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Review

Androgens and Bone

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Abstract. Androgen receptors are present at low densities in *osteoblasts*. Androgens are also metabolized in bone. (Non)aromatizable androgens probably induce proliferation of osteoblasts and differentiation. A direct effect of androgens on *osteoclasts* has not been demonstrated. Androgens may however inhibit bone resorption indirectly, by an inhibition of the recruitment of osteoclast precursors from bone marrow, by decreased secretion of interleukin-6 and/or prostaglandin E_2, and/or by an increased sensitivity of marrow cells or osteoblasts for bone resorption stimulating factors such as PTH. The recent demonstration of androgen receptors in bone marrow stromal and osteoclast-like cells opens new perspectives in this respect. During puberty, androgens stimulate bone growth both directly and indirectly. Observations in androgen-resistant animals clearly demonstrated that the sexual dimorphism of bone depends on the presence of a functional androgen receptor. Optimal peak bone mass seems related to an appropriately timed androgen secretion. In adults, androgens are also involved in maintenance of the male skeleton. Androgen replacement may prevent further bone loss in hypogonadal men, however, it seems difficult to fully correct bone mass in these men.

Osteoporosis represents a major health problem in elderly people [1–3]. The cumulative incidence of hip fractures—although lower than in women—is still substantial in elderly men (17% versus 32%) [3, 4]. Estrogen deficiency is a well-established cause of bone loss in postmenopausal women and a major risk factor for both spinal and hip fractures in women [1] whereas estrogen replacement prevents bone loss and reduces fracture risk [5]. Although hypogonadal men also have a lower bone density [6–14], it is not clear whether lower serum androgen concentrations observed in elderly men predispose to osteoporosis. Moreover, it has been difficult to provide evidence for direct androgen effects on bone cells [15, 16]. Nevertheless, in 1948, Allbright and Reifenstein [17] were already convinced that androgen deficiency leads to osteoporosis and that androgen replacement improves calcium balance. Recent research supports their hypothesis: the presence of androgen receptors, androgen metabolism, and androgen effects in bone cells are now established. Animal studies also demonstrated androgen effects on both skeletal growth and maintenance. However, androgens have the unique feature that they may be converted within the target cell into either the nonaromatizable 5α-dihydrotestosterone or into estrogens. The androgen effects on bone may therefore be expressed via activation of the androgen or the estrogen receptor.

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In vitro Evidence for Androgen Action in Bone Homeostasis

Androgen Receptors in Osteoblasts

Androgen, as well as other steroid receptors, is present in osteoblasts, as demonstrated at the protein and the mRNA level [18–21]. Receptor affinities are comparable with those of androgen receptors found in androgen target tissues such as the prostate. Receptor concentrations, however, are very low when compared with typical androgen target tissues. Androgen receptor characteristics in osteoblasts and osteoblast-like cells are summarized in Table 1.

Androgen Metabolism in Bone

Osteoblast-like cells are able to aromatize androgens into estrogens [22, 23]. Human osteoblasts also express 5α-reductase activity [24]. 5-Alpha-reductase activity however, could not be demonstrated in rat periosteal cells, suggesting that testosterone and not 5α-dihydrotestosterone stimulates periosteal bone formation in rats [25]. Earlier studies, however, suggested that 5α-reductase activity was present in crushed bones from both rat and human origin [26, 27]. Therefore, androgens may influence skeletal homeostasis both directly (as testosterone or 5α-dihydrotestosterone via the androgen receptor) and indirectly (after aromatization via the estrogen receptor).

Effect on Proliferation and Differentiation of Osteoblasts

Androgens stimulate proliferation of osteoblasts and osteoblast-like cells *in vitro* according to most [21, 28–30] but not all studies [20]. Androgens also stimulate differentiation of osteoblasts as measured by an increased secretion of collagenous proteins or by increased enzyme activities such as creatine kinase, whereas androgen effects on alkaline phosphatase were not consistent [20, 28–30]. However, the molecular biology of these androgen effects on osteoblasts remains largely unknown. Androgen effects on bone cells may also be sex-specific: only diaphyseal bone cells derived from growing male rats but not from female rats respond to androgens when tested in culture [30]. Androgens may therefore have direct effects on osteoblast proliferation and differentiation during growth in males. Androgen effects on osteoblast differentiation may be mediated by transforming growth factor-β [20, 31] whereas their effects on osteoblast

Table 1. Androgen receptors in osteoblast-like cells

Cell type (ref.)	Origin	Method	Receptor number	Receptor affinity (Kd)
Osteoblast [18]	Human	Nuclear binding assay Northern blot	821 ± 140/cell nucleus	NA
SaOS3 [19]	Human	Cytosol binding assay	1277/cell	1.6–2.5 nM
USO2 [19]	Human	Cytosol binding assay	1605/cell	NA
UMR-1060 [19]	Rat	Cytosol binding assay	74/cell	NA
TE85 [20]	Human	Cytosol binding assay Northern blot	2800/cell	0.66 nM
MC3T3-E1 [21]	Mouse	Whole-cell binding assay	14312 ± 1884/cell	1.12 ± 0.19 nM

NA: not available.

proliferation could be mediated by an increase of IGF-II receptors [31].

Effects on Bone Resorption in Vitro

Androgens inhibit bone resorption directly through the decrease of interleukin-6 production by osteoblasts or bone marrow cells [32–34], through inhibition of prostaglandin E_2 production in tissue culture [35], through inhibition of the parathyroid hormone (PTH) effect on osteoblasts [36], or through inhibition of osteoclastogenesis [32, 33]. The presence of androgen receptors in bone marrow stromal cells [32] and in osteoclast-like multinucleated cells [37] suggest that androgens could directly inhibit bone resorption. The *in vitro* evidence for a direct androgen effect on bone resorption is, however, still preliminary [38] in contrast with clear *in vivo* evidence for inhibitory effects on bone resorption (see Human and Animal Studies). However, inhibitory effects of estrogens on bone resorption seem well established *in vitro* [39–41]. As androgens may be converted into estrogens by skeletal aromatases [22,23,26], their *in vivo* effects may also depend upon their conversion into estrogens. Future *in vitro* studies dealing with androgen effects on bone resorption should therefore always compare the activity of nonaromatizable with aromatizable androgens. Figure 1 shows the different pathways that androgens may use to modulate osteoblasts, bone marrow cells, and osteoclasts.

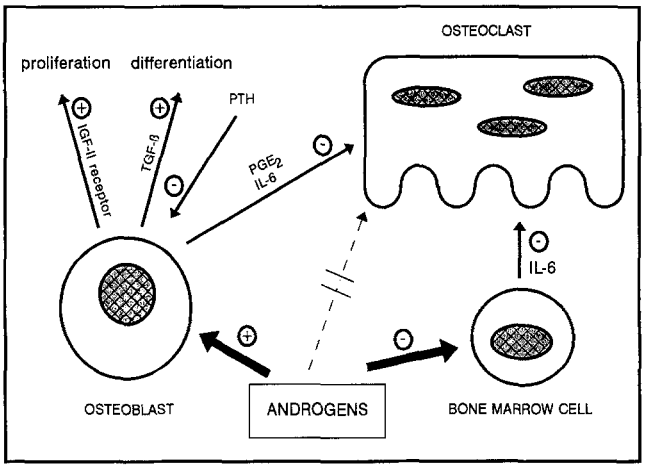

Fig. 1. Model of stimulatory (+) and inhibitory (−) effects of androgens on osteoblast bone marrow cells and on osteoclasts. Proliferation of osteoblast-like cells may be enhanced by increased IGF-II receptors and differentiation by increased TGF-β secretion. Bone resorption may be inhibited by decreased PGE_2 and IL-6 secretion and by decreased effects of PTH. Stromal bone marrow cells may provide and support differentiation of progenitor cells into mature osteoclasts.

Animal Data: Skeletal Effects of Androgen Deficiency and Replacement

Although skeletal effects of estrogen deficiency have received much more attention [42, 43], skeletal effects of androgen deficiency are also well established in animals; the orchidectomized (orch) male rat is certainly the most popular animal model in this regard [44–57]. This model has often been criticized because rats—in contrast to humans—continue to grow during their entire life-span. Therefore, the skeletal effects of androgen deficiency and replacement in rats have to be separated into effects on bone modeling (the combination of bone resorption and formation creating and shaping bone during growth) and bone remodeling (the replacement of old bone by new bone in a nongrowing skeleton). Skeletal growth slows down considerably in aged male rats: the skeleton of aged (more than 12 months old) rats therefore depends more on remodeling than on modeling [58]. Human-like remodeling also occurs in the skeleton of aged rats [59]. Table 2 emphasizes the most important differences between young growing orch rats [45–49, 53] and aged, nongrowing orch male rats [44, 50–52, 54–57]. In conclusion, the young orch male rat may be used as a model for the study of androgen effects on skeletal growth whereas the aged orch male rat model may represent androgen effects on skeletal maintenance.

Table 2. Skeletal changes in androgen-deficient and androgen-resistant rats compared with normal male littermates (references from personal work)

	Androgen-deficient rats		Androgen-resistant rats
	Young	Old	
Ref.	[60]	[43,54]	[59,60]
Body weight	↓	O	↓
Bone growth & modeling	↓	O	↓
Serum IGF-I	O	O	↓
Serum calcium homeostasis	O	O	O
Total bone mass	↓	↓	↓
Cortical bone density	O	↓	O
Cancellous bone density	↓	↓	O
Biomechanical properties	NA	O	O

↓: Decreases; O: Unchanged

Androgen deficiency induces a transient increase in cancellous bone remodeling and an imbalance between bone resorption and formation in both young [46] and aged [44]

male rats. Imbalance between bone resorption and formation results in cancellous and cortical bone loss [44, 45–49, 51, 52, 55, 56]. Whether these skeletal changes also result in biomechanical incompetence is not yet clear [52, 55]. Androgen replacement prevents both the early increase in bone turnover and late decrease of bone mass during androgen deficiency, and this without concomitant changes in bone growth, serum concentrations of calciotropic hormones, or serum IGF-I [44, 46]. 17β-estradiol also prevents bone loss in aged orch male rats suggesting that aromatization of androgens into estrogens may be involved in skeletal maintenance [44]. Moreover, skeletal changes during estrogen deficiency in the ovariectomized rat model and in postmenopausal women are similar to skeletal changes in androgen-deficient rats [43, 60]. Although estrogens may play a significant role in skeletal homeostasis, the nonaromatizable androgen 5α-dihydrotestosterone can also prevent bone loss in both young [46] and aged [44] orch male rats. Cortical thinning of the femoral shaft occurring during the normal aging of male rats [52, 55] is also prevented by androgen therapy [55], suggesting that the age-related decrease of serum testosterone in aged male rats may stimulate endosteal bone resorption. Besides inhibitory effects on bone resorption, androgens have also stimulatory effects on periosteal bone formation in growing male rats [53]. Table 2 summarizes the skeletal changes in orch male rats.

Sexual dimorphism may also explain differences in fracture incidence between sexes [1]. Studies in androgen-resistant rodents suggest that sexual dimorphism of the skeleton depends on sex steroids [61–63]. Although postnatal increase of serum androgen concentrations seems most important for skeletal morphogenesis and sexual dimorphism in mice [61], studies in rats demonstrate that sex steroids also continue to influence skeletal growth and maintenance after this postnatal period [30, 62, 63]. In this respect, androgens mainly stimulate (at least partly by direct stimulation) whereas estrogens inhibit skeletal growth. Testicular-feminized (Tfm), androgen-resistant male rats therefore have a female size skeleton [62]. Endogenous hyperproduction of estrogens [62] also prevents cancellous bone loss in mature Tfm androgen-resistant rats [63], again suggesting that aromatization of androgens into estrogens may be an important metabolic pathway for skeletal maintenance. Recent data (Vanderschueren D. and Bouillon R, Aromatase inhibitor; submitted for publication), showing that administration of an aromatase inhibitor for 4 months induces bone loss in aged male rats, also confirm that aromatization of androgens into estrogens may explain the protective effects of androgens on skeletal maintenance. However, administration of the anti-androgen, flutamide, also induces bone loss in female rats, suggesting that androgens have a direct protective effect on the female skeleton [90]. Skeletal growth, turnover, and maintenance therefore seem to depend on both estrogens and androgens in both female and male rodents.

Human Studies

Hypogonadism as a Risk Factor Compared to Other Risk Factors

Both spinal and hip fractures are less common in men than in women [1–3, 60] although many risk factors are similar for both sexes such as low calcium intake, low body weight, inactivity, heavy cigarette smoking, and excessive drinking whereas obesity seems protective in men as well as in women [64]. Spinal osteoporosis is also associated with underlying illnesses known to affect calcium or bone metabolism such as childhood rickets, gastrectomy, intestinal resection, the use of anticonvulsants, radiation therapy, and liver disease [64]. As in women, hypogonadism is also a risk factor for osteoporosis in men [64, 65]: the prevalence of hypogonadism was fivefold increased in elderly men with hip fractures [65] and was present in about 5% of men admitted for spinal osteoporosis [64]. According to some [12] but not all [66] studies, spinal osteoporosis in hypogonadal men may also be related to poor calcium absorption and low serum 1,25-dihydroxyvitamin D concentrations. The most important risk factor, however, certainly for hip fractures, remains aging [1–4]. Hypogonadism may also be more prevalent in elderly men [67] but is frequently associated with other risk factors such as low physical activity and low lean body mass. Therefore, hypogonadism is one, but only one, of the risk factor for osteoporosis in (elderly) men.

Androgens and Human Growth: Interactions with the Growth Hormone-IGF-I Axis

Androgens stimulate skeletal growth both directly [69, 70] and indirectly through stimulation of the growth hormone-IGF-I axis [71–75]. Indirect and direct androgen effects on skeletal growth have also clinical consequences for the acquisition of peak bone mass: delayed puberty was associated with decreased peak bone mass in men [68], suggesting that an appropriately timed androgen secretion is necessary for achieving optimal peak bone mass. Stimulatory effects of androgens on skeletal growth therefore seem associated with an increase of peak bone mass, explaining why men are not only taller but also have a higher peak bone mass than women protecting them against osteoporosis at older age. Moreover, these androgen effects on the acquisition of peak bone mass also explain why androgen replacement therapy, when started after closure of the growth plate, does not fully correct bone mass in hypogonadal men [10].

Bone Density in Normal Men and its Relationship with Androgens

Although hypogonadism represents a risk factor for osteoporosis in men, it is still not clear if there is a threshold concentration of serum-free testosterone associated with increased risk for osteoporosis. It is also not clear whether androgen replacement would prevent osteoporosis in elderly men although androgen replacement may decrease bone resorption [77]. Serum testosterone, however, does not correlate with bone density in eugonadal adult men [78]. Serum-free testosterone is weakly correlated with bone density in elderly men [79, 80] although this was not confirmed in all studies [81]. Many other variables may contribute to the decreased bone mass of elderly, mildly androgen-deficient men. As discussed earlier, however, studies in aging male rats suggest that the age-related thinning of the femoral cortex could be prevented by androgen replacement [55].

Recently, estrogen insensitivity was described in a 28-year-old male due to a point mutation in the estrogen receptor gene resulting in a premature stop codon. [76]. This syndrome was characterized by incomplete closure of the epiphyses. Although the serum androgen concentration was normal and the free estrogen concentration increased tenfold, his bone mineral density was nevertheless markedly decreased with biochemical indications of increased bone

turnover, indicating that aromatization of androgens is essential for normal bone homeostasis.

Bone Density in Women and its Relationship with Androgens

Serum androgen concentrations are positively correlated with bone density in pre- [82, 83], peri- [84]-, and postmenopausal [85–91] women. Androgen excess in women, however, is also associated with high body mass index and low sex hormone binding globulin concentrations. Increased bone density in hirsute women therefore may also be explained by their higher body mass index and higher free concentrations of sex steroids [83]. The protective effect of androgens on bone density in postmenopausal women may also be important although some studies could not demonstrate any correlation between serum androgens and bone density in these women [86, 89]. Furthermore, it is possible that the protective effects of androgens in women can be explained by their further local aromatization into estrogens [22, 23, 60].

Bone Density in Hypogonadal Men

Hypogonadal men have a low bone density [6–14]. Both cortical and cancellous density are decreased in these men when hypogonadism was present before peak bone mass is reached [10]. However, patients with Klinefelter's syndrome only have a lower cortical bone density [8], possibly related to variable degrees of hypogonadism or to their chromosomal abnormality. The lower cortical bone density seems difficult to correct by androgen replacement after puberty not only in Klinefelter patients [8], but also in patients suffering from hypogonadotropic hypogonadism or in patients suffering from hypogonadism secondary to heterogenous disorders [10, 11]. This suggests that puberty is a determining period for cortical peak bone mass. When hypogonadism occurs after reaching peak bone mass, bone turnover increases and cancellous density decreases [13, 14]. It is not clear what happens with the cortical bone. It is unlikely that bone loss in hypogonadal men is explained simply by changes in calciotropic hormones although hypogonadal men may have a decreased calcitonin release after stimulation [92–94] or may have concomitantly low $1,25(OH)_2D$ concentrations and decreased calcium absorption [12]. Treatment of elderly men suffering from benign prostate hyperplasia with the gonadotrophin-releasing agonist Decapeptyl also induces bone loss [14]. It seems therefore prudent to follow bone density in humans that need long-term antiandrogen treatment.

Conclusions

It is presently unclear to what extent physiological concentrations of androgens express their effect via the androgen receptor (with or without prior 5α-reduction into DHT) or via the estrogen receptor (with prior aromatization). Both pathways can clearly be activated by administration of exogenous sex hormones in animal models. Reduced bone density has however been observed in rats during prolonged treatment with a nonsteroidal aromatase inhibitor and in an adult man with estrogen-receptor deficiency and subsequent estrogen resistance, indicating that most of the androgen effects on bone turnover and bone mass occur via the estrogen receptor.

Whether hypogonadism increases the risk for osteoporotic fractures in men remains controversial. Hypogonadism is a relative rare finding in men and is due to a wide spectrum of disorders. However, clinical experience shows that spinal fractures are unusual in men unless an underlying disease is present, and an undiagnosed hypogonadal disorder is certainly one of the disorders that should be excluded in such circumstances.

Androgens may also influence skeletal homeostasis in women although their mode of action may again be related to their conversion into estrogens. In postmenopausal women, serum androgen concentrations may therefore have significant protective effects on bone mass.

It is still unclear whether or not a gradual and partial decrease of sex hormone concentrations in elderly men lowers their bone density or increases their fracture risk. A threshold concentration for serum testosterone concentrations associated with increased risk for osteoporosis is indeed not established. Moreover, bone density in elderly men may also be lower secondary to many intercurrent factors such as decreased physical activity, calcium intake, muscle mass, and decreased growth hormone-IGF-I secretion. Whether androgen replacement therapy could possibly prevent bone loss in elderly men (as is established for estrogen replacement in women) and even if positive, cost/benefit ratio may be doubtful.

Finally, the understanding of how androgens improve bone mass and protect men against osteoporotic fractures may help in the development of new strategies against osteoporosis.

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