the AGE-specific receptor (RAGE) [67]. Binding of AGE to its receptor reduces osteocalcin secretion (which regulates mineral crystal size, see above), and causes disruptions in cell-matrix interaction and cell adhesion [68], and increased osteoblast apoptosis [65], that ultimately affect bone formation [66]. Further, binding to RAGE activates NF-κB in osteoblasts and stimulates the production of cytokines such as IL-1β, IL-6, and TGF-α or β that alter both osteoclastogenesis and osteoclast activity and can promote resorption [69–71]. Paradoxically, however, others have reported that osteoclastic resorption is slowed in the presence of AGEs, in part, perhaps, because the solubility of collagen is reduced [72]. The AGE-RAGE binding interaction also upregulates the production of reactive oxygen species (ROS), which increase with age [73], and elevates inflammation in the bone microenvironment that can lead to bone loss [74–76]. Because ROS both antagonizes Wnt signaling in pre-osteoblasts [77], and promotes osteoclast differentiation [78,79], the effect of the AGE-RAGE complex on ROs likely exacerbates both increased bone resorption and reduced bone formation that accompany the aging process.

To summarize, AGEs accumulate with age in bone and have significant negative effects on bone matrix mechanical properties. With aging, AGE accumulation may decrease collagen's ability to deform by as much as 25% [8], which would impose more strain on the mineral platelets [80]. Furthermore, through interactions with the RAGE receptor, they alter the balance of resorption and formation in ways that can lead to bone loss.

3. Bone water

Although its role in bone is often ignored, water composes about 10–20% of the volume of cortical bone, but can drop to 5% in old age [81,82], which can have a profound negative impact on its mechanical behavior. As recently as 2012, the composition of bone lamellae was described as “a hierarchical composite of collagen fibrils, mineral and NCPs” [noncollagenous proteins], with no mention of a hydrated layer between the collagen and mineral [83]. Water at interfaces between the bone matrix components, or within them, can affect collagen fiber diameter and orientation, folding and other dynamic properties of protein formation and conformation [84–86]. Water in bone is found both in a free flowing form in the pores and spaces in bone tissue (vascular canals as well as in the lacunar-canalicular network), or bound to collagen or mineral (Fig. 3). The pore water is free-flowing and exchangeable on a nearly 1:1 basis with bone mineral (i.e. the more bone you have, the fewer pores and free water you have; [87]). Consequently, age-related increases in bone porosity in vivo can be estimated using short echo time magnetic resonance imaging [88], or by any means that can estimate a change in porosity. This extracellular bone fluid not only nourishes the cells but may also transmit signals from one cell to another through streaming potentials or as increased shear stress on cell walls. It also provides a site for the exchange of materials between the vascular system and the bone, and may be important in calcium exchange or in calcium signaling to the bone cells. The extracellular fluid space is large because of the large number of canaliculi connecting the cells in bone, and all of this space is available for ion exchange.

Estimates vary, but bound water may account for as much as 40–60% of the total water in bone. Most of the water in bone is bound to mineral or collagen [89]. There are three sub-compartments of matrix bound water, each playing a different role (Fig. 5). *Loosely bound water* is found at the collagen-mineral interface and is important to transfer load between collagen and mineral [90]. This allows sliding

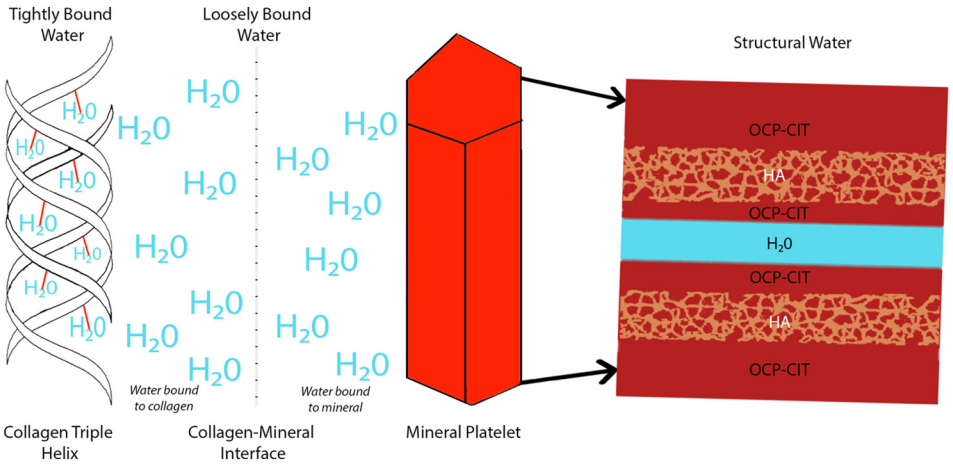

Fig. 5. Bound water in bone can be either tightly bound within the collagen triple helix, or loosely bound at the interface between collagen and mineral. Loosely bound water can either be associated with the collagen molecule (collagen-bound water), or associated with the mineral platelet itself (mineral-bound water). There is also a component of the bound water fraction that is found between the mineral platelets (structural water). The hydroxyapatite (HA) itself is surrounded by octacalcium phosphate-citrate (OCP-CIT) that separates the HA from the water between the plates. With aging, water is moved from the loosely bound compartment, to the more tightly bound compartments, causing bone to become stiffer and probably less tough.

between these two components of bone [91], dissipating energy and reducing shear stresses at the interface to increase bone's ductility [90]. The effect may be loading mode-dependent, having different mechanical effects in compression and tension [90]. Portions of the collagen molecule (e.g. hydroxyproline) are hydrophilic, promoting the binding of water to this surface [92]. Some of the water at the collagen interface is also mineral-bound [93], and may contribute to orientation of the mineral crystals in the c-axis direction through interaction with an amorphous calcium phosphate layer [94,95]. It also has been speculated that collagen-bound water is exchangeable with mineral as the newly formed bone (osteoid) becomes more mineralized with time [96], but because some of the water is mineral-bound, it is not completely exchangeable. Collagen-bound and mineral-bound water at this interface may contribute differently to the mechanical properties of bone, with the collagen-bound fraction associated positively with toughness, and mineral-bound water correlated negatively with elastic modulus [97]. It may be the water bound to mineral in the gap regions between collagen fibrils that has the greatest effect on bone toughness [98]. *Tightly bound water* is found within the collagen triple helix itself, and helps to stabilize the collagen. *Structural water* is found within the crystal lattice of the carbonated apatite, helping to bridge the octacalcium-phosphate citrate complex across mineral platelets, and regulating the accumulation of mineral [14]. Structural water also provides hydrogen-bonding bridges between ions in the apatite crystal, providing additional structural stability [93].

The precise ratio of bound and unbound water in bone depends on a number of factors, including age. The bound water fraction correlates positively with bone toughness [99,100], both decreasing with age [42,96,101,102]. The loss of water with age, and consequent reduction in toughness, may be partly dependent on changes to the glycosaminoglycans (GAGs, in particular chondroitin sulfate) and proteoglycans such as biglycan in bone. The GAG side chain of the proteoglycan molecule is negatively charged and known to be hydrophilic [103], so loss of proteoglycans would reduce the attraction of water. The biglycan, which binds to collagen in the peri-lacunar matrix and is also found in osteocytes, is thought to have shear sensing properties, suggesting that it may also have a role in mechanical adaptation that would affected by the loss of water. Wang et al. [104,105] found a 17% reduction in matrix GAGs between the third decade and 8th decades of life (approximately ages 26–73), associated with a 25–30% reduction in toughness. This was accompanied by a 40% decrease in the overall bound water fraction (Fig. 6). The reduction in bound water accounts for 71% of the age-related reduction in bone toughness [105]. In addition to binding water, GAGs may also regulate sclerostin function by interfering with the interaction between LRP5/6 and sclerostin [106]. Thus a reduction in sulfated GAG content could negatively affect Wnt signaling and be one cause for the lower bone formation that occurs

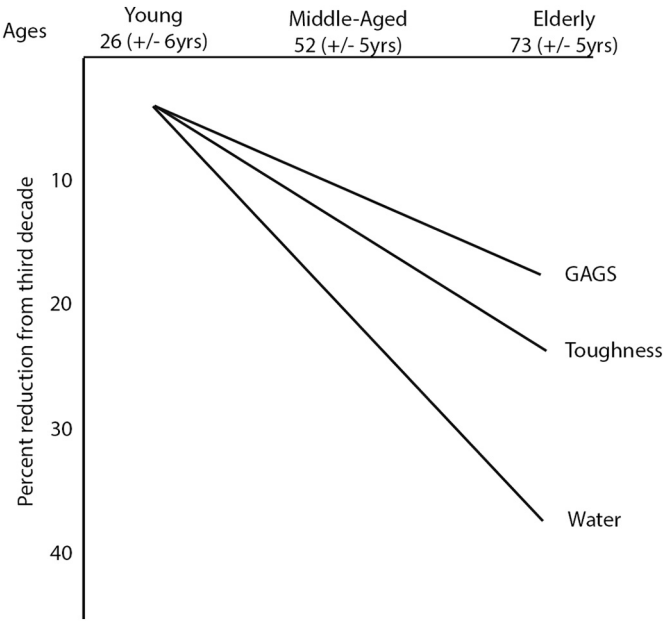

Fig. 6. Between young adults in their third decade, and elderly adults in their 8th, there is a 15–20% reduction in glycosaminoglycans, and a 40% decline in bound water, both of which contribute to a significant decline in bone toughness.
(Based on data from Wang et al. [105].)

with aging.

There also is a re-location of water from the loosely bound fraction to the more tightly bound fraction that may be more significant than the overall decline in bound water. The loosely bound water that exists at the collagen-mineral interface provides some stress relief at that interface as it allows collagen and mineral to slide against each other more easily [107,108], altering load transfer between collagen and mineral [109]. However, little is known about the age-related changes in residual stresses at the interface between collagen and apatite, nor whether age-related changes that may exist would affect fracture toughness [109]. Because some of the water is bound to mineral, though, this also may provide energy dissipation of the mineral crystals sliding across each other [108,110]. The interaction energies between collagen and mineral, or between mineral crystals themselves, are apparently much larger than the bonding between collagen and the mineral platelets [111,112]. The collagen-mineral and mineral-mineral sliding increases the ductility of the bone [113], increasing its plastic (or post-yield) potential for deformation, making it less likely to fracture by increasing its ability to deform [114]. It may also protect the

collagen from shear stresses during deformation [93], or provide stress relaxation in part by breaking collagen cross-links [108], which may act somewhat like “sacrificial bonds” [115,116]. Loss of water at this interface may also reduce collagen solubility [117], which in itself could have an independent effect on energy transfer between collagen and mineral. Consequently, the movement of water from this loosely bound fraction between the collagen and mineral components of the bone matrix, to the more tightly bound fraction, would reduce the bonding between collagen and mineral and may allow the collagen to fail in a quasi-brittle fashion. Loss of loosely bound water at this interface is associated with both reduced bending strength and energy to fracture [96].

Interestingly, there is a weak negative association between pentosidine, an AGE, and bound water [96]. Loss of water in bone tissue can initiate glycation reactions that cause the accumulation of AGEs [118], with consequences to bone toughness and stiffness. Alternatively, increasing the stability of the collagen framework by cross-linking could prevent the collagen from binding water [119]. Which change occurs first is not entirely clear, but what has been well-defined is that the processes are linked in some way. In any event, the decrease in toughness that occurs with age could be through a deterministic combination of factors that include increased AGE accumulation, and reduction in bound water, especially in the loosely bound fraction.

4. Non-collagenous proteins

Clearly, proteoglycans and GAGs are critical to bone's hydration, and contribute in positive ways to the mechanical properties of bone matrix. Other noncollagenous proteins (NCPs) such as osteocalcin and osteopontin affect matrix properties in part by co-regulating crystal size, shape and orientation in the hydroxyapatite mineral lattice, by regulating carbonate substitutions into the lattice, or by altering mineral nucleation onto the collagen scaffold [13]. Osteopontin, for example, is known to inhibit mineral formation and to delay nucleation [120], but has also been suggested as a bone matrix “glue” that contributes to intrafibrillar sacrificial bonds, allowing greater stress relaxation and deformation prior to failure [121,122]. What the balance of these various functions is on matrix mechanical properties is not clear. Besides binding water, GAGs are known to stabilize the collagen fibrils by binding them together [122]. NCPs likely contribute to load transfer at several hierarchical levels in bone matrix [83], in part because of their localization at the collagen-apatite interface [123,124]. The absence of both osteocalcin and osteopontin in genetically-modified mouse models is known to be associated with reduced fracture toughness [125]. Yet we know very little about how the quantity of NCPs (other than GAGs, which decrease) changes with age, or whether this contributes to the degradation of bone matrix quality in older individuals.

Osteocalcin (OCN) is secreted by mature osteoblasts and by osteocytes, and is known to boost calcium binding and regulate hydroxyapatite crystal growth. However, it may also promote osteoclast differentiation and stimulate osteoclast activity, increasing resorption. There is some evidence that circulating OCN decreases with age [126,127], with consequent declines in, and altered distribution of, the OCN found in bone matrix in both men and women [128], as well as in experimental animals such as mice and monkeys [129]. Bone with a younger mean tissue age (such as osteonal bone) has up to 20 times more OCN than bone with greater mean tissue age (e.g. interstitial bone) [130]. In mice, the lack of osteocalcin is associated with increased net bone formation, increasing trabecular bone volume and periosteal apposition [131]. The effect on bone matrix of these age-related changes in OCN is not known.

The evidence that does exist suggests that the quantity of some of the more ubiquitous SIBLING proteins, such as osteopontin (OPN), do not change with aging [132], but their composition may change, and this could have an effect on matrix properties. Sroga and Vashishth [132] recently reported that phosphorylation of bone matrix proteins in general declines by 20% by the age of 80, and phosphorylation of OPN may decline by as much as 30%. Interestingly, the changes were dimorphic, with elderly women showing reduced phosphorylation of all bone matrix proteins compared to older men (> 75 years old). There were slightly lower levels of OPN phosphorylation specifically in older women compared to men as well, though not significantly so. The reduction in OPN phosphorylation appears to begin in middle age (ages 55–75), and progress.

The significance of this is that bones with higher phosphorylation levels resisted crack growth to a greater degree (i.e., higher propagation toughness). Sroga and Vashishth [132] suggest that the decreased phosphorylation with aging would alter the negatively charged OPN molecule and negatively affect the process of OPN unfolding and replacement by calcium ions on the hydroxyapatite surface. This unfolding is an energy dissipative mechanism; its absence would limit energy dissipation and reduce the propagation toughness of bone to allow cracks to grow more easily.

5. Conclusion

Age-related changes in bone matrix affect different components of the matrix in different ways, but effects in one component can have profound implications for changes of other components, and ultimately for the deterioration of mechanical properties associated with aging. The carbonated apatite, collagen and its cross-links, water and non-collagenous proteins all interact with each other to create an overall mechanical environment, and alterations in one inevitably affect the others. The increased brittleness that occurs with age, contrary to previous thought, does not appear to be the product of increased mineralization or reduced mineral heterogeneity, but rather related to change to the collagen, and the interface between collagen and mineral. The accumulation of AGEs with age causes increased brittleness, and the reduction in loosely bound water at the interface of collagen and mineral reduces the capacity of older bone to dissipate energy and resist fracture. Preventing or reducing these two processes would maintain the ideal properties of young bone to resist fracture.

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