Androgens and oestrogens in skeletal sexual dimorphism

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ABSTRACT

Bone is an endocrine tissue expressing androgen and oestrogen receptors as well as steroid metabolizing enzymes. The bioactivity of circulating sex steroids is modulated by sex hormone-binding globulin and local conversion in bone tissue, e.g. from testosterone to oestradiol 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 knock-out 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 oestrogen deficiency have both direct and pleiotropic effects via interaction with e.g. 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; oestradiol; oestrogen receptor; male; osteoblast; osteoclast; osteocyte; osteoporosis; sex hormone-binding globulin; testosterone
1. INTRODUCTION

Bone is an endocrine tissue sensitive to androgens and oestrogens. The epidemiology and clinical approach to male osteoporosis have recently been reviewed elsewhere. Here, we will review the literature on the role of sex steroids in male bone health and more specifically in osteoporosis, the most common metabolic bone disease. We will focus on human data with support from recent evidence from knock-out mouse models. The latter are covered in more detail elsewhere in this issue [Ref Zajac AJA].

1.1 Men contribute considerably to the disease burden of osteoporosis

Although still perceived by the public, patients and most physicians as a typically female condition, men account for a substantial proportion of the burden of osteoporosis. At age 50, the remaining lifetime risk of osteoporotic fractures is 20-25% in Caucasian men versus 45-55% in women. About one third of the 9 million osteoporotic fractures worldwide occurs in men. For hip fractures, a review of worldwide epidemiological data recently showed that while incidence varied more than tenfold between countries, men almost invariably have twofold lower risk (except perhaps in regions where incidence is very low). Male osteoporosis is responsible for about 25% of the economic burden of osteoporosis in the United States ($ 17 billion total costs) and Canada (2.3 billion Canadian dollars). Nevertheless, osteoporosis remains even more underdiagnosed and undertreated in men compared to women, regardless of whether it is evidenced by low bone mineral density (BMD), a low-energy fracture or absolute 10 year fracture probability.

1.2 Basic sex steroid physiology in relation to bone health

1.2.1 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 oestradiol (E2, the principal oestrogen) 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 oestrogen 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 in dependent 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 testosterone (T), the principal circulating androgen, is bound to SHBG in normal men; only the non-SHBG-bound fraction is considered bioactive. Most of this fraction is bound non-specifically to albumin and other carrier proteins, while only about 2% of T and oestradiol (E2) and <1% of DHT circulates freely in normal men. According to the free hormone hypothesis however, this constitutes the most physiologically important fraction. 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 to 2% that is free.
1.2.2 Sex steroid nuclear receptor signalling

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 (AREs and EREs), recruit coactivators and other regulatory proteins, and influence gene transcription. Two types of AREs exist (classical and selective), but mice with abrogated AR binding to selective ARE only show a sex organ phenotype. Non-classical signalling 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 oestrogen deficiency also have important indirect effects via other signalling pathways in bone (e.g. insulin-like growth factor I (IGF-1)), potentially via AR and ER in other tissues like muscle, fat and the nervous system, or via e.g. decreased physical activity in AR knock-out (ARKO) mice. 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.

2. 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 turn-over 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.

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. However, caution should be used 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.

2.1 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. 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.

2.2 Peak bone mass
Bone mineral mass can be low in old age because insufficient PBM attainment during skeletal growth (modelling phase) or due to an imbalance in the normally coupled process of bone turnover (remodelling 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 area 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.\(^{39}\) There is considerable variation in the timing of male puberty, but neither early nor late normal puberty seem to compromise PBM.\(^{42}\)

### 2.3 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.\(^{1}\) 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.\(^{43, 44}\) 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.\(^{45}\)

Recent, mostly 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 (Fig. 1a) due to thicker (Fig. 1c), more plate-like trabeculae. On the other hand, cortical porosity is greater (not shown) and cortical vBMD slightly lower (Fig. 1f), while trabecular vBMD (not shown) and cortical thickness (Fig. 1e) are not yet greatly different.\(^{43, 46-48}\) Increasing cross-sectional area and cortical thickness, ongoing cortical mineralization and decreasing porosity contribute to ongoing aBMD and estimated strength increases during the third decade of life in men, whereas in women cortical perimeter and strength do not increase during young adulthood.\(^{40, 49}\)

Both cortical and trabecular vBMD losses are more pronounced with age in women, although rates of trabecular bone loss vary across sites.\(^{43, 50}\) 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 (Fig. 1e and Figure 2).\(^{43, 51-53}\) 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.\(^{51}\)

Cortical porosity is greater in young men but increases faster in women, especially after menopause, resulting in similar porosity after age 50.\(^{43, 44}\) Cortical vBMD decreases from midlife in women but
only after age 75 in men, whereas trabecular bone loss starts almost immediately after PBM in both sexes (Fig. 1a) but more so in women, with acceleration during perimenopause. Bone loss in men is more due to decreased formation rather than increased resorption, therefore trabeculae become thinner (Fig. 1c) but less perforated, disconnected and thus widely spaced (Fig. 1b,d) compared to postmenopausal women. Poor trabecular microarchitecture predisposes men to multiple and severe vertebral and peripheral fractures. At the femoral neck, men have higher aBMD despite lower vBMD, due to greater length and femoral neck area, showing the importance of geometry over mineralization in the decreased hip fracture risk in men. This also explains why men and women have the same strength for the same femoral neck aBMD: because of the offsetting effects of higher bone area and lower vBMD in men.

In conclusion, detailed imaging techniques have greatly improved our understanding of the structural basis for gender differences in bone strength. How and to what extent sex steroids regulate this sexual dimorphism in cortical and trabecular bone will be discussed below.

3. SEX STEROID SIGNALLING IS KEY TO MUSCULOSKELETAL SEXUAL DIMORPHISM

Are gender differences in bone phenotype due to sex steroid signalling or other pathways? Sex steroids and especially oestrogens were once considered a central hub on which bone signalling pathways converged in both genders, but this unitary oestrogen-centric view has been modified. Both at peak bone mass (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, 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 signalling 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 testosterone 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 oestrogen 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 androgen receptor mediated androgen action) are necessary for musculoskeletal sexual dimorphism in development and ageing, although they probably have important indirect actions on bone via aromatization, oxidative stress, pro-inflammatory cytokines, growth factors (e.g. transforming growth factor (TGF)-β, IGF-1), etc.

4. SEX STEROID REGULATION OF MALE BONE METABOLISM

4.1 Sex steroids control longitudinal bone size at the growth plate

It is generally accepted that delayed oestrogen-mediated closure of epiphyseal growth plate cartilage contributes to greater bone length in men. Testosterone on the other hand probably also stimulates height velocity mainly via aromatization and oestrogen-mediated pituitary growth hormone release. Non-aromatizable androgens increase growth rate in boys without altering serum GH/IGF-1, possibly via the androgen receptor in chondrocytes and local IGF-1 signalling in the growth plate. Pubertal
height velocity acceleration and subsequent growth plate closure seem to be absent in men with inactivating ERα mutations\textsuperscript{83} or aromatase deficiency\textsuperscript{84-87} who show steady, continues growth, suggesting that oestrogens 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).\textsuperscript{88} The fact that 46,XY girls with complete androgen insensitivity syndrome have a growth spurt similar in timing and amplitude as 46,XX girls\textsuperscript{89} is probably due to oestrogen effects, but it does not exclude a role for AR itself. Male ARKO mice have been anecdotally noted to have increased femur length,\textsuperscript{90} but in men the effects of increased oestrogen due to aromatization probably override any possible direct effect of testosterone on the growth plate. On the other hand, further research on androgen regulation of (growth plate) chondrocytes would be of interest.

### 4.2 Oestrogen deficiency: the primary mediator of bone loss in older hypogonadal men

The primary role of oestrogens in postmenopausal osteoporosis was proposed by Fuller Albright in 1941.\textsuperscript{91} Similarly, hypogonadal men with low T were noted to have increased risk of osteoporosis and fractures.\textsuperscript{92, 93} The historic dichotomous view that oestrogens 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.\textsuperscript{93} 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 oestrogens, not only in postmenopausal but also in male osteoporosis and even in the negative calcium balance and secondary hyperparathyroidism in age-related osteoporosis.\textsuperscript{58} This was based on seminal observations,\textsuperscript{94} 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.\textsuperscript{95-105} 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,\textsuperscript{96, 101, 106} while this has not been confirmed in younger cohorts of men.\textsuperscript{105, 107} 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.\textsuperscript{108} Nevertheless, several complimentary lines of evidence (Box 1) confirm that oestrogens are crucial to restrain bone turnover in ageing men. Thus, the finding that T blocks orchidectomy-induced bone loss in ERαKO mice\textsuperscript{109} has been contradicted by human experiments,\textsuperscript{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\textsuperscript{83}).

### 4.3 Low T and high SHBG pose additional risks

The overall conclusion thus appears to be that oestrogens are more important than androgens in maintaining bone health in ageing men. Yet low testosterone and high SHBG may still harbour 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.\textsuperscript{107, 122-124} Late-onset hypogonadism (LOH) as defined by the presence of three sexual symptoms according to criteria from the European Male Ageing Study (EMAS) appears to correlate more strongly than low T alone with low ultrasound-estimated BMD.\textsuperscript{125} Similarly, risk for hip fractures has been found highest in men with both low E2
and T. We have previously shown in mice that optimal effects of T require a functional AR. 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.

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. 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. The mechanisms by which SHBG may influence bone in this apparently paradoxical manner require further investigation.

4.4 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. Polymorphisms in the enzymes catechol-O-methyl-transferase (COMT, an oestrogen degrading enzyme) and uridine diphosphate glucuronosyltransferase 2B7 (which inactivates mainly androgens but also some oestrogens) have been associated with increased sex steroid levels and bone geometry in young men. In another large population-based study, the COMT polymorphism was independently associated with fracture risk but not BMD, and only in men. The activity of these and other steroid metabolizing enzymes, and whether they exert their influence mainly systemically or locally in bone, merits further study.

4.5 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. 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. 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. In contrast, male Srd5a1−/− mice have recently been characterized as having reduced bone mass and muscle strength despite normal androgen serum levels. Human RCTs however have not shown effects of dutasteride on bone metabolism, nor does dutasteride influence the anabolic effects of testosterone on muscle. 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 oestrogen deficiency in men with LOH. In a small randomized controlled trial (RCT), DHT gel treatment in older men with low T suppressed endogenous testosterone without affecting E2 or BTMs, while another RCT showed decreased E2 as well as spinal BMD. Therefore, the main effect of androgens or selective AR modulators (SARMs) may depend on their suppression of oestrogens. We have previously shown in rodents that androgens and oestrogens apparently act via different pathways and that their combination might be beneficial compared to each strategy alone, although this requires confirmation in men with LOH.

5. RELATIVE CONTRIBUTIONS OF AR, ERα AND ERβ 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 knock-out 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.  

Both AR and ER$\alpha$ are required for optimal periosteal bone expansion, although oestrogens limit periosteal expansion in early puberty, probably because of time-specific and independent effects on IGF-1. For trabecular bone development on the other hand, AR is solely responsible. Indeed, ARKO decreases cancellous bone, while systemic ER$\alpha$KO increases it (probably due to increased androgen levels in this animal model) and combined AR/ER$\alpha$KO does not decrease trabecular bone more than ARKO alone. Compared to wildtype 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. These results are confirmed in humans: oestrogen therapy in young aromatase-deficient men indeed improves cortical thickness and area however without increasing trabecular vBMD. Nevertheless, more recent animal studies using cell-specific ER$\alpha$KO models do also suggest a role for ER$\alpha$ in trabecular bone formation (see below). ER$\beta$ plays a role in female bone health but male ER$\beta$KO mice have normal bones and ER$\alpha$$\beta$KO show no difference to ER$\alpha$KO alone.

### 6. ACTION MECHANISMS OF AR AND ER$\alpha$ IN SPECIFIC BONE CELLS

#### 6.1 Androgens control osteoblasts and osteocytes, while oestrogens also regulate osteoclasts

Cell culture experiments from ARKO mice have suggested that AR controls mainly osteoblasts and their indirect control of osteoclastogenesis. 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 signalling 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 Zajac et al. in this theme issue).

##### 6.1.1. AR 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. Col2.3-Cre driven ARKO showed that mature osteoblasts contribute to trabecular bone maintenance. 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 periosteal and decreases endosteal bone formation, whereas osteogenesis was inhibited at both envelopes following AR overexpression in mature osteoblasts. Recently, we have shown in Dmp1-Cre mice that loss of AR in osteocytes results in similar, moderate impairment of trabecular bone maintenance. Importantly, AR expression increased with the maturation of osteoblasts towards osteocytes. These different phenotypes suggest that AR has a direct role across the entire osteoblast lineage.

##### 6.1.2. AR and ER$\alpha$ in osteoclasts

Clearly, AR signalling has important indirect restraining effects on osteoclasts, for example by controlling cytokine production in bone marrow stromal cells. 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. Several though not all studies have suggested that androgens also directly suppress in vitro osteoclast formation from haematopoietic precursors. 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), in a similar way as they and others have reported for osteoclast-specific ERαKO in female (but not male) mice. Thus, a direct in vivo role for AR in osteoclasts merits independent confirmation.

### 6.1.3. ERα 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. 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 oestrogens 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 knock-out 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).

### 6.2. Indirect effects of AR and ER 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 Zajac et al.

#### 6.2.1. 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.

#### 6.2.2. 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 increased gravitational loading. Fat mass is principally regulated by oestrogens 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 signalling in adipocytes modulates sex steroid effects on bone would be of interest.

6.2.3. Central nervous system control of bone mass

Central nervous system regulation of bone mass has been demonstrated recently with often opposite effects to peripheral signalling. This also seems to be the case for oestrogens, since neuron-specific ERαKO using nestin-Cre increases bone formation via leptin.\textsuperscript{187} 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.\textsuperscript{188} 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.\textsuperscript{26, 81, 189}

7. CONCLUSIONS

The musculoskeletal system is more robust in men, and sex steroid signalling 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 oestrogen 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 knock-out mouse models have recently refined our understanding of the contributions of AR and ERα in osteoclasts, osteoblasts and osteocytes to cortical 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, e.g. via interaction with the nervous system, IGF-1 and altered response to mechanical loading. A better understanding of the microarchitectural, cellular and molecular mechanisms of actions of androgens and oestrogens 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 Financial Interests

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

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Box 1. Evidence supporting the primary role of oestrogens in bone loss in older men

Animal models:
- E2 is a more effective bone-sparing agent than DHT in aged male rats\textsuperscript{111}

Human genetic evidence:
- polymorphisms in ER (but not AR) signalling negatively influence male bone\textsuperscript{112-114}
- longer CAG repeats diminish AR function but may be compensated by increased T levels, paradoxically stimulating bone via aromatization\textsuperscript{115}
- men with aromatase deficiency have high T but suboptimal bone mass which improves with oestrogen (but not androgen) therapy\textsuperscript{84-87}

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\textsuperscript{116} may be due to a loss of substrate for aromatization, since AR antagonists alone are not detrimental for bone\textsuperscript{117-119}

Human experiments:
- aromatase inhibition in older men increases T but decreases E2 and BMD\textsuperscript{120}
- gonadotropin inhibition and T or E2 add-back shows that E2 has the dominant antiresorptive effect\textsuperscript{121}
- T replacement with or without aromatase inhibition demonstrates that T alone cannot overcome male bone resorption in the absence of E2\textsuperscript{110}

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 \[46\].

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 \[51\].

Figure 3. Schematic illustration of the cellular targets of AR and ER\textsubscript{\alpha} on periosteal, endocortical and trabecular surfaces in male mice. Based on a µCT image of the proximal murine tibia.
AR

Periosteal apposition:
Increased through actions in immature osteoblasts

Endosteal bone formation:
Decreased through actions in immature and mature osteoblasts,
and decreased during puberty through actions in mineralizing osteoblasts

Endosteal bone resorption:
Inhibited by osteoclast ERα in females, but unaffected by actions in osteoclasts in male mice

Trabecular bone formation:
Maintained through actions in mineralizing osteoblasts and osteocytes

ERα

Periosteal apposition:
Increased through actions in immature osteoblasts

Trabecular bone formation:
Inhibited by osteoclast ERα in females, but unaffected by actions in osteoclasts in male mice