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ORIGINAL ARTICLE

Age-related Changes in the Collagen Network and Toughness of Bone

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The hypothesis of this study is that the mechanical integrity of the collagen network in bone deteriorates with age, and such adverse changes correlate with the decreased toughness of aged bone. To test the hypothesis, 30 human cadaveric femurs from donors ranging from 19 to 89 years of age were tested to determine the age-related changes in the mechanical properties of demineralized bone and fresh bone samples. Along with bone porosity, bone density, and weight fractions of the mineral and organic phases, collagen denaturation and concentrations of collagen cross-links (HP, hydroxylysylpyridinoline; LP, lysylpyridinoline; PE, pentosidine) were determined for these bone specimens as a function age. Analysis of variance (ANOVA) showed that age-dependent changes were reflected in the decreased strength, work to fracture, and fracture toughness of bone; in the decreased strength, elastic modulus, and work to fracture of the collagen network; as well as in the increased concentration of pentosidine (a marker of nonenzymatic glycation) and increased bone porosity. Regression analyses of the measured parameters showed that the age-related decrease in work to fracture of bone (especially its postyield portion) correlated significantly with deterioration in the mechanical integrity of the collagen network. The results of this study indicate that the adverse changes in the collagen network occur as people age and such changes may lead to the decreased toughness of bone. Also, the results suggest that nonenzymatic glycation may be an important contributing factor causing changes in collagen and, consequently, leading to the age-related deterioration of bone quality. (Bone 31:1–7; 2002)

Key Words: Bone; Toughness; Collagen; Denaturation; Cross-links; Aging.

Introduction

Over the years, loss of bone mineral or bone mass has been considered the major cause of age-related bone fractures.[36,38] However, the large overlap in bone density that exists between healthy individuals and patients who sustain bone fractures suggests that low bone density is not the only reason for the weakening of bone.[37] A recent study reported that the risk of bone fracture for older women (average 75 years old) is about 7%, whereas such a risk is only 1% for much younger individuals (average 45 years old), although they have a similar bone density level.[23] In addition, it has been found that, although elderly black Gambian women also experience loss of bone mass, they rarely suffer osteoporotic bone fractures as compared with their white counterparts.[2]

Bone is a natural composite comprising mineral (mainly hydroxyapatite), organic (mostly type I collagen), and water phases.[25,28] Thus, the biomechanical properties of bone are dependent on the quality and spatial arrangement of these constituents.[35] Recent studies have shown that the mineral predominantly contributes to bone stiffness,[42,43] whereas the quality of collagen matrix may predominantly determine the toughness of bone.[10,12,15,47,49] In addition, it was found that osteoporosis is not just a simple loss of bone mass, but involves significant changes in the biochemical and physical properties of the collagen network.[27] Thus far, only a few studies on the age-related changes in collagen and their correlation with the toughness of bone have been reported in the literature.[47,49] Although these studies have demonstrated the involvement of collagen in age-related changes in bone quality, the underlying mechanisms are still not clear.

The hypothesis of this study is that the mechanical integrity of the collagen network in human bone deteriorates with age, and such adverse changes correlate with the decreased toughness of aged bone. To test this hypothesis, we examined age-related changes in collagen molecular structures, the mechanical integrity of the collagen network, and the mechanical properties of bone. Finally, we attempted to explore the correlation of age-related changes in the collagen network with the toughness of bone.

Materials and Methods

Thirty fresh frozen human cadaveric femurs were acquired from the Musculoskeletal Transplant Foundation (Edison, NJ) and a local tissue bank, ranging between 19 and 89 years of age. All samples were screened carefully to avoid the influence of any bone-related pathologies. These samples were divided into three age groups: young adults (19–49 years old); middle-aged (50–69 years old); and elderly (>70 years old). Each group included ten samples (n = 10). Eight men and two women were included in both the young adult group and the middle-aged group, and four men and six women in the elderly group. For preparation of test specimens, a 40-mm-long section from the anterior aspect of the middiaphysis was excised from each femur. Two small bone coupons (1 mm × 4 mm × 4 mm) were used to

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assess the amount of collagen denaturation and the concentration of collagen cross-links, respectively. In addition, two bone coupons (30 mm × 4 mm × 2 mm) were obtained along the longitudinal axis of bone using a diamond saw and a benchtop milling machine under copious irrigation. One of them was used to perform fracture toughness tests in a three-point bending configuration to assess the transverse fracture toughness of bone. The other was used to determine the elastic modulus, yield strength, ultimate strength, and work to fracture of bone in three-point bending tests. All mechanical test specimens were kept moist throughout the preparation and testing, and were tested at room temperature.

To assess the fracture toughness of bone, a precrack was introduced in the middle of bone specimens using a circular saw and a sharp razor blade. Test specimens were supported in a custom-designed fixture with a support span of 16 mm. The specimens were loaded in the middle until fracture at a loading rate of 5 mm/min on materials testing machine (Model 1011, Instron Corp., Canton, MA). The curve of load vs. displacement was recorded, and fracture toughness values (critical stress intensity factor, K_{IC}) were calculated following ASTM Standard E399.[4] Because the precrack created in the specimens propagates in a direction perpendicular to the longitudinal axis of bone, the property measured in this test was actually the transverse fracture toughness of bone.

The bending properties of bone were determined by loading bone specimens to failure in a three-point bending configuration with a 16 mm support span. The loading rate was 5 mm/min. The elastic modulus and strength of bone were determined based on the load vs. deflection curve using the beam flexure theory as described in ASTM Standard D790-86.[3] Also, the work to fracture was calculated as the area under the curve of load vs. displacement. Because there were small variations (<2%) in the size of bending specimens used in this study, the work to fracture actually represented of the toughness of the specimens. Because of the limited availability of bone stock, specimen sizes in the fracture toughness and three-point bending tests were smaller than those recommended in the standards. However, a relative comparison between these specimens should be valid because they had the same shape and size and were tested under identical conditions.

Flat dog-bone-shaped tensile specimens were prepared to determine the mechanical behavior of the collagen network (demineralized bone). Before demineralization, 8 mm portions at both ends of the specimens were embedded in a plastic block to facilitate gripping of specimens during the test. The overall length of the specimen was 30 mm, and the gage length, width, and thickness were 10 mm, 2 mm, and 2 mm, respectively (**Figure 1**). Then, the bone samples were immersed in a buffered (pH 7.4) 0.5 mol/L ethylene-diamine tetraacetic acid (EDTA) solution at 4°C for 6 weeks with frequent changes of the solution until complete removal of the mineral phase. After demineralization, the specimens were washed three times in distilled water for 10 min and then preserved in a phosphorus-buffered saline solution with 0.02% sodium azide and 50 mmol/L EDTA. These specimens were tested under wet condition at room temperature and were extended to failure in the Instron materials testing machine (Model 1011) at a loading rate of 10 mm/min. The load-deformation curve was recorded and the elastic modulus (E), ultimate strength (σ_{sc}), work to fracture (W_{fc}), and strain to fracture (ε_{fc}) were determined as shown in **Figure 2**.

A selective digestion technique was used to determine the amount of denatured collagen molecules (percentage of the total amount of collagen), as described elsewhere.[9] Also, a simplified high-performance liquid chromatographic assay was used to quantify the pyridinium and pentosidine cross-links in the collagen network.[8] Briefly, bone samples were hydrolyzed at 110°C and vacuum dried. The hydrolysate then was dissolved in water containing 10 nmol pyridoxine/mL and 2.4 μmol homoarginine/mL as internal standards, and diluted fivefold in dilution solution (0.5% [v/v] heptafluorobutyric acid in 10% [v/v] acetonitrile). A two-isocratic-step chromatography was run together with a programmed fluorimeter. Finally, the concentrations of hydroxylysylpyridinoline, lysylpyridinoline, and pentosidine cross-links were measured and normalized by the total amount of collagen as moles per mole collagen. The column used was a Waters S5 ODS2 column (4.6 mm × 150 mm), packed with 5 μm spherical silica particles with 80 Å pores.

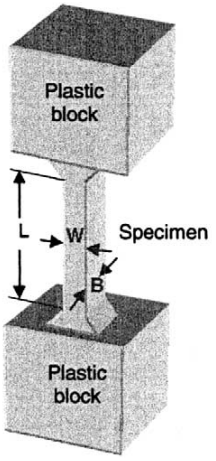

Figure 1. Schematic representation of the tensile test specimen for testing demineralized bone samples.

The weight fractions of mineral and organic phases of bone were determined using the specimens tested in the fracture toughness test. The bone specimens were weighed in air under wet and dry conditions to record wet and dry weights (dried in a vacuum oven overnight at 70°C). The bone samples then were ashed at 800°C for 3 hs.[40] The weight of ash residue was considered to be the weight of the bone mineral and the discrepancy between dry weight and ash weight was considered the weight of the organic phase. The weight fractions of mineral and organic phases were calculated by dividing the weight of ash

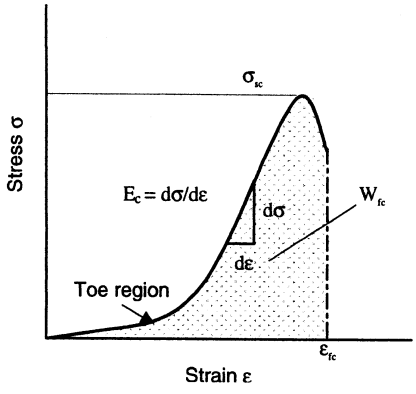

Figure 2. Determination of mechanical parameters for demineralized bone samples using their strain-stress curves (E_c, modulus of elasticity; W_{fc}, work to fracture; σ_{fc}, failure strength; ε_{fc}, strain at fracture of the collagen network).

Table 1. Mechanical properties of bone as a function of age (n = 10)

Age groups	σ_s (MPa)	σ_y (MPa)	E (GPa)	W_f (N · mm)	W_{fc} (N · mm)	W_{fp} (N · mm)	K_{IC} (MPa$\sqrt{m}$)
Young	281 ± 35.5	227 ± 23.8	11.5 ± 1.99	118 ± 35.3	44.2 ± 9.92	74.0 ± 28.0	5.09 ± 0.98
Middleaged	270 ± 30.8	221 ± 31.9	11.1 ± 0.78	95.0 ± 19.1[a]	40.4 ± 6.75	53.8 ± 19.8[a]	5.39 ± 0.82
Elderly	227 ± 35.7[a,b]	191 ± 35.9[a]	10.7 ± 1.34	71.8 ± 16.6[a,b]	31.8 ± 8.99[a]	40.0 ± 15.6[a,b]	4.28 ± 0.79[a,b]
ANOVA	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

KEY: σ_s, ultimate strength; σ_y, yield strength; ANOVA, analysis of variance; E, elastic modulus; K_{IC}, fracture toughness of bone; W_f, total work to fracture; W_{fc} and W_{fp}, elastic and postyield portions of W.
[a]Statistically significant difference compared with young group ($p < 0.05$).
[b]Statistically significant difference vs. middle-aged group ($p < 0.05$).

residue and weight of organic phase by wet weight of bone, respectively. Finally, the density of bone (d_b) was determined by dividing the wet weight by the volume of bone specimens.

The measurements of porosity were performed on the remaining half of the specimen used for the fracture toughness test. The cross section adjacent to the fracture site was embedded in a plastic resin and polished following standard procedures for engineering materials. An image of the cross section was digitized into a computer via a CCD camera attached to a light microscope. An image processing and computational code written in a macroprogramming language (NIH IMAGE, National Institutes of Health, Bethesda, MD) was then used to calculate the ratio of the area of cavities (haversian and vascular canals) to the entire cross-sectional area. This ratio was approximated as the porosity of bone. To ensure accuracy of the measurements, manual enhancement of contrast in the digitized images between pores and bone tissues were performed.

All experimental data were compiled as mean ± standard deviation. Analysis of variance (ANOVA) was performed to examine the effects of age on all parameters measured, and post hoc multiple comparisons (using Fisher's protected least significant difference test) were made to detect significant differences between the groups. In addition, Pearson's product moment correlation coefficients were calculated to account for the relationship between all age-dependent parameters measured in this study. The significance of the correlation between any two parameters was determined using Fisher's r-to-z transformation. Furthermore, a direct multiple regression was performed to determine the contribution of bone porosity and collagen network strength to bone biomechanical properties. Differences and correlations were considered significant at $p < 0.05$.

Results

ANOVA analyses indicated that age had significant effects on the mechanical integrity of bone (**Table 1**). Although neither the ultimate nor yield strength (σ_s and σ_y) of bone showed any significant difference for the young and middle-aged groups, they decreased significantly for the elderly group. In contrast, aging exhibited little effect on the elastic modulus (E) of bone. The work to fracture (W_f) of bone decreased with increasing age. Interestingly, it was observed that the resilience of bone (W_{fc}, total elastic energy absorbed in preyield deformation) was consistent for young and middle-aged bone samples, but decreased by almost 30% for elderly ones. However, the energy absorbed during postyield deformation (W_{fp}) appeared to decrease continuously with increasing age. Moreover, the transverse fracture toughness of bone showed a significant decrease only for elderly bone samples, with no significant changes between young and middle-aged ones.

As to the mechanical integrity of the collagen network in bone (**Table 2**), it was observed that its failure strength (σ_{sc}), elastic modulus (E_c), and work to fracture (W_{fc}) decreased significantly for elderly bone samples, with no significant difference between young and middle-aged groups. However, the strain to fracture (ε_{fc}) of the collagen network showed little change with increasing age.

The constituents and microstructure of bone also demonstrated significant changes with increasing age. The concentration of pentosidine (PE) cross-links (the marker of nonenzymatic glycation-induced cross-links) exhibited a significant increase with the increasing age, whereas the denaturation of collagen (%DC) and concentrations of the other two enzymatic collagen cross-links (i.e., hydroxylysylpyridinoline [HP] and lysylpyridinoline [LP]) showed few age-related changes (**Table 3**). Moreover, the porosity of bone demonstrated a significant increase with increasing age. Correspondingly, the weight fraction of the mineral phase exhibited a slight decrease for middle-aged and elderly bone samples. Finally, significant changes in bone density were observed only in middle-aged bone samples as compared with the young group (**Table 4**).

Pearson's product moment correlation coefficients were calculated to assess the correlation of mechanical integrity of the collagen network, porosity of bone, and pentosidine cross-links with the age-dependent biomechanical properties of bone (**Table 5**). The porosity of bone exhibited a significant correlation with strength, elastic modulus, and work to fracture (both elastic and postyield portions), but had little correlation with transverse fracture toughness of bone. In addition, bone porosity showed no significant correlation with the biomechanical properties of the

Table 2. Mechanical properties of the collagen network (demineralized bone) as a function of age (n = 10)

Age groups	σ_{sc} (MPa)	E_c (MPa)	W_{fc} (N · mm)	ε_{fc}
Young	23.8 ± 7.15	209 ± 36.1	63.3 ± 24.2	0.155 ± 0.052
Middle-aged	20.2 ± 7.68	207 ± 36.4	48.3 ± 29.1	0.141 ± 0.052
Elderly	15.4 ± 5.55[a,b]	143 ± 33.1[a,b]	35.8 ± 17.1[a]	0.135 ± 0.028
ANOVA	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$

KEY: ANOVA, analysis of variance; ε_{fc}, strain to fracture; σ_{sc}, failure strength; E_c, elastic modulus; W_{fc}, work to fracture of the collagen network.
[a]Statistically significant difference vs. young group ($p < 0.05$). [b]Statistically significant difference vs. middle-aged group ($p < 0.05$).

Table 3. Collagen denaturation and collagen cross-links as a function of age (n = 10)

Age groups	%DC	PE (mmol/mol)	LP (mol/mol)	HP (mol/mol)
Young	8.46 ± 1.81	0.44 ± 0.21	0.188 ± 0.058	0.376 ± 0.076
Middle-aged	8.73 ± 2.17	0.90 ± 0.23[a]	0.177 ± 0.041	0.376 ± 0.077
Elderly	8.85 ± 1.73	1.39 ± 0.29[a,b]	0.196 ± 0.061	0.395 ± 0.106
ANOVA	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$

KEY: ANOVA, analysis of variance; %DC, percent denatured collagen; HP, hydroxylysylpyridinoline; LP, lysylpyridinoline; PE, pentosidine.
[a]Statistically significant difference vs. young group ($p < 0.05$).
[b]Statistically significant difference vs. middle-aged group.

collagen network. In contrast, the mechanical integrity of the collagen network (e.g., σ_{sc}, E_c, and W_{fc}) exhibited little correlation with the strength and elastic modulus of bone, but correlated significantly with the work to fracture (W_f) of bone, especially with the postyield portion of W_f. Moreover, the concentration of PE demonstrated significant correlations with most mechanical properties of bone.

Using strength of the collagen network and bone porosity as independent variables, a direct multiple regression analysis was performed to determine their contributions to the mechanical properties of bone, because these two parameters are independent and representative of the collagen network and bone microstructural properties (**Table 6**). It was found that bone porosity contributes predominantly to the ultimate and yield strength (σ_s and σ_y), elastic modulus (E), and work to fracture (W_f) including both the elastic and postyield portions, but had little effect on the fracture toughness (K_{IC}). On the other hand, the mechanical integrity of the collagen network, represented by the failure strength (σ_{sc}), showed no significant correlation with ultimate and yield strength (σ_s and σ_y), elastic modulus (E), and fracture toughness (K_{IC}) of bone, but contributed significantly to the work to fracture (W_f), especially to the postyield portion (W_{fp}) of W_f.

Discussion

To test the hypothesis that the mechanical integrity of the collagen network in bone deteriorates with age, and such changes correlate with the age-related decrease in the toughness of bone, we measured the mechanical properties of the demineralized bone and bone samples (n = 30) acquired from a broad range of ages (19–89 years), and determined their compositional and microstructural characteristics. The experimental results of this study indicate that the mechanical integrity of the collagen network deteriorates with increasing age and correlates significantly with the decreased work to fracture (W_f) of aged bone, thereby supporting the hypothesis of the present study.

Several limitations exist for interpreting the results of this study. First, bone is an anisotropic material with collagen fibrils and mineral crystals oriented at various angles with respect to the long axis of bone.[21,29,30,48] Hence, age-related changes in collagen may be orientation-dependent, and may have different effects on the mechanical behavior of bone in other orientations.[31] In this study, however, only transverse fracture toughness of bone, longitudinal tensile properties of the collagen network, and transverse bending properties of intact bone samples were measured. Thus, the results of this study are meaningful only for the orientations tested. Second, the selective digestion technique has its limits in determining the amount of denatured collagen molecules. This technique requires that α-chymotrypsin be able to completely digest the denatured collagen molecules. However, some denatured collagen molecules may not be digested by α-chymotrypsin due to the varied concentration of collagen cross-links between the molecules. For instance, a preliminary study by our group has shown that ribose-treated demineralized bone samples exhibited far fewer denatured collagen molecules compared with controls (no treatment). This suggests that the nonenzymatic glycation-induced cross-links may inhibit the α-chymotrypsin digestion of denatured collagen molecules. In this study, an age-related increase in nonenzymatic glycation-induced crosslinks was observed. Thus, it is possible that the amount of denatured collagen in aged bone samples is underestimated. This issue is presently being investigated by our group. Finally, pentosidine is a senescent cross-link and the only measurable one of the advanced glycation end products. Thus, in this study pentosidine was used as a marker to assess the nonenzymatic glycation-induced cross-links. However, it is unclear whether the concentration of pentosidine is proportionally related to the concentrations of the other advanced glycation end products. Thus, only a rough and qualitative assessment can be made for the effect of the nonenzymatic cross-links in bone using the pentosidine concentration.

This study has revealed for the first time that the mechanical properties of the collagen network are age-dependent. Such changes are reflected by a 35% decrease in failure strength ($p < 0.05$), a nearly 30% decrease in elastic modulus ($p < 0.05$), an almost 50% decrease in work to fracture ($p < 0.05$), and a 10% but not statistically significant decrease in strain to fracture ($p > 0.05$) with increasing age. The mechanical properties of demin-

Table 4. Microstructural and compositional changes in bone (n = 10)

Age groups	PO (%)	WF_m (%)	WF_o (%)	d_b (g/cm^3)
Young	8.59 ± 1.86	57.9 ± 1.37	35.2 ± 0.94	1.95 ± 0.04
Middle-aged	12.7 ± 2.64[a]	55.7 ± 1.50[a]	35.3 ± 1.08	1.90 ± 0.04[a]
Elderly	13.2 ± 3.55[a]	56.0 ± 1.42[a]	34.9 ± 0.84	1.92 ± 0.05
ANOVA	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$

KEY: ANOVA, analysis of variance; d_b, bone density; Po, bone porosity; WF_m, weight fraction of the mineral phase; WF_o, weight fraction of the organic phase.
No significant differences in all the parameters between middle-aged and elderly groups.
[a]Statistically significant difference vs. young group ($p < 0.05$).

Table 5. Correlations between mechanical properties of collagen network and bone (n = 30)

	Bone properties						
	σ_s	σ_y	E	W_f	W_{fc}	W_{fp}	K_{IC}
Collagen network properties							
σ_{sc}	0.33	0.14	0.22	0.54*	0.38*	0.54[a]	0.34
E_c	0.28	0.19	0.09	0.36	0.25	0.36	0.12
W_{fc}	0.31	0.17	0.21	0.54*	0.36	0.54[a]	0.30
Po	−0.56[a]	−0.43[a]	−0.52[a]	−0.48*	−0.56[a]	−0.38[a]	−0.14
PE	−0.55[a]	−0.39[a]	−0.33	−0.62*	−0.54[a]	−0.58[a]	−0.48[a]

KEY: bone properties: σ_s, ultimate strength; σ_y, yield strength; E, elastic modulus; W_f, work to fracture; W_{fc}, elastic portion of W_f; W_{fp}, postyield portion of W_f; K_{IC}, fracture toughness of bone; Po, bone porosity; collagen network properties: σ_{sc}, failure strength; E_c, elastic modulus; W_{fc}, work to fracture; PE, pentosidine cross-link concentration.
[a]Statistically significant correlation ($p < 0.05$).

eralized bone samples determined in this study are in a good agreement with those reported elsewhere.[14] Thus far, numerous investigations have focused on age-related changes in the chemistry of collagen itself.[5,6,26,32] Very few have been directed toward the understanding of the role of collagen in the decreased toughness of aged bone.[17,47,49] A study by Danielsen et al. found that shrinkage temperature decreases with the chronological age of human bone collagen.[17] In addition, Danielsen et al. reported that ultimate strength and maximum stiffness of demineralized bone specimens of male rats decrease with age.[16] Similarly, Zioupos et al. measured the shrinkage temperature and the rate of contraction of demineralized bone samples during isometric heating, and found that age-related changes in the collagen network are manifested by the decreased shrinkage temperature and the decreased maximum changing rate of contraction force.[49] It is obvious that the mechanical integrity and thermal stability of the collagen network are interrelated. Hence, it is presumable that the integrity of collagen molecules and the concentration of cross-links in between the molecules are the dominant factors affecting the integrity of the collagen network in bone.

Age-related changes in mature enzymatic collagen cross-links have been controversial. For instance, several recent studies have reported that the concentration of hydroxylysylpyridinoline and lysylpyridinoline cross-links in human bone are not age-dependent.[5,49] However, another recent study demonstrated that the levels of hydroxylysylpyridinoline and lysylpyridinoline are age-related, and these pyridinium cross-links have a strong and positive correlation with bone strength.[39] The present study has demonstrated that these mature collagen cross-links are constant irrespective of increasing age. Also, our experiments have shown that hydroxylysylpyridinoline and lysylpyridinoline cross-links are very stable and may survive even when bone samples are heated at 200°C or gamma-irradiated at 60 kGy.[11] These results suggest that it is unlikely that these mature collagen cross-links are sensitive to the aging process of bone.

Bone is a composite consisting of mineral, organic, and water phases.[25] Thus, its mechanical properties are dependent upon the quality, spatial arrangement, and interaction of its constituents. It has been shown that the mineral phase most likely imparts stiffness to bone, whereas the more compliant collagen network contributes predominantly to the toughness of the tissue.[24,47,49] Evidence from previous studies also demonstrated that increased bone porosity (or bone mass loss) is a primary contributing factor in age-related bone fractures.[1,18] The results of the present study are consistent with previous studies, showing that bone porosity increases with age (Table 4), and correlates significantly with bone strength and stiffness. However, age-related changes in mechanical integrity of the collagen network also appear to have a significant effect on the work to fracture of bone, especially on the postyield property of bone (Table 6). It is noteworthy that neither bone porosity nor collagen integrity correlated significantly with the transverse fracture toughness of bone, suggesting that crack propagation in the transverse direction is not sensitive to either bone porosity or mechanical integrity of the collagen network. In fact, the toughening mechanism of bone has been reported to rely heavily on microcrack formation around the tip of propagating crack.[45]

The nonenzymatic cross-links in collagen are formed by a so-called Maillard reaction with sugar.[34,41] Thus far, pentosidine has been commonly used to assess the nonenzymatic glycation end products.[7,44] The effect of such nonenzymatic modifications of collagen on the mechanical integrity of bone has been reported to be a prominent feature of diabetes mellitus and aging.[19,20] The results of the present study again indicate that there is an age-related increase in nonenzymatic glycation, and it correlates with the deterioration of aged bone. Several studies have speculated that the modification of bone matrices with advanced glycation end products may play a role in the remodeling of senescent bone matrix tissues.[22,33] Also, some recent studies have demonstrated that the collagen in bone is susceptible to glycation-mediated changes by in vitro ribose treatments, and that the increased stiffness of the collagen network in bone due

Table 6. Regression coefficients of bone porosity and strength of collagen network obtained in direct multiple regression analyses (n = 30)

	Bone properties						
	σ_s (MPa)	σ_y (MPa)	E (GPa)	W_f (N · mm)	W_{fc} (N · mm)	W_{fp} (N · mm)	K_{IC} (MPa√m)
Collagen network properties							
Po (%)	−5.9[a]	−4.1[a]	−0.19[a]	−3.14[a]	−1.43[a]	−1.66[a]	−0.005
σ_{sc} (MPa)	0.99	−0.48	0.002	1.99[a]	0.19	1.58*	0.036
Regression	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$

KEY: bone properties: σ_s, ultimate strength; σ_y, yield strength; E, elastic modulus; W_f, work to fracture; W_{fc}, elastic portion of W_f; W_{fp}, postyield portion of W_f; K_{IC}, fracture toughness. σ_{sc}, strenght of collagen network properties: Po, bone porosity.
[a]Significant correlation ($p < 0.05$).

to such glycation may be related to the age-related increase in skeletal fragility and fracture risk.[13,46] However, the results of this study indicate that the stiffness of the collagen network decreases with age irrespective of the increased pentosidine level, suggesting that in vivo glycation-mediated changes are different from those induced by in vitro ribose treatments. The underlying mechanisms on the effects of nonenzymatic glycation on mechanical integrity of the collagen network and bone remain unclear and further investigations are needed.

In conclusion, the results of this study indicate that the mechanical integrity of the collagen network deteriorates with increasing age, and may lead to decreased work to fracture of aged bone. Moreover, the nonenzymatic glycation end products, assessed using pentosidine concentration, may be one of the major causes for age-related changes in the collagen network and bone quality.

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