Effects of preexisting microdamage, collagen cross-links, degree of mineralization, age, and architecture on compressive mechanical properties of elderly human vertebral trabecular bone.

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Effects of Preexisting Microdamage, Collagen Cross-Links, Degree of Mineralization, Age, and Architecture on Compressive Mechanical Properties of Elderly Human Vertebral Trabecular Bone

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ABSTRACT: Previous studies have shown that the mechanical properties of trabecular bone are determined by bone volume fraction (BV/TV) and microarchitecture. The purpose of this study was to explore other possible determinants of the mechanical properties of vertebral trabecular bone, namely collagen cross-link content, microdamage, and mineralization. Trabecular bone cores were collected from human L2 vertebrae ($n = 49$) from recently deceased donors 54–95 years of age (21 men and 27 women). Two trabecular cores were obtained from each vertebra, one for preexisting microdamage and mineralization measurements, and one for BV/TV and quasi-static compression tests. Collagen cross-link content (PYD, DPD, and PEN) was measured on surrounding trabecular bone. Advancing age was associated with impaired mechanical properties, and with increased microdamage, even after adjustment by BV/TV. BV/TV was the strongest determinant of elastic modulus and ultimate strength ($r^2 = 0.44$ and 0.55, respectively). Microdamage, mineralization parameters, and collagen cross-link content were not associated with mechanical properties. These data indicate that the compressive strength of human vertebral trabecular bone is primarily determined by the amount of trabecular bone, and notably unaffected by normal variation in other factors, such as cross-link profile, microdamage and mineralization. J Orthop Res 29:481–488, 2011

Keywords: bone strength; microdamage; microarchitecture; mineralization; collagen cross-links; vertebral trabecular bone

Several studies have shown that the mechanical properties of trabecular bone are influenced by bone volume fraction (BV/TV) and microarchitecture.[1,2] However, additional factors, such as degree of mineralization, preexisting microdamage, and collagen cross-link profile may also play a role, yet the contribution of these factors to human trabecular bone mechanical properties remains incompletely understood.

Increased mineralization of cortical bone is exponentially related to increased stiffness, but decreased energy absorption.[3] A similar trend was seen in human trabecular bone from the calcaneus, with a positive relationship between mineralization and elastic modulus, after adjusting for BV/TV.[4] It has also been suggested that a reduction in the heterogeneity of mineralization density distribution leads to increased bone fragility,[5] but there are few data to support this assertion.

Microdamage has been largely studied in animal models and in human cortical bone, with only a few studies reporting microdamage in human trabecular bone either naturally occurring[6–11] or due to loading ex-vivo.[12] In cortical bone, microdamage accumulation leads to decreased mechanical properties,[13,14] and therefore has been implicated in skeletal fragility and stress fractures.[15] However, the association between microdamage and cortical bone fracture toughness was weak,[16] raising questions as to the role of microdamage in cortical bone. Increased microdamage has also been associated with decreased strength in human vertebral cancellous bone;[12,13] however, Allen and Burr reported that in dogs treated with bisphosphonates, vertebral mechanical properties were unrelated to microdamage levels.[17] Thus, the contribution of microdamage to bone mechanical properties remains controversial. Recently, it has been suggested that microdamage levels and morphology may reflect alterations in collagen cross-linking.[18] Altogether, the relationships between in vivo microdamage and the mechanical behavior of vertebral trabecular bone remain ill-defined.

Collagen characteristics have also been shown to contribute to bone mechanical properties, often independently of bone mass and density.[19,20] In vivo, collagen cross-link abnormalities have been shown to contribute to skeletal fragility in studies using lathyric model, which is deficient in immature enzymatic and mature trivalent enzymatic cross-links, and in individuals suffering of mild hyperhomocysteinemia, who are thought to be deficient in enzymatic cross-links.[21,22] Furthermore, in vitro models showed that both enzymatic pyridinoline (PYD) and deoxypyridinoline (DPD) and the nonenzymatic advanced glycation end products (AGEs) contribute to bone mechanical behavior.[23–27] Whereas the content of PYD and DPD in bone matrix were related to bone strength in the lathyric model, in ex vivo studies, the role of these enzymatic cross-links remains unclear.[25,26,28,29] In comparison, the accumulation AGEs such as pentosidine (PEN) in the bone matrix is consistently associated with impairment of the postyield properties of trabecular and cortical bone.[24,25,29,30] Few of these studies were performed on human trabecular bone, thus the role of collagen cross-links on trabecular bone mechanical behavior remains unknown.

Altogether, the determinants of vertebral trabecular bone mechanical properties are not completely known.

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In particular, the relative contribution of microdamage, mineralization, and collagen cross-link content to vertebral trabecular bone strength are controversial. Thus, the primary aims of the present study are to determine whether the degree and heterogeneity of mineralization, the amount and type of preexisting microdamage and the collagen characteristics are related to compressive mechanical properties independently of trabecular bone volume in human vertebral trabecular bone from older donors.

MATERIALS AND METHODS

Specimen Preparation

L2 vertebrae were taken from 49 consecutive, recently deceased Caucasians donors 54–95 years of age (21 men, 27 women). The sex of one sample and the age of two subjects were not known. Bone samples were wrapped in gauze soaked with saline to keep them wet, then stored at −20°C. Vertebrae were screened using medial–lateral and anterior–posterior high-definition X-rays (Faxitron X-Ray, Lincolnshire, IL) to exclude prevalent fracture and significant bone diseases (e.g., metastasis, Paget's disease, osteochondritis). No additional information regarding donor disease status or medication history was available. Each vertebra was sectioned in half using a microsaw (Isomet 4000, Buehler GmbH, Düsseldorf, Germany) (Fig. 1). One hemi vertebra was used for mechanical tests; the other half was used to assess preexisting microdamage and mineralization. The hemi vertebrae for microdamage evaluation were bulk stained for 11 days at room temperature in 0.005 M xylenol orange (Sigma–Aldrich Corp., St. Louis, MO) based in 70% ethanol. In each hemi vertebrae, a cylindrical trabecular specimen (8.25 mm diameter) was removed in the supero-inferior direction from the anterior quadrant using a diamond tipped coring tool. The end plate of each vertebrae was removed, as previously described.[11] The remaining trabecular bone adjacent to the bone cores was used for quantification of collagen characteristics. Trabecular bone volume (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, μm), connectivity density (Conn.D, 1/mm^3), degree of anisotropy (DA), and structure model index (SMI), which reflects the rod- versus plate-like nature of the structure,

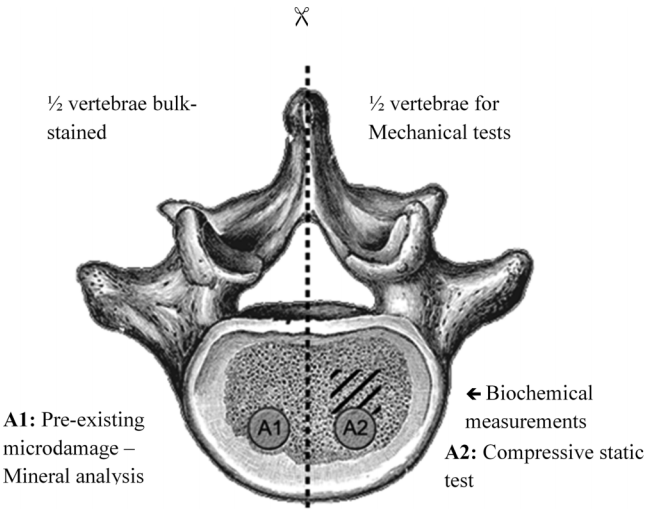

Figure 1. Study design. Specimens taken from vertebrae, view from the superior side (A, anterior); A1: core for preexisting microdamage, A2: core for compressive static test.

of the excised cores were assessed by microcomputed tomography, using an isotropic voxel size of 20 μm (μCT40, Scanco Medical AG, Brüttisellen, Switzerland).

Mechanical Testing

After μCT analyses, aluminum alloy endcaps were applied to the specimens (10 mm in height exposed specimen) to minimize the effects of end-artifacts.[31–34] The bone specimens were secured to the endcaps using a fast curing methyl methacrylate-based resin embedding media (Technovit® 3040, Kulzer, Wehrheim, Germany). The resulting gage length was ~10 mm. Quasi-static compression was performed to determine the trabecular bone mechanical properties: elastic modulus (MPa), the ultimate stress (MPa), and the energy to ultimate stress (MJ/m^3). Due to the nature of failure in compression, postyield measurements to failure were not possible. Specimens were tested at room temperature, and compressed at a constant displacement rate of 0.005 mm s^{-1} (equivalent to 0.0005 s^{-1} strain rate) at 1 Hz for 10 cycles from 0.1% to 0.3% strain. This compressive preload was followed by a destructive ramp to failure to 3% strain. Five samples did not reach failure. Because there was no initial nonlinear region in the stress–strain curves, elastic modulus values were calculated from a best-fit straight line to the stress–strain data in the 0–0.2% strain range, as previously recommended.[31–34] All mechanical tests were performed on a moving-magnet linear motor materials testing system (ELF 3300 Bose-Enduratec, Eden Prairie, MN), with a 2.25 kN load cell, and a displacement transducer (12.7 mm, accuracy 2 μm). Cores were kept hydrated during testing.

Microdamage Analysis

For detection of microdamage, trabecular cores were embedded in methylmethacrylate and cut parallel to the long axis to obtain at least three noncontiguous, parallel, 100 ± 5 μm sections for microdamage evaluation, as previously described.[11] Three sections per specimen—bulk stained with xylenol orange—were measured using fluorescence (excitation/emission wavelength of 440–570/610 nm) microscopy at ×200 magnification and morphometry software (Bone Morpho; Explora Nova, La Rochelle, France). Microdamage was categorized and quantified as linear microcracks or diffuse damage. Outcome assessments included the number of linear cracks (Cr.N, #), the number of diffuse damage areas (Dx.N, #), the linear crack density, defined as the number of linear microcracks per bone area (Cr.Dn, #/mm^2), and the diffuse damage density, defined as the number of diffuse damage regions per bone area (Dx.Dn, #/mm^2). Length and area of damage were expressed as mean linear crack length (Cr.Le, μm) and diffuse damage area (Dx.Ar, μm^2), whereas length and area density were expressed as linear length density (Cr.Le.Dn, μm/mm^2) and diffuse damage area density defined as diffuse damage area per bone area (Dx.Ar.Dn, %). The reproducibility of microdamage identification was assessed by two readers analyzing independently five sections from five different donors.[11]

Mineralization Measurements

The trabecular cores used for mineralization were also used for microdamage analysis. The mean degree of mineralization and heterogeneity of mineralization were assessed using quantitative microradiography, as previously described.[35,36] Briefly, thick sections (about 150 μm) were cut from embedded bone samples with a precision diamond wire saw (Well, Escil, Chassieu, France), progressively ground to a thickness of 100 μm, and polished with a diamond paste (1 μm). The

thickness of the section was measured with an accuracy of 1 μm using a precision micrometer (Compac, Geneva, Switzerland). Bone sections were then cleaned and microradiographed, as previously published.[36] Subsequently, the gray level values were converted into degree of mineralization after plotting a calibration curve based on the values obtained from the aluminum standard. Measurements included the mean degree of mineralization (DMB, g mineral/cm^3) and the heterogeneity index (HI, g mineral/cm^3), expressed as the widths at half-maximum height measured on the individual DMB curves.

Collagen Cross-Links Analysis: Pyridinoline, Deoxypyridinoline, Pentosidine

To avoid contaminating trabecular bone by other tissues as cortical bone or soft tissues, only the central part of the vertebral body around the coring containing exclusively trabecular bone was retained for biochemical analysis. The measurements of cross-links were carried out as previously described.[37,38] Briefly, defatted bone powder was hydrolyzed in 6 N HCl for 20 h at 110°C. Hydrolysates were then analyzed for the contents of cross-links (PYD, DPD, and PEN) and hydroxyproline on HPLC. The amount of collagen was determined using hydroxyproline HPLC assay (Bio-Rad, Munich, Germany). Collagen content was calculated using the dry weight of the bone assuming 14% hydroxyproline in type I collagen, and then used to calculate the cross-link levels as mol/mol of collagen. The content of both mature enzymatic cross-links PYD and DPD, and nonenzymatic cross-link PEN were detected by natural fluorescence by using our established reversed-phase HPLC system as previously described.[38]

Statistical Analysis

Data are reported as mean values, standard deviations, ranges, median, and interquartile range. As some variables were not normally distributed even after transformation, we used non-parametric tests. An unpaired t-test (Mann–Whitney rank sum test) was used to compare men versus women. No significant differences between men and women were observed ($0.24 < p < 0.97$), consequently men and women were pooled for regression and correlation analyses. We used Spearman correlation coefficients (r_{sp}) to assess the relationships between parameters, and partial spearman correlations to test whether associations between parameters were significant after adjusting for the contribution of BV/TV. All tests were two-tailed, and significance defined as $p \leq 0.05$.

RESULTS

Descriptive Statistics

The mean age of our donors was 78.7 ± 9.6 years old, and ranged from 54 to 95 years. Our architecture parameters reflected this age range, being in the lower range of values usually reported. Consequently, mechanical parameters were also in the lower range of values reported for human trabecular bone. But, microdamage, mineralization degree, and collagen cross-links were in the mean range met in the elderly population (Table 1).

Effect of Age on Mechanical Properties and Their Determinants

Mechanical properties (elastic modulus, ultimate stress, and energy), BV/TV, Tb.N, and connectivity decreased significantly with age, whereas Tb.Th, Tb.Sp, and SMI increased with age (Table 2). Collagen cross-link content and mineralization parameters were not related to age. After adjusting for BV/TV, only Tb.Th, linear microcrack number, linear microcrack density, linear length density remained positively correlated with age (Table 3).

Effect of Bone Volume Fraction and Microarchitecture on Mechanical Properties

Elastic modulus, ultimate strength and energy to ultimate strength were strongly correlated to BV/TV ($r_{sp} = 0.66$, 0.74, and 0.67, respectively; $p < 0.001$). Mechanical properties were also significantly correlated to all architectural parameters, except for Tb.Th (Table 2, and Fig. 2). After adjusting for BV/TV, trabecular microarchitecture was no longer associated with trabecular bone mechanical properties. The one exception to this finding was the degree of anisotropy, which remained significantly associated with elastic modulus after adjusting for BV/TV (Table 3).

Effect of Microdamage, Biochemical Characteristics, and Mineralization on Mechanical Properties

Neither linear microcrack nor diffuse damage traits were associated with mechanical properties of vertebral trabecular bone, with the exception that the mean linear crack length was positively associated with elastic modulus ($r_{sp} = 0.30$, $p = 0.034$, Table 2). Also, neither collagen cross-link content the degree of mineralization, or the mineralization heterogeneity index were related to trabecular bone mechanical properties (Table 2), either before and after adjustment for BV/TV (Table 3).

DISCUSSION

In this study, we aimed to determine the relative contributions of trabecular bone volume, architecture, preexisting microdamage, degree of mineralization, and collagen cross-link content to compressive mechanical properties of human vertebral trabecular bone. We found that the trabecular bone mechanical properties were strongly associated with BV/TV, but were not associated with the degree of mineralization, preexisting microdamage, or collagen characteristics, neither as bivariate associations nor after adjustment for trabecular BV/TV. From middle to old age, bone volume fraction and bone mechanical properties declined, whereas collagen cross-link content, the degree of mineralization, and the mineralization heterogeneity index did not vary.

Previously reported values for compressive elastic modulus and ultimate stress of vertebral trabecular bone are generally higher than our values. However, this is largely explained by the fact that the prior studies used samples from donors with lower mean age, and consequently higher BV/TV than ours.[39–43] Additionally, our low measurements could be due to differences in testing methodologies, anatomic site and/or donor age.[33,34,39–45] We note that our relationship between BV/TV and elastic modulus appears weaker than that in several other studies, where 60–80% of the variability in elastic modulus is explained by volume fraction. This

Table 1. Descriptive Statistics, Mean ± SD, Range, Median, and Interquartile Range (IQR)

	n	Mean ± SD	Range		Median	IQR
Age (years)	47	78.7 ± 9.6	54	95	79.0	12.75
Mechanical parameters						
Elastic modulus (MPa)	49	75 ± 32	10	139	67	45
Ultimate stress (MPa)	44	0.91 ± 0.63	0.05	2.8	0.68	0.71
Energy at ultimate stress (MJ/m^3)	44	0.011 ± 0.013	0.0004	0.066	0.007	0.011
Architecture	49					
BV/TV (%)		7.5 ± 2.3	2.84	13.62	6.9	3.8
Tb.Th (μm)		132 ± 19	100	191	132	28.9
Tb.Sp (μm)		1,169 ± 210	868	1,914	1,121	265
Tb.N (1/mm)		0.84 ± 0.13	0.52	1.08	0.85	0.20
Connectivity density ($1/mm^3$)		2.04 ± 0.84	0.73	4.54	1.89	1.33
SMI		1.79 ± 0.44	0.43	2.70	1.88	0.60
DA		1.68 ± 0.15	1.40	2.16	1.70	0.17
Mineralization degree	49					
DMB (g/cm^3)		1.14 ± 0.06	0.99	1.29	1.15	0.07
HI (g/cm^3)		0.29 ± 0.09	0.12	0.53	0.27	0.11
Microdamage	49					
Linear crack number (Cr.N, #)		23.4 ± 24.2	1	109	15	20
Diffuse damage number (Dx.N, #)		3.22 ± 4.9	0	31	2	3
Linear crack density (Cr.Dn, $\#/mm^2$)		1.49 ± 1.25	0.11	6.0	1.13	1.19
Diffuse damage density (Dx.Dn, $\#/mm^2$)		0.23 ± 0.42	0.0	2.86	0.13	0.20
Linear crack Length (Cr.Le, μm)		65.2 ± 17.3	37	106	61.7	25.3
Diffuse damage area (Dx.Ar, μm^2)		1,418 ± 1,663	0	6,957	986	1,359
Linear crack Length density (Cr.Le.Dn, $\mu m/mm^2$)		99 ± 94	7.7	456	70	70
Diffuse damage area density (Dx.Ar.Dn, %)		0.05 ± 0.14	0.0	0.91	0.01	0.03
Collagen cross-links	49					
PYD (mmol/mol coll)		233 ± 61	126	406	231	90
DPD (mmol/mol coll)		102 ± 25	61	192	103	32
PEN (mmol/mol coll)		20.4 ± 9.4	6.2	54.5	17.3	10.1
Collagen (%)		22.8 ± 2.5	15.3	28.7	23.3	2.9

may be due to the limited age range (and therefore bone density) of our donors compared to previously published studies, as a broader age (and density) range would likely lead to a higher correlation coefficient. By design, the mean age of our donors was higher than other studies, and the age range was smaller than previous studies.[33,39–44,46] Because we limited our sample to elderly donors, our results are not likely to be representative of the general population, but rather of an elderly population. This is important to consider when interpreting our results, as a study with a larger age range (i.e., young to old) may have different findings. Nonetheless, the sample that we studies was representative of those that suffer the most from fractures, and as such provides insight into the factors that are greatest contributors to trabecular bone strength in this age group.

Our mean microcrack length and diffuse damage area are consistent with published values.[8,47] Several factors may explain, in part, the disagreement between Wenzel et al. and the current study, including the age range of subjects (greater in Wenzel et al. than in current study), and the vertebral body examined (T12 vs. L2), and the location of the trabecular core within the vertebral body.[6]

Regarding our finding that microdamage was not associated with trabecular bone mechanical properties, Fazzalari et al.[8] also found no relationship between the amount of microdamage and the ultimate failure stress in trabecular bone specimens from human femurs. Furthermore, our data are also consistent with findings from Allen and Burr, who found no relationship between microdamage levels and compressive mechanical properties in vertebral bodies of dogs treated with vehicle, alendronate or risedronate.[17] We observed a negative relationship between microdamage and trabecular bone volume fraction, a finding consistent with previous studies.[11,48–50]

We did find a weak positive relationship ($r = 0.3$) between linear microcrack length and elastic modulus, suggesting longer cracks develop in stiffer bone. Microcracks seem to be associated with the most highly mineralized parts of the bone.[51] It may be possible that in trabecular bone a high elastic modulus could be more favorable to a crack extension (i.e., a longer crack) than to crack accumulation (a lot of tiny microcracks), though this needs further testing. Patterns of microdamage accumulation may also be influenced by the collagen cross-link profile of the bone matrix.[18]

The mean degree of mineralization of vertebral trabecular bone was similar to that of human trabecular bone from the calcaneus, and slightly higher than that in

Table 2. Bivariate Spearman Coefficient (r_{sp}) between Age, Architecture, Mineralization Degree, Mechanical Properties, Microdamage, and Collagen Characteristics

	Age	Elastic Modulus	Ultimate Stress	Energy
Age (years)	—	−0.36*	−0.39*	−0.39*
Mechanical parameters				
Elastic modulus	−0.36*	—	—	—
Ultimate stress	−0.39*	0.85**	—	—
Energy at ultimate stress	−0.39*	0.78**	0.97**	—
Architecture				
BV/TV	−0.39**	0.66**	0.74**	0.67**
Tb.Th	0.32*	0.18**	0.13	0.11
Tb.Sp	0.42**	−0.46**	−0.60**	−0.55**
Tb.N	−0.43**	0.43**	0.56**	0.51**
Connectivity density	−0.42**	0.33*	0.51**	0.46**
SMI	0.46**	−0.57**	−0.64**	−0.60**
DA	−0.17	0.46**	0.37*	0.37*
Mineralization degree				
DMB	0.05	0.12	0.12	0.05
HI	0.03	−0.04	0.02	0.0
Microdamage				
Cr.N	0.30*	0.07	0.20	0.20
Dx.N	0.10	0.19	−0.02	−0.04
Cr.Dn	0.35*	−0.10	−0.00	0.02
Dx.Dn	0.16	−0.12	−0.18	−0.19
Cr.Le	−0.02	0.30*	0.05	0.01
Dx.Ar	0.17	−0.10	−0.12	−0.10
Cr.Le.Dn	0.34*	0.03	−0.03	−0.02
Dx.Ar.Dn	0.27	−0.23	−0.24	−0.22
Collagen cross-links				
PYD	0.22	−0.06	0.060	−0.05
DPD	0.09	−0.06	−0.10	−0.06
PEN	0.032	−0.04	0.01	−0.01
% Collagen	−0.14	0.05	0.03	−0.006

*$p < 0.05$. **$p < 0.01$.

human iliac crest.[35] Mineralization heterogeneity index (HI) values from the current study of vertebral trabecular bone (0.29 ± 0.09 g/cm^3) are higher than those measured on iliac crest biopsies from healthy control subjects (0.19 ± 0.05 g/cm^3) but, similar to values obtained from osteoporotic patients (0.23 ± 0.06 g/cm^3).[35] Our lower range of HI overlapped values previously reported for bisphosphonate users (0.16 ± 0.03 g/cm^3).[52] In contrast to a previous report in human calcaneal trabecular bone,[4] we found no relationship between the mean degree of mineralization and mechanical properties. This could be explained in part by the fact that the variation of the mean degree of mineralization was nearly twofold greater in the calcaneal specimens (CV = 12.9%) than in the vertebral cores (CV = 5.3%). Also, despite a greater than fourfold range in the heterogeneity index in our samples (i.e., from 0.12 to 0.53 g/cm^3), there was no association between the heterogeneity index and trabecular bone mechanical properties. This indicates that the reported decrease in heterogeneity index due to bisphosphonate treatment[52–55] is not likely to be deleterious to trabecular bone mechanical properties.

The values of PYD, DPD, and PEN content were consistent with those previously reported for human vertebrae and femora.[25,56,57] In the current study, pyridinium cross-links did not explain the variation in biomechanical parameters either before or after adjustment for BV/TV. This result is consistent with most previous results obtained from ex vivo studies.[25,29,30] Only one ex vivo study performed in human vertebrae reported that the ratio of PYD/DPD in human vertebral trabecular bone was significantly associated with elastic modulus and ultimate strength, but the interpretation of the PYD/DPD ratio is challenging.[28] We also did not observe any association between PEN content and mechanical properties before or after adjustment for BV/TV. Several prior studies found that higher levels of AGEs are associated with decreased postyield properties of cortical and trabecular bone.[23,24,29,58] Moreover, in trabecular bone from human femora, Tang et al.[27] reported a negative relationship between PEN and postyield mechanical properties, but no association with preyield properties. We did not evaluate postyield properties in this study because they are problematic for compression tests since the trabecular bone compresses upon itself, thus our

Table 3. Spearman Partial Coefficient after Adjustment to BV/TV; Dependent Variables Shown along the Top and Independent Variables along the Side of the Table

Controlled by BV/TV	Age	Elastic Modulus	Ultimate Stress	Energy
Mechanical parameters				
Elastic modulus	−0.15			
Ultimate stress	−0.22	0.69**		
Energy at ultimate stress	−0.24	0.59**	0.95**	
Architecture				
Tb.Th	0.40**	0.14	0.04	0.03
Tb.Sp	0.17	0.16	0.09	0.08
Tb.N	−0.18	−0.19	−0.16	−0.16
Connectivity	−0.20	−0.23	−0.13	−0.13
SMI	0.24	−0.10	−0.14	−0.14
DA	−0.01	0.28*	0.14	0.16
Mineralization degree				
DMB	0.16	−0.03	−0.12	−0.18
HI	−0.05	0.12	0.20	0.14
Microdamage				
Cr.N	0.39**	−0.05	0.03	0.05
Dx.N	0.10	0.04	0.00	−0.03
Cr.Dn	0.35*	−0.07	−0.01	0.02
Dx.Dn	0.07	0.04	−0.01	−0.04
Cr.Le	0.10	0.20	−0.20	−0.21
Dx.Ar	0.15	−0.07	−0.11	−0.08
Cr.Le.Dn	0.37**	−0.02	−0.08	−0.07
Dx.Ar.Dn	0.20	−0.10	−0.11	−0.09
Collagen cross-links				
PYD	0.12	0.18	0.17	0.15
DPD	−0.01	0.12	0.20	0.21
PEN	0.06	−0.12	−0.16	−0.16
% Collagen	−0.07	−0.06	−0.09	−0.12

*$p < 0.05$. **$p < 0.01$.

finding that PEN was not associated with mechanical properties is consistent with previous studies.[27]

Nonetheless it is important to consider other possible reasons why we did not find an association between the collagen cross-link content (DPD, PYD, and PEN) and compressive mechanical properties. One possible explanation for the lack of such an association in our study may be due to the limited age range of our sample, and resulting lack of variation in collagen cross-link content. Specifically, in our sample of middle-to-older age donors (54–95 years) there was little variation in PYD and DPD contents; an observation that is in line with the literature since in human bone, the enzymatic trivalent mature cross-links content reach a plateau at 40 years of age, with little variability thereafter.[56,57] Similarly, the lack of association between PEN content and mechanical properties may be due in part to our limited age range, as Saito et al.[57] showed an exponential age-related

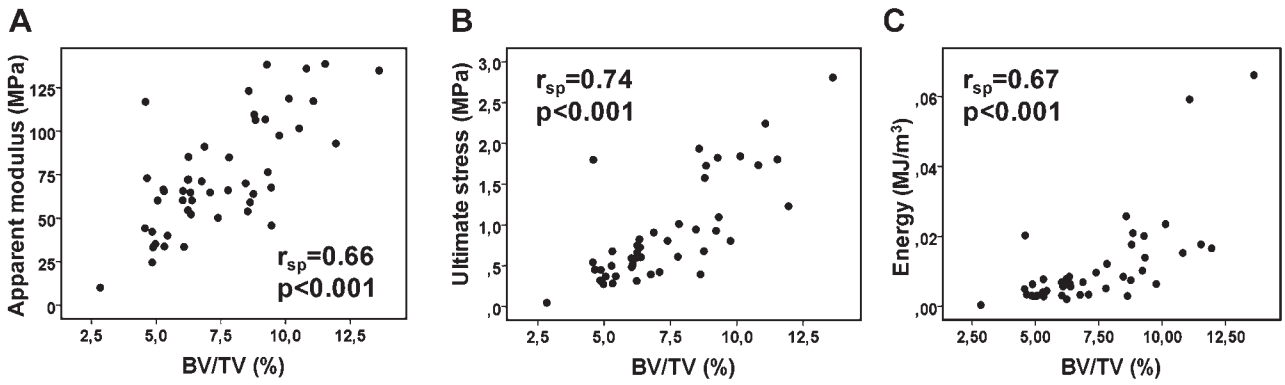

Figure 2. Positive correlation between vertebral BV/TV and the apparent elastic modulus (A), ultimate stress (B), and the energy at the ultimate stress (C).

increase of PEN content in human cortical femoral bone, with most of the changes occurring between young and middle ages. Therefore, the small variation in the pyridinium and AGEs content of our donors may have limited our ability to identifying the contribution of these cross-links on compressive mechanical properties of human vertebral bones. Nonetheless, our donors reflect well the age at which most fractures occur, and imply that in this age range, other factors are more important to mechanical properties that the normal variations in collagen cross-link concentration.

Our study has some limitations. First, we did not have access to the donor's medical history, and therefore we cannot rule out the presence of diseases or medications that may have influenced our outcome variables. However, the specimens were serologically tested, radiographed to exclude bone disease or tumors, and examined with bone histology to exclude specimens with osteomalacia. Though potentially interesting, we could not properly measure bone turnover status, due to a lack of double labeling. We acknowledge that many of our study subjects were old so our results might not be representative of the general population, but rather of an elderly population. This group; however, is most susceptible to fragility fractures. We did not quantify the reducible cross-links which may contribute to skeletal fragility and we only analyzed PEN, which is just one of many AGEs that accumulates with tissue age. It is possible that other AGEs could contribute to mechanical behavior of bone.[19,27] We assessed mechanical properties in quasi-static compression, and it may be that other loading modes that are relevant for vertebral trabecular bone, such as cyclic fatigue, may have a different association with bone architecture and matrix properties.[59]

Our study also has a number of strengths. First, we analyzed a relatively large sample (compared to other studies of cadaveric tissue) comprised of older-aged donors whose age distribution reflected the population in which the majority of fragility fractures occur. Power calculations show that we had 80% power to detect a correlation coefficient greater than $r = 0.35$, and therefore the study was sufficiently powered to detect meaningful correlations. Furthermore, this is among the few studies to assess mechanical properties, collagen cross-links, microdamage analyses, and mineralization in the same vertebral body.

In conclusion, we found that trabecular bone volume was the major determinant of the compressive mechanical properties of human vertebral trabecular bone from middle-aged to elderly donors. The amount of preexisting microdamage, the degree and heterogeneity of mineralization and the collagen cross-link profile were not related to the mechanical properties. These findings imply that age-related variation (from middle-to-old age) in these matrix-level properties have little impact on trabecular bone strength, and infer that noninvasive measures of trabecular bone density likely reflect the major variations in vertebral trabecular bone strength.

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