Sex steroids and the male skeleton: a tale of two hormones

Filip Callewaert, Steven Boonen and Dirk Vanderschueren

Center for Musculoskeletal Research, Leuven University Department of Experimental Medicine, Katholieke Universiteit Leuven, Herestraat 49, B-3000, Belgium

Traditionally, the stronger male skeleton was considered to result from higher androgen levels in men compared to women. However, the regulation of male bone growth by sex steroids appears more complex than originally anticipated. Based on clinical observations and studies in animal models, not only androgens and androgen receptor (AR), but also estrogens and estrogen receptor-α (not ERβ) are required for optimal bone mineral acquisition during male growth. In addition, both sex steroids are involved in the maintenance of male skeletal health. In fact, bone loss and fracture risk have been associated with estrogen exposure in elderly men. Overall, a compelling body of evidence suggests that both androgens and estrogens are crucial for male skeletal growth and maintenance.

Sex steroid action and bone: traditional concept

Gonadal sex steroids play a crucial role during skeletal growth and maturation in both men and women [1]. It is well-established that a decrease in bioavailable sex steroid concentrations is related to bone loss, not only in postmenopausal women but also in elderly men [2,3]. Gender differences in bone mineral acquisition are already established during puberty, resulting in a higher peak bone mass in men compared to women [4]. In addition, men not only gain more bone during growth, but they also lose less during aging because they do not experience an equivalent of menopause [5]. These skeletal gender differences were traditionally attributed to stimulatory androgen action in men versus inhibitory estrogen action in women. Indeed, the larger and stronger male skeleton was thought to result from androgen-mediated periosteal bone formation in men, as opposed to greater estrogen-related endocortical apposition in women [4,6]. However, more recently it became clear that androgens and estrogens cannot be considered as pure ‘male’ and ‘female’ hormones, respectively. Studies in rodents as well as experiments of nature challenge this traditional concept and have provided evidence for a role for estrogens in the accrual and maintenance of the male skeleton as well. In this review we discuss the most recent relevant clinical and experimental animal studies that have contributed to our current understanding of the relative roles of androgens and estrogens during male skeletal growth and maintenance.

Sex steroid metabolism

Androgens are C19 steroids secreted from the testes in men and from the adrenals in both men and women [3,7] (Figure 1). Testosterone, the most abundant circulating androgen in men, directly acts on its receptor, the androgen receptor (AR), that is present in bone cells (Figure 1) [3]. Moreover, in peripheral tissues including bone, testosterone can be irreversibly converted to the more potent dihydrotestosterone (DHT) by the enzyme 5α-reductase (Figure 1) [3,7]. In addition, testosterone can be converted into 17β-estradiol by P450 aromatase and subsequently activate estrogen receptor-α (ERα) or -β (ERβ) (Figure 1) [3,7]. The adrenals also secrete C19 androgens, such as dehydroepiandrosterone (DHEA) (Figure 1). Although these androgens have a weak androgenic potency, they are an important source of substrate for extragonadal conversion to estrone by aromatase or to testosterone by steroid sulfatase, 17-hydroxysteroid dehydrogenase (17-HSD) and/or 3-HSD (Figure 1) [3,7]. Therefore, androgens might either activate AR or ERs, depending on the relative activities of P450 aromatase, 5-reductase, 17-HSD, 3-HSD and steroid sulfatase.

These enzymes are all expressed in bone tissue, suggesting that local hormone synthesis might be important. As a result, target tissues might not rely on circulating estrogen levels. In fact, in men only about 20% of 17β-estradiol is directly secreted by the testes, whereas the other 80% is derived from aromatization in peripheral tissues, and primarily in adipose tissue (Figure 1) [7]. In human plasma, steroid hormones are bound with a greater affinity to sex hormone binding globulin (SHBG) than to albumin. SHBG is synthesized by the liver and is thought to regulate the access of sex steroids from the bloodstream to their target tissues. Binding to SHBG prevents bound hormones from diffusing out of the bloodstream, so preventing their binding to intracellular AR or ERα/β (Figure 1) [3,7]. Hence, non-SHBG-bound and albumin-bound hormones represent the biologically available fraction. Testosterone and estradiol bind with respectively higher and lower affinity to the same binding site on SHBG. For example, in men and women, between 40 and 65% of circulating testosterone and between 20 and 40% of circulating estradiol is bound to SHBG [3,7,8].

Effects of sex steroids on bone growth

Androgens

Sex steroids are important regulators of bone growth, as shown in animal studies. A pioneer study in male and
female rats in 1990 by Turner et al. led to the traditional concept that androgens in males stimulate, and estrogens in females inhibit periosteal bone formation [9]. Further evidence for a stimulatory role of androgens and AR activation on the periosteal bone surface was provided by studies in rats and mice with a natural mutation or a genetic manipulation of the AR gene [10–13]. In these animals, periosteal bone formation is impaired, leading to reduced cortical bone mass acquisition. In addition, trabecular bone resorption was higher. Along the same line, non-aromatizable androgens reversed orchidectomy-induced trabecular and cortical bone loss in male rodents. From a more clinical perspective, observations of low bone mass in hypogonadal men as well as in patients suffering from complete androgen insensitivity syndrome (cAIS) reinforced the traditional assumption that androgens were exclusively responsible for the typical male bone phenotype [14–16]. More recently, AR was selectively deleted in differentiated mineralizing osteoblasts of male mice [17]. Surprisingly, the periosteeum of these mice appeared not or only modestly affected, suggesting that androgens do not mediate periosteal growth through direct AR activation in mature osteoblasts. A potential limitation of this tissue-specific AR knockout model is that the deletion of AR might be incomplete. Moreover, AR disruption occurred only in later stages of osteoblast differentiation, whereas AR action might be more important in proliferating rather than mature osteoblasts. For instance, overexpression of AR in immature osteoblasts resulted in enhanced periosteal bone formation and decreased endocortical bone formation, whereas AR overexpression in mature osteoblasts decreased endocortical bone formation without affecting periosteal bone formation [18,19]. AR signaling in osteoblasts appears to determine trabecular bone growth and bone resorption. In fact, mice with osteoblast-specific AR deletion experienced trabecular bone loss [17], whereas AR overexpression in osteoblasts resulted in increased trabecular bone mass [18,19]. The potential role of AR in other bone cells such as osteoclasts or osteocytes remains currently unaddressed in vivo.

**Estrogens**

Estrogens were traditionally considered to be non-relevant for male skeletal homeostasis, because estrogen concentrations in men – in contrast with women – remain in the postmenopausal range, and with little age-related variation [20]. However, human experiments of nature firmly established the important role of estrogens in male skeletal homeostasis. Several case reports of men with a natural mutation in ERα, or of men with a total lack of estrogen synthesis (due to a mutation in the aromatase gene) provided evidence that estrogens are essential for male
skeletal homeostasis [21–25]. These patients – with undetectable estrogen levels – have severe osteopenia and still-open epiphyses, despite normal or elevated testosterone levels. Bone mass increased significantly in all of these aromatase-deficient men following estrogen administration [23–25]. Moreover, estrogens also induced epiphyseal closure and longitudinal growth in these patients, because men with aromatase deficiency experience no growth spur even in the presence of high testosterone concentrations or following testosterone treatment [23–25]. An AR-dependent effect on endochondral bone formation remains uncertain because bone length is unchanged in AR-disrupted mice and rats [3].

In contrast with the well-established dominant role in longitudinal growth, the impact of estrogen on the other growing bone envelopes is still uncertain in humans. A follow-up study of an adolescent aromatase-deficient boy during estrogen therapy suggested that estrogen stimulates cortical bone expansion at the outer periosteal surface [23]. Likewise, growing male rats treated with an aromatase inhibitor and mice with an ERα disruption provided further support for a role for estrogens in male bone growth [26–29]. In addition, only combined testosterone plus estradiol treatment in an aromatase-deficient patient with mild hypogonadism gave an optimal gain in bone mineral density (BMD) and cortical thickness, but no such improvements were obtained with either treatment alone [25]. Along the same line, a recent study in AR/ERα ‘double knockout’ mice showed that activation of both AR and ERα is required for periosteal bone apposition and optimal cortical bone mass acquisition [30]. Although these findings suggest that androgen action on periosteal growth is partly dependent on aromatization in estrogens, estrogen might also affect the endocortical bone surface in men as well. Recent studies showed that serum estradiol is inversely associated with endosteal circumference in young adult men [31]. In line with these findings, estrogen treatment of an adult with cAIS resulted in endosteal contraction [16]. In growing male mice with senile osteoporosis (senescence-accelerated mouse prone 6, SAMP6 mice), estrogen administration rescued their early failure of endosteal bone addition [32]. Similarly, transgenic male mice overexpressing the human aromatase gene in osteoblasts have a reduced endocortical perimeter [33]. It would seem therefore that estrogens – at least during pharmacological administration – stimulate endocortical bone expansion during growth as well. Furthermore, estrogens might also activate ERβ. However, in contrast with female ERβ knockout mice – that show an increased cortical bone mineral content and cross-sectional area – male mice lacking ERβ show no bone abnormalities [34].

In contrast with cortical bone, the role of estrogen and ERα seems less crucial for trabecular bone homeostasis, at least in male mice. Combined AR and ERα disruption did not further decrease trabecular bone mass compared to AR disruption alone [30]. Likewise, administration of an aromatase inhibitor had – in contrast to clear effects on cortical bone – no additional effect on the acquisition of trabecular bone mass in orchidectomized mice [11]. Accordingly, trabecular bone was not increased following estrogen therapy in the aromatase-deficient adolescent boy discussed above [23]. AR therefore seems sufficient for the development as well as for the maintenance of male trabecular bone mass. These observations are apparently in sharp contrast to the well-established role of estrogen and ERs in the female skeleton, as well as with the pharmacological action of estrogens in orchidectomized male rodents [35,36]. However, the relative role of estrogen with respect to skeletal homeostasis might be different in male mice compared to men. In fact, serum estrogen concentrations generally appear lower in male mice compared to men [20,30,33]. Concomitantly, overexpression of the human aromatase gene in male mice also increases trabecular bone mass, favoring a role of estrogen in trabecular bone remodeling [33]. These skeletal changes were apparent even without significant changes in circulating estradiol concentrations, again supporting the importance of local estrogen synthesis.

The skeletal effects of estrogen on bone growth are, at least in rodents, often associated with reduced levels of serum insulin-like growth factor-I (IGF-I). In this regard, the ERα-mediated effect on longitudinal growth might, at least in part, be attributed to changes in IGF-I [37]. Moreover, as IGF-I is essential for skeletal growth and periosteal bone expansion – evidenced by extremely short and thin bones in IGF-I disrupted mice [38,39] – part of the estrogen action on cortical bone might result indirectly from reduced serum IGF-I. It should be noted, however, that the amount of estrogen needed to maintain IGF-I during growth might be very low because administration of an aromatase inhibitor to orchidectomized mice and rats decreases both bone mass and serum IGF-I levels. Likewise, IGF-I levels were also decreased in combined AR- and ERα-disrupted mice without detectable changes in circulating estradiol levels [30]. Because IGF-I levels have not been monitored prospectively in aromatase-deficient men, it remains to be established if, and to what extent, estrogen action on bone growth in men is related to changes in the IGF-I axis.

Estrogens not only interact with IGF-I, but they also influence the mechanical sensitivity of the skeleton. Lanyon’s group provided evidence that ERα unequivocally influences the adaptive response of bone to mechanical loading in female mice [40]. However, recent findings were not able to report similar results for ERα in male mice, and even suggested an inhibitory role for AR in the mechanical sensitivity of the male skeleton [41].

**Sex steroids and bone maintenance**

**17β-estradiol**

Sex steroids are not only essential for bone growth, but also for maintenance of skeletal integrity as shown by skeletal changes following sex steroid deficiency in men and rodents [1,12,42]. A study in male transgenic mice lacking ERα or ERβ showed that both AR and ERα (but not ERβ) activation maintained trabecular bone mass, whereas only ERα preserved cortical bone [29]. In parallel, cortical bone appeared less or not responsive to supraphysiological doses of non-aromatizable androgens in aged male orchidectomized rats – again in contrast with estrogen [43].

In aging men, skeletal fragility is associated with reduced cortical thickness, trabecular thinning and decreased bone density [6]. Lower levels of endogenous
sex steroids might contribute to this observed bone loss [7,8,44,45]. Although most cohort studies indicate that estrogen concentrations are associated with bone density and turnover as well as bone loss in aging men [44,46–49], the impact of serum testosterone on bone health parameters appears less significant and uncertain [50–53]. In addition, recent studies have prospectively linked estrogen concentrations, as measured by mass spectrometry, to the occurrence of osteoporotic fractures even in the presence of normal testosterone levels [54]. According to some but not all of these studies, a critical threshold concentration for serum estradiol is apparent, below which bone loss and fracture risk is increased in elderly men [45,48,50,55–57]. This threshold might however be different according to the methodology of measurement (immunoassay versus mass spectrometry) as well as the outcome parameter of male skeletal health, but appears to be independent of testosterone concentrations. The clinical relevance of this estradiol threshold concentration was recently confirmed by a study in an aromatase-deficient man, showing that adequate estrogen levels are required to guarantee optimal bone maturation [24]. In addition, polymorphisms of enzymes involved in estrogen synthesis result in elevated estrogen levels and are associated with bone density and bone loss, even independently of circulating estradiol [58–60]. Such observations reinforce the concept that peripheral estrogen synthesis is important for maintenance of skeletal health. Moreover, according to findings of the European Male Aging Study, men with longer AR CAG repeats not only have higher testosterone levels, due to the weaker activity of their AR, they also have higher estradiol levels and bone health parameters [61], again indicating that most of the phenotypic effects of variation in testosterone are indirectly related to aromatization into estradiol.

However, the underlying mechanism of estrogen action in male skeletal maintenance is unclear. Aging of the male skeleton is characterized by increased net endosteal bone resorption, which is insufficiently matched by periosteal bone formation [6]. Trabecular thinning, more than disruption, characterizes male skeletal aging as well. But to what extent estrogens regulate these age-related dynamics at the different cortical and trabecular bone envelopes remains to be determined.

Testosterone

Although estrogens are currently believed to play a dominant role in maintaining the male skeleton, testosterone might still be important for male skeletal homeostasis. Hypogonadism is a well-recognized risk factor for osteoporosis in aging men [62]. Testosterone treatment effectively prevents bone loss in hypogonadal men, even at older age [63,64]. However, testosterone replacement studies in elderly men with lower testosterone levels have yielded conflicting results with respect to bone health outcome parameters [65]. In fact, human data indicate that only men below a certain threshold (200 ng/dl) will benefit from testosterone substitution [66]. According to the MrOS study, only about 12% of osteoporotic men are below this threshold [66], potentially explaining why testosterone concentrations do not significantly affect skeletal outcome, although the results of these cohort studies should be interpreted with caution because only the healthiest elderly men are likely to participate, whereas men suffering from disease might have lower testosterone concentrations that affect skeletal homeostasis [7].

Moreover, androgens might not only impact on osteoporosis by changes in bone density, but also by their indirect effects on muscle development and maintenance. In fact, testosterone therapy in elderly men might be beneficial for muscle strength, indirectly affecting fracture risk and falls independent of bone density [67,68]. In this regard, the MrOS study group reported that the risk of falls was higher in men with lower bioavailable testosterone levels, but the effect of testosterone appeared independent of physical performance [69]. Yet, the role of AR action on muscle might be more complex than originally anticipated [70].

Sex hormone binding globulin (SHBG)

An important but incompletely understood issue is the role of SHBG. Because SHBG concentrations rise during aging, it is generally accepted that this age-related increase in SHBG contributes to bone loss in aging men by limiting the biological active fraction of sex steroids [8,20]. The MrOS cohort clearly showed that testosterone, estradiol, and their free fractions all declined in men over age 65 [71]. Previous studies found that serum SHBG levels were negatively associated with bone density [44,72,73]. A study in Swedish men reported that SHBG gene polymorphisms associated with high SHBG levels were associated with high BMD [74]. Additionally, male mice overexpressing human SHBG have increased cortical bone mineral content [74]. Although these results are surprising, they should be interpreted with caution, because rodents do not express SHBG in the liver postnatally. Although most recent studies now suggest that SHBG is inversely and independently related to bone density, it remains uncertain to what extent the association of SHBG with male skeletal health outcome measures, including osteoporotic fractures in men, might be explained by decreasing bioavailability of sex steroids [44,52]. In this context, recent findings of MrOS and MrOS Sweden suggested that free estradiol and SHBG, but not free testosterone, were independently associated with fracture risk [54,75]. Similarly, de Ronde et al. reported that high SHBG levels are associated with lower levels of free estradiol, and with normal or even higher levels of free testosterone [76], indicating that SHBG might impact on peripheral aromatization. Thus, SHBG appears to affect bone mass in elderly men, but additional research is required to further elucidate the exact role of SHBG on the maintenance of bone mass in elderly men.

Conclusion

The regulation of male skeletal growth and maturation appears to be a tale of two sex hormone signaling pathways; these are depicted in Figure 2. Animal studies as well as relevant clinical observations provide evidence that estrogen and ERα activation stimulate longitudinal growth and induce epiphyseal growth plate closure at the end of puberty, whereas androgens appear to have no effect on longitudinal growth via the AR. In contrast, androgens and AR activation play a dominant role in the acquisition of male trabecular bone, although pharmaco-
logical estrogen action mediated through ERα impacts on trabecular bone mass during growth as well. Both AR and ERα are also required for optimal cortical bone mass acquisition during male growth. Importantly, the ERα-dependent stimulatory effects on longitudinal and cortical bone growth might be mediated in rodents, at least in part, through changes in IGF-I levels, whereas ERβ is of no importance. Sex steroids and their receptors appear to be not only essential for skeletal growth but also for the maintenance of skeletal integrity in males. Both AR and ERα, but not ERβ, are able to maintain trabecular bone mass, whereas ERα activation most effectively maintains cortical bone mass in orchidectomized rodents. Moreover, according to most of the recent cohort studies in elderly men, 17β-estradiol is more consistently associated with bone health parameters than testosterone.

Collectively, both clinical and translational observations have now established sex hormones as crucial regulators of male skeletal health. Future research challenges that should provide further insights in the mechanism of action of sex steroids and lead to the development of potential therapeutic strategies are listed in Box 1.

### Box 1. Outstanding questions and future research directions

- The long-term safety and efficacy of hormone replacement therapies in elderly men in the context of fracture risk, risk of falls and muscle frailty is currently lacking.
- Assessment of the potential independent effect of SHBG on bone health outcome parameters in elderly men.
- The role of mechanical loading and physical exercise for the prevention or restoration of age-related bone loss, alone or in combination with hormone replacement.
- The tissue-specific role and mechanism of action of AR signaling in osteoclasts and osteocytes.

### References

Review

31 Lapauw, B.M. et al. (2009) Serum estradiol is associated with volumetric BMD and modulates the impact of physical activity on bone size at the age of peak bone mass: a study in healthy male siblings. J. Bone Miner. Res. 24, 1075–1085
33 Sjogren, K. et al. (2009) Elevated aromatase expression in osteoblasts leads to increased bone mass without systemic adverse effects. J. Bone Miner. Res. 24, 1263–1270
41 Callawaert, F. et al. (2008) Androgen receptor disruption increases the osteogenic response to mechanical loading in male mice. J. Bone Miner. Res. 23, S40
42 Reim, N.S. et al. (2008) Cortical bone loss in androgen-deficient aged male rats is mainly caused by increased endocortical bone remodeling. J. Bone Miner. Res. 23, 694–704
43 Vandenput, L. et al. (2002) Evidence from the aged orchidectomized male rat model that 17beta-estradiol is a more effective bone-sparing and anabolic agent than 5alpha-dihydrotestosterone. J. Bone Miner. Res. 17, 2080–2086
49 Mellstrom, D. et al. (2006) Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J. Bone Miner. Res. 21, 529–535
54 Mellstrom, D. et al. (2008) Older men with low serum estradiol and high serum SHBG have an increased risk of fractures. J. Bone Miner. Res. 23, 1552–1560
70 Ophoff, J. et al. (2009) Androgen signaling in myocytes contributes to the maintenance of muscle mass and fiber type regulation but not to muscle strength or fatigue. Endocrinology 150, 3558–3566