INTRODUCTION

Bone is an endocrine tissue sensitive to androgens and estrogens. The bioactivity of circulating sex steroids is modulated by sex hormone-binding globulin and local conversion in bone tissue, for example, from testosterone (T) to estradiol (E2) by aromatase, or to dihydrotestosterone by 5α-reductase enzymes. Our understanding of the structural basis for gender differences in bone strength has advanced considerably over recent years due to increasing use of (high resolution) peripheral computed tomography. These microarchitectural insights form the basis to understand sex steroid influences on male peak bone mass and turnover in cortical vs trabecular bone. Recent studies using Cre/LoxP technology have further refined our mechanistic insights from global knockout mice into the direct contributions of sex steroids and their respective nuclear receptors in osteoblasts, osteoclasts, osteocytes, and other cells to male osteoporosis. At the same time, these studies have reinforced the notion that androgen and estrogen deficiency have both direct and pleiotropic effects via interaction with, for example, insulin-like growth factor 1, inflammation, oxidative stress, central nervous system control of bone metabolism, adaptation to mechanical loading, etc., This review will summarize recent advances on these issues in the field of sex steroid actions in male bone homeostasis.

Keywords: androgen receptor; bone mineral density; estradiol; estrogen receptor; male; osteoblast; osteoclast; osteocyte; osteoporosis; sex hormone-binding globulin; testosterone

Basic sex steroid physiology in relation to bone health

Metabolism and regulation of circulating sex steroids

Sex steroid serum concentrations are determined by their synthesis in the gonads and adrenals as well as by catabolic enzyme activity. Testosterone (T), the principal circulating androgen, can be aromatized to estradiol (E2, the principal estrogen) or 5α-reduced to dihydrotestosterone (DHT), a non-aromatizable androgen. Thus, in bone T may stimulate the androgen receptor (AR), either directly or as DHT, as well as estrogen receptors (ER) following aromatization. The synthesis of sex steroids is under hypothalamic-pituitary feedback control via gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)). Several high-profile reports have suggested that FSH contributes to hypogonadal bone loss in female rodents, but other groups have failed to confirm the presence of FSH receptors in bone cells or a physiological effect thereof. Gonadotropin inhibition also does not seem to influence bone turnover markers independent of sex steroid levels in men.

Serum bioactivity of sex steroids is restricted in humans by their high affinity binding to sex hormone-binding globulin (SHBG). About 45% of T, the principal circulating androgen, is bound to SHBG in normal men; only the non-SHBG-bound fraction is considered

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bioactive. Most of this fraction is bound nonspecifically to albumin and other carrier proteins, while only about 2% of T and E2 and <1% of DHT circulates freely in normal men.21 According to the free hormone hypothesis however, this constitutes the most physiologically important fraction.22 Others have pointed to the limitations of this theory and have pleaded to “stop looking backwards” and focusing exclusively on free sex steroid levels. Indeed, even if only a small portion of the protein-bound sex steroids is physiologically active, it is likely to have more influence than the 1%–2% that is free.23

**Sex steroid nuclear receptor signaling**

Sex steroids classically act via their nuclear receptors (NRs: AR, ERα and ERβ), which bind directly or indirectly via other proteins to cognate DNA response elements (androgen response elements and estrogen response elements), recruit coactivators and other regulatory proteins, and influence gene transcription.24 Two types of androgen response elements exist (classical and selective), but mice with abrogated AR binding to selective androgen response elements only show a sex organ phenotype.25 Nonclassical signaling pathways of these NRs have also been described but remain somewhat controversial (especially for AR). These have not yet been successfully translated (therapeutically) to studies in humans, and will therefore not be further discussed here.

Androgen and estrogen deficiency also have important indirect effects via other signaling pathways in bone (e.g. insulin-like growth factor I (IGF-1)26), potentially via AR and ER in other tissues like muscle, fat, and the nervous system, or via, for example, decreased physical activity in AR knockout (ARKO) mice.27,28 Therefore, caution needs to be exerted regarding the roles of AR and ER specifically in bone cells when interpreting the results of gonadectomy or ARKO/ERKO models. These limitations have been overcome recently using Cre/LoxP mouse models (see below). The fact that these cell-specific models show less dramatic bone phenotypes than their global ARKO/ERKO counterparts confirms the importance of pleiotropic actions of sex steroids on bone.

**STRUCTURAL BASIS OF MALE BONE STRENGTH**

The musculoskeletal system is sexually dimorphic, being on average (but obviously not in all cases) larger and more robust in men compared to women. Bone strength is determined by peak bone mass (PBM) acquisition in young adulthood and subsequent turnover in different compartments (cortical and trabecular bone), resulting in gender differences in bone length, bone mineral density (BMD), geometry, and microarchitecture. For other determinants like material properties, gender differences require further investigation. Osteoporotic fracture risk is not only determined by purely skeletal factors but also by risk of falls, which should also be evaluated and treated in osteoporotic men.29,30

In the following paragraphs, we will discuss the structural basis of male bone strength. It is well-known that men have higher peak areal BMD (aBMD) in young adulthood and aBMD declines slower as they age.31 However, this should be interpreted with caution because of the projectional nature of dual energy X-ray absorptiometry (DXA), which leads to potential confounding by bone size, geometry, and porosity rather than true volumetric density (vBMD). In this respect, quantitative computed tomography (qCT), especially high resolution peripheral qCT (HR-pqCT), has recently led to a major advance in our understanding of the gender dimorphism in the structural determinants of bone strengths.

**Bone size**

Men are on average taller due to the actions of sex steroids on the growth plate (see below). From a biomechanical point of view, longer bones (which are also wider and have greater bone mass) are more resistant to bending. However, several cohort studies have found a positive association between height and risk of low-energy fractures, although the results in men are scant and only bordered significance.32,33 This may be explained by greater loads when falling from a larger height, longer arms of moment, as well as by relatively thinner cortices and greater cortical porosity in larger bones; although definitive data in men are currently lacking.34

**Peak bone mass**

Bone mineral mass can be low in old age because insufficient PBM attainment during skeletal growth (modeling phase) or due to an imbalance in the normally coupled process of bone turnover (remodeling phase), either by excessive bone resorption or decreased bone formation. The risk of many chronic diseases which manifest in old age, including osteoporosis, is determined at least in part by childhood or even prenatal experiences. PBM is largely (60%–80%) genetically determined, but the remainder is determined by environmental factors, some of which (e.g. maternal age and vitamin D status during pregnancy) are potentially amenable to interventions.35,36 Theoretically, small increases in PBM translate into a large delay in osteoporosis onset,37 although it remains to be established whether bone mass increments early in life can be sustained into old age.38

The age of PBM or peak bone density differs between studies and depends on the measured site. In a prospective study with 15 years of follow-up, BMC in boys plateaued first at the femoral neck, followed by the total hip, lumbar spine, and whole body, respectively at the age of 2, 3, 4, and 6 years after peak height velocity (PHV, average age 14 in men).39 Hip aBMD decreases early after it peaks.40 Longitudinal studies across a wider age range have however estimated that lumbar spine BMD may peak as late as age 33 and 40 in men and women, respectively. Bone mass increments are followed 1–2 years later by mineralization, and there are little differences between men and women when the age of PHV is used as the reference.41 The peripubertal years (age 12–16) contributed 33%–46% to adult-level BMC, demonstrating the importance of this window of opportunity for interventions to optimize PBM.42 There is considerable variation in the timing of male puberty, but neither early nor late normal puberty seem to compromise PBM.42

**Bone geometry and microarchitecture**

Men develop wider bones even after adjustment for height because of greater periosteal apposition in the appendicular skeleton, whereas girls predominantly decrease their endosteal perimeter.43 As a result, cortical bone in men is placed further away from the neutral axis, providing greater resistance to bending, but no difference in size-independent biomechanical indices.44 Bone width predicts fractures in older men independently of aBMD, and men with low bone width combined with BMD T-scores <-1 have similar fracture incidence as men with T-scores <-2.45

Recently, most cross-sectional studies using HR-pqCT have documented cortical and trabecular bone across the lifespan in more detail (Figure 1).

At PBM, men have higher trabecular bone volume fraction (Figure 1a) due to thicker (Figure 1c), more plate-like trabeculae. On the other hand, cortical porosity is greater (not shown) and cortical vBMD slightly lower (Figure 1f), while trabecular vBMD (not shown) and cortical thickness (Figure 1e) are not yet greatly different.46–48 Increasing cross-sectional area and cortical thickness, ongoing cortical mineralization and decreasing porosity contribute to ongoing aBMD and estimated strength increases during
the 3rd decade of life in men, whereas in women cortical perimeter and strength do not increase during young adulthood.

Both cortical and trabecular vBMD losses are more pronounced with age in women, although rates of trabecular bone loss vary across sites. With ageing, endocortical resorption outpaces periosteal apposition leading to a thinner cortex in both sexes, but older women show dramatically greater medullary expansion and thus cortical thinning (Figures 1e and 3). This was nicely illustrated in one prospective pqCT study (Figure 2), which also demonstrated the limitations of capturing bone geometrical changes across the lifespan from cross-sectional data.

Cortical porosity is greater in young men, but increases faster in women, especially after menopause, resulting in similar porosity in both genders (difference compared to age 20 shown in blue), but this is much more pronounced in women, and ongoing periosteal apposition (difference compared to age 20 shown in red) in both genders is unable to compensate for the differences. On the other hand, bone is placed further away from the central axis in men at all ages, and this dramatically improves biomechanics. Adapted, with permission, based on data from Lauretani et al.

Figure 1: Structural determinants of bone strength in men based on high resolution peripheral computed tomography (HR-pqCT) at the ultradistal radius. Adapted, with permission, from Khosla et al. Tb.Th: trabecular thickness; BV/TV: trabecular bone volume/tissue volume; Tb.N: trabecular number; Tb.Sp: trabecular separation; Cort vBMD: cortical volumetric bone mineral density; Cl.Th: cortical thickness.

Figure 2: Schematic model of bone geometry changes at the tibia during adult lifespan in men and women. When comparing young women and men, men have greater cortical thickness due to greater periosteal perimeter (shown in black), while women mainly decrease their endocortical perimeter (also shown in black). With ageing, cortical thinning results from endocortical bone resorption in both genders (difference compared to age 20 shown in blue), but this is much more pronounced in women, and ongoing periosteal apposition (difference compared to age 20 shown in red) in both genders is unable to compensate for the differences. On the other hand, bone is placed further away from the central axis in men at all ages, and this dramatically improves biomechanics. Adapted, with permission, based on data from Lauretani et al.

SEX STEROID SIGNALING IS KEY TO MUSCULOSKELETAL SEXUAL DIMORPHISM

Are gender differences in bone phenotype due to sex steroid signaling or other pathways? Sex steroids and especially estrogens were once considered a central hub on which bone signaling pathways converged in both genders, but this unitary estrogen-centric view has been modified. Both at PBM and during ageing, BMD, bone geometry, bone turnover, and skeletal muscle mass in men have now been associated with multiple hormones (e.g., IGF-1 and IGF binding proteins), endogenous PTH, vitamin D, and thyroid hormone within the normal range), immunological, and other pathways in bone (e.g., CRP, RANK/RANKL/OPG), oxidative stress, and classical ageing pathways, etc. Mostly similar associations have however been reported in women, and a recent meta-analysis of genome-wide BMD association studies failed to identify any significant gene-by-sex interactions. Fundamental gender differences in key bone signaling pathways therefore seem unlikely.

Conversely, the musculoskeletal system of transsexual men shows a dramatic shift from the female to male phenotype after ovariectomy and prolonged T treatment. Similarly, XY women with complete androgen insensitivity syndrome due to inactivating AR mutations have reduced BMD and a bone geometry intermediate between male and female, and estrogen treatment does not induce periosteal bone apposition in these subjects. BMD is however much lower in both XY and XX women with gonadal dysgenesis, implying that gonadal status or sex steroids are more important than chromosomal determinants. Overall, we can conclude that androgens (or AR mediated androgen action) are necessary for musculoskeletal sexual dimorphism in development and ageing, although they probably have important indirect actions on bone via...
Sex steroids control longitudinal bone size at the growth plate

It is generally accepted that delayed estrogen-mediated closure of epiphyseal growth plate cartilage contributes to greater bone length in men. T on the other hand probably also stimulates height velocity mainly via aromatization and estrogen-mediated pituitary growth hormone release. Non-aromatizable androgens increase growth rate in boys without altering serum GH/IGF-1, possibly via the AR in chondrocytes and local IGF-1 signaling in the growth plate.92 Pubertal height velocity acceleration and subsequent growth plate closure seem to be absent in men with inactivating ERα mutations87 or aromatase deficiency44–47 who show steady, continuous growth, suggesting that estrogens have a dominant role in these processes. A recent study using ERαKO mice with no residual truncated isoforms confirmed the continuing longitudinal growth seen in the ERαKO (see next paragraph).88 The fact that 46, XY girls with complete androgen insensitivity syndrome have a growth spurt similar in timing and amplitude as 46, XX girls89 is probably due to estrogen effects, but it does not exclude a role for AR itself. Male ARKO mice have been anecdotally noted to have increased femur length,90 but in men the effects of increased estrogen due to aromatization probably override any possible direct effect of T on the growth plate. On the other hand, further research on androgen regulation of (growth plate) chondrocytes would be of interest.

Estrogen deficiency: the primary mediator of bone loss in older hypogonadal men

The primary role of estrogens in postmenopausal osteoporosis was proposed by Albright et al., in 1941.91 Similarly, hypogonadal men with low T were noted to have increased risk of osteoporosis and fractures.92,93 The historic dichotomous view that estrogens were important for bone health in women and androgens in men was however challenged by a unique report of a man with an ERα mutation.94 He presented with tall stature, incomplete epiphyseal closure in adulthood and markedly decreased bone density. In 1998, investigators from the Mayo Clinic proposed a unitary model for the pathogenesis of osteoporosis with a central responsibility for estrogens, not only in post-menopausal but also in male osteoporosis and even in the negative calcium balance and secondary hyperparathyroidism in age-related osteoporosis.95 This was based on seminal observations,96 subsequently confirmed in multiple prospective cohort studies, of a strong association between (calculated free or bioavailable) E2, bone turnover, and bone density in community-dwelling men; whereas, the same associations with T were variable, weak, absent, or disappeared after correcting for E2 or other variables.97–100 On the other hand, threshold effects (i.e., greater decreases in serum E2 in women compared to relatively normal androgen levels in ageing men) probably limit the conclusions that can be drawn from such cohort studies. Indeed, in several studies, E2 (and SHBG, see below) have been associated with bone loss below a certain threshold;96,101,102 while this has not been confirmed in younger cohorts of men.103,104 In the European Male Ageing Study (EMAS) of men aged 40–79 years, the prevalence of T between 11 and 8 nmol/L was 12.9%, while only 4.1% had T < 8 nmol/L, showing that most hypogonadism is mild in cohort studies.108 Nevertheless, several complimentary lines of evidence (Table 1)109–112 confirm that estrogens are crucial to restrain bone turnover in ageing men. Thus, the finding that T blocks orchidectomy-induced bone loss in ERαKO mice113 has been contradicted by human experiments,110 which is possibly due to limitations of the ERαKO mouse model (which shows high bone mass due to increased T, which also contrasts with the situation in an ERαKO man).83

Low testosterone and high sex hormone-binding globulin pose additional risks

The overall conclusion thus appears to be that estrogens are more important than androgens in maintaining bone health in ageing men. Yet low T and high SHBG may still harbor additional detriments. Free or bioavailable T has been associated with BMD (at predominantly cortical sites), bone area, muscle area and strength, reduced fat mass and hip, vertebral and non-vertebral fractures in different large studies in older men.107,122–124 Late-onset hypogonadism (LOH) as defined by the presence of three sexual symptoms according to criteria from the EMAS appears to correlate more strongly than low T alone with low ultrasound-estimated BMD.125 Similarly, risk for hip fractures has been found highest in men with both low E2 and T.126 We have previously shown in mice that optimal effects of T require a functional AR.127 These data are in agreement with findings from a man with concomitant aromatase deficiency and low T, in whom T and E2 replacement showed additive effects.128

In the largest study, low E2 and high SHBG were independent predictors of BMD losses and fracture risk in men, but risks were highest when both bioavailable E2 and T were combined with high SHBG.129,130 SHBG has been associated independently of bioavailable T and E2 with improved cortical bone development in young men, but accelerated bone losses with ageing and consequently increased fracture risk.106,111–114 The mechanisms by which SHBG may influence bone in this apparently paradoxical manner require further investigation.135

The role of catabolic enzymes

Not only synthetic but also catabolic enzymes control steady state sex steroid serum levels. In the Swedish MrOS study, specific androgen metabolites correlated with male BMD, while T itself did not.102 Polymorphisms in the enzymes catechol-O-methyl-transferase (COMT, an estrogen degrading enzyme) and uridine diphosphate glucuronosyltransferase 2B7 (which inactivates mainly androgens

| Table 1: Evidence supporting the primary role of estrogens in bone loss in older men |
|----------------------------------|--------------------------------------------------|
| **Animal models**                |                                                   |
| E2 is a more effective bone-sparing agent than DHT in aged male rats109    |
| **Human genetic evidence**       |                                                   |
| Polymorphisms in ER (but not AR) signaling negatively influence male bone110–112 | |
| Longer CAG repeats diminish AR function but may be compensated by increased T levels, paradoxically stimulating bone via aromatization113 |
| Men with aromatase deficiency have high T, but suboptimal bone mass which improves with estrogen (but not androgen) therapy64–67 |
| **Human observational studies**  |                                                   |
| Several large cohort studies confirm relationship between E2 and male bone metabolism, while the same relationship with T is weak, absent, or disappears after correcting for E2 (but not vice versa) |
| Increased bone turnover and fracture risk in older men receiving androgen deprivation therapy for prostate cancer may be due to a loss of substrate for aromatization, since AR antagonists alone are not detrimental for bone115–117 |
| **Human experiments**            |                                                   |
| Aromatase inhibition in older men increases T, but decreases E2 and BMD118 |
| Gonadotropin inhibition and T or E2 add-back shows that E2 has the dominant antiresorptive effect119 |
| T replacement with or without aromatase inhibition demonstrates that T alone cannot overcome male bone resorption in the absence of E2120 |

BMD: Bone Mineral Density; CAG: Cytosine adenine guanine; DHT: Dihydrotestosterone
but also some estrogens) have been associated with increased sex steroid levels and bone geometry in young men.156–159 In another large population-based study, the COMT polymorphism was independently associated with fracture risk but not BMD, and only in men.140 The activity of these and other steroid metabolizing enzymes, and whether they exert their influence mainly systemically or locally in bone, merits further study.

The contribution of 5α-reductase type 1

Only a small percentage of DHT enters the circulation, and this fraction does not correlate with male BMD.102 5α-reductase (encoded by Srd5a) type 2 is primarily expressed in male reproductive tissues including the prostate; whereas, type 1 and 3 are expressed in many tissues including reproductive tissues and bone.141 The 5α-reductase inhibitor finasteride inhibits type 2 and 3; whereas, dutasteride inhibits all three. It is therefore not unexpected that finasteride does not affect bone density, turnover, fracture risk, or muscle anabolic effects of androgens.142–148 In contrast, male Srd5a1−/− mice have recently been characterized as having reduced bone mass and muscle strength despite normal androgen serum levels.149 Human RCTs however have not shown effects of dutasteride on bone metabolism,145,150 nor does dutasteride influence the anabolic effects of T on muscle.151 Whether or not 5α-reductase type 1 activity contributes to male bone mass, thus merits further investigation.

Meanwhile, aromatizable androgens may be used preferentially to simultaneously compensate androgen and estrogen deficiency in men with LOH. In a small randomized controlled trial (RCT), DHT gel treatment in older men with low T suppressed endogenous T without affecting E2 or BTMs,152 while another RCT showed decreased E2 as well as spinal BMD.153 Therefore, the main effect of androgens or selective AR modulators (SARMs) may depend on their suppression of estrogens. We have previously shown in rodents that androgens and estrogens apparently act via different pathways and that their combination might be beneficial compared to each strategy alone,154,155 although this requires confirmation in men with LOH.

RELATIVE CONTRIBUTIONS OF ANDROGEN RECEPTOR, ESTROGEN RECEPTORα, AND ESTROGEN RECEPTORβ IN CORTICAL VS TRABECULAR BONE

Although observational studies in humans are important to determine the influence of sex steroids on male bone, the study of the respective contributions of AR and ER require knockout animal models as well as confirmation in rare human genetic disorders. These studies have revealed unexpected complexity regarding the roles of these NRs in different bone compartments.26

Both AR and ERα are required for optimal periosteal bone expansion,90,156 although estrogens limit periosteal expansion in early puberty, probably because of time-specific and independent effects on IGF-1.38,81 For trabecular bone development on the other hand, AR is solely responsible.90,156 Indeed, ARKO decreases cancellous bone, while systemic ERKO increases it (probably due to increased androgen levels in this animal model)121,157 and combined AR/ERKO does not decrease trabecular bone more than ARKO alone.90 Compared to wild-type females, male pubertal ARKO mice have equal length, decreased trabecular bone, and identical cortical bone parameters; showing that androgens are required for optimal bone development, especially trabecular but also cortical, but not for longitudinal growth.158

These results are confirmed in humans: estrogen therapy in young aromatase-deficient men indeed improves cortical thickness and area; however, without increasing trabecular vBMD.96 Nevertheless, more recent animal studies using cell-specific ERαKO models do also suggest a role for ERα in trabecular bone formation (see below). ERβ plays a role in female bone health,157,159,160 but male ERβKO mice have normal bones and ERβKO show no difference to ERαKO alone.154

ACTION MECHANISMS OF AR AND ERα IN SPECIFIC BONE CELLS

Androgens control osteoblasts and osteocytes, while estrogens also regulate osteoclasts

Cell culture experiments from ARKO mice have suggested that AR controls mainly osteoblasts and their indirect control of osteoclastogenesis.161 However, as mentioned above, global ARKO/ERKO models or gonadectomy/antagonist studies preclude definitive conclusions about the cellular targets of AR and ER in male bone. Indirect evidence suggested that AR and ER signaling targets mainly osteoblasts and osteoclasts, respectively. These assumptions have only recently been verified using Cre/LoxP mouse models, although this requires careful verification that neither the Cre driver nor the floxed genotypes have effects themselves (see Rana et al. in this theme issue).

Androgen receptor in the osteoblast lineage

Osteocalcin-Cre driven ARKO revealed that androgens stimulate mineralizing osteoblasts, thus indirectly inhibiting cortical and trabecular bone resorption, especially during times of bone accrual.162 Col2.3-Cre driven ARKO showed that mature osteoblasts contribute to trabecular bone maintenance.163 However, periosteal apposition was not influenced by AR deficiency in these mature cells, probably because the periosteum contains more pre- and proliferating osteoblasts. Indeed, AR overexpression in immature osteoblasts increases perios skeletal and decreases endosteal bone formation; whereas, osteogenesis was inhibited at both envelopes following AR overexpression in mature osteoblasts.164,165 Recently, we have shown in Dmp1-Cre mice that loss of AR in osteocytes results in similar, moderate impairment of trabecular bone maintenance.166 Importantly, AR expression increased with the maturation of osteoblasts towards osteocytes.167 These different phenotypes suggest that AR has a direct role across the entire osteoblast lineage.

Androgen receptor and estrogen receptor alpha in osteoclasts

Clearly, AR signaling has important indirect restraining effects on osteoclasts, for example by controlling cytokine production in bone marrow stromal cells.79 AR has been detected in vitro and with immunohistochemistry in rodent osteoclasts albeit at very low levels, and it may be absent in human osteoclasts.158,159 Several though not all studies have suggested that androgens also directly suppress in vitro osteoclast formation from hematopoietic precursors.160–167 Although they could not confirm the presence of AR in osteoclasts, the group of Kato found to their surprise that cathepsin K-Cre-driven ARKO also induces bone resorption (although this data has only been published in abstract),172 in a similar way as they and others have reported for osteoclast-specific ERKO in female (but not male) mice.173,174 Thus, a direct in vivo role for AR in osteoclasts merits independent confirmation.

Estrogen receptor alpha in osteoblasts

ERα has recently been established to have a role in osteoblasts and osteocytes too. It was already known that osteoblast-specific overexpression of aromatase increases bone mass in male mice.175 Prx1
Cre and Osterix-Cre mice have now been used to selectively excise ERα from pluripotent mesenchymal progenitors in the limb bud of the appendicular skeleton, and respectively, osteoblast progenitors. These mice showed mainly cortical bone deficits resulting from decreased periosteal bone formation, although cortical bone deficits were overcome during adulthood in Prx1-Cre ERαKO males. Deletion of ERα using the Col1a1 deleter did not affect cortical or trabecular bone. However, this should not be taken as evidence that ERα has no role downstream in osteoblast differentiation. Indeed, osteocalcin-Cre ERαKO decreased trabecular bone in males, and both trabecular and cortical bone in females. Dmp1-Cre ERαKO males also showed decreased bone formation and less trabecular bone, but there was no effect on cortical bone, or any effect in females. The authors concluded that the trabecular bone-sparing effects of estrogens are mediated by osteocyte ERα in males, but probably by osteoclast ERα in females. Interestingly, both ERαKO and ARKO have been known to influence the response to mechanical loading, but the response to mechanical loading was unaltered in the respective osteocyte-specific knockout mice.

In summary, these studies in male mice suggest that both AR and ERα are required for optimal cortical bone expansion via actions in immature osteoblasts, and trabecular bone maintenance via actions in more differentiated osteoblasts and osteocytes (Figure 3).

Indirect effects of androgen receptor and estrogen receptors on bone via muscle, fat, and the nervous system

The interaction of bone with muscle, adipose, and neural systems is increasingly studied. Tissue-specific ARKO models are discussed in more detail in the accompanying review by Rana et al. in this theme issue.

Bone-muscle interaction

Male ARKO mice have impaired muscle development, and additional ERαKO further reduces muscle mass. However, muscle-specific ARKO mice did not show altered bone metabolism and only slightly reduced peripheral skeletal muscle mass, possibly because only perineal muscles display high AR content and androgen regulation in mice. This contrasts with the well-known anabolic effects of androgens on human muscle. Thus, although the effects of androgens on bone in mice are unlikely to be mediated via the AR in muscle, it remains unclear whether sarcopenia in older men with LOH contributes directly or is merely associated with bone loss and fractures.

Bone-fat interaction

Clinical evidence suggests a positive association between bone and fat, but mainly in females, possibly because adipocyte aromatase activity influences circulating E2 or because of increased gastrointestinal loading. Fat mass is principally regulated by estrogens since double knockouts have similar adiposity compared to ERαKO alone, although androgens also have clear lipolytic activity, and male ARKO mice have increased adiposity. A link between glucose, insulin, and bone metabolism has also been suggested in mice, and male bone metabolism is altered in diabetes and the metabolic syndrome. Thus, whether AR or ER signaling in adipocytes modulates sex steroid effects on bone would be of interest.

Central nervous system control of bone mass

Central nervous system regulation of bone mass has been demonstrated recently with often opposite effects to peripheral signaling. This also seems to be the case for estrogens, since neuron-specific ERαKO using nestin-Cre increases bone formation via leptin. Conditional inactivation of AR in the nervous system of mice however disrupts the somatotropic axis as evidenced by growth retardation and twofold lower serum IGF-1, without relative differences in total bone mass or body composition. This reiterates that the skeletal sexual dimorphism resulting from sex steroids is dependent on indirect effects via growth hormone, IGF-1 and IGF binding proteins in brain, bone, and liver.

CONCLUSIONS

The musculoskeletal system is more robust in men, and sex steroid signaling remains essential for this sexual dimorphism. The advent of high resolution imaging has allowed better insights into the microarchitectural determinants of male bone strength. Specifically, androgens may promote trabecular bone development and thickness in young adulthood, as well as cortical consolidation in midlife and maintenance of cortical thickness and trabecular bone volume in older men by stimulating periosteal apposition and trabecular bone formation. Thus, although estrogen deficiency is the primary mediator of hypogonadal bone loss in men, high SHBG and low T probably pose additional detriments. The role of sex steroid catabolic enzymes and local lipid metabolism within bone (e.g. by 5α-reductase type 1) requires further investigation. Recent studies using knockout mouse models have refined our understanding of the contributions of AR and ERα in osteoclasts, osteoblasts, and osteocytes to cortical surfaces in male mice. Based on a μCT image of the proximal murine tibia.

Figure 3: Schematic illustration of the cellular targets of androgen receptor (AR) and estrogen receptor (ER) α on periosteal, endocortical, and trabecular surfaces in male mice. Based on a μCT image of the proximal murine tibia.
and trabecular bone development and maintenance. At the same time, they have reinforced the notion that sex steroids must have important pleiotropic effects on bone, for example, via interaction with the nervous system, IGF-I and altered response to mechanical loading. A better understanding of the microarchitectural, cellular and molecular mechanisms of actions of androgens and estrogens continues to be necessary to develop therapeutic strategies which can exploit their benefits for bone health without unwanted side effects in other tissues.

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COMPETING INTERESTS

Authors would declare that we have no financial or other competing interests.

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