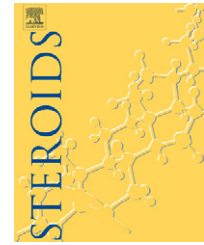


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## Androgens and bone<sup>☆</sup>

Bart L. Clarke<sup>a,\*</sup>, Sundeep Khosla<sup>b,1</sup>

<sup>a</sup> Mayo Clinic W18-A, 200 1st Street SW, Rochester, MN 55905, USA

<sup>b</sup> Mayo Clinic Endocrine Research Unit, Guggenheim 7, 200 1st Street SW, Rochester, MN 55905, USA

### ARTICLE INFO

#### Article history:

Published on line 17 October 2008

#### Keywords:

Androgens  
Androgen receptor  
Testosterone  
DHEA  
DHEAS  
Osteoporosis

### ABSTRACT

Testosterone is the major gonadal sex steroid produced by the testes in men. Testosterone is also produced in smaller amounts by the ovaries in women. The adrenal glands produce the weaker androgens dehydroepiandrosterone, dehydroepiandrosterone sulfate, and androstenedione. These androgens collectively affect skeletal homeostasis throughout life in both men and women, particularly at puberty and during adult life. Because testosterone can be metabolized to estradiol by the aromatase enzyme, there has been controversy as to which gonadal sex steroid has the greater skeletal effect. The current evidence suggests that estradiol plays a greater role in maintenance of skeletal health than testosterone, but that androgens also have direct beneficial effects on bone. Supraphysiological levels of testosterone likely have similar effects on bone as lower levels via direct interaction with androgen receptors, as well as effects mediated by estrogen receptors after aromatization to estradiol. Whether high doses of synthetic, non-aromatizable androgens may, in fact, be detrimental to bone due to suppression of endogenous testosterone (and estrogen) levels is a potential concern that warrants further study.

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## 1. Introduction

Androgens are critical for differentiation of male gonadal structures prior to birth, for sexual maturation during puberty, and for maintenance of male secondary sexual characteristics and genital function, including spermatogenesis, in adulthood. Because men have larger skeletons and greater muscle mass than women, it is assumed that this sexual dimorphism is due, at least in part, to increased androgen levels in men. The specific role of androgens in muscle, cardiovascular tissue, the central nervous system, and the immune system is being actively investigated, while the role played by androgens in bone is increasingly well understood due to many animal and human studies over the last three decades [1]. Albright and Reifenstein recognized that androgens help prevent bone loss and osteoporosis in aging men, and that androgens play

a role in building the skeleton in young adults [2]. Since that time, because osteoporosis affects more women than men, most research studies on gonadal sex steroids and bone have focused on the effect of androgens on bone. Testosterone is metabolized via the cytochrome P450 aromatase enzyme complex into 17 $\beta$ -estradiol, and increasing evidence indicates that at least part of the effect of androgens on bone is mediated by their aromatization to estrogens [3,4]. Epiphyseal closure in late puberty is dependent predominantly on estrogens in both men and women [5], and estrogens appear to play a greater role than testosterone in preventing bone loss in elderly men [6,7]. Because of these observations, it has been questioned whether androgens play a significant direct role in skeletal physiology, although androgen receptors are present on bone cells, and androgen receptor-mediated actions on bone have been known for several years [8,9].

<sup>☆</sup> Institution from which work originated: Mayo Clinic, 200 1st Street SW, Rochester, MN 55905, USA.

\* Corresponding author. Tel.: +1 507 266 4322; fax: +1 507 284 5745.

E-mail address: [Clarke.Bart@mayo.edu](mailto:Clarke.Bart@mayo.edu) (B.L. Clarke).

<sup>1</sup> Tel.: +1 507 285 6663; fax: +1 507 284 9111.

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doi:10.1016/j.steroids.2008.10.003

This review will summarize the currently recognized actions of androgens on the skeleton during development and aging, and review the available evidence that androgens affect skeletal physiology when used in physiological doses. Given the absence of data on effects of supra-physiological doses of androgens used to enhance athletic performance on bone, one can only speculate on whether these higher doses will have similar effects on bone or other, untoward actions.

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## 2. Androgen receptors in bone cells

Androgen receptors are expressed in cultured epiphyseal chondrocytes [10] and growth plate cartilage cells [11], as detected by immunohistochemistry, binding studies, and *in situ* hybridization. These receptors are expressed in all layers of the human growth plate at different ages [12], but only in the proliferative and early hypertrophic chondrocytes in sexually mature rats, and only in prehypertrophic chondrocytes in older rats [13]. No differences have been detected in androgen receptor expression in growth plate chondrocytes from human males or females [10,12], whereas male rats have increased androgen receptor expression in the growth plate and metaphyseal bone during sexual maturation compared to female rats [13]. In light of these findings, it is likely that androgens directly influence longitudinal bone growth during early puberty and epiphyseal growth plate closure in later puberty. Expression of ER $\alpha$  and ER $\beta$  in the human growth plate makes it possible that androgens also indirectly affect pubertal growth or epiphyseal closure via aromatization to estrogen [14,15].

Androgen receptors and ER $\alpha$  and ER $\beta$  are also reported in osteoblasts using more sensitive detection techniques. Early studies failed to detect these receptors in osteoblasts. Androgen receptors were first detected in cultured human fetal osteoblasts using a nuclear binding assay in 1989 [16]. Subsequent studies have detected androgen receptor mRNA and protein in both osteoblasts and osteocytes [11,17]. The number of binding sites per cell is reported to vary widely from 70 to 14,000 [18], depending on the assay used. This wide variation in binding site number per cell is within the range seen in other androgen target tissues.

Human osteoblastic cells from cortical bone express more androgen receptor mRNA and have greater androgen binding than cells from trabecular bone, but osteoblasts from males and females express similar number of androgen receptors [19]. Most, but not all, studies show that androgens upregulate the expression of androgen receptors in osteoblasts [17,19,20]. ER $\alpha$  and ER $\beta$  are also expressed in osteoblasts and osteocytes. There is no current consensus regarding the relative expression of androgen receptors and ER $\alpha$  and ER $\beta$  during osteoblast or osteocyte differentiation, or their localization within the skeleton.

Androgen receptors have been detected in avian [21] and mouse osteoclasts [22] *in vitro*, and in rat osteoclasts *in vivo* [23], but not yet in human osteoclasts *in vivo* [11]. Because of the decreased evidence for androgen receptors in osteoclasts to date, it is assumed that androgens exert most of their effects on osteoclastogenesis and bone resorption via osteoblasts or osteocytes. However, androgens have been shown to directly promote osteoclast apoptosis in several *in vitro* studies.

Androgen receptors have been found to be expressed by osteoblast precursor bone marrow stromal cells [24], as well as on megakaryocytes and endothelial cells within the bone marrow [25].

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## 3. Synthetic androgen actions on bone

There is little published information on the action or metabolism of synthetic androgens in bone cells, or the mechanism of action of synthetic androgens in bone. Synthetic androgens presumably exert receptor-mediated and nongenomic actions similar to gonadal androgens, although no studies have reported such an effect. Presumably synthetic androgens act via classical androgen receptors in bone similar to gonadal and adrenal androgens.

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## 4. Androgen effects on skeletal cells

Androgens likely stimulate longitudinal bone growth by their direct effects on growth plate chondrocytes. Under strict culture conditions, androgens regulate both proliferation and differentiation of cultured epiphyseal chondrocytes [10]. Testosterone injected directly into the rat growth plate increases the width of the growth plate [26]. Part of the effect of androgens on longitudinal bone growth may be mediated by indirect effects on pituitary function, because androgens modulate the kinetics of growth hormone secretion during puberty [27].

Both testosterone and 5 $\alpha$ -dihydrotestosterone stimulate proliferation of cultured osteoblast precursors in different species [28–30], but their effects on osteoblast differentiation are less clear. Studies have shown stimulatory or inhibitory effects, as well as no effect, on osteoblast expression of alkaline phosphatase, type 1 collagen, osteocalcin, and mineralization of extracellular bone matrix, but the weight of evidence suggests that androgens generally stimulate osteoblast differentiation [28,30,31]. Androgens have also been shown to decrease osteoblast and osteocyte apoptosis.

Androgen effects on bone may also be indirectly mediated by regulation of cytokines and growth factors expressed locally in bone. Androgens upregulate transforming growth factor (TGF)- $\beta$  and insulin-like growth factors (IGFs), which stimulate bone formation [29,30], and downregulate interleukin (IL)-6, which stimulates osteoclastogenesis [32]. Androgens inhibit parathyroid hormone (PTH)- or IL-1-induced prostaglandin (PG)E $_2$  production [33], and PTH-induced cAMP production [34]. Androgens stimulate IL-1 $\beta$  production [35] and enhance the mitogenic effect of fibroblast growth factor (FGF) in cultured osteoblasts [29]. Dihydrotestosterone has been shown to reduce osteoprotegerin (OPG) levels, which could potentially stimulate osteoclast activity [36].

Osteoclasts derived from the bone marrow colony forming unit-granulocyte macrophage (CFU-GM) hematopoietic cell lineage undergo proliferation after orchietomy, presumably mainly due to androgen deficiency. Osteoclast differentiation requires contact with stromal cells of the osteoblastic lineage in the bone marrow microenvironment, and stimulation by receptor activator of nuclear factor  $\kappa$ B ligand (RANKL)

expressed and secreted by osteoblastic cells, which binds to RANK on osteoclasts [37]. RANKL effects on osteoclasts are tightly regulated via secretion of osteoprotegerin (OPG), a decoy receptor for RANKL, by osteoblast precursors.

Androgens appear to exert their bone protective effects, in part, indirectly through osteoblastic cells. Orchiectomy normally results in increased osteoblast precursor cells that indirectly stimulate osteoclast proliferation and activation via RANKL expression, leading to bone loss. Dihydrotestosterone binds directly to androgen receptors on osteoclasts and blocks bone resorption by human, mouse, and avian osteoclasts *in vitro* [11]. In cell culture, androgens have been shown to directly regulate RANKL-induced osteoclast formation [38], osteoclast survival, RANK expression by preosteoclasts, and mature osteoclast function independent of bone marrow stromal osteoblast precursors. In conclusion, androgens appear to be capable of regulating osteoclastogenesis both indirectly and directly.

## 5. Effects of androgens on the human skeleton

### 5.1. Castration

Surgical or chemical orchiectomy, induced by GnRH agonist therapy, result in rapidly decreased gonadal sex steroid levels in men [39]. This decrease in gonadal steroids is associated with rapid bone loss, similar to that seen in women after surgical oophorectomy or early menopause, as well as decreased lean body mass and muscle mass. Rapid bone loss is particularly pronounced at trabecular sites in the skeleton with large remodeling surfaces [40], but also occurs at cortical sites, and is associated with greater increases in biochemical markers of bone resorption than bone formation [41] (Fig. 1). High-turnover bone loss after orchiectomy in men is prevented by bisphosphonates [41].

### 5.2. Male hypogonadism

Men with hypogonadism have decreased serum testosterone levels due to many different causes. There is controversy regarding the lower end of the normal range for testosterone, but levels less than 150 ng/dL are typically associated with signs and symptoms of hypogonadism. Men with low testosterone levels due to primary gonadal failure, secondary pituitary dysfunction, or hypothalamic malfunction, have significantly lower bone density than age- and sex-matched controls, especially at trabecular skeletal sites such as the lumbar spine [42]. Several studies have reported high bone turnover associated with hypogonadism, but other studies show low bone turnover [43,44]. Testosterone replacement may increase bone formation on histomorphometric biopsy [44]. Some studies show that hypogonadism is associated with decreased calcium absorption and decreased serum 1,25-dihydroxyvitamin levels [45].

Hypogonadal men with low testosterone levels with or without decreased bone density have been reported to have increased fracture risk [46–49]. Hypogonadal men with low estradiol levels have also been reported to have increased frac-

ture risk [50,51]. Men with spine or hip fracture have a higher prevalence of hypogonadism than expected [52], and men with hip fracture have increased bone resorption associated with hypogonadism [53]. Male hypogonadism is associated with a variety of disorders, each of which may have unique and independent effects on the skeleton. However, it appears that hypogonadism plays a more significant role in causation of bone loss than specific diseases [54]. Young men with delayed puberty have decreased DXA bone density at the lumbar spine, hip, and distal radius, as well as lower peak bone density [55]. However, volumetric bone density is normal in adult men with a history of delayed puberty, suggesting that the main effect of hypogonadism during skeletal growth is on bone size, rather than bone density [56].

Although estrogens are currently thought to play the dominant role in skeletal maturation and epiphyseal closure in both men and women, androgens play significant direct roles in skeletal development. It is not yet clear whether trabecular thickening or periosteal bone formation is directly affected by androgens, or primarily by estrogens aromatized from androgens [3]. Hypogonadism in men also results in estrogen deficiency of variable degrees, which may be partially compensated for by the ability to aromatize androgens [4]. However, hypogonadal men with very low androgen levels have only a limited ability to produce estrogens by aromatization.

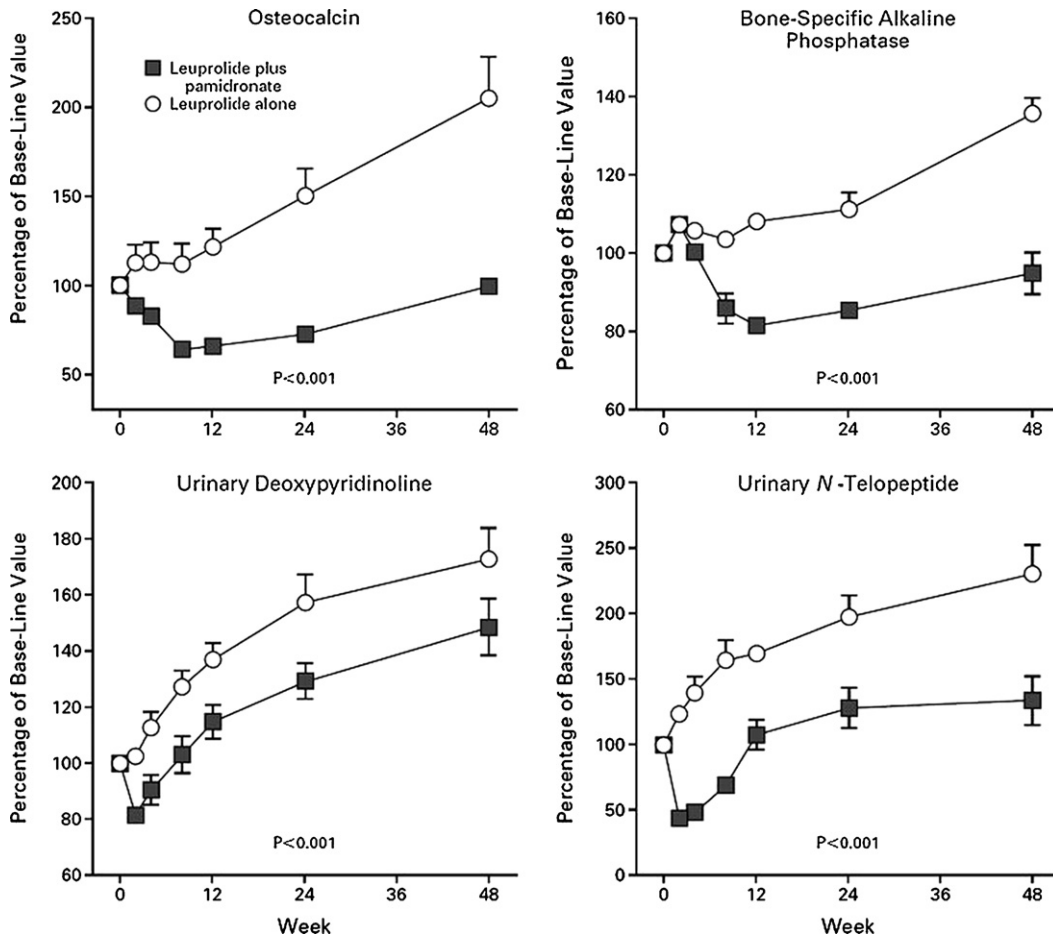
### 5.3. Androgen resistance in men

Men with complete androgen insensitivity syndrome have decreased areal bone density at the lumbar spine and hip compared to age- and sex-matched controls [57], suggesting that androgens exert direct skeletal effects via the androgen receptor, and not just indirectly via aromatization to estrogens (Fig. 2). However, surgical orchiectomy to prevent testicular tumors in this population, followed by hormone therapy, confounds bone density assessment in this population. Estrogen therapy typically increases bone density at the lumbar spine and hip in these men [58], although not all studies have adjusted bone density for the increased stature seen in these men. Androgen resistance is not associated with decreased longitudinal growth in men, but it is not known whether it is associated with decreased periosteal bone apposition.

### 5.4. Skeletal effects of androgens in women

The role of androgens in maintenance of female skeletal health has not been clearly defined. Serum androgen levels vary considerably in women, with serum testosterone levels considerably lower than in men. However, levels of weaker adrenal androgens such as DHEA-S and androstenedione are similar to those in men [59].

It is likely that androgens play a significant role in acquisition and maintenance of bone density in women, particularly at puberty. Women with polycystic ovary syndrome (PCOS), who develop variable hyperandrogenism at puberty [60], have increased peak bone density compared to age-matched controls as assessed by peripheral quantitative CT scanning, even after adjustment for increased body mass index [61]. Women with recognized PCOS commonly have an increased body



**Fig. 1 – Changes in serum concentrations of bone specific alkaline phosphatase and osteocalcin and urinary excretion of deoxyypyridinoline and N-telopeptide in men with prostate cancer treated with leuprolide alone or leuprolide and pamidronate. Values are expressed as the mean ( $\pm$ S.E.) percentage of the baseline value. P values are for the treatment effect according to the repeated-measures analysis of covariance controlled for the baseline value. Standard error bars that are not visible are covered by the symbol. Figure is reproduced from ref. [41] with permission from The Massachusetts Medical Society. Copyright © 2001 Massachusetts Medical Society. All rights reserved.**

mass index, changes in body composition, and menstrual irregularities that affect bone density independently of their hyperandrogenism. It is not yet clear whether androgens play a direct or indirect role in this situation. Obese PCOS patients have higher bone density than nonobese PCOS patients, possibly suggesting that aromatization of androgens in fat may play a significant role, or that increased biomechanical effects on the skeleton may be important. In addition, it is not yet clear whether low SHBG levels, which result in high bioavailable testosterone levels, or high insulin levels play a role in increasing bone density.

Androgen receptor blocker therapy, GnRH agonist therapy, or both for hirsutism, acne, or menstrual irregularities may worsen bone density in some women. In small uncontrolled studies, the androgen receptor blocker flutamide did not cause lumbar spine bone loss [62], whereas combination therapy with the androgen receptor blocker spironolactone and linestrenol caused lumbar spine bone loss [63]. GnRH agonist therapy causes bone loss in hirsute women unless they are simultaneously treated with gonadal hormone ther-

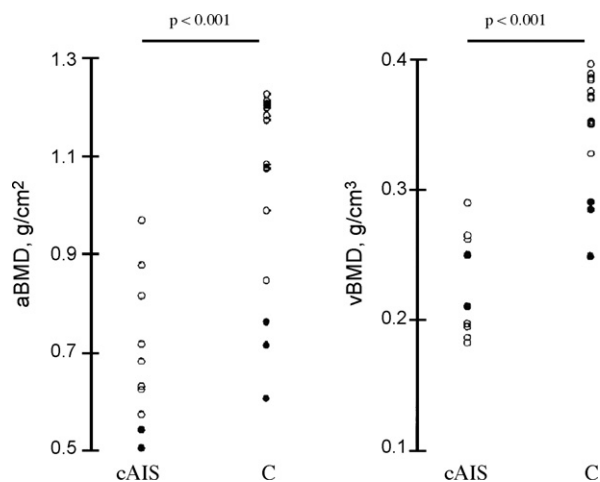
apy [64]. However, combination therapy with a GnRH agonist and spironolactone, but not flutamide, caused bone density to remain stable in hirsute women treated for 6 months [65].

Androgen effects in postmenopausal women are less well documented. Menopause is associated with a 70% decrease in adrenal androgens, including DHEA-S [59], but it is not yet clear that this decrease in weaker androgens contributes to bone loss or fractures. Several cross-sectional studies have not shown an association between circulating DHEAS levels and bone density [66,67]. Adrenal androgens may have direct effects on bone via the androgen receptor, or indirect effects via aromatization.

### 5.5. Skeletal effects of androgen replacement

The skeletal effects of androgen replacement have been most thoroughly studied in hypogonadal men. Testosterone increases skeletal calcium uptake in prepubertal boys [68]. Testosterone effects have also been investigated in small studies of men with delayed puberty, elderly men with partial





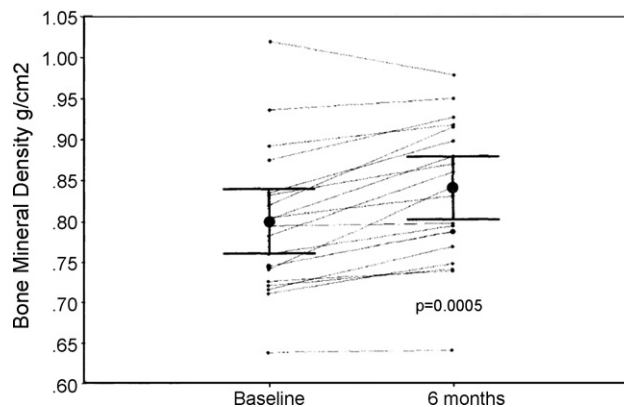
**Fig. 2 – Measured areal and volumetric BMD in patients with complete androgen insensitivity syndrome (cAIS) and in controls (C). In each group, dark circles are prepubertal patients, and open circles are pubertal patients. Figure is reproduced from ref. [57] with permission from S. Karger AG, Basel. Copyright © 1998 S. Karger AG, Basel.**

hypogonadism, eugonadal men, and men with glucocorticoid-induced osteoporosis.

Testosterone therapy in long-term prospective and retrospective studies of male hypogonadism has been shown to increase bone density over about 2 years, particularly at trabecular skeletal sites [69]. After two years, bone density stabilizes, similar to what is seen during therapy with antiresorptive osteoporosis drugs. The increase in bone density in hypogonadal men is highly variable [54,70], and not seen in all studies [71], possibly due to differences in treatment duration, skeletal sites assessed, and study methodology. Quantitative CT (QCT) or peripheral QCT scanning typically demonstrate greater increases in trabecular bone density than DXA assessment [70,72], even though the QCT methods are not able to correct for androgen-induced reduction in marrow fat.

Most studies demonstrate that testosterone treatment decreases markers of bone resorption in hypogonadal men [70,72], and some studies also show decreases in markers of bone formation [70,72]. However, some studies show an initial increase in markers of bone formation in hypogonadal men treated with testosterone [71,73] or human chorionic gonadotropin [74]. Intramuscular or transdermal testosterone therapy in hypogonadal men increases lean body mass [71,74] and muscle strength [73,75] in some studies, but not others [76]. Since intramuscular testosterone injections may produce serum testosterone levels above the normal range, supra-physiological effects on bone potentially could occur in some patients [76]. Transdermal testosterone produces more physiological levels of serum testosterone [75], and is less likely to cause supra-physiological effects. However, retrospective studies have shown no apparent differences in bone density or adverse events in hypogonadal men treated with these forms of testosterone [76] (Fig. 3).

Most studies of testosterone replacement in hypogonadal men have been uncontrolled, relatively short-term, and

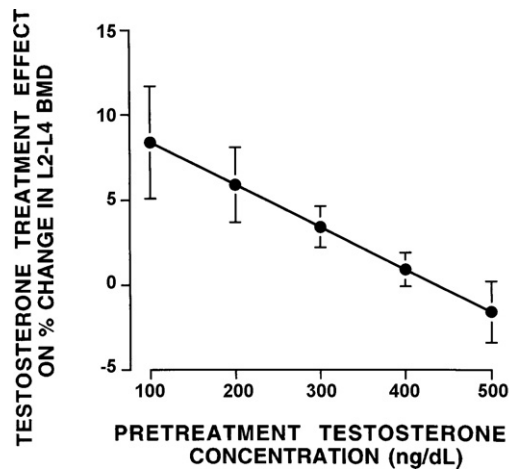


**Fig. 3 – Changes in lumbar spine BMD measurement in individual patients during treatment with testosterone. Error bars show mean  $\pm$ 95% confidence intervals. Figure is reproduced from ref. [76] with permission from Elsevier. Copyright © 1996.**

involved small numbers of subjects with a variety of causes for their hypogonadism. Because most studies are uncontrolled, it is difficult to compare the bone density effects of testosterone replacement to those of calcium and/or vitamin D supplementation alone. Some studies show that testosterone replacement does not prevent bone loss in Klinefelter's syndrome patients [77]. A large retrospective study showed that hypogonadal men with the lowest bone density gain the most bone density during testosterone treatment [69]. Men with idiopathic hypogonadotropic hypogonadism who do not gain bone density during puberty may not be able to replace this later with testosterone replacement [78].

Because almost all studies of hypogonadal men have used aromatizable androgens for hormone replacement, it is difficult to determine whether the benefits seen are due to the testosterone administered or to the estradiol aromatized from testosterone. In one uncontrolled study of eugonadal men, testosterone therapy appeared to exert its beneficial effects mainly via increased serum estrogen levels, because serum estrogen levels increased more than testosterone levels [76]. There have been no reported studies of the skeletal effects of nonaromatizable dihydrotestosterone in hypogonadal men.

Skeletal effects of androgen replacement for indications other than hypogonadism are less well studied. Elderly men with partial androgen deficiency might potentially benefit from testosterone therapy for bone density, muscle strength, general mood, sexual function, and/or hematopoiesis. However, the degree to which low levels of bioavailable testosterone contribute to bone loss in elderly men with partial hypogonadism is unclear, because several studies have failed to show strong or consistent associations between bioavailable testosterone and bone density [67,79,80]. Most studies have shown stronger correlations of estrogen with bone density in elderly men [3,4]. The randomized, double-blind, placebo-controlled trial by Snyder et al. [81] showed that transdermal testosterone therapy increased bone density over 36 months in elderly men with partial androgen deficiency, and that this increase was no greater than in the control group receiving calcium and vitamin D supplementation only. A



**Fig. 4 – The testosterone treatment effect on BMD change during 36 months of testosterone treatment in men over 65 years of age as a function of the pretreatment serum testosterone concentration. The lower the pretreatment serum testosterone concentration, the greater the effect of testosterone treatment on BMD. This relationship was derived from linear regression analysis of the pretreatment serum testosterone concentrations and the increments in BMD of all 108 men in the study who were treated with testosterone or placebo. The values shown are the mean ( $\pm$ S.E.) changes in BMD during the 36 months of treatment in the testosterone-treated subjects minus those in the placebo-treated subjects for pretreatment testosterone concentrations from 100 to 500 ng/dL. The testosterone treatment effect was statistically significant ( $P < 0.01$ ) for pretreatment serum testosterone concentrations of 100–300 ng/dL. Figure is reproduced from ref. [81] with permission from The Endocrine Society. Copyright © 1999, The Endocrine Society.**

significant portion of the men in this study was not truly hypogonadal, but had testosterone values in the lower part of the normal range. Interestingly, post hoc analysis revealed a significant correlation between the baseline testosterone level and increases in BMD following testosterone therapy (Fig. 4), suggesting that men with lower testosterone levels at entry were more likely to respond to testosterone replacement. In a similar study, Kenny et al. [82] showed that transdermal testosterone prevented bone loss, whereas calcium and vitamin D supplementation did not. Both studies showed that men with lower pre-treatment testosterone levels had greater improvement in bone density during testosterone treatment. Importantly, neither study gave convincing evidence for a threshold of serum testosterone necessary to protect bone density.

A single small 3-month study showed that treatment with nonaromatizable dihydrotestosterone did not decrease serum osteocalcin, a marker of bone turnover, in men with low-normal testosterone levels [83]. These findings imply that dihydrotestosterone does not have an effect on skeletal bone turnover and, as discussed later, may have implications for the skeletal impact of non-aromatizable androgens. Intra-

muscular testosterone therapy may be helpful in men with glucocorticoid-induced osteoporosis based on one study [84], but there are no long-term data on the effect of testosterone treatment on bone density or fractures in this population. A small study showed that low-dose testosterone supplementation in young men with delayed puberty caused increased bone density [85]. Combined androgen and estrogen therapy in postmenopausal women, with androgen given as low-dose testosterone or methyltestosterone, may have additional bone density benefit beyond the effect of estrogen alone [86], but sufficient safety and efficacy data are not currently available to recommend this form of combination therapy.

The weak androgen DHEA caused mild improvement in bone density and reduction in markers of bone resorption in a placebo-controlled, double-blind study in elderly women >70 years treated with DHEA 50 mg daily [87]. A small uncontrolled study showed a similar modest benefit of DHEA in men [88]. Subjects in these studies had variable serum DHEA levels at baseline, and serum testosterone and estradiol levels both increased with treatment in both studies, suggesting that DHEA might act as a prohormone for gonadal sex steroids. A more recent prospective randomized, double-blind, placebo-controlled clinical trial of DHEA treatment in 87 elderly men and 57 elderly women with decreased DHEAS levels over two years [89] showed that DHEA 75 mg daily in men and 50 mg daily in women caused <2% increases in femoral neck bone density in the men, and <2% increases in ultradistal radius bone density in the women. Men in the trial who received transdermal testosterone 5 mg daily had a comparable small increase in femoral neck bone density. Bone density did not change at other skeletal sites in either the men or women. These minimal findings suggest that DHEA does not play a major role in age-related bone loss in men or women.

### 5.6. Skeletal effects of selective modulation of androgen receptors in men

Relatively few studies have assessed the skeletal effects of androgen receptor antagonists, estrogen receptor antagonists, selective estrogen receptor modulators (SERMs), aromatase inhibitors, or type II 5 $\alpha$ -reductase inhibitors in men. Two small studies demonstrated that finasteride, a type II 5 $\alpha$ -reductase inhibitor, does not decrease lumbar spine bone density in men treated with finasteride for benign prostate hyperplasia [90,91]. These observations are likely due to the fact that osteoblasts predominantly express type I 5 $\alpha$ -reductase [92]. The aromatase inhibitor anastrozole has been shown to increase bone resorption in older men [93]. Doran et al. [94] showed that the SERM raloxifene did not reduce markers of bone turnover in elderly men treated for six months, although further analysis revealed that the subset of men with low endogenous estradiol levels may have a beneficial skeletal response to raloxifene.

Falahati-Nini et al. [6] showed that estradiol is the dominant gonadal sex steroid regulating bone turnover in healthy elderly men by treating men with physiological testosterone and estradiol replacement for three weeks, and then withdrawing both testosterone and estradiol, testosterone alone, estradiol alone, or neither for an additional 3 weeks. Estra-

diol prevented increased markers of bone turnover more effectively than testosterone in these men, although both testosterone and estradiol were important in maintaining bone formation. A similar study by Leder et al. [7] in younger men over 12 weeks of treatment showed that testosterone and estradiol both independently regulate bone resorption and formation.

### 5.7. Effects of supra-physiological doses of androgens on bone

As noted earlier, there are currently no data on the use of supra-physiological doses of androgens on bone. Thus, the issue of whether high doses of androgens have different effects on bone metabolism than those defined for relatively physiological doses in the above studies remains unclear at present and requires future study. It is unclear, for example, whether beneficial direct or indirect (via aromatization to estrogen) effects of androgens on bone plateau at high androgen levels or not. However, one can speculate that these effects likely plateau since the replacement studies described above have found beneficial skeletal effects of androgens generally only in significantly hypogonadal men and marginal benefits, if any, in men with borderline testosterone levels. Moreover, given the increasing evidence that many of the effects of androgens on bone are due to aromatization to estrogens, it is possible that synthetic, non-aromatizable androgens may even have negative effects on bone metabolism due to suppression of endogenous testosterone (and estrogen) levels. This issue clearly requires further study and is a potential significant concern with steroid doping with non-aromatizable androgen compounds.

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## 6. Summary

Androgens affect bone directly via interactions with androgen receptors, and indirectly via binding to ER $\alpha$  and ER $\beta$  after aromatization in fat or other tissues, or at the local tissue level, with effects dependent on the skeletal site, sex, age, degree of skeletal maturation, and species. Both androgens and estrogens preserve trabecular bone in males and females, regardless of age or species. Androgens and estrogens preserve trabecular bone primarily by decreasing osteoclastogenesis after interacting with bone marrow osteoblast precursors and possibly osteoclasts, and both prevent osteoblast apoptosis and stimulate osteoclast apoptosis. It has been difficult to assess the proportion of trabecular bone loss prevented by androgens and estrogens, but it appears that androgens exert their major effect on trabecular bone by local aromatization to estrogens. However, androgens have direct effects on trabecular bone mediated by androgen receptors in the absence of ER $\alpha$  or ER $\beta$ , at least in rodents. It appears that most, if not all, effects of estrogens on trabecular bone are mediated by ER $\alpha$ , although ER $\beta$  may modulate the action of ER $\alpha$ . ER $\beta$  may mediate estrogen effects on longitudinal and radial bone growth.

Androgens stimulate cortical bone formation by increasing longitudinal bone growth at the epiphyseal growth plate and radial bone growth by periosteal apposition. Androgens

and estrogens have similar biphasic effects on endochondral bone formation. Androgens stimulate bone formation and growth early in puberty, and epiphyseal closure later in puberty, with androgen effects on growth plate closure mediated by aromatization to estrogens and binding to ER $\alpha$ . Low estrogen concentrations in men stimulate longitudinal bone growth, whereas higher estrogen levels inhibit longitudinal bone growth in women. It is likely that estrogen receptors mediate longitudinal bone growth, since there is not much evidence that androgen receptors mediate longitudinal bone growth in any species. Androgens stimulate radial bone growth by increasing periosteal apposition, whereas estrogens decrease such apposition. Most of the evidence for this comes from growing rodents, with post-pubertal effects less clear. In humans, androgens continue to stimulate periosteal bone growth later in life, thereby causing increased bone size relative to muscle mass in older age, and consequently increased bone strength. Given that trabecular and cortical volumetric bone density appear to be only mildly increased in younger women compared to younger men, and mildly decreased in older women compared to older men [95], larger bone size may explain the majority of the increased bone strength in men.

It is not clear whether androgen effects on cortical bone are mediated directly via cortical osteoblasts or indirectly via biomechanical strain due to increased body size and muscle mass. Both the androgen receptor and ER $\alpha$  appear to play a role in radial bone growth in males, with the androgen receptor playing the dominant role, based on data from rats with complete androgen resistance and androgen receptor knock-out mice. However, ER $\alpha$  stimulation causes increased periosteal bone formation in the absence of ER $\beta$ , and treatment of rats with normal androgen levels and normal androgen receptors with aromatase inhibitors inhibits periosteal bone growth. It may be that radial bone growth is mediated predominantly by the androgen receptor, and to a lesser degree by ER $\alpha$ , and inhibited by ER $\beta$  when stimulated by increased estrogen levels. This could explain why skeletal size stops increasing earlier in girls than boys, and why periosteal bone growth increases in postmenopausal women. The GH-IGF-1 system also plays a role in stimulating bone growth.

Treatment of marked hypogonadism in men with testosterone increases bone density and strength, but it is not yet clear that treatment of partial hypogonadism with androgen has the same effect. Selective androgen receptor modulators with stimulatory effects on bone and inhibitory effects on prostate would be beneficial for some men, and might also have application in certain metabolic bone diseases in some women. Tissue selective estrogen receptor modulators with preferential ER $\alpha$  activity in trabecular bone also might be beneficial in some men.

In summary, testosterone and other androgens exert major beneficial effects on bone cells and skeletal growth and homeostasis, with actions mediated by both the androgen receptor and estrogen receptors after aromatization of androgens. Both androgen and estrogen receptors play important roles during bone remodeling. ER $\alpha$  appears to play the dominant role in longitudinal growth of bone, whereas both the androgen receptor and ER $\alpha$  play a role in radial growth of bone. Whether supra-physiological doses of androgens have similar effects

on bone as the physiological effects described here remains unresolved. In addition, whether high doses of synthetic, non-aromatizable androgens may, in fact, be detrimental to bone due to suppression of endogenous testosterone (and estrogen) levels is a potential concern that warrants further study.

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