0163-769X/07/$20.00/0
Printed in U.S.A.

Endocrine Reviews 28(2):151–164

doi: 10.1210/er.2006-0029

Insights into Material and Structural Basis of Bone Fragility from Diseases Associated with Fractures: How Determinants of the Biomechanical Properties of Bone Are Compromised by Disease

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Minimal trauma fractures in bone diseases are the result of bone fragility. Rather than considering bone fragility as being the result of a reduced amount of bone, we recognize that bone fragility is the result of changes in the material and structural properties of bone. A better understanding of the contribution of each component of the material composition and structure and how these interact to maintain whole bone strength is obtained by the study of metabolic bone diseases. Disorders of collagen (osteogenesis imperfecta and Paget's disease of bone), mineral content, composition and distribution (fluorosis and osteomalacia); diseases of high remodeling (postmenopausal osteoporosis, hyperparathyroidism, and hyperthyroidism) and low remodeling (osteopetrosis, pycnodysostosis); and other diseases (idiopathic male osteoporosis, corticosteroid-induced osteoporosis) produce abnormalities in the material composition and structure that lead to bone fragility. Observations in patients and in animal models provide insights on the biomechanical consequences of these illnesses and the nature of the qualities of bone that determine its strength. (*Endocrine Reviews* 28: 151–164, 2007)

I. Introduction

BONE MUST BE stiff, able to resist deformation, so that loading is possible. If bone is not sufficiently stiff, *i.e.*, too flexible for the loads imposed on it, it will deform beyond its peak strain and crack. Bone must also be flexible, able to deform to allow energy absorption during impact loading. If bone is not sufficiently flexible, *i.e.*, too brittle, the energy imposed on it will be released in the only way possible—by cracking because it cannot deform "enough" to absorb it when loaded. Bone must also be light to allow movement (1).

These seemingly contradictory properties, stiffness yet flexibility, and lightness yet strength, are determined by bone's material composition and how this material is fashioned into a three-dimensional structure with geometric properties that confer structural strength. A change in the material or structural components of bone or the inability of bone modeling and remodeling to adapt these material and structural properties to the prevailing loads produces bone fragility (Fig. 1).

The relative importance of each component to whole bone strength, and the cellular mechanisms that adapt one component to compensate for abnormalities in another are incompletely understood. In this review, we analyze the effect of diseases on the material composition, structure, and cellular mechanisms of modeling and remodeling and demonstrate how these diseases shed light on the qualities of bone and mechanisms leading to fragility fractures (Table 1).

First Published Online December 19, 2006

Abbreviations: BMD, Bone mineral density; BMU, basic multicellular unit; BSU, basic structure unit; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand.

***Endocrine Reviews* is published by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

II. The Material Composition of Bone: Collagen and Mineral

Bone is a specialized connective tissue composed of an organic matrix of type I collagen. It is specialized in that it is mineralized with an inorganic phase comprising calcium hydroxyapatite-like crystals. The organic matrix provides

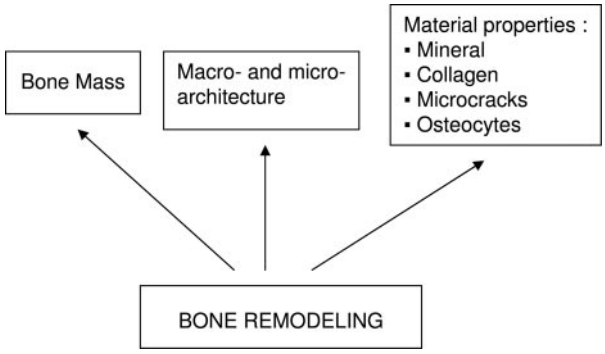

FIG. 1. The determinants of the bone strength.

flexibility, whereas increasing amounts of mineral confer increasing degrees of material stiffness (2, 3). The mineralized tissue is organized into basic structure units (BSUs) that are the footprint of a remodeling event.

The BSUs are not uniformly mineralized; younger more recently completed BSUs are less densely mineralized than older BSUs that have had more time to undergo secondary mineralization (crystal enlargement) (4–6). Even within a BSU, the organization is formed as a composite characterized by alternating higher and lower density lamellae with collagen fibers oriented in different directions conferring the birefringence under polarized light. This heterogeneity creates a structure that serves to prevent the occurrence of cracks and limits crack progression.

Energy is needed for cracks to propagate, and heterogeneity in collagen orientation, tissue mineral density, and numbers of osteons and cement lines around each osteon provides obstructions to crack progression, a process that requires energy that is absorbed by these obstructions rather than driving the crack forward. Loss of the lamellar organization as occurs in woven bone in Paget's disease and loss of heterogeneity in tissue mineral density as occurs with prolonged bisphosphonate therapy may compromise bone's ability to prevent crack occurrence and progression (7, 8).

When a load is imposed on bone, it deforms elastically, *i.e.*, it absorbs the energy imposed by loading shortening and widening in compression, lengthening and narrowing in tension, then returning to its original length when unloaded. If bone is deformed beyond its peak elastic strain, it can absorb more energy but at the price of developing permanent change in length produced by microcracks. This is the plastic zone of deformation and when unloaded the bone does not return to its original length. Microdamage in this postyield region is the last resort available before the only means of energy release is structural failure. The collagen phase determines the tissue flexibility and the postyield properties of bone, its toughness or ability to absorb energy before it fails (9, 10).

In bone tissue, collagen fibrils are stiffened by integration of the mineral phase (11). Collagen molecules are staggered within the fiber to provide spaces within the fiber for nucleation of the calcium apatite crystals. The presence of the organic phase increases bone strength; but in woven bone, which is constituted by unorganized collagen fibrils, the mechanical properties are decreased despite a high mineral content (12). Thus, the orientation of collagen fibers is important in determining the mechanical properties. Furthermore, the orientation of collagen fibers is related to the direction of load. The strength of bone is higher in the direction of physiological loading that corresponds to the orientation of osteons in the cortical bone (13, 14).

The posttranslational modifications of collagen influence the mechanical properties. The presence of crosslinks in collagen maintains the helixes, but if there are too many, the ability to absorb energy decreases. In lathyrism, which is characterized by a decreased bone strength and abnormalities of joints and blood vessels, the activity of lysyl oxidase is inhibited, and the concentration of pyridinoline crosslinks is very low (15–17). The higher ratio pyridinoline/deoxypyridinoline, the greater the stiffness and maximal strength (18). In contrast, increased concentration of pentosidine with aging correlates with the decrease in bone strength (19).

III. The Structural Design of Bone

The structural design of the skeleton is achieved using minimal mass by taking advantage of the strength of bone achieved through its geometric properties. Long bones are levers needed for loading and movement—rigidity is favored over flexibility. Fashioning the long bone with a medullary cavity achieves strength yet lightness. By shifting the cortical shell outward using a marrow cavity, the expanded marrow space effectively creates a void space and achieves lightness, whereas the further displacement of the cortical shell from the neutral axis increases bending strength. Minimizing the need for material to build wider bones is achieved by having wider bones with a narrower cortex so that a given cross-section of bone has a constant bone area (Fig. 2).

Long bones grow in length by endochondral apposition and in width by deposition of bone on its outer or periosteal surface. Resorptive excavation of a marrow cavity shifts the thickening cortex away from the neutral axis, thereby increasing resistance to bending. The conical metaphyses are fashioned by periosteal bone resorption and formation, whereas endochondral bone formation forms the trabecular network. External and internal contours differ at each point along and around the shaft, reflecting local modeling and remodeling in response to regional loading needs.

The expanded marrow space achieves lightness in trabecular regions such as the vertebral body, and the material used is fashioned like a porous sponge to function more like a spring than lever. Interconnecting trabecular plates achieve lightness and favor structural flexibility over stiffness. These structures can absorb more energy than long bones by deforming before cracking, but they sacrifice the ability to tolerate large loads. Males and females generally have similar vertebral trabecular volumetric density and similar vertebral heights; the larger vertebral cross-sectional area in males contributes to sex differences in bone strength.

The number, thickness, spacing, distribution, and connectivity (*i.e.*, connection) of trabeculae reflect the trabecular network and determine bone strength. For the same deficit in trabecular density, loss of connectivity has more deleterious effects on strength than thinned but well-connected

TABLE 1. Abnormalities of the various components of bone strength in bone diseases

Disease	Bone mass	Turnover	Formation	Resorption	Trabecular architecture	Cortices	Collagen	Mineral
Abnormal collagen								
OI	↓	↑ In children, ↓ in adults	↓	↔ or ↑	↓ Tb.N, ↑ Tb.Th	↓ Ct.Th	Type I collagen mutation: abnormal pro-α1(I) chains, low amount of procollagen	↑ Mineral density (adults), ↓ degree of mineralization (children), ↓ crystal size
Paget	↑	↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ Tb.Th	↑ Ct.Th	Woven bone	?
Change of bone mineral								
Osteomalacia	↔ ↓ MdV/TV	↔ or ↑	↑	↔ or ↑ (if secondary HPT)	↔		Normal	Mineral content: ↓ on bone surfaces, ↔ in center of trabeculae
Fluorosis	↑	↓	↑	↑		↑ Ct.Th, ↑ Ct.Po	Area of woven bone in lamellar bone	Linear mineralization defects: periosteocytic mottled lacunae, ↓ degree of mineralization, ↑ crystal size
Modification of the bone turnover								
PM-OP	↓	↑	↑	↑ ↑	↓ Tb.N	↓ Ct.Th, ↑ Ct.Po	↑ Lysine hydroxylation, ↓ DHLNL, HLNL	↔ or ↓ mineral content, ↑ crystallinity
Male OP	↓	↓	↓	↓	↓ Tb.Th	↓ Ct.Th		
GIOP	↓	↓	↓ ↓	↑	↓ Tb.N, ↓ Tb.Th			
HPT	↔ or ↓	↑ ↑	↑ ↑	↑ ↑	↓ Tb.Th	↓ Ct.Th, ↑ Ct.Po		↓ Degree of mineralization
Hyperthyroidism	↓	↑	↑ ↑	↑ ↑ ↑		↓ Ct.Th, ↑ Ct.Po		
Osteopetrosis	↑	↓	↔	↓ (Deficiency of osteoclast activity)	↑ ↑ Tb.Th	↑ Ct.Th	Mineralized cartilage	↑ Mineral content, ↓ crystal size
Pycnodysostosis	↑	↓ ↓	↓	↓ (No osteoclast activity)	↑ ↑ Tb.Th	↑ Ct.Th	Abnormal lamellar texture, mineralized cartilage	↑ Crystal size, ↑ degree of mineralization

OP, Osteoporosis; PM-OP, postmenopausal OP; GIOP, glucocorticoid-induced OP; HPT, hyperparathyroidism; HLNL, hydroxylysinonorleucine; DHLNL, dihydroxylysinonorleucine; MdV/TV, mineralized bone volume; Tb.N, trabecular number; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; Ct.Po, cortical porosity. ↑, Increase; ↓, decrease; ↔, no increase or decrease. Single arrow, Small increase/decrease; double arrows, medium increase/decrease; triple arrows, high increase/decrease.

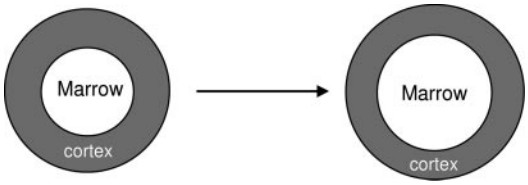

FIG. 2. Modification of the external bone size and thinning of the cortex with a constant bone area.

trabeculae (20–22). Women with osteopenia with vertebral fractures have four times as many unconnected trabeculae as women without fracture, despite a similar bone mineral density (BMD) (23).

IV. Bone Modeling and Remodeling: The Cellular Machinery of Bone

The cellular activities of bone modeling and remodeling determine the material composition and structure of bone. Bone modeling refers to the deposition of new bone without prior bone resorption. Bone remodeling is characterized by the appearance of focally and temporally distinct regions of resorption followed by bone formation that constitutes the basic multicellular units (BMUs). The purpose of bone modeling and remodeling during growth is to build peak bone strength. After the completion of growth, bone modeling continues in adulthood modestly to increase bone size further, whereas bone remodeling maintains bone strength by removal of microdamage.

The bone remodeling is initiated on a bone surface usually covered by a very thin layer of unmineralized matrix and lining cells. These cells may respond to stimuli (hormones, cytokines) that initiate the remodeling. The differentiation of osteoclasts is stimulated, and they start to resorb bone. The stimulation of the osteoclast activity requires an interaction with the osteoblastic cells (24). Receptor activator of nuclear factor-κB (RANK) ligand (RANKL) is expressed and secreted by osteoblast precursor cell and binds RANK expressed by osteoclasts, thus promoting the differentiation and activity of the osteoclasts. Osteoblasts secrete osteoprotegerin (OPG), which binds to RANKL and inhibits the RANK-RANKL interaction. RANKL knockout mice exhibit a severe osteopetrosis with narrowing or occlusion of the marrow space. They lack osteoclast, but osteoprogenitors are present. It has been suggested that the increased expression of RANKL may explain the increased resorption in multiple myeloma. In contrast, OPG-deficient mice show a severe osteoporosis resulting from an increased osteoclast formation and activity (25). After the resorption phase, osteoblasts secrete the bone matrix, which refills the resorption lacunae. Under normal conditions, the remodeling process of resorption followed by formation is closely coupled in BMU and results in no change of bone mass when the amounts of resorbed and newly formed bone are similar. The coupling between resorption and formation is controlled by several factors that are poorly defined. The absence of coupling, *i.e.*, formation occurring without prior resorption, is observed only under pathological conditions such as bone metastasis. The frequency of initiation of a new remodeling sequence characterized the bone turnover.

Abnormalities in the rate and balance of bone remodeling play a pivotal role in the pathogenesis of bone loss and structural decay. A high remodeling rate contributes to bone fragility by reducing the time available for secondary mineralization; bone is removed and replaced with new, less densely mineralized bone, which reduces its material stiffness. High bone remodeling itself also alters collagen composition by impairing isomerization, maturation, and cross linking. The high remodeling rate produces stress concentrators—excavated regions of bone that concentrate stress predisposing to microdamage. In the presence of a negative BMU balance produced by an increase in the volume of bone resorbed, a decrease in the volume of bone formed or both, each remodeling event during the high remodeling rate after menopause, and in disease states, accelerates bone loss and structural decay producing trabecular thinning, tunneling in the trabeculae, cortical thinning, and porosity (1).

The resorptive phase of the remodeling cycle is responsible for the removal of microdamage, whereas the formation phase replaces bone and restores its material composition and structure. Suppression of bone remodeling after high dose bisphosphonate favors microcrack accumulation and may reduce bone toughness (26). Osteocytes play a role in the detection of microdamage and in the targeted bone remodeling, and their death by apoptosis may be a means of determining the location of damage and its extent (27).

V. Bone Fragility Primarily due to Abnormal Collagen

A. Osteogenesis imperfecta (OI)

OI is an inherited disorder characterized by increased bone fragility with recurrent fractures that leads to skeletal deformities in severe cases. The phenotypic expression is heterogeneous with the most severe forms being fatal in the perinatal period to mild forms diagnosed only in adulthood (28–30). OI is characterized by a low bone mass, a reduced trabecular thickness and number (Fig. 3A), and a decreased bone formation at the cellular level (31, 32). The bone turnover is increased in children but decreased in adults (31–33).

Animal studies and studies in human subjects suggest that skeletal fragility in OI is due to the defect in collagen synthesis, whereas the abnormalities in bone turnover and mineral are inconsistent. The collagen abnormalities are the result of a mutation of the two type I collagen genes, COLIA1 and COLIA2. Over 200 mutations have been reported. Two main classes of type I collagen mutations have been described. The first "null allele" mutation affects the pro-α1 (I) or pro-α2 (I) alleles that impair transcription and mRNA stability and produce low amounts of the secreted heterodimer. The procollagen is normal in composition. The second mechanism results in the secretion of structurally abnormal pro-α1 (I) chains. The abnormal heterodimers are incorporated into the matrix, resulting in a quantitative and qualitative abnormal bone matrix.

Whatever the mutation, there is less bone synthesized (34). An increase in the degree of hydroxylation of lysine in type

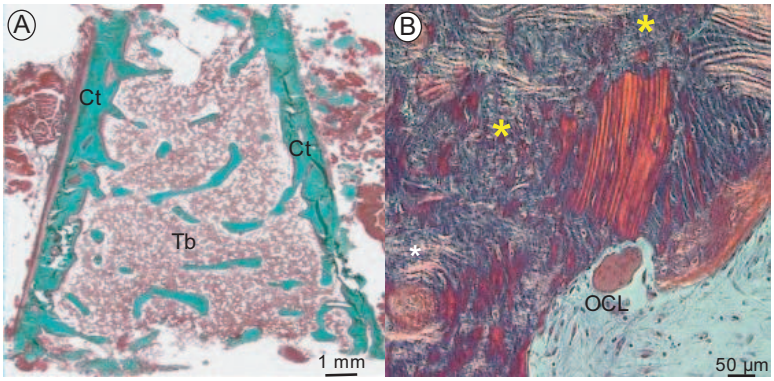

FIG. 3. Abnormal collagen. A, OI in an 11-yr-old girl, characterized by a low bone mass with a loss of the trabecular connectivity. Tb, trabecular bone; Ct, cortical bone. Goldner's trichrome staining. B, Paget disease of bone characterized by voluminous osteoclasts containing numerous nuclei (OCL). Under polarized light, the texture of bone is abnormal with area of woven bone (*). Solochrome cyanin staining.

I collagen has been reported, but the mature cross-linking amino acids are normal (35, 36). The data concerning the size, structure, and three-dimensional organization of collagen are inconsistent (37–39). The concentration of dermatan sulfate and chondroitin sulfate may vary according to the type of OI and may influence the collagen fibril diameter (40). These abnormal collagen fibrils may be unable to provide nucleating and scaffolding sites for mineral propagation (39). Furthermore, extrafibrillary mineral crystals are sometimes larger but collagen-associated crystals are smaller than in normal bone (6). In severe forms of OI, the length of mineral crystals is decreased (37). The Ca/P ratio is lower in OI bones than in controls (37), but the distribution of the mineral density evaluated by back-scattered electrons is increased in children with OI (41). However, in children, the mean degree of mineralization measured by quantitative microradiography is slightly decreased compared with age-matched controls (32).

The abnormalities of the organic and inorganic phases may explain the brittleness of the bone. A mouse model having well-defined genetic mutations on the COLIA2 gene produces α1(I) collagen homotrimers and nonfunctional proα2(I) chains has been described. These *oim/oim* mice show OI phenotypes characterized by spontaneous fractures and limb deformities (42). The mechanical properties of *oim/oim* femurs are decreased: failure and stiffness are 40 and 30% lower, with a collagen content reduced by 20%, a mineral content unchanged, but with a decreased mineral crystallinity (43). An increased anisotropy has been suggested in *oim/oim* mice, reflected by a decrease of the elastic modulus along the length not width of femurs (44). These changes indicate an altered stress distribution and a modified elastic response to loads in *oim/oim* mice. The brittleness may be related to a reduction of the ultimate tensile strain of the abnormal collagen (45). Collagen abnormalities are probably the cause of a decrease in the intermolecular cross-linking, mainly of immature ketoimine cross-links that alter the tensile properties of collagen and the mineral crystallinity of bone (46). An increased mineral density has been reported in *oim/oim* mice and may be linked to the brittleness of *oim* bone. Crystals appear thinner and less well aligned (47, 48). However, the hardness is increased for *oim* bones (48).

Another animal model of human OI is the *Mov13* mouse. Incorporation of a provirus prevents the initiation of transcription of one of the α1(I) collagen genes. This mutation is associated with a reduction in type I collagen content, increased brittleness (49), and a reduced stress level (50) at which damage appears. The reduced collagen content and the altered collagen-matrix organization with an increased proportion of woven bone in *Mov13* mice disturb the ability to detect damage (51). In this model, the material abnormality is compensated for by structural adaptation with periosteal apposition that maintains whole bone strength (52). In contrast, in the brittle IV mouse, another animal model of OI, adaptation results from the mineral:collagen ratio (53).

B. Paget's disease of bone

Paget's disease of bone is a localized disease characterized by increased bone remodeling, bone hypertrophy, and abnormal bone structure. The illness occurs in 2–3% of individuals over age 60. The consequences are pain, bone deformities, fractures of long bones or vertebrae, secondary osteoarthritis from deformity of bone near joints and neurological complications. Paget's disease may affect only one bone or may involve several bones (54).

Bone fragility in Paget's disease probably results from the accelerated bone turnover and the consequent disorganization of the matrix. Fragility occurs despite an increase in bone density/size at most skeletal sites. In affected bone, resorption is dramatically increased with abnormal osteoclasts containing numerous nuclei (Fig. 3B). Bone formation is also increased with numerous osteoblasts actively synthesizing bone matrix, which is rapidly mineralized. The accelerated bone turnover leads to the formation of abnormal woven bone with an irregular arrangement of collagen fibers that are not deposited in a lamellar fashion (55) (Fig. 3B). The excessive bone formation results in the bone hypertrophy and osteosclerosis with thick and numerous trabeculae (55). The woven bone is not specific for Paget's disease but reflects an extremely high rate of bone turnover. This immature woven bone is also characterized by an alteration of the degree of β-isomerization of the C-terminal telopeptide of α1 chains of type I collagen (56). In addition, an abnormal distribution of noncollagenous matrix proteins has been reported (57).

The alteration of the bone texture due to abnormal turnover is likely to impair bone strength. Heritable disorders characterized by an accelerated bone turnover resulting from mutations of the RANKL/OPG/RANK/nuclear factor-κB signaling have been described. Familial expansile osteolysis results from an activation of RANK due to a duplication in its gene. Juvenile Paget's disease is caused by an inactivating

mutation of OPG gene (58). The OPG knockout mouse reproduces this phenotype. In OPG-deficient mice, accelerated bone remodeling is associated with disorganized collagen matrix and bone fragility (59). In human fetal bones, the age-related bone maturation corresponds to more cross-linked and ordered collagen fibrils that correlate with the increase in mechanical properties (60).

VI. Bone Fragility Primarily due to Abnormal Mineral Density

A. Primary mineralization defect: osteomalacia

Osteomalacia is characterized by a defect of mineralization due to calcium and phosphate deficiencies that mainly result from a poor gut absorption due to vitamin D deficiency. Most common is vitamin D deficiency due to lack of sunlight exposure or intestinal malabsorption, but disorders of vitamin D metabolism contribute (defect of hydroxylation, increased renal excretion, and increased catabolism by anticonvulsants). Osteomalacia may be drug-induced (fluoride or etidronate) or the result of aluminum exposure in parenteral nutrition or hemodialysis. Clinical features are pain, fissures, and fractures, which may occur after minimal trauma (61).

The consequence of vitamin D deficiency is a fall of blood calcium concentration that induces a secondary hyperparathyroidism. The effect of hypocalcemia is a defect of bone mineralization, and the effect of secondary hyperparathyroidism is an increase in bone turnover. In contrast, in phosphate deficiency, parathyroid level and vitamin D status are normal, and the consequence of hypophosphatemia is osteomalacia. Recently, a new phosphaturic factor, FGF23, has been identified. High levels of FGF23 are observed in X-linked phosphatemic rickets, autosomic dominant hypophosphatemic rickets, and oncogenic osteomalacia, which are characterized by hypophosphatemia and low 1,25-hydroxyvitamin D (62).

The reduced mechanical properties of osteomalacic bone results from the delayed primary mineralization, which is the cause of the small amount of mineralized tissue (Fig. 4A). Bone tissue is characterized by an accumulation of osteoid (Fig. 4B) and a decrease in mineralization rate (Fig. 4C) with a prolongation of the mineralization lag time, *i.e.*, the delay between the deposition of the matrix and the onset of the mineralization (63). In hypophosphatemic osteomalacia, bone tissue mass may be increased but the mineralized bone volume is decreased. Infrared imaging of calcified tissue shows that the matrix is undermineralized, with differences in mineral content within trabeculae but without evidence of an abnormal organic matrix or alteration in the crystal size and perfection (64).

Animal models of rickets and osteomalacia have been developed to reproduce vitamin D deficiency by a mutation of 25-hydroxyvitamin D-1 α hydroxylase gene (65). These hypocalcemic mice have an osteomalacia with a decreased ultimate load and stiffness, which are corrected by the treatment with 1,25-hydroxyvitamin D_3 (65). In *Hyp* mouse, an animal model of X-linked hypophosphatemia, a genetic disorder characterized by hypophosphatemia and osteomalacia, the altered mechanical properties have been attributed to the reduced quantity of mineral (66).

B. Abnormal mineral: fluorosis

The effects of fluoride on bone tissue depend on the cumulative dose: at very low dose, less than 1.5 mg F^-/d, fluoride prevents the development of dental caries; at doses of about 23 mg F^-/d, fluoride was proposed in the treatment of osteoporosis but no beneficial effects on the fracture rate were observed (67, 68). Skeletal fluorosis results from a prolonged ingestion of fluoride causing bone pain, stiffness, and rigidity and possible deformities of the spine and limbs (69).

Despite the increased bone tissue mass, the bone strength is reduced due to several abnormalities in mineral. The femoral and lumbar compressive strength of fluoride-treated rats is lower than controls (70, 71), and the fluoride treatment increases BMD in ovariectomized rats but decreases bone strength and stiffness (72). *In vitro*, fluoride exposure of mouse femora induces reductions in ultimate load and rigidity and an increase in ductility (73).

Histomorphometric analysis of iliac crest biopsies from patients with fluorosis shows that osteosclerosis is the result of unbalanced coupling in favor of bone formation (Fig. 5A). Mineralization defects are present in calcified tissue or around the osteocytic lacunae (Fig. 5B), and some area of woven bone may be observed but the bone texture is mainly lamellar (74). Despite an increased bone mass, fluoride-treated mice have decreased bone strength (71).

Fluoride induces the production of an altered organic matrix and reduced mineralization. Some modifications of the amino acid composition of collagen (decreased hydroxypro-

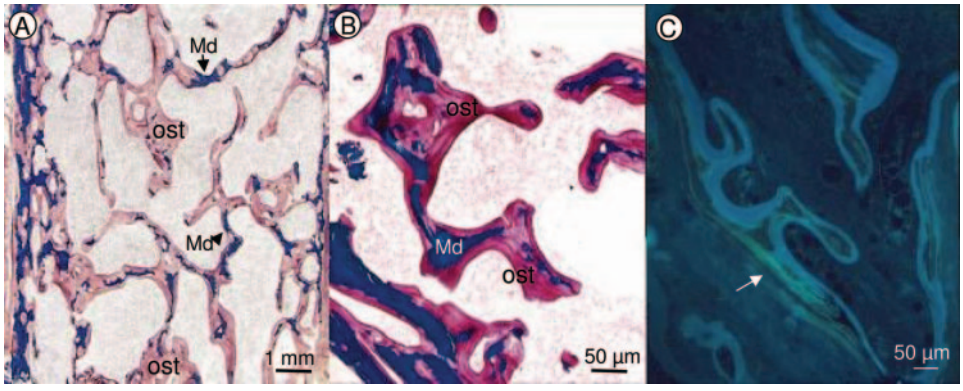

FIG. 4. Primary mineralization defect: osteomalacia. A, Bone mass is normal with a decreased mineralized bone volume (Md) and an increased osteoid volume (ost). B, Accumulation of the osteoid tissue with an increase in the osteoid seam width. C, The tetracycline labels (*arrow*) are diffused due to a severe decrease of the mineralization rate. A and B, Solochrome cyanin R staining; C, unstained section under ultraviolet light.

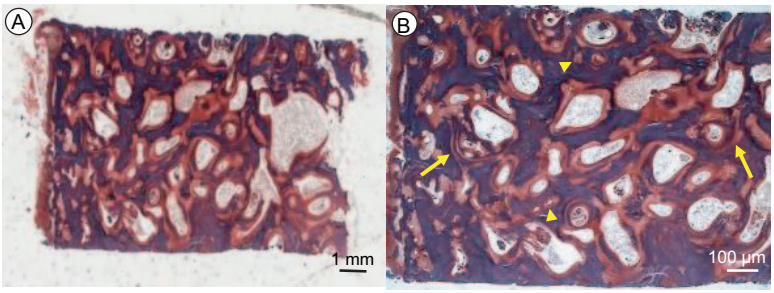

FIG. 5. Abnormal mineral: skeletal fluorosis. A, Increased bone mass with large trabeculae bordered by extended and thick osteoid seams. B, Linear formation defects (*arrows*) and mottled periosteocytic lacunae (*arrowheads*). Solochrome cyanin R staining.

line and lysine residues, increased proline residues) and of collagen cross-links disturb the mineralization process and consequently reduce the bone strength (75). Fluoride is incorporated into the crystalline lattice of hydroxyapatite, making the lattice more stable and less soluble and increasing the crystallinity of the bone mineral. The effect of fluoride on the crystal size is controversial (74, 76). In fluoride-treated rabbits, the length of hydroxyapatite crystals is unchanged, but the width is increased (77). Measured by quantitative microradiography, the mean degree of mineralization in skeletal fluorosis is decreased, but the intraindividual heterogeneity index of mineralization reflecting variation of the secondary mineralization is normal (78). In bone from fluoride-treated osteoporotic women and fluorotic patients, x-ray scattering shows crystals too large to be located inside the collagen fibrils (79). A tissue with a large amount of extrafibrillar mineralization is probably harder but more brittle. In addition, fluoride alters the interface between mineral and collagen that may influence the mechanical properties of bone (80).

VII. Bone Fragility Primarily due to Abnormal Remodeling Rate and Balance (Turnover)

A. High bone turnover with a negative BMU balance

A balance between the volumes of bone resorbed and formed in each BMU maintains bone mass. When the volume of bone resorbed increases and/or the volume of bone deposited in each BMU decreases, a negative BMU balance results producing bone loss and structural decay. Bone loss and structural decay are amplified when remodeling rate increases. A negative BMU balance is found in postmenopausal, corticosteroid-induced osteoporosis and in some endocrine diseases.

1. Postmenopausal osteoporosis. Osteoporosis is defined by a BMD lower than 2.5 SD from the young adult mean (81). This decreased BMD variably reflects the contributions of growth and age-related deficits in bone size, tissue mass, and the degree of mineralization of the bone. Fragility fractures occur in up to 50% of postmenopausal women, but half of the fractures occur in persons without osteoporosis, confirming that bone density is not the only determinant of bone strength in postmenopausal osteoporosis (82). Bone fragility in postmenopausal osteoporosis is the result of a decrease in bone mass and architectural decay in cortical and trabecular bone. There is an increase in bone remodeling and, within each remodeling unit, a reduced bone formation and increased bone resorption (Fig. 6, A and B). Abnormalities in the material properties of bone (collagen and mineral) may contribute, but their relative contribution to skeletal fragility in postmenopausal osteoporosis is unclear. Increased bone remodeling after menopause in women, hypogonadism in some men, and secondary hyperparathyroidism in both sexes accelerates bone loss because each BMU has a negative bone balance due to an imbalance in the volumes of bone resorbed and formed and a decrease in the duration of bone formation by the osteoblasts (83).

Bone turnover shows a wide spectrum in osteoporosis, and different groups of patients have been described with high, low, and normal remodeling (83–87). Furthermore, an increase in bone remodeling at the corticoendosteal envelope contributes to bone loss (88). Osteoporotic trabecular bones are characterized by the transformation of trabecular plates to rods and trabecular perforations corresponding to a thin-

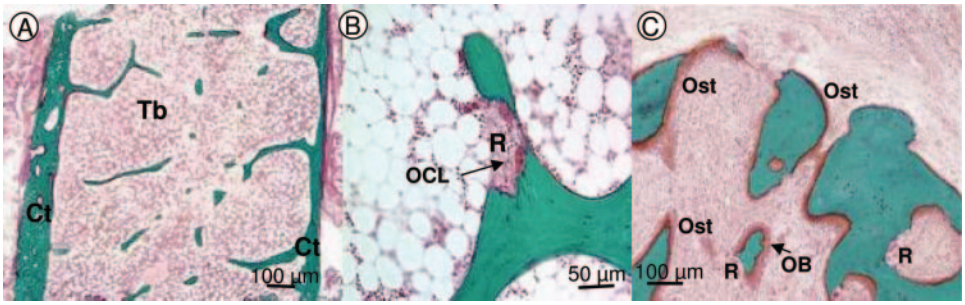

FIG. 6. High bone turnover with a negative BMU balance. A and B, Postmenopausal osteoporosis. A, The decreased bone mass is associated with a loss of the trabecular connectivity. Tb, Trabecular bone; Ct, cortical bone. B, The increased bone resorption (R) contributes to the thinning and tunneling of the trabeculae. C, Primary hyperparathyroidism is characterized by a high bone turnover with increased eroded (R) and osteoid (ost) surfaces. OCL, Osteoclast; OB, osteoblast. Goldner's trichrome staining.

ning of the trabeculae and a loss of connectivity (83, 84, 89). Several studies have shown the relationship between the connectivity and the risk of fracture (23, 90, 91).

As bone loss proceeds in the endosteal surface, reduced periosteal apposition fails to compensate for bone loss so that cortices thin more than would otherwise occur if there had been periosteal compensation as suggested in one study (92) but not in another (93).

Modifications of collagen matrix and mineral occur during aging. Collagen fibrils have been found to be smaller in osteoporotic bone, and an increase in the lysine hydroxylation has been reported (94–96). Concentrations of the reducible collagen cross-links decreased by 25–30% are suggested to reduce bone strength although the collagen density is unchanged (97). An alteration of the degree of type I collagen isomerization reflected by an increase in the ratio α/β urinary C-telopeptide may predict an increased risk of fracture in postmenopausal women (98). Increased fracture risk associated with the Ss polymorphism in the COL1A1 (Sp1) gene is attributed to an increased ratio of α1(I) relative to α2(I) protein (99). Furthermore, a possible link between vitamin D receptor polymorphism and the risk of fracture and bone mass has been found (100).

The mean degree of tissue mineralization measured by backscatter is influenced by the rate of bone remodeling in patients with vertebral fractures. Although the mean is similar to controls, the frequency distribution is different, suggesting a greater heterogeneity in osteoporotic patients than in controls (101). A decreased mineral content and an increased mineral crystallinity have been found in osteoporosis with high bone turnover (102). In contrast, a lower mean degree of mineralization measured by quantitative microradiography has been reported in postmenopausal women with vertebral fractures when compared with postmenopausal controls with a same heterogeneity index, which reflects the variation of the degree of mineralization among the different BSUs of bone, in both groups (103). Overall, the contribution of mineral abnormalities to fragility in postmenopausal osteoporosis is unclear.

2. Hyperparathyroidism. Primary hyperparathyroidism is common and usually asymptomatic. The skeletal manifestations are variable and include bone pain and fractures at several sites including vertebral, distal radius, and pelvis (104). Osteopenia at various degrees may be observed and localized on the cortical bone (105, 106), trabecular bone, or both (107). Very few studies have evaluated fracture incidence in primary hyperparathyroidism. A retrospective study has shown an increased risk of vertebral and nonvertebral fractures in 5766 person-years of observation (104).

The major consequence of primary hyperparathyroidism is an increase in the rate of bone remodeling (Fig. 6C). Increased bone resorption is shown by the extended resorption surfaces and increased osteoclast number. The augmentation of formation associates an increase in the osteoid surfaces, osteoblast number, and mineral apposition rate, which is the rate of the primary mineralization (108–113). Despite this accelerated bone remodeling, cancellous bone volume is maintained (114, 115) with a thinning of trabeculae but a preservation of connectivity (115, 116). In contrast to postmenopausal osteoporosis, the coupling between resorption and formation remains balanced in primary hyperparathyroidism with an augmentation of the osteoblastic activity and lifespan or a decreased erosion depth that results in a normal or increased balance at the BMU level (114).

In contrast, cortices are thinner and more porous (117). Long bones are affected by increases in inner and, to a greater extent, in outer diameters resulting from the stimulation of the subperiosteal apposition and endocortical resorption. The reduction in bone density, which may explain the increased fracture risk at cortical sites, may be partly counteracted by the increase in the cross-sectional bone area (118). Quantitative microradiography shows a decrease of the mean degree of mineralization related to the high turnover, with no change of the heterogeneity index (119). Thus, bone strength in hyperparathyroidism is a function of many variables, such as bone density, bone size, and microarchitecture (118).

3. Hyperthyroidism. Bony manifestations of thyrotoxicosis include bone pain and fractures mainly of vertebrae and femoral neck but also affecting other sites (120–122). The increased risk of fracture results from a decreased BMD, but fractures occur at an earlier age in patients with an history of hyperthyroidism (123–126). Hyperthyroidism is characterized by bone loss at the trabecular and cortical levels with an increased cortical porosity. Bone loss results from a marked increase in bone turnover with an increase in both the resorption and formation surfaces associated with an imbalanced coupling in favor of resorption and a shortening of the formation period (120, 121, 127). Thus, thyrotoxicosis is a typical example of skeletal fragility due to excessive and imbalanced bone turnover with increased resorption. There is no mineralization defect. The microarchitecture, the mineral and collagen phases have not been investigated.

B. Other abnormalities of bone turnover with a negative BMU balance

1. Osteoporosis in men. Fragility fractures occur in men. The incidence of fractures is higher in men than women from adolescence through middle life as a result of more severe trauma, but bone fragility may also contribute to the fracture risk. After 50 yr, the incidence of fractures increases with aging in men (128). The age-adjusted incidence of both hip and vertebral fractures in men is about half of that in women (128). In addition, several other factors may contribute, including nutritional deficiencies, inactivity, hypogonadism, or alcoholism.

Men with vertebral or hip fractures have reduced bone size. As in women, osteoporosis in men is characterized by decreased bone mass with a similar magnitude associated with a reduced cortical thickness and an increased porosity (129–135). Modifications of bone microarchitecture have been reported in osteoporotic men with vertebral fracture independently of BMD when compared with osteoporotic men without fracture (136).

The bone microarchitecture is characterized by a lower trabeculae number and an increased trabecular separation (136). In contrast, in another study performed in younger

men, no significant difference in trabecular architecture was observed between men with crush fractures and controls (137), except for a trend in decreasing number of free-ends (23). The reduced bone size may be due to reduced periosteal apposition during growth, aging, or both (138). Bone loss results mainly from a decreased bone formation (132–135). In addition, increased bone resorption contributes (128, 131, 134, 135, 139, 140).

2. Corticosteroid-induced osteoporosis. Bone loss and fracture risk are related to the dose and duration of glucocorticoid exposure. Bone loss is rapid during the first 12 months, with a significant decrease of lumbar spine BMD since the third month of treatment, observed even with a low dose (10 mg/d) of prednisone (141–142). The fracture risk increases rapidly: the vertebral fracture incidence has been reported to be 2-fold higher in a large cohort of corticosteroid-treated patients compared with controls but decreases after cessation of therapy (143). Fracture risk is greater at predominantly trabecular sites such as the vertebrae and ribs, and the risk of hip fracture is also doubled in glucocorticoid-treated patients (144). However, the prevalence of fractures in corticosteroid-induced osteoporosis is higher than expected from the decreased BMD (145), suggesting that the low bone strength induced by glucocorticoid may be partly independent of the changes of BMD (146, 147).

Glucocorticoids induce apoptosis of osteocytes in animals as in humans (148–152). The number of apoptotic osteocytes is increased on femoral sections obtained from patients undergoing hip arthroplasty for glucocorticoid-induced osteonecrosis in contrast to patients with alcoholic or traumatic osteonecrosis (153). The mechanism by which osteocytes contribute to bone strength is still unknown, but osteocytes have been hypothesized to play a major role in the targeted remodeling, acting as mechanosensor and transducer in bone, and to be involved in the detection and repair of microcracks (146, 147). The loss of viable osteocytes disturbs the osteocyte-canaliculi network resulting in a failure to detect microcracks and consequently to induce their repair (147, 153, 154). In patients with hip fracture, the number of viable osteocytes is decreased by 25%, and no fracture callus is observed when the osteocyte viability is low (155).

The main effect of glucocorticoids on bone is a dramatic inhibition of the osteoblastic activity that results in a decreased wall width of trabecular packets (156) and consequently a thinning of trabeculae. An increased bone resorption has also been reported (157–159). Experimental studies have shown that corticosteroid may promote osteoclastogenesis by inhibiting OPG and concurrently stimulating the expression of RANKL by the osteoblasts (160). This stimulatory effect of corticosteroid on the osteoclast differentiation may be observed at the beginning of the exposure and explain that osteoclastic surfaces are not always increased in corticosteroid-induced osteoporosis (161).

At low cumulative doses (<10 g prednisone), trabeculae are thinner, but connectivity is preserved (159, 162). At high doses (>10 g of prednisone), trabeculae are thin, less numerous, and disconnected (159). A decreased trabecular connectivity has been shown when the bone volume is lower than 11% in corticosteroid-induced osteoporosis (163), contributing to bone fragility. In female growing rabbits, prednisolone induced a decreased BMD and a histomorphometric profile of corticosteroid-induced osteoporosis with low bone mass, thin trabeculae, and decreased bone strength (164).

C. Low bone turnover

Skeletal fragility in osteopetrosis and pycnodysostosis appear to result mainly from the profound decrease in bone turnover that may lead to the lack of microdamage removal. There is, however, no direct evidence of microdamage accumulation.

1. Osteopetrosis. This is a heterogeneous group of inherited metabolic bone diseases characterized by diffuse osteosclerosis. Two major forms are described: the malignant autosomal recessive infantile type, and the autosomal dominant adult type (165). Infantile osteopetrosis manifests in the first year of life with severe bone deformity, fractures, cranial nerves deficit due to the extended size of bones, recurrent infections, anemia, and bleeding due to insufficient marrow space. Adult osteopetrosis is characterized by x-ray abnormalities, fractures of long bones, and vision or hearing deficits. An intermediate form of osteopetrosis with short stature, fractures, cranial nerve deficit, and moderate anemia has also been described (165).

Histological observations confirm an increased bone mass with a large cortical thickness and thick trabeculae. In osteopetrosis, deficient osteoclast activity explains the remnant of calcified cartilage included in mature bone and the presence of osteoclasts with numerous nuclei but without ruffled borders or clear zone (166, 167). Type II may be differentiated from type I by an increased resorptive surface with numerous and multinucleated osteoclasts and a decreased resorption at the cell level (167, 168). These two types present a depressed bone resorption activity because osteoclasts are absent or inactive. Reduced resorption results in decreased bone formation because of the coupling between resorption and formation (169). In mice lacking *c-fos*, a critical gene for osteoclast differentiation, the osteoclasts are absent and both formation and resorption are reduced (167).

Human type II is due to inactivating mutation of the chloride-7-channel gene necessary for acidification of the resorptive site and dissolution of mineral (170). The collagen and mineral contents are normal in osteopetrotic bone (167, 171). In osteopetrotic (*ia/ia*) rats, Boskey and Marks (172) reported higher mineral:matrix ratios than controls due to the absence of remodeling. The continued accretion of mineral leads to the formation of less perfect crystals. Ultrasonic velocity measurements have confirmed the decreased biomechanical strength in human osteopetrotic bones (173). Another animal model of the severe human autosomal recessive form of osteopetrosis is the gray-lethal (*gl*) mouse. In the *gl* mutation, multinucleated osteoclasts are fully differentiated but show a defective cytoskeletal organization and an underdeveloped ruffled border and consequently a marked defect of the osteoclast function. The *gl* phenotype appears independent of c-src or TRAF-6 expression, two signaling factors of the osteoclast differentiation (174, 175).

2. Pycnodysostosis. This is an extremely rare autosomal recessive bone disease due to a deficient activity of cathepsin

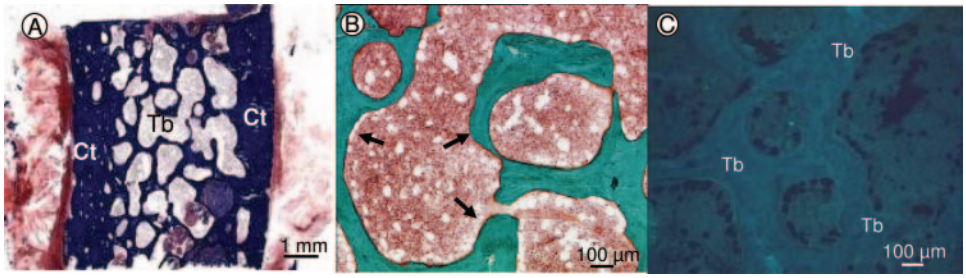

FIG. 7. Pycnodysostosis. A, Increased bone mass with thick cortices and trabeculae. Tb, Trabecular bone; Ct: cortical bone. B and C, The trabecular surfaces show no evidence of active bone turnover (*arrows*). B, No osteoblastic surface and no active osteoclastic resorption. C, Absence of tetracycline label. A, Solochrome cyanine R staining; B, Goldner's trichrome staining; C, unstained section under ultraviolet light.

K, the key enzyme for the degradation of bone collagen. Pycnodysostosis is diagnosed during childhood because of short stature with dysmorphic features of the skull, narrow thorax, short hands and fingers, and increased lumbar lordosis. Recurrent fractures involve the lower limbs. Radiographic examination shows a generalized osteosclerosis with fractures and delayed closure of cranial sutures and fontanels (165).

Histological observations show thick cortices and high bone mass with thick trabeculae (Fig. 7A). The collagen texture is mildly disorganized with the inclusion of some mineralized cartilage within the calcified bone. Bone turnover is markedly decreased without any sign of active osteoblasts (176) (Fig. 7B) and low tetracycline uptake (Fig. 7C). Fringes of nondigested but demineralized matrix are described adjacent to osteoclasts on the trabecular surfaces (176, 177). The orientation of the mineral particles is highly variable, reflecting the disorganization of the collagen fibrils. The size of mineral crystals is augmented as observed in low bone turnover states (176). Cathepsin K knockout mice have an osteopetrosis with an altered osteoclast and osteoblast function, an abnormal bone matrix organization, and very brittle bones. Besides the suppressed bone turnover, a possible direct effect of cathepsin K on bone matrix formation has been hypothesized (178).

VIII. Summary and Conclusions

Bone is a composite material, and the integrity of each component contributes to bone strength. From the size of bones to the levels of collagen molecules and mineral crystals, any modification of these determinants influences bone strength. The ability of bone to resist failure depends on the ability of its material and structural properties to absorb energy imposed during loading and to release it when unloaded. The relative contribution of each determinant in the occurrence of fractures remains unknown, but the study of disease provides avenues to identify and explore the pathogenesis of these defects. Bone fragility in OI results from abnormal quantity and quality of collagen synthesis, which disturb the mineral crystal size. Modifications of the mineral phase, either of its amount as in osteomalacia or of its quality as in fluorosis, decrease bone strength. The level of the bone remodeling influences tissue mineral density and collagen cross-linking, producing structural abnormalities such as stress risers, whereas an imbalance in the volume of bone resorbed and formed compromises the structure of bone. Depressed bone remodeling and subtle abnormalities of mineral and collagen matrix appear to be responsible for skeletal fragility in osteopetrosis and pycnodysostosis, despite an increase in bone density. A better knowledge of the relative importance of the different determinants of the bone "quality" (intrinsic properties of bone matrix, bone architecture, and turnover) in the determination of skeletal strength and fragility will improve the understanding of the pathogenesis of bone fragility in metabolic bone diseases.

Acknowledgments

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Disclosure Statement: The authors have nothing to disclose.

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