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Review

Sex steroid metabolism in the regulation of bone health in men☆

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ABSTRACT

The growth and maintenance of both the female and the male skeleton are influenced by sex steroids. Although the regulation of the female skeleton by estrogens is well established, the relative importance of androgens and estrogens for the male skeleton remains uncertain. Evidence from cross-sectional and longitudinal studies suggests that serum estradiol levels are more strongly associated with bone mineral density, bone turnover and bone loss than testosterone levels are in adult men. In addition, it appears that a threshold level of serum estradiol exists for optimal skeletal maturation and prevention of both bone loss and fractures. Also, the specificity of the assay technique should be considered when examining serum sex steroid levels in epidemiological cohorts, with a preference for the gold standard mass spectrometry. Additionally, serum levels of sex steroid metabolites, rather than the bio-active sex steroids, may be better markers of local sex steroid action at the target tissue level. In this respect, serum levels of glucuronidated androgen metabolites appear to provide additional information as markers of local androgenic activity in bone than the bio-active androgens. Taken together, even though an important role of testosterone is not excluded, estradiol is an important regulator of bone health in men.

Contents

1. Introduction

The growth and maintenance of both the female and the male skeleton are influenced by sex steroids [1]. In women, it is well established that estrogens are implicated in the regulation of bone metabolism, illustrated by the considerable loss of bone mass following natural menopause [2]. Likewise, androgens have a major influence on the skeleton in men, as demonstrated by the skeletal effects of sex steroid deficiency [3]. On the basis of these data, estrogens and androgens were traditionally considered the main sex steroids that influence bone maturation and maintenance in women and men, respectively. Testosterone (T), the major androgen, can act either directly through the androgen receptor or indirectly via aromatization to estradiol (E2) and activation of estrogen receptor-α and/or -β. Alternatively, T can be irreversibly converted to the more potent 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase (Fig. 1). Because of this possible dual mode of

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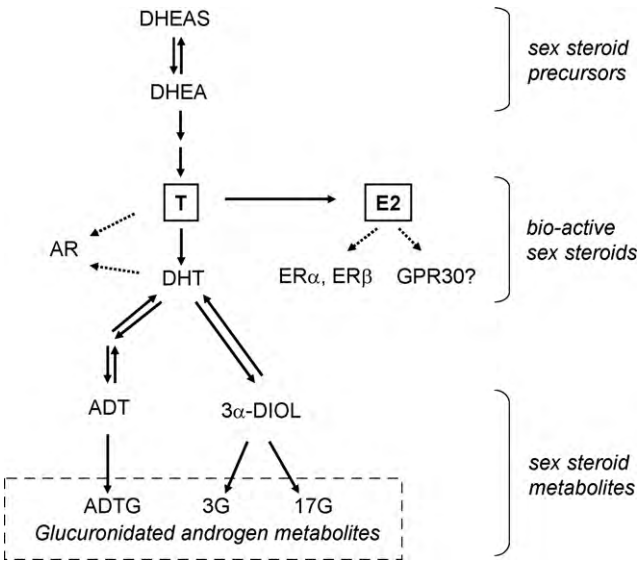

Fig. 1. Simplified overview of androgen metabolism. DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; T, testosterone; E2, estradiol; ER, estrogen receptor; AR, androgen receptor; DHT, dihydrotestosterone; ADT, androsterone; ADTG, ADT-glucuronide; 3α-DIOL, androstane-3α,17β-diol; 3G, 3α-DIOL-3glucuronide; 17G, 3α-DIOL-17glucuronide.

action of androgens, the contribution of androgens and estrogens in the regulation of the male skeleton remains complex. All three sex steroid receptors are expressed in bone cells [4], and experimental animal studies have shown that each of these three receptors mediates site-specific skeletal effects of sex steroids [5–12]. In addition, recent *in vitro* studies suggested that the membrane G protein-coupled receptor GPR30 might also be a potential estrogen receptor (Fig. 1) [13]. Using GPR30-inactivated mice, we showed that GPR30 is not a functional ER in most estrogen-responsive tissues, but is required for a normal estrogenic response in the growth plate and mediates E2-induced insulin release [14,15].

2. Sex steroid metabolism

The bio-active sex steroids are secreted either directly by the gonads or synthesized locally in target tissues from their precursors dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), which are secreted in large amounts by the adrenal cortex (Fig. 1). All the enzymes necessary for the conversion of the precursors DHEA and DHEAS into the bio-active sex steroids T, DHT and E2 are present in the various target cells. The production of potent sex steroids in peripheral target tissues, with the locally produced sex steroids exerting their action in the same cell as where the synthesis took place, is referred to as intracrinology [16,17].

The biological action of sex steroids in target tissues, such as bone, is dependent on the local synthesis and degradation of these sex steroids. The bio-active sex steroids can be inactivated locally into more water-soluble compounds (mainly glucuronides and sulfates), which are secreted in the general circulation where they can be measured. An alternative pathway for androgens is the transformation of DHT by hydroxysteroid dehydrogenase enzymes resulting in androstane-3α,17β-diol (3α-DIOL) and androsterone (ADT) (Fig. 1). These metabolizing steps are reversible and do not lead to the termination of the androgenic signal. In a next, irreversible step, the 5α-reduced metabolites of DHT are inactivated via glucuronidation. 3α-DIOL can be glucuronidated either at the 3α-hydroxy position (3α-DIOL-3glucuronide, 3G) or at the 17β-hydroxy position (3α-DIOL-17glucuronide, 17G) (Fig. 1). The three enzymes responsible for the glucuronidation of all androgens and their metabolites in humans are uridine glucuronosyl transferase (UGT) 2B7, UGT2B15 and UGT2B17. Conjugation of sex steroids with glucuronic acid is thought to play a major role in the regulation of the intracellular levels of unconjugated steroids and their biological activities in tissues [18]. It has been suggested that measurement of the glucuronidated androgen metabolites is the only approach that permits an accurate estimate of the total androgen pool [19].

3. Sex steroid precursors and bone health in men

The adrenal hormones DHEA and DHEAS are a major source of androgens and estrogens in older men [17]. Serum levels of DHEA and its sulfate decline considerably with aging [20], but it remains unclear if these changes contribute to the age-related bone loss. Cross-sectional studies reported conflicting results regarding the association between serum levels of DHEA/DHEAS and BMD measurements [21–24]. Replacement studies with subjects selected for having low DHEAS levels at baseline reported significant beneficial effects on BMD, but these changes were usually small and specific to a certain bone site [25–27]. Jankowski et al. suggested that the increase in hip BMD in older men undergoing DHEA treatment was mediated primarily by an increase in serum E2 levels [26]. When evaluating DHEA replacement in healthy subjects with normal DHEAS levels at baseline, no beneficial effect on bone was seen in men [28–30]. These findings seem to suggest that DHEA is not a major mediator of the age-related bone loss in men.

4. Sex steroids and bone health in men

4.1. Sex steroids and bone maturation

The traditional assumption, with estrogens and androgens being the main sex steroids affecting bone maturation and maintenance in women and men, respectively, was challenged in the 1990s by the description of several experiments of nature. The case reports of a man with a naturally occurring mutation in the estrogen receptor-α gene [31] and men with complete estrogen deficiency [32,33], due to mutations in the aromatase (*CYP19*) gene, provided evidence that estrogens are crucial for bone growth and maturation in men. These patients had almost identical skeletal phenotypes – severe osteopenia, increased bone turnover, and continued linear growth into adulthood – despite having normal or elevated T levels. The aromatase-deficient men, in contrast to the estrogen-resistant male, did respond to estrogen treatment [33,34]. Serum E2 levels around 20 pg/ml are proposed to normalize bone mass and bone turnover, and fuse the growth plates [35,36].

These case reports have established a principal role for estrogens in the final phase of skeletal maturation and mineralization. Nevertheless, androgens are also considered to have an impact on bone growth, since prepubertal hypogonadism is clearly associated with decreased peak bone mass [37,38]. In addition, androgens are believed to be partly responsible for the sexual dimorphism of the skeleton [39]. By stimulating periosteal bone formation, androgens are thought to increase bone size, at least according to studies in rodents [9,40]. In young adult men, Lorentzon et al. reported that serum free T is positively associated with cortical bone size [41].

Besides having direct effects on bone growth, sex steroids may also interact with the growth hormone – insulin-like growth factor I axis to regulate bone size [4,42–44].

4.2. Sex steroids and maintenance of bone mass

With aging, serum levels of free/bioavailable androgens and estrogens decrease in men, and this coincides with an age-related increase of sex hormone-binding globulin (SHBG) levels [22,24,45].

Table 1
Serum levels of estrogens according to CYP19 rs2470152 genotype in young adult and elderly men.

	AA	AG	GG	*P*	GG versus AA
GOOD	*n* = 241	*n* = 514	*n* = 286		
E2 (pg/ml)	17.5 ± 6.2[a,b]	18.8 ± 6.1[a]	19.8 ± 6.2	1×10^{-5}	+13%
E1 (pg/ml)	21.8 ± 7.4[a]	22.5 ± 7.5[a]	24.0 ± 8.2	3×10^{-3}	+10%
MrOS Sweden	*n* = 551	*n* = 1249	*n* = 768		
E2 (pg/ml)	19.7 ± 7.5[a,b]	21.1 ± 8.0	21.9 ± 8.1	4×10^{-6}	+11%
E1 (pg/ml)	31.8 ± 12.8[a,b]	33.8 ± 14.2[a]	36.8 ± 17.2	1×10^{-9}	+16%
MrOS US	*n* = 484	*n* = 944	*n* = 494		
E2 (pg/ml)	21.6 ± 7.9[a,b]	22.8 ± 8.6	23.4 ± 8.2	4×10^{-4}	+8%
E1 (pg/ml)	31.3 ± 13.4[a,b]	34.1 ± 15.7[a]	35.8 ± 15.8	1×10^{-6}	+14%

Values are adjusted for age, body mass index and race (in the MrOS US cohort), and are given as mean ± SD, unless otherwise indicated. E2, estradiol; E1, estrone. *P* values are for comparison of the three genotypes (ANOVA with Tukey's post hoc test). Adapted from Eriksson et al. [60] with permission from the Endocrine Society ©.

[a] $P < 0.05$ versus GG.

[b] $P < 0.05$ versus AG.

These changes in sex steroid levels may in turn affect bone remodeling and maintenance in adult men. In addition, average levels of serum E2 in elderly men appear to be markedly higher than in postmenopausal women [46,47], arguing for a physiologically important role of estrogens in skeletal health in men. Cross-sectional, observational studies in adult and elderly men indicated that serum levels of E2, and especially bioavailable E2, were more strongly associated with bone mineral density (BMD) at various sites than total or bioavailable T levels [22–24,48–53], even across different racial and ethnic groups [54]. Slemenda et al. found that BMD measurements at the hip and spine correlated positively with serum E2 levels in older men (correlation coefficients varying from 0.21 to 035, $P = 0.01$–0.05), and negatively with serum T levels (correlation coefficients varying from −0.20 to −0.28, $P = 0.03$–0.10) [23]. Moreover, prospective studies showed that serum E2 was a better predictor than serum T of both the increase in bone mass in young men [46] and the bone loss in elderly men [46,55,56]. Also, treatment of elderly men with an aromatase inhibitor resulted in significant increases in bone resorption, together with decreases in bone formation markers [57]. Importantly, Khosla et al. proposed a threshold level for bioavailable E2 of 11 pg/ml, corresponding to a total E2 level of 31 pg/ml, below which the rate of bone loss at the radius and the ulna was clearly associated with bioavailable E2 levels in elderly men [46]. Above this level, no apparent association between the rate of bone loss and bioavailable E2 levels was found. Similar threshold levels for serum E2 have since been reported in elderly men [52,56]. When relating this threshold to E2 levels and bone loss in general, Khosla et al. described the E2 threshold value in men as being higher than the average E2 levels in postmenopausal women. A significant proportion of elderly men, as much as 50%, is believed to have E2 levels below this threshold and these men are thus likely to be at greatest risk for osteoporosis and related fractures [46].

Since the majority of estrogens in elderly men do not originate from the testes but from peripheral conversion from androgens [20], the extent of peripheral aromatase activity may also influence serum E2 levels. Elderly men with a high number of repeats in the aromatase gene had higher E2 levels, decreased rates of bone loss and tended to have fewer fractures compared to men with a low number of repeats [58]. A study by Van Pottelbergh et al. also reported a significant association between this CYP19 repeat polymorphism and BMD change [55]. These findings suggest that a genetic variation in the aromatase gene may predispose men to increased age-related bone loss and fracture risk, by modulating bone metabolism either directly or indirectly via altered serum E2 levels. This notion is supported by our finding of associations between CYP19 polymorphisms and BMD and cortical bone size in young adult Swedish men [59]. By extensive evaluation of 604 single nucleotide polymorphisms (SNPs) in 50 sex steroid-related candidate genes, we recently identified a SNP in the I.4 promoter region of the aromatase gene that associated significantly with serum estrogen levels (both E2 and estrone) in young adult and elderly men (Table 1) [60]. Interestingly, subjects with the GG genotype of this CYP19 polymorphism did not only have markedly elevated E2 levels but also higher lumbar spine BMD and fewer prevalent fractures than subjects with the GA or AA genotypes. Moreover, the European Male Ageing Study reported that the CAG repeat length of the androgen receptor associated with calcaneus ultrasound parameters and this was related to increased estrogen rather than decreased androgen action [61].

In males, estrone is mainly derived from the adrenal androgen precursor androstenedione and only a limited amount of estrone is converted into E2 [62]. Therefore, androstenedione does not seem to have an important role as a precursor of serum E2 in men. This is supported by less strong correlations between serum androstenedione and E2 compared to serum androstendione and estrone [23] and lack of associations of androstenedione with bone density or bone turnover parameters in men [52,63]. In elderly men, estrone concentrations are higher than E2 levels [47,64]. However, the few studies reporting serum estrone levels in older men did not find stronger associations for estrone with bone density measurements or bone turnover markers than for E2 [23,46,51]. In addition, we evaluated serum levels of both estrone and E2 as predictors of fracture risk in the MrOS Sweden cohort; when combining both variables in the same proportional hazards regression model, only serum E2 was a significant independent predictor of risk of first fracture [64].

These studies, altogether, suggest that, in adult men, E2 plays a dominant role in regulating bone homeostasis. An important direct role of androgens is, however, not excluded (for review see [12,65]), especially since they provide the necessary substrate for aromatization. The skeletal effects of T replacement are most evident in hypogonadal men [66–68]. Nevertheless, one should take into account that hypogonadism is usually characterized by low levels of both T and E2. Similarly, the skeletal response to T replacement might reflect the action of estrogens derived from T, and this could compromise the analysis of the relative contributions of T and E2 to bone maintenance in men.

4.3. Sex steroids and fractures

The ability of androgens and estrogens to predict fracture risk in older men remains unresolved. Cross-sectional studies reported inverse associations between both serum E2 [53,69] and T [53] and prevalent fractures. The predictive role of serum E2 and T for fracture risk in prospective studies remains contradictory [70–73]. These conflicting results might be explained by the fact that these prospective studies were underpowered, as they included few inci-

dent fractures. Also, most of them [70–72] analyzed the baseline sex steroid levels using immunoassay-based techniques that are thought to have reduced specificity in the lower concentration ranges [74,75]. Gas chromatography combined with mass spectrometry (GC–MS) or liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) assay methods are generally considered the gold standard for sex steroid measurements. These methods are increasingly used in epidemiological studies because of their accuracy and sensitivity [76].

Recently, we analyzed the predictive role of serum E2 and T levels for incident fracture risk in the Osteoporotic Fractures in Men (MrOS) Sweden study, the largest population-based cohort so far with sex steroid levels at baseline measured with the GC–MS technique [64]. Both serum E2 and T levels were inversely associated with risk of all fractures (including fractures at the hip, distal radius, proximal humerus and pelvis, and clinical vertebral fractures) when analyzed separately. Yet, in multivariate analyses, serum free E2 was an independent predictor of all fractures in these elderly men (hazard ratio (HR) per SD decrease 1.37, 95% confidence interval 1.10–1.71), whereas serum free T was not (HR per SD decrease 1.03, 0.84–1.27). This relation between serum E2 and fracture risk was recently confirmed in the MrOS US study, which also analyzed baseline sex steroid levels with the MS technique [77]. Older men with low bioavailable E2 levels at greater risk for nonvertebral fractures, even after adjustment for bioavailable T levels [77]. When analyzing the effect of having low serum levels of E2 and or low levels of T, subjects with low E2 levels were at increased risk for fracture. In contrast, subjects with low serum levels of T but normal E2 levels were not at higher risk for fracture [64]. In addition, the inverse relationship between serum E2 levels and fracture risk appeared to be nonlinear, with a strong relationship at E2 levels below 16 pg/ml (Fig. 2). This finding further substantiates the concept of a threshold E2 level for skeletal health in men [12,78]. The threshold level of serum E2 for fracture risk described in the MrOS Sweden study [64] is slightly lower than the thresholds previously described for other bone phenotypes (bone maturation, BMD and markers of bone resorption) [35,46,52,56]. This discrepancy might be explained by the fact that in the latter studies, serum E2 was analyzed using immunoassay-based techniques, whereas it was determined by GC–MS in our Swedish cohort. Alternatively, the mechanisms involved in the effect of E2 on bone maturation, BMD and bone resorption might, to some extent, differ from those that confer the effect of E2 on fracture risk.

We recently demonstrated that polymorphisms in the promoter region of the SHBG *gene* associated with serum levels of SHBG and hip BMD in older men [79]. Also, serum SHBG levels have been shown to be directly related to prevalent fractures in men [80,81]. In the MrOS Sweden study, men with high serum levels of SHBG have an increased risk of fracture (HR per SD increase 1.41, 1.22–1.63) [64], confirming previous findings in the Tromso study [72] and the Dubbo Osteoporosis study [73]. Interestingly, both low E2 and high SHBG independently predicted risk of fractures in the MrOS Sweden study, with the highest fracture risk seen in men with both low E2 and high SHBG. Similar findings were again reported for the MrOS US cohort; older men with low bioavailable E2 or high SHBG levels were at increased risk of nonvertebral fractures, and men with low bioavailable E2, low bioavailable T and high SHBG were at highest risk [77].

Even though serum T levels are not consequently independently associated with incident fracture risk, T may influence other factors related to fracture risk, including bone geometry, bone microarchitecture, neuromuscular function, or balance. This latter option is supported by findings from the MrOS US cohort indicating that the risk of falling was higher in men with lower bioavailable T levels [82].

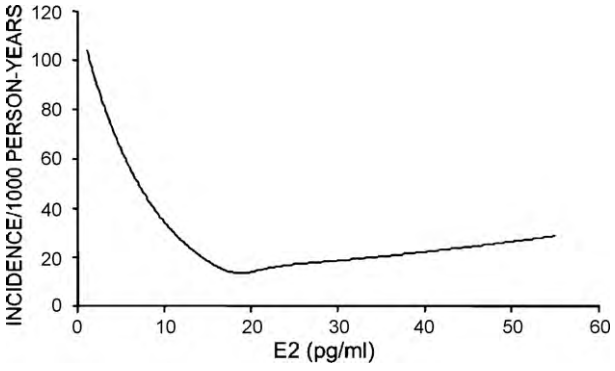

Fig. 2. Yearly incidence of fractures in relation to serum E2 levels. Poisson regression models were used to determine the relation between serum E2 and fracture risk (based on all validated fractures) in elderly men participating in the MrOS Sweden study. Adapted from Mellstrom et al. [64] with permission form the American Society for Bone and Mineral Research.

5. Androgen metabolism and bone health in men

When GC–MS and LC–MS/MS assay methods are used to measure sex steroids, a more complete panel of steroid metabolites can be assessed in the same run [76]. We used the availability of additional measurements of androgen metabolites to test whether these metabolites would be more closely associated to BMD than the bio-active steroid T. Previous studies reported only weak or no correlation between BMD and serum T levels in men [22–24,50,51]. This might be related to the process of intracrinology, where androgens made locally in the target tissues from the adrenal precursor DHEA act in the same cells where synthesis took place and are not released in significant amounts in the circulation [16,17]. This would make the measurement of serum T levels unreliable as a marker of total androgenic activity, since these levels do not reflect T production and androgen action at the target tissue level. An example of the poor predictability of serum T levels is provided in castrated men where castration causes a 90–95% decrease in serum T levels whereas the intraprostatic concentration of DHT and the serum levels of ADTG and 3α-DIOL glucuronides are only reduced by 50–70% [83].

Using the specific LC–MS/MS technique, it is possible to separate the 3-glucuronidated form (3G) from the 17-glucuronidated form (17G) of the 3α-DIOL glucuronides [84] (Fig. 1). The separate measurement of the two 3α-DIOL glucuronides allows investigating of tissue-specific glucuronidation of androgens and a possible association with biological parameters in men. In a sub-cohort of the MrOS Sweden study, the serum levels of the glucuronidated androgen metabolites ADTG, 3G and 17G associated stronger with BMD measurements at several sites than the levels of the bio-active androgens T and DHT [85]. Both 3G and 17G associated independently with BMD measurements, and the sum of both (3G + 17G) explained a larger part of the variance in BMD than either of the two glucuronidated androgen metabolites separately. To determine if the predictive role of glucuronidated androgen metabolites for local androgenic activity is specific for bone, we evaluated the associations in another androgen-responsive tissue. Interestingly, serum levels of 17G associated with prostate volume, explaining 4.5% of the variance, whereas the serum levels of the bio-active androgens showed no significant associations [85]. These findings suggest that the glucuronidated metabolites are more valid estimates of androgen activity in bone and prostate than the bio-active androgens.

The polymorphisms in the genes encoding the enzymes responsible for the degradation of androgens by glucuronidation, UGT2B7,

UGT2B15 and UGT2B17, were also examined in relation to bone phenotypes. The UGT2B7 $H^{268}Y$ polymorphism, which associated with serum levels of T, DHT, 3G and 17G, independently associated with cortical bone size in young adult men participating in the GOOD study, as a result of an effect on the periosteal circumference [86]. The $D^{85}Y$ polymorphism in the *UGT2B15* gene and a deletion polymorphism in the *UGT2B17* gene were not associated with any bone parameters.

6. Conclusions

The findings from all these cross-sectional and longitudinal epidemiological studies provide proof of an essential role for estrogens in bone health in men, even though an important role for T in male skeletal homeostasis is not excluded. In addition, accumulating evidence indicates that a threshold level for serum E2 exists which is necessary for optimal skeletal maturation and for preventing bone loss as well as fractures in men. The specificity of the assay technique used should be taken into consideration when examining serum sex steroid levels in epidemiological cohorts, with a preference for the gold standard MS. Additionally, serum levels of sex steroid metabolites may be more valid estimates of local sex steroid action at the target tissue level than the serum levels of the bioactive sex steroids. In this respect, serum levels of glucuronidated androgen metabolites appear to provide additional information as markers of local androgenic activity in bone than the serum levels of the bio-active androgens.

Competing interests

The authors declare they have no competing interests.

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