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Sex Steroids and Bone

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Compston, Juliet E. Sex Steroids and Bone. *Physiol Rev* 81: 419–447, 2001.—Sex steroids are essential for skeletal development and the maintenance of bone health throughout adult life, and estrogen deficiency at menopause is a major pathogenetic factor in the development of osteoporosis in postmenopausal women. The mechanisms by which the skeletal effects of sex steroids are mediated remain incompletely understood, but in recent years there have been considerable advances in our knowledge of how estrogens and, to a lesser extent androgens, influence bone modeling and remodeling in health and disease. New insights into estrogen receptor structure and function, recent discoveries about the development and activity of osteoclasts, and lessons learned from human and animal genetic mutations have all contributed to increased understanding of the skeletal effects of estrogen, both in males and females. Studies of untreated and treated osteoporosis in postmenopausal women have also contributed to this knowledge and have provided unequivocal evidence for the potential of high-dose estrogen therapy to have anabolic skeletal effects. The development of selective estrogen receptor modulators has provided a new approach to the prevention of osteoporosis and other major diseases of menopause and has implications for the therapeutic use of other steroid hormones, including androgens. Further elucidation of the mechanisms by which sex steroids affect bone thus has the potential to improve the clinical management not only of osteoporosis, both in men and women, but also of a number of other diseases related to sex hormone status.

I. INTRODUCTION

Osteoporosis is defined as a condition characterized by reduced bone mass and disruption of bone architecture, resulting in increased bone fragility and increased fracture risk (294). These fractures, which particularly affect the hip, spine, and wrist, are a major cause of morbidity and mortality in elderly populations (65, 247, 248). Clinically, osteoporosis may be recognized by the presence of fragility fractures, but recently, diagnostic criteria based on bone mineral density measurements have been proposed (397), based on the well-documented inverse relationship between bone mineral density and fracture risk (70, 115, 160, 235, 390). According to this classification, osteoporosis is defined as a bone mineral density in the spine and/or proximal femur 2.5 or more standard deviations below normal peak bone mass. The term *established osteoporosis* is used when one or more fragility fractures have occurred.

The recognition, by Fuller Albright in 1948, of the central role of estrogen deficiency in the pathogenesis of postmenopausal osteoporosis (7) provided a major stimulus to research into this hitherto neglected condition and into the mechanisms by which estrogens affect bone. The advances that followed have been paralleled by a rapid growth in understanding of bone physiology and biochemistry; together, these have been responsible for significant improvements in the clinical management of patients with osteoporosis over the past two decades. In particular, Albright's fundamental observation provided the rationale for the use of estrogen replacement therapy in the prevention of postmenopausal osteoporosis and altered the widely held perception that osteoporosis was an inevitable and untreatable consequence of ageing.

Sex steroids play an essential role in the maintenance of bone health throughout life, and adverse effects of hormone deficiency can be seen in the young and old and in men and women. The mechanisms by which these effects are mediated remain incompletely understood and are the subject of enormous research effort. The potential therapeutic implications of progress in this field are, however, considerable and extend beyond osteoporosis. In this review relevant aspects of bone physiology and biochemistry are discussed, and current knowledge of the skeletal effects of sex steroids is reviewed.

II. BONE COMPOSITION, STRUCTURE, AND FUNCTION

The skeleton provides structural support for the body, protecting internal organs and housing the bone marrow. It also functions as a reservoir of calcium and phosphate ions and plays a major role in the homeostasis of these minerals. Bone consists of an extracellular matrix, the organic phase of which is composed of type I collagen, proteoglycans, and noncollagenous proteins including osteocalcin, bone sialoprotein, osteonectin, thrombospondin, and osteopontin. Bone matrix also contains growth factors and cytokines that have an important regulatory role in bone remodeling. The inorganic phase of bone matrix is composed mainly of calcium hydroxyapatite.

Approximately 80% of the skeleton is composed of cortical bone, which is found mainly in the shafts of long bones and surfaces of flat bones. It is composed of compact bone, which is laid down concentrically around central canals or Haversian systems, which contain blood vessels, lymphatic tissue, nerves, and connective tissue. Cancellous or trabecular bone is found mainly at the ends of long bones and in the inner parts of flat bones and consists of interconnecting plates and bars within which lies hematopoietic or fatty marrow. The surface-to-volume ratio of cancellous bone is much greater than that of cortical bone, and the potential for metabolic activity is correspondingly higher.

A. Bone Cells

Three cell types are found in bone, namely, osteoblasts, osteoclasts, and osteocytes. However, the close proximity of the bone marrow exposes bone to the influence of other cell types that play a vital role both in the production of osteogenic cells and in the regulation of bone modeling and remodeling.

1. Osteoblasts

Osteoblasts are responsible for the formation and mineralization of bone. They are derived from pluripotent mesenchymal stem cells, which can also differentiate into chondrocytes, adipocytes, myoblasts, and fibroblasts (279, 280) (Fig. 1). The mechanisms by which commitment to the osteoblast phenotype is achieved are not fully established, but the core binding transcription factor Cbfa1 (also known as osteoblast stimulating factor 2 or Osf2) has recently been shown to be essential for osteoblast differentiation; thus loss of function mutant mice exhibit complete lack of ossification of cartilage (197, 273), and heterozygous loss of function causes cleidocranial dysplasia (255), a condition associated with patent fontanelles, abnormal dentition, short stature, and hypoplastic clavicles. In addition, a number of other factors are required for normal osteoblast differentiation including fibroblastic growth factors (FGFs), transforming growth factor-β (TGF-β), bone morphogenetic factors (BMPs), glucocorticoids, and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (216).

In situ, osteoblasts actively involved in bone formation appear as monolayers of plump cuboidal cells in

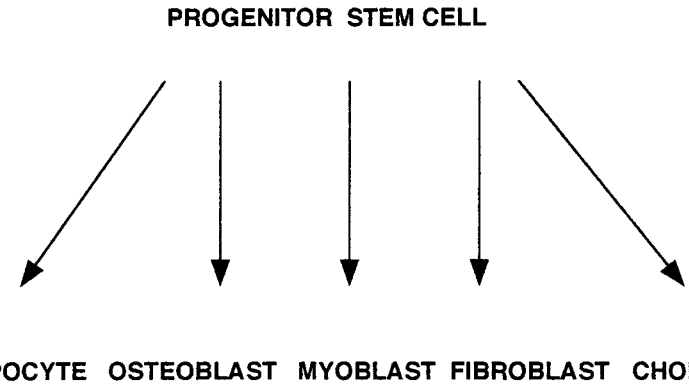

FIG. 1. Possible differentiation pathways of the pluripotent mesenchymal stem cell.

close juxtaposition to newly formed unmineralized bone (osteoid). Structural characteristics include a round nucleus at the base of the cell, a strongly basophilic cytoplasm, and a prominent Golgi complex (44). Cytoplasmic processes extend from the secretory side of the cell into the bone matrix and communicate with the osteocyte canalicular network. There are also gap junctions, composed of proteins called connexins, that connect the cytoplasm of adjacent cells (343, 410). Developing and mature osteoblasts express a number of products including type I collagen, alkaline phosphatase, osteopontin, and osteocalcin that may be used to identify the osteoblastic phenotype in vivo and in vitro.

Actively forming osteoblasts may subsequently undergo apoptosis or become bone-lining cells or osteocytes; both the latter are believed to represent further stages of maturation. Bone-lining cells are flat elongated cells with a spindle-shaped nucleus that lie along the endosteal membrane covering quiescent bone surfaces. Lining cells, together with the endosteal membrane, form a protective layer over the bone surface; their function is not well understood, but they may play a role in the activation of bone remodeling (32).

2. Osteocytes

Osteocytes are small flattened cells within the bone matrix and are connected to one another and to osteoblastic cells on the bone surface by an extensive canalicular network that contains the bone extracellular fluid (1). The cytoplasmic projections within the canaliculi communicate via gap functions and enable osteocytes to respond to mechanical and biochemical stimuli (83, 308). Osteocytes are terminally differentiated and may ultimately undergo apoptosis or be phagocytosed during the process of osteoclastic resorption.

Osteocytes are believed to play a central role in the response to mechanical stimuli, sensing mechanical strains and initiating an appropriate modeling or remodeling response via a number of chemical messengers including glucose-6-phosphate dehydrogenase, nitric oxide, and insulin-like growth factors.

3. Osteoclasts

Osteoclasts are large, multinucleated bone-resorbing cells derived from hematopoietic precursors of the monocyte/macrophage lineage. They are formed by the fusion of mononuclear cells and are characterized by the presence of a ruffled border, which consists of a complex infolding of plasma membrane, and a prominent cytoskeleton. They are rich in lysosomal enzymes, including tartrate-resistant acid phosphatase (TRAP). During the process of bone resorption, hydrogen ions generated by carbonic anhydrase II are delivered across the plasma membrane by a proton pump to dissolve bone mineral. Subsequently, lysosomal enzymes including collagenase and cathepsins are released and degrade bone matrix. Attachment of osteoclasts to the bone surface is an essential prerequisite for resorption and is mediated by integrins, particularly avβ3, which bind matrix proteins containing the motif Arg-Gly-Asp (153); potential ligands include osteopontin, bone sialoprotein, thrombospondin, osteonectin, and type 1 collagen. Morphologically, attachment of the osteoclast to the bone surface is seen as an actin-containing ring (211) that surrounds completely the ruffled membrane.

It has long been known that osteoblastic or stromal cells are essential for osteoclastogenesis, and the identity of the factor concerned, termed "osteoclast differentiation factor" or ODF, has recently been reported as receptor activator of NFκB ligand (RANKL), a new member of the tumor necrosis factor (TNF) ligand family, also termed TRANCE (TNF-related activation-induced cytokine) or osteoprotegerin ligand (OPGL) (413). The signaling receptor for RANKL is RANK, a type 1 transmembrane protein expressed by osteoclasts (9), whereas osteoprotegerin (OPG), a novel member of the TNF receptor superfamily, acts as a soluble decoy receptor that prevents RANKL from binding to and activating RANK on the osteoclast surface (198). The interaction of RANKL with RANK activates a cascade of intracellular events that involve activation of NFκB and the protein kinase JNK, and interaction with TNF receptor-associated factors (TRAFs) (147). Macrophage-colony stimulating factor

TABLE 1. *Loss of function gene mutations resulting in osteopetrosis*

Gene Mutations
PU.1
M-CSF
c-*fos*
c-*src*
Cathepsin K
TRAP
Carbonic anhydrase
NFκB
RANKL
TRAF 6
$\alpha v\beta 3$
H^+-ATPase

M-CSF, macrophage-colony stimulating factor.

(M-CSF) production by osteoblastic/stromal cells is also essential for osteoclastogenesis (415), although unlike RANKL, it does not appear to have effects on osteoclast activity (364).

Osteoclast apoptosis is an important determinant of osteoclast activity. Like osteocytes, osteoclasts are terminally differentiated cells with a limited life span. The cytokines interleukin-1, TNF-α, and M-CSF all reduce osteoclast apoptosis (348), thus prolonging the viability of these cells. In contrast, as discussed in section IV*C*, estrogen increases apoptosis of osteoclasts (158), an effect which is associated with increased production of TGF-β and reduced expression of NFκB-activated genes. Loss of function gene mutations associated with osteopetrosis, a group of disorders caused by osteoclast dysfunction, are shown in Table 1.

B. Bone Modeling and Remodeling

Bone modeling involves both the growth and shaping of bones. It occurs during the first two decades of life in humans and in animals species while growth plates remain open. In the mature adult skeleton, modeling may occur in response to altered biomechanical stress such as that induced by vigorous exercise, although the capacity of the skeleton to respond in this way decreases with increasing age. Modeling also occurs as part of the fracture healing process. The process of bone modeling involves both bone formation and resorption; the former exceeds the latter and is not coupled to it temporally or spatially as in bone remodeling.

Like bone modeling, bone remodeling is a surface phenomenon. Remodeling serves to maintain the mechanical integrity of the adult skeleton and also provides a mechanism by which calcium and phosphate ions may be released from or conserved within the skeleton. It consists of the removal, by osteoclasts, of a quantum of bone followed by the formation by osteoblasts within the cavity so created of osteoid, which is subsequently mineralized. In normal adult bone, the processes of resorption and formation are coupled both in space and time; thus bone resorption always precedes formation (coupling), and in the young adult skeleton, the amounts of bone formed and resorbed are quantitatively similar (balance) (Fig. 2). The sites at which bone remodeling occurs are termed basic multicellular units (BMUs) or bone remodeling units. The life span of each remodeling unit in humans is believed to be between 2 and 8 mo, with most of this period being occupied by bone formation (287). In normal

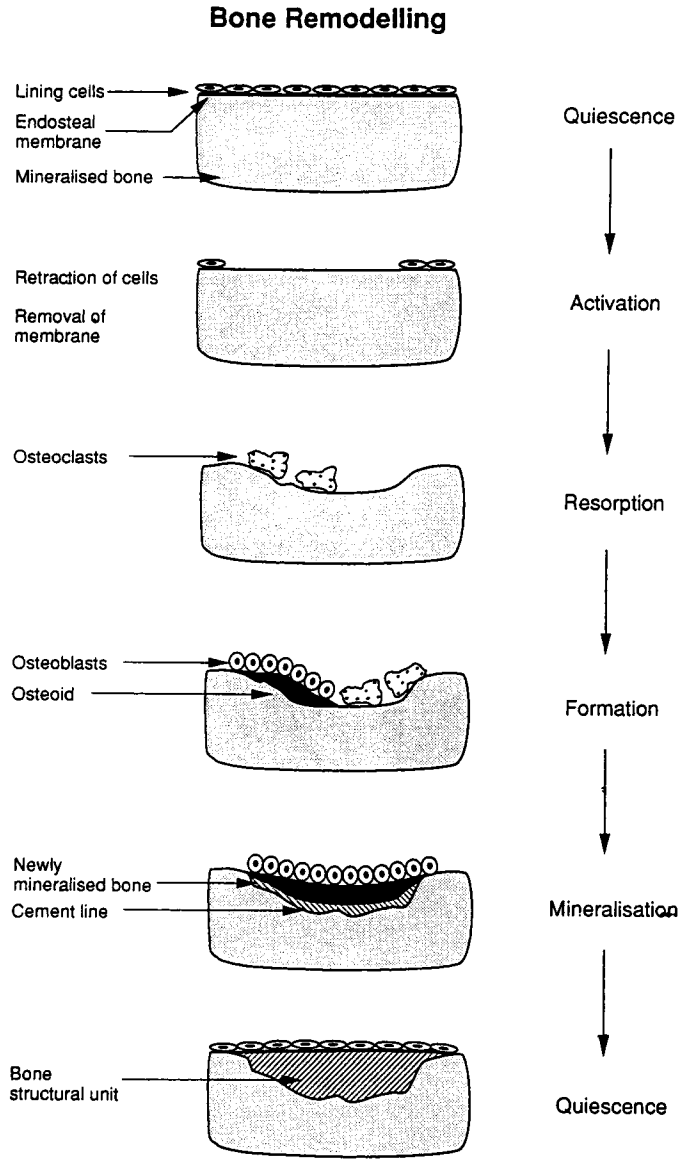

FIG. 2. Schematic representation of bone remodeling. (From Compston JE. Bone morphology: quality, quantity and strength. In: *Advances in Reproductive Endocrinology. Oestrogen Deficiency: Causes and Consequences*, edited by Shaw RW. Carnforth, Lancs, UK: Parthenon, 1996, vol. 8, p. 63–84.)

human adults, ~20% of the cancellous bone surface is undergoing remodeling at any given time.

The first stage in bone remodeling involves activation of the quiescent bone surface before resorption. Although the process of activation is not well understood, it is believed to involve retraction of lining cells and digestion of the endosteal membrane, the latter possibly occurring as a result of the production of collagenases by the lining cells (32). Osteoclast precursors are then attracted to the exposed mineralized bone surface and fuse to become functional osteoclasts that resorb bone. Exposure of the mineralized bone surface by this process of activation is thought to be an essential prerequisite for osteoclastic resorption. The presence of capillary sinusoids close to sites of bone remodeling suggests that circulating osteoclasts may pass through the vessel wall before bone resorption rather than being directly recruited from bone marrow (288). There is a close interdependence between angiogenesis and osteogenesis in developing bone (125, 151), a relationship which may also exist in adult bone.

The determinants of the sites at which bone remodeling is initiated have not been fully elucidated. However, it is likely that the location of activation and the subsequent remodeling process is critically dependent on mechanical factors, and sites of trabecular thinning may thus be favored. (60)

C. Cellular and Structural Mechanisms of Bone Loss in Osteoporosis

At the tissue and cell levels, there are two possible mechanisms of bone loss in osteoporosis (59) (Fig. 3). Quantitatively, the most important is an increase in the activation frequency (also termed high bone turnover) in which the number of remodeling units activated on the bone surface is increased; this results in a greater number of units undergoing bone resorption at any given time and is potentially reversible provided that bone remodeling is coupled and that remodeling balance is maintained. The second mechanism, which often coexists with increased bone turnover, is that of remodeling imbalance, in which the amount of bone formed within individual remodeling units is less than that resorbed due either to an increase in resorption, decrease in formation, or a combination of the two. This form of bone loss is irreversible once the remodeling cycle has been completed, at least in terms of that remodeling unit.

These mechanisms of bone loss can be quantitatively assessed using histomorphometric techniques. The administration of two, time-spaced doses of a tetracycline compound before bone biopsy enables identification of actively forming bone surfaces (111) and calculation of bone turnover and activation frequency. The amounts of bone formed and resorbed within individual bone remodeling units can also be measured; the former is known as the wall width (72) and is a measure of osteoblast function. The erosion depth and other indices of resorption cavity size can be assessed after computerized or manual reconstruction of the eroded bone surface (55, 118).

The alterations in bone remodeling responsible for bone loss determine the accompanying changes in bone architecture, an important determinant of the mechanical strength of bone (62). In cancellous bone, either trabecular thinning or trabecular perforation and erosion may occur; these two processes are to some extent interdependent. Trabecular thinning is associated with better

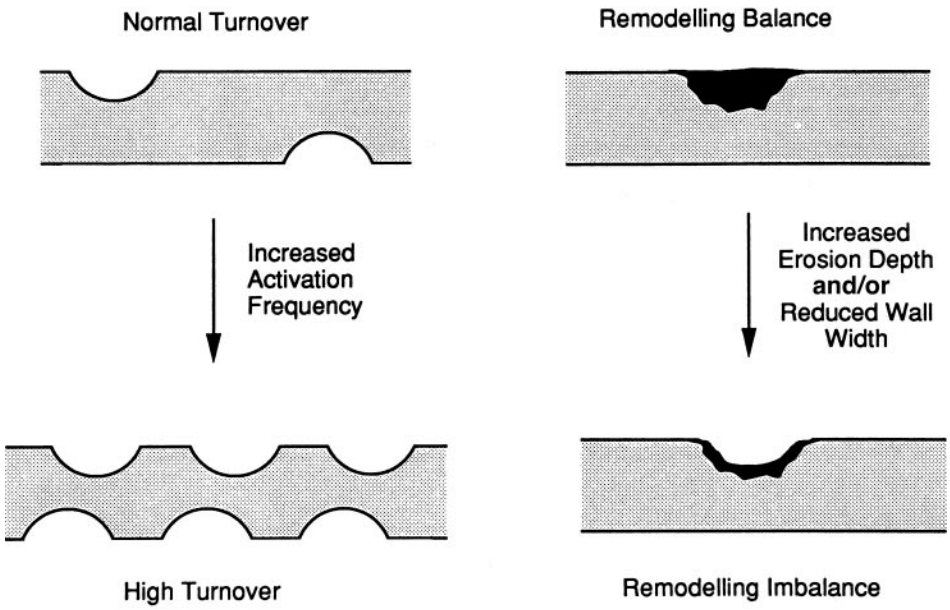

FIG. 3. Mechanisms of bone loss in osteoporosis. (From Compston JE. The skeletal effects of oestrogen depletion and replacement: histomorphometrical studies. In: *Annual Review of the Management of Menopause*, edited by Studd J. Carnforth, Lancs, UK: Parthenon, 2000, p. 287–296.)

preservation of bone architecture than penetration and erosion of trabeculae, the latter having the greater adverse effects on bone strength. Increased activation frequency and/or increased resorption depth predispose to trabecular penetration and erosion, whereas low bone turnover states favor trabecular thinning.

A number of approaches to the quantitative assessment of cancellous bone structure have been described. In histological sections of bone, trabecular width and spacing can be measured directly or calculated from area and perimeter measurements (289) and indirect assessment of connectivity made by the technique of strut analysis (119) or measurement of trabecular bone pattern factor (138) or marrow star volume (381). Finally, a number of techniques have been used to generate three-dimensional images of bone; these include reconstruction of serial sections; scanning and stereo microscopy; volumetric, high-resolution, and microcomputed tomography; and magnetic resonance imaging (124, 231). Such approaches enable direct assessment of connectivity and measurement of anisotropy, but their application in vivo is currently restricted by limited resolution, partial volume effects, and noise.

D. Regulation of Bone Remodeling

The regulation of bone remodeling involves a complex interplay between systemic hormones, mechanical stimuli, and locally produced cytokines, growth factors, and other mediators (Fig. 4). Much of our knowledge in this area is derived from in vitro experiments and may not always be relevant to the control of bone remodeling in vivo.

1. Mechanical factors

Mechanical stresses are a major determinant of bone modeling and remodeling, and it is generally believed that osteocytes are the major mechanosensory bone cell. Intermittent loading at physiological levels of strain results in rapid metabolic changes in osteocytes, one of the earliest manifestations of which is an increase in the production of glucose-6-phosphate dehydrogenase activity (293). The mechanisms by which osteocytes sense mechanical loading have not been fully established, but it is believed that the deformation resulting from strain stimulates the flow of interstitial fluid through the osteocyte canalicular network (299). Electrokinetic streaming potentials and/or fluid shear stress may then modulate production by the osteocyte of mediators such as prostaglandins and nitric oxide (264). These may then stimulate the production of other cytokines and growth factors, for example, insulin-like growth factor (IGF) (214).

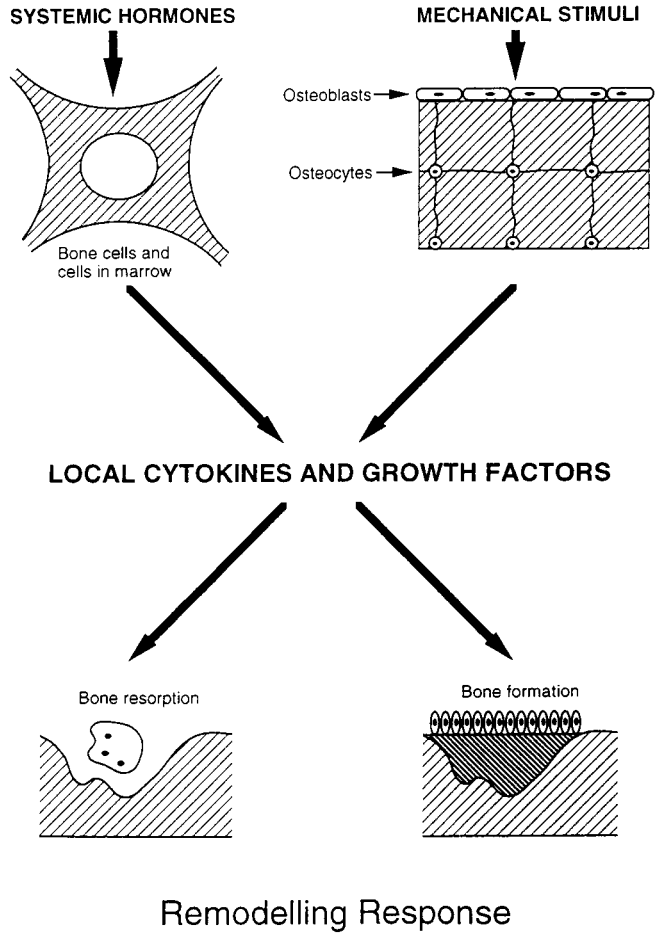

FIG. 4. Control of bone remodeling. (From Compston JE. Hormone replacement therapy for osteoporosis: clinical and pathophysiological aspects. *Reprod Med Rev* 3: 209–244, 1994.)

2. Systemic hormones

Many systemic hormones influence bone modeling and remodeling. In addition to the sex steroids, these include parathyroid hormone (PTH), thyroid hormones, growth hormone, glucocorticoids, and $1,25(OH)_2D$. Many of these act via the production of locally produced factors and may also interact with mechanical stimuli to affect bone modeling and remodeling.

3. Locally produced factors

Bone is a rich source of cytokines and growth factors (Fig. 5, Table 2) and also other mediators such as prostaglandins and nitric oxide. In addition, cells in the bone microenvironment play a major role in the regulation of bone remodeling, both as a source of bone cell precursors and by the production of bone active cytokines and growth factors. Table 2 lists the major cytokines and growth factors known to be implicated in bone metabolism. Those known to play an important role in mediating

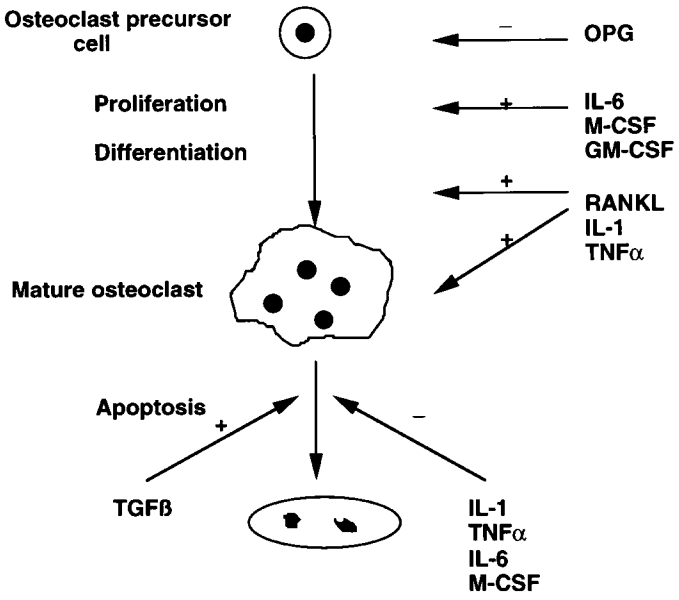

FIG. 5. Effects of cytokines on osteoclast production and activity. TGF-β, transforming growth factor-β; IL, interleukin; TNF-α, tumor necrosis factor-α; M-CSF, macrophage-colony stimulating factor; GM-CSF, granulocyte/macrophage-colony stimulating factor.

the effects of estrogen on bone are described in greater detail below.

Interleukin (IL)-1α and -1β are potent stimulators of bone resorption in vitro and in vivo (34, 129, 324). These effects are mediated both by an increase in the proliferation and differentiation of osteoclast precursors and also by increased osteoclastic activity (297, 354), the latter resulting at least in part from inhibitory effects on osteoclast apoptosis. Some of the effects of IL-1 on osteoclasts result from an increase in prostaglandin synthesis (34). IL-1 also has effects on osteoblasts, which are probably dependent on whether administration is continuous or intermittent (130, 335). In the former situation, inhibitory effects on bone formation are seen, whereas intermittent administration is associated with an increase in osteoblast proliferation and differentiation. The IL-1 receptor antagonist (IL-1ra) is a constitutively occurring inhibitor of IL-1 (139), inhibiting IL-1-induced stimulation of bone resorption both in vitro (330) and in vivo (136). TNF-α and lymphotoxin (TNF-β) are also potent stimulators of bone resorption (22, 173) and appear to act in a similar way to IL-1.

IL-6 also stimulates bone resorption, although by different mechanisms. Its production in bone is increased by other bone-resorbing cytokines and systemic hormones (for example, PTH) (101), and it also acts synergistically with these agents, increasing their bone resorptive effects (75). The effects of IL-6 in vivo may be modulated by the circulating levels of IL-6 soluble receptor (350).

Granulocyte/macrophage-colony stimulating factor (GM-CSF) acts on the early development of hematopoietic precursor cells, including osteoclasts (210). Unlike M-CSF, it is not essential for osteoclastogenesis, although it supports the differentiation of osteoclast precursors. GM-CSF has also been reported to increase the proliferation of osteoblastic cells in vitro (74) and in vivo (352), probably by an indirect action.

The TGF-β superfamily includes the TGF-β isoforms, the activins and inhibins, and BMPs (28). TGF-β is present in a latent, biologically inert form in bone matrix, its active form being released in the process of bone resorption (298). It is a potent stimulator of bone formation (267), stimulating osteoblastic differentiation and the synthesis of bone matrix proteins and their receptors, while inhibiting the synthesis of proteases. Most data support inhibitory effects on osteoclastic bone resorption (29, 233) due to effects both on osteoclast formation and activity, the latter effect being mediated by stimulation of osteoclast apoptosis (157). Three main TGF-β receptors exist (50): type I and type II, which are transmembrane serine/threonine kinases and function as signaling receptors (109), and type III, betaglycan, which is nonsignaling (389). It is believed that TGF-β binds directly to the type II receptor, which is constitutively active, and that this complex is then recognized by the type I receptor to form a complex, with phosphorylation of the type I receptor by the type II receptor (401).

The BMPs are members of the TGF-β superfamily. They possess osteoinductive properties, inducing differentiation of osteoblastic and chondroblastic precursor cells, and are similar to but not identical to TGF-β in terms of their structure and activity (400). BMPs act as morphogens during embryogenesis, with the pattern of production of BMPs 2, 4, and 6 indicating a role in bone and cartilage formation. The regulation and precise functions of the BMPs remain to be elucidated, but estrogen-

TABLE 2. *Cytokines and growth factors affecting bone*

Cytokine/Growth Factor	Abbreviation
Stimulators of bone resorption	
Interleukins-1, -6, -8, -11	IL-1, -6, -8, -11
Tumor necrosis factors	TNFs
Epidermal growth factor	EGF
Platelet-derived growth factor	PDGF
Fibroblast growth factors	FGFs
Leukemia inhibitory factor	LIF
Macrophage-colony stimulating factor	M-CSF
Granulocyte/macrophage-colony stimulating factor	GM-CSF
Inhibitors of bone resorption	
Interferon-γ	IFN-γ
Interleukin-4	IL-4
Stimulators of bone formation	
Insulin-like growth factors	IGFs
Transforming growth factor-β	TGF-β
Fibroblast growth factors	FGFs
Platelet-derived growth factor	PDGFs
Bone morphogenetic proteins	BMPs

induced stimulation of the production of BMP-6 mRNA and protein has been demonstrated in human osteoblastic cell lines (311).

IGFs exist in two forms: IGF-I and -II. In the circulation, they form a large-molecular-weight complex with binding proteins (IGFBPs), and in the case of IGFBP3 and -5 complexes an acid-labile subunit (309). IGFs stimulate bone formation, their production by bone cells being regulated by a number of systemic hormones and locally produced factors (45). They increase proliferation of osteoblast precursors and enhance the synthesis and inhibit the degradation of type I collagen (145, 241). There are at least six IGFBPs (45, 212), all of which are expressed by bone cells in various in vitro systems (319). All IGFBPs bind IGFs with high affinity, preventing their interaction with the receptor. However, because of posttranslational modifications that result in changes in both structure and function, the IGFBPs may exert either stimulatory or inhibitory effects; thus, for example, IGFBP-1 and -3 have both stimulatory and inhibitory potential, IGFBP-2 and -4 are inhibitory, and IGFBP-5 is stimulatory (251). IGFBP-6 is inhibitory and exhibits a selective affinity for IGF-II over IGF-I. The complexity of the IGF axis is further increased by the action of IGFBP proteases, which affect the binding affinity of the binding proteins for IGFs and may themselves be regulated by IGFs (64, 85).

III. LIFETIME CHANGES IN BONE MASS: EFFECTS OF SEX STEROIDS

A. Pattern of Lifetime Changes in Bone Mass

Bone mass increases throughout childhood and adolescence (30, 31, 126); in prepubertal children, there is a close relationship between bone mass and body height, but this becomes less evident during puberty. In girls the rate of increase in bone mass decreases rapidly after the menarche, whereas gains in bone mass in boys persist up to 17 yr of age (30, 353) and are closely linked to pubertal stage and androgen status (200). Although by the age of 17 or 18 in both sexes the vast majority of peak bone mass has already been achieved, small increases in bone mass during the third decade of life have been demonstrated in several studies (31, 116, 290, 310); however, this finding has not been consistently reported (159, 236, 266). Peak bone mass is attained in the third decade of life and maintained until the fifth decade, when age-related bone loss commences both in men and women, thereafter persisting throughout life (140, 174, 239, 240, 313, 314, 318) (Fig. 6).

The onset of age-related bone loss has not been well defined. In cross-sectional studies, bone loss has been documented in healthy premenopausal women at the spine, proximal femur, and forearm (14), and this finding has also been confirmed in prospective studies (13, 54, 337, 340). In women there is an acceleration in the rate of bone loss at the time of the menopause, the duration of which has not been well characterized but is probably between 5 and 10 yr (16, 88, 123, 161, 265). In men, relatively few data are available, but bone loss is generally believed to begin during the fifth decade of life; thereafter, both in women and men, bone loss continues throughout life (140, 174, 239, 240, 313, 314, 318).

Genetic factors are important determinants of peak bone mass, and up to 60–80% of its variance is genetically determined (51, 78, 182). The basis of this effect has not been fully defined, and a number of genetic polymorphisms are likely to be involved. A polymorphism in the regulatory region of the collagen 1AI gene at a recognition site for the transcription factor Sp1 has been demonstrated to correlate with bone mineral density and fracture in several populations (131, 366); there are many other potential candidates including the vitamin D recep-

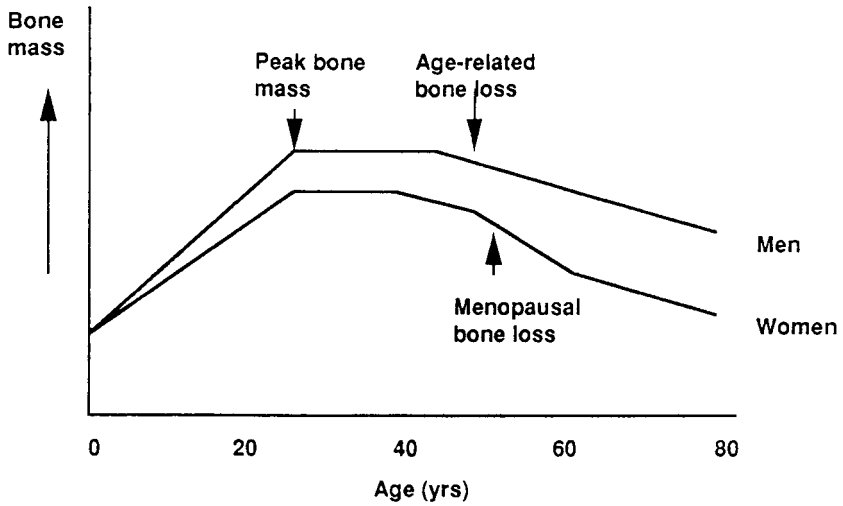

FIG. 6. Lifetime changes in bone mass. (From Compston JE. Osteoporosis, corticosteroids and inflammatory bowel disease. *Aliment Pharmacol Ther* 9: 237–250, 1995.)

tor gene, estrogen receptor gene, and genes for many cytokines and growth factors (306). Other determinants of peak bone mass include nutrition, calcium intake, physical activity, and hormonal status.

B. Effects of Sex Steroids on Growth and Peak Bone Mass

Sex steroids play an important role in bone growth and the attainment of peak bone mass. They are responsible for the sexual dimorphism of the skeleton, which emerges during adolescence (369); the male skeleton is characterized by larger bone size (even when corrected for body height and weight) with both a larger diameter and greater cortical thickness in the long bones. Volumetric bone mineral density is, however, very similar in young adult men and women (183), but the larger bone size in men confers significant biomechanical advantages and, in part, explains the lower incidence of fragility fractures compared with women. Estrogen is essential for normal closure of the growth plates in both sexes; thus estrogen resistance and aromatase deficiency in men are associated with delayed bone age and tall stature despite normal or high circulating concentrations of testosterone (252, 336).

Hypogonadism has adverse effects on the attainment of peak bone mass both in men and women. Late menarche has been associated with reduced bone mineral density (321, 340) and premenopausal amenorrhea resulting from anorexia nervosa (24, 315), excessive exercise (84, 234), and hyperprolactinemia (23), and a variety of other disorders (73) also result in low bone density. Reduced spinal bone mineral density has been reported in women with asymptomatic disturbances of ovulation (i.e., without amenorrhea) (305), although this finding has not been universal (79, 388), and premature menopause, whether natural or induced, is a major risk factor for osteoporosis (12). Low bone mineral density values have also been reported in Turner's syndrome, predominantly reflecting the smaller bone size associated with this condition (260, 263, 322), which is believed to be due to resistance to growth hormone (374).

The role of androgens in growth of the male skeleton during puberty is supported by several observations. Androgen deficiency due to hypogonadotropic hypogonadism is associated with low bone mineral density (103), while administration of testosterone before epiphyseal closure leads to increases in bone mass (102) and testosterone administration to prepubertal boys results in increased bone calcium accretion (238). The timing of puberty may also be important, with some studies indicating that late puberty is associated with reduced bone mineral density and peak bone mass later in life (21, 104); in these subjects, increases in bone mineral density were reported in response to testosterone therapy. Notwithstanding these observations, however, the effects of estrogen resistance and aromatase deficiency on skeletal mass (253, 336) indicate that estrogens also play an important role in skeletal development in males during adolescence; furthermore, it is uncertain to what extent the skeletal effects of androgens are mediated by local metabolism to estrogens. Finally, there is evidence that androgens also have effects on the attainment of peak bone mass in women (42, 43, 71), conditions of androgen excess in women being associated with higher bone mineral density (42, 81).

C. Age-Related Bone Loss and Relationship to Sex Steroids

Estrogen deficiency is a major pathogenetic factor in the bone loss associated with the menopause and the subsequent development, in some women, of postmenopausal osteoporosis. Estrogen replacement at or after menopause, whether natural or induced, prevents menopausal bone loss and characteristically results in an increase in bone mineral density during the first 12–18 mo of treatment (52, 96, 218, 259, 346). This increase, which is typically between 3 and 5% but may be as much as 10% (53, 219), is attributed to the simultaneous reduction in activation frequency and formation of new bone within existing resorption cavities when an antiresorptive agent is administered in high turnover states. There is evidence, almost exclusively from observational studies, that estrogen replacement is associated with a reduction in fracture risk at the hip, spine, and wrist (162, 187, 249, 261, 285, 396); however, such studies are biased by the better health status of women who choose to take estrogens as opposed to those who do not and are thus likely to overestimate any benefit (58).

Even in postmenopausal women, the small amounts of estrogen produced endogenously are determinants both of bone mineral density and fracture risk. In a large population-based study it was demonstrated that women aged 65 yr or older with serum estradiol levels between 10 and 25 pg/ml had significantly higher bone mineral density in the hip, spine, calcaneus, and proximal radius than those with estradiol levels below 5 pg/ml (97). Furthermore, women with undetectable serum estradiol levels had a significantly increased risk of hip and vertebral fractures compared with those with levels above 5 pg/ml, and this risk was further increased in the presence of high serum concentrations of sex hormone binding globulin (68). These interesting and unexpected data challenge the perception that endogenous estrogen production in postmenopausal women does not have physiological skeletal effects and emphasize the potential functional significance of relatively low concentrations of the hormone in

the bone microenvironment. In this respect, the presence in human osteoblastic cells of 17β-hydroxysteroid dehydrogenases (17β-HSDs), which interconvert estradiol, and the relatively inactive estrone (and testosterone) may be relevant, providing a mechanism for the local regulation of intracellular ligand supply for estrogen receptors (82). Four isoforms of this enzyme have been cloned (6, 122, 228, 409), with 17β-HSD I and III being mainly involved in the reduction of estrone to estradiol and testosterone to dihydrotestosterone and 17β-HSD II and IV in the oxidation of estradiol to estrone.

The relationship between the age-related decline in serum testosterone levels and reduction in bone mineral density in men is less well documented, and although some studies have demonstrated such a correlation (106, 257), this finding has not been universal (244). However, hypogonadism is believed to be an important pathogenetic factor in male osteoporosis (272, 341); in the majority of such cases, there are no overt clinical manifestations of hypogonadism, the diagnosis being established by the presence of low free serum testosterone levels. Klinefelter's syndrome is associated with low bone mineral density (107, 152), and castration in adult men is followed by rapid bone loss with evidence of increased bone turnover (345), similar changes being described after the administration of gonadotrophin-releasing hormone analogs (127). The extent to which conversion of androgens to estrogen in bone is responsible for the effects of androgens in adult men is unclear; some studies have reported closer correlations between bone mineral density and estrogen than androgen status (134, 199). Furthermore, prevention by estrogens of bone loss associated with cyproterone acetate in trans-sexual men has been reported (220), and there is indirect evidence that the beneficial effects of testosterone on bone mineral density in eugonadal men with osteoporosis may be partly mediated by conversion to estrogens (10).

IV. SKELETAL EFFECTS OF ESTROGEN: MECHANISMS OF ACTION

Estrogen has a diverse range of actions involving growth, differentiation, and function in many target tissues. The mechanisms by which these actions are achieved have not been fully established, but it is thought that many of the effects of estrogen are mediated by a genomic pathway involving ligand/receptor interaction. The importance of nongenomic mechanisms, in which the ligand interacts with plasma membrane receptors, is increasingly recognized in the mediation of rapid responses to estrogen (39, 393) and in the ROS osteoblastic cell line rapid activation of mitogen-activated protein kinase by estrogen has recently been reported (89). In addition, there is evidence for nongenomic effects of estrogen on osteoclasts, rapid tyrosine phosphorylation of several proteins, including src, being reported in avian osteoclasts after administration of 17β-estradiol (38).

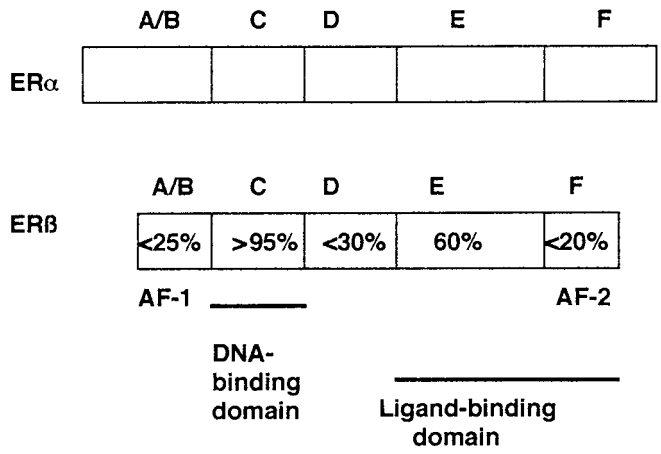

FIG. 7. Structure of estrogen receptors (ER) α and β. The percentage figures indicate the degree of structural homology for each domain between the two receptor subtypes; these are similar in the rat, mouse, and human.

A. Estrogen Receptors

Estrogen receptors (ERs) belong to a family of steroid hormone receptors that include receptors for glucocorticoids, androgens, progestins, and mineralocorticoids (135) and can be considered as ligand-regulated transcription factors. ERs consist of several domains, defined according to their function (Fig. 6). The AF-1 and AF-2 sites (activation functions 1 and 2) activate gene transcription, with the AF-1 being constitutively active and responsible for promotor-specific activation, independent of the presence of ligand, whereas AF-2 is ligand specific (20, 392). The C region contains the highly conserved DNA-binding domain with two zinc fingers that are essential for DNA binding (208). The classical estrogen response element (ERE) consists of an inverted hexanucleotide repeat (A/GGGTCA) separated by three nucleotides. The hormone binding domain is in the COOH terminus of the molecule and is responsible for specific ligand recognition and binding. The E region, and possibly also the C region, contains a 90-kDa heat shock protein function (229).

At least two main ER subtypes exist, namely, ERα and ERβ. ERα was originally cloned from the uterus (133) and, more recently, ERβ was cloned, initially from a rat prostate cDNA library (90, 204, 254, 358). The ERβ shows close structural homology with the ERα molecule, especially in the DNA binding domain and, to a lesser extent, in the ligand binding domain (Fig. 7). The binding affinities of estradiol and other ligands including SERMs and phytoestrogens for the two ER subtypes are very similar

(203). Several isoforms of the ERβ and at least two of ERα, created by alternative splicing or alternative initiation of translation, have been demonstrated (mainly at mRNA level); one of these does not bind estrogen and may act as a dominant negative inhibitor of ER-mediated activity (207).

Mice with loss of function mutations of the ERα gene (ERKO) show only minor skeletal abnormalities with reduced longitudinal bone growth, particularly in females, and modest reductions in bone mineral density which are, in contrast, more prominent in males (66, 286). These changes differ from those observed in human males with ER resistance (336) or aromatase deficiency (253), in which longitudinal growth is increased. In the ERβ knock-out model (BERKO), increased cortical bone mineral content and periosteal diameter have been reported in females, but the males exhibit a normal skeletal phenotype (383). No effect on ovariectomy induced bone loss was demonstrated in these mice; this observation, together with the normal trabecular bone mineral density in the intact females, indicates that ERβ does not mediate the protective skeletal effects of estrogen in this species. To date, therefore, the knock-out models do not indicate a major role for either of the two known ER subtypes in mediating estrogen-induced effects on the skeleton, possibly reflecting the presence of other, as yet unidentified ERs.

The tissue distribution of the ERs is overlapping but not identical, and at least in some tissues where both receptor subtypes exist, they are cell specific, possibly indicating different functions (202). In keeping with the diverse actions of estrogen, ERs are widely distributed and are found in the central nervous system, heart, blood vessels, mammary gland, uterus, testis, epididymus, bladder, ovary, kidney, intestine, prostate, and bone (90, 203, 204, 206, 254, 292, 358). However, it should be recognized that current knowledge of the tissue distribution of the two receptor subtypes is based mainly on localization of mRNA rather than protein.

The presence of ER (presumably ERα) on rat and human osteoblastic cells was first reported in 1988 (91, 276) and subsequently extended to osteoclasts (295) and osteocytes (35). However, the relative proportion and distribution of the two receptor subtypes in bone remains to be established. ERβ mRNA has been reported on rat osteoblastic cells (268) and also in a human osteoblast cell line, SV-HFO (11). Recently, Vidal et al. (382) reported the presence of ERβ mRNA in human osteoblast cell lines and cultures and have also demonstrated the presence of ERβ protein in these cells, both in vitro and in vivo. Furthermore, ER protein was identified in osteocytes, where the staining was nuclear, and in osteoclasts, in which staining was predominantly cytoplasmic. Interestingly, these workers noted the presence of nuclear and cytoplasmic staining for ERβ in some bone marrow cells, an observation consistent with the recent report of ERβ expression in megakaryocytes in human bone marrow (33).

ERα protein has also been demonstrated in the growth plates of rodents and rabbits, where it is localized in the proliferative and early hypertrophic zone (184). The observation in rats that loss of expression at sexual maturity is associated with failure of epiphyseal closure is consistent with the well-documented role of estrogen in this process.

In target cells, 17β-estradiol diffuses through the plasma membrane and binds to the ER (Fig. 8). On binding, heat shock proteins dissociate, and the receptor undergoes a conformational change and dimerization (164, 229). The receptor/ligand complex then binds to response elements within the promotor area of target genes, resulting in transcriptional activation and modulation of gene

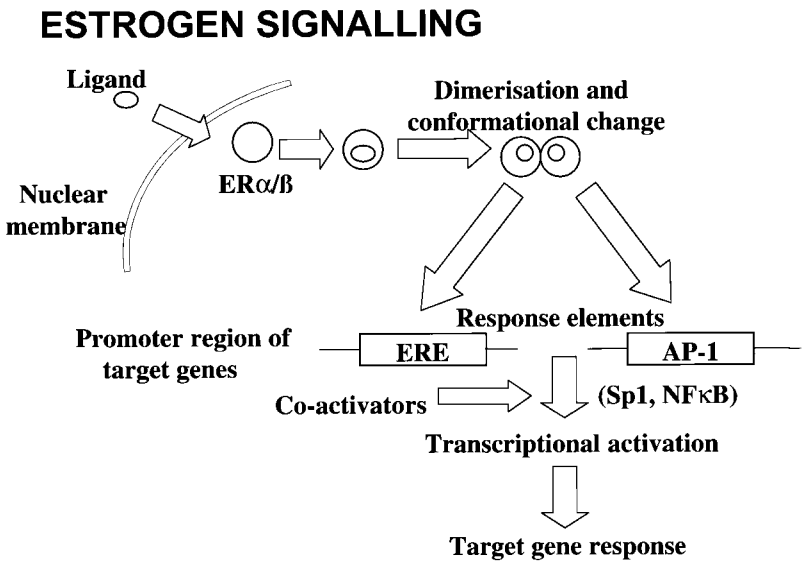

FIG. 8. Estrogen signaling pathways. The ligand 17β-estradiol is transported to the nucleus where it forms a complex with the estrogen receptor (ER). This subsequently undergoes dimerization and conformational change resulting in the formation of a transcriptionally competent complex that binds to response elements in target estrogen-sensitive genes. In addition to the classical ERE and the AP-1 site shown in the diagram, other transcription factors such as NFκB and Sp1 can interact with the ER and modulate gene transcription.

expression. In addition, ERs can regulate the transcription of genes that lack classical EREs in their promotor region by modulating the activity of other transcription factors such as AP-1, NFκB, and Sp1 (120, 302, 342). The conformational change that occurs in the ligand-binding domain of the receptor enables the AF-2 function of the ER to interact with coactivators and corepressors in a ligand-dependent manner; in the case of 17β-estradiol, this results in the formation of a transcriptionally competent complex and the initiation of gene transcription (154, 165, 195).

B. Effects of Estrogen on Osteoblastic Cells

A number of estrogen-induced effects on gene expression in osteoblasts have been described (275). These include induction of TIEG, a TGF-β-inducible gene that inhibits DNA synthesis (351), IGF-I (93, 94), and TGF-β (274, 276). Increased BMP-6 mRNA expression has also been reported in response to estrogen in a fetal osteoblastic cell line (311). Reports on the effects of estrogen on DNA synthesis and proliferation and bone matrix protein production have produced conflicting results, possibly as a result of differences in the in vitro systems investigated and, in particular, the stage of differentiation of osteoblasts in these systems (275). Thus, in osteoblastic cells, for which estrogen acts as a mitogen, increased expression of alkaline phosphatase and type I collagen has been reported (230, 416), whereas in cells that show no proliferative response to estrogen, stimulation of type I collagen and osteocalcin expression have been demonstrated with no increase in alkaline phosphatase (181). Third, in systems in which estrogen has antiproliferative effects, stimulation of alkaline phosphatase expression has been reported, with suppression of osteocalcin and variable effects on type I collagen expression (317). Estrogen also increases expression of the receptors for $1,25(OH)_2D$ (95), growth hormone (163), and progesterone (334); modulates PTH responsiveness in osteoblastic cells (93, 112); and increases expression of IGFBP-4, as well as reducing its proteolytic breakdown (180).

C. Effects of Estrogen on Osteoclast Differentiation and Activity

The report by Pensler et al. (295) that ERs were present on osteoclasts has since been confirmed by a number of groups in bone from humans (155, 277), chicks (276), mice (150, 250), and rabbits (232). Levels of the ER on osteoclasts are generally low and, as discussed below, the antiresorptive effects of estrogen may largely be mediated by modulation of cytokine production by cells in the bone microenvironment rather than by direct effects on osteoclasts. However, estrogen-induced reduction in the expression of mRNAs and secretion of several lysosomal enzymes, including cathepsin L, β-glucuronidase, and cathepsin K have been reported in osteoclasts in vitro (201, 278).

The bone-preserving action of estrogen is mediated predominantly if not solely through effects on osteoclast number and activity, the latter encompassing both resorptive activity per se and the life span of the cell. Studies in ovariectomized rodents have demonstrated an increase in the proliferation and differentiation of osteoclast precursors (168, 169), increased numbers of stromal/osteoblastic cells (170, 190), and reduced osteoclast apoptosis (158). These effects are, in turn, believed to be largely mediated via cytokines involved in the regulation of osteoclastogenesis and osteoclastic activity. Studies in postmenopausal women have demonstrated increased production of IL-1, GM-CSF, and TNF-α by monocytes in the bone microenvironment after natural or surgical menopause, these changes being abrogated by the administration of exogenous estrogen (281, 282, 307). In support of these observations, treatment with TNF binding protein prevents bone loss in ovariectomized rats but has no effect in estrogen-replete animals (189, 194). The increase in IL-1 activity associated with estrogen deficiency is a result not only of increased IL-1 synthesis but also of decreased production of IL-1ra (283); thus treatment of ovariectomized rats with IL-1ra decreases bone loss (191) by blocking the proliferation and differentiation of osteoclast precursors (188). Mice that are unable to synthesize or respond to either IL-1 (8) or TNF-α (301) do not exhibit the bone loss seen in normal animals after ovariectomy, and simultaneous inhibition of IL-1 and TNF activity is required completely to prevent bone loss after ovariectomy in normal mature rats (189). However, these animals have a normal bone phenotype with no evidence of abnormal remodeling activity when sex hormone status is normal (167). These observations emphasize the interdependent nature of cytokine regulation; IL-1, IL-6, and TNF-α not only induce their own synthesis but also have synergistic autocrine effects, TNF-α and IL-1 acting to increase production of TNF and IL-6, and PTH synergizing with TNF to stimulate IL-6 production (80, 108, 167, 291).

Estrogen also inhibits the production of IL-6 by blocking the activity of the transcription factors NFκB and CCAAT/enhancer binding protein β that are required for activation of the IL-6 promotor (114, 209, 303, 342). In vivo studies in ovariectomized mice have demonstrated increased production of IL-6 from bone marrow cells (168) and increased expression of the IL-6 soluble receptor IL-6R, through which the effects of IL-6 are mediated, may also contribute (217). Transgenic mice overexpressing IL-6 do not exhibit osteopenia or increased osteoclastogenesis (193, 349, 399), and IL-6-deficient mice exhibit a normal bone phenotype, although they are protected from

ovariectomy-induced bone loss (301). The role of IL-6 in the pathogenesis of menopausal bone loss in women remains to be fully established.

Effects of estrogen on stromal/osteoblastic cells, which support osteoclastogenesis, have been reported. Thus estrogen deficiency is associated with an increase in this cell population (170), and increased synthesis of M-CSF and osteopontin has been reported in vitro and in ovariectomized animals (100, 190, 411). Recently, it has also been shown that estrogen increases levels of OPG mRNA and protein in osteoblastic cells (148). In addition, estrogen plays an important role in the regulation of osteoclast activity. The cytokines IL-1, IL-6, TNF-α, and M-CSF have all been shown to inhibit apoptosis in osteoclasts (156, 172), whereas TGF-β, the production of which is decreased in estrogen deficiency states, stimulates apoptosis (158). Estrogen may also directly stimulate apoptosis by decreasing expression of NFκB-activated genes that normally suppress apoptosis (171). Interestingly, the reverse effect has been reported for osteocytes, acute estrogen withdrawal in humans being associated with increased apoptosis of osteocytes (357).

Evidence for a role of nitric oxide in bone loss associated with estrogen deficiency is provided by the observation that nitroglycerine, a nitric oxide donor, alleviates bone loss induced by ovariectomy in rats and that in the presence of N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase (NOS), estrogen was ineffective in reversing bone loss (398). This is consistent with earlier studies in the guinea pig demonstrating estrogen-induced regulation of the constitutive NOS enzymes, epithelial NOS and neuronal NOS (394), and with the inhibitory effect of high nitric oxide concentrations on osteoclastogenesis and osteoclastic activity (although there is some evidence that lower concentrations of NO have a stimulatory effect on bone resorption) (98). Interestingly, functional ERs have been demonstrated in bone endothelial cells in vitro (36), supporting a role for estrogens in angiogenesis and hence, potentially, access of osteoclasts to remodeling bone surfaces (288).

The role of estrogen in the regulation of osteoclast activity is thus mediated via effects on osteoclast number and activity. The former action is determined both by direct cytokine-induced effects on osteoclast proliferation and differentiation and by modulation of the stromal/osteoblastic cell population that supports osteoclastogenesis. Changes in osteoclast activity are probably mediated predominantly through effects on apoptosis.

D. Skeletal Effects of Estrogen in Animal Models

Ovariectomy leads to the development of rapid cancellous bone loss in some species, particularly the rat, with an increase in osteoclast and osteoblast number and also an increase in osteoclast size (408). In young rats, much of the apparent cancellous bone loss occurs as a result of increased resorption of calcified cartilage by chondroclasts (405). Bone formation rates are increased, consistent with high bone turnover, and these changes persist for at least 1 yr after ovariectomy (407). Studies of cancellous bone architecture in the ovariectomized rat have demonstrated that bone loss is accompanied by osteoclastic perforation and erosion of trabecular plates without trabecular thinning (77), indicating that both the number and activity of osteoclasts are increased in estrogen-deficient states. In cortical bone, increased bone resorption results in an increase in the volume of the medullary canal in the tibiae (175); however, there is also an increase in bone formation at the periosteal surface that may exceed endocortical resorption in rapidly growing rats (362). Osteoclast numbers are increased at the endocortical surface. These changes, both in cancellous and cortical bone, can be prevented by administration of estrogen (362, 406).

It is important to emphasize that sexually mature rodents should be used for these models to avoid confounding effects of estrogen deficiency on longitudinal growth (192). Other animals that have been studied as models of estrogen deficiency-induced bone loss include mice, ferrets, dog, sheep, swine, and monkeys. These species vary in their skeletal responsiveness to estrogen depletion and are less well established than the rat model (121, 192).

E. Effects of Estrogen in the Human Skeleton

Histomorphometric data on the skeletal changes associated with menopausal bone loss are sparse and restricted to cross-sectional studies in relatively small numbers of women. Some of these studies have provided evidence for an increase in bone turnover during the menopause, both in cortical and cancellous (37, 86, 377), although this finding has not been universal (246). These somewhat conflicting data contrast with results obtained from kinetic and biochemical measurements of bone turnover, which have invariably demonstrated an increase in bone turnover during menopause (143, 365). Furthermore, estrogen replacement therapy is associated with a return to premenopausal values of biochemical markers of bone resorption and formation. The failure of histomorphometric studies to demonstrate unequivocally an increase in bone turnover in association with menopause is likely to be attributable to several factors including the small numbers studied, lack of prospective data, and the large measurement variance associated with bone histomorphometry.

A consistent finding in untreated postmenopausal women has been a reduction in wall width, indicating

reduced bone formation at the cellular level and hence a reduction in osteoblast activity (221, 377). The age at which this reduction occurs is uncertain. Thus Lips et al. (221) reported an age-related reduction in mean wall width in 22 men and 14 women aged between 18 and 82 yr, whereas in another study, the age-related reduction in women and men appeared to begin after the age of 50 yr (377). However, the cross-sectional design of both these studies makes it difficult to determine accurately the age of onset of change. Whether this change is specifically related to estrogen deficiency is uncertain; similar changes occur in men, and conventional estrogen replacement at menopause has not been demonstrated to reverse this change. In women, an age-related decrease in wall width has also been reported in cortical bone in some, but not all, studies (37, 110, 166). Measurement of resorption depth has demonstrated a small decrease or no change in postmenopausal women, suggesting that the negative remodeling balance is primarily due to reduced bone formation (67, 92). However, studies of acute estrogen deficiency in premenopausal women, induced by administration of gonadotrophin releasing hormone analogs, suggest that there may be a transient increase in resorption depth (63). In these women, rapid and significant disruption of cancellous bone architecture was observed after 6-mo therapy; these changes are unlikely to be due solely to increased bone turnover and would be consistent with an early and transient increase in osteoclastic activity, resulting in increased cavity depth and trabecular penetration and erosion. Furthermore, in cortical bone, an increase in resorption depth within Haversian systems was demonstrated in these patients (17).

The greater age-related disruption of cancellous bone architecture in women than in men (60, 245) also supports the contention that estrogen deficiency is associated with increased erosion depth. Studies of cancellous bone structure in women have clearly demonstrated a reduction in trabecular continuity and loss of whole trabeculae after menopause. Whether there is significant trabecular thinning is less certain; some studies have reported significant or nonsignificant decreases in trabecular width, whereas others have found no change (2, 25, 61, 386, 395). The increase in trabecular separation that has consistently been demonstrated in postmenopausal women may thus mainly reflect loss of whole trabeculae rather than trabecular thinning. It is also possible that there is preferential erosion of thin trabeculae so that the contribution of trabecular thinning to bone loss is underestimated.

There have been relatively few bone histomorphometric studies of the effects of hormone replacement therapy. Evidence that hormone replacement reduces bone turnover was first reported by Riggs et al. (312) in a prospective study of 17 women with established osteoporosis. Iliac crest bone biopsies were obtained before and either 2.5–4 mo (short-term) or 26–42 mo (long-term) after estrogen replacement. After 2.5–4 mo, there was a significant reduction in bone-resorbing but not bone-forming surfaces, both of these being evaluated by microradiography; in contrast, after 26–42 mo, there was a significant reduction in both resorbing and forming surfaces. These data thus indicate that estrogen replacement reduces bone turnover, a suppressive effect on bone resorption being followed by a later decrease in bone formation.

A more detailed histomorphometric analysis of the effects of hormone replacement therapy on bone remodeling was later reported in a study of postmenopausal women with established osteoporosis (344). Bone formation rate at tissue level and activation frequency, both indices of bone turnover, were significantly decreased at 1 yr to ~50% of the pretreatment value, but no significant changes were observed in resorption depth or wall width, suggesting that remodeling balance was unchanged. However, because of the long life span of the bone remodeling unit in humans and, in particular, the time required for formation to be completed, a period of at least 2 yr is required to demonstrate changes in wall width induced either by disease or treatment. In contrast, because the resorptive component of the remodeling cycle is relatively rapid, changes may be seen over a much shorter period of time. Similar changes in bone turnover were reported in osteoporotic postmenopausal women after a 1-yr treatment with transdermal estrogen (226). Activation frequency and bone formation rate were both significantly lower in the posttreatment biopsies, bone turnover being suppressed to well below pretreatment values. A reduction in activation frequency, but not bone formation rate, was also reported in a study of postmenopausal women with low bone mineral density after treatment for 1 yr with percutaneous estradiol therapy (149). Finally, in a 2-yr prospective treatment study in postmenopausal women with osteopenia or osteoporosis, a significant reduction in bone turnover was observed; in addition, there was a trend toward decreased resorption cavity size after treatment, consistent with suppression of osteoclastic activity by hormone replacement therapy and a small reduction in wall width, possibly reflecting compensatory changes in response to the reduction in resorption cavity size (379). In this cohort, there was no significant change in cancellous bone structure during the study period, indicating that hormone replacement therapy preserves existing bone microstructure but does not reverse previously induced structural disruption (378).

These studies thus provide strong evidence that hormone replacement therapy, whether given as estrogen alone or combined with a progestin, preserves bone mass predominantly by reducing bone turnover. The relative contribution to this action of effects on the process of activation per se and those on osteoclast number and activity have not been established; a role for the latter

mechanism is supported by the well-documented effects of estrogen on osteoclast proliferation, differentiation, and activity demonstrated in vitro. The effects of estrogen administration on remodeling balance remain to be fully defined, but there is at present no evidence that, when given in conventional doses, estrogens increase bone formation at the cellular level. It is therefore possible that the age-related decrease in wall width may be an estrogen-independent phenomenon. Conversely, there is some evidence that estrogen replacement reduces resorption cavity size and hence improves this component of remodeling imbalance.

Evidence from animal studies indicates that high doses of estrogens have anabolic skeletal effects (87, 356), but until recently, it was unknown whether similar effects occur in the human skeleton. Percutaneous estrogen implant therapy has been reported to be associated with higher bone mineral density levels than oral or transdermal hormone replacement, an observation that may be related to the higher serum estradiol concentrations associated with parenteral treatment (117, 323, 328, 347). Many of these studies, however, were cross-sectional and involved the coadministration of testosterone implants, thus providing only indirect evidence for an anabolic skeletal effect of estrogen.

Recently, Wahab et al. (385) reported high bone mineral density values in a cohort of women who had received long-term high-dose estradiol implant therapy, without testosterone. A histomorphometric assessment of iliac crest bone from a subgroup of this cohort was performed, and the values obtained compared with those of healthy premenopausal women (375), based on the rationale that significant age-related bone loss had not occurred in the patient group before estradiol replacement and that any differences between the two groups would therefore reflect effects of high dose as opposed to physiological estrogen replacement. The results of this study demonstrated a significantly higher wall width in the implant-treated group (Fig. 9), providing direct histological evidence that high-dose estrogens produce anabolic skeletal effects in postmenopausal women and indicating that these are achieved by stimulation of osteoblastic activity, resulting in increased bone formation at cellular level and hence a more positive remodeling balance.

These findings have recently been confirmed in a prospective study of women undergoing treatment with estradiol implant therapy (185). In this study, not only was a significant increase in wall width observed, but changes indicative of increased connectivity of cancellous bone structure were also demonstrated. This raises the interesting possibility that the anabolic skeletal effects associated with high-dose estrogen therapy in postmenopausal women may result not only from improvement in remodeling balance but also de novo bone formation; the latter mechanism has been described in mice (326), but further studies are required to investigate its potential contribution to the observed changes in the human skeleton.

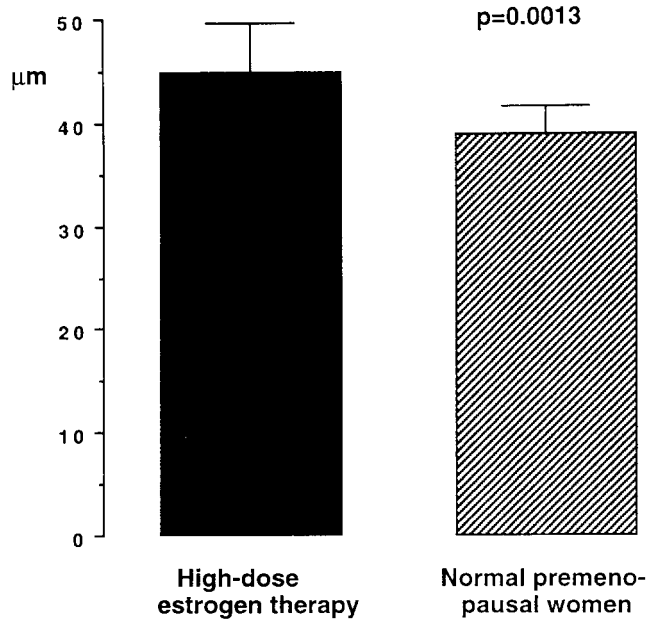

FIG. 9. Wall width in women treated with high-dose, long-term estradiol and normal premenopausal women. High-dose estradiol therapy was associated with a significantly higher wall width than that found in normal premenopausal women, reflecting increased bone formation at the cellular level due to increased osteoblastic activity. Data are shown as means ± SD. (From Compston JE. The skeletal effects of oestrogen depletion and replacement: histomorphometrical studies. In: *Annual Review of the Management of Menopause*, edited by Studd J. Carnforth, Lancs, UK: Parthenon, 2000, p. 287–296.)

V. EFFECTS OF PROGESTERONE ON BONE

Relatively little is known about the effects of progestins on bone metabolism. Normal human osteoblastic cells express progesterone receptors (196), and stimulation of the proliferation and differentiation of these cells has been reported in response to relatively high doses of progesterone (46). In the ovariectomised rat model, progesterone was reported to have similar effects to estrogen in one study (15) but antagonistic actions in another (360).

Menopausal estrogen therapy in women with an intact uterus is combined with a progestin to prevent increase in endometrial cancer risk associated with the use of unopposed estrogen. Some of the progestrogens used in these formulations, particularly 19-nortestosterone derivatives, may independently have beneficial effects on bone mass, although the evidence in this area is conflicting (3, 5, 316, 331). Thus preservation of bone mineral density in postmenopausal women treated with norethisterone was demonstrated in metacarpal cortical bone (3),

but Hart et al. (142) reported that norgestrel therapy was associated with significant bone loss at this site in a similar cohort. In a study of the effects of medroxyprogesterone in early postmenopausal women, Gallagher et al. (114a) demonstrated preservation of total body bone mineral density (reflecting predominantly cortical bone) but significant losses at the spine, forearm, and metacarpal cortex. Consistent with these findings, Adachi et al. (5) were unable to demonstrate any beneficial effect of medroxyprogesterone on bone mineral density in the lumbar spine or proximal femur in postmenopausal women taking estrogen replacement therapy. However, increases in bone mineral density have been reported in premenopausal women treated with cyclic medroxyprogesterone for menstrual disturbances (304).

The issue of whether decreased ovarian progesterone production is associated with changes in bone mineral density is also controversial. Prior et al. (305) reported decreased spinal bone mineral density in women with anovulatory cycles or cycles with short luteal phases, both of which are associated with reduction in endogenous progesterone production. Serum estradiol levels were reportedly normal in these women, indicating a role for progesterone deficiency in the pathogenesis of low bone mineral density. However, other studies in which documentation of ovulatory and hormonal status was more accurate and detailed (79, 144, 388) indicate that, provided that adequate estradiol status is maintained throughout the menstrual cycle, reduced progesterone production resulting from shortened luteal phases does not adversely affect bone mineral density. There is no evidence that combined estrogen/progestin therapy is more effective in reducing fracture risk than estrogen alone (404).

VI. SKELETAL EFFECTS OF ANDROGENS: MECHANISMS OF ACTION

Androgens have important effects on bone development and homeostasis. Increasing recognition of the morbidity and mortality attributable to osteoporosis in men has stimulated considerable interest in recent years in the mechanisms by which androgens act on bone. Nevertheless, knowledge in this area remains relatively sparse compared with the rapid advances that have been made in understanding estrogen-induced effects on the skeleton, and the treatment of osteoporosis in men remains largely unexplored.

A. Androgen Receptor

The androgen receptor was cloned in 1988 (49, 225), and its presence was subsequently demonstrated in rat and human osteoblastic cell lines and normal human osteoblast cells in vitro (56, 270, 369) and in human bone in situ (4). In the latter study, receptors were expressed in hypertrophic chondrocytes, osteoblasts, osteocytes, mononuclear cells, and endothelial cells of blood vessels in the bone marrow. The binding affinity appears to be similar for testosterone and dihydrotestosterone (DHT) (19).

B. Local Metabolism of Sex Steroids

Although testosterone is the major circulating androgen, there is evidence that its skeletal effects are at least partially mediated by metabolites produced by enzymes present in bone (Fig. 10). Thus the presence both of aromatase (262, 414), which converts testosterone to estradiol and androstenedione and dehydroepiandrosterone (DHEA) to estrone, and 5α-reductase (329, 384), which reduces testosterone to androstenedione and DHT, has been reported in bone. In addition, androstenedione can be converted locally to testosterone by 17β-HSD (40). Case reports of a male with ER resistance and of patients with aromatase deficiency emphasize the importance of normal aromatase activity for bone health in both sexes. Thus, in a 28-yr-old man with a point mutation of the ER gene, complete estrogen resistance was associated with a severe defect of skeletal growth resulting in delayed epiphyseal closure and bone age, tall stature, increased bone turnover, and severely reduced bone mineral density for his chronological age, although not for bone age (336). Manifestations of aromatase deficiency in females include pubertal failure and delayed bone age (253), whereas in a male with a homozygous mutation and severe aromatase deficiency, the phenotype was characterized by tall stature, delayed skeletal maturation, and osteopenia (253). Subsequently, another male with aromatase deficiency and similar clinical features has been described; estrogen therapy was associated with a large increase in bone mineral density and closure of the epiphyses (48). These clinical observations demonstrate that estrogens have an important physiological role in the male skeleton, but do not exclude a role for androgens (368).

C. Effects of Androgens on Osteoblastic Cells

Effects of androgens on osteoblastic cells have been demonstrated both in animals and humans. Stimulation of proliferation of these cells and possibly also of their differentiation has been reported (178) with increased expression of TGF-β mRNA (19, 177) and increased responsiveness to FGF and IGF-II (177). Other reported effects on osteoblastic cells include inhibition of the cAMP response to PTH or PTH-related peptide (113, 132), reduced prostaglandin production in stimulated calvarial organ cultures (300), and inhibition of IL-6 production by stro-

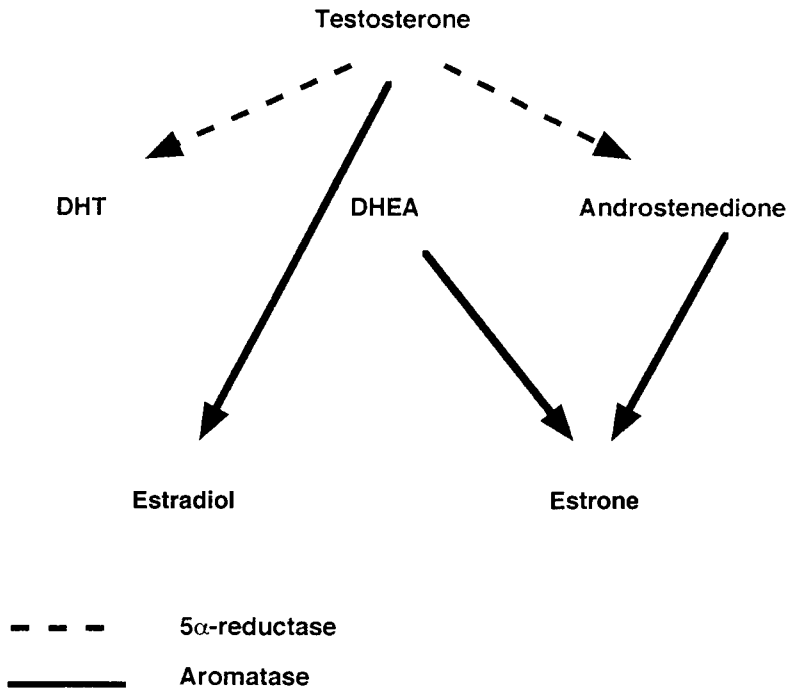

FIG. 10. Local metabolism of androgens and estrogens in bone cells by 5α-reductase and aromatase enzymes. DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

mal cells (18). Increased production of type I collagen has also been reported (19, 132), although this finding has not been universal (46, 300).

D. Skeletal Effects of Androgens in Animal Models

In vivo animal studies have shown that androgens promote chondrocyte maturation, metaphysial ossification, and the growth of long bones; this contrasts with the effect of estrogens that promote epiphysial closure and hence reduce longitudinal growth (271). The effects of androgens on bone growth are manifest particularly by an effect on bone size, with male animals having both larger bones and thicker cortices than their female counterparts (179, 361). In growing male rats and mice, castration is associated with a reduction in cortical and cancellous bone mass (146, 269, 370), probably due to an increase in bone turnover and in osteoclastic activity (360). Unlike the response to ovariectomy in female animals, however, the reduction in cortical bone mass appears to be predominantly due to decreased periosteal bone formation (360, 363). In mature rats, castration is also associated with cortical and cancellous bone loss (137, 367), with evidence of increased bone turnover in the first few months after castration followed by a lower turnover state (137, 371, 380).

A number of studies support the contention that both estrogens and androgens are required for normal skeletal health in males and females. Thus the administration of flutamide, a specific androgen receptor antagonist, to female rats results in osteopenia, indicating a role for androgens in the female skeleton (128). In support of these findings, Lea et al. (213) reported that the antiandrogen compound Casodex inhibited the protective effects of androstenedione on ovariectomy-induced bone loss, whereas administration of an aromatase inhibitor was ineffective. Furthermore, in female rats, nonaromatizable androgens have been shown to prevent or reverse bone loss induced by ovariectomy, these effects being mediated by a reduction in bone turnover in cancellous bone and increased periosteal and endosteal bone formation (355, 363). The skeletal effects of castration in male animals can be prevented by the administration both of testosterone and nonaromatizable androgens, indicating that aromatization of androgens to estrogen cannot be wholly responsible for androgenic skeletal effects (176, 338, 363, 387). Administration of the type II 5α-reductase inhibitor finasteride, which blocks conversion of testosterone to 5α-dihydroxytestosterone, has no effect on bone density in rodents or humans (237, 320), although these findings may be explained in part by the presence of type I 5α-reductase in bone (325). Estrogens have also been reported to prevent orchidectomy-induced bone loss in rats (372). Finally, in the testicular feminized (Tfm) rat, which is androgen receptor deficient, cancellous bone volume is similar to that of normal male littermates, but orchidectomy, which removes the source of estrogen production, prevents the attainment of normal cancellous bone volume, suggesting a role for estrogen in bone development in growing animals (371, 373).

E. Effects of Androgens in the Human Skeleton

The mechanisms by which androgen depletion and repletion affect the human skeleton have been little studied. Studies in men undergoing orchidectomy or rendered hypogonadal by administration of gonadotrophin releasing hormone analogs (127) have shown rapid bone loss associated with an increase in biochemical markers of bone resorption and formation, indicating increased bone turnover. However, in the absence of histomorphometric data, it is not possible to ascertain the effects of androgen deficiency on remodeling balance or on cancellous or cortical bone architecture. Similarly, the mechanisms underlying age-related bone loss in men have not been clearly established, although the wall width falls with age (376), indicating reduced osteoblastic activity, and the better preservation of bone architecture than that observed in ageing women indicates that increased activity of osteoclasts may be less prominent, although there may be some increase in bone turnover (67).

Similarly, data on the mechanisms by which exogenously administered androgens affect the skeleton are very sparse. Those that exist indicate that androgens preserve bone mass predominantly by reducing bone turnover (10), but this finding has not been universal and further studies are required.

There is also evidence that androgens play an important role in the female skeleton (333, 369). Thus in females affected by the androgen insensitivity syndrome, there is resistance to androgens, and endogenous estrogen production is also reduced. Low bone mineral density is a frequent finding in these patients (339) even in those women treated with long-term estrogen replacement (215, 256, 258). Furthermore, the addition of testosterone to estrogen replacement in normal postmenopausal women has been reported to result in higher bone mineral density values than treatment with estrogen alone (356), and there is some evidence that age-related bone loss in women is related to serum androgen levels (222, 223).

VII. SELECTIVE ESTROGEN RECEPTOR MODULATORS

A. Early Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are compounds that exhibit tissue specificity, with estrogenic effects in some tissues and antiestrogenic effects in others. The first of these compounds developed for clinical use was clomiphene, which is used in the treatment of infertility in women, but it was the example of tamoxifen, which was developed as an antiestrogen for the treatment of breast cancer and subsequently shown to have estrogenic effects on the skeleton and endometrium, which particularly illustrated the potential therapeutic benefits of SERMs (57). Tamoxifen is widely used in the treatment of breast cancer and also prevents bone loss in postmenopausal women (224); histomorphometric studies indicate a similar mechanism of action to that of estrogen, the predominant effect being a reduction in bone turnover (402, 403). However, its use in the management of osteoporosis in healthy women is precluded by estrogenic effects on the endometrium, which result in an increased risk of endometrial cancer (105). Concurrent with and subsequent to the development of tamoxifen, other compounds were investigated with the aim of producing the pharmacological profile of the "ideal" estrogen, namely, one that exerts the beneficial effects of estrogen, for example, in the skeleton and cardiovascular system without its adverse effects, particularly in the breast and endometrium. A significant step in this direction has been the development of raloxifene, a synthetic benzothiophene, which is licensed in many parts of the world for prevention and treatment of postmenopausal osteoporosis. The chemical structure of raloxifene is shown in Figure 11.

RALOXIFENE

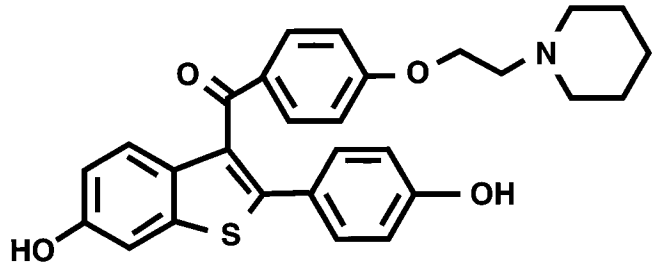

FIG. 11. Chemical structure of raloxifene.

B. Skeletal Effects of Raloxifene

Studies both in animals and humans have shown beneficial effects of raloxifene in bone, similar to those observed with estrogen. Thus, in the ovariectomized rat model, raloxifene has protective skeletal effects both when given at the time of ovariectomy and after bone loss has become established (27, 327, 359). In keeping with its antiresorptive mechanism of action (99), the main effect is to prevent rather than restore bone loss. In postmenopausal women, raloxifene prevents bone loss at multiple skeletal sites both at the perimenopause (76) and in later years (95, 227) and, furthermore, significantly reduces vertebral fracture risk in women with osteoporosis (227, 360). Although there are no published reports directly comparing the effects of estrogen and raloxifene on bone mineral density, the increases observed with raloxifene in

the spine and femur of 1.6 and 1.2% at 2 yr in healthy perimenopausal women and 2.4 and 2.1%, respectively, in women with postmenopausal osteoporosis treated for 3 yr are generally lower than those reported in studies conducted in similar populations with hormone replacement therapy. This may indicate that raloxifene has weaker effects on the skeleton than estrogen, although whether these differences in bone mineral density have a significant impact on fracture reduction is uncertain, since no adequately powered prospective randomized studies of the effects of estrogen on vertebral fracture have been reported, and evidence for protection against nonvertebral fracture is almost exclusively based on observational studies. It is, however, of interest that no reduction in nonvertebral fracture has been demonstrated for raloxifene in women with postmenopausal osteoporosis, since trials in comparable populations with another group of drugs, the bisphosphonates, have shown such reductions in smaller trials in which the nonvertebral fracture rate in the control group was comparable to that seen in the raloxifene study (26, 141).

Unlike estrogen and tamoxifen, raloxifene does not have agonistic effects on the endometrium, thus avoiding unwanted vaginal bleeding and increased risk of endometrial cancer. Furthermore, a highly significant reduction in breast cancer has been observed in women treated with raloxifene for a median of 40 mo (69). Other potential long-term benefits of raloxifene (and estrogen replacement) include protection against cardiovascular disease and improvement in cognitive function, but these have not been firmly established for either estrogens or SERMs, although they are currently being investigated in large prospective clinical studies.

C. Mechanisms for Tissue Specificity of SERMs

The mechanisms by which SERMs exhibit tissue specificity have not been clearly established, but recent progress in defining estrogen signaling pathways has provided some insight as to potential modes of action (202, 229). The existence of at least two ER subtypes with a differential tissue distribution and, in cells where both are present, the ability (demonstrated in vitro but not in vivo) to form either homodimers or heterodimers provides a potential mechanism for tissue specificity that could be ligand specific (90, 203, 205, 284, 296). Furthermore, depending on the ligand and response element, the two ER subtypes may signal in different ways; thus, at AP-1 sites, 17β-estradiol interacts with ERα to activate transcription, whereas with ERβ, this ligand inhibits transcription. Conversely, tamoxifen and raloxifene activate transcription with both ERα and ERβ at AP-1 sites (284). Both estrogen and raloxifene stimulate transcription of the TGF-β3 gene, but raloxifene is considerably more potent in this respect; it has been shown that the TGF-β3 gene contains a response element termed the raloxifene response element (RRE) to which raloxifene binds after the interaction of the ERα with additional "adaptor" protein(s) (412). Third, ligands may have differential effects at the AF-1 and AF-2 sites. Thus, in some cell lines, tamoxifen acts with the ERα as an AF-1 agonist and an AF-2 antagonist (16, 243, 391) (although this is not seen with ERβ), and while the AF-1 domain is required for estrogen- but not raloxifene-induced activation of the TGF-β3 gene, deletion of the AF-2 domain inhibits raloxifene-induced activation but not that due to estrogen (186). Finally, ligand-specific conformational changes in the ligand-binding domain of the receptor determine the surfaces by which the ER interacts with regulatory proteins and thus affects gene transcription (41, 242, 332). In the case of raloxifene, for example, it has been shown that the alkylaminoethoxy side chain interacts directly with aspartate-351 of the ERα, displacing helix 12 and thus preventing the AF-2 from activating gene transcription (41).

VIII. CONCLUSIONS AND FUTURE PERSPECTIVES

The last few decades have seen significant advances in our understanding of how estrogens affect bone, and these have been translated into improvements in the management of osteoporosis. However, many issues remain unresolved, and recent discoveries about bone physiology and biology pose further questions. The challenge for the immediate future is to define more clearly the mechanisms by which estrogens affect bone cell formation and activity and to make progress in the relatively unexplored area of androgens and bone.

In the past few years major new areas of research have emerged. The realization that estrogen is essential for skeletal health in men has led to a reexamination of the etiology of male osteoporosis and the metabolism of sex steroids in the bone microenvironment. The demonstration, in animals, that high doses of estrogens have anabolic effects in bone has been extended to the human skeleton and may lead to a better understanding of the mechanisms by which such effects can be achieved. Third, the recognition that compounds developed as antiestrogens could exhibit tissue specificity, with a mixture of agonistic and antagonistic effects, has provided a basis for the concept of SERMs; the subsequent and ongoing discoveries related to estrogen signaling indicate the potential for improvement of the pharmacological profile of these compounds. The goal of the "ideal" estrogen, which provides protection against many of the major diseases of the postmenopause, has not yet been realized but is becoming a possibility. Furthermore, the lessons learned from the SERMs should be applicable to other steroid

hormones, such as androgens and glucocorticoids, the therapeutic value of which is currently limited by adverse effects.

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