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Sex Steroids and the Construction and Conservation of the Adult Skeleton

B. LAWRENCE RIGGS, SUNDEEP KHOSLA, AND L. JOSEPH MELTON, III

Division of Endocrinology and Metabolism (B.L.R., S.K., L.J.M.), Department of Internal Medicine, and Section of Clinical Epidemiology (L.J.M.), Department of Health Sciences Research, Mayo Clinic and Mayo Foundation, 200 First Street SW, Rochester, Minnesota 55905

Here we review and extend a new unitary model for the pathophysiology of involutional osteoporosis that identifies estrogen (E) as the key hormone for maintaining bone mass and E deficiency as the major cause of age-related bone loss in both sexes. Also, both E and testosterone (T) are key regulators of skeletal growth and maturation, and E, together with GH and IGF-I, initiate a 3- to 4-yr pubertal growth spurt that doubles skeletal mass. Although E is required for the attainment of maximal peak bone mass in both sexes, the additional action of T on stimulating periosteal apposition accounts for the larger size and thicker cortices of the adult male skeleton. Aging women undergo two phases of bone loss, whereas aging men undergo only one. In women, the menopause initiates an accelerated phase of predominantly cancellous bone loss that declines rapidly over 4–8 yr to become asymptotic with a subsequent slow phase that continues indefinitely. The accelerated phase results from the loss of the direct restraining effects of E on bone turnover, an action mediated by E receptors in both osteoblasts and osteoclasts. In the ensuing slow phase, the rate of cancellous bone loss is reduced, but the rate of cortical bone loss is unchanged or increased. This phase is mediated largely by secondary hyperparathyroidism that results from the loss of E actions on extraskeletal calcium metabolism. The resultant external calcium losses increase the level of dietary calcium intake that is required to maintain bone balance. Impaired osteoblast function due to E deficiency, aging, or both also contributes to the slow phase of bone loss. Although both serum bioavailable (Bio) E and Bio T decline in aging men, Bio E is the major predictor of their bone loss. Thus, both sex steroids are important for developing peak bone mass, but E deficiency is the major determinant of age-related bone loss in both sexes. (*Endocrine Reviews* 23: 279–302, 2002)

Abbreviations: AR, Androgen receptor; ArKO, aromatase knockout; BERKO, ERβ knockout; Bio, bioavailable; BMD, bone mineral density; BMU, basic multicellular units; DERKO, double ER knockout; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; DXA, dual energy x-ray absorptiometry; E, estrogen; E_1, estrone; E_2, estradiol; ER, E receptor; αERKO, ERα knockout; HSD, hydroxysteroid dehydrogenase; M-CSF, macrophage colony-stimulating factor; OHE_1, hydroxyestrone; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; OPG, osteoprotegerin; PGE_2, prostaglandin E_2; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; T, testosterone.

I. Introduction

INVOLUTIONAL OSTEOPOROSIS IS one of the most serious diseases facing the aging population. Age-related bone loss is universal, affecting older women and men in

every population. By one analysis, 35% of postmenopausal white women and 19% of white men have osteoporosis as assessed by bone mineral density (BMD) measurements at the hip, spine, or distal forearm (1). Nonwhite men and women are affected to a lesser, but still substantial, degree (2). In the United States alone, osteoporosis leads to an estimated 1.3 million fractures each year, at a cost to the health care system at least 14 billion dollars annually for only direct expenditures (3). Moreover, the lifetime risk for these fractures is 40% for women and nearly 15% for men, and these figures will rise substantially based on projected increases in life expectancy (4). Few serious diseases have such a high penetrance in their target populations.

Sixty years ago, Albright *et al.* (5) related the causation of postmenopausal osteoporosis to estrogen (E) deficiency and found that E treatment improved calcium balance in postmenopausal women. These pioneering studies were validated some 30 yr later by densitometric studies demonstrating that the accelerated bone loss induced by ovariectomy could be prevented by E therapy (6, 7). Although the menopause came to be well accepted as a cause of postmenopausal bone loss, a number of other age-related factors were also implicated in both women and men. These included secondary hyperparathyroidism (8), impaired vitamin D metabolism (9), and impaired osteoblast function (10). In addition, nutritional vitamin D deficiency (11) and inadequate calcium intake (12) were found to cause bone loss in subsets of the aging population. Thus, E deficiency was believed to be but one of the multiple causes of involutional osteoporosis and its effect largely limited to bone loss in women during the first decade after menopause.

In 1998, however, we (13) proposed a new unitary model for the pathophysiology of involutional osteoporosis that identified E deficiency as the major cause of both the early, accelerated and the late, slow phases of bone loss in postmenopausal women and as a contributing cause of bone loss in elderly men. We now update this model based on new data that have been published subsequently and extend it by examining the effect of sex steroids on skeletal growth and maturation and on the sensing of biomechanical strain by bone cells.

II. Skeletal Effects of Sex Steroids

A. Synthesis and metabolism of sex steroids

In premenopausal women, more than 95% of serum estradiol (E_2) and most of serum estrone (E_1) is derived from ovarian secretion. Peripheral conversion of steroid precursors accounts for the remainder in premenopausal women and for almost all of the circulating estrogens in postmenopausal women. Also, in men, more than 95% of the major potent circulating androgen, testosterone (T), is derived from testicular secretion. For serum T in premenopausal women, 25% is derived from ovarian secretion, 25% from adrenal secretion, and 50% from peripheral conversion, and the sources are similar in postmenopausal women except that ovarian secretion of T decreases. In many target tissues, 5α-dihydrotestosterone (DHT), formed from T through the action of the enzyme 5α-reductase, is the main source of androgenic activity. 5α-Reductase is present as two isoforms. Almost all of the circulating DHT arises from back diffusion into the circulation from this extragonadal conversion, rather than from direct gonadal secretion. In addition, the adrenal cortex and, to a lesser extent, the gonads secrete large amounts of C19 androgens, chiefly dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), and Δ^4-androstenedione. Although only weakly androgenic themselves, they are an important source of substrate for the extragonadal synthesis of potent sex steroids [see reviews (14, 15) for details of sex steroid biosynthesis]. Table 1 gives mean values for circulating sex steroids in young adult and elderly women and men based on the data of Labrie *et al.* (16) and of Khosla *et al.* (17).

The levels of circulating active sex steroids are functions of both their rates of production and removal. Although other hydroxylation pathways exist, the two main ones for the removal of circulating E involve 2-hydroxylation and 16α-hydroxylation. The 2-hydroxylated estrogens are inactive or, in some experimental systems, antagonistic, whereas the 16-hydroxylated estrogens retain E activity (18). The major pathway for degradation of circulating T is oxidation to 17-ketosteroids.

Extragonadal biosynthesis plays a minor role in sex steroid biosynthesis in lower mammals (except for the brain in most mammals and the placenta in some ruminants), but in humans and higher primates, extragonadal biosynthesis is remarkably well developed (19, 20). Thus, multiple peripheral tissues including bone can synthesize E_1 from circulating C19 steroids, and E_2 and DHT can be synthesized directly from T. The concentrations of circulating C19 precursors are high. For example, serum DHEA-S levels in adult men and women are 100- to 500-fold higher than T and 1,000- to 10,000-fold more than E_2 (19). Thus, although the conversion rate is only 1–2%, the quantity of active new steroids generated extragonadally is appreciable. The principal site of this conversion is adipose tissue. The rate of extragonadal biosynthesis is increased in obese persons and is also increased in aging postmenopausal women (20).

Labrie *et al.* (16) have given the name "intracrinology" to the process by which active steroids are synthesized by a

TABLE 1. Mean serum levels of sex steroids and percursors in young women and untreated elderly postmenopausal women and men

Variable	Women		Men	
	20–30 yr	70–80 yr	20–30 yr	70–80 yr
DHEA (nmol/liter)	24	7	23	5
DHEA-S (nmol/liter)	6	2	12	2.5
AND-4 (nmol/liter)	3.7	1.5	3.5	1.7
AND-5 (nmol/liter)	3	1.5	5	2.5
Total T (nmol/liter)	1.4	1.1	20	16
Bio T (nmol/liter)	0.3	0.2	6.6	3.3
DHT (nmol/liter)	1.0	0.8	2.8	3.2
Total E_1 (pmol/liter)	221	133	150	130
Total E_2 (pmol/liter)	338	78	124	121
Bio E_2 (pmol/liter)	108	20	70	43

Data are from Labrie *et al.* (16) and Khosla *et al.* (17). All samples in premenstrual women were taken during the first 5 d of the follicular phase of the menstrual cycle. See text and Fig. 1 for definition of abbreviations.

peripheral target cell, in which the action of the steroid is exerted without its release into the extracellular fluid. The extragonadal intracrine tissues that synthesize E_1 and E_2 utilize the same enzymatic pathways that are employed for gonadal synthesis except that they are unable to synthesize C_{19} steroids and must depend on circulating precursors for substrate. Figure 1 shows the major pathways for the extragonadal synthesis of the potent sex steroids. The key enzymes involved in this process—the seven isoforms of 17β-hydroxysteroid dehydrogenase (17β-HSD) (21), aromatase (CYP19) (22–24), steroid sulfatase (25), 3β-hydroxysteroid dehydrogenase (3β-HSD) (26), and 5α-reductase (27, 28)—are present in osteoblast-lineage cells. Aromatase is also expressed in chondrocytes (29). The sex steroids synthesized extragonadally undoubtedly also have paracrine actions.

Recently, gonadal secretion and extragonadal synthesis of E and T have been shown to play interactive roles in sex steroid action. Of active sex steroids in peripheral tissues, Labrie *et al.* (16) estimated that 50% of total androgens in adult men, 75% of estrogens in premenopausal women, and nearly all of the estrogens in postmenopausal women originate from extragonadal synthesis. The biosynthesis of sex steroids is highly tissue specific: thus, for the gonads, the major estrogen synthesized is E_2; for adipose tissue, it is E_1; and, for the placenta, it is estriol. Expression of the aromatase gene at these various sites is under control of tissue-specific promoters that are regulated by different transcription factors and cytokines. The intracrine biosynthesis of sex steroids is economical because only the concentration required by the cell is synthesized, and the large dilution in the extracellular fluids that occurs after endocrine secretion is avoided. Moreover, there is evidence that extragonadal E production is a regulated process. IL-6 has been shown to regulate the activity of 17β-HSD in breast cancer cells (30) and IL-1β and TNFα have been shown to regulate the activity of CYP19 aromatase in osteoblasts (20). Interestingly, however, the production of these same proinflammatory cytokines in the bone microenvironment is increased by E deficiency (31). Finally, Eyre *et al.* (21) demonstrated that the rat osteoblastic cell line, ROS 17/2.8, could synthesize E_1, E_2, and T from Δ^4-androstenedione and that these syntheses could be up-regulated by 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and down-regulated by glucocorticoids.

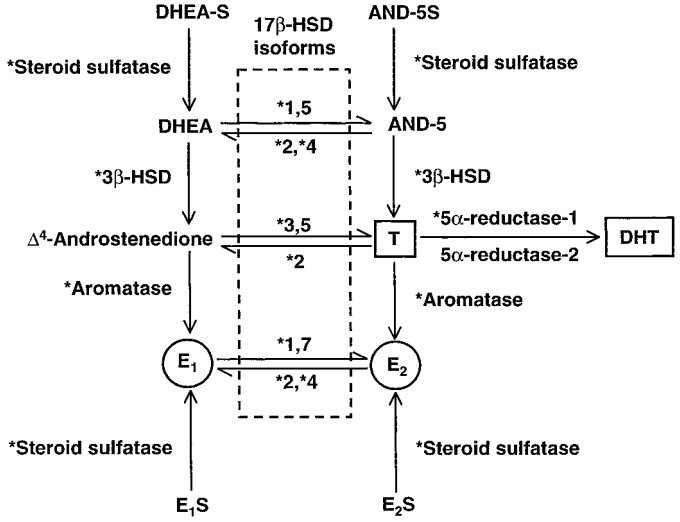

FIG. 1. Main pathways for extragonadal synthesis of active androgens (*boxes*) and potent estrogens (*circles*) in humans. The various 17β-HSD isoforms are given as *numbers within the broken outline*. The *arrows* indicate whether the reaction is reversible or irreversible. *Asterisks* indicate the enzymes that have been shown to be present in osteoblasts. S, Sulfate; AND, androstenediol. See text for derivation of other abbreviations. [Adapted with permission from F. Labrie *et al.*: *J Mol Endocrinol* 25:1–16, 2000 (19). © the Society for Endocrinology.]

B. Physiological effects of estrogen

E has specific functions at the organ, tissue, and cellular levels of the skeleton. At the organ level, E acts to conserve bone mass. Indeed, the actions of E and those of biomechanical strain are the major physiological mechanisms for bone mass conservation. In fact, with a few exceptions, such as states of corticosteroid excess, major decreases in bone mass do not occur unless one of these two homeostatic mechanisms is affected. At the tissue level, E tonically suppresses bone turnover and maintains balanced rates of bone formation and bone resorption (as reviewed in Ref. 32). At the cellular level, E affects the generation, lifespan, and functional activity of both osteoclasts and osteoblasts. E decreases osteoclast formation and activity and, by increasing apoptosis, it decreases osteoclast lifespan (33). As will be discussed later, controversy exists about the action of E on osteoblasts. Some evidence suggests that E increases osteoblast formation, differentiation, proliferation, and function, although results have varied among different model systems (34–36). Recently, two groups (32, 37) have demonstrated that E antagonizes glucocorticoid-induced osteoblast apoptosis and, thus, extends osteoblast lifespan.

As originally pointed out by Frost (38), the activities of osteoclasts and osteoblasts are combined into functional assemblies called basic multicellular units (BMUs). A remodeling cycle begins with formation of a new BMU on a previously inactive surface of bone. The lining cells disappear and are replaced by multinucleated osteoclasts that construct a resorption lacunae on the endosteal surface of bone over a 2-wk interval. The resorption phase then is terminated, probably by osteoclast apoptosis, and after a brief reversal phase, a team of osteoblasts is recruited that fill in the resorption cavity with new bone. In cortical bone, osteoclasts form the leading edge of a cutting cone that creates a resorption tunnel, and osteoblasts follow in their wake to convert it into a structural osteon [Haversian system (for reviews, see Refs. 32 and 39)].

E deficiency affects remodeling in several ways. First, it increases the activation frequency ("birth rate") of BMUs, which leads to higher bone turnover. Second, it induces a remodeling imbalance by prolonging the resorption phase [osteoclast apoptosis is reduced (33)] and shortening the formation phase [osteoblast apoptosis is increased (32)]. Also, increased osteoclast recruitment extends the progression of the BMU. As a consequence of these changes, the volume of the resorption cavity is increased beyond the capacity of the osteoblasts to refill it. In cancellous bone, the extended osteoclast lifespan increases resorption depth, leading to trabecular plate perforation and loss of trabecular

connectivity (39–41). In cortical bone, the rapid phase is associated with subendocortical cavitation, and eventually, the inner third of the cortex may assume cancellous-like characteristics (39). The consequences of the effects of perforative resorption on cancellous bone are shown in Fig. 2, which compares the three-dimensional microstructure of lumbar spine bone samples from an E-replete premenopausal woman with those from an E-deficient woman with postmenopausal osteoporosis. In contrast to the osteoclast-mediated disruption of the cancellous bone microarchitecture during the rapid phase, the subsequent slow phase of bone loss is characterized by trabecular thinning in which impaired osteoblast activity plays a prominent causal role (39).

C. Physiological effects of androgens

As with E, the major action of T at the tissue level is to reduce bone resorption (42). However, much of this action is indirect via aromatization of T to E (43). As with E, T also increases the lifespan of both osteoblasts (32) and osteoclasts (44) by affecting apoptosis. T also has a modest effect on osteoblast proliferation (45). Both effects of T (stimulation of proliferation and inhibition of apoptosis) contribute to its action on enhancing bone formation. Moreover, T may also differ from E by acting at different stages of osteoblast differentiation, and T and E may affect osteoblasts differently at various skeletal locations. Thus, T increases periosteal apposition of bone (46), whereas E opposes it (47). This differential effect accounts, in part, for the larger skeleton achieved by the male during puberty. More research is needed to clarify the relative effects of T and E on bone cells.

D. Transduction by sex steroid receptors

Before 1988, sex steroids were believed to affect the skeleton only indirectly by regulating secretion of systemic calcitrophic hormones. However, it now is firmly established that osteoblasts (48, 49), osteoclasts (50, 51), and osteocytes (52, 53) contain functional E receptors (ERs), although their concentration is lower than in reproductive tissues. In addition to the classical ERα, a genetically distinct second receptor, ERβ, has recently been discovered that has extensive homology with the ligand and DNA binding domains of ERα (54). ERα/ERβ heterodimers also have been described (55). ERα mediates most of the actions of E on bone cells, whereas ERβ, in some circumstances, can act as a dominant negative antagonist to ERα (56, 57). Bone cells contain both receptors, although their distributions within bone differ. Immunohistological studies of developing human bone have demonstrated that ERα is the predominant species in cortical bone but that ERβ is the predominant species in cancellous bone (58). Moreover, variation in the sequence of expression of ERα and ERβ during osteoblast differentiation could contribute to developmental differences in expression of ER-responsive genes. Thus, in human fetal osteoblastic cells, ERα mRNA increases only slightly (3-fold), whereas ERβ increases markedly (20-fold) and exponentially during osteoblast differentiation (59). Chondrocytes in human growth plate cartilage also contain both ERα and ERβ (60, 61). Finally, both osteoblasts (62) and osteoclasts (63) also contain high affinity androgen receptors (ARs).

Much has been learned from studies of the skeletal phenotypes of ERα knockout (αERKO) (64), ERβ knockout (BERKO) (65), and double ER knockout (DERKO) (65) mice. However, the findings in these mutant mice do not completely reproduce those of E-deficient women. For example, the αERKO and DERKO mice have shortened femoral length (64, 65), whereas E-deficient girls and the single reported case of a male with homozygous null mutations of the ERα gene (64) have elongated limb bones due to failure of the epiphyseal growth plate to fuse. In αERKO or DERKO mice, there is a decrease in appendicular bone growth (associated with and possibly due to a decrease in serum IGF-I levels) that is greater in females than in males (64, 66). However, αERKO mice have a cortical osteopenia and increased bone turnover that is greater in the male than in the female (64). In contrast, the skeletal phenotype in BERKO males is similar to that of the wild-type males (65). However, the BERKO females have an increase in cortical bone associated with increased periosteal apposition that develops during growth (3–6 months old) and is maintained in adults (12–13 months old; Refs. 67 and 68). The adult BERKO females are also protected against

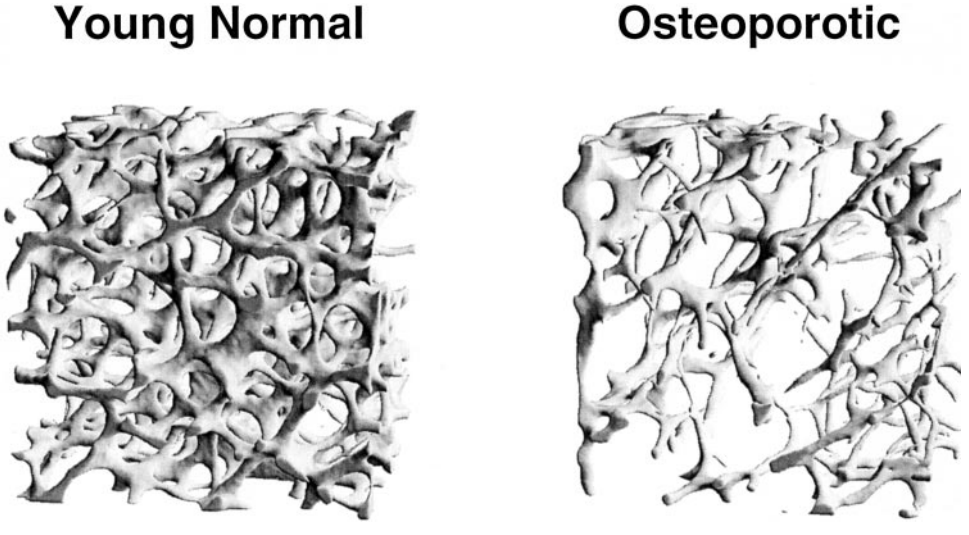

FIG. 2. Three-dimensional reconstruction by microcomputed tomography of a lumbar spine sample from a young-adult normal woman and from a woman with postmenopausal osteoporosis. In the osteoporotic woman, not only is bone mass reduced, but there is microarchitectural deterioration of bone structure. Whereas the rod-like structure in the normal case is very isotropic, the structure in the osteoporotic case shows preferential loss of horizontal struts and a concomitant loss of trabecular connectivity. These changes lead to a reduction in bone strength that is more than would be predicted by the decrease in BMD. Images courtesy of Ralph Müller, Ph.D., Beth Israel Deaconess Medical Center and Harvard Medical School (Boston, MA).

the age-related cancellous bone loss that occurs in the wild-type mice, and although the growth plate width is unaffected, histological indices of formation and resorption are decreased (67, 68). Interestingly, after ovariectomy, adult DERKO females undergo the same degree of cancellous bone loss as wild-type females, and the bone loss can be prevented by E treatment, but at a 5-fold higher dosage than is required to prevent bone loss in wild-type mice (69). The reason for these observations is unclear at present, but they could be caused by the persistence of ERα splice variants in the DERKO mice, or possibly, by a sex-nonspecific, nongenomic mechanism involving the AR (70).

Although the exact meaning of the data from these mutant mice is unclear and more studies are needed, several tentative interpretations can be made. First, most of E-dependent bone growth is mediated through ERα because growth is disrupted in the αERKO and DERKO mice, but not in the BERKO mice. Second, ERβ may account for at least part of the sexual dimorphic changes in the skeleton because BERKO females, but not BERKO males, have larger cortical bone width than the wild-type females. These changes may be the result of ERβ-antagonism of ERα-stimulated periosteal bone formation. Third, because 1-yr-old BERKO females have more cancellous bone than do wild-type females, ERβ may be permissive for age-related bone loss in females, possibly by stimulating bone resorption on cancellous and endocortical surfaces or by inhibiting a stimulatory effect of ERα on bone formation. Alternatively, the deletion of ERβ may lead to enhanced sensitivity of bone to ERα and, hence, to an increase in E action despite age-related decreases in serum E levels.

The testicular feminized male (TFM) rat has a spontaneous homozygous null mutation of the AR gene, resulting in androgen resistance (71). The animals have a female skeletal phenotype, but are not osteoporotic. The aromatase knockout (ArKO) mouse is E deficient because of targeted deletion of the CYP19 aromatase gene. Both ArKO males and females are osteoporotic. However, as assessed by histomorphometry and serum osteocalcin levels, the ArKO females have high bone turnover, whereas ArKO males have decreased bone turnover (72). The explanation for this sexually dimorphic response in the ArKO mice is not clear at present.

E. Molecular mediators of sex steroid action on bone cells

During the last decade, but especially during the past 3 yr, major progress has been made on elucidating the molecular mechanisms of E action on bone cells. Early studies focused on the role of E deficiency in increasing the production in bone of the proinflammatory cytokines IL-1, IL-6, TNFα, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor (M-CSF), and prostaglandin-E_2 (PGE_2). These cytokines increase bone resorption, mainly by increasing the pool size of preosteoclasts in bone marrow (31, 32, 73). Moreover, ovariectomy-induced increases in osteoclastogenesis are attenuated or prevented by measures that impair the synthesis or response to IL-1, IL-6, TNFα, or PGE_2 (31, 32, 74). E also up-regulates TGF-β (75), an inhibitor of bone resorption that acts directly on osteoclasts to decrease their activity (33) and rate of apoptosis (32).

However, E regulation of bone resorption must now be re-evaluated because of the discovery of three new members of the TNF ligand and receptor signaling family that are the final effectors of osteoclast differentiation and function (76, 77). The long-sought osteoblast-derived paracrine effector of osteoclast differentiation was identified as the receptor activator of nuclear factor-κB ligand (RANKL), which is expressed by stromal-osteoblastic lineage cells. Cell-to-cell contact between these cells and osteoclast lineage cells allows RANKL to bind its membrane receptor, RANK, potently stimulating all aspects of osteoclast function: in response to RANKL signaling, osteoclast differentiation and activity increase and osteoclast apoptosis decreases. Indeed, RANKL is both necessary and sufficient for osteoclast formation, provided that permissive concentrations of M-CSF are present. The stromal-osteoblast lineage cells also secrete osteoprotegerin (OPG), a soluble decoy receptor that neutralizes RANKL. E increases OPG (78) and decreases M-CSF (79) and RANK (80). However, part of its effect on this signaling system may be indirect through E-stimulated intermediaries. Thus, IL-1 and TNFα increase RANKL, OPG, and M-CSF, whereas PGE_2 increases RANKL and decreases OPG (31, 76, 77). E has not yet been shown to regulate RANKL or RANK directly. E also blocks the activity of Jun NH_2-terminal kinase and the resulting production of c-Jun and JunD in osteoclast lineage cells (80, 81). Thus, it seems likely that E inhibits bone resorption by inducing small but cumulative changes in multiple E-dependent regulatory factors, as is shown by the model in Fig. 3.

Less is known about the molecular mechanisms of androgen action on bone cells. Androgens stimulate osteoblast proliferation (45) and osteoblast differentiation (82, 83) in primary and transformed osteoblastic cell lines. At least one of the paracrine mediators of these anabolic effects is IGF-I. 5α-DHT increases IGF-I mRNA by up to 6-fold in osteoblastic cells, and the induction of osteoblast proliferation by 5α-DHT can be blocked by cotreatment with a neutralizing antibody to IGF-I (84). 5α-DHT also increases the number of IGF-II receptors, thereby potentiating the mitogenic effects of IGF-II on osteoblastic cells (82). Finally, androgens increase TGF-β production and activity, and orchiectomy decreases all three TGF-β isoforms by 80% (82, 85, 86).

The effects of androgens on inhibiting bone resorption may be mediated, at least in part, by decreased IL-6 production, because 5α-DHT suppresses constitutive and cytokine-stimulated IL-6 production in murine marrow stromal cells (42) and in human osteoblastic cells (87), and this inhibition is quantitatively similar to that achieved with E. Both in osteoblastic cells *in vitro* and in elderly men *in vivo*, androgen decreases (88, 89) and E increases (78, 89) OPG production, which may partly explain why the antiresorptive action of T is weaker than the antiresorptive action of E.

Finally, Kousteni *et al.* (70) have reported that the antiapoptotic effects of E and T on osteoblasts and osteocytes may be mediated by rapid, nongenomic, and sex-nonspecific signaling through the ligand binding domain of ERα, ERβ ,or AR. This action is distinct from the classical actions of these receptors, which are sex-specific, genomic, and transcriptional.

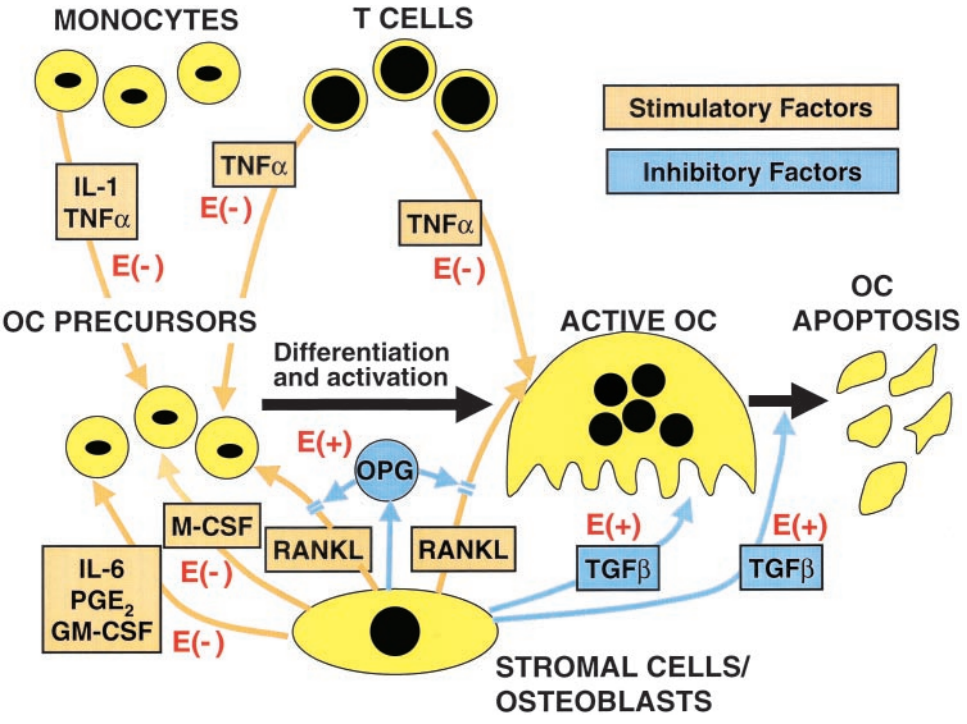

FIG. 3. Model for mediation of effects of E on osteoclast formation and function by cytokines in bone marrow microenvironment. Stimulatory factors are shown in *orange* and inhibitory factors are shown in *blue*. Positive (+) or negative (−) effects of E on these regulatory factors are shown in *red*. The model assumes that regulation is accomplished by multiple cytokines working together in concert. [Modified with permission from B. L. Riggs: *J Clin Invest* 106:1203–1204, 2000 (73).]

Thus, the autocrine and paracrine basis for sex steroid action on bone cells has recently come into sharper focus. However, how to weigh the importance of the various cytokines and how to quantify their complex interactions in mediating the sex steroid effects are subjects for further research.

F. Interaction with biomechanical forces

Frost (90) has emphasized the role of biomechanical strain, especially that induced by muscle contraction, in determining the level of bone mass. He suggests that the strain is sensed by an internal skeletal mechanostat that initiates changes in bone remodeling to adjust bone mass and distribution to a level that is appropriate for the ambient biomechanical forces. At normal adult activity levels, bone remodeling is maintained by what he terms a conservation mode that suppresses BMU activity on all bone surfaces. The higher strain levels associated with growth or extreme physical activity will induce a modeling mode that increases bone mass by accretion on bone surfaces. However, chronically low strain levels will induce a disuse mode of bone remodeling. In this mode, there is increased bone turnover on all bone surfaces, but on endosteal surfaces, which are in contact with bone marrow, more bone is resorbed than is formed. From these observations, Frost (91) theorized that the remodeling imbalance on endosteal surfaces induced by inactivity was mediated by a factor released by bone marrow termed rho. He also noted that the histomorphometric changes induced by either E deficiency or the disuse mode were very similar. In both, the bone loss is confined to the endosteal surface and does not involve the intracortical and periosteal surfaces (92). Thus, he hypothesized that E deficiency alters the set point of the mechanostat by decreasing the sensing of strain signals. This then switches bone remodeling from the conservation to the disuse mode. The bone loss will continue until a new steady state is reached where once again strain is sensed as high enough to return to the conservation mode of bone remodeling. This model is illustrated by the schematic in Fig. 4.

Although Frost based these insights largely on theoretical considerations, several experimental studies now support them. First, there is evidence that the cells sensing biomechanical strain ("the mechanostat") are, at least in part, the osteocytes (93), which do contain ERs (52, 53). Second, rho appears to be comprised of a cascade of marrow-derived cytokines that include both pro-inflammatory cytokines and the OPG/RANKL/RANK regulatory system. Moreover, these cytokines regulate the coordinated differentiation of osteoclasts and osteoblasts from precursor cells in bone marrow (31, 73), and precursors of both cell types in bone marrow are responsive to E (31, 80, 94). Thus, it is plausible that bone marrow cytokines could mediate the effects of both E and biomechanical strains and, depending on the signal, will give rise to either the conservation or disuse modes. Third, the interaction of mechanical forces and E action has now been demonstrated experimentally. Westerlind *et al.* (95) studied intact and ovariectomized rats on earth and in the orbiting space station and concluded that both E and mechanical strain shared common elements in their respective signal transduction pathways. The Lanyon group (96) found that the effects of mechanical strain and E-stimulated proliferation of osteoblasts cultured on plastic strips were additive, and that the effect of mechanical strain could be blocked by cotreatment with the ER antagonists ICI 182,780 or tamoxifen. Also, using a similar *in vitro* system, they have demonstrated that mechanical strain and E share a common transduction pathway involving activation of ERα, MAPK and ERK-1 signaling molecules, and the estrogen response

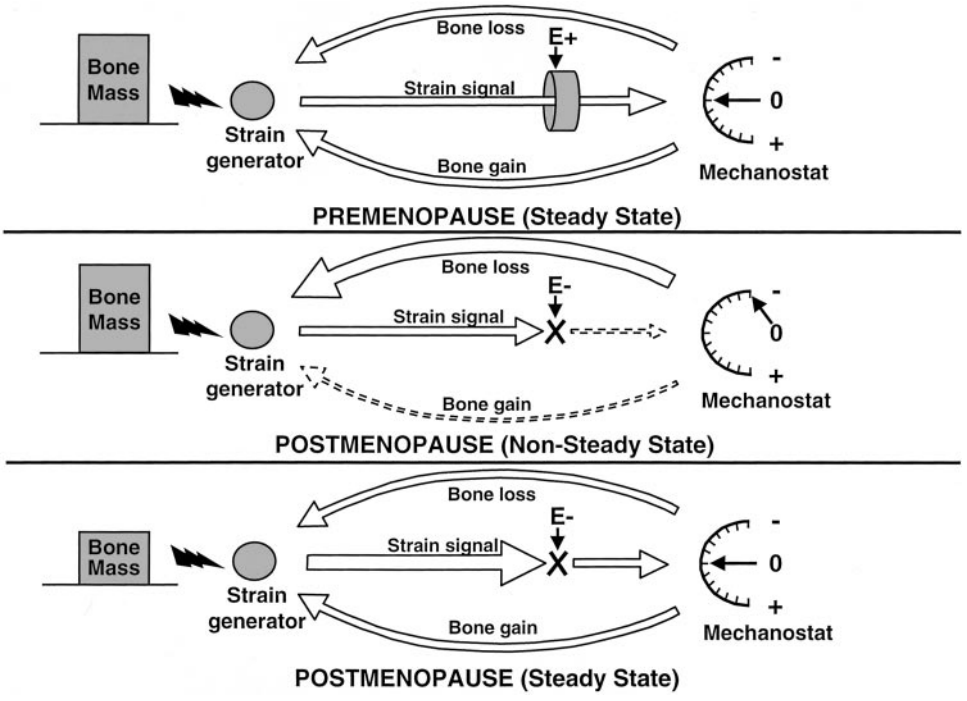

FIG. 4. Schematic of mechanism of E interaction with biomechanical strain to regulate bone mass based on publications of Frost (90–92). Internal mechanostat in bone senses strain generated by muscle contraction or skeletal loading. Mechanostat provides cybernetic feedback by activating bone cells that induce either bone gain or bone loss until a new steady state is achieved. High bone mass is associated with low strain signals, and low bone mass is associated with high strain signals. However, the sensing of strain by the mechanostat is modulated by ambient E concentration in the bone microenvironment. At menopause, low E levels lead to impaired sensing. Because of the resultant low strain signals, the mechanostat erroneously senses that bone mass is increased. This leads to rapid bone loss that continues until strains are sensed as being the same as those present before menopause, at which point bone loss ceases.

element of DNA (97–98). These data suggest that the interrelationship between the effects of mechanical strain and E on osteoblast function occur because they both share a common afferent pathway. Thus, although additional studies are needed, major advances have been made in defining the cellular and molecular basis of the mechanostat and its interaction with E action.

III. Patterns of Skeletal Growth and Maturation

Sex steroids are responsible for the maturation and the sexual dimorphism of the skeleton. Skeletal size and volumetric BMD are similar in prepubertal girls and boys. Between the onset of puberty and young adulthood, however, skeletal mass doubles (100). The rates of increase in statural height and bone remodeling are greatest in early puberty and then decline progressively (100–104). In contrast, maximal increases in volumetric BMD occur 2 yr later—at menarche in girls and late puberty in boys. The pattern of growth of boys differs from that of girls in two ways: boys have two more years of prepubertal growth because of their later puberty (age 14, rather than age 12 as in girls), and their pubertal growth spurt lasts for 4 yr rather than the 3 yr that it lasts in girls (100–104). These differences largely account for the 10% greater statural height and the 25% greater peak bone mass achieved by males. For the most part, the greater bone mass in males is due to their greater bone size.

Skeletal growth occurs mainly by modeling that increases the size and shape of bones. Linear bone growth occurs by ossification of the endochondral growth plates. Radial bone growth occurs by periosteal apposition, and the marrow cavity size increases by endosteal resorption. Prepubertal growth is proportionately greater in the legs, whereas pubertal growth is proportionately greater in the trunk (105). The excess in periosteal bone apposition over endosteal bone resorption that occurs during the pubertal growth spurt increases both the size and the volumetric BMD (the total bone mass contained within a volume of bone) of the extremities (106). Puberty is terminated by epiphyseal plate closure, by which time volumetric BMD has reached about 90–95% of peak mass. A process termed "consolidation" then brings the skeleton to its maximal values by continued periosteal apposition and, possibly, also by trabecular thickening. Undoubtedly, part of the increase in BMD during consolidation relates to the decline in the high intracortical porosity associated with the rapid pubertal phase of bone growth. How long consolidation continues is disputed: some find that it lasts only until the end of the second decade (101), whereas others find that for vertebral BMD it may last until the end of the third decade (107).

IV. Role of Sex Steroids in Skeletal Maturation

Before puberty, basal levels of the GH/IGF-I axis maintain slow, but continuous, bone growth. Puberty is triggered by increased pulsatile secretion of GnRH by the hypothalamus, leading to increases in serum gonadotropins and, thus, to increases in gonadal secretion of sex steroids (108). The increases in serum E enhance pulsatile GH secretion in both sexes by 1.5- to 3.0-fold; these increases, in turn, increase circulating, and possibly osteoblast, IGF-I concentrations by 1.5- to 3.0-fold (109, 110). The increases in GH, IGF-I, and E act coordinately to support the pubertal growth spurt. The high pubertal levels of GH and IGF-I are maintained during the 3–4 yr of rapid growth but then gradually decrease to prepubertal levels over several years, although the serum sex steroids remain at adult levels (109). However, it is the increase in serum E that is responsible for the pubertal growth spurt. Males with homozygous mutations in ERα or aromatase genes do not undergo rapid adolescent growth, de-

spite normal or increased levels of serum T (110–114). Moreover, it is the continued rise in serum E levels during puberty that is the probable cause of epiphyseal closure in both sexes, because young adult males who are unable to respond to E because of homozygous mutations of the ERα gene (111) or the aromatase gene (112–114) have open epiphyses, whereas men with testicular feminization due to null mutations of AR achieve epiphyseal closure (115, 116). Experimental studies in juvenile ovariectomized rabbits have demonstrated that E accelerates the programmed senescence in the proliferation rate and number and size of chondrocytes, leading to epiphyseal plate fusion (60). Thus, E both initiates the pubertal growth spurt and then ends it by inducing epiphyseal closure. Sex steroids also appear to increase bone mass during skeletal maturation independently of the effects of circulating levels of GH and IGF-I. The 25% greater bone mass in postpubertal boys over postpubertal girls is likely due mainly to the pubertal increase in serum T, because increases in GH secretion and IGF-I production are similar or even greater in girls than in boys. However, as will be reviewed later, E contributes substantially to volumetric BMD in both sexes. Based on measurements made in a young adult male who was unable to synthesize E because of a genetic defect in aromatase activity, BMD was reduced by 25–40% of predicted values at various skeletal scanning sites (114). Thus, both T and E have substantial effects on bone size and volumetric BMD, although E appears to play the dominant role.

V. Patterns of Age-Related Bone Loss

As pointed out by Seeman (106), the compartments (endosteal, intracortical, and periosteal) of bone may change differentially during bone growth and maturation and during bone loss. However, the changes in the individual compartments cannot be assessed by dual energy x-ray absorptiometry (DXA), the most commonly employed method for assessing bone density, which provides only an integral measure of BMD. Moreover, DXA provides data as areal BMD (g/cm^2), which overestimates volumetric BMD (g/cm^3) in larger bones by failing to account for the variable dimension of depth. Finally, bone size is ignored in the results of conventional assessments of BMD despite biomechanical data showing that, for a given bone mass, larger bones are stronger (117, 118). Thus, sex steroid sufficiency and deficiency exert differential effects on BMD and bone size that are not captured by most clinical measurements. Age and sex differences in bone size and shape are shown schematically in Fig. 5.

In women, there may be bone loss, especially from the proximal femur, during the 5 yr before menopause when sex steroid levels begin to decline (119). Whether substantial premenopausal bone loss occurs at other sites in women, or whether bone loss occurs in young men, is controversial. In women, some (107, 120), but not other, cross-sectional studies suggest that premenopausal bone loss may occur at the vertebrae, and a 16-yr longitudinal study demonstrated that it occurred at the hip (121). If these findings are correct, they suggest that a remodeling imbalance begins to occur in women after the cessation of growth and consolidation, which is subsequently enhanced by menopause.

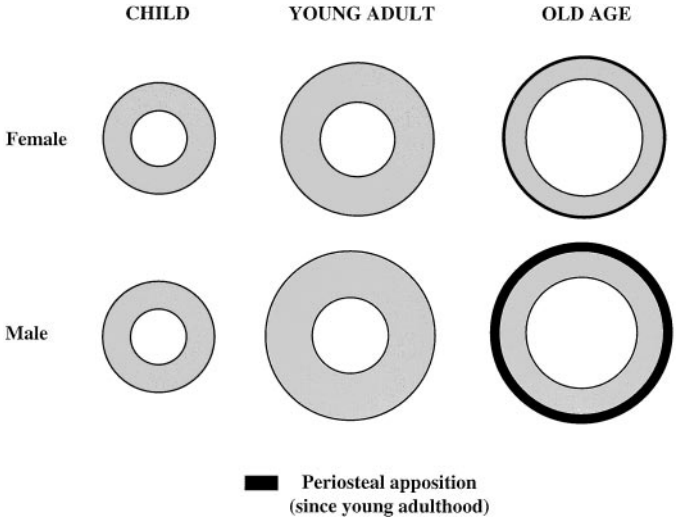

FIG. 5. Schematic representation in cortical bone of the differential effects of gender on growth and maturation and on bone loss with aging. Before puberty there are no differences in cortical bone between sexes. The bone of the prepubertal female and that of the adult female are similar in proportions, but the bone of the latter is larger. However, owing to a greater rate of periosteal apposition during puberty, the cortical bone of the young-adult male is larger and the cortex is thicker. By old age, there has been extensive loss of cortical bone due to endosteal resorption in both sexes, but because women undergo menopause, they lose more cortical bone than men do. The endosteal resorption with aging is partially offset by a continued periosteal apposition that is 3-fold greater in men than in women. Not shown in the cartoon are age-related increases in intracortical porosity that present in both sexes, but more so in women because of menopause. Cartoons are based on published data (105, 141).

A. Patterns in women

Women undergo two major phases of involutional bone loss: an early, but transient, accelerated phase that begins at menopause and a slow, continuous phase. The early phase declines exponentially over 4–8 yr to merge asymptotically with the subsequent slow phase. This phase accounts for losses of 20–30% in cancellous bone, but for only 5–10% in cortical bone. Because natural menopause occurs at different ages, the skeletal consequences of menopause are most clearly apparent after ovariectomy. In a 2-yr longitudinal study of middle-aged women who underwent ovariectomy, Genant *et al.* (7) found losses of 18% in cancellous bone by quantitative computed tomography but of only 4% in cortical bone by single photon absorptiometry. By contrast, Recker *et al.* (122) and Guthrie *et al.* (123) followed cohorts of 75 and 224 perimenopausal women, respectively, across natural menopause. In the 3 yr preceding the cessation of menses, Recker *et al.* (122) found losses of 4% in BMD of the lumbar spine and proximal femur associated with declining serum E levels, whereas Guthrie *et al.* (123) found losses of only 1–2%. After cessation of menses, Recker *et al.* reported losses of about 7% over 3 yr when an asymptote was reached. In their more extended follow-up, Guthrie *et al.* reported that the total excess bone loss was 14% and that the asymptote was not reached until after 8–10 yr. There are two reasons for the higher rate of bone loss in the study of Genant *et al.* (7) as compared with the latter two investigations (122–123). First, the more precipitous fall in serum E and, to a lesser

extent, T, after ovariectomy led to a more rapid rate of bone loss. Second, the high rate of bone loss found by Genant *et al.* was determined using quantitative computed tomography that specifically measured the more responsive cancellous bone in the vertebral centrum as compared with DXA that measured overall (both cortical and cancellous) vertebral bone loss in the latter two studies.

B. Patterns in men

Men do not undergo the equivalent of menopause and, thus, lack the early, accelerated phase of bone loss experienced by women. Castrated men (male sex offenders in Czechoslovakia) have a pattern of rapid bone loss similar to that of women after menopause (124). However, aging men exhibit a slow phase of bone loss that is virtually identical with the late slow phase that is experienced by postmenopausal women, leading to overall losses of about 20–25% in both cortical and cancellous bone. Periosteal apposition in the appendicular skeleton continues through life in both men and women, but men add 3-fold more bone by this process than do women (106). This increases the width of the long bones, including the proximal femur, and the same amount of bone distributed over a wider area is stronger. Thus, the greater biomechanical strength afforded by the wider bones partially compensates for age-related decreases in BMD. Indeed, Beck *et al.* (117) reanalyzed data from DXA measurements of the proximal femur on a non-Hispanic, white subgroup (2719 men and 2904 women) of subjects from the third National Health and Nutrition Examination Survey (NHANES). As a result of the increased bone width, they calculated that the femoral neck section modulus, an index of mechanical strength, was reduced over life by 14% in elderly women and by 6% in elderly men. However, these analyses did not include measurements of cancellous bone in the proximal femur and, thus, did not take into account the decrease in mechanical strength due to cancellous bone loss that occurred concomitantly with the changes in bone size. Thus, bone strength was undoubtedly reduced more than they estimated.

VI. Mechanism of the Early Accelerated Phase of Postmenopausal Bone Loss

This phase begins at the menopause, can be prevented by E replacement (6, 7), and clearly results from loss of ovarian function. During the 2- or 4-yr menopausal transition, serum E2 levels fall to 10–15% of the premenopausal level, although levels of serum E_1, a 4-fold weaker E, fall to about 25–35% of the premenopausal level (125). Also, serum T decreases after menopause because of decreases of ovarian T production (126), but this decrease is only moderate, because T continues to be produced by adrenal cortex and by the ovarian interstitium. As assessed by biochemical markers, bone resorption increases by 90% at the menopause, whereas bone formation markers increase by only 45% (127). The increase in bone turnover and remodeling imbalance lead to accelerated bone loss, particularly on the endosteal surface of bone. Although the menopause induces rapid bone loss, part of the decrease in BMD that is measured by bone densitometry relates to an increase in the remodeling space induced by the large increase in BMU numbers (128). The rapid bone loss in this phase produces an increased outflow of calcium from bone into the extracellular pool, but hypercalcemia is prevented by compensatory increases in urinary calcium excretion (129) and decreases in intestinal calcium absorption (130), and by a partial suppression of PTH secretion (13). Although bone responsiveness to infused PTH is enhanced during this phase (131), this may reflect only the overall increase in BMU numbers.

As reviewed earlier, it is possible that the early rapid phase of bone loss results from a reduced sensing of biomechanical strain by bone cells induced by acute E deficiency. If this concept is correct, it would rationalize the otherwise difficult to explain observation that the rapid phase of postmenopausal bone loss subsides after 4–8 yr. Thus, when bone mass is reduced to such a level that the mechanostat again senses bone strains as similar to those present before menopause, when E was sufficient, rapid bone loss will cease. Indeed, Heshmati *et al.* (132) found that reducing serum E among postmenopausal women to virtually undetectable levels by administration of an aromatase inhibitor resulted in increased bone resorption and decreased serum PTH, which is evidence that the rapid phase of bone loss had been reactivated. Had the effect of increased E deficiency been on external calcium homeostasis, aromatase treatment would have increased serum PTH further. Also, had the mechanism terminating the rapid phase of bone loss been a high degree of cancellous bone depletion, induction of a more severe degree of E deficiency should not have reactivated the rapid phase of bone loss. Nonetheless, an effect of reduced bone mass *per se* on tapering the rate of bone loss cannot be excluded.

VII. Mechanisms of the Late, Slow Phase of Age-Related Bone Loss in Women

A. Secondary hyperparathyroidism

The late, slow phase of bone loss is associated with progressive increases in levels of serum PTH and in biochemical markers of bone turnover (Fig. 6), and these increases correlate with each other (13). Moreover, when serum PTH levels were suppressed by a 24-h calcium infusion in groups of young premenopausal and elderly postmenopausal women, the increases in biochemical markers in the postmenopausal women that were present on the control day were no longer present on the calcium infusion day, strongly suggesting that the increased serum PTH was the cause of the increase in bone turnover (133). Because the increases in serum PTH are not associated with increases in serum ionic calcium levels or major abnormalities in renal function, they are indicative of secondary hyperparathyroidism caused by age-related abnormalities in extraskeletal calcium homeostasis. Indeed, many studies have shown that age impairs calcium absorption (134) and especially impairs the ability to adapt to a lower calcium intake by increasing intestinal calcium absorption (135). Aging also impairs renal calcium conservation (133, 136). Both abnormalities lead to external calcium wasting. Thus, unless dietary calcium is substantially

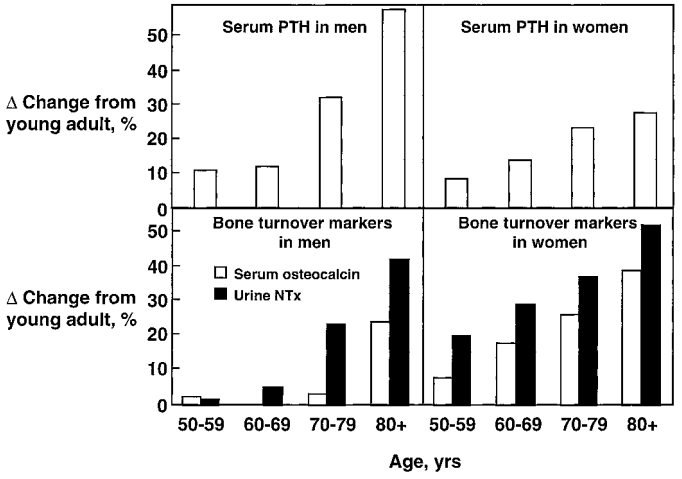

FIG. 6. Changes in serum PTH (*upper panels*) and in bone turnover markers (*lower panels*) as a function of age in men and women over the age of 50. Data are from a population sample from Rochester, Minnesota (17). Results are expressed as the percentage change from young-adult values. Serum osteocalcin is a marker for bone formation, and urine N-telopeptide of type I collagen (NTx) is a marker for bone resorption. For changes in markers of bone turnover, note that increases in women begin at menopause and continue progressively with aging. In men, the increases begin later in life. Note also that the increase in bone resorption exceeds that of bone formation at all ages, indicating a persistent remodeling imbalance. Serum PTH levels increase in both sexes. Although the proportional increase in men over midlife values is greater than in women, absolute values late in life are similar in both sexes. This discrepancy occurs because the change over life in men is parabolic. There are higher values in young adulthood, which decrease in midlife and then increase in old age. In contrast, the increase in serum PTH levels in women begins earlier and increase continuously.

increased to offset these losses, PTH secretion increases to maintain normal levels of serum ionic calcium by resorption of bone that contains 99% of body calcium stores. If the hypothesis that calcium wasting is the cause of the secondary hyperparathyroidism and increased bone resorption associated with aging is correct, these abnormalities should be corrected by calcium supplementation. In fact, many studies have now shown that calcium supplementation in elderly women retards bone loss and, possibly, also reduces fracture occurrence in late, postmenopausal women (12). Moreover, McKane *et al.* (137) demonstrated that a chronically high calcium intake reduced the elevated levels of serum PTH and bone turnover markers in elderly women to within the normal range for premenopausal women (Fig. 7). However, the level of calcium intake (2400 mg/d) in the treatment group of that study was far higher than the average calcium intake among American postmenopausal women of 700 mg/d found in the National Health and Nutrition Examination Survey (NHANES) survey (138).

The serum PTH begins to increase in women about 10–15 yr after the menopause (Fig. 6), which is 5–10 yr after the rapid phase of bone loss has subsided. Thus, there may be a transitional interval before the processes leading to secondary hyperparathyroidism become dominant over the direct effect of E deficiency on bone cell function. Thereafter, serum PTH increases throughout life, a progression that may be due, at least in part, to abnormal parathyroid gland function. Ledger *et al.* (139) conducted formal studies of parathyroid secretory dynamics by sequential infusions of calcium or EDTA. Compared with young adult women, they found that elderly women had greater basal, maximal, and nonsuppressible levels of PTH secretion without alterations in the set-point (Table 2). These abnormalities are similar to those found in patients in early renal failure associated with secondary hyperparathyroidism and parathyroid hyperplasia and are consistent with a histological autopsy study showing a trend to parathyroid hyperplasia in elderly women and men (140).

B. Effects of estrogen deficiency on extraskeletal calcium metabolism

Tradition holds that the slow phase of bone loss in elderly women is caused largely by age-related abnormalities in extraskeletal calcium metabolism and that E deficiency plays either no role or only a minor one. However, in 1998 (13), we proposed that E deficiency is, in fact, the principal cause of both the abnormal extraskeletal calcium metabolism and the

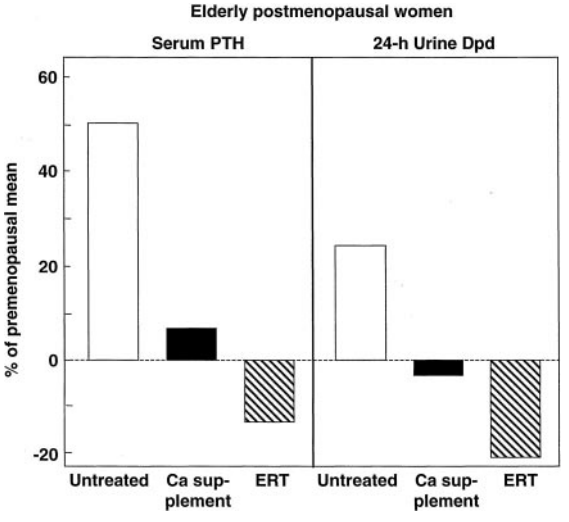

FIG. 7. Levels of serum PTH and bone resorption [assessed by urinary excretion of deoxypyridinoline (Dpd)] are increased ($P < 0.001$ for both variables) in elderly postmenopausal women as compared with premenopausal women. Either a high calcium (Ca) intake (2400 mg/d over 3 yr) or chronic E therapy reduced values to levels that were not significantly different from premenopausal women. Values were reduced to a greater degree after E replacement therapy (ERT) than after Ca supplementation. [Data are from W. R. McKane *et al.*: *J Clin Endocrinol Metab* 81:1699–1703, 1996 (137), and *Proc Assoc Am Physicians* 109:174–180, 1997 (142).]

TABLE 2. PTH secretory dynamics in young and elderly women

Variable	Mean ± SE		*P*
	Young	Elderly	
n	10	10	–
Age, yr	30.3 ± 0.8	73.7 ± 0.6	–
Basal PTH, pM	2.7 ± 0.4	3.8 ± 0.5	<0.05
Set point, mM	1.19 ± 0.01	1.18 ± 0.01	NS
Maximal PTH, pM	12.8 ± 1.0	16.6 ± 1.1	<0.05
Minimal PTH, pM	0.4 ± 0.1	0.8 ± 0.1	<0.001

Adapted from Ledger *et al.* (139).
NS, Not significant.

secondary hyperparathyroidism and, thus, is the ultimate cause of the slow phase of bone loss. The compelling data that supported this hypothesis are found in two studies by our group (125, 142) demonstrating that elderly postmenopausal women receiving long-term E treatment had levels of serum PTH and bone turnover markers that were identical with those of young premenopausal women, whereas the untreated controls had the expected high levels for both variables (Fig. 7).

We have attempted to resolve the apparent paradox of how E deficiency produces opposite types of parathyroid function in the two phases of bone loss (reviewed above) by hypothesizing that there are two types of E action on bone—a direct action on bone cells and an indirect action that is mediated by changes in PTH secretion resulting from E effects on extraskeletal calcium metabolism. E increases intestinal calcium absorption both in experimental animals (143, 144) and in humans (130, 145), acting through intestinal ER (143). E also increases renal calcium conservation (136, 146) by enhancing tubular calcium resorption (146). Thus, the loss of the direct actions of E on the gut and kidney will result in continued calcium wasting. Unless these losses are compensated for by very large increases in dietary calcium intake, they will lead to secondary hyperparathyroidism and will contribute to the slow phase of bone loss.

C. Relationship between the direct and indirect mechanisms of estrogen deficiency on bone and the resultant two phases of bone loss

Although both phases of postmenopausal bone loss are caused by E deficiency, the mechanisms by which the E deficiency produces the bone loss differ. We suggest that this accounts for the different patterns observed in the two phases of postmenopausal bone loss. The major characteristics of the early, rapid phase are that it is self-limiting and induces disproportionate cancellous bone loss. As reviewed earlier, both of these characteristics can be explained by E deficiency resetting the mechanostat. When bone strain is sensed as "normal" by the reset mechanostat, the accelerated bone loss ceases. The remodeling characteristics of this phase of bone loss follow what Frost (90–92) has termed the "disuse mode," which affects mainly bone on endosteal surfaces. Because of its greater proportion of surfaces interfacing with the bone marrow, cancellous bone, rather than cortical bone, is preferentially lost in this mode.

The major characteristics of the late, slow phase are that it continues indefinitely and that there are similar or even greater losses of cortical than of cancellous bone. Because the bone loss is driven by the PTH excess, rather than by the sensing of biomechanical strain by bone cells, it will continue as long as the secondary hyperparathyroidism persists. The action of PTH also determines the remodeling characteristics, and the bone loss is not restricted to the endosteal-marrow interface but affects all bone surfaces. These remodeling characteristics are consistent with those observed in patients with mild primary hyperparathyroidism who maintain cancellous volume and structure but lose cortical bone (147, 148). They are also consistent with the findings that transgenic mice expressing constitutively active PTH receptors in osteoblasts have increased density of cancellous bone but decreased density of cortical bone (149). The relative sparing of cancellous bone may be due to the anabolic action of PTH that is manifested in certain circumstances (150).

D. Effects of decreased bone formation

Although increased bone resorption is the predominant cause of bone loss in postmenopausal women, decreased bone formation also contributes. Because the components of bone turnover are tightly coupled, an increase in bone resorption will not cause substantial bone loss unless the compensatory increase in bone formation is impaired. In both phases of postmenopausal bone loss in women, however, bone resorption at the tissue level is higher than formation, indicating impaired compensation (Refs. 125 and 127 and Fig. 6). Moreover, Lips *et al.* (10) have demonstrated by histomorphometry that late postmenopausal women have decreased wall thickness of trabecular packets, which is strong evidence of decreased bone formation at the cellular level.

These abnormalities generally have been attributed to age-related factors, particularly to decreases in paracrine production of growth factors (151) or to decreases in circulating levels of GH (109, 152) and IGF-I (153–155). However, if E stimulates bone formation, postmenopausal E deficiency could also be a contributing cause. Indeed, impaired bone formation becomes apparent soon after menopause (156). E increases production of IGF-I (157), TGF-β (75), and procollagen synthesis by osteoblastic cells *in vitro* (157) and increases osteoblast lifespan by decreasing osteoblast apoptosis (32, 37). Direct evidence that E can stimulate bone formation after cessation of skeletal growth was provided by Khastgir *et al.* (158), who obtained iliac biopsies for histomorphometry in 22 elderly women (mean age, 65 yr) before and 6 yr after percutaneous administration of high dosages of E. They found a 61% increase in cancellous bone volume and a 12% increase in the wall thickness of trabecular packets. Tobias and Compston (159) have reported similar results. It is unclear whether these results represent only pharmacological effects or are an augmentation of physiological effects of E that are ordinarily not large enough to detect.

Thus, accumulating data implicate E deficiency as a contributing cause of decreased bone formation with aging. Nonetheless, there is not a clear consensus on whether E stimulates osteoblast function, and, if it does, what is the relative contribution of increased proliferation and decreased apoptosis.

VIII. Mechanism of Age-Related Bone Loss in Men

A. Age-related bone loss and osteoporosis in men

Although osteoporosis is often considered to be mainly a disease of women, men lose half as much bone with aging and have one third as many fragility fractures that women do (13). Except in the infrequent older man who develops overt hypogonadism, levels of total serum E and T decrease in men only slightly with aging. Thus, the prevailing opinion has been that sex steroid deficiency is not a major cause of

age-related bone loss in men. However, in the last few years, thinking on this issue has undergone a paradigm shift.

B. Changes in serum sex steroids with age

It is now clear that the failure of earlier studies to find major decreases in serum levels of total sex steroid in aging men was due to their failure to account for the confounding effect of a 2-fold age-related rise in levels of serum SHBG (Ref. 160 and Table 3). Circulating sex steroids that are bound to SHBG have restricted access to target tissues, whereas the 1–3% fraction that is free and the 35–55% fraction that is loosely bound to albumin are readily accessible. Although there is controversy about the reliability of bioavailable (Bio; non-SHBG-bound) sex steroid measurements, they correlate well with the more well accepted measurement of free levels. Several groups have reported substantial decreases in serum levels of free or Bio sex steroid levels with aging (17, 160, 161). Figure 8 and Table 3 show changes with age of serum SHBG, Bio E, and Bio T in 350 women and 350 men of a population-based, age-stratified sample from Rochester, Minnesota (161). In aging men, Bio T and E are decreased substantially due to progressive increases in serum SHBG. The physiological importance of these decreases is reinforced by the reciprocal increases in serum FSH and LH.

C. Mechanisms of the age-related decreases in bioavailable estrogen and testosterone

Although Bio E and Bio T decrease with aging in both sexes (17), the mechanism of the decrease differs: in women, it is caused by menopausal ovarian failure, whereas, in men, it is caused by the progressive age-related increase in serum SHBG. Changes in the regressions of SHBG and Bio sex steroid levels on age are shown in Fig. 8. Although the testis does not fail suddenly, as the ovary does, stimulation studies with clomiphene citrate have established that aging men have a decreased testicular secretory reserve capacity (162). Because T decreases the hepatic production of SHBG, decreased secretion of T with aging will increase levels of serum SHBG. In addition, decreases in circulating levels of Bio E in aging men will negatively feed back on the hypothalamus to reduce GH pulsatile secretion further (163). This then will decrease the production of IGF-I and IGF-binding protein 3 (164) that will increase SHBG synthesis still further (165). The increased serum SHBG binds tightly to serum T, rendering a progressively larger fraction unavailable to tissues. Although the decrease in Bio T increases gonadotropin secretion, the aging testis is unable to respond by increasing serum levels of Bio T and E to within the young adult range. Thus, as shown schematically in Fig. 9, a vicious cycle is initiated that leads to progressive age-related decreases in the Bio levels of both sex steroids in men.

Although aging women also have decreased secretion of GH and decreased levels of serum IGF-I and IGF-binding protein 3, serum SHBG levels do not increase as they do in men. This can be explained by the different actions of the two sex steroids: T decreases SHBG synthesis, whereas E increases it. Thus, the effect of age-related decreases in GH and IGF-I production on stimulating SHBG production is largely offset by postmenopausal decreases in serum E that reduce it. These offsetting effects account for the parabolic relationship of serum SHBG to age in women (Fig. 8).

TABLE 3. Sex-specific changes over life in serum sex steroids and gonadotropins

Hormone	Men (% change)	Women (% change)
Bio E	−47**	−83**
Bio T	−64**	−28*
SHBG	+124**	−1
LH	+285**	+731**
FSH	+505**	+1805**

Adapted from Khosla *et al.* (17).
*, $P < 0.05$, **, $P < 0.005$.

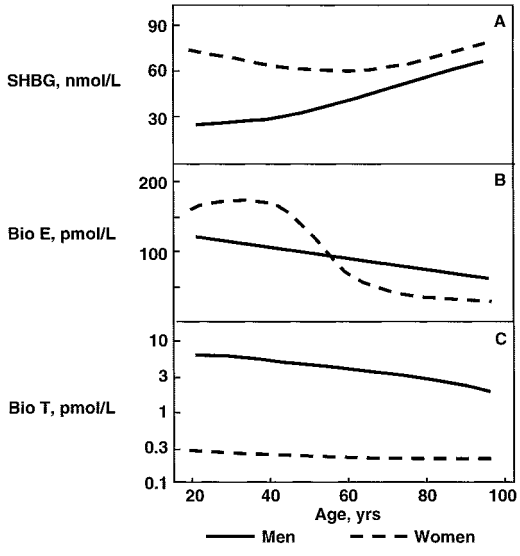

FIG. 8. Patterns of age-related changes in serum values for SHBG (panel A), Bio E (panel B), and Bio T (panel C) among an age-stratified sample of Rochester, Minnesota, men (*solid lines*) and women (*dashed lines*). Note that changes in serum Bio T are plotted logarithmically to accommodate large differences in levels between sexes. [Data are from Khosla *et al.*: *J Clin Endocrinol Metab* 83:2226–2274, 1998 (17).]

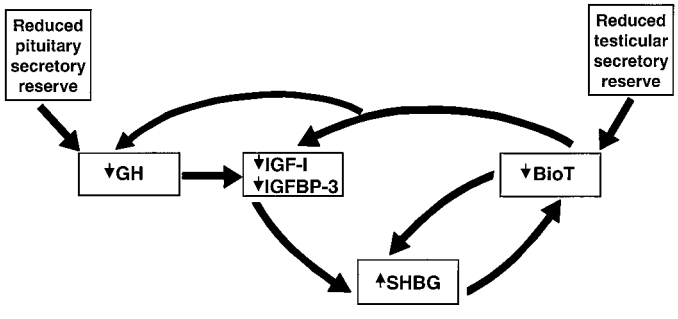

FIG. 9. Model for causation of increases in serum SHBG in aging men. This is a complex interaction driven by a reduced secretory capacity of GH by the pituitary and T by the testes. Because T decreases SHBG synthesis, the progressive decreases in Bio T lead to higher values, which further reduce Bio T. Decreased GH secretion reduces IGF-I production that also reduces SHBG synthesis. This leads to a vicious cycle: as SHBG increases progressively, it will further reduce circulating levels of Bio T.

D. Relative effects of estrogen and testosterone on the male skeleton

The traditional beliefs that bone mass is regulated by androgens in men and by E in women have recently been called into question by three "experiments of nature." Smith *et al.* (111) reported that a 28-yr-old man with homozygous mutations of the ER gene was eunuchoid, had unfused epiphyses, and was severely osteopenic despite normal levels of serum T and elevated levels of serum E. Carani *et al.* (113) and Bilezikian *et al.* (114) each studied a young adult male with homozygous null mutations of the gene for the P-450 enzyme, aromatase, which is required for E synthesis from androgen precursors. Both men had undetectable levels of serum E, elevated levels of serum T, unfused epiphyses, and osteopenia. In both, E treatment fused the epiphyses and increased BMD. Thus, either impaired responsiveness of bone to E or impaired E synthesis leads to osteopenia in young adult men despite T sufficiency. These important case reports fulfill Koch's postulates for a major effect of E on the male skeleton in humans.

In a relevant experimental study in aged male rats, Vanderschueren *et al.* (166) found that both orchiectomy and treatment with an aromatase inhibitor produced comparable decreases in bone density, suggesting that the aromatization of androgens to E was playing a major role in skeletal maintenance. Moreover, targeted deletion of the gene for either ERα (65, 167) or aromatase (72) results in decreased BMD in male mice. In rats, the nonaromatizable androgen DHT decreased biochemical markers of bone turnover and urinary calcium excretion in immature rats, although it is unclear whether these effects were due to its skeletal or extraskeletal actions (169). In an *in vitro* system, E, T, and DHT stimulated osteoblast proliferation. However, the ER antagonist ICI 182,780 blocked the effects of E and T, but not that of DHT (96). Finally, T has been shown to prevent orchiectomy-induced bone loss in ERα-knockout mice (170). One possible interpretation of these data is that aromatization of T to E followed by binding of E to the ER is the preferred pathway for androgen action, but when this is blocked or when a high dosage of an androgen is administered, the AR-mediated pathway is used as a default pathway to modulate bone cell function.

Nonetheless, eight recent, community-based, observational studies (17, 160–162, 171–175) have uniformly demonstrated by multivariate analysis that E, rather than T, was the main predictor of BMD at all sites, except for certain cortical bone sites in the appendicular skeleton. However, because the prevailing BMD of elderly men is the algebraic summation of the amount of bone that is gained during growth and maturation and the amount lost with aging, these correlations could reflect either or both processes. In a population-based cohort, Khosla *et al.* (176) found that the 4-yr rates of loss from the radius and ulna in aging men correlated with Bio E rather than with Bio T, confirming a major role for E deficiency in the bone loss of aging men. Moreover, they found that this inverse correlation occurred only when the baseline level of serum Bio E_2 was below a level of 40 pmol/liter [11 pg/ml; serum total E_2, 114 pmol/liter (31 pg/ml)]. Also, when 50 elderly men were treated for 6 months with raloxifene or placebo, Doran *et al.* (177) found that a baseline serum E_2 level of 96 pmol/liter (26 pg/ml) delineated a nonbeneficial from a beneficial effect of raloxifene therapy: when values were above this level, raloxifene therapy tended to increase biochemical markers for bone resorption, whereas when they were below this level, they tended to decrease them and did so with an inverse relationship to serum Bio E_2 levels. One interpretation of these data is that a serum E_2 level of 96–114 pmol/liter (26–31 pg/ml) represents the threshold level below which the ERs in bone cells are unoccupied by E, leading to functional skeletal E deficiency. Interestingly, population-based studies (160) show that only about half of men aged 70 are below this level, whereas almost all postmenopausal women are. This may explain, in part, why all aging women lose bone but only some aging men do.

Finally, Falahati-Nini *et al.* (43) assessed the relative effects of E and T on bone turnover (and, by inference, on inducing bone loss) by direct intervention. Fifty-nine elderly men (mean age, 68 yr) were made pharmacologically hypogonadal by administration of the GnRH agonist leuprolide and had the conversion of androgens to E blocked by administration of the aromatase inhibitor letrozole. During a 3-wk lead-in, all subjects received replacement dosages of T and E by patch. The sex steroids were then withdrawn and the subjects were randomly assigned to treatment groups of E alone, T alone, both, or neither. Bone turnover markers were assessed before randomization and after 3 wk of treatment. By two-factor ANOVA, E prevented the increase in the bone resorption markers, whereas T had only a small, nonsignificant effect. Based on these data, we inferred that E accounted for at least 70% of the effect of sex steroids on bone resorption and that T accounted for no more than 30% of the effect. An effect of androgens on bone resorption is consistent with the presence of AR in human osteoclasts (63). For bone formation markers, however, serum osteocalcin was maintained by both E and T, whereas serum COOH-terminal type I procollagen peptide was maintained only by E. Because osteocalcin is a late marker of osteoblast differentiation, these data are consistent with the observation *in vitro* by Kousteni *et al.* (70) that both E and T regulate apoptosis in mature osteoblasts via a rapid nongenomic action. Collectively, these results (Fig. 10) strongly suggest that E is the dominant sex steroid regulating bone resorption, but that both E and T may be important in maintaining bone formation. Thus, age-related decreases in serum Bio E may be the major cause of bone loss and osteoporosis in aging men. Finally, Lanyon and Skerry (178) have suggested that the effect of bone strain on maintaining bone mass in men also is mediated by the ER.

E. Direct skeletal effects of testosterone in men

If E, rather than T, is mainly responsible for regulating bone resorption and if both E and T regulate bone formation, how can sexual dimorphism of the skeleton occur? The answer to this question appears to be that osteoblasts in different regions of the skeleton will respond differentially to one or the other sex steroid. Tetracycline-based studies in rats have shown that periosteal bone formation is inhibited by E but is stimulated by T (46, 47). These findings are consistent

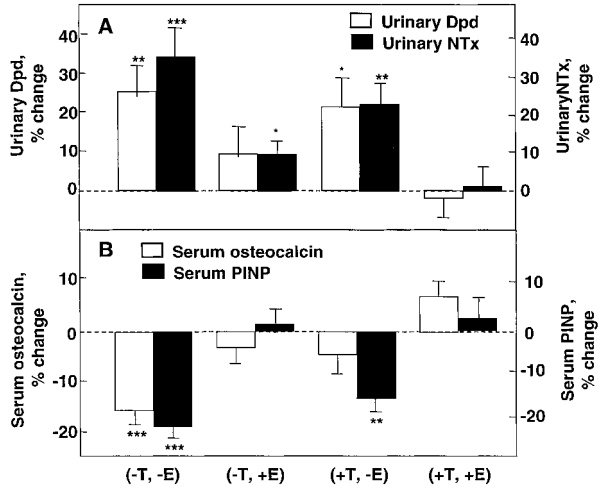

FIG. 10. Experimental testing of the relative importance of E and T in suppressing bone turnover in elderly men. After suppression of sex steroid production by GnRH agonist treatment and blocking conversion of androgens to E with aromatase inhibition, subjects were randomly assigned to groups treated with T, E, both, or neither. Panel A shows the effects of treatment on the resorption markers, urinary deoxypyridinoline (Dpd) and N-telopeptide of type I collagen (NTx). By ANOVA, E, but not T, prevented increases in bone resorption markers. The possibility of a small effect on T on opposing this increase cannot be excluded. Panel B shows the effects on bone formation markers, serum osteocalcin, and N-terminal extension peptide of type I procollagen (PINP). Levels of serum bone alkaline phosphatase did not change (data not shown). Withdrawal of E and T leads to a decrease in markers (indicating that bone formation was being stimulated). For serum osteocalcin, a marker of late osteoblast function, ANOVA showed that T and E were equally effective in reversing decreases, whereas for serum PINP, a marker of all stages of osteoblast function, E, but not T, was effective. For significance of change from baseline: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. [Reprinted with permission from A. Falahati-Nini *et al.*: *J Clin Invest* 106:1553–1560, 2000 (43).]

with the observation that one of the major skeletal differences between sexes is that men have larger appendicular bones and thicker cortical widths. Moreover, it is interesting that the homozygous null mutations of the ERβ gene result in increased skeletal size in female mice but not in male mice (179). Thus, it is also possible that the smaller bone size of the female skeletal phenotype is mediated by the greater concentrations of serum E in females acting through ERβ. In addition to these direct skeletal effects, androgens also increase intestinal calcium absorption (180), although it is unclear whether they enhance renal calcium homeostasis as E does.

IX. Causes of Individual Differences in Skeletal Responsiveness

The patterns of bone gain and bone loss described earlier and the mechanisms that cause them occur in everyone. Yet only about one in two women and one in six men develop fractures due to osteoporosis. Even accounting for the occurrence of falls and trauma that predispose certain individuals to fracture, there remains a wide variability of peak bone mass and of rates of bone loss with aging. Because of the overriding importance of sex steroids in determining and in maintaining bone mass in both sexes, variability in the levels of serum sex steroids, in the responsiveness of bone to a given serum level, or both could contribute to the variability of bone gain during puberty and bone loss with aging.

A. Differences in serum sex steroid levels

The relationship between individual differences in sex steroid levels and rates of bone acquisition during puberty needs further examination. Cadogan *et al.* (104) found that 64% of the variance in total body bone mineral in pubertal girls could be explained by serum E_2 levels and lean body mass. However, Lorentzon *et al.* (181) were unable to relate changes in linear growth to serum E levels in pubertal boys. See the review by Grumbach (110) for more details.

There also is insufficient information about the relationship between individual differences in sex steroid levels and the rate and duration of the accelerated phase of bone loss in the early postmenopause. We have hypothesized that women who develop vertebral or distal forearm fractures during the first 15–20 yr after menopause are those who have experienced disproportionate cancellous bone loss. We have termed this clinical syndrome "type I osteoporosis" and have suggested that it may be caused by E deficiency plus some additional factor(s) that increases the rate or extends the duration of the accelerated, early phase of postmenopausal bone loss (182). This contrasts with type II osteoporosis, which occurs in the entire population of aging women and men, is associated with hip and other fractures later in life, and can be attributed to the effects of the slow phase of bone loss. Women with type I osteoporosis have higher bone turnover and a larger remodeling imbalance (183) but do not have consistently lower levels of serum sex steroids (184) as compared with non-osteoporotic control women. However, these earlier studies could be criticized because the assays for assessing sex steroid levels then available were relatively insensitive. Thus, we have recently (185) reexamined this issue using new ultrasensitive assays in 40 typical type I osteoporotic women with vertebral fractures and in 40 age-matched control women. We found that serum levels of E_2, E_1, and T were indistinguishable between the groups, whereas bone turnover markers were increased by up to 50% in the osteoporotic group. Previous studies (186) have shown that E replacement will normalize bone turnover in these patients. Thus, the data are consistent with the hypothesis that the type I osteoporosis fracture syndrome is mainly the result of increased responsiveness of bone to E deficiency that is evident in the presence of low serum E levels but that is overcome by restoring premenopausal high serum E levels. This is likely to be caused by a genetically determined change such as polymorphism(s) of a gene or genes involved in receptor or post-receptor sex steroid signaling (see next section). Moreover, it is possible that these same polymorphisms also may lead to impaired E enhancement of skeletal growth and maturation, resulting in reduced peak bone mass.

More information is available on the relationship between late postmenopausal bone loss and serum E levels assessed by ultrasensitive assays. In three nested case-control studies from the Study of Osteoporotic Fractures, elderly women with lower levels of serum E and higher levels of serum

SHBG had lower cross-sectional BMD values at the calcaneus, proximal radius, proximal femur, and lumbar spine (187); higher rates of bone loss from the calcaneus and proximal femur (188); and increased risk for vertebral and hip fractures (189) after adjusting for age. We have reanalyzed our population-based data from Rochester, Minnesota. After adjusting for age, we also find a positive correlation between proximal femur BMD and serum Bio E_2 ($r = 0.31$, $P < 0.001$) in untreated elderly women.

Although the correlations between serum E levels and bone loss in late postmenopausal women were significant, they may underestimate the restraining effect on bone loss of extragonadal E synthesis, which is virtually the exclusive source of circulating E levels in women after menopause. Depending on the gradient between circulating concentrations and intracellular concentrations in intracrine cells, local synthesis could play a major role in sex steroid action. Based on the effect on bone turnover markers induced by administration of the potent aromatase inhibitor letrozole to postmenopausal women, Heshmati *et al.* (132, 190) estimated that the remodeling imbalance present in postmenopausal women would be 50% higher except for the presence of aromatase-dependent extragonadal E synthesis.

Moreover, because the process is substrate limited, it is likely that the large age-related decreases in levels of circulating C19 adrenal precursors reduce extragonadal E synthesis and, thus, further enhance bone loss. As shown in Table 1, these decrease by as much as 75% between young adulthood and old age in both sexes. Interestingly, the decline begins in young adulthood and, for the most part, continues throughout life (191). This raises the possibility that part of the bone loss that has been documented to occur in premenopausal women (120, 121) may relate to these decreases. Finally, it is possible that changes in the pattern of serum E metabolism could affect bone loss. After the reversible 17β-HSD-mediated conversion of E_2 to E_1, there is a mutually exclusive C-2 or C-16α hydroxylation. Using the ovariectomized mouse model, Westerlind *et al.* (18) demonstrated that the 2-hydroxyestrone (2-OHE_1) metabolite was inactive, whereas the 16α-OHE_1 metabolite was equipotent with E_2. Indeed, Lim *et al.* (193) reported that women with postmenopausal osteoporosis had a low 16-OHE_1/2-OHE_1 ratio, although the validity of these measurements has been challenged (194).

B. Differences in bone responsiveness to sex steroids

After T or E binds to its respective receptor, the hormone-receptor complex disassociates from heat shock proteins, dimerizes, forms complexes with various coactivator proteins (195), and binds to E or T response elements in DNA directly or by protein-protein bridging to other DNA binding sites. Binding of the various E/ER complexes to DNA activates genes involved in several signaling systems. Genetic polymorphisms could modify any step of this complex pathway, thus affecting the responsiveness of bone cells to E. Also, individual differences in the concentration or ratio of ERα and ERβ in bone cells could alter bone responsiveness to E, but this has not been systematically studied.

Recently, a number of investigators have related various allelic variants in the ERα gene to BMD or to fracture risk. In a multivariate analysis in healthy adolescent boys, Lorentzon *et al.* (181) found that the *Xba*I and *Pvu*II genotypes independently predicted volumetric BMD of the lumbar spine, and that the PP allelic variant predicted statural height. In postmenopausal women, a TA repeat polymorphism was associated with lower BMD (197, 198) and increased risk for osteoporotic fractures (198). Others (199, 200) found that the Px haplotype was associated with significantly lower BMD values in postmenopausal women and, in one study (196), accounted for 16% and 23% of the observed variance in BMD at the lumbar spine and proximal femur, respectively. In a group of 322 early postmenopausal Finnish women followed for 5 yr, Salmen *et al.* (201) found that those expressing the pp variant of the *Pvu*II genotype lost less bone than did those expressing the Pp or PP variants. In a group of Italian postmenopausal women with normal BMD, osteopenia, or osteoporosis, the combination of the PPXX ERα and vitamin D receptor AABBtt genotypes (202) predicted lumbar spine BMD best. Others, however, have been unable to demonstrate a relationship between ERα polymorphisms and BMD (203, 204). Allelic variants of the ERβ gene have thus far not been reported to increase the risk for osteoporosis. Although these associations are intriguing, they are far from conclusive. Moreover, there is, as yet, no direct experimental evidence that polymorphisms of the ERα gene alter phenotype such as has been demonstrated between the polymorphic binding site of the collagen type I A1 gene and altered collagen synthesis *in vitro* (205).

Finally, individual differences in the effect of E deficiency on extraskeletal calcium metabolism could affect osteoporosis risk. This possibility was suggested by Heshmati *et al.* (206), who found that a group of 20 women with postmenopausal osteoporosis had impaired renal tubular reabsorption of calcium as compared with 20 postmenopausal women without osteoporosis. Whether this defect is part of an alteration in the E-signaling system is not known. Variability in the concentration of serum T, the rate of aromatization of T to E, or bone responsiveness to T also could affect the rate of bone loss in aging men. Polymorphisms of the aromatase gene have been related to both female (207) and male (208) osteoporosis.

X. Causes of Bone Loss Other Than Sex Steroid Deficiency

A. Age-related decreases in muscle mass

As a corollary to his mechanostat hypothesis, Frost has suggested in various publications (summarized in Refs. 209 and 210) that the loss of muscle mass with aging is the principal cause of involutional osteoporosis. He cites studies of 1066 persons by three groups that show high correlations ($r = 0.89–0.94$) between lean body mass and total body bone mineral (210). Indeed, in a population sample, Proctor *et al.* (211) found that physical activity declined by 34% and 38% and lean body mass declined by 18% and 17% with aging in women and men, respectively. Moreover, Center *et al.* (171) have shown that a 1 SD decrease in quadricep muscle strength is associated with a 3-fold increase in the risk of fracture.

Nonetheless, there are a number of reasons for believing that the action of sex steroids on bone is of equal, or of even greater, importance to the conservation of bone mass. First, the high correlation between total skeletal muscle mass and total body bone mass is an overestimate because both variables are highly correlated with body size. In our age-stratified, population-based sample of 348 adult men and 351 adult women from Rochester, Minnesota, when both height and body mass index were entered into the multivariate model, total skeletal muscle mass accounted for 18% of the variance in proximal femur BMD in both women and men (211). In the same multivariate model, serum sex steroid levels accounted for 21% and 20% of the variance of proximal femur BMD in women and men, respectively (160). Second, part of the high correlation between muscle and bone mass may be related to changes in age-related factors that affect both correlates, such as decreases in serum T, GH, and IGF-I. Third, E therapy initiated soon after menopause essentially prevents significant bone loss for at least 8 yr after menopause (212), but a regular exercise program has not been demonstrated to do so. Indeed, in a 2-yr intervention study in early postmenopausal women, the rate of change in distal forearm BMD in the group receiving only a formal exercise program was −2.6%, whereas in the group receiving both exercise and E therapy, it was +2.7% (213). Even older postmenopausal osteoporotic women receiving E therapy will gain 12% in lumbar spine BMD over 3 yr (214), whereas exercise programs in older postmenopausal women generally increase BMD by only 1–3% (215). Finally, elite woman distance runners who become amenorrheic develop severe bone loss despite subjecting their skeleton to large biomechanical loads (216). This is consistent with the concept that the major function of the mechanostat is to add bone during growth but that it is less able to add bone to the adult skeleton (217). It should be also noted that T has a direct effect on increasing muscle mass and strength (218). Studies should be made to quantify the independent effects of biomechanical strains and sex steroid action on the maintenance of bone mass and, especially, to determine *in vivo* the interaction between these two positive determinants.

B. Other endocrine abnormalities

Other than changes in serum levels of sex steroids and PTH, the two most important causes of age-related bone loss are abnormalities in the vitamin D-endocrine and in the GH-IGF-I regulatory systems. Reduced serum concentrations of both of the active vitamin D metabolites—25-hydroxyvitamin D [25(OH)D] and 1,25-$(OH)_2$D—occur with aging in both sexes. In several population-based studies, 25(OH)D, an indicator of vitamin D nutrition, decreased by 30–60% (219). Nutritional vitamin D deficiency may contribute to the secondary hyperparathyroidism and bone loss with aging because decreases in serum 25(OH)D correlate inversely with serum PTH levels and directly with BMD (125). However, nutritional vitamin D deficiency is unlikely to be the major cause of secondary hyperparathyroidism in most elderly women because, as mentioned earlier, E replacement normalizes the increase in serum PTH levels (125, 142). Nonetheless, house-bound persons with inadequate exposure to UV radiation and poor nutrition, especially populations who reside in countries with higher latitudes, such as Great Britain and France, and where dairy products are not fortified with vitamin D, may be at risk for vitamin D deficiency bone loss. Chapuy *et al.* (11) found that supplementing the diet of elderly, house-bound French women with 800 U/d of vitamin D and 1000 mg/d of calcium decreased the incidence of hip fracture by 43% over the next 18 months. However, Lips *et al.* (220) could not demonstrate this in a Dutch cohort receiving somewhat smaller supplements. Serum levels of the physiologically active vitamin D metabolite, 1,25-$(OH)_2$D, also decrease with aging, at least relative to the concomitant increases in serum PTH (134). Elderly women infused with PTH had a blunting of the stimulated increases in serum 1,25-$(OH)_2$D levels relative to changes in young adults (221). Thus, reduction in the activity of 25(OH)D 1α-hydroxylase, the renal enzyme that is responsible for the conversion of 25(OH)D to 1,25-$(OH)_2$D, may also contribute to the secondary hyperparathyroidism and increased bone resorption associated with aging.

Aging decreases the amplitude and frequency of GH secretion (152), which leads to decreased hepatic production of IGF-I. Indeed, serum IGF-I levels decrease by 60% with aging in women, and there are also smaller decreases in serum IGF-II levels (153, 154). Thus, decreased systemic and skeletal production of IGF-I may contribute to decreases in bone formation with aging.

Other changes in endocrine function with aging appear to make smaller contributions to bone loss. Among the weak adrenal androgens, levels of serum DHEA and DHEA-SO4 decrease by about 80% (16). Because cortisol secretion remains constant or increases throughout life, the decrease in adrenal androgenic steroids leads to an increase in the catabolic/anabolic ratio of circulating adrenal steroid hormones with aging that could also contribute to bone loss (222, 223).

C. Peak bone mass

Those persons who achieve a higher peak bone mass in young adulthood are less likely to develop osteoporosis as age-related bone loss ensues, whereas those with low levels are clearly at greater risk (105, 111). The relative contribution of peak bone mass and bone loss to the BMD in an elderly woman or man is unclear. Some have estimated, however, that about half of the variance in cancellous BMD and one third of the variance of cortical BMD in women by age 70 is due to bone loss (224–226). In a study of women with vertebral fractures and their daughters, Tabensky *et al.* (227) found that the daughters had half of the deficit in Z-scores that the mothers did, indicating an important contribution of peak bone mass. As reviewed in *Section IX*, differences in serum levels of or responsiveness to sex steroids could contribute to the large variability in peak bone mass. Nonetheless, the combined effects of nonhormonal factors such as heredity, activity, calcium intake, and protein and caloric nutrition are substantial (228).

D. Genetic polymorphisms not affecting sex steroids

The heritable component of peak bone mass has been estimated to be about 50–70% and that for age-related bone

loss is thought to be much less. In addition to genetic polymorphisms involving sex steroids that affect BMD, other allelic variations have been described. These include polymorphic variants for the vitamin D receptor gene, the TGF-β gene, and the Sp1 binding site in the collagen type I A1 gene. In addition, studies in inbred mice have made quantitative trait localizations of additional genes that affect bone density, size, and structure [see the review by Nguyen *et al.* (229) for details].

E. Behavioral and environmental causes

Sporadic factors that affect some, but not other, members of the aging population may contribute to fracture risk in about 40% of men and 20% of women (230). These include use of certain drugs such as corticosteroids; diseases such as malabsorption, anorexia nervosa, and renal hypercalciuria; and behavioral factors such as smoking, alcohol abuse, and inactivity. Some of these sporadic factors, however, may exert their effect on bone by impairing production of sex steroids. For example, smoking increases the catabolism of E (231) and anorexia nervosa (232) or excessive exercise (216) may result in hypothalamic-induced amenorrhea. It is important to recognize that bone loss from these secondary factors is superimposable on that induced by decreases in sex steroid production.

XI. Summation of Mechanisms

We propose that E deficiency is the major cause of both the early, accelerated and the late, slow phases of bone loss in postmenopausal women and contributes substantially to the continuous phase of bone loss in aging men. To this can be added the important roles of E and T in the development of peak bone mass during and after the pubertal growth spurt. Although E deficiency is the primary cause of the two phases of bone loss in women and the single phase in men, the downstream mediators differ, as is shown schematically in Fig. 11. The acute loss of the restraining effect of E on bone turnover and remodeling imbalance at menopause initiates the early, accelerated phase of bone loss. The predominantly cancellous bone loss may be mediated by a resetting of the mechanostat induced by E deficiency, leading to a reduction in bone mass to such a level that bone strain is again sensed as normal. When this level is reached after 4–8 yr, further bone loss from this mechanism ceases. During the accelerated bone loss, the outpouring of calcium from bone into the extracellular fluids is compensated for by decreases in PTH secretion and 1,25-$(OH)_2$D production, leading to excretion of the excess calcium (Fig. 11A).

In the late, slow phase of bone loss in aging women, there is continued cortical bone loss but smaller losses or a maintenance of cancellous bone. This phase also is mainly caused

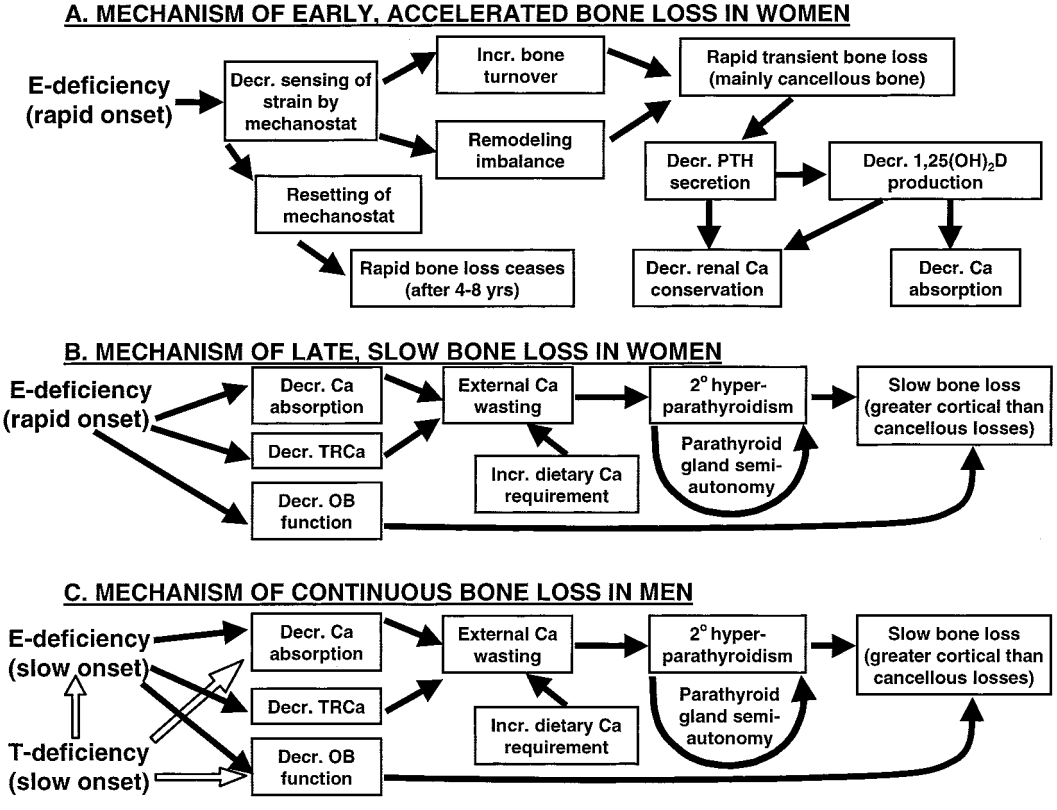

FIG. 11. Flow diagram of the suggested mechanisms for the two phases of bone loss in elderly women and the single phase in elderly men. They are similar in that all three are driven by E deficiency, but the downstream effects differ. In panel A (early postmenopausal women), the loss of the direct inhibitory effects of E on bone leads to increased outflow of bone and inhibition of PTH secretion and 1,25-$(OH)_2$D production. In panel B (late postmenopausal women), the loss of effects of E on external calcium metabolism leads to calcium wasting and to increased PTH secretion that secondarily induces bone loss. In panel C (aging men), the mechanism is the same as in phase B in women except that there are additional effects due to T deficiency (*open arrows*). OB, Osteoblast; TRCa, renal tubular reabsorption of calcium.

by E deficiency, although age effects may also contribute, especially the decrease in bone formation. In contrast to the preceding rapid loss phase, the primary mechanism in this phase is the loss of the extraskeletal actions of E on stimulating intestinal calcium absorption and renal calcium conservation. This leads to external calcium losses that, unless restored by large increases in dietary calcium intake, will lead to compensatory increases in PTH secretion. This resultant secondary hyperparathyroidism is progressive because, after a number of years, the parathyroid gland function becomes semiautonomous (139). The age-related impairment of osteoblast function that prevents compensatory increases in bone formation from fully offsetting increases in bone resorption also is due, at least partially, to E deficiency. Because the slow phase of bone loss is mediated by increased PTH secretion, rather than by a resetting of the mechanostat by E deficiency, it continues indefinitely. That calcium supplementation is ineffective in preventing bone loss in the early, rapid phase (233) but has a substantial effect in the late, slow phase (12) is further evidence for two independent mechanisms. The sequence of changes leading to the slow phase of bone loss is given in Fig. 11B. There is indirect evidence that those women who develop vertebral or distal forearm fractures in the first 15–20 yr after menopause (type I osteoporosis) have an increase in the rate or an extended duration of the early, accelerated phase of bone loss. This could be caused by a genetically determined increased responsiveness to the effects of E deficiency.

In aging men, the continuous phase of bone loss with increases in serum PTH and bone resorption markers has a pattern that is similar to that of the late, slow phase in aging women. Because men do not have the equivalent of menopause, they lack the early, accelerated phase that is induced in women by the precipitous fall in serum estrogens soon after menopause. However, serum levels of both Bio E and Bio T decrease substantially in aging men, although the decrease in Bio E appears to be the dominant cause of their bone loss. A major difference between the mechanisms of bone loss in aging men and that of the slow phase of bone loss in aging women, however, is the added effects of T deficiency, as shown in Fig. 11C. First, and not shown in the figure, young adult men have larger, denser bones because of the added effect of T during the pubertal growth spurt, and thus, men will have more bone late in life than women for a similar rate of loss. Moreover, because larger bones are stronger, men have additional protection against fractures than women who have smaller skeletons. Second, because aromatase can convert T to E_2, T can be considered to be a prohormone for E (20). Thus, a deficiency in T will exacerbate E deficiency by reducing substrate. Third, T clearly enhances bone formation through its antiapoptotic effect on osteoblasts and, possibly, through increased osteoblast proliferation. Moreover, T deficiency decreases the rate of periosteal bone apposition, whereas E deficiency opposes it. Fourth, T has an antiresorptive action, although it is not as great as that of E. Some of the antiresorptive effect of T is probably mediated by enhancing calcium absorption (180). However, because osteoclasts contain functional AR (63), undoubtedly there is also a direct effect.

Thus, sex steroids play key roles in the construction and in the conservation of the adult skeleton, and E deficiency is an important cause of involutional osteoporosis in both sexes.

XII. Epilogue

The view that E plays a central role in the regulation of bone mass in both sexes leads to a number of unanswered questions. Why are sex steroids rather than the primary calcitrophic hormones the major regulators of bone mass? Why does E have major regulatory effects on external calcium homeostasis? Why is E, rather than T, the major regulator of bone mass in men? Why are the onset of puberty and the accelerated bone growth leading to adult bone mass so closely linked? And why is the overall system so complex?

It is often said that biology can be understood only in the context of evolution. We speculate that the major role of E in regulating bone mass evolved from its primary role in supporting reproduction. When a new function is needed, the evolutionary process characteristically adapts an existing mechanism rather than developing a completely new one. The therapsips (mammal-like reptiles) of the Triassic era were the immediate ancestors of mammals and were oviparous. In birds, up to 38% of bone mineral is mobilized to supply calcium for eggshell mineralization (234). Once egg laying is completed, bone mineral of the skeleton is rapidly restored by enhanced utilization of dietary calcium and reduced renal calcium excretion. When mammals became viviparous, it is likely that this earlier system was co-opted to provide calcium for mineralizing the fetal skeleton and for subsequent lactation. Once in place in females, it could also be used to conserve bone mass in males. Indeed, ER appears to have been the first steroid nuclear receptor to evolve in vertebrates and was present long before the AR evolved (235). Moreover, even the role of the sex steroids in inducing and supporting the pubertal growth spurt can be understood in evolutionary terms. The tight coupling between the onset of puberty and the skeletal growth spurt ensures that reproduction cannot occur until there is sufficient skeletal mass to support pregnancy. Finally, because reproductive success is the keystone of natural selection, the surprising complexity of sex steroid regulation of bone mass in mammals can be explained by its evolutionary linkage to ancient reproductive mechanisms.

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Address all correspondence and requests for reprints to: B. Lawrence Riggs, M.D., Mayo Clinic and Mayo Foundation, 200 First Street SW, North 6 Plummer, Rochester, Minnesota 55905.

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