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Androgens and Bone

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Loss of estrogens or androgens increases the rate of bone remodeling by removing restraining effects on osteoblastogenesis and osteoclastogenesis, and also causes a focal imbalance between resorption and formation by prolonging the lifespan of osteoclasts and shortening the lifespan of osteoblasts. Conversely, androgens, as well as estrogens, maintain cancellous bone mass and integrity, regardless of age or sex. Although androgens, via the androgen receptor (AR), and estrogens, via the estrogen receptors (ERs), can exert these effects, their relative contribution remains uncertain. Recent studies suggest that androgen action on cancellous bone depends on (local) aromatization of androgens into estrogens. However, at least in rodents, androgen action on cancellous bone can be directly mediated via AR activation, even in the absence of ERs.

Androgens also increase cortical bone size via stimulation of both longitudinal and radial growth. First, androgens, like estrogens, have a biphasic effect on endochondral bone formation: at the start of puberty, sex steroids stimulate endochondral bone formation, whereas they induce epiphyseal closure at the end of puberty. Androgen action on the growth plate is, however, clearly mediated via aromatization in estrogens and interaction with ERα. Androgens increase radial growth, whereas estrogens decrease periosteal bone formation. This effect of androgens may be important because bone strength in males seems to be determined by relatively higher periosteal bone formation and, therefore, greater bone dimensions, relative to muscle mass at older age. Experiments in mice again suggest that both the AR and ERα pathways are involved in androgen action on radial bone growth. ERβ may mediate growth-limiting effects of estrogens in the female but does not seem to be involved in the regulation of bone size in males.

In conclusion, androgens may protect men against osteoporosis via maintenance of cancellous bone mass and expansion of cortical bone. Such androgen action on bone is mediated by the AR and ERα. (*Endocrine Reviews* 25: 389–425, 2004)

Abbreviations: AF, Activation function; ANDRKO, AR knockout; AR, androgen receptor; ArKO, aromatase knockout; BERKO, ERβ knockout; BMD, bone mineral density; cAIS, complete androgen insensitivity syndrome; DERKO, double ER knockout; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, 5α-dihydrotestosterone; DPA, dual photon absorptiometry; DXA, dual energy x-ray absorptiometry; E_2, estradiol; ER, estrogen receptor; ERKO, ERα knockout; 17β-HSD, 17β-hydroxysteroid dehydrogenase; IGFBP, IGF binding protein; IHH, isolated hypogonadotropic hypogonadism; KS, Klinefelter's syndrome; LBD, ligand-binding domain; OPG, osteoprotegerin; orch, orchidectomized or orchidectomy; ovx, ovariectomized or ovariectomy; PCOS, polycystic ovary syndrome; pQCT, peripheral QCT; QCT, quantitative computed tomography; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; SERM, selective ER modulator; SPA, single photon absorptiometry; T, testosterone; Tfm, testicular feminized male.

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I. Introduction

ANDROGENS INDUCE MALE sexual differentiation before birth and sexual maturation during puberty; in adult men, they maintain the function of the male genital

system, including spermatogenesis. However, the role of androgens in other target organs—including muscle tissue; the cardiovascular, central nervous, and immune systems; and bone—is less well-established (1).

In the early 1940s, Albright and Reifenstein were among the first to refer to the antiosteoporotic and anabolic properties of androgens (2). From a public health perspective, osteoporosis is a greater problem in women than in men (3). This explains why most research efforts to explore skeletal effects of sex steroids have been devoted to estrogens. Moreover, androgens may be converted into estrogens via the P450 aromatase enzyme complex and may therefore act as prohormones for estrogens. In this respect, there is increasing evidence that at least part of the effects of androgens in men can be explained by their aromatization into estrogens (4, 5). Epiphyseal closure at the end of puberty, for example, is now generally accepted to be estrogen-dependent in both genders (6). In recent years, a specific role of androgens in skeletal homeostasis has even been questioned, although androgen receptors (ARs) in bone cells and AR-mediated actions on bone have been documented for more than a decade (7, 8).

The aim of this review is to address the question whether and how (through which receptors and/or pathways) androgens may affect bone strength and provide protection against osteoporosis. The clinical relevance of this question results from the recognition that, even in men, fractures due to skeletal fragility represent a huge public health problem.

Elderly men maintain cancellous bone integrity in comparison with postmenopausal women, although their bone trabeculae become thinner. From a biomechanical perspective, compromised bone strength in men is the result of gender-related differences in the loss of bone mass during aging and, even more importantly, in the accumulation of bone mass during childhood and adolescence (9) (Table 1). During puberty, men develop a bigger bone size than women, due to increased periosteal apposition. In females, on the other hand, estrogens have an inhibitory effect on periosteal bone formation, whereas endocortical apposition is stimulated, narrowing the medullary cavity. Estrogens also stimulate epiphyseal closure earlier in women, resulting in longer bones in men. After puberty, the amount of bone formed on the periosteal surface is still greater in men (10, 11), whereas endocortical bone resorption is similar in both sexes, so that net bone loss is less in men (Table 1). The end result is a skeletal sexual dimorphism, characterized by a greater bone length, larger outer and inner bone perimeters, and a larger cortical volume in men compared with women. Therefore, adult men have greater bone mass than women, but this is due to a greater bone volume and not to a greater volumetric density. The greater areal bone mineral density (BMD) in males is thus only an artifact of the dual energy x-ray absorptiometry (DXA) software by expression of bone mass as projected areal (grams per square centimeter) instead of true or volumetric density (grams per cubic centimeter). In the current review, we will focus on the potential mechanisms through which androgens may prevent bone loss, increase bone mass (size), and improve bone strength.

TABLE 1. Sexually dimorphic age-related changes in humans

	Men	Women
Puberty		
Epiphyseal closure		Earlier
Endocortical bone apposition		↑
Periosteal bone formation	↑	
After puberty		
Trabecular perforation		↑
Trabecular thinning	↑	
Periosteal bone formation	↑	

↑, Increased *vs.* the opposite sex.

In this manuscript, *in vitro*, experimental animal data and clinical human data with respect to skeletal androgen action will be reviewed in an attempt to define the possible impact of androgen action [through the estrogen receptor (ER) and AR pathways] on different aspects of skeletal homeostasis during growth and aging. Recent clinical and experimental data have indeed provided evidence that at least some of the skeletal androgen actions are not solely ER-dependent. Furthermore, we will discuss potential indirect nonbone cell-mediated effects of androgens on the skeleton.

Finally, the potential clinical benefits of androgen replacement in the context of male hypogonadism as well as in different patient groups will be discussed. Recent studies have explored different modes of action of androgens via the AR and ER pathways and may ultimately contribute to the potential use of selective AR or ER modulators in selected male target populations.

II. General Aspects of Androgen Action

A. Androgen metabolism

Androgens are C-19 steroids secreted primarily from the testes and the adrenals. The synthesis and metabolism of sex steroids have been extensively reviewed in several recent publications (4, 12). Therefore, we will just briefly mention the major pathways to facilitate the interpretation of experimental animal and clinical studies described in this review. The major gonadal androgen in males is testosterone (T), which is bound in the circulation to albumin and SHBG. It can be irreversibly converted in peripheral tissues by the enzyme 5α-reductase to the more potent 5α-dihydrotestosterone (DHT). Both DHT and T can activate the AR (Fig. 1). T can also be converted to estradiol (E_2) by an enzyme complex known as estrogen synthetase or aromatase followed by activation of the ERs. The adrenal cortex secretes large amounts of C-19 androgens including dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-S), and androstenedione. These C-19 androgens can be metabolized either directly or indirectly in a rather complex pathway to estrone by the aromatase enzyme or to T by steroid sulfatase, 17β-hydroxysteroid dehydrogenase (17β-HSD) and/or 3β-HSD (Fig. 1). Thus, depending on the relative activity of aromatase, 5α-reductase, 17β-HSD, 3β-HSD, and steroid sulfatase (13), T and C-19 androgens may predominantly activate either the AR or the ERs (Fig. 1). Several recent publications have demonstrated that aromatase (14–25), 5α-reductase (14, 17, 18, 22, 26–30), 17β-HSD (14–17, 19, 23, 27, 31), 3β-HSD (14, 32), and steroid sulfatase (13–15, 17, 19) are expressed in bone tissue, supporting the notion that local metabolism of androgens in bone tissue might be of physiological importance.

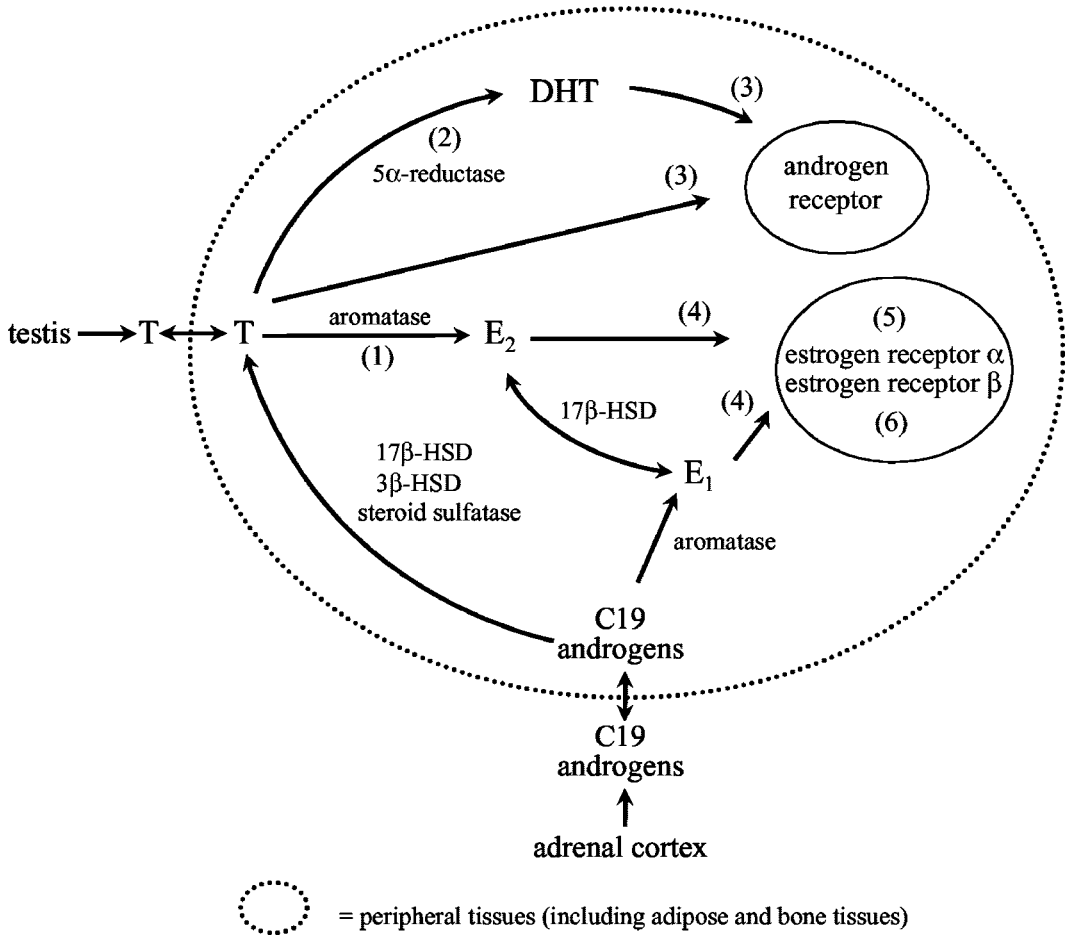


FIG. 1. Simplified overview of the metabolism and action of sex steroids in men. 3β-HSD, 3β-Hydroxysteroid dehydrogenase. The sites of action of the different specific inhibitors of androgen and estrogen action, which are discussed in the present review, are indicated as follows: 1) aromatase inhibitor or aromatase-inactivated mice; 2) 5α-reductase inhibitor; 3) AR antagonist or AR-inactivated rodents; 4) ER antagonist; 5) ER α-inactivated mice; and 6) ERβ-inactivated mice.

Thus, sex steroids are partly synthesized locally in peripheral tissues, providing individual target tissues with the means to adjust formation and metabolism of sex steroids to their local requirements (Fig. 1). To elucidate the relative importance of androgen metabolism and action in bone, a variety of specific inhibitors/antagonists have been extensively used, including 5α-reductase inhibition (33–35), AR antagonists (36–40), AR inactivation in rats (41) and mice (42–46), aromatase inhibitors (47–49), aromatase gene inactivation [in humans (50–53) and mice (54–56)], ER antagonists (36, 40, 57–60), ERα gene inactivation [in humans (61) and mice (46, 62–67, 69–74)], and ERβ inactivation in mice (64–67, 73, 75–81) (Fig. 1). A large part of this review will describe and discuss the results of these different approaches to improving our understanding of the mechanism of action of androgens in bone tissue.

B. Mechanism of action of androgens

In this section, we will discuss the relative importance of the AR, ERα, and ERβ in mediating the effect of androgens on bone. The AR was cloned in 1988 (82, 83). ERα was cloned in 1986 (84, 85) and a second ER, ERβ, in 1995 (86). All of these receptors belong to the nuclear receptor family. They are all composed of three independent but interacting functional domains; the NH_2-terminal or A/B domain, the C or DNA-binding domain, and the D/E/F or ligand-binding domain (LBD) (87) (Fig. 2). The sex steroid receptors are DNA-binding proteins that have the capacity to interact with specific DNA sequences, the androgen response element for the AR, and the estrogen response element for the ERs. The sequence homology in the DNA-binding domain is high among the sex steroid receptors (87). The N-terminal domain of these receptors encodes a ligand-independent activation function (AF-1), a region of the receptor involved in protein-protein interactions, and transcriptional activation of target gene expression (87) (Fig. 2). The COOH-terminal region, or LBD, mediates ligand binding, receptor dimerization, nuclear translocation, and transcription of target gene expression (87) (Fig. 2). The classical mechanism of sex steroid action involves interaction with intracellular receptors, which are either cytoplasmic or nuclear. Binding of the sex steroids to their respective receptors leads to conformational changes of the protein that allow it to interact with the transcriptional machinery: directly or indirectly via protein-protein interactions with different transcription factors (88). The transcriptional activity of androgen-bound AR and estrogen-bound ERs is affected by tissue-specific coregulators,

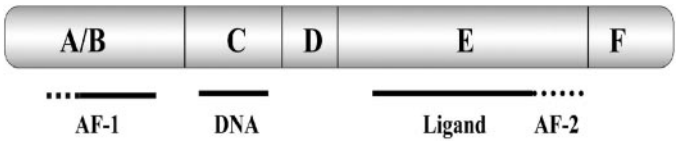


FIG. 2. Diagramatic representation of the domain structure of nuclear receptors. The A/B domain at the NH_2 terminus contains the AF-1 site where other transcription factors interact. The C/D domain contains the two-zinc finger structure that binds to DNA, and the E/F domain contains the ligand binding pocket as well as the AF-2 domain that directly contacts coactivator peptides. [Reproduced with permission from S. Nilsson *et al.*: *Physiol Rev* 81:1535–1565, 2001 (87).]

including factors enhancing transactivation (coactivators) and factors reducing transactivation (corepressors) (87, 89).

C. Nongenomic effects of sex steroids

It was initially thought that the only mechanism for androgens/estrogens to affect transcription was by direct binding of activated AR to androgen response element or ERs to estrogen response element. But transcription can also be affected by protein-protein interactions with, for instance, the specificity protein-1, activation protein-1, and nuclear factor κB proteins (87). Furthermore, a variety of cell types respond to estrogens rapidly (within seconds/minutes), making a classical genomic mechanism of action unlikely (90). The importance of nongenomic mechanisms, in which the ligand interacts with plasma membrane/cytosolic receptors, is increasingly recognized to mediate the rapid responses to sex steroids (8, 91, 92). Nongenomic rapid effects of estrogens *in vitro* have been described for both osteoblasts and osteoclasts (8, 88, 91, 93). Furthermore, a plasma membrane ER is reported to partly mediate a nongenomic apoptotic effect of estrogens on osteoclasts (94).

The group of Manolagas (88) has demonstrated that the antiapoptotic effect of estrogens and androgens on osteoblasts *in vitro* is mediated by Src/Shc/ERK signaling via a nongenomic action of the classical receptors and is sex nonspecific. This action is mediated by the LBD and is eliminated by nuclear targeting of the receptor protein (Fig. 3A). More recently, the same research group presented *in vitro* as well as *in vivo* data suggesting that the nongenomic effect of sex steroids involves kinase-mediated regulation of common transcription factors (95). Thus, nongenomic effects of sex steroids alter the activity of Elk-1, CCAAT enhancer binding protein-β (C/EBPβ), and cAMP-response element binding protein, or c-Jun/c-Fos by an extranuclear action of the ER or AR, resulting in activation of the Src/Shc/ERK pathway or down-regulation of c-Jun N-terminal kinase, respectively (95). Interestingly, a synthetic ligand, estren, which reproduces the nongenomic effects of sex steroids without affecting classical transcription, increases BMD in both ovariectomized (ovx) females and orchidectomized (orch) male mice, without affecting reproductive organs (uterus and seminal vesicles) (Fig. 3B). Such ligands merit investigation as potential therapeutic alternatives to hormone replacement for osteoporosis, in both women and men (88, 95, 96).

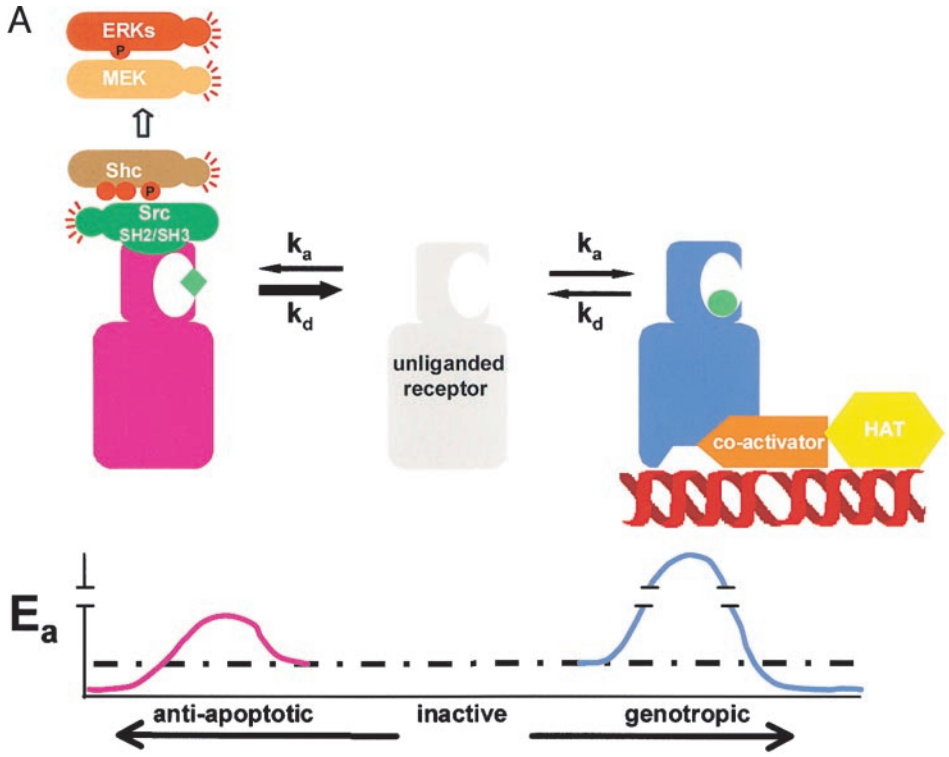
Based on *in vitro* studies, Manolagas and co-workers (88, 97) have proposed that ERα, ERβ, and the AR can transmit the antiapoptotic effect of sex steroids with similar efficiency, irrespective of whether the ligand is an estrogen or an androgen. In contrast, however, several *in vivo* studies of transgenic mouse models do not support the notion that estrogens have important AR-mediated physiological effects on cancellous BMD or the concept that nonaromatizable androgens exert bone-sparing effects on cancellous BMD through direct activation of the ERs (42, 45, 66, 67, 70, 98) (see also *Sections IV.E* and *IV.F*). Although the hypothesis by Manolagas *et al.* that the bone-sparing effect of sex steroids is mediated via nongenomic mechanisms is extremely interesting and provocative, additional investigation and confirmation by others are required before it can be fully accepted (99).

D. Expression of androgen and estrogen receptors in the skeleton

It is generally believed that an important part of the effect of androgens or their metabolites on the skeleton is exerted via a direct stimulation of the AR, ERα, and/or ERβ expressed locally in the skeleton. In this section, we will summarize *in vitro* and *in vivo* studies investigating the expression of these three receptors by growth plate chondrocytes, osteoblasts, osteocytes, osteoclasts, and/or by other bone-related cells.

1. Growth plate cartilage. Androgens exert important effects on pubertal growth, and a local effect on the growth plate is supported by the fact that both cultured epiphyseal chondrocytes (100) and growth plate cartilage cells *in vivo* express AR (101–105) as detected by immunohistochemistry (101–105), binding studies (100), and *in situ* hybridization (105) (Table 2). The AR has been detected in all layers of the human growth plate at different ages (101–104), whereas in the rat it was expressed in proliferative and early hypertrophic chondrocytes at sexual maturation and only in prehypertrophic chondrocytes in older rats (105). Male rats displayed a higher AR expression in the growth plate and metaphyseal bone than female rats during sexual maturation (105). In contrast, no major sex difference regarding AR expression has been observed in human growth plate chondrocytes (100–102).

Several studies have detected ERα (102, 104, 106–112) and ERβ (104, 106, 110, 113) protein in the human, rabbit, and rat growth plate by using immunohistochemistry (Table 2), and the results regarding ERα have been confirmed by *in situ* hybridization (107). Most studies have detected ERα expression in all layers of the human and rabbit growth plate during both fetal stage and puberty (102, 104, 108, 110). In contrast, Kennedy *et al.* (111) detected ERα expression in the growth plate of immature but not mature rats. In addition, it is clear that the ERβ protein is expressed in the growth plate, but the expression pattern varies between studies. The first study by Nilsson *et al.* (113) localized ERβ mainly to the hypertrophic chondrocytes, whereas later studies have detected it in all layers of the growth plate (104, 106, 110) (Table 2). In conclusion, AR, ERα, and ERβ are all expressed in the growth plate, indicating that androgens, either directly or after aromatization, might influence the pubertal growth spurt and growth plate closure via a direct interaction with local sex steroid receptors. Future experiments, using growth plate-specific inactivation of the different sex steroid receptors, are



B

	BMD		Uterine Weight	Seminal vesicles Weight
	Males	Females		
Estrogen	I	I	I	NC
DHT	I	I	NC	I
Estren	I	I	NC	NC

FIG. 3. Nongenomic effects on BMD of the synthetic ligand estren. A, Model for ligand-induced dissociation of antiapoptotic from classical genomic activity of sex steroid receptors. The *three diagrams* depict conformational states of the receptor protein before and after interaction with the ligands, which are required to effect either the genomic (genotropic) or the antiapoptotic responses. The inactive unliganded receptor is depicted in the *middle* in *gray*. The change in conformation induced by interaction with a ligand that preferentially triggers transcriptional activity is depicted in the *right* in *blue*. The change in conformation induced by interaction with a ligand that preferentially triggers the antiapoptotic activity of the receptor (*e.g.*, the estren) is depicted in the *left* in *magenta*. The *green circle* and *green diamond* represent the two ligands; please note the perfect and imperfect fit within the binding pocket, respectively. Ligands such as E_2 will of course induce both conformations. Although in the antiapoptotic model we show direct contact between the receptor and Src, it is possible that adaptor protein(s) may bridge the interaction between the two molecules. Corresponding activation energy (E_a) of the receptor protein in the unliganded state (*broken line*), progressing to either the genotropic conformation (*blue line*) or the antiapoptotic conformation (*magenta line*), is shown at the *bottom*. B, Summary of the recent results presented by Kousteni *et al.* (96) and earlier studies (66, 70) regarding the effects of estrogens (E_2), androgens (DHT), and the nongenomic acting synthetic ligand estren in gonadectomized mice. Estren reproduces the nongenomic effects of sex steroids without affecting classical transcription, and it increases BMD without affecting the reproductive organs. Thus, these results indicate that its effects on BMD are exerted via nongenomic effects, whereas the reproductive effects require genomic effects. I, Increase; NC, no change. [Panel A is reproduced with permission from S. Kousteni *et al.*: *Cell* 104:719–730, 2001 (88) with permission from Elsevier Science.]

needed to investigate whether these locally expressed receptors are of functional importance for the regulation of longitudinal bone growth.

2. Osteoblasts/osteocytes. Despite the obvious importance of androgens and estrogens in the regulation of adult bone metabolism, it has been difficult to detect AR and ERs in osteoblasts. Therefore, osteoblasts were for a long time not generally considered as primary target cells for sex steroids. The development of new and more sensitive techniques has resulted in the detection of the AR as well as ERα and ERβ expression by osteoblasts and osteocytes. The expression of the AR in cultured osteoblasts was first described in 1989 by Colvard *et al.* (114) using a nuclear binding assay. Several *in vivo* and *in vitro* studies have confirmed that the AR mRNA and protein are expressed by osteoblasts and osteocytes (14, 18, 101, 103, 105, 114–122) (Table 3). AR binding has been demonstrated *in vitro* in osteosarcoma cell lines, osteoblast-like cell lines, and primary osteoblasts from several different species including human, rat, and mouse (18, 115, 118–120, 123–126)

TABLE 2. Expression of ARs and ERs in growth plate cartilage

Receptor	Species	*In vivo*/*in vitro*	Protein/mRNA	Technique	Characteristics	Refs.
AR	Human	*In vivo*	Protein	IH	Pubertal, predominantly hypertr.	101
	Human	*In vivo*	Protein	IH	Fetal, all layers	102
	Human	*In vivo*	Protein	IH	Pubertal, all layers	104
	Human	*In vivo*	Protein	IH	Infant, rim between prolif. and hypertr.	103
	Human	*In vitro*	Protein	Binding	Fetal, cultured chondrocytes	100
	Rat	*In vivo*	Protein/mRNA	IH/ISH	Sexual maturation, prolif. and early hypert. Older, prehypertr.	105
ER	Rabbit	*In vitro*	Protein	Binding	Articular chondrocytes	399
	Rat	*In vivo*	Protein	Binding	Growth plate cartilage	112
ERα	Human	*In vivo*	Protein	IH	Neonatal, prolif. and prehypertr.	106
	Human	*In vivo*	Protein	IH	Fetal, all layers	102
	Human	*In vivo*	Protein	IH	Pubertal, all layers	104
	Human	*In vivo*	Protein	IH	Fetal, all layers	108
	Human	*In vivo*	Protein/mRNA	IH/ISH	Pubertal children	107
	Pig	*In vivo*	Protein	IH	Cartilage above the growth plate	109
	Rabbit	*In vivo*	Protein	IH	Several ages, all layers	110
	Rabbit	*In vivo*	mRNA	ISH	Growing rabbits	107
	Rabbit	*In vivo*	Protein	IH	Sexual maturation, prolif. and early hypertr.	111
	Rat	*In vivo*	Protein	IH	Growth plate cartilage	112
	Rat	*In vivo*	Protein	IH	All layers	110
	Rat	*In vivo*	Protein	IH	In immature but not mature rats, prolif. and hypertr.	111
ERβ	Human	*In vivo*	Protein	IH	Pubertal, hypertr.	113
	Human	*In vivo*	Protein	IH	Neonatal, prolif. and prehypertr.	106
	Human	*In vivo*	Protein	IH	Pubertal, all layers	104
	Rabbit	*In vivo*	Protein	IH	Several ages, all layers	110
	Rat	*In vivo*	Protein	IH	Several ages, all layers	110

ER, estrogen receptor binding and not possible to distinguish between ERα and ERβ; IH, immunohistochemistry; Binding, binding studies; ISH, *in situ* hybridization; prolif., proliferative layer of growth plate; hypertr., hypertrophic layer of growth plate.

(Table 3). The number of binding sites per cell appears to vary greatly from 70 to 14,000 binding sites per cell (127), depending on the assay technique, but it is in a range seen in other androgen target tissues. Human osteoblastic cells, isolated from cortical bone, expressed higher AR mRNA levels and AR binding than cells isolated from cancellous bone, whereas no major differences in AR expression in osteoblasts derived from males compared with osteoblasts derived from females were found (118). Most studies (116, 117, 119, 125), but not all (118, 128), indicate that androgen up-regulates the expression of its own receptor in osteoblasts.

ER expression in bone cells was first reported in 1988 when specific binding sites for estrogens were identified in nuclear extracts from rat and human osteoblasts (129–131) (Table 3). The number of estrogen binding sites in different studies varies between 60 and 4,500 binding sites per osteoblast, which is lower than in estrogen-responsive reproductive cells such as uterine and breast cells (132). Estrogens regulate osteoblast proliferation and expression of genes encoding enzymes, bone matrix proteins, hormone receptors, transcription factors, as well as growth factors and cytokines (133). However, conflicting results have been presented regarding the *in vitro* effects of estrogens on proliferation and differentiation of osteoblasts, which might be due to differences in the number of receptors per cell, animal species, skeletal localization of the osteoblasts, the stage of osteoblast differentiation, and the relative concentration of ERα *vs.* ERβ in the osteoblasts (132, 133). It is now well-established that both ERα and ERβ are expressed by osteoblasts and osteocytes, as studied both *in vivo* and *in vitro*; in several different species including human, rat, mouse, pig, and guinea pig; and using several different techniques including Western blot, Northern blot, RNase protection assay, PCR, *in situ* hybridization, and immunohistochemistry (Table 3). Some studies indicate that ERα expression increases with the increasing stage of differentiation of cultured osteoblasts (132, 134–136). ERβ mRNA levels have been shown to either increase (135) or remain constant (134) with advancing cellular development. Thus, the ratio of ERα to ERβ and the estrogenic response may vary as the cells progress from preosteoblasts to mature osteoblasts (132). In a recent paper, the relative expression of ERα, ERβ, and the AR was followed simultaneously during differentiation of cultured osteoblasts (121), demonstrating that ERα levels were elevated during matrix maturation and then declined during mineralization. ERβ expression was relatively constant throughout differentiation, whereas AR levels were lowest during proliferation and then increased throughout differentiation, with highest levels in the most mature mineralizing cultures (121). One study in human developing bone demonstrated that ERα immunoreactivity is strong in osteoblasts adjacent to the periosteal surface of the cortical bone, whereas ERβ immunoreactivity is dominant in osteoblasts in cancellous bone (106). Taken together, the AR and both ERα and ERβ are expressed by osteoblasts, but there is no consensus about their relative expression during differentiation and their localization within the skeleton.

TABLE 3. Expression of ARs and ERs in osteoblasts

Receptor	Species	*In vivo*/*in vitro*	Protein/mRNA	Technique	Characteristics	Refs.
AR	Human	*In vivo*	Protein	IH	Osteoblasts/osteocytes	101, 103
	Human	*In vitro*	Protein/mRNA	Binding/Northern/PCR	Osteoblast-like cells	14, 114, 118, 124, 125
	Human	*In vitro*	Protein/mRNA	Binding/Western/RPA	Osteoblastic cell line	117, 119, 123
	Human	*In vitro*	Protein/mRNA	Binding/Northern/IH/RPA	OS	115, 116, 120, 125
	Rat	*In vivo*	Protein/mRNA	Western/IH/ISH/PCR	Osteoblasts/osteocytes	105, 121
	Rat	*In vitro*	Protein	Binding	Osteoblastic cell line	123
	Mouse	*In vitro*	Protein/mRNA	Western/IH/Binding/Northern/PCR	Osteoblastic cell line	18, 122, 126
ER	Human	*In vivo*	Protein	Binding	Osteoblasts, osteoblast-like cells, OS	114, 124, 129–131, 400–402
	Rat	*In vitro*	Protein	Binding	Osteoblast-like cells, OS	130, 403
	Mouse	*In vitro*	Protein	Binding	Osteoblastic cell line, OS	126, 159, 404
ERα	Human	*In vivo*	Protein/mRNA	IH, RT-PCR, *in situ* PCR	Osteoblasts/osteocytes	106, 109, 131, 144, 405
	Human	*In vivo*	Protein/mRNA	IH/ISH	Osteoblasts, not osteocytes	107
	Human	*In vitro*	Protein/mRNA	Enzyme immunoassay/Northern/PCR	Osteoblast-like cells	14, 114, 129, 406
	Human	*In vitro*	mRNA	PCR	Osteoblastic cell line	135
	Human	*In vitro*	Protein/mRNA	Western/IH/Northern	OS	130, 407–409
	Pig	*In vivo*	Protein	IH	Mainly in osteocytes	109
	Guinea pig	*In vivo*	Protein	IH	Mainly in osteocytes	109
	Rabbit	*In vivo*	mRNA	ISH	Osteoblasts, not osteocytes	107
	Rat	*In vivo*	Protein/mRNA	IH/PCR/RT-PCR	Osteoblasts/osteocytes	134, 157, 158
	Rat	*In vitro*	Protein/mRNA	Western/Northern/PCR	Osteoblast-like cells	121, 136, 158, 403
	Rat	*In vitro*	Protein	IH	Osteoblastic cell line	122
	Rat	*In vitro*	mRNA	Northern/PCR	OS	130, 134, 410
	Mouse	*In vitro*	Protein/mRNA	IH/PCR	Osteoblastic cell line	122
	Mouse	*In vitro*	mRNA	Northern	OS	404
ERβ	Human	*In vivo*	Protein/mRNA	IH/Western/RPA	Osteoblasts/osteocytes	106, 150, 151
	Human	*In vitro*	mRNA	PCR	Osteoblastic cell line	135
	Human	*In vitro*	Protein/mRNA	Western/RPA	Osteoblast-like cells	150
	Human	*In vitro*	Protein/mRNA	Western/RPA	OS	150
	Rat	*In vivo*	mRNA	PCR/RT-PCR	Bone tissue	134, 157
	Rat	*In vivo*	Protein/mRNA	IH/PCR/ISH	Osteoblasts	153, 158
	Rat	*In vitro*	Protein/mRNA	Western/PCR	Osteoblast-like cells	121, 134, 158
	Rat	*In vitro*	Protein	IH	Osteoblastic cell line	122
	Rat	*In vitro*	Protein/mRNA	Western/PCR	OS	134, 150
	Mouse	*In vivo*	Protein/mRNA	IH	Osteoblasts/osteocytes	150
	Mouse	*In vitro*	Protein/mRNA	IH/PCR	Osteoblastic cell line	122

ER, Estrogen receptor binding and not possible to distinguish between ERα and ERβ; IH, immunohistochemistry; Binding, binding studies; ISH, *in situ* hybridization; RT-PCR, quantitative PCR; Western, Western blot; Northern, Northern blot; RPA, RNase protection assay; OS, osteosarcoma cell line.

3. Osteoclasts. AR expression has been detected in avian (137) and mouse (138) osteoclasts *in vitro* and in rat osteoclasts *in vivo* (17), whereas no expression has been detected in human osteoclasts *in vivo* (101, 103) (Table 4). Thus, the available data are contradictory regarding AR expression in osteoclasts, and it is generally believed that the major part of the effect of androgens on osteoclastogenesis and bone resorption is mediated by cells of the osteoblast lineage (139). However, some recent *in vitro* studies demonstrate that androgens can act directly on osteoclasts to promote their apoptosis (88, 96, 140). Furthermore, in bone marrow cell preparations, sex steroids have identical effects on osteoclastogenesis in the presence or absence of cells of the osteoblastic lineage (140). An effect of estrogens, produced by aromatization of androgens, via ERs localized in osteoclasts is possible because most (106, 141–151), but not all (107, 152, 153), studies have identified ERα and ERβ in osteoclasts (Table 4). In two separate studies, preosteoclasts, but not mature osteoclasts, were found to express ERα (154, 155). Taken together, the conflicting results regarding ER expression in osteoclasts suggest that osteoclasts express a low number of ERs, which may be close to the detection limit of the different assays used. This would be consistent with the fact that most but not all studies have detected ERs on osteoclasts. Although the expression and the physiological role of sex steroid receptors in osteoclasts remain controversial, the available evidence suggests that the inhibitory effect of estrogens on osteoclastogenesis is largely mediated indirectly by cells of the osteoblast lineage, and not via a direct interaction with ERs on osteoclasts.

4. Other bone-related cells. It is generally believed that osteoblasts originate from pluripotent mesenchymal stem cells in the bone marrow. Several studies have demonstrated that bone marrow stromal cells express the AR (122, 156) as well as both ERα (122, 154, 157–160) and ERβ (122, 157, 158) (Table 5). Furthermore, both AR and ERs have been detected on megakaryocytes (151, 156) and endothelial cells (101, 151, 156) within the bone compartment. Thus, besides growth plate chondrocytes and osteoblasts, it is clear that several other types of cells within the skeleton express sex steroid receptors, which may be involved in mediating the effect of androgens on the skeleton.

III. Effects of Androgens *in Vitro* on Skeletal Cells

The effect of androgens on skeletal growth and on adult bone metabolism is exerted via direct effects on the different types of cells located within the bone compartment. Indirect effects via muscle or vascular cells may also be operative.

TABLE 4. Expression of ARs and ERs in osteoclasts

Receptor	Species	*In vivo/in vitro*	Protein/mRNA	Technique	Characteristics	Refs.
AR	Human	*In vivo*	Protein	IH	No expression in osteoclasts	101, 103
	Chicken	*In vitro*	Protein	Binding	Osteoclasts	137
	Rat	*In vivo*	Protein/mRNA	IH/ISH	Osteoclasts	17
	Mouse	*In vitro*	Protein	IH	Osteoclast-like cells	138
ER	Human	*In vitro*	Protein	Binding	Preosteoclastic cell line	141
	Avian	*In vitro*	Protein	Binding	Isolated osteoclasts	142, 143
ERα	Human	*In vivo*	Protein/mRNA	ISH	Osteoclasts	106, 144, 146
	Human	*In vivo*	Protein/mRNA	IH/ISH	No expression in osteoclasts	107
	Human	*In vivo*	Protein	IH	Osteoclast-type giant cells in granulomas in 10 of 26 patients	145
	Human	*In vitro*	Protein/mRNA	Western/Northern	Osteoclasts from giant cell tumors	147
	Human	*In vitro*	mRNA	PCR	Preosteoclastic cell line	141
	Human	*In vitro*	mRNA	PCR	Primary purified osteoclasts	148
	Human	*In vitro*	mRNA	PCR/ISH	No expression in cells from human giant cell tumors	152
	Human	*In vitro*	Protein/mRNA	IH/ISH	In preosteoclasts but not in mature osteoclasts	154
	Rabbit	*In vivo*	mRNA	ISH	No expression in osteoclasts	107
	Rabbit	*In vitro*	mRNA	Northern	Osteoclasts	149
	Avian	*In vitro*	Protein/mRNA	Western/Binding/ Northern	Isolated osteoclasts	142, 143
	Rat	*In vivo*	mRNA	ISH	In mononuclear precursors, not in multinuclear mature osteoclasts	155
ERβ	Human	*In vivo*	Protein	IH	Osteoclasts	106, 150, 151
	Rat	*In vivo*	mRNA	ISH	No expression in osteoclasts	153
	Murine	*In vivo*	Protein	IH	Osteoclasts, cytoplasmatic	150

No expression, No expression was detected; ER, estrogen receptor binding and not possible to distinguish between ERα and ERβ; IH, immunohistochemistry; Binding, binding studies; ISH, *in situ* hybridization; Western, Western blot; Northern, Northern blot; RPA, RNase protection assay.

TABLE 5. Expression of ARs and ERs in other bone-related cells

Receptor	Species	*In vivo/in vitro*	Protein/mRNA	Technique	Characteristics	Refs.
AR	Human	*In vivo*	Protein	IH	Mononuclear and endothelial cells in BM	101
	Human	*In vivo*	Protein	IH	BM stromal cells, macrophages, endothelial cells, megakariocytes	156
	Mouse	*In vitro*	Protein/mRNA	IH/PCR	BM cells	122
ER	Mouse	*In vitro*	Protein	Binding	BM stromal cells	159
ERα	Human	*In vitro*	Protein/mRNA	ISH/Northern	BM stromal cells	154
	Rat	*In vivo*	mRNA	PCR	Calvarian periosteum	411
	Rat	*In vitro*	mRNA	RT-PCR	BM stromal cells	157, 158
	Mouse	*In vitro*	Protein/mRNA	IH/PCR	BM stromal cells	159, 160
	Mouse	*In vitro*	Protein	IH	Cultured BM cell line	122
ERβ	Human	*In vivo*	Protein	IH	Megakariocytes, capillary blood vessel cells	151
	Rat	*In vitro*	mRNA	RT-PCR	BM stromal cells	157
	Rat	*In vitro*	mRNA	RT-PCR	BM cells	158
	Mouse	*In vitro*	Protein/mRNA	IH/PCR	BM cells	122

ER, Estrogen receptor binding and not possible to distinguish between ERα and ERβ; IH, immunohistochemistry; Binding, binding studies; RT-PCR, quantitative PCR; Northern, Northern blot; BM, bone marrow.

A. Growth plate chondrocytes

Androgens probably have direct effects on growth plate cartilage and thus on longitudinal bone growth. It has been documented, at least when using very strict culture conditions, that androgens regulate both proliferation and differentiation of cultured epiphyseal chondrocytes (100, 161–164), supporting a direct effect of androgens on growth plate cartilage. A direct effect of androgens on epiphyseal growth and maturation is also supported by the fact that T, injected directly into the growth plate of rats, increases the growth plate width (165). Androgens, however, also have an important effect on GH secretion and its pulsatility during puberty, and this may indirectly mediate their effects on linear growth (166).

B. Osteoblasts/osteocytes

Most *in vitro* studies (18, 126, 167–172), but not all (115, 173, 174), demonstrate that both DHT and T increase cell proliferation of cultured osteoblast progenitors derived from different species. The effects on osteoblast differentiation are rather controversial, including stimulatory, no effect, and inhibitory effect on alkaline phosphatase, type I collagen, osteocalcin, and mineralization of extracellular bone matrix (18, 115, 125, 167, 169, 170, 173–177). These conflicting results might be due to differences in receptor concentration, animal species, skeletal localization of the osteoblasts, or the stage of osteoblast differentiation. However, in our opinion, most studies indicate that androgens induce a more differentiated osteoblast phenotype. Recent studies demonstrate that androgens decrease osteoblast and osteocyte apoptosis (88, 96). Thus, most *in vitro* studies support the notion that androgens stimulate proliferation of osteoblast progenitors and differentiation of mature osteoblasts while inhibiting apoptosis of osteoblasts.

Some of the local effects of androgens on bone might, as previously described for estrogens (4), be mediated via a regulation of cytokines and growth factors expressed locally in bone. The three most discussed androgen-regulated locally expressed factors include TGFβ, IGFs, and IL-6. TGFβ and the IGFs are involved in bone formation, whereas IL-6 increases osteoclastogenesis and androgens may therefore theoretically preserve bone via either an induction of TGFβ and IGFs or an inhibition of IL-6. We will here discuss results regarding androgen-induced regulation of these factors, but it should be emphasized that the functional role of these regulations is complex and full interpretation is not yet possible. TGFβ is highly expressed in bone tissue (the largest reservoir for TGFβ), and it is a mitogen for osteoblasts (178, 179). Several studies, both *in vivo* and *in vitro*, indicate that androgens increase TGFβ expression and/or activity (115, 169, 180, 181). Furthermore, orch reduces bone content of TGFβ, whereas T treatment increases TGFβ content (181). It remains unclear to what extent this effect of T is mediated through the AR or ERs. In contrast, Hofbauer *et al.* (173) found that androgens decrease TGFβ mRNA levels in a human osteoblastic cell line. IGFs and IGF-binding proteins (IGFBPs) exert important effects on osteoblast proliferation and differentiation (182, 183). Androgens have been shown to regulate the expression and/or the activity of IGFs either directly by regulating IGF expression or indirectly via a regulation of the expression of IGFBPs in several (168, 175, 184), but not all, studies (18, 185). IL-6 is a cytokine believed to be involved in the bone loss associated with sex steroid deficiency. It increases osteoclastogenesis and bone resorption. Orch increases IL-6 secretion by bone marrow cells (186). DHT and T suppress the IL-6 production in both cultured bone marrow stromal cells and osteoblasts (187–189). Furthermore, androgens inhibit the expression of the gp80 and the gp130 subunits of the IL-6 receptor (190). Orch increases osteoclastogenesis, which is inhibited by androgens or IL-6 neutralizing antibody (188), and IL-6 knockout mice do not lose bone after orch (188), supporting the notion that inhibition of IL-6 production is at least partly involved in the antiresorptive effect of androgens on bone. Thus, TGFβ, IGFs, and IL-6 are three major factors believed to be involved in the bone-sparing effect of androgens. However, other possible pathways for androgen regulation of bone metabolism have also been investigated. Androgens inhibit PTH- or IL-1-induced prostaglandin E_2 production (176) and PTH-induced cAMP production (170, 191), whereas they increase IL-1β production (192) and the mitogenic effect of fibroblast growth factor (168) in cultured osteoblasts. A recently published study demonstrates that DHT decreases osteoprotegerin (OPG) levels (193), whereas it has previously been shown that estrogens increased OPG expression in cultured osteoblasts (194, 195). It remains to be investigated *in vivo* whether DHT and estrogens have opposite effects on OPG expression and, if so, whether it is of any physiological importance for the regulation of bone homeostasis.

C. Osteoclasts

Osteoclasts are derived from hematopoietic precursor cells of the colony-forming unit granulocyte-macrophage lineage within the bone marrow. The proliferation of these colony-forming unit granulocyte macrophages is up-regulated after orch. The terminal differentiation into mature osteoclasts requires close interaction and also cell-to-cell contact with stromal cells of the osteoblastic lineage in the bone marrow under tight control of the receptor activator of nuclear factor κB-ligand (RANKL)/OPG system (196). The osteoblastic stromal cells also appear to be essential for the bone-sparing action of androgens. This notion is supported by the fact that in the SAMP6 mouse model, in which osteoblast function is impaired due to an age-related decrease in osteoblast progenitors (197), the rise in remodeling after orch is blunted. Their failure to up-regulate osteoclastogenesis is secondary to defective osteoblast formation. Thus, orch-induced increased osteoclastogenesis is dependent on osteoblast/preosteoblast-derived signals (139). Therefore, the removal of testes-derived sex steroids by orch in mice primarily leads to bone marrow changes. These changes, mediated by cells of the osteoblast lineage, are characterized by an increase of osteoblast precursors, which in turn indirectly stimulates osteoclastogenesis (198). A direct effect of androgens on preosteoclasts/osteoclasts is more controversial. However, Pederson *et al.* (137) have demonstrated that osteoclasts express AR and that DHT inhibits the resorptive capacity of

isolated human, murine, and avian osteoclasts *in vitro*. Furthermore, recent *in vitro* data suggest that androgens may also directly modulate RANKL-induced osteoclast formation, independently of the bone marrow cells (199). Additionally, androgens, like estrogens, may regulate osteoclast survival, RANK expression in preosteoclasts, and activity of mature osteoclast independently of their effects on bone marrow stromal cells, at least *in vitro*. However, the contribution of these *in vitro* observations to *in vivo* activity of androgens remains to be clarified. A direct effect of estrogens on osteoclasts *in vivo* is supported by the finding that E_2 promotes apoptosis of murine osteoclasts (200). These results indicate that osteoclast precursors as well as osteoclasts are able to respond directly to androgens *in vitro* and thus are potential androgen target cells *in vivo* (137). In conclusion, it is apparent that some of the effect of androgens on osteoclastogenesis is indirectly mediated via cells of the osteoblasts lineage, although further investigation is needed to characterize a possible direct effect of androgens on osteoclasts *in vivo*.

IV. Effects of Androgens on the Rodent Skeleton

A. The rodent as a model for the study of skeletal androgen action

The rat is the best-characterized animal model for the study of skeletal androgen action. The skeleton of young and mature rats is mainly dependent on modeling. This modeling process involves both growing and shaping of the bones. It is a highly synchronized process of bone formation at one site and resorption at another, with the former exceeding the latter. Longitudinal bone growth occurs through endochondral bone formation, whereas radial bone growth is the result of periosteal apposition. The expansion of the medullary cavity is a combination of endocortical bone resorption and formation.

Young rats have been widely used as a model for the growing skeleton. The skeleton of rats, though, differs from the human skeleton because the growth plates never fully close (201). This should not be overinterpreted because by 12 months of age, the growth plate characteristics have stabilized, with no further significant change up to 24 months (202). This allows aged rats to be used as a model for human skeletal remodeling. Bone remodeling maintains the mechanical and structural integrity of the skeleton after puberty. Coupling of osteoblast and osteoclast actions ensures that the processes of bone resorption and formation occur at the same time and place, which allows old bone to be replaced by new bone. It is also important to mention that rodents do not experience spontaneous fractures. Rodent studies will therefore not answer the question whether androgens protect against osteoporotic fractures, but they may still contribute to our knowledge of how androgens influence skeletal structure and density.

Several experimental procedures have been used to evaluate skeletal androgen action and metabolism in male and female rodents. These include (surgical and chemical) castration and administration of AR antagonists, ER antagonists, aromatase inhibitors, selective ER modulators (SERMs), and type II 5α-reductase inhibitors, either alone or in combination with sex steroid replacement. Because the skeletal effects of these experimental conditions often differ between cortical and cancellous bone compartments, these compartments will be considered separately. Some of these interventions may also induce extraskeletal effects—including changes in body composition, growth, and food intake—that may indirectly interfere with skeletal homeostasis (see *Section V*). Tables 6–8 summarize the most important findings concerning androgen action in male and female rats, respectively.

More recently, mice have been introduced as a model to study skeletal androgen action. Mice with targeted disruption of the different receptors or enzymes involved in androgen action have been described, and their skeletal phenotype will be reviewed. At the end of each section, we will indicate to what extent these animal data may contribute to our understanding of how androgens affect skeletal structure and density.

B. Skeletal consequences of gonadectomy in rodents

1. Skeletal effects of gonadectomy in rats. Surgical castration, as induced by ovx in the female rat and orch in the male rat, represents the most frequently used procedure to study skeletal sex steroid action. Chemical castration, as induced by GnRH agonists, has similar skeletal effects as surgical castration in female rats (37), but its impact on bone has not been studied in male rats. Both orch and ovx dramatically reduce serum levels of T and E_2 in male and female rats. However, these procedures do not totally eliminate E_2 production, because adrenal androgens can be transformed into estrogens after aromatization. In both genders, castration has considerable impact on cancellous and cortical bone compartments. However, whereas this response appears to be similar in cancellous bone, it is different in cortical bone.

Skeletal cancellous changes are characterized by an increase in cancellous bone turnover, resulting in bone loss in gonadectomized rats, irrespective of gender, age, or strain (203–211) (Table 6). Cancellous bone loss after gonadectomy can be detected not only by histomorphometry but also by peripheral quantitative computed tomography (pQCT) and microcomputed tomography. Biochemical markers of bone resorption (*e.g.*, urinary deoxypyridinoline) and formation (*e.g.*, serum osteocalcin) reflect the early increase of cancellous bone turnover after gonadectomy in both genders (40, 204, 209, 211–214). These changes in the cancellous bone compartment are reminiscent of high-turnover osteoporosis

TABLE 6. Skeletal effects of orch and ovx in rats

	Body weight gain	Appendicular skeletal growth	Cancellous bone mass	Cortical bone area
Male rats				
Growing	↓, =	↓, =	↓	↓, =
Aged	=	=	↓	↓
Female rats				
Growing	↑, =	↑, =	↓	↑, =
Aged	↑	NA	↓	NA

The main effects of sex hormone deficiency on body weight and the different bone compartments are summarized. ↓, Decreased; ↑, increased; =, no change; NA, not available. For references, see text.

after menopause (215) and explain why the ovx female and the orch male rat model have gained wide acceptance as animal models for osteoporosis (216). Also in line with observations in postmenopausal osteoporosis in humans, the number of osteoclasts is increased after ovx (39, 203, 207, 212, 217, 218) or orch (208, 210, 211, 219). Osteoblast number, surface, and mineral apposition rates are up-regulated in an attempt to fill the increased number of resorption cavities created by these osteoclasts (203, 208, 210, 211, 220). Although relative changes in histomorphometric and biochemical indices of bone resorption and formation indices cannot predict final outcomes on cancellous bone mineral content, orch-induced bone resorption tends to be increased more and longer than formation, even at the level of the individual remodeling unit (211, 220). Therefore, and despite increased bone formation at the tissue level, net cancellous bone loss occurs as a result of an imbalance with bone resorption exceeding bone formation in each bone mineral unit. The microanatomical mechanism responsible for this cancellous bone loss after orch in rats is a reduction in trabecular number (33, 211, 220) and thickness (211).

In contrast to the human skeleton, the rodent cortical skeleton has no Haversian canals, which explains why intracortical bone loss is not readily observed in ovx and orch rodents. Some studies have reported increased cortical porosity in older orch rats (205, 221), probably due to orch-induced medullary expansion through increased resorption at the endocortical site. In contrast to the changes in cancellous bone, the responses to castration at the periosteal site and growth plate are essentially sexually dimorphic, especially in young growing rats (Table 6). Periosteal and longitudinal bone formation appear to be increased in some (40, 57, 58, 222–224), but not all (203, 204, 207), studies on ovx and decreased in the orch model (207, 210, 213, 219, 224) (Fig. 4). The end result will be cortical bone expansion and increased bone length in the ovx female but decreased cortical bone volume and bone length in the orch male. This increase/decrease in cortical bone volume after gonadectomy (in female and male rats, respectively) is primarily due to a relative increase/decrease in periosteal bone formation and not (or to a lesser extent) to significant changes of the inner endocortical perimeter (225). The implication is that sex hormone deficiency may have a more severe impact on the integrity of the male skeleton compared with the female skeleton.

TABLE 7. Skeletal effects of sex steroids or sex steroid-related agents in orch and ovx rats

	Body weight gain	Appendicular skeletal growth	Cancellous bone mass	Cortical bone area
orch + T	=, ↑	=, ↑	↑	↑
orch + DHT	=, ↓	=, ↑	↑	=, ↑
orch + E_2/phytoestrogens	=, ↓	=	↑	=, ↑
orch + SERM	↓	NA	↑	↑
ovx + T	=	NA	↑	=
ovx + ADIONE	=	↓	↑	NA
ovx + DHEA	=	NA	↑	=
ovx + DHT	=, ↑	=, ↑	↑	=
ovx + E_2/phytoestrogens	↓	↓	↑	NA
ovx + SERM	↓	NA	↑	NA
ovx + tibolone	↓	↓	↑	=

The reported effects on body weight and the different bone compartments are summarized. ↓, Decreased; ↑, increased; =, no change; NA, not available; ADIONE, androstenedione. For references, see text.

TABLE 8. Skeletal effects of selective pharmacological modulation of androgen and estrogen action in intact male and female rats

	Body weight	Appendicular skeletal growth	Cancellous bone mass	Cortical bone area
Male rats				
Aromatase inhibitor	↓	=, ↓	↓	=, ↓
ER antagonist	=	=	=	=
SERM	↓	NA	↑	=
AR antagonist	↓	NA	(↓)	=
5α-reductase inhibitor	=	=	=	=
Female rats				
AR antagonist	=, ↑	=	=, (↓)	=
ER antagonist	=	=	↓	=

The effects of selective pharmacological modulation of androgen and estrogen action on body weight and the different bone compartments are summarized. ↓, Decreased; ↑, increased; =, no change; NA, not available. Changes between *parentheses* indicate that bone loss was only documented by changes in total calcium or ash content and not by methods evaluating decreases of cancellous bone specifically. For references, see text.

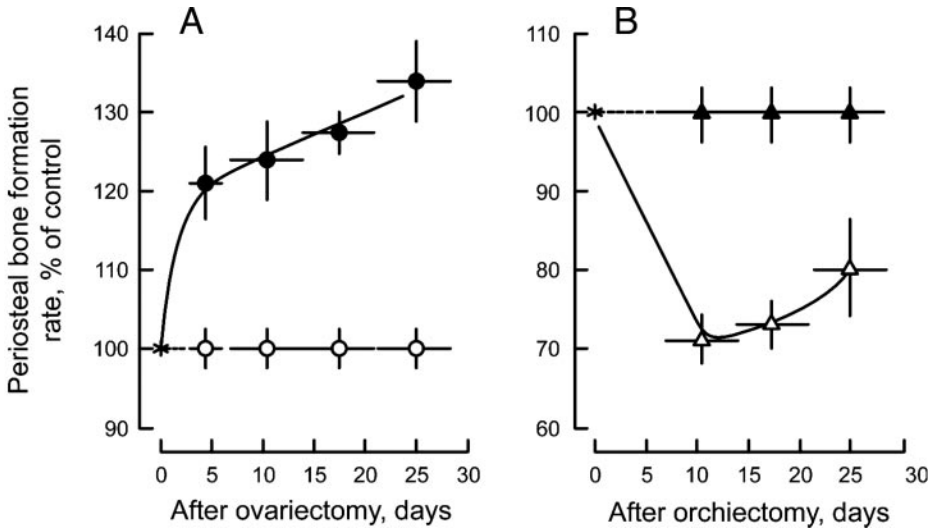


FIG. 4. A, The effect of ovx on periosteal bone formation rate. The mean ± SE (*vertical bar*) and tetracycline labeling period (*horizontal line*) for intact controls (○) and ovx (●) rats are shown as a function of time after ovx. $P < 0.01$ for all ovx time points compared with intact controls. B, The effect of orch on periosteal bone formation rate. The mean ± SE and tetracycline labeling period for intact controls (▲) and orch (◇) are shown as a function of time after orch. $P < 0.01$ for all orch time points compared with the same labeling period in intact controls. [Reproduced from R. T. Turner *et al.*: *J Orthop Res* 8:612–617, 1990 (234) with permission from Orthopaedic Research Society.]

Gender-related differences in the response of periosteal bone to gonadectomy also explain why methods that measure areal rather than volumetric bone density, such as DXA, tend to overestimate bone loss in the orch male while underestimating bone loss in the ovx female. Moreover, cortical bone loss in the orch rat model, in contrast to changes in cancellous bone, is due to failure to gain new bone and not to net bone loss. These gender-specific responses in cortical bone volume are particularly important during active growth but less significant in elderly, slow-growing rats (209, 220) (Table 6). In older rats, longitudinal bone growth does not decrease after orch (205, 206, 209).

As summarized in Table 6, castration induces postmenopausal-like bone loss in the cancellous bone compartment in both genders, irrespective of age or strain of the rat. The intracortical bone compartment is relatively resistant to surgical castration, whereas the responses at the periosteal site and the growth plate appear to be sexually dimorphic, especially in younger rats, with androgens stimulating and estrogens inhibiting periosteal bone expansion.

2. Skeletal effects of gonadectomy in mice. Reduced cortical bone growth, as described in rats, has also been observed in male orch mice (70, 226, 227). Female mice, in contrast to rats, do not experience significant cortical bone expansion after ovx (228, 229).

In cancellous bone, both ovx (228–231) and orch (66, 70, 188, 227, 231) induce bone loss. This cancellous bone loss is associated with elevated markers of bone turnover (70, 229), and ovx and orch mice are therefore increasingly being accepted as animal models for the study of steroid action in postmenopausal-like osteoporosis (139).

C. Skeletal effects of androgen replacement in rodents

1. Skeletal effects of androgen replacement in rats

a. Effects of aromatizable and nonaromatizable androgens. The aromatizable androgen T is a very effective bone-sparing agent. T not only fully prevents cancellous bone loss in orch rats (208, 209, 221) but is also bone-sparing in ovx rats (232), even at subphysiological concentrations (233) (Fig. 5) and irrespective of age. In addition, T antagonizes periosteal expansion in ovx rats (234), but increases periosteal bone formation in orch rats (209, 221, 234) (Table 7). Weaker aromatizable androgens, such as DHEA (223, 235) and androstenedione (218, 236), are evidently bone-sparing in the ovx rat model but have not been studied in male orch rats (Table 7). In the ovx rat model, bone protection by these androgens is exerted through the AR and not the ERs, as illustrated by the fact that the effect is blunted by concomitant administration of AR antagonists but not by aromatase inhibitors or ER antagonists (218, 235, 236).

The nonaromatizable androgen DHT also exerts bone-sparing action in orch (208, 209, 234, 237) and ovx rats (235, 238, 239) (Table 7). However, DHT appears to be less effective than T in the elderly orch rat model (208, 237), in particular on cortical bone (Fig. 5). In this model, higher doses of DHT than T are indeed needed to obtain at least some bone-protective action on cancellous bone, but this occurs at the expense of side effects like hypertrophy of the ventral prostate and seminal vesicles. Moreover, high-dose DHT fails to prevent orch-induced cortical thinning (237).

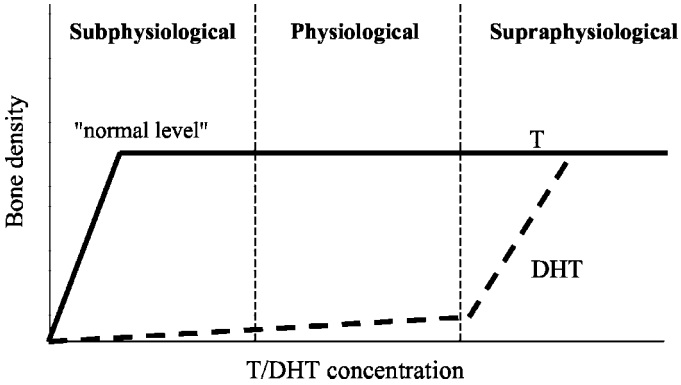


FIG. 5. In the aged orch male rat model, subphysiological T replacement, which only partially prevents atrophy of ventral prostate and seminal vesicles, is already bone-sparing (233). In contrast, only supraphysiological doses of DHT, resulting in hypertrophy of androgen-sensitive organs, prevent cancellous bone loss (237). The difference in bone-sparing capacity between T and DHT may be related to the fact that T can be aromatized in bone and activates one or both ERs.

In conclusion, in both ovx female and orch male rats, aromatizable and nonaromatizable androgens show bone-protective action, especially at cancellous bone sites. Nonaromatizable androgens seem to be less effective than aromatizable androgens. A possible explanation for the relative lack of efficacy of nonaromatizable androgens compared with aromatizable androgens may be the aromatization of the latter via estrogens and stimulation of the ERs.

b. Effects of estrogens. The bone-sparing effects of aromatizable androgens may depend on activation of the AR, the ERs, or both. Estrogens (including phytoestrogens) have well-documented bone-sparing effects, not only in ovx rats (204, 232, 235, 239–242) but also in orch rats (209, 213, 237) (Table 7).

Overall, in gonadectomized rat models, aromatizable and nonaromatizable androgens and estrogens (Table 7) appear to be bone-sparing in the cancellous bone compartment, irrespective of gender or age. Table 7 also indicates that sex steroid action on cortical bone is not only less well documented but also less consistent. Both androgens and low-dose estrogens tend to stimulate cortical bone, however only in male rodents.

2. Skeletal effects of sex steroids in mice. T effectively prevents cancellous bone loss in orch mice (70, 188). DHT (96) and estrogens (66, 70, 96, 228, 231) also appear to be bone-sparing after castration, in both genders. Similar bone-sparing effects have been observed with phytoestrogens in ovx (243) and orch mice (244). So, in accordance with experiments in rats, androgens and estrogens both protect against cancellous bone loss in mice, irrespective of gender. Additionally, T and estrogens increase the cortical area in orch mice (226, 245).

D. Skeletal effects of selective manipulation of estrogen and androgen action in rodents

1. Effects of selective pharmacological modulation in rats. Administration of AR antagonists, ER antagonists, SERMs, and

aromatase inhibitors will selectively interfere with the AR, the ERs, and the aromatization of androgens into estrogens, respectively (Fig. 1).

Aromatase inhibitors impair both skeletal development (cortical expansion) and maintenance (integrity of the cancellous compartment) in male rats (47, 48) (Table 8). The bone-phenotypic changes induced by administration of an aromatase inhibitor are thus similar to those observed after orch, although bone turnover seems to be less elevated. Moreover, E_2 prevents bone loss induced by an aromatase inhibitor in male rats, supporting the concept that pharmacological administration of estrogen is protective for male bone (49).

Administration of a selective ER antagonist, ICI 182,780, which induces cancellous bone loss in female intact and estrogen-repleted ovx rats (40, 57, 58), does not impair skeletal homeostasis in T-supplemented orch rats (59) or intact male rats (60) (Table 8). Yet, SERMs, some of which are being used in the treatment of postmenopausal osteoporosis, have been reported to restore ovx-induced bone loss in female rats (246, 247) (Table 7) as well as orch-induced (Table 7) and age-related bone loss (Table 8) in male rats (248, 249). Similarly, tibolone, a drug with mixed androgenic, estrogenic, and progestogenic properties, prevents bone loss in ovx rats (212) (Table 7). The bone-sparing effect of tibolone is reversed by concomitant administration of an antiestrogen but not by an antiprogestogen or antiandrogen, suggesting that bone protection by tibilone after ovx occurs through the ER pathway (232).

The skeletal effects of AR antagonists in rats are not consistent (Table 8). In older studies, AR antagonists like flutamide or cyproterone acetate were found to induce bone loss in rats, irrespective of gender; however, in these studies, bone mass was only assessed by bone calcium content or kinetics (36, 37). Several histomorphometric studies have reported significantly reduced bone formation or an increase in bone resorption after AR antagonist administration, but without concomitant bone loss (38–40). Confirmation of AR antagonist-induced osteopenia by methods such as histomorphometry is therefore required. Finally, finasteride, a type II 5α-reductase inhibitor that blocks the conversion of T into DHT, does not interfere with skeletal homeostasis in male rats (33) (Table 8).

In conclusion, selective modulation of the AR and ER pathways generally supports the concept of a dual mode of androgen action (through both the AR and ER pathways), at least in cancellous bone, both in male and female rat models (Table 8). Effects of AR antagonists in rats, on the other hand, are less well-established.

2. Effects of selective pharmacological modulation in mice. In intact male mice, the AR antagonist casodex does not affect bone density, whereas another AR antagonist, epitestosterone, which is also a 5α-reductase inhibitor, decreases bone density (250). The AR antagonist cyproterone acetate prevents orch-induced bone loss, suggesting that this agent may act as an AR agonist in bone (251). SERMs also prevent orch-induced (227, 231) and ovx-induced bone loss (231, 252) in mice. Overall, these studies (although limited in number) provide further evidence for a similar action of sex steroids in cancellous bone. Along these lines, the activator of nongenomic estrogen-like signaling, estren, has a similar (or even greater) anabolic effect on (especially cortical) bone than DHT in male orch mice or than E_2 in female ovx mice, despite a much lower affinity for the ER (96) (see also *Section II*). These effects of estren, although obtained with a much higher dose than with E_2 and DHT, occur without stimulation of reproductive organs in either sex.

E. Skeletal effects of selective manipulation of androgen and estrogen action in transgenic mice

1. Description of transgenic animal strains. The cyp19 aromatase gene has been inactivated [aromatase knockout (ArKO) mice] by two independent groups (253, 254), and a similar skeletal phenotype has been reported (54–56). ERβ has been inactivated by three independent groups [ERβ knockout (BERKO) mice] (79, 255, 256), and the skeletal phenotypes of all three mouse strains are rather similar (64–67, 73, 75–81, 98). Two independent groups have inactivated ERα [ERα knockout (ERKO) mice] (256, 257). The first and most studied ERKO mouse strain was generated in the laboratories of Korach and Smithies (257). A recent study indicates that these mice are not completely ERα-inactivated (258), as supported by the observation that they express one or two N-terminally modified ERα transcripts associated with minor ER activity regarding uterine weight and endothelial nitric oxide production (258). The remaining ERα activity is suggested to be mediated via a truncated ERα with remaining AF-2 activity, whereas there is no AF-1 activity left (258). This truncated ERα isoform has been detected in bone of these mice and has been shown to have effects on gene transcription in cultured human osteoblasts (259). These mice will be referred to as ERKO$^{AF\text{-}1-/-}$ because they do not have any remaining AF-1. The second ERα-inactivated mouse model was developed in the laboratory of Chambon (256). There is no remaining ERα activity in these mice, and both AF-1 and AF-2 activity are absent in these animals, which will be referred to as ERKO$^{AF\text{-}1/AF\text{-}2-/-}$. The skeletal phenotype has been reported for both the ERKO$^{AF\text{-}1-/-}$ mice (62–67, 69, 260, 261) and the ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ mice (46, 71–74, 98). Most skeletal phenotypes (including the male skeletal phenotype and the skeletal responses to estrogen treatment in female ovx mice) are identical for the ERKO$^{AF\text{-}1-/-}$ (66, 67, 70) and the ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ mice (72, 73). However, a clear difference between the two ERKO models is seen in female gonadal intact mice (see *Section IV.E., 2* and *3*). Possible factors that might explain the differences between the two ovarian intact female ERKO models include: 1) differences in genetic background; 2) differences in dietary/environmental estrogen; 3) differences in the feedback regulation of sex steroids between the ERKO$^{AF\text{-}1-/-}$ and ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ mice, in which both E_2 and T are dramatically increased but the magnitude of this disturbance might differ between the two ERKO models; 4) remaining AF-2 activity in ERKO$^{AF\text{-}1-/-}$ mice; and 5) other unknown reasons.

Mice with inactivated ERα and ERβ [double ER knockout (DERKO)], developed in two independent laboratories, have been reported to have rather similar skeletal phenotypes, both in intact mice and in estrogen-treated gonadectomized

mice (64–67, 72, 73, 98). AR knockout mice (ANDRKO mice) have recently been developed by two independent groups using the cre/loxP system (42–44), and their skeletal phenotypes have recently been described (42–44, 262, 263).

It is of importance to note that the sex steroid levels (both E_2 and T) are increased in ERKO and DERKO, due to disturbed feedback regulation (65, 73, 264). In contrast, the skeletal phenotype of single ERβ inactivation is not influenced by altered sex steroids levels. By comparing the skeletal phenotype of wild-type, ArKO, BERKO, ERKO, DERKO, and ANDRKO mouse strains, it may be possible to gain new insight in the relative importance of the AR, ERα, and ERβ in mediating the skeletal effects of androgens. The different transgenic mouse strains described in this section are inactivated from birth, which results in disturbed skeletal growth and maturation. Thus, the adult skeletal phenotype in these mice is a combination of effects on growth/maturation and adult bone remodeling. Future transgenic mouse models with inducible gene inactivation will be useful in separating the effects on growth/maturation from the effects on adult bone remodeling and will therefore be more relevant to further explore the protective effect of sex steroids on the adult skeleton. Nevertheless, the currently available transgenic mouse models have already been very informative. In the following section, the effects on longitudinal appendicular skeletal growth as well as the effects on cortical and cancellous bone in these mice will be discussed.

2. Longitudinal appendicular skeletal growth

a. Females. ERβ-inactivated female mice develop increased femoral length after sexual maturation (65, 73, 75, 78). Actually, the normal length difference between males and females is not observed in young adult female BERKO mice (65, 75, 76, 78) (Fig. 6A). An altered length of femur is often associated with a disturbed GH-IGF-axis (265–268). Interestingly, serum levels of IGF-I are increased in young adult female BERKO mice and correlate to the effect on femoral length, which might indicate that ERβ is an inhibitor of the GH-IGF-I axis (65). However, GH secretion has not yet been studied in these mice. Several independent studies using female ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ mice have demonstrated that these mice have a reduced length of the femur, which develops during sexual maturation and is still present at 18 months of age (62, 63, 65, 260). In contrast, in one recent study in female ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ mice, no effect on femoral length was seen at 16 wk of age (73). The length of the femur is largely unchanged in adult female ArKO (54) and DERKO (65, 73) mice. In summary, ERβ represses longitudinal appendicular skeletal growth during sexual maturation in female mice. Some (but not all studies) in ERKO models indicate that ERα may stimulate appendicular skeletal growth in female mice. Finally, a normal appendicular skeletal growth is observed in the absence of the opposing effects of ERα and ERβ activation as seen in female ArKO and DERKO mice.

b. Males. The longitudinal appendicular skeletal growth is unchanged in male BERKO mice (64, 73, 75) (Fig. 6A), although several independent studies using male ERKO$^{AF\text{-}1-/-}$ mice have clearly demonstrated that these mice have a reduced appendicular skeletal growth during sexual maturation (62–64). The decreased appendicular skeletal growth of these mice is associated with decreased serum levels of IGF-I (64). The appendicular growth of the male ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ mice has been analyzed by two independent groups with conflicting results, demonstrating unchanged growth in one study (73) and, similar to all the ERKO$^{AF\text{-}1-/-}$ studies, decreased growth in another study (46). Thus, most available data indicate that the appendicular growth is decreased in male ERKO mice. Femoral length is also decreased in male ArKO (54) and DERKO (65, 73) mice, whereas bone length is reported to be unchanged in male ANDRKO mice (263). In summary, ER activation, but not AR activation, is involved in the regulation of male longitudinal appendicular skeletal growth in mice. Most studies indicate that the effect of estrogens is mediated via ERα and not via ERβ (46, 62–64, 75) (Fig. 6).

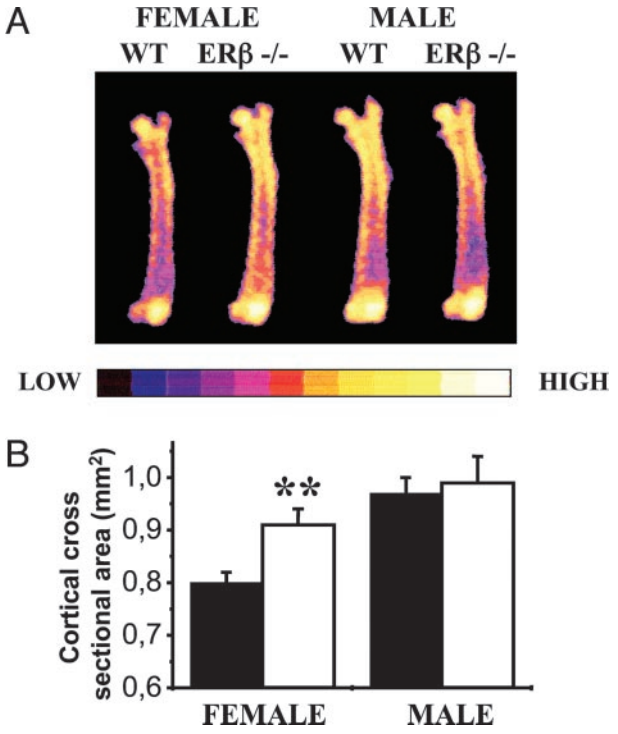


FIG. 6. Increased length (A) and increased cortical cross-sectional area (B) of the femur in young adult female but not male BERKO mice (*open bars*) compared with wild-type mice (*filled bars*). Thus, the skeletal sexual dimorphism is diminished in the ERβ −/− mice compared with wild-type mice. LOW, Low BMD; HIGH, high BMD. [Panel A is reproduced with permission from S. Windahl *et al.*: *J Clin Invest* 104:895–901, 1999 (75).]

3. Cortical bone

a. Females. Studies in two different BERKO mouse strains have demonstrated an increase in cortical cross-sectional bone area due to increased radial cortical bone growth (Fig. 6B). This effect is seen after sexual maturation (65, 75, 78, 81). In contrast, no significant effect on cortical bone parameters was found in female BERKO mice, developed in a third laboratory (73). In line with the effects on appendicular skeletal growth, the effect on cortical thickness differs dramatically between the gonadal intact female ERKO$^{AF\text{-}1-/-}$ mouse model (65), a mouse model with increased thickness, and the gonadal intact female

ERKO$^{AF\text{-}1/AF\text{-}2-/-}$ (73) with decreased thickness. The cortical thickness is similar in the female ERKO and DERKO mice. Female ArKO mice have a decreased cortical cross-sectional bone area (56). In summary, it is clear that ERβ activation decreases cortical thickness in female mice and that estrogen deficiency, due to aromatase inactivation, results in a decreased cortical area in female mice. The role of ERα in the regulation of cortical bone in gonadal intact female mice is unclear, because conflicting results have been presented.

b. Males. Cortical bone parameters reflecting the size of the cortical bone, including periosteal circumference, endocortical circumference, cortical thickness, and cortical cross-sectional area, are decreased in male ERKO (64, 70, 73), DERKO (64, 73) (Fig. 7), and ArKO mice (56). The decreased amount of cortical bone is associated with a reduced periosteal apposition rate (73). In contrast, the cortical bone parameters are unchanged in male BERKO mice (64, 73, 75). Recent data indicate that male ANDRKO mice have a decreased cortical bone area (44, 263). In summary, ERα and AR but not ERβ enhance cortical radial bone growth in male mice.

4. Cancellous bone in gonadal intact mice

a. Females. Surprisingly, female BERKO mice are protected against age-related cancellous bone loss (73, 76, 78, 80). This finding indicates that ERβ, in the presence of low age-related estrogen levels, might act as a competitor for the stimulatory effect of ERα on cancellous bone in old female mice. An inhibitory role of ERβ in the presence of ERα is supported by the observation that ERβ reduces ERα-regulated gene transcription (69). However, the estrogenic response to pharmacological treatment with estrogen in gonadal intact female BERKO mice is not significantly altered compared with wild-type mice (81). Unexpectedly, female gonadal intact ERKO mice have an increased amount of cancellous bone (65, 73), probably caused by increased serum levels of T acting via the AR (98). Furthermore, female ERKO mice have masculinized livers, which also might affect bone mass accrual through circulating IGF-I (269). Female gonadal intact DERKO (65, 73) and ArKO mice (54–56) have a decreased amount of cancellous bone. These findings indicate that ER activation as well as AR activation has the capacity to preserve the amount of cancellous bone in gonadal intact female mice.

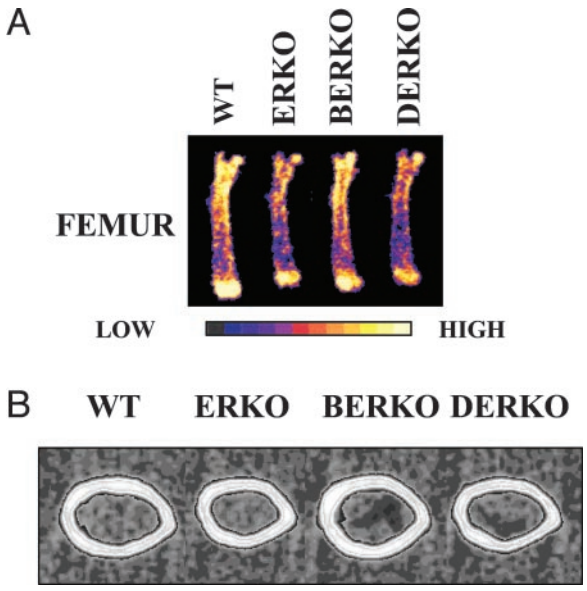


FIG. 7. Effects of ER inactivation on skeletal growth in gonadal intact male mice. Representative DXA scans (A) and middiaphyseal pQCT scans (B) of femora in young adult wild-type, ERKO, BERKO, and DERKO mice. LOW, Low BMD; HIGH, high BMD. Femoral length (A), a measure of appendicular growth, and cross-sectional area (B) are significantly reduced in ERKO and DERKO mice. [Reproduced with permission from O. Vidal *et al.*: *Proc Natl Acad Sci USA* 97: 5474–5479, 2000 (64). © National Academy of Sciences, USA.]

b. Males. Cancellous bone is unaffected in male BERKO mice (64, 73, 75, 76). Unexpectedly, the amount of cancellous bone is increased, associated with reduced bone turnover in adult gonadal intact male ERKO and DERKO mice (66, 70, 73). The increased cancellous bone mass is caused by elevated T levels acting via the AR, because treatment with an antiandrogen results in cancellous bone loss in the gonadal intact male ERKO mice (66, 73, 98). Male ArKO mice (55, 56) and ANDRKO mice (42–44, 263) have reduced cancellous bone mass. In the ANDRKO model, this osteopenia is associated with an increased bone turnover (42–44, 263). Regarding male ArKO mice, one study has reported increased bone resorption (56), whereas another group has documented decreased bone turnover markers (both formation and resorption) (55). Taken together, these data suggest that the AR but not ERβ is required for the maintenance of cancellous bone mass in males. The specific role of ERα is difficult to determine using the gonadal intact male ERKO mice because their T levels are increased. Indeed, all studies on gonadal intact animals with mutated sex steroid receptors have to be considered carefully because endogenous sex steroid levels might be modified.

5. Effect of sex steroids on cancellous bone in gonadectomized mice. In the experiments described in this section, adult mice of the different genotypes were gonadectomized, and the effects of E_2 (activating the ERs), T (potentially activating both the AR and the ERs) or DHT (activating the AR) on cancellous bone were investigated.

a. Females. Ovx-induced cancellous bone loss in BERKO mice is prevented by treatment with either E_2 or DHT (Refs. 67, 72, 98; and M. K. Lindberg, and C. Ohlsson, unpublished personal data). In contrast, physiological E_2 replacement therapy exerts no effects (67) or minor effects (72, 98) on cancellous bone in ovx ERKO mice. However, pharmacological treatment of ovx ERKO, reaching very high concentrations of E_2, results in an increased amount of cancellous bone (98). The amount of cancellous bone is not increased by physiological E_2 treatment in ovx DERKO mice (67, 98), whereas this treatment restores the cancellous bone in female ArKO mice (56). Both DHT and T increase the cancellous bone mass in ovx ERKO and DERKO mice (Ref. 98; and M. K. Lindberg, and C. Ohlsson, unpublished personal data). These findings seem to indicate that the cancellous bone-sparing effect of physiological levels of estrogens in ovx mice is mainly mediated via

ERα. AR activation has the capacity to increase cancellous bone mass in ovx mice, but the physiological role of this effect is unclear (Refs. 96 and 98; and M. K. Lindberg, and C. Ohlsson, unpublished personal data).

b. Males. Orch-induced cancellous bone loss is prevented by either E_2 or DHT in BERKO mice and by either T or DHT, but not by E_2, in ERKO and DERKO mice, demonstrating that (in contrast to ERβ activation) ERα and AR have the capacity to increase the amount of cancellous bone in male mice (66, 70, 98, 245). T (in contrast to DHT) increases the amount of cancellous bone in male gonadectomized ANDRKO mice, supporting the concept that ER activation preserves the cancellous bone in the absence of a functional AR (42, 262, 263). These findings suggest that the AR and ERα, but not ERβ, can independently mediate the cancellous bone-sparing effects of sex steroids in male mice.

6. Conclusion of studies in sex steroid-related transgenic mouse models. Both ERα and the AR are involved in mediating the effects of androgens on cortical cross-sectional area and adult cancellous bone remodeling, although only ERα regulates longitudinal appendicular growth in male mice (Tables 9 and 10). ERβ, however, is not involved in the regulation of the male skeleton. In contrast, both ERα and ERβ are of importance for the regulation of the female skeleton. The ERα is the main receptor responsible for the cancellous bone-sparing effect of physiological levels of estrogens in female mice. However, ERβ activation suppresses the cancellous bone mass in old female mice, indicating that ERβ suppresses the cancellous bone-sparing effect of ERα in female mice with low serum levels of estrogens. The AR has the capacity to preserve cancellous bone in female mice, but its physiological role in female bone metabolism is unclear.

F. Skeletal effects of androgen resistance in rodents

1. Androgen resistance in rats. Experiments in androgen-resistant testicular feminized male (Tfm) rats provide further support for the concept of a dual mode of action of sex steroids. Tfm rats are unresponsive to androgens due to a single base mutation in the LBD of the AR gene. These genotypic male but phenotypic female rats have a more female-like (cortical) bone structure (270). In contrast to orch rats, they do not experience spontaneous cancellous bone loss. Vanderschueren *et al.* (41) have hypothesized that bone loss in these Tfm rats is prevented by the increased serum levels of estrogens. Indeed, after orch, Tfm rats lose bone, suggesting the importance of T as a precursor for estrogens in the protection of bone (41). Again, androgen-induced extraskeletal effects may have important indirect skeletal consequences in these rats. In the Tfm rat, GH secretion follows a more female-like pattern because of lack of neonatal androgenization (270) (see also *Section V*). This female-like GH profile may explain, at least partly, the more female type of bone structure. Moreover, high estrogen concentrations in Tfm rats may further inhibit growth.

The hypothesis that androgens may prevent cancellous bone loss via both AR and ER pathways is thus supported by experiments in the Tfm rat model. According to the Tfm phenotype, cortical bone structure depends on a functional AR. In Tfm rats, however, female-like GH secretion and an increased degree of aromatization may be confounding factors creating a growth-inhibitory environment.

2. Androgen resistance in mice. Tfm mice, on the other hand, have a high-turnover cancellous bone phenotype (226), similar to the phenotype observed in the ANDRKO mouse

TABLE 10. Summary of the role of ERα, ERβ, and AR activation on skeletal parameters in mice with different sex steroid-related gene inactivations

	ERα	ERβ	AR
Female mice			
Longitudinal skeletal growth	?	−	ND
Cortical bone area	?	−	ND
Effect on cancellous bone	+	(−/+)	+
Male mice			
Longitudinal skeletal growth	+	0	0
Cortical bone area	+	0	+
Effect on cancellous bone	+	0	+

+, Stimulation; −, inhibition; (−/+), stimulatory in the absence of ERα but inhibitory in old mice in the presence of ERα; 0, no effect; ?, conflicting results presented depending on which female gonadal intact ERKO model analyzed; ND, not determined. For references, see text.

TABLE 9. Summary of the skeletal phenotypes in mice with different sex steroid-related gene inactivations

		Longitudinal skeletal growth	Cortical bone area	Cancellous bone			
				Intact mice	Effect of E in gx	Effect of T in gx	Effect of DHT in gx
Female	BERKO	+	+	(+)	Yes	ND	Yes
	ERKO	?	?	+	Minor	Yes	Yes
	DERKO	0	?	−	No	ND	Yes
	ARKO	0	−	−	Yes	ND	ND
Male	BERKO	0	0	0	Yes	ND	Yes
	ERKO	−	−	+	No	Yes	Yes
	DERKO	−	−	+	No	ND	Yes
	ArKO	−	−	−	Yes	ND	ND
	ANDRKO	0	−	−	ND	Partial	No
	Tfm	?	?	−	Yes	Partial	ND

+, Increased; (+), increased in old mice; −, decreased; 0, no effect; minor, effect of very high but not physiological concentrations of estrogen; ?, conflicting results presented depending on which gonadal intact female ERKO model analyzed and the results are difficult to interpret due to increased sex steroid levels; ND, not determined; gx, gonadectomy; E, treatment with physiological levels of estrogen; T, treatment with physiological levels of testosterone; DHT, treatment with physiological levels of 5α-dihydrotestosterone. For references, see text.

model (42, 43, 262). In line with observations in ANDRKO mice, Tfm mice have low levels of T and E_2 (as opposed to the high levels of T and E_2 in the Tfm rat), which may confound both models. Sex steroid replacement in orch Tfm mice allows further investigation of the role of the AR in mediating T action in this model. In orch Tfm mice, cortical bone appears to be completely unresponsive to T action, whereas cancellous bone is preserved by T (226) (Table 9). Again, these data suggest that T action on cortical bone is at least partly AR-dependent, whereas T action on cancellous bone may depend on both AR and ER activation.

G. Animal data in support of a dual mode of androgen action on the skeleton

Androgens (both aromatizable and nonaromatizable) are able to maintain skeletal integrity of the cancellous compartment in rodents via the AR, irrespective of age and gender. Estrogens also prevent cancellous bone loss (through activation of the ERα only) in male rodents, even at low concentrations. This has led to our concept of a dual mode of action of androgens on bone via both ERα and the AR, although the relevance of ERβ activation remains uncertain. In addition, androgens are able to stimulate bone formation at the outer periosteal bone surface, at least in male rodents and during skeletal growth. Again, in this process of androgen-mediated periosteal bone formation, both the AR and ERα are involved. In female rodents, on the other hand, both ERα and ERβ are of importance for skeletal development and maintenance.

V. Indirect Mechanisms of Action of Androgens with Skeletal Implications

Sex steroids may differentially and sex-specifically regulate longitudinal and radial bone growth in rodents, leading to the typical sexually dimorphic skeletal changes (longer and broader bones in males) at the end of puberty (see *Section IV*). These opposite effects of androgens and estrogens may depend on stimulation of sex steroid receptors expressed locally in the skeleton (see *Section II.D*). Alternatively, androgens may also indirectly affect skeletal homeostasis through interactions with body growth, body composition, and the GH-IGF-I axis.

A. Androgens, body growth, and body composition

Androgen-induced changes in growth and body composition may have an important impact on skeletal changes: in growing rats, gain in body weight (associated with increased food intake) is impaired after orch in most (33, 47, 205, 207, 219, 224, 271), but not all studies (208, 210, 234), whereas the opposite is observed in the ovx rat (57, 58, 203, 204, 271, 272) (Table 6). These opposite changes in body weight are fully prevented by androgens in male and by estrogens in female gonadectomized growing rodents (Table 7). It is important to emphasize that body weight remains mostly unchanged in older gonadectomized rats (205, 206, 221, 272) (Table 6). Male rats also lose muscle mass and gain fat mass after orch (233, 237). It is therefore tempting to speculate that orch-induced muscle loss may lower mechanical strain. Decreases of longitudinal and radial bone growth after orch might therefore essentially represent an adaptation of skeletal modeling during growth to orch-induced changes in mechanical strain rather than a direct action of sex steroids on bone. The effects of gonadectomy-induced changes in body weight and composition on skeletal modeling and remodeling remain hypothetical but can and should be further explored. Conversely, increased skeletal growth in response to androgens (Table 7) might reflect increased mechanical loading. In line with this assumption, actions of T on bone and lean body mass (a surrogate for muscle mass) seem to be closely interrelated, at least in orch rodent models (233).

In addition, several links exist between adipocytes and bone. There is increasing evidence for some adipocytokines, such as leptin, to be the mediators via the central nervous system, in particular the nucleus paraventricularis (273). The communication between cells of the immune system occurs via very similar cytokines and receptors (*e.g.*, RANK-RANKL-OPG) as the ones used by the different bone cells. Moreover, there are several arguments for a direct interaction between immune cells (*e.g.*, B and T lymphocytes) and bone cells (274). Because sex steroids have a direct influence on immune cells (androgens and estrogens influence hematopoiesis and notably B lymphopoiesis), indirect sex steroid effects on bone via immune cells might be plausible but need further exploration.

In humans, androgens stimulate longitudinal growth in the male, whereas estrogens appear to have a biphasic effect in both sexes (6). Low-dose estrogens stimulate the pubertal growth spurt, but higher concentrations inhibit linear growth and promote growth plate closure. Males may therefore be taller and have a higher peak bone mass because they are exposed to relatively lower estrogen concentrations during a longer prepubertal and pubertal period. Although it is assumed that sex steroids have similar actions on radial as on longitudinal growth, this remains to be established in humans.

Puberty in males is characterized not only by increased longitudinal growth but also by a greater gain in muscle mass compared with females (275). Increased muscle mass may result in enhanced mechanical loading, which is considered to be the most important stimulus for skeletal modeling (276). It is therefore tempting to speculate that changes in body composition (more muscle, less fat) contribute to greater bone size in men. However, the extent to which androgens increase muscle and thereby indirectly stimulate skeletal modeling remains unknown. Although T supplementation stimulates fat-free mass and muscle size in hypogonadal men (277), the skeletal implications of this type of anabolic response remain to be established. *In vitro* data support the concept that mechanical strain and sex steroids might impact on bone through similar modes of action. ERα has indeed been shown to mediate the strain-related proliferation of osteoblasts of both sexes (278, 279), but the relevance of this interaction between mechanical strain and sex steroids *in vivo* remains unclear.

B. Androgens and the GH-IGF-I axis

Postnatal growth is primarily regulated by the GH-IGF-I axis. Inhibition of IGF-I action—either directly (280, 281), or indirectly via disruption of the GH receptor (282, 283)—dramatically decreases growth rate in male and female mice, similar to the growth delay observed in GH-deficient or GH-resistant humans (265). This decrease in growth rate affects cortical bone (280, 283) but has no effect on cancellous bone mass (280, 283, 284). Interaction with the GH-IGF-I axis may therefore provide yet another indirect mechanism by which sex steroids regulate sexually dimorphic skeletal changes, including longitudinal bone growth and radial cortical bone growth (166, 285). The concept that a functional GH-IGF-I axis is essential for skeletal sexual dimorphism is supported by observations in the GH receptor or IGF-I knockout mice, which are both characterized by the absence of gender differences in growth rate and skeletal size. Therefore, the male cortical phenotype requires a functional and active GH-IGF-I axis. This axis is already differently imprinted in males and females during the neonatal period (286). Such imprinting depends on neonatal androgen secretion. The importance of androgen programming of the GH-IGF-I system for male growth is supported by observations in androgen-resistant Tfm rats; in these androgen-resistant male rats without neonatal androgen secretion, female-like GH profiles are associated with female-like growth rates and bone size (285) (see also *Section IV*).

During puberty, a rise in GH-IGF-I activity occurs in both sexes. After puberty, serum IGF-I levels tend to be higher in men and male rats compared with females (287, 288), whereas no gender-related difference has been observed in mice (65, 76, 280).

High-dose estrogens lower serum levels of IGF-I in the rat (49, 209, 289), whereas low doses, via a stimulation of GH secretion, are stimulatory in both sexes (237). In humans, androgens indirectly stimulate IGF-I secretion after aromatization into estrogens (290). Furthermore, aromatase inhibitors lower serum IGF-I levels (49), whereas antiestrogens like ICI 182,780 that do not penetrate the blood-brain barrier, do not affect serum IGF-I levels in male rats (59). These findings indicate that androgens are centrally aromatized, followed by an ER-mediated regulation of GH secretion. Estrogens stimulate GH via ERα, as indicated by the lower serum IGF-I in both genders of ERKO mice (see *Section IV*). In addition, the growth-limiting effect of high-dose estrogens seems to depend more on an interaction with the GH-IGF-I axis than the growth-stimulatory action of androgens. Indeed, the expected stimulation of radial bone growth does not occur after ovx in GH-deficient and hypophysectomized female rats, whereas periosteal bone formation and growth rate decrease after orch in GH-deficient male rats (224, 225, 291). In addition, the nonaromatizable androgen DHT may stimulate longitudinal growth in boys suffering from delayed puberty without concomitant increases of serum IGF-I (292).

Taken together, both human and animal data (although limited in number) support the concept that androgens, apart from a direct action on bone cells, also stimulate (skeletal) growth indirectly after aromatization into estrogens and stimulation of pituitary GH secretion.

VI. Effects of Androgens on the Human Skeleton

A. Skeletal consequences of castration, male hypogonadism, and androgen resistance in men

1. Skeletal effects of castration in men. Surgical (orch) or chemical (administration of GnRH agonists) castration induces a complete and sudden decline in the levels of sex steroids in men. Such androgen deprivation therapy in adult men with advanced prostate cancer is followed by rapid bone loss (293), similar to the loss of skeletal integrity in women after surgical ovx or during early menopause. Lumbar spine bone density decreases by about 5–10% within 1 yr after castration (294–302), with a continuing further decrease of bone loss thereafter. More recently, significant bone loss, albeit to a lesser extent, has also been confirmed at appendicular skeletal sites, including the hip (296, 298–301, 303–305) and radius (299, 306, 307). Recent data suggest that many men with prostate carcinoma may already have reduced bone densities before androgen deprivation therapy (308). In these patients, additional bone loss after castration is likely to further increase their risk of osteoporotic fractures (303, 309–312) (Fig. 8). In addition to the decline in bone density, androgen deficiency-induced changes in body composition, including decreased lean body and muscle mass (299, 300, 313), may further enhance fracture risk.

Castration increases bone turnover, as assessed by biochemical parameters of bone resorption and formation (294, 295, 298, 299, 301, 302, 307). These observations indicate that the mechanism of bone loss in androgen-deficient men is similar to that induced by gonadal insufficiency of women or animals. An imbalance in favor of bone resorption induces net bone loss, especially at cancellous bone sites with large remodeling surfaces. Histomorphometric confirmation of this high turnover bone loss in these men is still lacking, but suppression of bone turnover preserves bone density. In particular, the use of bisphosphonates—including etidronate (301), iv pamidronate (298), or zoledronic acid (314)—has been shown to prevent bone loss in patients with prostate carcinoma and should therefore be considered after castration. Overall, these findings support the concept that sex

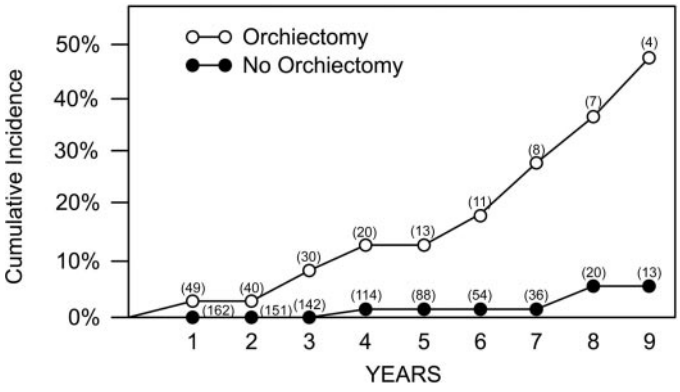


FIG. 8. Cumulative incidence of first osteoporotic fractures in men with prostate cancer with and without orchidectomy. Intervals are times from castration to last follow-up or to first osteoporotic fracture and from diagnosis, respectively. *Numbers in parentheses* indicate men remaining at various intervals. [Reproduced with permission from H. W. Daniell: *J Urol* 157:439–444, 1997 (303).]

steroid deficiency in men, as in women, induces high-turnover osteoporosis.

2. Skeletal effects of male hypogonadism. Hypogonadism is often defined as T levels well below the normal values. Still, this definition of hypogonadism may cause problems in some cases due to the wide range of T levels observed. In this section, we will discuss the skeletal effects of overt male hypogonadism, characterized by sustained low T concentrations (due to testicular and/or hypothalamic-pituitary dysfunction) and clinical manifestations of hypogonadism. In *Section VI.C.2,* the skeletal impact in partially hypogonadal men will be addressed. Men with hypogonadism have significantly lower bone density (in particular at cancellous bone sites like the spine) than age-matched controls (315, 316). Little is known, however, about the impact of male hypogonadism on bone remodeling. Some data suggest that bone resorption and, to a lesser extent, bone formation may be increased in adult hypogonadal men, in line with observations in postmenopausal women (317), whereas other reports have provided histomorphometric evidence for low bone formation in the context of male hypogonadism (318, 319). Deterioration of the trabecular bone architecture of the distal tibia, as determined by high-resolution magnetic resonance micro-imaging, was recently reported in untreated hypogonadal men (320). In the study by Baran *et al.* (318), follow-up biopsies showed increased bone formation during supplementation with T, supporting the concept that T stimulates bone formation. In addition to histomorphometric evidence for a low rate of bone formation, Francis *et al.* (319) also observed calcium malabsorption and low serum 1,25-dihydroxyvitamin D_3 concentrations in hypogonadal men. These findings contrast with other studies, documenting normal calcium and 1,25-dihydroxyvitamin D_3 metabolism in hypogonadal men (317) and suggesting that male hypo-gonadism may affect skeletal homeostasis independently of calcium and vitamin D. Experiments using the orch rat model support this assumption (209).

The extent to which low bone density in the context of male hypogonadism is associated with an increased risk for fracture remains to be clarified. Case control studies report a higher than expected prevalence of hypogonadism in men with spine or hip fracture (321, 322). In men with hip fractures, increased bone resorption (but not the low formation rate) has even been documented to be a direct consequence of their T deficiency (323). However, prospective evidence to establish a cause-and-effect relationship between hypogonadism and future fracture risk is currently lacking.

Male hypogonadism can be due to a variety of diseases, which may all have a specific impact on skeletal integrity. Nevertheless, comparative data suggest similar impairment in bone density in men with different etiologies of hypogonadism, suggesting that hypogonadism *per se* and not the primary disease entity is responsible for the bone loss (324).

In young hypogonadal men, skeletal growth is impaired and, in line with animal data, bone mass and density will be even more severely affected. Decreased radial, spinal, and femoral bone densities and lower peak bone mass have been reported in adult men with delayed puberty (325–327), but areal bone density assessment based on projectional methods should be interpreted with caution. These methods may overestimate the bone mineral deficit during growth because of the associated failure to expand bone during delayed puberty. In this regard, measurements of volumetric density using pQCT are more appropriate. In adult men with a history of late puberty, pQCT fails to show decreased bone density, indicating that impairment of bone size may be more important in these men than changes in bone tissue composition (328).

Isolated hypogonadotropic hypogonadism (IHH) represents the most complete and early form of male hypogonadism. In contrast to most other types of hypogonadotropic hypogonadism, men with IHH have isolated sex steroid deficiency without other metabolic abnormalities, making IHH a good model to examine the effects of sex steroids and sex steroid deficiencies in men. Compared with age-matched controls, patients with IHH have lower bone density at the spine and radius, not only before but also after growth plate closure (329). Interestingly, both areal and volumetric bone density are reduced. This suggests that true bone composition is impaired in the context of IHH. In these patients, assessing bone turnover has produced inconsistent results, demonstrating histomorphometric evidence for low-turnover osteoporosis in some patients (330) but increased levels of markers of bone formation and resorption in others (331).

Lower bone density (at radius and spine) is well documented in hyperprolactinemic hypogonadal men as well (332). Moreover, reversal of the hypogonadism significantly increases cortical bone density, irrespective of the serum levels of prolactin, suggesting that it is T deficiency and not prolactin excess that impairs skeletal homeostasis in these patients (333).

Finally, Klinefelter's syndrome (KS) is the most frequent form of hypergonadotropic hypogonadism. According to most studies, with one exception (334), bone density is decreased in KS (335–339). Low bone density has even been reported in patients who have already been receiving long-term T replacement (338, 339), questioning the role of androgen deficiency as the cause of the bone deficit (as well as the role of androgen replacement to maintain bone density in this patient group) and suggesting other, disease-specific effects on bone that may be independent of the associated hypogonadism. Varying degrees of hypogonadism have been observed in patients with KS, and only those with severe hypogonadism may experience bone loss (334, 337). However, because of the small numbers of subjects and the lack of appropriate control groups in most studies dealing with KS, controversy is likely to persist until large prospective and controlled studies document the skeletal impact of different degrees of hypogonadism (and their response to T replacement).

Recently, the pivotal role of estrogens with respect to skeletal development and maintenance in both sexes has received much attention (4, 5). It is now well-established that estrogens are responsible for skeletal maturation and epiphyseal closure, not only in women but also in men. However, the extent to which other components of bone formation, including trabecular thickening and periosteal formation, are regulated by aromatization of androgens into estrogens or directly by androgens remains unknown. Moreover, the de-

gree of estrogen deficiency during male hypogonadism may vary according to the capacity to aromatize androgens. Patients with very low androgens also have limited capacity for aromatization. Hypogonadism should therefore be regarded as a combination of varying degrees of androgen and estrogen deficiency, which may impact bone differently.

3. Skeletal effects of androgen resistance in men. According to most (340–343) but not all (344) studies, patients with the complete androgen insensitivity syndrome (cAIS) have lower areal bone density at the spine and hip when compared with age- and sex-matched controls. These findings suggest that androgens may also directly stimulate bone density via the AR and not only indirectly, after aromatization into estrogens. However, bone density values in androgen-resistant patients may be confounded by surgical castration and hormone replacement therapy. In line with this assumption, Marcus *et al.* (344) reported that compliance with estrogen is an important determinant of bone density in this patient group (Fig. 9). In addition, estrogen increases bone density at the spine and hip in most patients with cAIS (342, 343), although these measurements have not always been adjusted for their tall stature. Finally, androgen resistance in humans is not associated with impaired longitudinal growth, providing further evidence for the pivotal role of estrogens in longitudinal bone growth and epiphyseal closure in men. Whether androgen resistance in men affects periosteal bone formation, as would be expected from animal research, is not known.

Overall, bone studies in hypogonadal and androgen-resistant men are confounded by varying states of androgen and/or estrogen deficiency. Currently available data are particularly hampered by the inability of areal bone density measurements to distinguish between bone size and composition. The specific role of estrogens and androgens in the regulation of periosteal bone formation in humans also remains unknown (345). Only recently, Ahlborg *et al.* (346) have reported that the increased bone loss after menopause in women is associated with increased periosteal apposition,

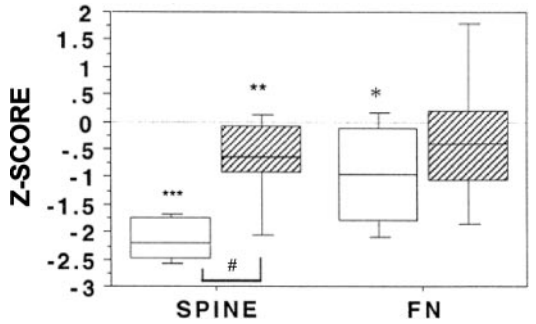


FIG. 9. Box plots of lumbar spine and femoral neck (FN) BMD z-scores for 18 women with cAIS by degree of compliance with estrogen replacement therapy. Values were compared with nominal population means by single sample *t* tests. *Open boxes*, Poor and fair compliance; *hatched boxes*, good and excellent compliance. The *vertical lines of the box* define the 25th and 75th percentiles, and the *error bars* denote the 10th and 90th percentiles. *, $P = 0.06$; **, $P = 0.02$; ***, $P = 0.0001$ (compared with normative mean z-score of 0). #, $P = 0.005$ (good *vs.* fair compliance). [Reproduced with permission from R. Marcus *et al.*: *J Clin Endocrinol Metab* 85:1032–1037, 2000 (344). © The Endocrine Society.]

and accordingly, increased bone size (346, 347). Because bone size is a major determinant of bone strength, further research in this area is urgently required.

B. Skeletal effects of androgens in women

The role of androgens in female skeletal homeostasis has not been well-established. In women, serum androgen concentrations vary considerably; although T concentrations are lower than in men, serum concentrations of other, weaker androgens like androstenedione and DHEA-S are similar (348). Androgens may therefore contribute to clinically relevant differences in bone density between women.

Androgens might stimulate skeletal development during puberty. Most data in support of such a bone-stimulatory action are based on a number of studies, providing evidence for increased density in women with polycystic ovary syndrome (PCOS) (Ref. 349 and references therein). One of the limitations of the PCOS model of androgen excess is the potential confounding by differences in body mass index, body composition, and menstrual irregularities (oligo- and amenorrhea) that may affect skeletal homeostasis independently of the androgen excess and are difficult to control in the case-control design of these studies. Moreover, androgen excess is often defined clinically by the presence of hirsutism, which represents only a rough determinant of androgen exposure in women. Despite these limitations, studies in PCOS have provided increasing and consistent evidence that hirsute women have higher peak bone density than age-matched controls, even after correction for body mass index (350, 351). It is important to note that this increase in bone density has been confirmed by cancellous pQCT and thus reflects real changes in bone tissue composition, rather than changes in bone size. Whether these positive effects relate to AR-mediated action or to aromatization of androgens in estrogens remains unknown. Obese patients with PCOS tend to have higher bone densities than their nonobese counterparts, suggesting that aromatization within fat tissue may be important. It remains to be clarified whether and to what extent other typical metabolic abnormalities associated with PCOS, including low levels of SHBG (with correspondingly increased bioavailable hormone concentrations) and high concentrations of insulin, also affect skeletal homeostasis in PCOS. Finally, differences in body composition may be additional, clinically important mechanical determinants of skeletal integrity. In line with this assumption, site-specific differences in bone density have been observed in patients with PCOS (352).

One particular area of concern relates to the bone safety of some of the treatment regimens in hirsute women, in particular GnRH agonists and AR antagonists, alone or in combination. The AR antagonist flutamide, when administered alone, does not appear to induce changes in lumbar spine density (353), whereas another AR antagonist, spironolactone, when combined with linesterol, induces a decline in bone density at the same site (354). However, these findings are based on uncontrolled, short-term studies. Suppression of estrogen and androgen levels using GnRH agonist therapy induces postmenopausal-like bone loss in hirsute women. As expected, this bone loss is prevented by estrogen-progestin

replacement therapy (355). More surprisingly, the AR antagonist spironolactone (but not flutamide) maintained bone density in hirsute women treated with GnRH agonists during a 6-month study (356). The mechanism of this bone-sparing effect remains unclear and is in contrast to the earlier reported bone loss during spironolactone treatment in women who are not receiving a GnRH agonist (354).

In postmenopausal women, a potential bone-sparing action of androgens is less documented. Menopause is associated with a 70% decline in the circulating levels of adrenal androgens (especially DHEA-S) (348, 357), but the extent to which this decline contributes to bone loss and fracture risk has not been assessed prospectively. In cross-sectional studies, no consistent relationship has emerged between serum levels of DHEA-S and bone density (358, 359). Moreover, it remains unclear to what extent these adrenal androgens have direct AR-mediated skeletal effects or mainly represent a source for aromatization into estrogens.

C. Skeletal effects of androgen replacement

Although the skeleton of prepubertal boys already responds to T, as shown in an elegant calcium kinetics study by Mauras *et al.* (360), the benefits of T replacement have been most extensively documented in hypogonadal men. The interest of the scientific community in the potential benefits of androgen administration currently extends to young men with delayed puberty, elderly men with partial androgen deficiency, eugonadal men and glucocorticoid-treated men suffering from osteoporosis, and even postmenopausal women. Therefore, we will discuss the skeletal effects of T in the well-established indication of male hypogonadism and other new potential indications separately.

1. Skeletal effects of androgen replacement in male hypogonadism. Long-term prospective and retrospective data with respect to the efficacy of T replacement in male hypogonadism indicate that, after a 2-yr initial increase, bone density is maintained (Fig. 10). However, the reported gain in bone density is highly variable (324, 333, 361–366), and an increase has not even been documented in all studies (367). This large variation in effects on bone density may relate to differences in treatment duration as well as to different methodologies and sites to assess bone density. Table 11 summarizes the effects of androgens on bone in hypogonadal men. Overall, cancellous bone sites like the spine are more responsive than cortical sites like the radius or hip (363, 365), and measurements based on pQCT or QCT (which do not fully correct for possible androgen-induced changes in fat) show much greater responses than approaches using DXA, dual photon absorptiometry (DPA), or single photon absorptiometry (SPA).

The overall impression is that T, similar to estrogen in postmenopausal women, primarily acts as an antiresorptive rather than an anabolic agent. Not only are the reported increases and the pattern of bone density gain in line with this assumption, but also the observed responses of biochemical bone turnover markers. Most studies show a consistent decline in bone resorption after T replacement in hypogonadal men (331, 363, 365–367). A similar decrease in the levels of markers of bone formation, as would be expected during treatment with a typical antiresorptive agent, has been observed in some studies (363, 365). However, in various trials, markers of bone formation have been reported to initially increase after T replacement in hypogonadal men (362, 366–368) or human choriogonadotropin (331). In addition to its effects on bone turnover, androgen replacement in hypogonadal men may have beneficial effects on body composition, which in turn may protect bone. Several studies have reported increases in lean body mass (331, 363, 367, 369) or muscle strength (277, 367, 368, 370) after im or transdermal T replacement, but again, other trials have been unable to confirm these findings (365, 371).

The mode of T administration may be an important determinant of its efficacy and safety. Although im administration of T may result in supraphysiological effects on bone, as suggested by a 5% (uncontrolled) gain of lumbar bone density within 2 yr in eugonadal T-treated men with osteoporosis (372), transdermal administration tends to reach more physiological concentrations of T (369). Preliminary retrospective data have not revealed differences in efficacy (or safety) between these two modes of administration (364),

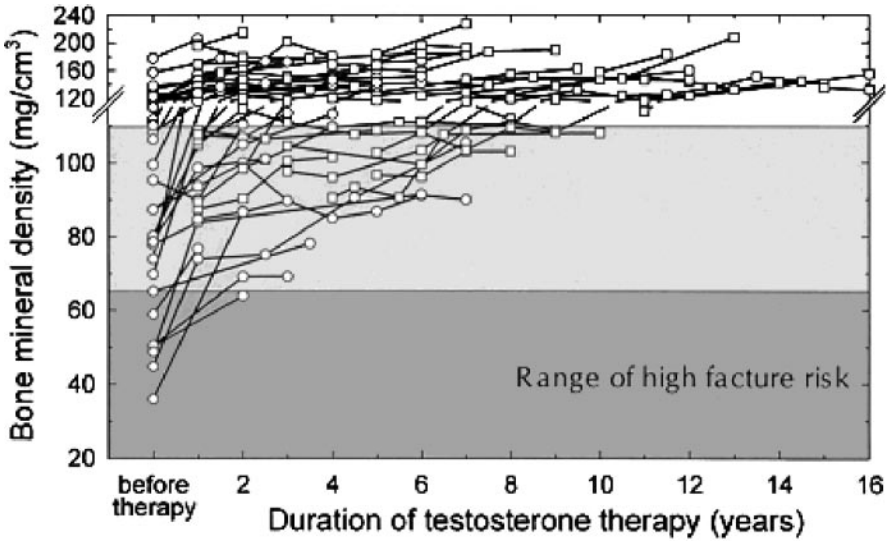


FIG. 10. Increase in BMD during long-term T substitution therapy up to 16 yr in 72 hypogonadal patients. *Circles* indicate hypogonadal patients with first pQCT measurement before initiation of T substitution therapy; *squares* show those patients already receiving T therapy at the first QCT. The *dark-shaded area* indicates the range of high fracture risk, the *unshaded area* indicates the range without significant fracture risk, and the *light-shaded area* indicates the intermediate range in which fractures may occur. [Reproduced with permission from H. M. Behre *et al.*: *J Clin Endocrinol Metab* 82:2386–2390, 1997 (364). © The Endocrine Society.]

TABLE 11. Effects of androgen replacement on bone density in hypogonadal men

First author, year (Ref.)	Study group (n)	Duration (yr)	Type of replacement	Method of measurement	Effect on BMD
Prospective					
Finkelstein *et al.*, 1989 (330)	IHH (21)	1–3	Testosterone enanthate 200 mg every 2 wk, im or hCG or GnRH	QCT spine	↑ (14%) (n = 6, 19–26 yr) = (n = 15, 24–52 yr)
Greenspan *et al.*, 1989 (333)	Hyperprolactinemic hypogonadism (10)	0.5–4	Correction of hyperprolactinemia and hypogonadism by bromocriptine	SPA radius QCT spine	↑ (3%) =
Devogelaer *et al.*, 1992 (361)	Mixed (16)	1–10	Sustanon 250 mg every month, im	SPA radius	↑ (6%) (only cancellous site when closed epiphysis)
Isaia *et al.*, 1992 (362)	Mixed (6)	0.25	Sustanon 250 mg every week, im	DPA spine	↑ (13%)
Katznelson *et al.*, 1996 (363)	Mixed (29)	1.5	Testosterone enanthate 100 mg every week, im	SPA radius QCT spine DXA spine	= ↑ (14%) ↑ (5%)
Wang *et al.*, 1996 (367)	Mixed (34)	0.5	Testosterone cyclodextrin 5 mg 3 times daily, sublingually	DXA total body, hip, spine	=
Snyder *et al.*, 2000 (365)	Mixed (14)	3	Testoderm transdermally, Ca + vit D	DXA spine DXA hip	↑ (8%) ↑ (4%)
Wang *et al.*, 2001 (366)	Mixed (227)	0.5	Androgel 50 or 100 mg or Androderm transdermally	DXA spine DXA hip	↑ (2%) ↑ (1%) (only with 100 mg gel)
Retrospective					
Wong *et al.*, 1993 (338)	KS (14)	2–12	Testosterone undecanoate 3 times daily, orally, or testosterone enanthate every 3 wk, im	DXA total body, hip, spine	Lower than controls
van den Bergh *et al.*, 2001 (339)	KS (52)	1–32	Testosterone undecanoate orally and/or Sustanon im	DXA hip, spine	Lower than controls
Behre *et al.*, 1997 (364)	Mixed (72)	1–16	Testosterone enanthate im, Testoderm transdermally, testosterone undecanoate orally	QCT spine	↑
Leifke *et al.*, 1998 (324)	Mixed (32)	1–7	Testosterone enanthate 250 mg, im	QCT spine	↑

↑, Increased; =, no change; n, number of patients in study group; hCG, human choriogonadotropin.

but prospective comparative trials have not been performed as yet.

For ethical reasons, most T replacement studies in hypogonadal men have been uncontrolled. Consequently, there is little or no information about the natural evolution of bone density in these patients or about the skeletal benefits of T in addition to the benefits provided by calcium and vitamin D. Moreover, the effects of androgen replacement have been studied in small-sized studies of patients with different etiologies and variable degrees of estrogen and androgen deficiency. Lack of efficacy of T replacement in maintaining bone mass has been suggested in studies including KS patients (338, 339). However, this assumption was based on subnormal bone densities found in a small number of patients with KS who were receiving long-term T therapy. It is likely that the need for T replacement and the patient's response in terms of bone density gain will depend on his pretreatment levels of circulating T and bone density. Indeed, according to a large retrospective study, patients with the lowest initial BMD gain most during therapy (364). Of particular importance are findings that the loss of skeletal integrity may be partially irreversible. This is probably the case in patients with IHH who experienced a failure to gain normal bone mass during puberty. In these patients, T is likely to maintain bone density without completely restoring peak bone mass (330).

Finally, the question remains whether the impact of androgen supplementation relates primarily to activation of the AR. Most density-endpoint studies have used T replacement. Because T is an aromatizable androgen, all of these studies may reflect both androgen and estrogen replacement and, thus, do not allow us to draw specific conclusions regarding direct AR-mediated action only. An uncontrolled study in eugonadal men suggests that the beneficial effects of T on bone density may be related more to increases in E_2 levels than to changes in T concentrations (372). No studies on the skeletal effects of the nonaromatizable androgen DHT have been reported in hypogonadal men.

Overall, the beneficial effects of the aromatizable androgen T on bone density are well-established in the context of male hypogonadism. In addition to the bone-sparing effect, a T-induced gain in lean body mass, as reflected in increased muscle mass and strength, may provide an additional benefit. However, it remains unknown to what extent these positive effects of T replacement will ultimately protect hypogonadal men against osteoporosis and osteoporotic fracture occurrence.

2. Skeletal effects of androgen replacement in other indications. Although the overall benefits of T replacement are well-documented in hypogonadal men and although T may not only affect bone and muscle mass but also mood, sexual function, and hematopoiesis (373, 374), the potential benefits of T replacement in indications other than overt hypogonadism are not well-established and remain the subject of considerable debate.

The large group of elderly men with modest or partial degrees of T deficiency, as reflected by low levels of bioavailable T, represents the most important potential target population for T replacement. Aging men are increasingly at risk of bone loss and osteoporotic fracture. However, the extent to which low levels of bioavailable T contribute to age-related bone loss in men remains unknown. In contrast to well-documented correlations between bioavailable E_2 and bone density (4, 5), studies in elderly men have failed to show strong or consistent associations between bioavailable T and bone density (359, 375, 376). Small studies have reported favorable changes on bone markers after T replacement in these men, but again, findings have not been consistent and their clinical significance remains unclear (377–379). Because the relationship between bone density or bone turnover and fracture risk is complex and, in men, largely unsettled, additional research is required to examine whether and to what extent beneficial effects on bone turnover or density may ultimately translate in clinical benefits in terms of fracture risk reduction.

Two randomized double-blind placebo-controlled studies in elderly men with partial androgen deficiency have been large enough and of sufficient duration to evaluate the effects of T on bone density (Table 12). In the trial by Snyder *et al.* (378), the use of transdermal T during 36 months increased bone density, but no additional benefit could be documented compared with the control group receiving calcium and vitamin D supplementation (Fig. 11). In a study by Kenny *et al.* (379), transdermal T maintained bone density, whereas significant bone loss was observed in the placebo group, despite calcium and vitamin D supplementation. It is possible that both studies tend to underestimate the potential beneficial effect of T. As indicated, the placebo groups in both studies were supplemented with calcium and vitamin D, and this may have partially blunted any age-associated bone loss. In addition, a significant proportion of the men included in the Snyder study (378) were not really hypogonadal but had T levels in the low normal range. A *post hoc* analysis has highlighted the importance of pretreatment androgen status on treatment outcome: the lower the pretreatment serum T levels, the greater the effect of T on bone density. In addition, both studies were performed with transdermal T, which might be less effective than im T, the more commonly used

TABLE 12. Effects of androgen replacement on bone density in men with partial androgen deficiency

First author, year (Ref.)	Study group (n)	Duration (yr)	Type of replacement	Method of measurement	Effect on BMD
Snyder *et al.*, 1999 (378)	50	3	Testoderm transdermally, Ca + vit D	DXA spine	↑ (Also in placebo group)
				DXA hip	=
Kenny *et al.*, 2001 (379)	44	1	Androderm transdermally, Ca + vit D	DXA total body, spine, hip	= (No further loss)

↑, Increased; =, no change; vit, vitamin; n, number of patients in study group.

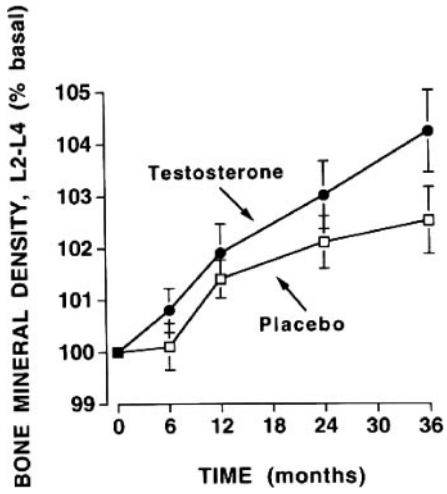


FIG. 11. Mean (± SE) BMD of the lumbar spine (L2–L4) as a percentage of the basal value in 108 men over 65 yr of age who were treated with either T or placebo (54 men each). BMD increased significantly ($P < 0.001$) from 0–36 months in both groups, but the increase was not significantly different between the two groups at 36 months. [Reproduced with permission from P. J. Snyder *et al.*: *J Clin Endocrinol Metab* 84:1966–1972, 1999 (378). © The Endocrine Society.

androgen in hypogonadal men. Finally, these studies do not answer the critically important question about a potential threshold serum T level that may warrant T replacement in elderly men. Animal data suggest that it may be lower than the lower range of young normal men (233).

More recently, the effects of the nonaromatizable androgen DHT have also been evaluated in men with serum T levels in the low normal range. However, this study was of short duration (3 months) and only evaluated one marker of bone turnover (osteocalcin), which was not affected (371).

T replacement might be of more benefit to men who have both T deficiency and an additional increase in the risk for osteoporosis. A placebo-controlled cross-over study in glucocorticoid-treated men suffering from osteoporosis reported a 5% gain in lumbar spine bone density after 1 yr of im administration of T (380). However, no long-term data are available on the effects of T supplementation on bone loss and fracture risk in steroid-induced osteoporosis.

Delayed puberty represents another potential indication for T therapy. A 6-month study in a small number of adolescents with delayed puberty suggests that treatment with a low dose of T may increase bone density. However, the ultimate impact of this treatment on adult bone density was not documented (326).

Finally, in postmenopausal osteoporosis, the potential role of T administration remains to be defined as well (381, 382). The combination of low-dose T (or methyl-T) and estrogen administration may reverse the inhibitory effects of estrogen on biochemical markers of bone formation (383). Limited data also suggest that combined estrogen and T replacement may result in an additional increase in bone density compared with estrogen alone (382, 384). However, the potential side effects of long-term androgen replacement are a concern and, as long as long-term safety and efficacy data are lacking, androgen and estrogen combination therapy cannot be recommended in postmenopausal women.

In recent years, several studies have addressed the skeletal effects of the weaker androgen DHEA. A placebo-controlled double-blind study in elderly women (>70 yr) suggested that 50 mg daily of DHEA increased serum levels of DHEA-S, with a concomitant decline in biochemical markers of bone resorption and a modest increase in bone density at some sites (385). Such beneficial effects of DHEA have not been confirmed in men (385), except in one uncontrolled small study (386). The women and men included in these studies had variable concentrations of DHEA-S at baseline. It is plausible that women and men with the lowest serum levels of DHEA would have the greatest benefit, but this remains to be established. In these trials, DHEA treatment increased T and E_2 concentrations (385–387), indicating that DHEA may act as a prohormone for these more potent sex steroids. However, further prospective controlled long-term research has to determine the bone-sparing potential of DHEA, alone or in combination with established osteoporotic treatments.

In summary, current scientific evidence does not allow recommending T replacement or other androgens to promote bone health in indications other than male hypogonadism.

D. Skeletal effects of selective modulation of androgen and estrogen action in men

In line with published animal data, AR antagonists, ER antagonists, SERMs, aromatase inhibitors, and type II 5α-reductase inhibitors all potentially interfere with male skeletal homeostasis. However, few studies have investigated the potential skeletal benefits and side effects of these drugs in men.

Finasteride, a type II 5α-reductase inhibitor, does not decrease vertebral bone density (388) or increase bone turnover markers (35) in men suffering from benign prostate hyperplasia, according to two small-sized short-term studies. In accordance, the recent finding that type I 5α-reductase is predominantly expressed in osteoblasts (30) supports the absence of effects on bone in these clinical studies with a type II 5α-reductase inhibitor. These findings suggest that selective interference with the AR pathway in men may be relatively safe with respect to bone, but data remain scarce. Therefore, surveillance of bone density still remains advisable, especially in men with clinical risk factors for osteoporosis.

The skeletal risks and benefits of selective interaction with the ER pathway in men remain unsettled as well. Interfering with estrogen production in men is likely to be associated with deleterious effects on bone, as reflected in the animal studies. In an uncontrolled study, administration of the aromatase inhibitor anastrazole to elderly men for 9 wk was associated with an increase in bone resorption (389). In contrast, estrogen suppression via administration of anastrazole to young male adolescents did not affect calcium kinetics (390). Long-term studies should further clarify the impact of aromatase inhibitors on skeletal integrity in men.

In agreement with animal data, high-dose estrogens are bone-sparing in selected populations such as patients with prostate carcinoma after surgical castration (306) and male-to-female transsexuals receiving chemical castration (391, 392). In elderly men, preliminary evidence suggests that

short-term low-dose estrogen may reduce bone resorption as assessed by biochemical markers (393). However, because of its potential feminizing side effects, this type of therapy cannot be recommended. In contrast, SERMs could potentially have a more acceptable risk-benefit profile in men. However, Doran *et al.* (394) recently failed to show a significant bone-sparing effect of raloxifene in elderly men, but this conclusion was based on a short-term evaluation of bone turnover over a 6-month period. Further studies are needed to explore the potential skeletal benefit of selective stimulation of the ER pathway in men. It remains a tempting hypothesis that selected populations of elderly men, particularly those with low serum estrogen concentrations, would benefit from treatment with SERMs. This assumption is supported by the observation that raloxifene suppresses bone resorption in elderly men with low E_2 levels (394). A potential alternative is the recently reported gender-neutral synthetic steroid, estren, which increases bone mass without affecting reproductive organs in both male and female rodents (395, 396). Whether estren will have similar beneficial effects for the treatment of osteoporosis in humans remains to be determined.

The relative importance of androgens and estrogens for bone turnover in men still remains unresolved. Falahati-Nini *et al.* (397) first studied elderly men under conditions of physiological T and E_2 replacement and then assessed the impact on bone turnover of withdrawing both T and E_2, withdrawing either T or E_2, or continuing both during 3 wk. Their findings establish E_2 as the dominant sex steroid regulating bone resorption in normal elderly men, whereas both T and E_2 independently maintain bone formation. More recently, an analogous pharmacological intervention study was designed by Leder *et al.* (398), inducing combined T and E_2 deficiency, T and E_2 sufficiency, or selective E_2 deficiency in young men during a longer experimental period (12 wk). Both androgens and estrogens appeared to be independent mediators of bone resorption in young adult men and may have similar effects on osteoblast function as well. The differences reported between the two studies most likely represent differences in study subjects or study design, or the (in)ability to separate direct effects of sex steroid deprivation on osteoblast function from indirect ones (remodeling-coupled increases in osteoblast activity). Longer-term studies are needed for a comprehensive picture of the relative role of sex steroids in regulating bone turnover in men.

VII. General Conclusions

Bone development and growth are similar in boys and girls up to the start of puberty. Thereafter, skeletal sexual dimorphism evolves with a greater bone mass in adult males than in adult females. The volumetric or true density of bone is, however, similar in both sexes. Men have more bone because of greater bone volume as a result of higher periosteal bone formation rates. A similar sexual dimorphism is also observed in many other species (*e.g.*, rodents), and a wide variety of data suggest androgens and estrogens to be the hormones responsible for this sexual dimorphism.

After a period of peak bone mass, age-related bone loss occurs in both genders, but men experience less age-related net bone loss, again in contrast to the accelerated bone resorption in women. Androgen deficiency in men induces cancellous bone loss similar to estrogen deficiency in postmenopausal women. The histological and biochemical changes induced by castration in men are, again, similar to changes observed in postmenopausal women; the rate of bone remodeling is increased after loss of sex steroids, resulting in enhanced osteoclastogenesis and an increase in the number of osteoblast progenitors, with the former exceeding the latter. This imbalance between resorption and formation, together with a delay of osteoclast apoptosis, is responsible for a decrease in trabecular bone volume, thickness, and connectivity (198, 395).

Recent studies using various sex steroid-related transgenic mouse models as well as selective pharmacological modulations of the different pathways of androgen action have expanded our understanding of the relative roles of the AR, ERα, and ERβ in mediating the effects of androgens on the skeleton. Androgen action on bone differs according to the skeletal compartment, species, sex, and maturation or age (Table 13). In all species investigated thus far, androgens as well as estrogens maintain cancellous bone mass and integrity, regardless of age or sex. Sex steroids reduce cancellous bone turnover primarily via a down-regulation of osteoclastogenesis after interaction with bone marrow osteoblast precursor cells (and maybe also via direct action on osteoclasts). Moreover, recent data indicate that androgens as well as estrogens also induce apoptosis of osteoclasts and prevent osteoblast apoptosis. Although androgens via the AR and estrogens via the ERs can induce these effects, it is less clear what the relative contribution of each system is in the normal life cycle of male and female animals and humans. Recent

TABLE 13. General overview of androgen action on bone in males

	Cancellous bone	Cortical bone	
		Longitudinal growth	Radial growth
Effect of androgen action	Bone-sparing	Growth-stimulating	Growth-stimulating
Sex specific	No	?	Yes (only documented in rodents)
Age specific	No	During puberty	During puberty/after puberty?
Species specific	No	Yes (epiphyseal closure only in humans)	?
Mechanism of action	Similar to estrogens: decrease of cancellous bone turnover	Similar to estrogens: stimulation of endochondral bone formation/ epiphyseal closure (biphasic effect)	Opposite to estrogen: stimulation of periosteal bone formation
Receptors	AR and ERα	ERα	AR and ERα

?, Conflicting results reported. For references, see text.

data suggest that androgen action on cancellous bone depends (only) on (local) aromatization of androgens into estrogens. However, at least in rodents, androgen action on cancellous bone can be directly mediated via AR activation, even in the absence of ERs. These data establish the dual mode of action of T via both the AR and ERα in males (Table 13). In contrast, estrogen effects on cancellous bone are always mediated via ERα. In females, ERβ is involved in longitudinal and radial bone growth as well as in the regulation of cancellous bone.

Androgens increase cortical bone size via stimulation of both longitudinal and radial growth. First, androgens, like estrogens, have a biphasic effect on endochondral bone formation; at the start of puberty, sex steroids stimulate endochondral bone formation, whereas they induce epiphyseal closure at the end of puberty. Androgen action on growth plate closure is, however, clearly mediated via aromatization in estrogens and interaction with ERα (Table 13). Indeed, the bone phenotype of men suffering from estrogen deficiency (due to a mutation in the aromatase gene) or resistance (secondary to a mutation in the ERα gene) is characterized by the absence of a pubertal growth spurt and delayed epiphyseal closure. In growing rodents, who do not experience epiphyseal closure, androgens and estrogens seem to stimulate longitudinal growth via ERα in the growth plate. Low concentrations of estrogens (as present in males) appear to be stimulatory, whereas higher concentrations of estrogens (as in females) are inhibitory for longitudinal bone growth. The role, if any, of AR stimulation with respect to longitudinal growth and epiphyseal closure is not well-established in any species.

Androgens increase radial growth, whereas estrogens decrease periosteal bone formation. However, such action has only been documented in rodents and poorly or only partially in humans (346). Androgens stimulate periosteal bone formation in male rodents during puberty (Table 13). Whether androgens also stimulate periosteal bone formation after puberty remains to be clarified. This latter effect of androgens may be important because bone strength in males seems to be determined by relatively higher periosteal bone formation and, therefore, greater bone dimensions, relative to muscle mass at older age. Indeed, because volumetric bone density is similar in both genders and bone fragility and subsequent fracture incidence is greater in females, bone size is a major determinant of bone strength. Understanding the hormonal and humoral mechanisms of periosteal bone growth or, more generally, the growth and maintenance of the cortical area is of major importance. However, little is known about the differences in cellular behavior of the two major bone compartments (cancellous and cortical), and the lack of understanding of the effects of sex steroid hormones on these two compartments is only a reflection of this statement. Indeed, it remains unclear to what extent androgens directly interact with (periosteal) osteoblasts or their precursors or, alternatively, stimulate osteoblasts secondary to mechanical loading via androgen-mediated increases of body growth and muscle. The relative importance of direct effects of androgens via local sex steroid receptors in osteoblasts might soon be further clarified because mice with osteoblast-specific inactivations of the AR, ERα, and ERβ are currently being developed. Experiments in mice suggest that both the AR and ERα pathways are involved in androgen action on radial bone growth in males, but the relative importance and interaction between both pathways is unclear (Table 13). There are conflicting data from rodent studies; most data indicate that the AR is the mediator of periosteal expansion (Tfm rats and ANDRKO mice). However, ERα stimulation (in the absence of ERβ) can also stimulate periosteal growth and aromatase inhibitors in the presence of the AR, and normal androgen levels inhibit periosteal growth in rats. A possible scenario, still to be confirmed, would be that periosteal expansion can be achieved by AR activation and, to a lesser extent, by ERα but is inhibited by ERβ activated by relatively high estrogen concentrations. This hypothesis could explain why bone volume is arrested earlier in girls than boys during puberty and why periosteal expansion reoccurs in postmenopausal women. The molecular mechanism of action, even whether this occurs via direct or indirect mechanisms, and the role of the GH-IGF-I axis all have to be further clarified.

Treatment of severe androgen deficiency with androgen replacement therapy is beneficial for bone. Whether replacement therapy is also warranted in partially androgen-deficient elderly men is not unequivocally shown, and the overall effects on all target tissues and quality of life and survival should be further explored. Selective AR modulators, combining agonistic effects on bone and other target tissues (endothelium, brain, *etc.*) and antagonistic or neutral effects on prostate, might be equally attractive as SERMs for women. In view of the essential role of ERα on cancellous bone, some SERMs with the appropriate tissue selectivity might even be useful for elderly men with osteoporosis. Vice versa, selective AR modulators might be equally interesting for metabolic bone diseases in women. To achieve such goals, the molecular targets of ligand-activated AR/ERs mediating their action on bone morphology and metabolism have to be much better identified. New technologies of genetics and proteomics combined with the availability of a whole set of cell-specific transgenic mice may soon provide new insights.

In summary, the AR is present in nearly all bone cells, and the major androgen, T, has a major impact on skeletal growth and maintenance, not only via signaling through the AR, but also via ERs. During skeletal remodeling, both receptor pathways generate similar and additive effects on bone. For skeletal longitudinal growth, ERα is the crucial pathway, whereas for periosteal growth, activation of both the AR and ERα appears to be relevant.

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