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Review

Sex hormones and bone health in males

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ABSTRACT

Sex steroids play a key role in maintaining skeletal integrity lifelong, through a complex variety of endocrine, but also paracrine and possibly autocrine actions. The current knowledge that androgens may act as pro-hormones for estrogens has seriously challenged many traditional views, so that, at least for their skeletal actions, these can no longer be considered exclusively "male" or "female" hormones.

A brief summary on androgen synthesis, metabolism and transport, in relation to clinical assessment of hypogonadism

Androgens are C19 steroids secreted by the testes in men and the adrenals in both men and women. Testosterone is the most abundant circulating androgen in men, 95% of which is secreted by the testis. Serum testosterone concentration is considerably lower in women, whose levels of weaker adrenal androgens such as dehydroepiandrosterone (DHEA),[1] its sulfate (DHEA-S) and androstenedione are instead similar to those in men [1]. The adrenal androgens can be converted to testosterone by steroid sulfatase, 17β- and 3β-hydroxy-steroid dehydrogenases (17β-HSD and 3β-HSD). Serum testosterone levels vary significantly as a result of circadian rhythm with peak levels in the morning, and this rhythm is maintained also in healthy elderly men [2]. Therefore, it has been suggested that serum samples for total testosterone assessment should always be taken in the morning before 11 am [3]. Circulating total testosterone consists of unbound or free testosterone (about 2%), testosterone bound with high affinity to sex hormone binding globulin (SHBG) (40–50%), and testosterone loosely bound to other proteins, mainly albumin (about 50%) [4,5]. In both sexes, 20–40% of estradiol is bound with lower affinity to the same binding site of SHBG. The hormone tightly bound to SHBG is biologically inactive, while that loosely bound to albumin can rapidly dissociate. All non-SHBG-bound testosterone (i.e. albumin-bound and free testosterone), having rapid access to target tissues, represents the fraction available for biological actions, termed bioavailable testosterone [6]. The measurement of serum free testosterone requires equilibrium dialysis, so that local laboratories usually do not accurately and reliably measure free testosterone. For clinical use free and bioavailable testosterone levels can be estimated from serum total testosterone, SHBG, and serum albumin concentrations [7,8].

Though men do not experience an abrupt drop of sex steroids equivalent to the menopause, serum testosterone levels decline progressively in aging men at a rhythm of about 1–2% per year [9]. Thus 20% of men over 60, 30% over 70, and 50% over 80 years attain total testosterone levels below 325 ng/dL, i.e. below the reference range for young adult males [10]. On the other hand, aging is also associated with an increase (longitudinally at about 1.3% per year) in SHBG concentration, so that the age-related decrease of free and bioavailable testosterone levels occurs to a greater degree than is reflected by the total testosterone level [11]. This in turn implies that, considering free testosterone levels, the proportion of elderly men fulfilling a biochemically defined diagnosis of hypogonadism would be even higher [12]. Moreover, there is still not general agreement even concerning the levels of serum total testosterone below which a diagnosis of late onset hypogonadism may be made, some Authors favoring a testosterone level below

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[1] *Abbreviations used:* DHEA, dehydroepiandrosterone; 17β-HSD, 17β-hydroxy-steroid dehydrogenase; 3β-HSD, 3β-hydroxy-steroid dehydrogenase; SHBG, sex hormone binding globulin; AR, androgen receptor; DHT, dihydrotestosterone; BMU, basic multicellular units; BMD, bone mineral density; BMP, bone morphogenetic protein; QCT, quantitative computed tomography; PSA, prostate specific antigen.

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300 ng/dL, others a level below 200 ng/dL [3]. Adrenal androgens progressively decline with age too, reaching 15% of peak levels by the ninth decade [13].

Testosterone may act on the androgen receptor (AR) both directly and after its irreversible conversion to the more potent, non-aromatizable, androgen dihydrotestosterone (DHT) operated by the enzyme 5α-reductase in peripheral tissues [14]. In adjunct, both testosterone and adrenal androgens have the unique feature that they can be converted by the cytochrome P450 enzyme aromatase to the C18 estrogenic steroids 17β-estradiol (E_2) and estrone (E_1). Through this enzymatic system, testosterone contributes more than adrenal androgens to the total amount of circulating estrogens [15]. Only about 15–20% of the circulating estrogen is directly secreted by the testes in men. The remaining 80–85% is derived from peripheral aromatization of androgens in several tissues, as adipose tissue, brain, skin, endothelium, and bone. In fertile women large amounts of aromatase activity are expressed by the ovaries, which secrete 95% of 17β-estradiol. In postmenopausal women instead circulating estrogen comes solely from the conversion of adrenal androgen by the peripheral aromatase activity. So, in postmenopausal women estrogen concentrations are an order of magnitude lower than testosterone and are generally even lower than those of elderly men, being the availability of androgen precursor for aromatization in women much lower than in men [16]. Moreover, both inherited and acquired variations in aromatase efficiency could result in qualitative and/or quantitative differences in the enzyme activity, with possible consequences on bone and other tissues [17]. Collectively considering the mentioned enzymatic activities, it is conceivable that, depending on the relative expression of the P450 aromatase, the 5α-reductase, the 17β-HSD, the 3β-HSD, and the steroid sulfatase, testosterone and adrenal androgens may predominantly act either directly or indirectly, as precursor hormones for estrogens. In other words, apart from their contribution to the circulating pool, local sex steroids synthesis could regulate their concentration at the target tissue level, adapting it to local requirements [18].

Apart from the documented effects on bone tissue, androgen may also indirectly affect skeletal homeostasis through several mechanisms. For instance, androgens maintain a positive calcium balance, since they may enhance intestinal calcium absorption by increasing active vitamin D synthesis [19], and increase calcium reabsorption by the kidney [20]. Furthermore, in humans androgens indirectly stimulate GH-IGF-1 secretion after aromatization to estrogen, in turn acting on ERα. Moreover, androgens contribute to sustain the defense against oxidative stress in bone [21]. Finally, androgens exert a relevant direct trophic effect on muscle mass and strength, also independently of GH-IGF-1 axis [22], which in turn promotes bone strength [23].

Sex steroids receptors and action at the cellular and tissue level

Androgen receptor (AR) as well as estrogen receptors (ERα and ERβ) belong to the nuclear receptor family. These receptors are composed by three functional domains: the NH_2-terminal domain, the ligand-binding domain, and the DNA-binding domain. In the absence of sex steroids these receptors are inactive, and kept sequestered in the cytoplasm in protein complexes with chaperone molecules. After binding with the sex steroids, the receptors undergo conformational changes, dissociate from the chaperone proteins, and translocate into the nucleus. Here the hormone-bound receptor dimerizes and interacts with specific DNA sequences (either androgen or estrogen response elements) into the regulatory region of the target gene. The subsequent effects on transcriptional activity are either enhanced or reduced by tissue-specific co-regulators (co-activators and co-repressors, respectively). This classical genomic machinery seems to be the most important mechanism of action of sex steroids in the skeleton, taking at least 30–45 min to ensue and hours to produce significant levels of proteins [1,18]. But, sex steroids may also elicit rapid responses (within seconds) in several tissues and cells in a non-genomic manner, involving plasma membrane receptors and second messenger signal transduction [24]. Such non-sex-specific rapid actions seem to mediate antiapoptotic effects of androgens and estrogens on osteoblasts [25].

All these receptors are expressed in growth plate chondrocytes. Androgen receptors, as well as estrogen receptors (ERα and ERβ) are expressed in all layers of human growth plate, with no differences in expression in chondrocytes from males and females [1]. Therefore, conceivably, androgens, either directly acting on AR or after aromatization seem capable both to influence longitudinal bone growth in early puberty [1]. Moreover, androgens also indirectly contribute to the epiphyseal growth plate closure in later puberty, via aromatization to estrogens [26]. The influence on linear growth in both sexes has to be partly attributed to the estrogen-dependent modulation of GH/IGF-1/IGFBP system, namely through the activation of ERα [27].

Androgen receptors, ERα, and ERβ are expressed as well in osteoblasts, osteocytes, and osteoclasts. Thus, they significantly influence the process of bone remodeling, a continuous process that renews old bone through the activity of teams of osteoclasts and osteoblasts, called basic multicellular units (BMU). This way, on average, about 10% of bone volume is replaced per year. Osteoclasts are multinucleated cells derived from hematopoietic precursors which are highly specialized to remove old bone (bone resorption). The tissue is then replaced with new bone by teams of osteoblasts, a progeny of the mesenchymal stem cells lineage, which produce and mineralize the bone matrix (bone formation). At the end of this phase, all osteoclasts and 60–80% of osteoblasts die via apoptosis. The remaining osteoblasts either become lining cells, covering quiescent surfaces, or remain individually entrapped into lacunae of the mineralized matrix, and become osteocytes. The latter cells, through a canalicular network of cytoplasmic processes, are linked to neighboring osteocytes, as well as to the lining cells at bone surface, and to bone marrow and endothelial cells.

Androgen, both directly and through aromatization to estrogen, might influence bone marrow stromal cells, pre-adipocytes, endothelial cells within bone marrow, osteoblastic progenitors, which express AR and/or ERα receptors [28].

In particular, androgens directly stimulate the proliferation of osteoblast precursors and also the differentiation of osteoblasts via the Wnt signaling [29]. Moreover, after aromatization to estrogen, androgen might also mediate osteoblastic response to loading. In fact, interestingly, the Wnt/β-catenin signaling pathway seems to play a relevant role in bone adaptive responses to mechanical stimulation, and, noteworthy, ERα of osteoblastic cells facilitates this adaptive response to strain [30]. This way androgen could indirectly increase the sensitivity of bone to the mechanical stimuli provided by muscle contractions, and this also explains why the set point of the mechanostat is lower in girls [31]. Androgens also directly decrease osteoblast and osteocytes apoptosis [32,33].

AR, as well as ERα and ERβ expression has been detected in mature osteoblasts and osteocytes. In particular, as like as in other target tissues, the number of androgen binding sites varies widely in osteoblasts, whose expression of AR is upregulated by androgens. Despite this, osteoblasts from men and women express similar number of androgen receptors. It is noteworthy that osteoblasts from cortical bone have higher AR mRNA expression and androgen binding than cells from trabecular bone [32]. Androgen effects may be also partly mediated through the regulation of the skeletal expression of several cytokines, such as transforming growth factor-β, insulin-like growth factors, interleukin-6, interleukin-1β, fibroblast growth factor, osteoprotegerin [33].