The influence of gonadal hormones on skeletal health in men

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Summary
Osteoporosis in men has only recently begun to receive more attention despite it being estimated that about one third of all osteoporotic fractures occur in men and that the residual lifetime fracture risk in a man aged 60 years may be as high as 30%. Accrual of bone mass and age-related bone loss in aging healthy men are multifactorial processes involving hormonal, environmental, and genetic factors. This review will summarize the effects of gonadal steroids on bone turnover and bone mass in both, men with over hypogonadism and healthy elderly men with age-related androgen deficiency.

Sex hormone metabolism and action
Androgens are synthesized from cholesterol through several enzymatic pathways in which the side chain of cholesterol is shortened through oxidation from 27 carbons to 19 carbons. In men, androgens are secreted almost exclusively from the testes as testosterone. The adrenal glands also secrete dehydroepiandrosterone (DHEA), which is a minor androgen that also serves as a substrate for peripheral aromatisation to estradiol. Testosterone is either converted by 5α-reductase to dihydrotestosterone (DHT), or metabolized to E2 by aromatase, a widely distributed microsomal cytochrome P450 enzyme. The former pathway amplifies androgen action locally while the latter pathway diversifies androgen action (1). Hence, enzymatic androgen activation leads to testosterone acting directly or via its more potent metabolite DHT through the androgen receptor (AR) or indirectly via aromatization to estradiol through the estrogen receptors (ER) (Fig. 1). Thus, testosterone functions as a precursor for peripheral conversion into biologically highly active hormones. Estradiol, which is thought to play a major role in bone metabolism in men, is largely synthesized by extratesticular aromatisation of circulating testosterone with only a small proportion of E2 (approx. 15–20%) being directly secreted by the testes (2). Depending on the relative activity of aromatase, 5α-reductase, and dehydrogenases, and the relative distribution of AR and ERs in peripheral tar-
get tissues, testosterone and its metabolites may predominantly activate either the AR or the ER. In bone tissue, the expression of aromatase, 5α-reductase, 17β-HSD and 3β-HSD has been documented supporting the concept of tissue-specific peripheral activation of gonadal hormones.

The AR has been identified in most bone cells, including osteoblasts, osteocytes, and osteoclasts. Estrogen action on bone, in men and women, is mediated via estrogen receptors. These nuclear hormone receptors are also expressed in osteoblasts, osteoclasts and osteocytes. Two ERs have been identified: ERe is predominantly expressed in cells resident to cortical bone, whereas ERβ shows higher levels of expression in cells found in cancellous bone. Alternate, non-genomic pathways have also been described in which ARs and ERs modulate transcription indirectly, via protein-protein interactions.

**Age-related changes in gonadal hormones in men**

Aging in men is associated with a gradual, progressive decrease in circulating testosterone (3, 4). Longitudinal population-based studies show that serum total testosterone concentrations decline by approximately one percent per year in men, but the importance of such a decline remains unclear. A variety of derived testosterone measures (“free”, “bioavailable”), which putatively reflect various binding, and tissue availability, of testosterone to carrier proteins, have been postulated to reflect androgen action more closely, however the underlying free hormone hypothesis lacks adequate empirical verification (5). For example, while “free” testosterone (that is the fraction of testosterone that is unbound, particularly to albumin or SHBG) is reported to fall more rapidly due to a concomitant twofold rise in SHBG binding capacity, it is unclear whether this represents more or less net androgen action at a tissue level since the unbound hormone fraction is more accessible both to sites of hormone action as well as degradation (6).

Although an age-related fall in blood estrone has been observed in men (7), similar reductions in E2 have not been well documented. This may be due to increased aromatase activity with age, which in turn is attributed to the age-associated increase in fat mass (8). In analogy to testosterone, non-SHBG bound E2 levels decrease with age (by about 50% over six decades) as a consequence of increasing SHBG concentrations (9). However, the biological validity of this derived estradiol measure remains to be established since binding to SHBG is competitive with testosterone, which is present in 100-fold higher molar concentrations. While the available evidence indicates that estradiol has significance for the male skeleton, the circulating blood estradiol concentration in men are comparable with estrogen-deficient post-menopausal women raising the paradoxical issue of why male bone does not acquire the osteoporotic state of post-menopausal women.

**Gender-specific effects of sex hormones on bone geometry**

There are little differences in bone size and volumetric bone mineral density (vBMD) between girls and boys until puberty, when sexual dimorphism begins. During puberty skeletal mass doubles. The increase in stature height are greatest in early puberty and then declines in both sexes, whereas the maximal increases in vBMD occur at the menarche in girls and in late puberty in boys. The pattern of growth differs in boys from that of girls. In boys puberty occurs approximately two years later than in girls and thus boys have two more years of prepubertal growth. Furthermore the pubertal growth spurt lasts for four years in boys compared to three years in girls (10, 11). These differences account for the ten percent greater height and the 25% greater peak bone mass achieved by boys. The greater bone mass in boys is for the most part due to their greater bone size. Peak vBMD is not different in young men and women.

The endosteal and periosteal bone compartment change differentially during bone growth as well as during bone loss. These differences in changes cannot be assessed by dual energy x-ray absorptiometry (DXA), which provides in integral measure of BMD. Furthermore DXA provides areal BMD (g/cm²), which is overestimating volumetric BMD (g/cm³) in larger bones by not taking in account the variable dimension of depth.

Testosterone and estrogen may affect osteoblast function differently at various skeletal sites. Testosterone increases peristomal apposition whereas estrogen opposes it (12, 13). This difference in effect accounts for the larger bone achieved in boys during puberty. Estrogen in girls inhibits periosteal bone formation and limiting the expansion of the outer diameter and simultaneously stimulates endosteal bone formation and so narrowing the inner diameter. In boys androgen production during puberty increases periosteal apposition, bone diameter and cortical thickness. Age and sex differences in bone size and shape are shown schematically in Figure 2 and Figure 3.
In women two major phases of bone loss can be distinguished, an early, accelerated loss that begins at the menopause and a slow, continuous phase. The early accelerated bone loss declines exponentially over five to ten years and thereafter a the continuous slow phase of bone loss occurs with advancing age. During the early phase 20–30% of cancellous bone is lost, but only five to ten percent of cortical bone. The early rapid bone loss is due to estrogen deficiency, whereas the slow phase is due to aging and calcium and vitamin D deficiency.

Men do not experience the equivalent of menopause and therefore the early phase of accelerated bone loss does not occur. However in castration or antiandrogen therapy for prostate cancer the same pattern of accelerated bone loss has been found. Men exhibit with advancing age a slow phase of bone loss, similar to the late phase observed in women, leading to an overall loss of about 20 to 25% in both cortical and cancellous bone. Loss of trabecular bone during aging is similar in men and women. However the pattern of loss differs, in men there is mainly thinning of trabeculae (reduced bone formation) whereas in women there is loss of connectivity (accelerated bone loss due to estrogen deficiency). As endosteal bone loss proceeds, periosteal apposition in the appendicular bone continues throughout life in both sexes, but is greater in men than women (11, 13). Thus men loose less cortical bone, but have less endosteal resorption and therefore bone loss is less in men. The increase in bone size and bone geometry results in an increase in mechanical strength. As a consequence this change in bone size compensated partially the age-related loss in women and men, but more so in men.

There is evidence that estrogen deficiency plays a role in bone loss with advancing age in both sexes. Testosterone, however, accounts for the sexual dimorphism of the skeleton that develops during puberty and later in life by stimulating growth of periosteal bone. Estrogen deficiency leads to an increase in endocortical remodelling and more bone is removed than replaced leading to endocortical bone loss. At the same time estrogen deficiency removes a constraint on periosteal apposition and this partially compensates for endocortical bone loss.

Estrogen deficiency is also important in men. This is illustrated with skeletal changes in men with estrogen resistance due to loss of function mutation in the ERα gene. Although serum estrogen levels are elevated, affected men present with continued linear growth, incomplete epiphyseal closure, low BMD and increased bone resorption (14, 15).

**Skeletal health with various degrees of androgen deficiency**

**Consequences of male hypogonadism**

Hypogonadism is present in 15% to 36% of men with documented osteoporosis although this may be an over-estimate given the non-specific effects of acute or chronic illness on blood testosterone concentrations. Nevertheless, it is clear that normal gonadal function is crucial for the development and maintenance of male bone integrity. Androgen deprivation therapy (surgical or chemical castration), which is often required in adult men with advanced prostate cancer for example, results in a profound decline in circulating gonadal hormones. Similar to changes observed in women with surgical ovariotomy or during early menopause the rapid decline in sex steroids after castration is followed by accelerated and unbalanced bone turnover which results in net bone resorption. Bone turnover, as assessed by biochemical markers of bone resorption and formation is increased and results in rapid and sustained bone loss in hypogonadal men (16). Bone mineral density is pre-

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**Fig. 2** Metacarpal width increases more in males than females, and medullary width contracts more in females, net cortical thickness is similar; adapted from (10)

**Fig. 3** Change in bone shape and size during growth and aging. Effect of sex hormones on periosteal and endosteal bone formation and resorption; adapted from (10)
dominantly reduced at the lumbar spine which decreases by about five to ten percent within the first year of androgen deprivation therapy. Ultimately, bone loss after castration results in an increased risk of osteoporotic fractures (17).

Men with overt hypogonadism due to organic testicular or hypothalamic-pituitary dysfunction present with less dramatic changes in BMD and bone turnover, largely due to less profound and more variable degrees of androgen deficiency. Most men with hypogonadism have significantly lower bone density than age-matched controls. Luisetto et al., however, observed that bone mass was comparable to healthy controls in men with Klinefelter’s syndrome (18). This discrepancy is most likely due to the moderate decreases in testicular function and reflects the wide phenotypic spectrum of Klinefelter’s syndrome (19, 20). In contrast to these mostly uniform alterations in BMD, changes in bone turnover, specifically in bone formation, are less clear. While bone resorption is accelerated in hypogonadal men as compared to controls, bone formation may be either decreased or increased when assessed either by biochemical indices of bone formation or histomorphometric studies (16).

Effects of androgen replacement in male hypogonadism

Most studies of androgen administration in hypogonadal men have reported beneficial effects on BMD, although the gain in bone density is highly variable amongst studies. This may, at least in part, be related to differences in the adequacy of testosterone replacement regimens (21), variable degrees of underlying testosterone deficiencies, pharmacogenetic characteristics, as well as to different methods used to quantify bone density (DXA, QCT). As reported by Behre et al., the most significant increase in BMD is seen during the first year of testosterone treatment, and is greatest in those with the lowest initial BMD. Therefore, bone density is maintained during long-term testosterone administration (22), as long as adequate testosterone dosage is maintained (21).

Data from prospective and retrospective studies on the effect of androgen replacement on bone density in hypogonadal men indicate that mostly cancellous bone sites (e.g. spine) are more responsive than predominately cortical sites (e.g. radius, hip), and that measurements based on QCT show much greater responses than studies using DXA, dual or single photon absorptiometry (23). These differences may be in part due to androgen-induced changes in body composition (i.e. fat mass), which are not corrected for when QCT measures are used to evaluate BMD responses. Furthermore, the adequacy of testosterone administration is an important determinant of its efficacy (21). Intramuscular testosterone administration may result in supraphysiological circulating T levels, while transdermal (24, 25) or buccal (26) testosterone administration increases serum T levels within the physiological range.

The effect of androgen replacement on BMD is largely accounted for by its effect on bone turnover. In hypogonadal men, testosterone administration decreases bone resorption and increases bone formation. However, beneficial effects on body composition, specifically on muscle mass, are important contributors to the increase in BMD.

Consequences of partial androgen deficiency in elderly men

Male reproductive health, specifically androgen-deficiency in aging men, has become an issue of growing interest not only to physicians but also to the wider society. Putative somatic consequences of gradually falling testosterone concentrations, including changes in bone mass, have become the rationale for wider use of testosterone treatment of middle-aged and older men with apparent age-related but no other form of overt androgen deficiency.

Several cross-sectional and longitudinal studies have investigated the association between sex hormones, biochemical markers of bone turnover and bone mass in elderly men. In a cross-sectional study, Khosla et al. reported inverse correlations between urinary NTX levels and both “bioavailable” E2 and “bioavailable” testosterone (9). However, no correlation with total estradiol or testosterone was detected. In contrast, Szulc et al. found that only bioavailable E2 levels were negatively correlated with bone turnover, but no associations were observed with total E2, or any testosterone measure (27). The association between E2 and bone resorption markers has been confirmed in another study where significant correlations were found only with markers of bone resorption (serum and urinary NTX), but not with biochemical indices of bone formation (OC, BAP) (28). The available data emphasize that low levels of circulating E2 are associated with increased bone resorption, and that this increase is only partly compensated by a concomitant rise in bone formation (29).

Elderly men, especially over 70 years of age, are increasingly at risk of bone loss and osteoporotic fractures. However, the extent to which low levels of testosterone contribute to age-related bone loss in men remains unclear. In analogy to markers of bone turnover, several cross-sectional and longitudinal studies have documented significant correlations between serum levels of “bioavailable” or total E2 and bone density (12, 13) or change in bone mass during follow-up (28, 30, 31). In contrast, however, studies have failed to show consistent associations between “bioavailable” testosterone and BMD or bone loss (9, 32, 33).

All above-mentioned studies focus on the association between sex hormone levels and bone turnover markers and BMD as surrogate markers of bone integrity. However, while bone mineral density (BMD) and risk of falls appear to be important determinants of osteoporotic fracture risk in older men, the relationship between sex hormones and incident osteoporotic fracture risk remains less clear. A study from Sweden has claimed that free testosterone within the normal range is an independent predictor of prevalent osteoporotic fractures in elderly men (34). A subset analysis from the Rotterdam study failed to detect an association between sex hormones and incident fracture risk in older men (35), while recent studies observed significant associations between both, decreased circulating estrogen and testosterone levels, and incident fracture risk (36–40) (Fig. 4). Hence, whether, and if so, to what extent sex hormone levels contribute to fracture risk in men, and whether this effect is inde-
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**Effect of hormone replacement in men with age-related androgen deficiency**

The effects of testosterone treatment on bone in elderly men remain inconclusive and there is no evidence from long-term randomised studies to indicate that androgen treatment reduces bone fractures in men (41). So far four randomized placebo-controlled studies in healthy men over 50 years have examined the impact of androgenic supplementation on bone health including bone turnover markers (42–45) and bone density (43–45). All studies treated otherwise healthy, non-osteoporotic men with transdermal testosterone or IM testosterone injections. Irrespective of baseline blood testosterone concentrations (range from 10.1 to 13.5 nmol/L), no study showed significant changes in bone turnover markers after three to 36 months of treatment. Only in an early small cross-over study by Tenover (42) did urinary excretion of OHP decrease in testosterone treated men while the respective levels remained unchanged in the placebo group. The relevance of this singular finding using a non-specific marker of collagen turnover remains unclear.

Two placebo-controlled studies investigating the effect on BMD have differed in outcome: whereas the study by Snyder et al. showed no benefit of treatment (43), the study by Kenny et al. showed that testosterone is able to prevent on-going age-related bone loss in one of five bone sites (45). Post-hoc analysis of the larger study by Snyder et al. suggested that bone density gains were inversely related to pre-study baseline levels of blood testosterone (43), consistent with the idea that the benefits depend on the degree of underlying androgen deficiency. In a recent study in which testosterone enanthate was administered at a higher dose reported significant increases in BMD at the lumbar spine and the hip after 36 months of treatment (44); however, a reduction in dose for polycythemia was required in 25% of the participating men.

In summary, there appears to be no consistent effect of exogenous testosterone treatment on bone turnover and limited, dose-dependent effect on BMD in older men with low-normal circulating testosterone (46). Results from placebo-controlled trials including elderly men with consistently lower baseline testosterone levels (i.e. below 8 nmol/L) are needed to unravel the effects of testosterone replacement on bone surrogate markers, such as BMD and bone turnover markers, and ultimately fracture risk, morbidity and mortality. In the interim, there is no basis for empirical testosterone treatment for men with idiopathic or age-related osteoporosis unless there is concomitant evidence of overt androgen deficiency (47).

**Differential effect of testosterone and E2 on skeletal metabolism in men**

Short-term randomized pharmacological studies have dissected the relative contributions of androgenic and estrogenic action in maintenance of bone turnover (48–51). These studies suggest that bone resorption is largely regulated by local E2 action, although how much of this effect is due to systemic vs. local aromatization of blood testosterone remains unclear. Falahati-Nini et al. assessed the relative effects of testosterone alone, or E2 alone) or complete (testosterone and E2) sex hormone replacement in 59 elderly men, who prior to the study were rendered acutely androgen and estrogen deficient by combined treatment with a GnRH agonist and an aromatase inhibitor (48). Bone resorption markers increased significantly in men after acute hormone withdrawal but remained unchanged in subjects with combined testosterone and E2 replacement therapy. Interestingly, E2 alone almost completely prevented the increase in bone resorption markers, whereas testosterone alone was much less effective in this respect. In another study with pharmacological hormone withdrawal, testosterone replacement alone was partially able to prevent the increase in bone resorption markers (U-DPD, but not S- and U-NTX), whereas in men with combined sex hormone replacement, bone resorption remained unaltered (51). Although bone resorption seems to be under the predominant control of E2 in men, an E2-independent effect of androgens on bone resorption seems conceivable as androgen receptors were found on human osteoclasts (52), and in vitro and animal data suggest crucial roles for both estrogens and androgens in male bone metabolism. Due to limited tolerance for chronic androgen deficiency, both of these studies were short-term so the effect of longer term hormonal regul-
lation of bone turnover and density remains unclear.

Androgen receptors are found on osteoblasts, suggesting that testosterone per se may have important effects on bone formation. In keeping with this concept, Falahati-Nini et al. observed that markers of bone formation (OC, PINP) decreased significantly in men during acute sex hormone withdrawal. In contrast, these markers remained unchanged in men receiving testosterone and E2 replacement therapy (48). Of note, the decrease in serum OC was prevented equally well by either testosterone or E2, while serum PINP levels were affected primarily by E2 but not by testosterone. In contrast, no such effect on bone formation was observed in a study of sex steroid withdrawal for a period of twelve weeks, which might be due to the longer study duration with an overall increase in bone turnover that had occurred by the time the measurements were made (51). In addition, the potential effect of E2 on osteoblastic differentiation was not only observed during selective E2 replacement in hypogonadal men, but also as a result of supraphysiological E2 treatment (hCG treatment) in elderly men with otherwise adequate androgen exposure (50). These data would suggest that it is principally E2 that regulates osteoblastic differentiation, and both, testosterone and E2 may be important in modulating late osteoblastic function.

In summary, results from interventional studies support the findings from observational studies of bone development that estradiol is important for bone turnover in elderly men. These findings suggest that aromatization of testosterone to E2 plays a significant role in the regulation of bone metabolism, and, ultimately, influences age-related bone loss in elderly males (29). Just how important relative to blood testosterone for maintenance of bone in older men and whether the principal effects are due to local aromatization in bone or from circulating estradiol remain contentious.

Conflict of interest
The corresponding author declares that there are no conflicts of interest.

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