Preface

Bone biology and skeletal health are topics of great interest and extensive scientific activity. It therefore seems logical to initiate a series of review volumes that describe current developments in bone biology and the treatment of bone diseases. New knowledge as reported in a wide array of primary publications is evaluated, summarized and its significance explored. To accomplish this effectively requires topical focus and expertise. This first volume in the series ***Topics in Bone Biology*** is focused on *Bone Formation,* the cells that initiate this process, its regulation and its disorders. Subsequent volumes will focus on *Bone Resorption* and *Engineering of Functional Skeletal Tissues.* We hope that our readers will find this first volume both useful and exciting, as we have in putting it together.

The first chapter in this volume, authored by Bukka, McKee and Karaplis, deals in molecular terms with the differentiation of the bone-forming cells. It describes how undifferentiated mesenchymal cells condense to assume the shape of skeletal elements and then follow one of two paths to form the two types of bone, trabecular and membranous. Until recently, knowledge of pertinent gene expression and the mechanisms of osteoblast differentiation was fairly limited, but now a number of factors regulating bone cell differentiation have been identified and characterized. The authors describe specific genes that alter a precursor cell's commitment to a particular lineage, and then discuss specific transcription factors that determine the fate of each cell type. Proteins specific to osteoblasts, such as osteocalcin, and regulation of their expression, are topics discussed in the remainder of the chapter.

Karin and Farach-Carson, in the second chapter, deal with a topic of special interest to experimentalists, namely osteoblast culture *in vitro*. Bone cell culture is essential to understanding what these cells can do, how they respond to regulatory molecules, to stress, and under what conditions they thrive or fail to thrive. At the same time, the authors emphasize that an event observed *in vitro* must be demonstrated to occur *in vivo* in order to assign it functional significance, a lesson that in the excitement of discovery may be forgotten. Experimentalists will appreciate the large amount of cell culture and cell line information that is made available by the authors in the form of extensive tables not readily available elsewhere.

The regulators of bone formation and remodeling are many, both systemic and local. Hurley and Lorenzo, in the third chapter, discuss how growth hormone and the insulin-like growth factor 1 modulate bone formation, as do thyroid hormone, the gonadal hormones, glucocorticoids, and vitamin D, which, its name notwithstanding, effectively acts like a steroid hormone. Parathyroid hormone, PTH, as is well known, is a major regulator of both rapid and long-term bone cell responses, mediated by the PTH receptor of the osteoblast. The more recently discovered parathyroid-related protein,

PTHrP, which binds to the same receptors as PTH, plays a critical role in both intramembranous and endochondral bone formation and development. Local regulators that are discussed include prostaglandins, transforming growth factor-beta, platelet-derived and fibroblast growth factors, as well as a series of newer factors, such as the core-binding factor, osterix, and RANK, RANK-ligand, and osteoprotegerin. Because bone formation and resorption are so closely linked, especially in bone remodeling, the authors discuss the role many of these systemic and local factors play in osteoclast formation and bone resorption.

The skeleton contains virtually all of the body's calcium. Initiating the deposition of the bone mineral, principally calcium phosphate, is a major function of the osteoblasts. Calcium is deposited into cartilage, thus leading to the formation of trabecular or endochondral bone. It is also directly deposited in membrane (or intramembranous) bone, yet the mechanisms of calcification are still not fully understood. Puzas, in the fourth chapter, discusses in detail mineralization and the signals that circulate between osteoblasts and osteoclasts, thus assuring mineralization, on the one hand, and mineral dissolution, on the other. Matrix vesicles, spatial localization of matrix formation, and cellular recognition, as by osteoblasts of the osteoclast lysosomal enzyme, are among the topics that are discussed.

The skeleton fulfills two major functions, metabolic and mechanical, with the mechanical or support function clearly an evolutionary goal. Turner, in Chapter 5, discusses the interrelationship between mechanical usage and metabolic response. For instance, weight bearing or, in engineering language, mechanical loading, causes stress, with maximum stress in a given bone site leading to enhanced bone formation. Stress also causes trabeculae and collagen fibers to align in the direction of the stress. Static loading, however, does not lead to enhanced bone formation. It may be evident that bone cells are the sensors that transduce stress, but the precise way this occurs has only recently been explored and is discussed in detail by Turner. The chapter is enriched by a large number of illustrations.

Martin and Seeman, in Chapter 6, discuss in detail the role played by reduced bone formation in the pathogenesis of bone disease. They thus move in their discussion from the cell to the organ and illustrate how current concepts of bone formation have been developed from *in vitro* studies of cell and organ culture and from *in vivo* studies of mutant mice. They emphasize that the reduction in bone mass associated with aging is related to a reduction in bone formation and further that the increase in bone remodeling that occurs as a result of the decrease in estrogen at the menopause leads to a negative bone balance. This in turn contributes to trabecular and cortical thinning, and increased cortical porosity. Thus research aimed at treating bone fragility should focus on understanding and modulating bone formation, as well as on minimizing bone resorption.

In recent years significant progress has been made in identifying the genetic bases of many diseases affecting bone. In Chapter 7, McLean and Olsen describe in detail diseases involving excess bone formation, many of which are rare. In a number of instances the genetic basis of the disease has been elucidated, although the mechanism by which the genetic defect is translated into the specific disease remains unknown. One example discussed by McLean and Olsen is endosteal hyperostosis (van Buchem type). This disease begins at puberty and affects a large number of bones. The genetic region that has been linked to the disease also may be linked to sclerosis and involves a large (52 kb) deletion flanked by two genes whose dysregulated expression may cause van Buchem disease. McLean and Olsen list many of

these diseases in table form, thereby enabling the reader to understand similarities and differences more readily.

In Chapter 8, Peterlik reviews the means by which osteoblast function is impaired in certain bone diseases (growth retardation, osteomalacia, rickets, metabolic bone diseases, and osteoporosis) and then analyzes a variety of therapeutic and preventative approaches that can minimize or cause the disease to regress. For example, physical exercise, through its strengthening of muscle, also strengthens bone. Peterlik describes the cellular mechanisms by which this occurs. At the same time he calls attention to the observation that estrogens facilitate transduction of mechanical stimuli into skeletal responses. This therefore adds to the more widely appreciated effects of estrogen on bone health, discussed in detail in an earlier section of the chapter. Throughout Peterlik provides insight into the molecular pharmacology of the various agents and drugs that can modulate osteoblast function and thus be used to treat bone diseases selectively.

As editors, we want to thank our author colleagues for the wealth of information contained in their contributions, their willingness to adapt their chapters to make this volume into a meaningful beginning of the series, and their readiness critically to incorporate recent findings in their final texts. We thank Springer UK for sharing our enthusiasm for this volume and the series as a whole.

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Reduced Bone Formation in the Pathogenesis of Bone Fragility

T. John Martin and Ego Seeman

Introduction: Bone Modeling and Remodeling on its External and Internal Envelopes

The mineralized skeleton is defined externally by its periosteal surface and internally by the endocortical, trabecular and intracortical components of its endosteal surface [66, 68]. Cellular activity on these surfaces modifies the external size and shape, internal architecture, total mass, and so the material and structural strength of the skeleton. Bone modeling, the change in size and shape of the skeleton, is achieved by subperiosteal bone formation during growth and aging and defines the whole bone's cross-sectional area in old age. Bone remodeling (reconstruction) on each of the three components of the endosteal (inner) envelope is achieved by teams of osteoclasts that resorb a volume of bone at temporally and spatially discrete sites and by teams of osteoblasts that deposit bone in the same sites. The surface extent of remodeling and the balance between the volumes of bone resorbed and formed in each basic multicellular unit (BMU) on the endocortical surface determine the proximity of the endocortical and periosteal surfaces. This in turn determines the cortical thickness and the distance the cortical shell is placed from the neutral or long axis of the bone. This geometric feature determines the bending strength of the whole bone [77]. Bone remodeling within the cortical shell determines the number of secondary osteons and the porosity of the cortex, while bone remodeling on the trabecular surfaces determines the thickness and connectivity of the trabecular network of plates and sheets.

Provided bone remodeling remains balanced, with the same volumes of bone removed and formed within each BMU, no change in cortical thickness, trabecular number, thickness and connectivity occurs. The necessary and sufficient structural requirement for bone to be irreversibly "lost" is that the volume of bone resorbed is greater than the volume of bone formed [66, 68]. This may result from an increase in the volume of bone resorbed, a reduction in the volume of bone formed, or from both.

Bone Formation and Growth

The processes of bone resorption and bone formation are controlled by circulating hormones, locally produced cytokines and growth factors. Matrix proteins contribute both cytokines and growth factors through specific signaling processes and serve as structural scaffolding. During fetal bone development and during bone repair, woven bone is produced, being characterized by a random (woven) organization of its collagen. It is then remodeled to form lamellar bone, the form which constitutes most of the mature skeleton. In compact

(cortical) bone, lamellar bone is formed as a solid mass enclosing the marrow cavity and constituting approximately 80% of the total bone mass. In cancellous (trabecular) bone the mass is a spongy one, traversing the marrow cavity. It is mainly found in the vertebrae, the flat bones and in the juxta-articular epiphyses of the long bones, forming part of the stromal microenvironment of hemopoietic marrow. The original skeletal matrix of the fetus is transformed into bone by either of two fundamental processes, endochondral or intramembranous ossification.

Most flat bones (skull, mandible and maxilla) are formed by *intramembranous bone formation*, which also contributes to the growth of short bones and the thickening of long bones. In this process a group of mesenchymal cells within a highly vascularized area of the embryonic connective tissue differentiates directly into preosteoblasts and then into osteoblasts, which begin the synthesis and secretion of osteoid, forming a network of spicules and trabeculae at sites of ossification. The collagen fibers at these sites are randomly oriented and calcification quickly follows osteoid formation, resulting in the trapping of some osteoblasts, which then become osteocytes. Their cytoplasmic extensions shrink to form the fine processes, which can be recognized within canaliculi. Continuous division of mesenchymal cells provides a supply of undifferentiated osteoprogenitor cells, which form osteoblasts that lay down more bone. As the network of trabeculae is established, the primitive mesenchymal tissue among these trabecular branches differentiates into the myeloid (hemopoietic) tissue of the bone marrow. The addition of trabeculae to the periphery increases the size of the forming bone. Those regions of the mesenchymal tissues that remain uncalcified differentiate into the periosteum and endosteum of developing intramembranous bone.

Most of the long and short bones of the body develop by *endochondral bone formation.* The first step in this process is that a miniature hyaline cartilage model is formed, following which this cartilage model continues to grow, serving as a structural scaffold for bone development, with the scaffold then resorbed and replaced by bone. Growth of the cartilage model occurs mainly by perichondral apposition and by interstitial chondrocytic mitosis to form an elongated dumb-bell shaped mass of cartilage, consisting of a shaft (diaphysis) and future articular portions (epiphyses) surrounded by perichondrium [92].

Within the shaft of the cartilage model, chondrocyte hypertrophy results in enlargement of the lacunae and reduction of the intervening cartilage matrix septa, which become calcified. At the same time the overlying perichondrium of the shaft develops osteogenic potential and assumes the role of periosteum. This takes effect as the perichondrium at the middle of the diaphysis of cartilage becomes vascularized and the chondrogenic cells become osteoprogenitor cells forming osteoblasts. These manufacture bone matrix, forming the subperiosteal bone collar on the surface of the cartilage template by intramembranous bone formation. The consequent restriction of nutrient diffusion to the hypertrophic chondrocytes causes them to die, leaving empty, confluent lacunae. These form large concavities – the future marrow cavity – in the center of the cartilage model. An osteogenic bud consisting of osteoprogenitor cells, hemopoietic cells and blood capillaries penetrates the spaces left by the degenerating chondrocytes within the cartilage model. More osteoprogenitor cells invade the area, and therefore primary bone synthesis takes place over the remnants of calcified cartilage, with the bone matrix eventually becoming calcified.

These events constitute a dynamic continuum that is completed over a number of years as bone growth and development progress towards the growing epiphyses at each end of the bone. At the same time the bone is constantly being remodeled. Bone grows in length from the growth plate and the metaphyseal cartilage of the epiphyseal growth plate is replaced by a plate of calcified cartilage/calcified bone complex. The latter is resorbed by osteoclasts, and the marrow cavity of the diaphysis becomes confluent with the bone marrow cavity of the epiphysis. Once the epiphyseal growth plate is resorbed, growth in length is no longer possible, although widening may still occur [41, 50].

Growth in girth of the diaphysis takes place by periosteal membranous bone formation along the periphery and by osteoclastic resorption along the inner medullary surface. The deposition of new bone on the endosteum exceeds resorption so that the cortex thickens even as the shaft diameter and marrow cavity widen. Bone growth involves the coordination of many cellular activities in specific sites whose onset and rates vary among bones and even within a

single bone during its development. Control of these processes is achieved by many humoral and local factors whose relative concentrations, sites and sequences of appearance vary during development.

Transcriptional Controls

A number of experimental, clinical and therapeutic advances of the last few years have informed us of bone formation mechanisms. A most important discovery has been that of Cbfa1 (runx2), a transcription factor that appears to be the earliest transcriptional regulator of osteoblast differentiation. It also controls bone cell function by maintaining the differentiated phenotype of the osteoblast in maturity. Transgenic overexpression of a dominant negative form of runx2 postnatally in mice led to decreased production of runx2, as well as diminished expression of genes required for the differentiated osteoblast [24]. Runx2 is central to replenishment of osteoblasts after bone loss, a key requirement in restoring bone.

The search for transcription factors other than runx2 has led to a recent exciting development. Nakashima et al. (2002) used subtractive hybridization between untreated C2C12 cells and those treated with bone morphogenetic protein 2 (BMP-2) [60]. They identified a novel zinc finger-containing transcription factor, called osterix (Osx), which is specifically expressed in developing bones and not in other tissues. Mice rendered null for the *osx* gene did not develop a mineralized skeleton, but, like the runx2-/- mice, had an entirely cartilaginous skeleton and died at birth [60]. In Osx-/- mice, runx2 mRNA was at the same level as in the wild type, but the mutant mice had no Osx mRNA. This suggests that Osx is an important transcription factor in osteoblast differentiation that functions downstream from runx2. The authors suggest that runx2 activity may induce partial differentiation of mesenchymal cells to a pre-osteoblast stage, at which time Osx is required, acting perhaps cooperatively with runx2. The runx2-/- mice have no osteoclasts but the Osx-/- mice do. It seems therefore that osteoclasts are associated with hypertrophic chondrocytes, cells that are not formed in runx2 mice. This is an intriguing discovery, which will surely lead to significant further insights. In the early 1990s the BMPs were the main class of proteins thought to be involved in the regulation and maintenance of skeletal formation. The field has now expanded. BMPs are important in osteoblast differentiation through their paracrine role in bringing into play signaling mechanisms that are crucial for the process [93], including sonic and indian hedgehog, and indeed runx2, the production of all of which is enhanced by BMP-2.

However important runx2 is in osteoblast differentiation and bone formation, it is clearly subject to regulatory mechanisms mediated by other transcription factors, growth factors and hormones [46]. This regulatory pathway undoubtedly contains a series of molecular steps that can serve as targets for the development of drugs to influence bone formation. There can be little doubt that defects in the runx2 pathway result in impaired bone formation and the control of runx2 appears to be so important that defects could contribute to states of impaired bone formation.

Insights from Genetics

Intriguing insights into the control of bone mass have come from recent discoveries of mutations in a gene associated with the osteoporosis pseudoglioma syndrome and the high bone mass syndrome. The genetic abnormalities consist of mutations that lead to inactivation of the gene for low density lipoprotein receptor-related protein 5 (LRP5). This results in impaired bone mass and severe osteoporosis. Heterozygous carriers also have reduced bone mass and an increased incidence of osteoporotic fractures [34]. An activating mutation of the same gene is responsible for the high bone mass syndrome [53]. Further light on this pathway comes from the findings in yet another kindred with greatly increased bone mass resulting from an activating mutation of LRP5 [14]. *In vitro* studies showed that the normal inhibition of Wnt signaling by another protein, the Wnt antagonist Dickkopf-1 (Dkk-1) was defective in the presence of the mutant LRP5. This provides a molecular explanation for the increased activity of the Wnt signaling pathway.

LRP5 is a single pass membrane receptor that forms part of a complex necessary for activation in the Wnt signaling pathway. LRP5 interacts with the Wnt-frizzled ligand-receptor complex, resulting in inhibition of beta-catenin

phosphorylation by glycogen synthetase kinase-3 (GSK-3). Since GSK-3 activity facilitates the ubiquitin-mediated breakdown of beta-catenin, the LRP5 effect is to prevent this, allowing translocation of beta-catenin to the nucleus, where it interacts with TCF/LEF transcription factors to activate gene expression. Dkk-1 and Dkk-4 are both inhibitors of the LRP5 interaction with the Wnt receptor complex, whereas Dkk-2 and Dkk-3 are both stimulators.

New approaches to understanding the bone formation process will undoubtedly come from further study of the Wnt signaling pathway, a pathway that had not previously been identified with the regulation of bone cell function.

Neural Control

An exciting example of the power of mouse genetics is the discovery that leptin deficient (ob/ob) and leptin signaling deficient (db/db) mice have greatly increased bone mass, compared to wild type, notwithstanding their hypogonad and hypercortisol state [25]. This work has raised the intriguing possibility that leptin, acting in the brain, is a central regulator of bone formation by reducing the release into the circulation of a promoter of bone formation. To add to the concept of central nervous system regulatory control of bone formation, neuropeptide Y (NPY) also may have a role [5]. NPY, known to be a downstream modulator of leptin action in the central nervous system, was found to cause bone loss when administered into the cerebral ventricles of mice. Furthermore, in the same study, Y2 receptor deficient mice were found to exhibit a two-fold increase in the amount of trabecular bone, and selective deletion of hypothalamic Y2 receptors in conditional Y2 knockout mice resulted in an increase in trabecular bone.

Lessons from Therapeutic Experiments

In considering the topic of diseases of impaired bone formation it is useful to consider what might be learned from at least two important observations in therapy. One is the decrease in bone formation that occurs with glucocorticoid treatment and contributes to the severe osteoporosis that so often accompanies this. The other is the dramatically beneficial effect on bone formation of intermittent therapy with parathyroid hormone (PTH).

The fact that intermittent injections of PTH stimulate bone growth was already recognized in the 1930s. Not surprisingly, PTH-related protein (PTHrP) exerts similar actions, with PTHrP(1-34), (1-36) and (1-74) having all been shown to be anabolic in studies with rodents [28, 36, 82, 91]. Recent clinical studies have established the fact that PTH has a powerful anabolic effect, revealing it as the first therapy that is clearly capable of restoring lost bone [61].

Insulin-like growth factor-1 (IGF-1) and TGF beta are two of the major growth factors of bone. The bone matrix stores large amounts of latent TGF beta, which requires activation to produce its effects. IGF-1 is also provided in a latent form in matrix because of the regulated production of several specific binding proteins which sequester it [16, 17]. PTH has been shown to activate TGF beta [94] and to enhance IGF-1 production by osteoblasts and by bone in organ culture. Such mechanisms could contribute to the PTH anabolic effect [16].

The discovery of PTHrP production in bone raised the intriguing possibility that this molecule has important local actions in bone, perhaps even being the primary ligand for the receptor it shares with PTH (PTH1R).The latter possibility would imply that circulating PTH adds a systemic endocrine regulatory step to what is primarily the result of local, paracrine effects [84]. A function of PTHrP that has been studied extensively is its role in endochondral bone formation. Targeted disruption of the genes for PTHrP or the common PTH/PTHrP receptor (PTHR1) in mice resulted in death in the perinatal period with gross skeletal abnormalities consistent with chondrodysplasia [44]. Histological studies of tibiae from homozygous PTHrP null mutants showed significant shortening of epiphyseal growth plates as a result of: a markedly reduced number of proliferating chondrocytes, distortion of the orderly columnar arrays of hypertrophic chondrocytes by clusters of non-hypertrophic chondrocytes, alteration in the orderly program of cell death normally occurring in the metaphyseal region, and aberrant differentiation of periosteal progenitor cells. These observations suggest that PTHrP plays a central role in fetal endochondral bone formation by maintaining a pool of

proliferating chondrocytes, by inhibition of terminal chondrocyte differentiation, by retardation of cartilage matrix mineralization, and by inducing differentiation of periosteal mesenchymal precursors into cells of the chondrocytic or osteoblastic lineages.

It seems likely that PTHrP is also involved in intramembranous bone formation. In the rabbit, cells of the osteoblast lineage expressed PTHrP mRNA and protein throughout the entire sequence of intramembranous bone formation, with prominent production by cuboidal, actively synthesizing osteoblasts, and weaker expression in lining cells on the mineralized trabeculae [47]. These observations, together with those of others [2, 45], all support a role for PTHrP in the differentiation of mesenchymal precursors to the osteogenic lineage.

Further investigations of PTHrP mutant mice provided evidence to suggest that PTHrP is equally important for the orderly commitment of precursor cells to the osteogenic lineage and subsequent maturation and/or function. In the PTHrP(-/-) mice, osteoblastic progenitor cells (as with chondrocytes) contain inappropriate accumulations of glycogen. This indicates that as a consequence of PTHrP deficiency, there arises a metabolic or other defect in cells of the osteogenic lineage [3]. Heterozygous PTHrP(+/-) mice, while phenotypically normal at birth, by three months of age exhibit a form of osteopenia that is characterized by a marked decrease in trabecular thickness and connectivity [2]. Moreover, the bone marrow of these mice contains an abnormally high number of adipocytes. Since the same pluripotent stromal cells in the bone marrow compartment can give rise to adipocytes and osteoprogenitor cells [65], the increased number of adipocytes and the osteopenia in these mice may be attributed to altered stem cell differentiation resulting from PTHrP haploinsufficiency. Alternatively, changes in apoptosis, which is known to be modulated by PTHrP, may have important implications for the development of osteopenia in these animals. Whatever the mechanism, PTH1R-mediated processes appear to play a significant part in the promotion of bone formation. Specific ablation of *PTHrP* or *PTH1R* gene sequences in osteoblasts will undoubtedly lead to a better understanding of the role PTHrP plays in adult bone metabolism.

Because PTHrP mimics the actions of PTH by binding to the common receptor and activating adenylate cyclase, PTHrP causes an increase in the production and activity of osteoclasts, thereby promoting bone resorption [47].

PTHrP also reproduces the anabolic effect of PTH [36]. The ability of PTH (and PTHrP) to promote bone formation is dependent upon the hormone being administered intermittently in a way that yields blood level peaks that are not maintained. In that circumstance, processes are initiated in bone which result in anabolic effects, presumably as a result of the activation of genes responding specifically to a rapid increase in PTH or PTHrP. On the other hand, if PTH or PTHrP is infused, or administered in such a way that elevated plasma levels are maintained, the dominant effect is stimulation of osteoclast formation and bone resorption, which overrides any anabolic response. Recent *in vivo* studies in the rat have shown that PTH infusion caused a robust and sustained increase in RANKL and a decrease in OPG production. In addition there occurred a rapid depletion of OPG stores in the bone matrix. All of these changes preceded hypercalcemia and enhanced osteoclast formation. Furthermore, sustained elevated levels of PTH resulted in decreased expression of genes associated with the bone formation phenotype of the osteoblast [54]. These included cbfa1, osteocalcin, bone sialoprotein and type I collagen. In the case of single injections of PTH, on the other hand, even though they triggered a rapid and transient increase in the RANKL/OPG ratio, they led to increased bone formation and enhanced expression of the genes associated with bone formation [64].

The results of the mouse genetic experiments favor the idea that there is a central role for PTHrP in bone development and growth. What are the ways in which PTHrP, as a paracrine/autocrine factor in bone, can contribute? The pharmacologic effects of intermittent versus sustained PTH/PTHrP treatment are striking and very different. Since the circulating level of PTH is a function of the extracellular calcium concentration, its concentration is unlikely to go up and down so as to stimulate osteoblasts and bone formation. This role could be filled by PTHrP, whose local production is regulated in turn by various hormones, cytokines and/or perhaps even neural transmission. Moreover, other regions of the PTHrP molecule could have added influence on local events, either directly or by modifying actions through the PTH1R.

Glucocorticoid Inhibition of Bone Formation

Glucocorticoid-induced osteoporosis results largely from inhibition of bone formation; in the early stages bone resorption is also promoted. The inhibition of bone formation constitutes a target for therapy, but the mechanism of inhibition may also give insight into disorders of bone formation.

Glucocorticoids induce the differentiation of preosteoblasts to more mature cells [8], but paradoxically decrease the function of mature differentiated cells, as reflected by decreased transcription of osteocalcin and type I collagen. Decreased synthesis of growth factors, particularly IGF-1 and IGF-2, may contribute to the inhibition of bone formation. Glucocorticoids inhibit IGF-2 synthesis and decrease IGF-1 synthesis in osteoblasts by inhibiting transcription. In addition, glucocorticoids regulate production by osteoblasts of IGFBP-3, -4 and -5 in osteoblasts [62]. IGFBPs, all of which are expressed by osteoblasts [62], enhance or inhibit IGF action. Prednisolone given to male mice reduced bone mineral density (BMD), vertebral cancellous bone area, trabecular width and histological indices of bone formation, including osteoid area, perimeter, width, mineralizing perimeter and mineral apposition rate. Production of osteocalcin, the marker of bone formation, decreased while excretion of desoxypyridinoline, a marker of bone resorption, remained unchanged. High dose prednisolone increased osteoblast and osteocyte apoptosis in femoral cortical bone [55, 90]. This work suggests that decreased production of osteoclasts explains the corticosteroid-induced reduction in bone remodeling, and that decreased production and increased apoptosis of osteoblasts explains the decline in bone formation and trabecular width.

The early accelerated loss of bone may also be the result of corticosteroid-induced prolongation of the lifespan of existing osteoclasts by reducing apoptosis [90]. Cancellous osteoclast number increased even though osteoclast progenitor number decreased. *In vivo*, glucocorticoids prevent the proapoptotic effect of bisphosphonates on osteoclasts, thereby maintaining osteoclast numbers. Bone loss occurs despite bisphosphonate treatment.

Structural Abnormalities in Women and Men with Fragility Fractures and Reduced Bone Formation

Reduced bone formation plays an important role in the pathogenesis of the bone fragility characteristic of osteoporosis, the most common metabolic bone disease. The section to follow describes the structural abnormalities found in patients with fractures and the ways in which abnormalities in bone formation during growth and aging may produce these abnormalities.

Women and men with fragility fractures have reduced BMD at most regions of the skeleton, but the deficits tend to be most severe at the site of fracture. Patients with spine fractures have greater deficits at the spine than hip, patients with hip fractures have greater deficits at the hip than spine, patients with forearm fractures have greater deficits at the forearm than spine [80].

Women and men with spine fractures have reduced vertebral BMD for two reasons. The cross-sectional area and height of the vertebrae are smaller [21, 89]. As a result the bone mass is decreased, and the cortices are thin and porous, particularly on the inner third near the bone marrow. In addition, the trabecular plates and sheets are thinned, and many are rodlike or have disappeared. This causes loss of connectivity [1]. In men, trabecular thinning rather than loss of connectivity tends to dominate, but men with osteoporosis and fractures have greater loss of connectivity than men who have osteoporosis but no fractures [51].

Women and men with hip fractures have vertebrae of normal size and modest deficits in vertebral BMD. In the women, femoral neck diameter may be reduced, normal or increased [11, 18, 23, 45]. BMD is reduced due to thinning of cortices which contain large intracortical cavities. The diameter of the femoral neck is reduced in these men, as is their BMD, probably due to cortical thinning [80].

These abnormalities are usually attributed to "excessive bone loss". However, the term "bone loss" is ambiguous. Excessive or more rapid bone loss may be the result of a more negative bone balance in the BMU. This in turn may be due to a greater volume of bone having been resorbed in each BMU. It may also be due to a

lower volume of bone formed in each BMU, or to a combination of more bone being lost and less formed. Alternatively, excessive bone loss may be due to a higher remodeling rate than in the controls. Histomorphometric evidence for higher resorption in the BMU, lower bone formation in the BMU, or higher remodeling rate in fracture cases than in controls has indeed been reported in many studies [24, 29, 37, 49, 59, 63]. A higher group mean for indices of resorption or a lower group mean for indices of bone formation in fracture cases has also been reported. However, many patients have normal or reduced bone resorption rates, whereas many others have reduced bone formation rates. Some others have no histological parameters outside the reference range.

For example, 63 postmenopausal women aged 55 to 75 years with vertebral crush fractures had a 20–30% reduction in cortical width and cancellous bone volume. The number, but not the thickness, of the trabeculae was reduced in the patients, compared to 25 age-matched normal controls. However, the patients exhibited no increase in activation frequency, or increased bone resorption, or reduced bone formation relative to controls. This suggests that bone loss proceeded at a rate that was no different from that of controls [83]. Similarly, in a study of 146 osteoporotic women aged 45 to 75 years, 58% had decreased osteoblastic and osteoclastic activity, 22% had decreased osteoblastic and increased osteoclastic activity, 16% had an increase in both osteoblastic and osteoclastic activity, 5% had no bone surface cell activity and 4% had normal osteoblastic and osteoclastic activity [72]. Likewise, lower mean bone formation and a higher mean bone resorption has been reported in women with vertebral fractures, compared with controls selected by having normal bone mass [27]. The remodeling parameters varied widely, with many subjects having low, high or normal indices of bone formation and many having high, low or normal indices of bone resorption. Similar observations have been reported with remodeling markers [15, 26]. This variability in indices of remodeling reflects heterogeneity in the pathogenesis of the structural abnormalities that produce bone fragility in old age.

Reduced Bone Formation During Growth, Young Adulthood, Midlife and Old Age

Reduced Endosteal and Periosteal Bone Formation During Growth

Cortical thickness is determined by the relative growth of the periosteal and endocortical surfaces. The extent of periosteal expansion determines the peak diameter of the long bone. The movement of the endocortical surface relative to the periosteal surface is determined by the extent of endocortical bone resorption and formation. If both are equal, the endocortical surface will not move during growth and cortical thickness will be determined entirely by the extent of periosteal apposition. For any given degree of periosteal expansion, cortical thickness may be reduced if endocortical bone mass is reduced, either when resorption exceeds formation, or formation is reduced more than resorption.

In males the endocortical surface of the metacarpals moves little. As a result resorption, is matched by an equal amount of apposition, resulting in little net change in the medullary canal diameter. At weight-bearing sites like the femur, there is net endocortical resorption, causing the medullary cavity to be enlarged. At puberty in males, periosteal bone formation increases, probably due to androgen and growth hormone (GH) and IGF-1 dependent mechanisms [95]. In females, estrogen inhibits periosteal expansion. This accounts for the smaller long bone diameter in females than males. However, estrogen, metabolites of estrogen, and perhaps progestins may inhibit endocortical bone resorption and stimulate endocortical apposition. As a result, the medullary diameter of the narrower long bone is reduced so that final cortical thickness is similar in men and women. However, in men it is largely the result of periosteal apposition. In women it is the result of both periosteal apposition and endocortical contraction (85%:15%).

Differences in bone mass are the result of species-specific and sex-specific differences in the growth of the periosteal and endocortical surfaces of long bones. For example, the peak

bone mass of the SAM P6 mouse is reduced because the cortices are thin compared with those of the P2 control [39, 42]. The thinner cortices are the result of reduced endocortical bone formation during growth, so the resulting bone has a wider medullary cavity. Periosteal bone formation in the mutants was no different from that in the P2 control (the P6 and P2 have similar bone diameter). The reduction in endocortical bone formation is due to a decrease in the osteoblast progenitor population. Replacement of the marrow in the mutants by marrow cells from the P2 controls restores bone formation [86, 87].

The mechanisms causing the accelerated aging of the colony forming units which induce osteoblast progenitor formation are not known. Whatever the mechanism, the reduction in osteoblasts was confined to the endocortical surface adjacent to marrow, and did not involve periosteal osteoblasts. External bone size is the same in C3H and B6 mouse strains because periosteal bone formation is similar. The C3H mice have higher femoral and tibial bone mineral content (BMC) than the B6 because their endocortical bone formation rate (BFR) is greater, producing a thicker cortex than in the G6 mice. The ratio of femoral periosteal BFR/bone surface was also slightly higher in C3H than in C57 mice in this study [73].

Differences in periosteal bone formation may produce differences in bone size within and between species. Mice made GH receptor protein (GHRP) deficient have a reduced femoral BMC, because their periosteal apposition rather than endocortical remodeling is reduced [81]. The defect can be reversed by administration of IGF-1. The MOV 13 mouse, a transgenic strain, carries a provirus that prevents the initiation of transcription of the alpha 1(I) collagen gene. Thus the resulting bone fragility is the result of a form of abnormal osteogenesis [9, 10]. Heterozygotes for the null mutation survive into adulthood and their fibroblasts make less collagen type I. In this mouse, there is skeletal adaptation that compensates for the bone fragility; bending strength is increased by compensatory periosteal apposition. In this model of osteogenesis, continued periosteal bone formation is compensated for by the marked reduction in type I collagen synthesis, with bending strength maintained relative to that of the wild-type controls.

It is not known whether the reduced bone size or thinner cortices found in the spines of men and women with fractures can be explained by specific abnormalities that limit periosteal apposition, or as having come about because of excessive endocortical resorption or reduced endocortical bone formation. The larger diameter of the femoral neck in women with hip fractures and in their daughters is likely to be growth related [85], but the mechanisms are not known. As estrogen deficiency during growth results in persistence of periosteal apposition, sex hormone deficiency during this time may partly account for the findings [95].

Reduced Formation of Trabeculae and Reduced Thickening of Trabeculae During Growth

Trabecular BMD (the amount of mineralized bone within the periosteal envelope of the whole bone) is determined by the number and thickness of trabeculae. Trabecular numbers do not increase during growth but remain constant [69]. The increase in trabecular volumetric BMD is the result of thickening of existing trabeculae. At puberty, trabecular BMD increases at the same rate in males and females of a given ethnic group. The increase in trabecular BMD is the result of further thickening of existing trabeculae, rather than of an increase in the numbers of trabeculae. African Americans and Caucasians have the same number and thickness of trabeculae before puberty, but the increase in trabecular BMD at puberty is greater in African Americans than in Caucasians [32, 33]. It seems likely, therefore, that race-specific factors regulate the increase in trabecular BMD that occurs at puberty, but the increase is not sex specific, being the same in males and females of a given race. As yet the genetic factors conferring a higher or lower volumetric BMD are not known, but knowing them could be important because an individual's peak volumetric BMD in young adulthood determines the volumetric BMD over the next many years.

Rosen et al. [76] have reported that the serum IGF-1 levels were 35% higher in C3H mice than in B6 mice. The F1 progeny had IGF-1 and femoral BMD values intermediate between the parental strains. The F2 progeny with the highest BMD had the highest IGF-1 levels, while IGF-1 values in calvaria, tibia, femora were also ~30% higher in C3H than B6 mice. The authors inferred that the difference in the volumetric

BMD between strains may be the result of differences in skeletal IGF-1 synthesis levels between the two mouse strains. Whether this is indeed the case is as yet unclear, nor is it known whether the variance in peak volumetric BMD in human subjects can be explained at least in part by differences in sex steroid levels and differences in the GH and IGF-1 endocrine system.

The lower volumetric BMD in women and men with spine and hip fractures is likely to be due to lower peak volumetric BMD, rather than to excessive bone loss during aging. Data supporting this explanation come from studies of the offspring of these patients [78, 85]. The volumetric BMD values of the spine of daughters of women with spine fractures are about half as low as those of their mothers. This is consistent with a genetic basis. In other words, excessive bone loss during aging need not be invoked to explain the deficit in the mothers with fractures. Daughters of women with hip fractures have deficits in volumetric BMD that are less than half the deficits observed in their mothers. This suggests that environmental factors, such as illness or immobility, may also account in part for these deficits. Why women who sustain spinal fractures have thinner or fewer trabeculae and thinner cortices, and the mechanisms responsible for this, has not been studied.

Reduced Bone Formation in Bone Loss in Young Adulthood, Menopause and Old Age

There is compelling evidence from several sources to support the view that trabecular bone mass begins to decline shortly after bone mass has attained its peak in the second or third decade of life, perhaps even while periosteal and endocortical bone is still accruing [31, 43, 57, 74]. The precise time or point when trabecular bone loss begins is unclear because of the absence of data on cross-sectional or longitudinal histomorphometric measurements in young adults. Yet this early loss of trabecular bone is probably not due to an increase in activation frequency or in the volume of bone resorbed in each BMU. This is because activation frequency declines from its highest value, attained in the first few years of life, to reach a nadir after puberty and thereafter remains low until the menopause in women and old age in most men [69]. At the same time, as the remodeling rate declines on the endocortical and trabecular endosteal surfaces, osteoblast lifespan increases and the BMU balance becomes positive as a result of the increase in cortical width, trabecular thickness (not number), and mean wall thickness.

The likely mechanism for trabecular bone loss in young adulthood is a progressive age-related decline in bone formation in each BMU, so that the bone balance, initially positive, becomes zero for a period of time and then becomes negative. Lips et al. [52] have reported a linear decrease in mean wall thickness across age in a small sample of men and in women. Although the regression line suggests a linear decline from 20 years of age, the data seem to favor a period of stability, followed by a decline after the age of around 50 years. The decline in mean wall thickness reflects a drop in the volume of bone that is formed in each BMU. It is this decline that is responsible for the lifelong fall in trabecular bone mass in men and in women before they reach the menopause.

At the menopause bone loss accelerates in women because estrogen withdrawal leads to an increased remodeling rate, with many more remodeling foci on the endosteal surfaces. Each of these foci produces bone loss because the BMU balance is negative. The accelerated loss of bone that occurs initially is due to the rapid fall in bone mineral, which comes about because of the large increase in the number of BMUs in negative balance. This in turn increases the porosity of cortical bone [40] and produces thinning and loss of trabeculae. The rapid fall in bone mass is partly a consequence of the delay in bone formation within each of the increasing number of remodeling sites, many of which are new [35, 67].

Following completion of the remodeling cycle by bone formation in the large number of remodeling sites, perimenopausal bone loss slows, but continues at a faster rate than before menopause. This is because of the persistence of the high remodeling rate and the worsening of the BMU balance. Estrogen deficiency increases the rate of remodeling and the lifespan of osteoclasts, but decreases the lifespan of osteoblasts. As a result, less bone is formed [38, 55]. The increased number of remodeling sites and the deeper resorption lacunae produce loss of trabecular connectivity, which contributes to the increase in intracortical porosity on the inner third of the cortex in women.

Men do not undergo a midlife acceleration in bone remodeling. The loss of trabecular bone in men involves the thinning of trabeculae, but does not lead to the complete loss of trabecular plates seen in women [1]. Bone loss is the result of a reduction in the volume of bone formed, rather than an increase in the volume of bone removed. This helps maintain trabecular connectivity in men. When trabeculae are lost, the trabecular surface available for remodeling decreases. Trabeculae are better preserved in men than in women. Therefore, in old age men continue to lose bone from the trabecular compartment for a longer time than women. Yet the overall rate of loss of trabecular bone in men and women is quite comparable.

Late in life, endocortical and intracortical remodeling increase and bone is lost primarily from cortical bone. This is because remodeling is surface-based and the surfaces within cortical bone increase as a result of the increase in intracortical porosity [12]. Initially the number of intracortical pores increases with age, but as pores coalesce, the cavities become larger but fewer in number. This predisposes to stress (like eddy currents around a rock in a river). In turn this may lead to microfractures at cortical sites such as the proximal femur. Cortical bone effectively becomes "trabecularized", particularly on its inner third. The total surface available for bone remodeling does not diminish with age, it moves from the trabecular to cortical compartment. Secondary hyperparathyroidism may increase intracortical remodeling in elderly men and women. This accelerates the loss of cortical bone mass but not of trabecular bone mass, which seems better preserved in this condition [70].

Thus, bone loss accelerates in old age because the already thinner porous cortices, and the thinner and fewer trabeculae, are subjected to the same or higher intensity of remodeling; the *same or a larger* volume of bone is removed and less is formed in each of the many BMUs that remodel *a diminishing total volume* of cortical bone on its intracortical and endocortical endosteal surfaces. Consequently, structural damage and bone fragility increase out of proportion to the reduction in bone mass.

Loss of bone *mineral* mass occurs in old age also out of proportion to the loss of bone mass because the high rate of remodeling leads to a drop in mineral content of the ever-declining bone tissue mass. The high remodeling rate replaces older, more completely mineralized bone with younger bone that has undergone primary, but less complete secondary mineralization. Consequently, both material and structural properties of bone decline as people age [19].

Reduced Periosteal Bone Formation During Aging

Periosteal bone formation is vigorous during growth and accounts for about 90% of the increase in cortical thickness in men and 75% of the increase in cortical thickness in women, with endocortical bone formation accounting for the rest. After the completion of growth, periosteal bone formation continues in both sexes as endosteal bone loss proceeds, but much more slowly than during growth. The reasons for this are not understood.

Periosteal bone formation plays an important role in the maintenance of bone mass and in providing bending strength to bone. It does this by increasing cross-sectional area (CSA) of bone and by partly offsetting the endosteal bone loss [6, 7, 22, 77]. Cortical bone loss is smaller in men than in women, because periosteal bone formation is greater, *not* because endosteal resorption is lower. Thus, bone "loss" is the *net* result of the difference between the periosteal bone formed during aging and all the bone irreversibly removed from the endosteal surface. The latter is a function of the size of the negative bone balance in each BMU and of the number of BMUs (the remodeling rate). The hormonal factors that determine the greater periosteal apposition in men than in women have not been studied.

The factors regulating the extent of periosteal bone formation in men and women of different races and at different sites in the skeleton are virtually unstudied. Conceivably the periosteal apposition rate is in part an adaptive response to the effects of increased loads on the subperiosteal bone surface. As trabeculae disappear and cortices become more porous and thinner, the strains produced by loads increase significantly. Evidence to support this notion comes from studies with the transgenic mouse strain, Mov13. This strain has a form of osteogenesis imperfecta, in which less collagen type I is produced, but compensatory periosteal apposition offsets the increased bone fragility by increasing cortical thickness. As a result bending strength is equal to or above that of the wild type [4, 9, 10].

Concluding Remarks

Reduced bone formation plays a central role in the pathogenesis of bone loss and bone fragility. Patients with fractures are likely to have a variety of structural abnormalities that are the result of abnormalities in several of the hierarchical steps in bone remodeling and modeling, with genetic and environmental components that require study [88]. The resulting abnormalities in the structural and material properties of bone produce a decline in bone strength. Antiresorptive agents meet many of the requirements of a drug needed to prevent progression of bone fragility [13]. They reduce bone remodeling, reduce the extent of bone resorption in the BMU and may increase the extent of bone formation in the BMU to some degree by increasing the lifespan of the osteoblast. These drugs increase the extent of mineralization of the bone tissue [56, 78]. As these drugs do not restore the architecture of bone, there is also a need for agents that can stimulate bone formation at the periosteal surface, within the BMU, and on quiescent bone surfaces, so as to restore more completely the structure of the skeleton. There seems to be progress in this direction [71, 79].

Summary

Bone formation is carried out by osteoblasts generated from mesenchymal precursor cells that acquire the osteoblast phenotype through differentiation processes controlled by hormones, cytokines and growth factors. Transcription factors that govern gene expression during differentiation have been discovered recently, as have other genetic control mechanisms identified with the use of mouse genetics or gene mutations in states of deficient or excessive bone formation. Current concepts of the regulation of bone formation have been developed from *in vitro* studies in rodent cell or organ culture, in addition to the insights from mouse and human genetics. At any one time, many stages of osteoblast differentiation and activity occur at different sites throughout the skeleton; this leaves the challenge of translating these events into molecular explanations of site- and age-specific changes in bone growth and bone mass.

Reduced bone formation plays a central role in the age-related decline in bone mass. The term "bone loss" obscures the contribution of reduced bone formation to the *net* loss of bone. The latter is a function of the volumes of bone resorbed *and* formed in each basic multicellular unit (BMU) on its endosteal envelope, the remodeling rate, *and* the amount formed beneath its periosteal envelope. Trabecular bone mass declines in the third or fourth decade in both sexes, probably because of a reduction in bone formation in each BMU. This bone loss is slow because the remodeling rate remains low until menopause, when estrogen deficiency accelerates remodeling and worsens the negative BMU balance by reducing osteoblast lifespan and increasing osteoclast lifespan. Bone loss continues at a higher rate because the remodeling rate is higher and the BMU balance becomes more negative than before menopause. There then result trabecular thinning, disappearance and loss of connectivity, cortical thinning, and increased intracortical porosity. Concurrent age-related subperiosteal bone formation partly offsets endosteal bone loss, but this process, so vigorous during growth, is limited during adulthood. Patients with fractures are likely to represent a heterogeneous group with one or more of abnormalities in bone remodeling and modeling during growth and aging. Research efforts and treatments aimed at reducing the progression and reversing bone fragility should be directed at the resorptive and formative aspects of bone remodeling, as well as at the formative aspects of bone modeling.

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