REVIEW

The role of estrogens for male bone health

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Abstract

Sex steroids are important for the growth and maintenance of both the female and the male skeleton. However, the relative contribution of androgens versus estrogens in the regulation of the male skeleton is unclear. Experiments using mice with inactivated sex steroid receptors demonstrated that both activation of the estrogen receptor (ER) α and activation of the androgen receptor result in a stimulatory effect on both the cortical and trabecular bone mass in males. ER β is of no importance for the skeleton in male mice while it modulates the ER α -action on bone in female mice. Previous *in vitro* studies suggest that the membrane G protein-coupled receptor GPR30 also might be a functional ER. Our *in vivo* analyses of GPR30-inactivated mice revealed no function of GPR30 for estrogen-mediated effects on bone mass but it is required for normal regulation of the growth plate and estrogen-mediated insulin-secretion. Recent clinical evidence suggests that a threshold exists for estrogen effects on bone in men: rates of bone loss and fracture risk seem to be the highest in men with estradiol levels below this threshold. Taken together, even though these findings do not exclude an important role for testosterone in male skeletal homeostasis, it is now well-established that estrogens are important regulators of bone health in men.

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Introduction

Peak bone mass is higher in men than in women. After peak bone mass is reached, both men and women lose bone but the bone loss is accelerated after menopause in women. Compared with women, men have greater bone strength, resulting in fewer fractures. This is mainly due to a larger cortical bone size in men compared with women, resulting from more bone deposition at the periosteal bone surface in men during sexual maturation. Sex steroids are important for the skeletal growth and maintenance of both the female and the male skeleton (1). The effects of testosterone (T) can be exerted either directly through the androgen receptor (AR) or indirectly via aromatization to estradiol (E₂) and activation of estrogen receptor (ER) α and/or β (listed as ESR1 and ESR2 in the HUGO Database). All three of these sex steroid receptors are expressed in bone (2–4). In addition, it has recently been suggested from *in vitro* experiments that the membrane G protein-coupled receptor GPR30 (listed as GPER in the HUGO Database) is also a functional ER (Fig. 1) (5). In this review, some of our experimental animal and clinical studies exploring the role of estrogens for the skeleton in males will be discussed.

Estrogen receptors and the male skeleton: lessons from mouse studies

Cortical bone

The larger cortical bone size in males compared with females is, at least partly, due to differences in sex steroid exposure during sexual maturation. Experiments by Venken *et al.* using male AR^{-/-} mice have clearly demonstrated that AR activation results in cortical radial bone expansion (6). In addition, we found that male ER α ^{-/-} but not ER β ^{-/-} mice displayed reduced cortical radial bone growth during sexual maturation, demonstrating that ER α but not ER β activation is also required for a normal cortical radial bone expansion in males during growth (Fig. 2) (7). As the cortical radial bone growth was affected during sexual maturation in the male ER α ^{-/-} mice, one might speculate that the GH/insulin-like growth factor I (IGF-I) axis is involved in this effect. In this context, we and others have shown that a major part of serum IGF-I is liver-derived and that male ER α ^{-/-} mice had a similar cortical bone phenotype as mice with liver-specific IGF-I inactivation (7–11). Importantly, serum IGF-I levels were reduced in the ER α ^{-/-} mice, suggesting that these decreased serum IGF-I levels might mediate the reduced cortical radial bone growth seen in male ER α ^{-/-} mice during

Figure 1 Summary of possible pathways for androgens to regulate bone mass in males. Experimental animal studies using mouse models with inactivation of the androgen receptor (AR), estrogen receptor α (ER α), ER β or the recently suggested ER GPR30 have demonstrated that the AR and ER α , but not ER β or GPR30, are involved in the regulation of cortical and trabecular bone in males.

sexual maturation (Fig. 2). We therefore, proposed that a normal pubertal cortical bone expansion in males might be dependent on an early ER α -mediated stimulation of the GH/IGF-I axis.

Trabecular bone

Similar to that seen in cortical bone, both AR and ER α but not ER β activation regulate trabecular bone mass in male mice (Fig. 1) (12–14). To directly compare the effect of ER activation on trabecular bone *in vivo* with the effect of AR activation, orchidectomized wild type (WT) and ER-inactivated mice were treated with the non-aromatizable androgen dihydrotestosterone (DHT), E $_2$ or vehicle. Both ER α and AR but not ER β activation preserved the amount of trabecular bone. ER α activation resulted both in preserving the thickness

Figure 2 Reduced cortical radial bone growth and serum IGF-I levels in male ER α ^{-/-} and ER α ^{-/-} β ^{-/-} double knock out but not ER β ^{-/-} mice during sexual maturation. The upper panel displays representative mid-diaphyseal peripheral quantitative computer tomography sections of the femur, and in the lower panel, serum IGF-I levels of mice from the different genotypes are shown. * $P < 0.05$, ** $P < 0.01$ versus wild type (WT). (Adapted from Vidal *et al.* (7) with permission from the National Academy of Sciences, USA).

and number of trabeculae. By contrast, AR activation exclusively preserved the number of trabeculae (13). Furthermore, the effects of E $_2$ could not be mediated by the AR, and the effects of DHT were not reduced in ER-inactivated mice (Fig. 3). Thus, the *in vivo* bone-sparing effect of ER α activation is distinct from the bone-sparing effect of AR activation in adult male mice. Because these two pathways are clearly distinct from each other, we proposed that a combined treatment of selective ER and AR modulators might be beneficial in the treatment of male osteoporosis (13). In contrast to the IGF-I - mediated effects of E $_2$ on cortical bone during sexual maturation, the effects of E $_2$ on trabecular maintenance were not associated with the GH/IGF-I axis and seem to result from direct action of E $_2$ on bone.

Estrogen receptor β modulates ER α activity in female but not male mice

Similar to that seen in males, ER α is the principal ER for the regulation of both trabecular and cortical bone in female mice (15–18). However, female ER β inactivated mice have an increased cortical bone area, resulting in a loss of feminization of the cortical bone size (Fig. 4A) (19). Furthermore, female ER β ^{-/-} mice are partly protected against age-related trabecular bone loss (Fig. 4B) (20), suggesting that an ER β antagonist might be useful in the treatment of post-menopausal osteoporosis. Thus, although ER α is the principal ER for the female skeleton, its activity can be modulated by ER β . Interestingly, when we evaluated the effect of E $_2$ in female bone using global gene expression analyses, we found that the magnitude of the stimulatory effect of E $_2$ on estrogen-regulated transcripts was in general more pronounced in the ER β ^{-/-} mice than in the WT mice (21). These data were the first to demonstrate *in vivo* that ER β has the capacity to reduce ER α regulated gene transcription in a global manner. This inhibitory role of ER β on ER α activity is now generally believed to be a major function of ER β in several tissues, including for the regulation of tumorigenesis. These data support a 'yin yang' relationship between ER α and ER β in the female bone and possibly in other tissues. It remains, however, to be determined why ER β modulates the effect of ER α on the skeleton in female but not male mice.

Evaluation of GPR30 as a potential ER in bone

In vitro studies suggest that, besides the two known nuclear ERs α and β , the membrane GPR30 is also a functional ER (5, 22). GPR30 was shown to bind E $_2$ with high affinity *in vitro* (5, 22) and to mediate estrogen-promoted proliferative signaling in an estrogen-sensitive but ER-negative breast cancer cell line

Figure 3 The *in vivo* trabecular bone-sparing effect of ER activation is distinct from the trabecular bone-sparing effect of AR activation in males. To directly compare the effect of ER activation on trabecular bone *in vivo* with the effect of AR activation, 9-month-old orchidectomized (orx) wild type (WT) and ER-inactivated mice (ERα^{-/-}β^{-/-}) were treated with the non-aromatizable androgen dihydrotestosterone (DHT), E₂ or vehicle (V). Trabecular volumetric BMD of the femur is shown, demonstrating that the effects of E₂ could not be mediated by the AR, and the effects of DHT were not reduced in ER-inactivated mice. Values are given as means ± s.e.m. ***P* < 0.01 versus orx + V; ††, *P* < 0.01; †, *P* < 0.05 versus sham. (Reproduced from Movérare *et al.* (13) with permission from the National Academy of Sciences, USA).

(23, 24) and human endometrial cells (25). Furthermore, it was shown *in vitro* to mediate estrogen-dependent kinase activation as well as transcriptional responses (26, 27). However, it remains to be determined if GPR30 is an ER *in vivo* for bone metabolism or other phenotypes. We therefore, recently investigated the possible role of GPR30 as a functional ER for the regulation of skeletal parameters using GPR30 inactivated mice (28). The estrogenic responses on most of the investigated parameters, including increase in bone mass (total body bone mineral density (BMD), spine BMD, trabecular BMD, cortical thickness), increase in uterine weight, fat mass reduction, and increase in bone marrow cellularity, were similar for all the investigated E₂ doses in WT and GPR30 inactivated mice, demonstrating that GPR30 is not required for normal estrogenic responses on several major well-known estrogen-regulated parameters. On the other hand, high dose E₂ treatment reduced longitudinal bone growth, reflected by decreased femur length and distal femur growth plate height, in the WT mice but not in the GPR30 inactivated mice, supporting a role of GPR30 for a normal estrogenic response in the growth plate. In addition, we found that GPR30 mediates E₂-stimulated insulin release *in vivo* (29).

Figure 4 A role of ERβ for the skeleton in female mice. (A) Female ERβ^{-/-} mice have an increased cortical bone area, resulting in a loss of feminization of the cortical bone size. Cortical cross-sectional bone area in the mid-diaphyseal region of the femur in 11-week-old mice. **P* < 0.01 versus wild type (WT). (Adapted from Windahl *et al.* (19)). (B) Female ERβ^{-/-} mice are partly protected against age-dependent trabecular bone loss. Histomorphometric analyses of bone volume/total volume (BV/TV) for the distal metaphyseal region in the femur of 11- and 52-week-old female mice. **P* < 0.05 versus WT. Values are means ± s.e.m. (Adapted from Windahl *et al.* (20) with permission from the American Society for Bone and Mineral Research).

Thus, although GPR30 does not seem to be a functional ER in several major ER-responsive tissues, it mediates E₂-stimulated insulin release and is required for a normal estrogenic response in the growth plate (28, 29).

Serum E₂ and bone growth in men

The traditional view was that estrogens and androgens were the main sex steroids influencing bone maturation and maintenance in women and men respectively. This concept has, however, been challenged in the 1990's by the description of several 'experiments of nature'. In 1994, Smith *et al.* described a 28-year-old man with a naturally occurring mutation in the ERα gene, making him resistant to estrogen (30). This patient had undergone normal early growth and developed normal male secondary sexual characteristics, but had unfused epiphyses and a markedly delayed bone age of 15 years. He was tall and had experienced no pubertal growth spurt, suggesting continued linear growth into adulthood. Moreover, despite normal serum T levels and elevated E₂ levels, he had severe osteopenia associated with elevated markers of bone remodeling. Henceforth, two men with estrogen deficiency, due to mutations in the aromatase (CYP19) gene, were described (31, 32). These individuals had undetectable E₂ levels and almost identical skeletal phenotypes as the estrogen-resistant man, regardless of normal or elevated T levels. But, contrary to the estrogen-resistant male who had no response to estrogen therapy, these aromatase-deficient men responded to estrogen treatment with a significantly increased bone mass, suppression of bone resorption and growth plate closure (32, 33).

Since then, several new cases of aromatase deficiency have been reported, all with similar baseline skeletal phenotypes as the landmark case report by Smith *et al.* (30). Estrogen replacement therapy, using different doses of E_2 , in patients with aromatase deficiency has indicated that serum E_2 levels above a threshold of ~ 20 pg/ml are required to complete bone maturation and mineralization (32–35).

Serum E_2 and bone maintenance in men

In general, observational studies reported that serum E_2 correlated better with BMD at various sites than T (36–45). Moreover, prospective studies have shown that serum E_2 was the best predictor of both the increase in bone mass in young men (46) and the decrease of bone density in elderly men (46–48). In addition, treatment of elderly men with an aromatase inhibitor resulted in significant increases in bone resorption, together with decreases in bone formation markers (49). This pivotal role of estrogen with respect to male skeletal metabolism has since been confirmed in several other studies. Importantly, Khosla *et al.* (46) suggested that, in elderly men, a threshold exists for bioavailable E_2 of 11 pg/ml (40 pmol/l), corresponding to a total E_2 level of 31 pg/ml (114 pmol/l), below which the rate of bone loss at the radius and the ulna was clearly associated with bioavailable E_2 levels. Above this level, no apparent association between the rate of bone loss and bioavailable E_2 levels was found. Similar thresholds for serum E_2 were reported in elderly men by Gennari *et al.* (48) for bone loss at the femoral neck and lumbar spine and by Szulc *et al.* (43) for changes in biochemical markers of bone turnover.

The majority of estrogens in elderly men do not originate from the testes but from peripheral conversion from androgens (50). Therefore, the extent of peripheral aromatase activity may also influence serum E_2 levels. Elderly men with a high number of repeats in the aromatase gene have been reported to have higher E_2 levels and decreased rates of bone loss compared with men with a low number of repeats (51). In addition, this CYP19 repeat was significantly associated with BMD change in elderly community-dwelling men (47). These findings suggest that a genetic variation in the aromatase gene may predispose men to increased age-related bone loss, by modulating bone metabolism either directly or indirectly via altered serum E_2 levels. This notion is supported by our earlier report that CYP19 polymorphisms were associated with BMD and cortical bone size in young adult Swedish men (52). Recently, our extensive evaluation of 604 single nucleotide polymorphisms (SNPs) in 50 sex steroid-related candidate genes identified an SNP (rs 2470152) in the I.4 promoter region of the aromatase gene to be clearly associated with serum E_2 levels in both young adults and elderly men ($P = 10^{-14}$) (53).

Importantly, subjects with the GG genotype of this CYP19 polymorphism did not only have markedly elevated E_2 levels but also higher lumbar spine BMD and fewer prevalent fractures than subjects with the GA or AA genotypes. Interestingly, the G to A transition of rs2470152 is predicted to alter a potential binding site for the transcription factor cyclic AMP response element binding protein, which is thought to be important in regulating aromatase expression (54). Further studies are required to test the functional significance of this CYP19 promoter polymorphism on the expression of the CYP19 gene in bone and other relevant tissues, and to evaluate the impact of this polymorphism on sex steroid-related disorders (53). In addition, a recent paper from the European Male Aging Study reported that the CAG repeat length in the AR correlated to calcaneus ultrasound parameters and this was associated with increased estrogen rather than decreased androgen action (55).

Serum E_2 and fractures in men

Even though the above-mentioned studies provide strong evidence for an important role of estrogens for bone metabolism in men, little is known about the relative role of androgens and estrogens on fracture risk in men. In cross-sectional studies, inverse associations between both serum E_2 (44, 56) and T (44) and prevalent fractures have been shown. Still, the roles of serum E_2 and T as predictors of fracture risk in men analyzed in large prospective studies remain contradictory (57–60). Previous conflicting results might be due to the fact that these earlier prospective studies have been underpowered, including few incident fractures, and most of them (57–59) have analyzed the baseline sex steroid levels using immunoassay-based techniques, which have been shown to have a questionable

Figure 5 A threshold for the inverse relation between serum E_2 and fracture risk. Yearly incidence of fractures in relation to total E_2 . Poisson regression models were used to determine the relation between serum hormone levels and fracture risk (based on all validated fractures) in elderly subjects from the MrOS Sweden cohort. (Adapted from Mellström *et al.* (61) with permission from the American Society for Bone and Mineral Research).

specificity, especially at lower concentrations. Most recently, we analyzed the predictive role of serum E₂ and T levels for incident fracture risk in the MrOS Sweden study, the largest population-based cohort so far with sex steroid levels measured at baseline with the mass spectrometry technique (61). We found that both serum E₂ levels and T levels were inversely associated with fracture risk when analyzed separately. However, in multivariate analyses, serum free E₂ was independently of free T, a predictor of all fractures in these elderly men. Moreover, when analyzing the effect of having low E₂ and/or low T levels, subjects with low serum E₂ levels had an increased risk of fractures, independent of T status. By contrast, subjects with low T levels but normal E₂ levels were not at higher risk for fracture. In addition, the inverse relationship between serum E₂ levels and fracture risk was nonlinear, with a strong relationship at total E₂ levels below 16 pg/ml (59 pmol/l; Fig. 5). This observation further confirms the concept of a threshold E₂ level for skeletal health in men (62). The threshold E₂ level for fracture risk described in the MrOS Sweden Study (61) is slightly lower than those previously described for bone maturation, BMD and markers of bone resorption (35, 43, 46, 48). This difference could be due to the fact that in the latter studies, serum E₂ was analyzed using immunoassay-based techniques, while it was analyzed by mass spectrometry in the Swedish cohort.

Conclusions

Experimental animal studies have demonstrated that both activation of ER α and AR result in a stimulatory effect on bone mass in males. Recent human studies have gathered substantial evidence for the existence of a threshold E₂ level in men for bone maturation, bone loss as well as fracture risk. Even though these findings do not exclude an important role for T in male skeletal homeostasis, they do provide proof of an important role for estrogen in bone health in men.

Declaration of interest

The authors have no conflicts of interest.

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