

REVIEW

Bone composition: relationship to bone fragility and antiosteoporotic drug effects

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The composition of a bone can be described in terms of the mineral phase, hydroxyapatite, the organic phase, which consists of collagen type I, noncollagenous proteins, other components and water. The relative proportions of these various components vary with age, site, gender, disease and treatment. Any drug therapy could change the composition of a bone. This review, however, will only address those pharmaceuticals used to treat or prevent diseases of bone: fragility fractures in particular, and the way they can alter the composition. As bone is a heterogeneous tissue, its composition must be discussed in terms of the chemical makeup, properties of its chemical constituents and their distributions in the ever-changing bone matrix. Emphasis, in this review, is placed on changes in composition as a function of age and various diseases of bone, particularly osteoporosis. It is suggested that while some of the antiosteoporotic drugs can and do modify composition, their positive effects on bone strength may be balanced by negative ones.

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Introduction

Bone is a heterogeneous composite material consisting, in decreasing order, of a mineral phase, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) (analogous to geologic 'hydroxyapatite'),¹ an organic phase (~90% type I collagen, ~5% noncollagenous proteins (NCPs), ~2% lipids by weight)² and water. Proteins in the extracellular matrix of bone can also be divided as follows: (a) structural proteins (collagen and fibronectin) and (b) proteins with specialized functions, such as those that (i) regulate collagen fibril diameter, (ii) serve as signaling molecules, (iii) serve as growth factors, (iv) serve as enzymes and (v) have other functions. The relative amount of each of these constituents present in a given bone varies with age,³ site,⁴ gender,⁵ ethnicity⁶ and health status.⁷ The amount, proper arrangement and characteristics of each of these components (quantity and quality) define the properties of bone. The tendency of bones to fracture depends on the quantity of mineralized tissue present (size and density) often measured by clinicians as bone mineral density or BMD⁸ and several other factors, grouped together as 'bone quality'.^{8,9} 'Bone quality' factors include composition (weight percent of each component), mineralization (organization of the mineral and its crystallite size and perfection), collagen content and collagen crosslinks, morphology,¹⁰

microarchitecture¹¹ and the presence of microcracks.¹² Each of these factors varies with health, disease and drug therapies. Their distribution in the heterogeneous tissue also varies with these perturbations. The focus of this review will be on the composition of bone and its site-specific variation. Materials present, their characteristics and their distribution will be discussed here. Readers are referred to the references above for more information on morphology, microarchitecture and the presence of microcracks, which will not be discussed.

Bone Mineral

Hydroxyapatite is the principal component of the mineral phase of bone. This was demonstrated more than 60 years ago using X-ray diffraction, now viewed as the 'gold standard' for such determinations.¹ The quantity of mineral present in bone can be determined by a variety of techniques¹³ including gravimetric analyses (ash weight determination), analysis of calcium and phosphate contents, spectroscopic and densitometric analyses including bone mineral density distribution (BMDD), bone mineral density (BMD) and micro-computed tomography (micro-CT). Such methods show that the mineral content of bone ranges from ~30%/dry weight (in the skate or ray appendicular skeletal element, the propterygium) to 98%/dry

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weight in the stapes of the human ear. Most bones have ~60–70% mineral/dry weight, depending upon site, species and stage of development (Figure 1).^{13,14}

Variation in the distribution of mineral and its properties in bone can be illustrated by a variety of imaging techniques, discussed here, including BMDD, Raman and infrared spectroscopic imaging. It can also be determined by microprobe or synchrotron radiation-induced micro-X-ray fluorescence elemental analysis and mapping¹⁵ including trace elements such as strontium, aluminum, zinc or lead. In contrast, backscattered electron imaging in the scanning electron microscope is highly sensitive to the average atomic number of the bone material that is dominated by calcium. This technique is not a tool to identify specific elements in bone. Quantitative backscattered electron imaging is used for mapping the calcium concentrations and for the determination of bone mineralization density distribution (frequency distribution of Ca concentrations within the bone sample, BMDD; Figure 2).¹⁶ Parameters obtained from BMDD include the average and mode Ca content and the full-width at half-maximum of the BMDD peak, which is a measure of the heterogeneity of mineralization. Deviations from normal calcium distributions have been reported to date in: osteomalacia,¹⁷ osteoporosis¹⁸ and idiopathic osteoporosis¹⁹ (peak shifted to the left of normal), classical and new forms of osteogenesis imperfecta^{16,20} (peak shifted to the right of normal) and treatment with some but not all bisphosphonates examined by this technique.^{18,21,22}

Variation in phosphate distribution is visualized by both Fourier transform infrared microscopic imaging (FTIRI)²³ and Raman microscopy and imaging (Raman).^{24,25} These types of vibrational spectroscopic imaging describe the distribution of any elemental pair or larger moiety that vibrates when excited by incident light. Relevant vibrations for bone are those in phosphate, protein and lipid groups. The precise location of the vibrations, often given in wave numbers or reciprocal wavelength, reflects the molecular environment in which the vibrating ions are found. In addition, as with BMDD, the line width at half-maximum of any of the broadened peaks indicates the heterogeneity (number of pixels with different values in the section

analyzed) for that particular vibration. The spatial resolution of the FTIRI experiment, unless synchrotron radiation is used, is ~7 μm. Raman spectroscopy, in contrast, has a spatial resolution of ~1 μm. Raman spectral data is not affected by the presence of water, making the analysis of non-dehydrated samples, not possible for FTIRI, yet possible using Raman. Vibrations that are strong in the infrared spectra are weak in Raman spectra, and vice versa. These two are complimentary techniques, providing information on both the mineral phosphate and the organic matrix distribution in tissues (Figures 3–5). The data from these images can be presented as ‘chemical photographs’ (as in Figures 3 and 5) or as numerical averages (Figure 6). Data in Figure 6 compares the composition of cancellous bone in patients treated with bisphosphonate followed by 1 year of teriparatide (PTH) treatment, as determined by Raman spectroscopy. The lower half of Figure 6 shows baseline values as determined by FTIRI in an on-going study of female patients with and without fragility fractures. The mean heterogeneity for each of these parameters is also shown. An important observation is that the fracture cases had reduced heterogeneities relative to unfractured controls. We believe (discussed later) that this loss of compositional heterogeneity may allow microcrack propagation resulting in a weakened, more brittle tissue as the microcracks spread. Whether this change in heterogeneity is due to oversuppression or other mechanisms is yet to be determined.

Using FTIRI spectroscopy, biopsies from patients with low-energy (fragility) fractures were found to exhibit differences in their mineral composition, relative to the same tissue in fracture-free controls of similar age and sex. Using these FTIRI analyses of 54 iliac crest biopsies from patients ranging in age from 30 to 84 years, with and without fragility fractures,²⁶ models were constructed to determine which FTIRI parameters were associated with fracture. The mineral parameters significantly associated with fracture in the constructed model were high cortical mineral/matrix ratio (increased fracture risk) and high cancellous crystallinity (increased fracture risk). Carbonate-to-phosphate ratio was increased in both areas, however, not significantly. Raman analysis performed on femoral cancellous

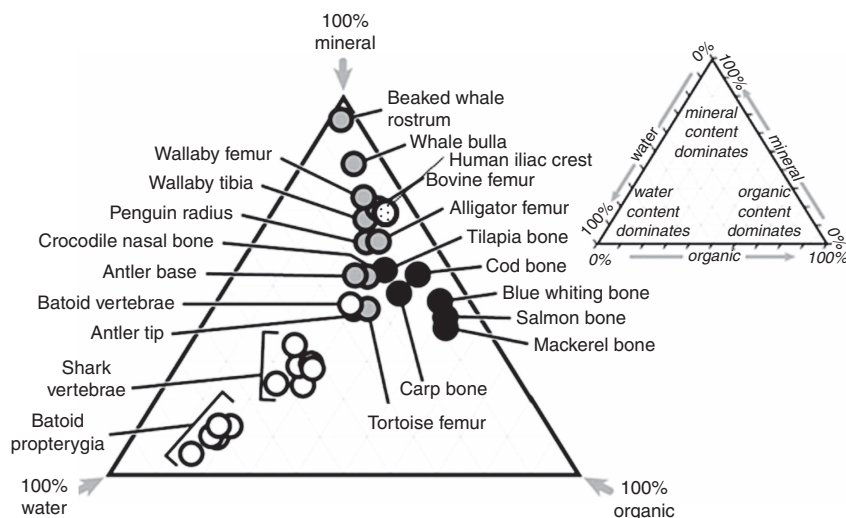


Figure 1 Bone composition. Ternary diagram illustrating the composition of mature bones in different species (adapted from Currey J.D. ‘Bones’. Princeton University Press: Princeton, NJ, 2002, p 436.) and reproduced with permission from *Journal of Experimental Biologists*, Figure 7B in ‘Comparison of structural, architectural and mechanical aspects of cellular and acellular bone in two teleost fish’.¹⁴ The speckled circle is the average of data from five iliac crest biopsies of females aged 69–75 years.

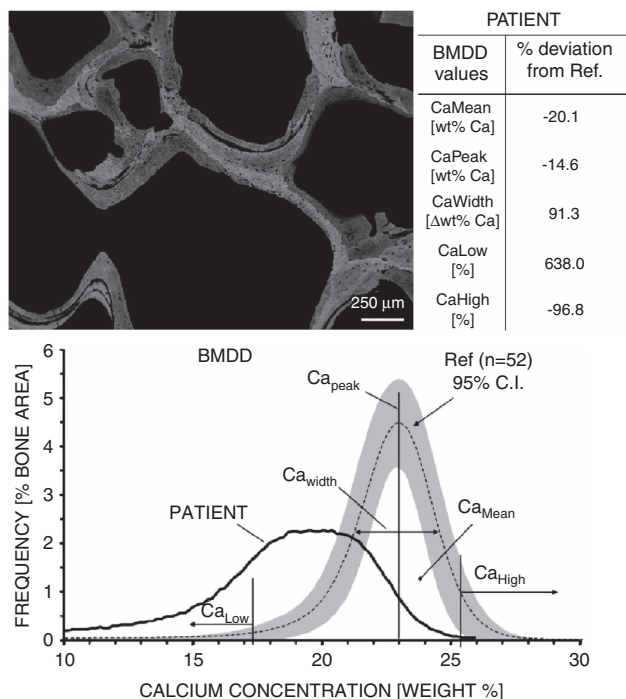


Figure 2 BMDD distribution of bone. Measurement of bone mineralization density distribution (BMDD) using quantitative backscattered electron imaging (qBEI) in a transiliac bone biopsy sample (left insert) from a 39-year-old woman with coeliac disease (PATIENT). The qBEI image shows a detail of the cancellous bone compartment. Bright gray levels mean high and dark gray levels mean low mineral content. The trabecular features display severe under mineralization and mineralization defects. Thus, the BMDD curve is extremely shifted to the left toward lower matrix mineralization (decreased CaMean, CaPeak, CaHigh and increased CaLow) compared with the normal reference BMDD (Ref.). In addition, the peak width of the BMDD (CaWidth) is distinctly enlarged, indicating an enormous heterogeneity in mineralization of the bone matrix. Examinations by histological staining techniques revealed a severe osteomalacia combined with signs of secondary hyperparathyroidism. Description of the individual BMDD indices derived from the BMDD curve: *CaMean, the weighted mean calcium concentration of the bone area obtained by the integrated area under the BMDD curve; *CaPeak, the peak position of the histogram, which indicates the most frequently occurring calcium concentration (mode calcium concentration); *CaWidth, the full-width at half-maximum of the distribution, describing the variation in mineralization density (heterogeneity); *CaLow, the percentage of bone area that is mineralized below the 5th percentile of the reference BMDD of normal adults, that is, below 17.68 weight percent calcium. This parameter corresponds normally to the amount of bone area undergoing primary mineralization. *CaHigh, the percentage of bone area that is mineralized above the 95th percentile of the reference BMDD of normal adults that is above 25.30 weight percent calcium. This parameter corresponds to bone matrix having achieved plateau level of normal mineralization. Figures courtesy of P Roschger.

bone near the fracture site of women with fractures similarly demonstrated a higher carbonate/amide I area ratio than in those without fractures. Iliac crest biopsies in those fractured patients also revealed a higher carbonate/phosphate ratio in cortical bone samples of women with fractures.²⁷ These compositional changes and the loss of heterogeneity reflect the persistence of older more mature bone (increased mineral/matrix ratio and carbonate/phosphate ratio and a lower acid phosphate substitution) along with the absence of new bone formation.

Changes in mineral composition also occur in other bone diseases associated with increased fracture risk in addition to

osteoporosis. In most types of OI (or brittle bone disease), as in osteoporosis, the mineral content (mineral/matrix ratio) is increased.²⁸ In osteoporosis, this increase is attributable to the lack of osteoid associated with increased remodeling and decreased bone formation, whereas in OI there is a lesser amount of collagen because OI patients make an improper collagen matrix.²⁸ In osteomalacia, the mineral/matrix ratio is unchanged when mineralized tissue alone is examined.²⁹ Crystallinity (measured based on different vibrations in FTIRI and Raman³⁰) generally shows parallel trends. These trends, reflecting the changes in mineral properties in different diseases, are summarized in **Table 1** along with other compositional parameters measured by FTIRI and Raman. Carbonate/phosphate ratio, based on the same vibrations in both techniques varies similarly. The extent of acid phosphate substitution³¹ varies inversely with crystallinity. For example, in chronic kidney disease, abnormalities in phosphate transport and clearance lead to osteoporosis and increased crystallinity when bone turnover is high, with no significant changes occurring when bone turnover is low.³²

Bone Matrix

Protein composition of bone was classically determined following demineralization of the tissue and isolation and characterization of the component proteins. Collagen, predominantly type I, accounted for the majority of the matrix, but other proteins, the so-called NCPs, accounted for ~5% of the total bone weight. The major components of the NCPs were identified as belonging to the SIBLING (small integrin-binding N-glycosylated), SLRP (small leucine-rich proteoglycans), GLA protein (γ -carboxyglutamic acid protein) and CCN protein (small secreted cysteine-rich protein) families. Today, using proteomics and gene expression profiling, it is known that there are thousands of proteins in the bone matrix, some of which (based on whole-genome analysis) have been associated with changes in BMD, but most are yet to be identified and their functions determined. Major structural proteins and NCPs will be reviewed here.

Structural proteins. Collagen. The most abundant protein in the bone matrix is type I collagen, a unique triple helical molecule consisting of two identical amino-acid chains and one that is different. The collagen molecules that consist of repeating glycine-X-Y residues are often hydroxylated and glycosylated. This gives rise to some of collagen's unique crosslinking ability, in turn, making the collagen lattice ideal for its functions. These include: providing elasticity to the tissues, stabilizing the extracellular matrix, supporting or templating initial mineral deposition and binding other macromolecules. In different types of OI, mutations in the collagen genes are reflected in the inability of the OI bones to mineralize properly. Reviewed elsewhere,²⁸ the origin of the mineralization defect is unknown. This defect may be in the altered structure of the collagen itself, or in the inability of extracellular NCPs, which regulate the mineralization process, to bind to the defective collagen, and hence regulate mineralization.

Chemical analyses of the crosslinks in bone collagen have demonstrated two types of crosslinks, those formed enzymatically and those that occur by glycation.³³ Both types of crosslinks (enzymatic and non-enzymatic) increase with age,

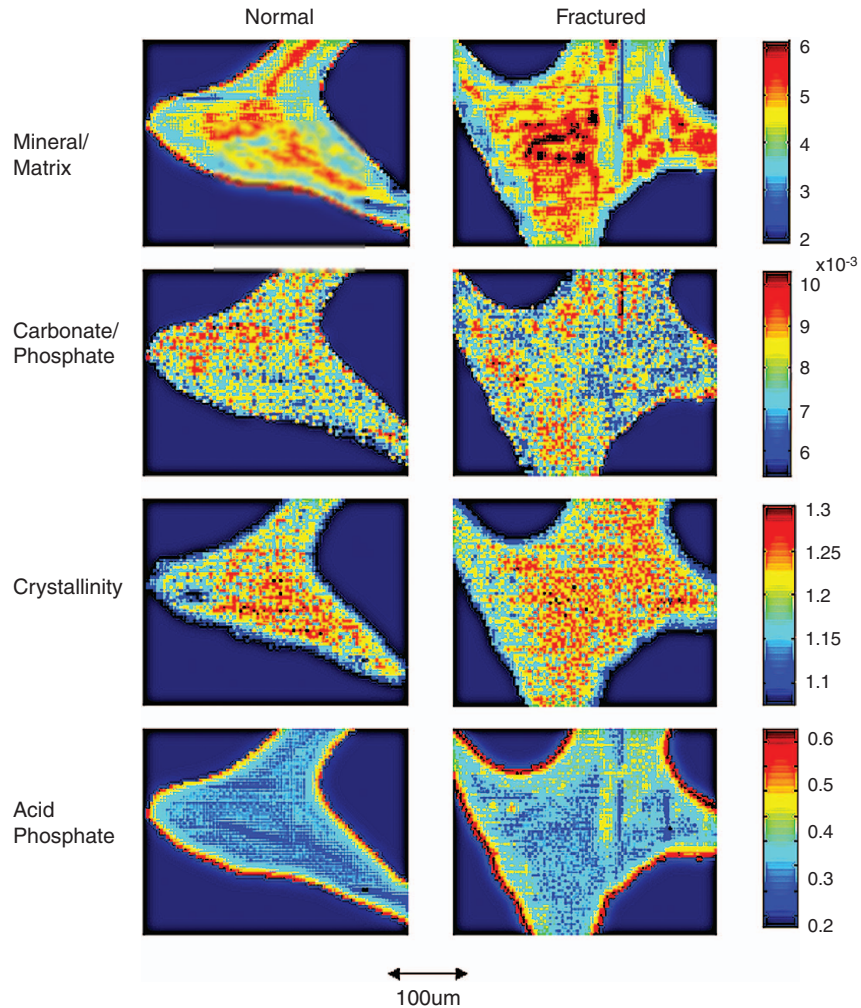


Figure 3 FTIR images. Typical FTIR images of mineral compositional parameters in normal and osteoporotic bone trabeculae from iliac crest biopsies of a woman with fractures and her age-matched control. Left side, normal; right side, osteoporotic (fractured). Images are shown for: mineral/matrix ratio, carbonate/phosphate ratio, crystallinity ($1030/1020\text{ cm}^{-1}$) and acid phosphate substitution. The arrow indicates the size scale for all images. The color scales for each parameter for both normal and fractured images are shown.

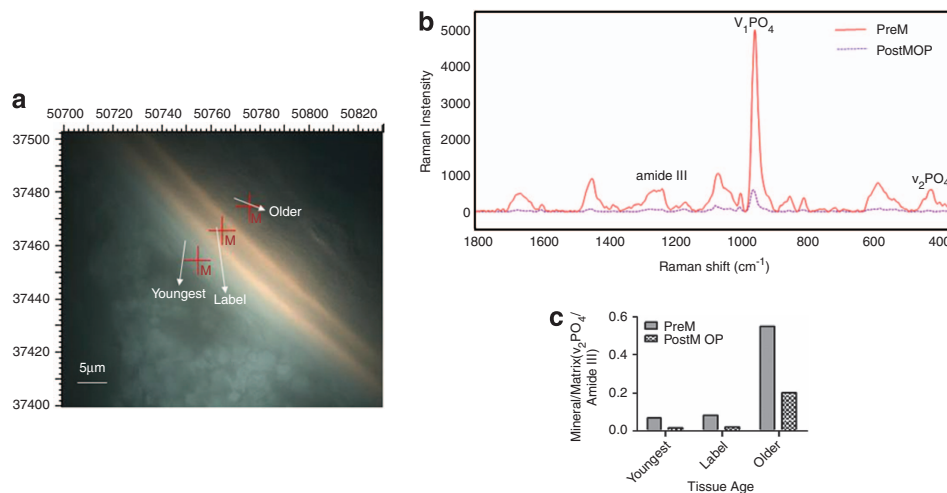


Figure 4 Raman analysis. (a) Image as seen under the Raman microscope showing the double tetracycline labels used to define newly formed trabecular bone, the three areas examined are shown. (b) Typical Raman spectra of bone from a premenopausal woman and a woman with osteoporosis. (c) Mean values for the indicated parameters calculated from the Raman spectra of these two patients. Figure courtesy of Dr Lefteris Paschalis.

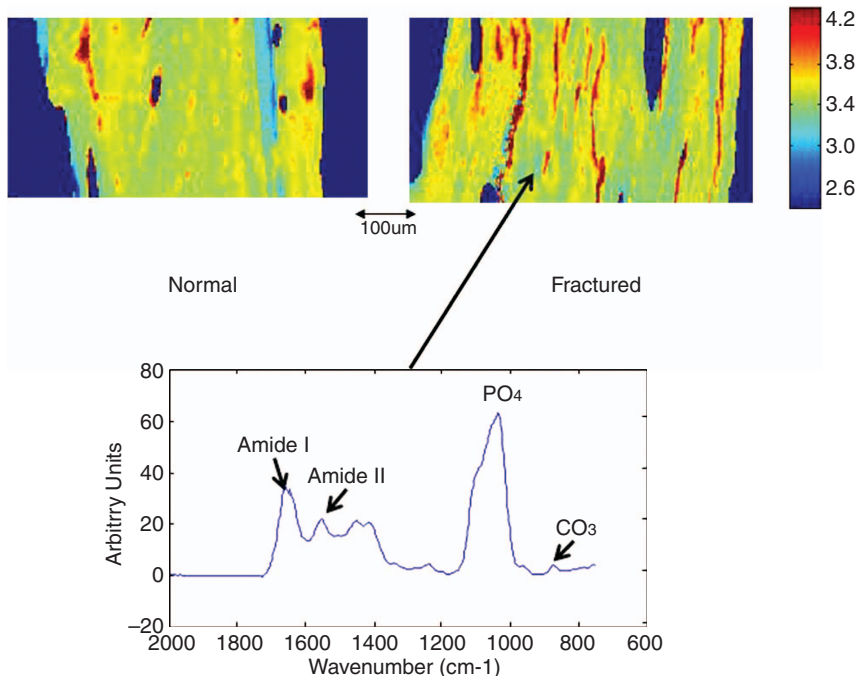


Figure 5 FTIR image of the organic matrix in osteoporosis. Typical cortical bone image of collagen maturity (ratio of intensity of 1660/1690 cm^{-1}) in iliac crest biopsies from age-matched women without and with a fracture. The magnification is indicated by the arrow. The infrared spectra corresponding to the pixel at the tip of the arrow is shown. The major peaks used in the analysis of mineral and matrix are indicated in this figure.

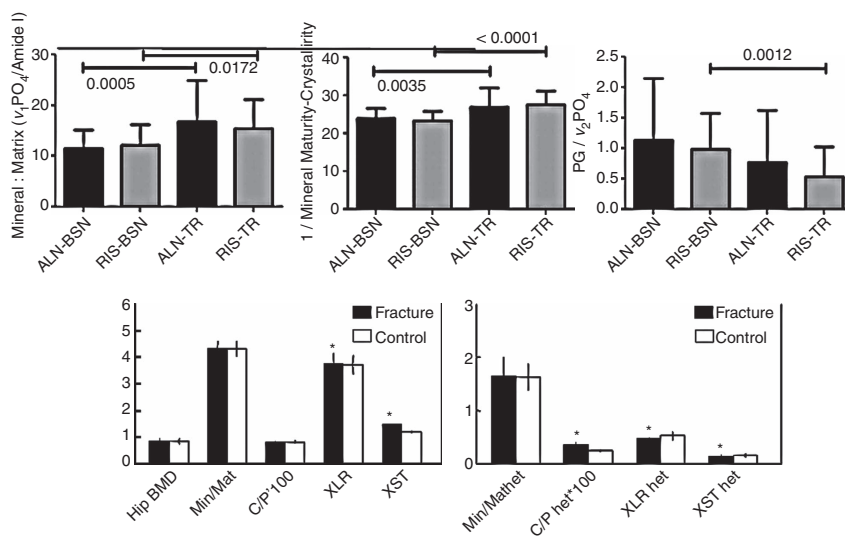


Figure 6 Compositional changes in bone as detected by Raman and FTIR. Top row of figures show Raman compositional data indicating changes from baseline in newly formed trabecular bone caused by 1 year treatment with teriparatide (TP) following longer treatment with alendronate (ALN) or risedronate (RIS). Differences between baseline and TP treatment are indicated. Mineral-to-matrix, inverse of crystallinity and proteoglycan-to-mineral ratios are shown (adapted with permission from Figures 2a, 3 and 4 in Gamsjaeger *et al.*⁵¹). Lower row shows FTIR lack of compositional changes in iliac crest biopsies from a group of 48 women, half with fractures and half without who had comparable hip BMD values. The first figure shows mean and standard deviation in cancellous bone. The second figure illustrates the mean and standard deviation of the heterogeneity of these parameters. * $P < 0.05$.

are altered in disease (**Table 2**) and affect the mechanical strength of the collagenous matrix. Enzymatic crosslinks are believed to enhance mechanical strength, whereas the advanced glycosylation end-products, which are elevated in uncontrolled diabetics and in oxidative stress, make for a more brittle bone. Distribution of total crosslinks in the bone matrix can be visualized in FTIR data (**Figure 5**). Such analyses agree

with the high-performance liquid chromatography chemical analysis of crosslinks.³⁴ The number of these crosslinks, which reflect collagen maturity, is increased in osteoporotic individuals, especially in the center of the remaining cancellous bone. The heterogeneity of this distribution is decreased in consequence of age, osteoporosis and treatment with bisphosphonates.³⁵

Table 1 Mineral property distribution in diseased human bone revealed by FTIRI and Raman analysis

Parameter/condition	Compared with healthy age-matched similar site							
	Cortical				Cancellous			
	Min/matrix	CO ₃ /P	XST	HPO ₄	Min/matrix	CO ₃ /P	XST	HPO ₄
Aging ⁵² primates	Inc	Inc	Inc	Dec	Inc	Inc	Inc	Dec
Osteogenesis imperfecta ^{53,54,a}								
Type I	NC	Dec	Dec	NA	NC	Dec	Dec	NA
Type III								
Type IV								
Type VII	Inc	NC	Dec	NA	Inc	NC	Dec	NA
Type VIII	Inc	NC	Dec	NA	Inc	NC	Dec	NA
Osteomalacia ^b	NC	NA	NC	NA	NC	NA	NC	NA
Osteoporosis ^c	Inc	NC	Inc	NA	NC	NC	Inc	NA
Renal osteodystrophy ^{32,55}	NC	NC	NC	NA	Dec	NC	NC/INC	NA

Abbreviations: CO₃/P, carbonate-to-phosphate ratio; Dec, decreased relative to appropriate control; HPO₄, acid phosphate substitution; Inc, increased relative to appropriate control; Min/mat, mineral-to-matrix ratio; NA, not measured; NC, unchanged relative to appropriate control. XST = crystallinity.

^aA Boskey, E Carter, CL Raggio, unpublished data. ^bSee Faibish *et al.*²⁹ ^cSee Gourion-Arsiquaud *et al.*^{26,35}

Table 2 Variation in collagen crosslinking in human bones with age, disease and therapy (relative to control values)

	FTIRI collagen maturity	Enzymatic crosslinks	AGEs
Age ^a	Increases ^b	Increases ⁵⁶	Increases in cortical bone ⁵⁶
Osteogenesis imperfecta ^c	Increased ^b	No change ⁵⁷	No change ⁵⁷
Osteomalacia ^c	No change ^d	ND	ND
Renal osteodystrophy ^c	No change ⁵⁵	Increased ⁵⁸ (rats)	Increased ⁵⁸ (rats)
Osteoporosis ^c	Increased ⁵⁹	Increased ^e	Increased ^e
+ Alendronate ^f	No change ^{60,61}	No change (dogs) ⁶²	Decreased (dogs) ⁶²
+ Risedronate ^f	No change (dogs) ⁶¹	No change (dogs) ⁶²	Decreased (dogs) ⁶²
+ Zoledronate ^f	No change ^b	ND	ND
+ PTH ^f	No change ^b	Increased (monkeys) ⁶³	Decreased (monkeys) ⁶³
+ Estrogen ^f	Increased ⁶⁴	ND	ND
+ Raloxifene (SERM) ^f	No change	ND	Decreased

Abbreviations: AGE, advanced glycosylation end-products; FTIRI, Fourier transform infrared microscopic imaging; ND, not determined; PTH, parathyroid hormone; SERM, selective estrogen receptor modulator.

^avs Younger individuals, ^bA Boskey, E Carter, CL Raggio, unpublished data, ^cvs Age-matched control, ^dSee Faibish *et al.*²⁹, ^eSee Vashishth.³³, ^fvs Untreated osteoporotic.

Fibronectin. Fibronectin, a minor constituent of bone matrix, is one of the first proteins produced by osteoblasts, and directs the initial deposition of collagen fibrils.³⁶ Continued presence of fibronectin is also required to maintain the integrity of the collagenous matrix.³⁷ Studies with a variety of different conditional knockout animals demonstrated that while osteoblasts produce fibronectin, they are not responsible for the presence of the same in the bone extracellular matrix; rather, the bone matrix fibronectin is derived from circulating liver fibronectin.³⁸ In primary biliary cirrhosis, the incidence of osteoporosis is markedly elevated. This higher incidence is due to an increased production of a fibronectin isoform that lessens osteoblastic bone formation.³⁹

Noncollagenous proteins. There are several families of proteins that account for a small proportion of the extracellular matrix, which, as reviewed elsewhere,⁴⁰ serve important functions in matrix organization, cell signaling, metabolism and mineralization. Other than early studies showing a reduced NCP content of osteoporotic bone,⁴¹ little has been written on how these proteins change in expression or distribution in osteoporosis or other bone diseases, with or without anabolic or antiresorptive therapies. Known changes in the expression of NCPs in health and disease are summarized in **Table 3**. As

shown in **Table 3**, recent gene-wide association studies have identified several NCPs that may be related to fracture risk. In terms of actual measurement of protein content, findings to date are that osteocalcin and osteopontin are important for fracture resistance,⁴² their concentrations are reduced in older osteonal bone⁴³ and osteopontin may retard crack propagation.⁴² As a number of NCPs can and do interact with collagen fibrils,⁴⁰ they may function as 'glue', enhancing bone's resistance to fracture.⁴² Recent studies have shown compositional differences between lamellae and interlamellar areas of cortical bone. The interlamellar areas have lower collagen content and increased concentration of NCPs.⁴⁴ The significance of such differences is as yet unknown. No published studies to date have examined changes in the expression of enzymes and signaling factors in the matrix of patients with metabolic bone disease.

Lipids. Less than 3% of the total bone matrix is fat soluble. Lipids are important for cell function, surrounding the cell body, regulating the flux of ions and signaling molecules into and out of the cell. The distribution of lipids in the matrix can be observed from histology, based on sudanophilia, from FTIR and Raman analysis or by nuclear magnetic resonance (NMR).⁴⁵ There are no recent published studies on lipid composition

Table 3 Variation of noncollagenous bone protein concentrations in healthy and diseased human and animal bones^a

Protein	Bone conc. in osteoporosis	Bone conc. in OI (types I-IV)	Bone conc. after drug treatment
Albumin	Reduced ⁶⁵	Increased	?
Alpha 2-HS Glycoprotein (fetuin)	Unchanged ⁶⁵	Increased ⁶⁶	?
Bone Gla protein (osteocalcin)	Reduced ⁶⁷	Increased ⁶⁶	?
Fibronectin	?	Increased ⁶⁸	?
Matrix Gla protein	Reduced ⁶⁹	?	+ ALN not affected (mice) ⁷⁰
Large proteoglycans	?	Decreased ⁶⁷	+ ZOL reduced ^{71,72}
SLRPS			
Biglycan	Depleted ^{72,73}	Decreased ⁶⁸	?
Decorin	Depleted ⁷²	No change ⁶⁸	?
Osteoadherin	No change ⁷⁴	?	?
SIBLINGS			
BSP	Associated with BMD ⁷⁵	No change ⁷⁶	+ ALN (rats) reduced ⁷⁷ + PTH (mice) reduced ⁷⁸ + Sr ranelate no effect ⁷⁹ + Ca supplement increased ⁸¹
DMP1	Increased (mouse) ⁸⁰	No change ⁷⁶	+ Ca supplement decreased ⁸¹
DPP	?	Decreased ⁸⁰	?
MEPE	Reduced ^{82,83}	No change ⁷⁶	+ Ca supplement decreased ⁸¹
Osteopontin	Associated with BMD ⁸²⁻⁸⁴	No change ⁷⁶	+ PTH serum levels lowered ⁸⁶
Osteonectin	Reduced ⁸⁵	Reduced ⁶⁸	+ ALN (rats) reduced ⁷⁷
Thrombospondins	Reduced (mouse) ⁸⁷	Reduced ⁶⁸	
	Associated with BMD in males ⁸⁸	TSP1 increased ⁷⁶	?
	Associated with BMD ^{89,90}		
Matrix metalloproteases			
MMP13	Reduced; ^{81,84} associated with BMD ⁸²	?	+ ALN (rats) reduced ⁹¹
ADAMTS18	Associated with BMD in Japanese women ⁸²	?	?
Phosphatases			
FAM210A	Reduced; ⁸² associated with BMD ⁸⁴	?	?
Alkaline phosphatase	Associated with BMD ⁹⁰	Decreased staining ⁹¹	+ ZOL-enhanced staining ⁹²
Tartrate-resistant acid phosphatase	No change in staining (rats) ⁹¹	Increased staining (mice) ⁹⁵	+ Estrogen reduced ⁹²
	Increased ^{93,94}		

Abbreviations: ADAMTS18, A Disintegrin And Metalloprotease with ThromboSpondin repeats; 'Associations', refer to gene expression studies; ALN, alendronate; BMD, bone mineral density; BSP, bone sialoprotein; Conc., relative concentration; DMP1, dentin matrix protein 1; DPP, dentin phosphoprotein; ?, no data available; MEPE, matrix extracellular phosphorylated glycoprotein; MMP13, matrix metalloproteinase 13; OI, osteogenesis imperfecta; PTH, parathyroid hormone; SIBLING, small integrin-binding N-glycosylated; SLRPS, small leucine-rich proteoglycans; TSP1, thrombospondin-1; ZOL, zoledronate.

^aSee review by Boskey and Robey⁴⁰ for the function of these proteins and animal models in which the diseases mentioned are noted. Items without reference numbers are discussed in that text.

associated with fragility fractures or other bone diseases in humans. Thirty years ago, we did analyze the lipid composition of femoral heads from patients with avascular necrosis, reporting increased cholesterol content.⁴⁶

Water. The water content of bone may be demonstrated by proton NMR and can be assessed quantitatively by Raman spectroscopy⁴⁷ and gravimetric methods.⁴⁸ Water serves many functions, including filling the pores, interacting with collagen fibrils and binding to mineral crystals.⁴⁹ Unfortunately, the precise role of water in determining the mechanical competence of bone has not been determined. Analysis of water content shows a direct relationship between water and cortical porosity, which occurs with aging and osteoporosis (**Figure 7**) and is a key feature of renal osteodystrophy⁵⁰ and its associated osteoporosis. It is assumed, but not yet demonstrated, that decreases in porosity caused by bisphosphonate treatment will result in a lesser water content in both osteoporosis and renal osteodystrophy.

Composition Changes in Aging and Disease

Bone is a dynamic as well as a heterogeneous tissue; therefore, it is not surprising to see changes in composition as a function of age.³ The types of compositional changes that have been

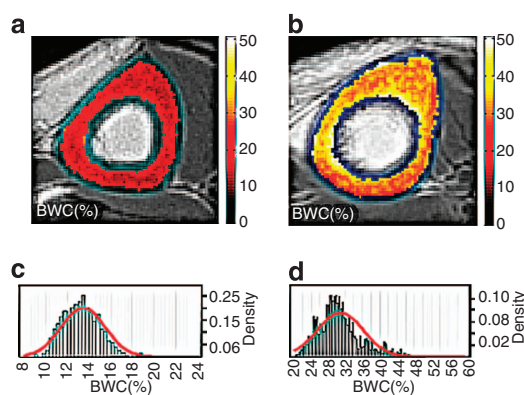


Figure 7 Water distribution in midshaft tibia cortical bone is demonstrated by three-dimensional ultrashort echo-time magnetic resonance imaging. (a) A young 33-year-old woman without osteoporosis, and (b) is a 64-year-old woman. Note the marked increase in bone water content (BWC) shown by both the concentration images (on the top) and their histograms (c and d, respectively, on the bottom) (reproduced from Yoder *et al.*⁴⁹ (Figure 5) with permission from John Wiley & Sons Ltd).

reported are summarized in **Table 4**. These are distinct from the changes discussed above that are associated with bone disease, fragility fractures or treatments to prevent such

fractures. Important is the observation that some age-dependent changes in composition are due to alterations in cell activity and protein expression as well as changes in the concentration and post-translational modification⁴⁰ of those NCPs that regulate matrix composition and mineralization. Therapies to limit the extent of bone disease are directed against these very same cells.

Compositional changes induced by therapies for osteoporosis. The interesting and important observation is that treatments for osteoporosis while decreasing fracture incidence do not consistently correct the above compositional abnormalities. Therapies currently used (**Table 5**), other than supplements of calcium, vitamin D and phosphate, fall into two classes: anabolic agents and antiresorptive agents. These therapies all increase or maintain BMD. They each, however, have distinct effects on compositional properties and heterogeneity of their distribution. The most informative of the studies in **Table 5** are those based on biopsies before and after treatment. Not every study examined the same tissue site, dose of drug or duration of treatment, and many did not compare the resulting data to the same tissue site in an untreated control. The

few instances where this comparison was carried out, and the therapy returned the parameter in question to healthy control values are emphasized in **Table 5**. Where no human data exists, animal data is included, with the caveat that this does not mean the same alterations will occur in man. Bisphosphonates, alendronate in particular, decreased the heterogeneity of the tissue, rather than increasing it, as would be appropriate for a mechanically strong tissue. Parathyroid hormone, an anabolic agent, and strontium ranelate both increase heterogeneity, determined by FTIRI, Raman and micro-CT measurements. Calcitriol's effect on bone composition has not been determined, neither were the effects of sclerostin antibodies. Odanaticib, the cathepsin K inhibitor, has been shown to affect composition based on BMDD measurements.

Most of the antiresorptive therapies for osteoporosis (estrogen, bisphosphonates, calcitonin, cathepsin K inhibitors) increase mineral/matrix ratio, decrease crystallinity and return other FTIR and Raman parameters to less osteoporotic values without returning these values to normalcy. The anabolic agent PTH and strontium-ranelate, which may have both catabolic and anabolic properties, correct many of these FTIRI properties and increase tissue heterogeneity. Many of the therapies lead to retention of existing 'older' bone. Older bone has increased collagen maturity and increased crystal size. Anabolic agents, in contrast, stimulate new bone formation and the tissue acquired has characteristics of younger bone.

Loss of material heterogeneity, in fracture mechanics, is associated with an increase in brittleness, hence a greater risk of fracture. The importance of heterogeneity is seen in lumber structure and in development of stronger cements and plastics. Bisphosphonate treatment usually results in an increase of bone mineral density and bone volume, or in its maintenance. Bisphosphonate treatment is often accompanied by a decrease in heterogeneity. The reason for this event is as yet uncertain. It may be that there is a balance in these two opposing effects. The use of bisphosphonates results in an increase in BMD and bone volume, hence an increase in bone stiffness and strength. The use of bisphosphonates also causes a decrease in bone heterogeneity, which in turn increases bone brittleness. If this

Table 4 Age-related changes in healthy bone composition (cortical and cancellous considered together)

Bone mineral density ^a and tissue mineral density ^b	Increase with age
Mineral to organic matrix ratio ^c	Increase with age
Calcium-to-phosphate ratio ^d	Increase with age
Carbonate-to-phosphate ratio ^c	Increase with age
Crystal size and perfection (crystallinity) ^{c,e}	Increase with age
Acid phosphate substitution ^c	Decrease with age
Matrix heterogeneity ^{b,c}	Decrease with age
Total collagen crosslinks (collagen maturity) ^c	Increase with age
Collagen enzymatic crosslinks ^d	Increase with age
Collagen AGEs ^d	Increase with age

Abbreviations: AGE, advanced glycation end-products; BMDD, bone mineral density distribution; DXA, dual photon absorptiometry; FTIRI, Fourier transform infrared microscopic imaging; XRD, X-ray diffraction.

^aDetermined by DXA, ^bDetermined by microcomputed tomography, ^cDetermined by FTIRI, BMDD and Raman spectroscopy, ^dDetermined by chemical analyses, ^eDetermined by XRD.

Table 5 Effects of current therapies on cancellous bone compositional properties

	Ca content (BMDD)	Min/mat	BV/TV	TbN	TbS	XLR	XST	Heterogeneity
Antiresorptives								
Estrogen	N ⁹⁶	I ⁶⁴	N ⁹⁶	N ⁹⁶	N ⁹⁶	I ⁶⁴	D ⁶⁴	NA
Calcitonin	NA	D ⁹⁷	I ⁹⁷	I ⁹⁷	D ⁹⁷	NA	NA	NA
Ibandronate	NA	I ⁹⁸	N ⁹⁹	N ⁹⁹	N ⁹⁹	NA	I ⁹⁸	N ⁹⁹ D ⁹⁸
Alendronate	I ^{18,22,100}	N ⁶⁰	I ¹⁰¹	N ¹⁰¹	N ¹⁰¹	N ⁶⁰	N ⁶⁰	D ^{18,60,98,100} N ^{22,99}
Odanaticib	I ¹⁰²	I ¹⁰²	NA	NA	NA	NA	NA	D ¹⁰²
Risedronate	I ¹⁰²	D ^{*103}	NA	NA	NA	D ^{*103}	N ^{*103}	D ¹⁰⁴
Zoledronate	I ¹⁰³	I ^{105,106}	I ²¹	I ²¹	D ²¹	NA	D ^{*105,106}	D ⁷¹
Anabolics								
PTH	D ¹⁶	D ^a	N ¹⁰⁷	N ¹⁰⁷	N ¹⁰⁷	D	D ^a	I ^{16,a}
Antisclerostin	I ¹⁵	NA	I ¹⁵	NA	NA	NA	NA	NA
Other								
SrRAN	I ^{108,109}	N ^{109,110}	N ¹¹¹	N ¹¹¹	N ¹¹¹	N ¹¹⁰	N ¹¹¹	I ¹⁰⁸

Abbreviations: BMDD, bone mineral density distribution; BV/TV, bone volume fraction; D, decreased; I, increased; Min/mat, mineral/matrix ratio; N, no change; NA, not measured; TbN, trabecular number; TbS, trabecular separation; XLR, collagen maturity; XST, crystallinity. I, D, N and NA show changes relative to untreated osteoporotic patients. Differences in reported values may be because of site, duration of treatment or method of analysis. Where no human data was available, other species are shown in italics. Bold indicates treatments that were reported to normalize indicated property to that in healthy controls.

^aA Boskey, E Carter, CL Raggio, unpublished data.

balance is disturbed, as in 'oversuppression' of bone turnover, failure may occur. Thus, the composition of bone in the healthy individual must be maintained and adjusted, similar to the structure described by Wolff's law, so as to optimize the function of bone.

Conclusions

This review of mineral and matrix properties in healthy and diseased bones demonstrates that these properties show both age- and disease-dependent changes. Bone disease and therapies for these diseases also affect the composition of bone. Bisphosphonates increase bone quantity but their effects on bone quality are variable. The effects of many other agents used in the treatment of osteoporosis are still under investigation. Some bisphosphonates decrease tissue heterogeneity, which may in turn increase brittleness. The origins of this effect and the significance of the alteration in bone quality remain to be determined.

Conflict of Interest

Dr Boskey's work on FTIR and osteoporosis has been funded by the NIH. The author declares no conflict of interest.

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