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REVIEW

A concise review of testosterone and bone health

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Abstract: Osteoporosis is a condition causing significant morbidity and mortality in the elderly population worldwide. Age-related testosterone deficiency is the most important factor of bone loss in elderly men. Androgen can influence bone health by binding to androgen receptors directly or to estrogen receptors (ERs) indirectly via aromatization to estrogen. This review summarized the direct and indirect effects of androgens on bone derived from in vitro, in vivo, and human studies. Cellular studies showed that androgen stimulated the proliferation of preosteoblasts and differentiation of osteoblasts. The converted estrogen suppressed osteoclast formation and resorption activity by blocking the receptor activator of nuclear factor κ-B ligand pathway. In animal studies, activation of androgen and ERα, but not ERβ, was shown to be important in acquisition and maintenance of bone mass. Human epidemiological studies demonstrated a significant relationship between estrogen and testosterone in bone mineral density and fracture risk, but the relative significance between the two remained debatable. Human experimental studies showed that estrogen was needed in suppressing bone resorption, but both androgen and estrogen were indispensable for bone formation. As a conclusion, maintaining optimal level of androgen is essential in preventing osteoporosis and its complications in elderly men.

Keywords: androgen, men, osteopenia, osteoporosis, estrogen, skeleton

Introduction

Osteoporosis is a progressive metabolic bone disease characterized by reduced bone mass and destructive bone microstructural changes, resulting in bone fragility and increased fracture risk.[1,2] It is a significant global health care issue with an expanding prevalence of nine million patients suffering from osteoporotic fractures, in which 1.6 million are suffering from hip fractures, 1.7 million from lower arm fractures, and 1.4 million from vertebral fractures.[3] Osteoporotic fracture-associated morbidity and mortality have significant societal impact due to the various medical, social, and financial implications on the patients.[4]

Osteoporosis is a common condition that afflicts both genders, with the lifetime risk of fracture at ≥50 years being 50% for women and 20% for men.[5–7] The major cause of osteoporosis in women is estrogen deficiency due to menopause, while in men, age-related testosterone deficiency.[8] Despite the higher prevalence in women, men are more prone to be associated with disability and death due to osteoporotic fractures compared to women.[9] The Dubbo Osteoporosis study demonstrated that death rate 5 years after fracture and second fracture in men aged 60 years (standardized mortality ratio [SMR]: 1.78, 95% confidence interval [CI]: 0.96–3.31) was higher compared to their women counterpart (SMR: 1.41, 95% CI: 1.01–1.97).[10] The incidence of osteoporotic fractures in men increases exponentially later in their life.[11] This is accompanied by a parallel decrease in their bioavailable testosterone level.[12] Osteoporosis is also an important side effect of androgen deprivation therapy aimed to treat prostate cancer

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in men.[13,14] These observations highlight the importance of testosterone in maintaining optimum bone health in men. This review gathered important evidence from cellular, animal, and human studies to present a comprehensive view on the role of testosterone in maintaining bone health, particularly in elderly men.

Androgen and androgen receptor

"Androgen" is a broad term encompassing testosterone and its precursors which are C19 metabolites of cholesterol. The predominant gonadal androgen in men is testosterone, 95% of which is secreted by the testes. The remaining 5% is produced by the adrenals by the conversion of dehydroepiandrosterone.[15] Testosterone is bound by sex hormone-binding globulin (SHBG) and albumin with strong and weak bonding in blood subsequently.[16] Effects of testosterone on the body are mediated by its local conversion to 5α-dihydrotestosterone by peripheral tissues, which has a greater affinity for androgen receptors (ARs).[17,18]

Androgen synthesis is controlled by the pulsatile release of hypothalamic gonadotropin-releasing hormone (GnRH). At the pituitary gland, GnRH stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the general circulation. Both FSH and LH are released by the anterior pituitary gland. FSH stimulates Sertoli cells to support spermatogenesis and secrete inhibin B, which negatively regulates FSH secretion. Meanwhile, LH is needed for the Leydig cells in the testes to produce testosterone. Testosterone stimulates sperm production and virilization, in addition to providing feedback to the hypothalamus and pituitary to regulate GnRH secretion via negative feedback mechanism (Figure 1).[19]

Testosterone possesses strong androgenic and anabolic effects that are important for both women and men, despite that men produce significantly more testosterone than women.[20] For example, bone growth and maintenance are significantly influenced by testosterone. Previous studies showed that testosterone administration increased the width of epiphyseal growth plate of growing rats directly.[21,22] This effect was independent of growth hormone and insulin-like growth factor-1.[21,22] The effects of testosterone in maintaining bone mineral density (BMD) in elderly men are well known and have been summarized by previous authors.[16,23,24] Thus, androgens take part in building the skeleton of young men and help to prevent bone loss in the elderly men.[25] Furthermore, testosterone is metabolized via the cytochrome P450 aromatase enzyme into 17β-estradiol. The aromatization process is also important for bone.[26] Several case reports indicated that men with mutations in the estrogen receptor (ER) or aromatase genes suffered from severe osteoporosis,[27,28] suggesting that at least part of the activities of testosterone on the male skeleton are exerted by its aromatization to estradiol.[29]

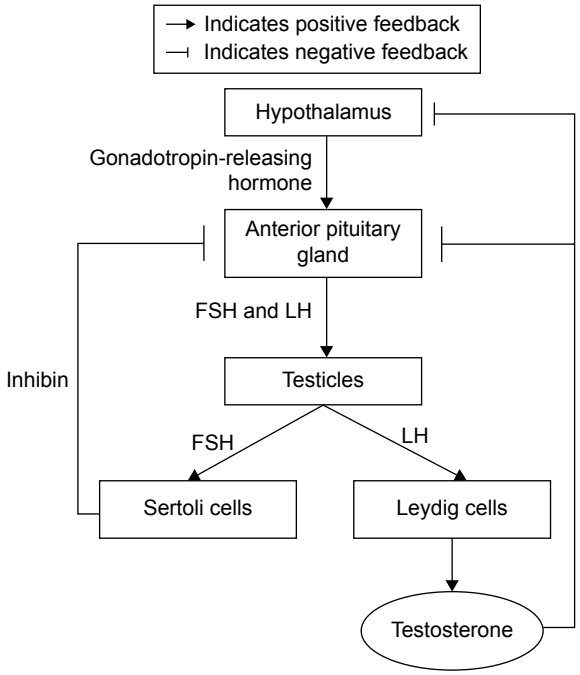

Figure 1 The synthesis of testosterone.
Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Sex steroid hormones act on their target cells by binding to members of the nuclear hormone receptor superfamily. Androgens bind to the AR, while estrogens bind to ERα or ERβ. ARs are expressed in bone marrow cell and growth plate.[30,31] In a study, ARs were expressed in the proliferative and early hypertrophic chondrocytes of sexually mature rats, and only in prehypertrophic chondrocytes in older rats.[32] Male rats had increased AR expression in the growth plate and metaphyseal bone with higher mRNA- and protein-staining intensities, as well as preferential nuclear staining during sexual maturation, compared to female rats.[32] This suggests that direct actions of androgens in chondrocytes and in bone-forming cells may be involved in establishing the gender differences in the skeleton. However, there is no difference in receptor expression in the growth plate chondrocytes of human males or females.[33,34] ERs α and β are expressed in the human growth plate. This suggested that androgens possibly affect pubertal growth or epiphyseal closure indirectly via aromatization to estrogen.[35,36]

The effects of testosterone on bone cells

Integrity of our skeletal system is maintained by an intricate process named remodeling which in turn is governed by

three major types of bone cells: bone-forming osteoblasts, bone-resorbing osteoclasts, and mechanosensor/mediator osteocytes. These bone cells are sensitive to signaling conveyed through hormones, cytokines, minerals, and dietary components. Some of these cells express ARs and ERs, and thus are responsive to both sex hormones. Dysfunction of these cells leads to dysregulation of the remodeling process. In the case of osteoporosis, the rate of bone resorption is greater than bone formation, leading to net bone loss.[37,38]

Osteoblasts

Osteoblasts are specialized bone-forming cells that have an essential role in bone remodeling, such as production of bone matrix proteins as well as bone mineralization.[39] Osteoblasts are developed from pluripotent mesenchymal stem cells under the direction of a characterized suite of regulatory transcription factors, such as osterix and runt-related factor 2.[40] The term "osteoblasts" actually refers to a diverse cellular population that includes immature osteoblast lineage cells as well as differentiating and mature matrix-producing osteoblasts. The mature osteoblasts are responsible for matrix mineralization, while the immature ones regulate formation of osteoclasts.[41–43]

ARs have been identified in cultured human fetal osteoblasts utilizing a nuclear-binding assay.[44] Subsequent studies identified AR mRNA and protein in osteoblasts. Almost all studies demonstrated that androgen upregulated the expression of ARs in osteoblasts.[40,45] Stromal cells (precursors of osteoblasts), megakaryocytes, and endothelial cells in the bone marrow express ARs.[30,31] Studies showed that both testosterone and 5α-dihydrotestosterone stimulated proliferation of cultured osteoblast precursors in distinctive species.[46,47] In addition, androgens have been shown to suppress the apoptosis of osteoblasts.[48,49] Androgens stimulated interleukin-1β production and enhanced the mitogenic effect of fibroblast growth factor in cultured osteoblasts.[50,51] Further studies demonstrated that androgens exerted stimulatory, inhibitory, or no effects on the expression of osteoblast markers, such as osteocalcin, collagen type 1 alpha 1, and alkaline phosphatase, and mineralization of extracellular bone matrix. However, the evidence generally suggested that androgens stimulate differentiation of osteoblasts.[52–55]

Osteoclasts

Osteoclasts originate from the colony-forming unit-granulocyte macrophage hematopoietic cell lineage in the bone marrow. Osteoclast differentiation entails contact with stromal cells of the osteoblastic lineage in bone marrow microenvironment, and stimulation by receptor activator of nuclear factor κ-B ligand (RANKL) released by immature osteoblasts, which binds to RANK on osteoclasts.[56] The effects of RANKL on osteoclasts are tightly regulated by osteoprotegerin (OPG), a decoy receptor of RANKL, also secreted by osteoblast precursors.[57]

The proliferation of osteoclasts after orchidectomy is most likely due to androgen deficiency. Androgens exert their bone-defensive effects indirectly through osteoblastic cells. Orchidectomy will cause the proliferation of osteoblast precursors which secrete RANKL that stimulates osteoclast proliferation and activation, thus resulting in bone loss.[58] In vitro studies showed that dihydrotestosterone interacted with ARs on osteoclasts and inhibited bone resorption in human, murine, and avian osteoclasts.[59] In other cell culture studies, androgens were shown to regulate osteoclast formation and survival associated with RANKL pathway, and also to inhibit OPG mRNA levels and protein secretion by osteoblastic cells.[38,57,59]

Osteocytes

During bone formation, a portion of osteoblasts undergoes terminal separation and entombment by mineralized osteoid, subsequently transforming to osteocytes.[60] Osteocytes are cased in liquid-filled holes (lacunae) inside the mineralized bone and are found in abundance, representing 90%–95% of the total bone cells.[61] Osteocytes possess long dendrite-like processes that cooperate with other osteocytes inside the mineralized bone and interact with osteoblasts on the bone surface.[62] Osteocytes modulate bone remodeling by responding to mechanical stimuli to prevent accumulation of bone microdamage.[63,64]

In vitro studies suggested that ERα or ERβ translated mechanical forces into prosurvival signals in osteocytes and osteoblasts, independent of estrogens.[65] Estradiol was shown to prevent osteocytes apoptosis and enhance the production of transforming growth factor-alpha which inhibits osteoclastic bone resorption.[66] Thus, estrogens converted from testosterone in male could contribute to the anabolic action of osteocytes.

The effects of androgens and estrogens on bone cells are summarized in Figure 2.

The effects of testosterone on animals

Many animal studies have been performed to further investigate the relative effects of androgen and estrogen on bone. Orchidectomized rodent is a well-characterized animal model for osteoporosis. Following orchidectomy, bone resorption increases at cancellous and endocortical surfaces,

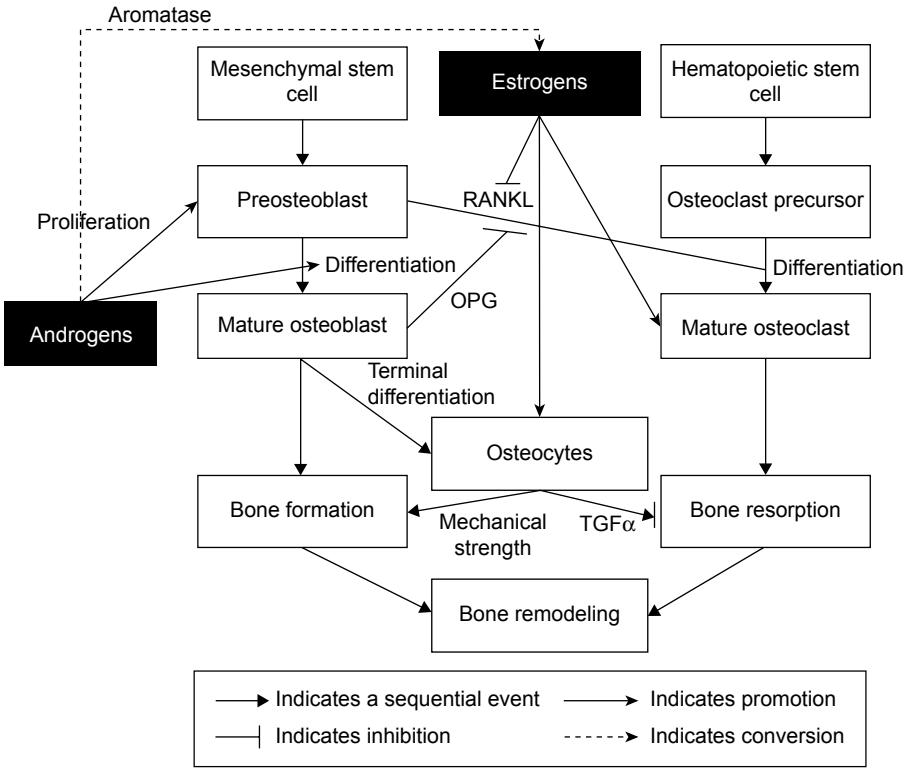

Figure 2 The effects of testosterone on bone cells.
Abbreviations: RANKL, receptor activator of nuclear factor κ-B ligand; OPG, osteoprotegerin; TGFα, transforming growth factor-alpha.

thus reducing cancellous and cortical bone volume. Besides, periosteal bone formation during growth is decreased in orchidectomized rodents. These effects translate to a lower bone strength.[67]

Several studies investigated the bone phenotypic changes induced by androgens, nonaromatizable androgens, and estrogens in orchidectomized rodents. These studies revealed that nonaromatizable androgens stimulated periosteal bone formation and inhibited cancellous bone resorption in orchidectomized rats, although the effect is lesser compared to testosterone and estradiol.[68] Despites this, it is unclear to what extent the effects of these hormones are pharmacological or physiological, and whether these physiological effects can be extrapolated to the human condition, as estradiol concentration is higher in mice.[69]

Other animal studies on bone phenotype of transgenic male animals with knockout (KO) of AR, ERα or ERβ, or both have been investigated. These studies manipulated the genetic conditions of the mice, in combination with orchidectomy and replacement of androgens and estrogens. The evidence suggests that activation of ER but not AR is involved in longitudinal appendicular skeletal growth in male mice.[70] Besides, studies in knockout mice again suggest that both ERα and AR are involved in enhancing cortical radial bone growth. Both AR and ERα can independently mediate the cancellous bone-sparing effects of sex steroids in male mice, but not ERβ.[71,72] These studies have greatly increased the understanding of the role of ERs and ARs in male skeletal growth. The dual mode of action of testosterone is exerted either directly by the AR or indirectly by the ERα through aromatization.

In conclusion, animal evidence suggests that testosterone exerts anabolic effects on different bone surfaces of AR with activation of both ERα and AR on cortical bone and muscle mass; ERβ, on the other hand, seems not to be significantly related to bone growth and maintenance in male mice.

The relationship between testosterone and bone in human studies

The effects of testosterone on bone health in humans can be measured in terms of BMD and fracture risk. On the other hand, bone formation and resorption markers offer insight on the individual processes that constitute bone remodeling. While association obtained in observational studies is hypothesis generating at best, some experimental studies have been performed to give us direct evidence on the importance of bone health in humans.

Many prospective studies have demonstrated that the risk of fragility fracture increases progressively as BMD decreases.[73] The Framingham cohort studies established that femoral and radial BMD decreased significantly with age in both men and women (n=1,154, aged 68–98 years).[74] The decline of BMD is probably due to age-related loss of trabecular and cortical bone.[75] However, prior to this slow phase of bone loss, degeneration of the cancellous bone is sexually dimorphic, in which rapid-accelerated bone loss after menopause only occurs in women.[76] In men, cancellous bone loss occurs much later and gradually in life, especially after the age of 70 years.[77]

The associations between circulating testosterone and estrogen, and BMD and fracture in elderly men have been demonstrated in many studies. The US cohort of the Osteoporotic Fractures in Men Study examined the associations between nonvertebral fracture risk and bioavailable testosterone, bioavailable estradiol, and SHBG of elderly men (n=5,995, aged ≥65 years, follow-up duration =4.7 years). Each sex hormone was associated with fracture risk, but the combination of low bioavailable testosterone, low bioavailable estradiol, and high SHBG predicted fracture risk the best.[78] In another cohort study of elderly men in the People's Republic of China (n=1,448, aged ≥65 years, follow-up duration =4 years), associations between serum total testosterone, free testosterone, estradiol, bioavailable estradiol, SHBG, BMD, and incident fractures were examined. The results showed that low serum estradiol levels were associated with elevated bone loss and increased risk of fractures.[79] Similar to the American cohort, the combination of the lowest quartile of free testosterone and bioavailable estradiol and the highest two quartiles of SHBG predicted incident fracture the best.[79] This suggests that the association between sex hormones and bone health is similar across different populations. The relative importance of androgens and estrogens in maintaining bone health in the elderly remains debatable. Serum testosterone and estrogens were found to be associated significantly with fracture in elderly men (n=609, median follow-up duration =5.8 years) in the Dubbo Osteoporosis Epidemiology Study. After adjustment for confounding factors, only the relationship between serum testosterone and fracture remained significant.[80] The Sweden cohort of the Osteoporotic Fractures in Men Study found otherwise, whereby low level of free estradiol, but not free testosterone, was significantly associated with fracture risk in older men (n=2,639, average follow-up duration =3.3 years).[81]

Interestingly, the relationship between SHBG and bone is dependent on age in men. A study showed that increase in SHBG with age was associated with bone loss in elderly men.[78] However, in younger men, the relationship between peak bone mass and SHBG was positive rather than negative.[82–84] We postulate that the higher level of SHBG in young men is reflective of higher circulating testosterone level, thus explaining the positive relationship. However, a higher SHBG level is not accompanied by a higher testosterone level in older men because the negative feedback mechanism is dysregulated. Thus, the ensuing decrease in the bioavailability of testosterone causes the deterioration of bone health. Results on testosterone concentrations were less consistent compared to estradiol level in relationship with bone loss or fractures in men, except for very low levels in hypogonadal men, especially those undergoing chemical and surgical castration. They had an increase in bone turnover, bone loss, and fracture risk.[85]

While association obtained in observational studies is hypothesis generating at best, experimental studies in human have been performed to provide direct evidence on the importance of bone health in humans. In an experimental study, young men aged between 20 and 44 years were divided into three groups. The first group received only a GnRH analog to suppress endogenous sex hormone production, while the second group received a GnRH analog plus testosterone, and the third group, a GnRH analog plus an aromatase inhibitor, to prevent the conversion of testosterone to estrogen. The results showed that bone resorption markers were increased in the groups receiving GnRH analog alone and GnRH analog plus aromatase inhibitor, indicating that estrogen is necessary in suppressing bone resorption. The level of bone formation markers increased greatly in the group receiving GnRH analog alone than the group receiving GnRH analog and testosterone. Overall, these findings suggest that both estrogens and androgens play independent and fundamental roles in regulating bone resorption in men.[86]

Another study showed that bone resorption markers increased significantly in the absence of both hormones and were unchanged in men when receiving both testosterone and estrogen. Although estrogen prevented the increase in bone resorption markers, testosterone did not exert similar effects. Meanwhile, the bone formation marker, serum osteocalcin, decreased in the absence of both hormones, and its level reverted back to normal with replenishment of either estrogen or testosterone. This study postulated that estrogen may be essential in regulating bone resorption, and both estrogen and testosterone may be critical in maintaining bone formation.[87]

Besides, a study demonstrated that a 12-week administration of aromatase inhibitor in elderly men with hypogonadism

increased testosterone and reduced estrogen levels but did not affect biochemical markers of bone formation (osteocalcin and amino-terminal propeptide of type 1 collagen), serum OPG, and total body BMD significantly.[88] However, administration of aromatase inhibitor for 12 months resulted in a decrease in BMD.[89] Thus, it proved the important role of estrogens and androgens in male bone metabolism (bone biochemical markers) when induced with aromatase inhibitor.

Conclusion

The current evidence suggests that circulating androgens and estrogens are protective of bone. However, the relative importance between androgens and their derived estrogens in bone is still debatable. Experimental data suggest that androgens influence bone directly via interactions with ARs, and indirectly via binding to ERα and ERβ after aromatization in adipose or different tissues. Androgens, directly or indirectly through estrogens, preserve trabecular bone principally by diminishing osteoclastogenesis, and both hormones counteract osteoblast apoptosis and stimulate osteoclast apoptosis.

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Disclosure

The authors report no conflicts of interest in this work.

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