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Review article

Does the GH/IGF-1 axis contribute to skeletal sexual dimorphism? Evidence from mouse studies

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ABSTRACT

The contribution of the gonadotropic axis to skeletal sexual dimorphism (SSD) was clarified in recent years. Studies with animal models of estrogen receptor (ER) or androgen receptor (AR) null mice, as well as mice with bone cell-specific ablation of ER or AR, revealed that both hormones play major roles in skeletal acquisition, and that estrogen regulates skeletal accrual in both sexes. The growth hormone (GH) and its downstream effector, the insulin-like growth factor-1 (IGF-1) are also major determinants of peak bone mass during puberty and young adulthood, and play important roles in maintaining bone integrity during aging. A few studies in both humans and animal models suggest that in addition to the differences in sex steroid actions on bone, sex-specific effects of GH and IGF-1 play essential roles in SSD. However, the contributions of the somatotropic (GH/IGF-1) axis to SSD are controversial and data is difficult to interpret. GH/IGF-1 are pleotropic hormones that act in an endocrine and autocrine/paracrine fashion on multiple tissues, affecting body composition as well as metabolism. Thus, understanding the contribution of the somatotropic axis to SSD requires the use of mouse models that will differentiate between these two modes of action. Elucidation of the relative contribution of GH/IGF-1 axis to SSD is significant because GH is approved for the treatment of normal children with short stature and children with congenital growth disorders. Thus, if the GH/IGF-1 axis determines SSD, treatment with GH may be tailored according to sex. In the following review, we give an overview of the roles of sex steroids in determining SSD and how they may interact with the GH/IGF-1 axis in bone. We summarize several mouse models with impaired somatotropic axis and speculate on the possible contribution of that axis to SSD.

1. Introduction

What are the characteristics of skeletal sexual dimorphism (SSD)? The short answer is that during growth males show *greater gains* in *bone strength* than females, and during aging males show *lesser declines* in *bone strength* than females. Males grow longer and wider bones that are stronger than female bones. The increases in linear growth in males depend largely on the increase in the height of the long bones, while the increase in the height of the vertebrae is smaller, and its contribution to the sex differences in height is modest. The sex differences in bone strength are established during puberty. In humans, pubertal growth in boys starts approximately 1 year later than girls, lasts a little longer, and growth velocity is greater than in girls [1,2]. These temporal differences in males contribute to their longer and wider bones.

Bone strength is largely determined by bone size, geometry, and quality. The amount of mineral per unit volume of bone, referred as volumetric bone mineral density (vBMD), is not different between the two sexes either for appendicular or axial skeletons [3,4]. Thus, differences in bone size are the major contributor to the sex differences in bone strength [5]. The contribution of bone quality to sex differences in bone strength is less well known. We note that changes in bone-size, -geometry, and -quality, continue to occur during growth, puberty and adulthood, and are largely determined by genetic and hormonal factors.

Bone size is regulated by the activity of osteoblasts (bone building cells) and osteoclasts (bone resorbing cells) on the periosteal and endosteal surfaces of the bone, processes collectively referred as bone modeling (during growth). Periosteal bone apposition in both sexes results in increased bone diameter, an important factor determining bone strength. Pubertal-growth in males associates with enhanced periosteal bone apposition with increased endocortical bone resorption and little endosteal bone expansion, processes that contribute to wider bones with proportionally thicker cortex. In females, however, periosteal bone apposition during pubertal growth slows down, while endosteal bone expands as a consequence of reduced resorption at the endosteal surface resulting in smaller marrow cavity [6]. During aging periosteal apposition in males continues at a slow rate, while in females the periosteal surface is inactive [7]. Both males and females show increases in endosteal bone resorption (bone remodeling). However, since males

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have continuous periosteal bone apposition (though at a slower rate), the *net* cortical bone loss is smaller than females. Sex differences during aging are also apparent in the trabecular bone compartment. Males loose trabecular bone during aging via thinning of the trabeculae [3,4]. Females, however, remodel trabecular bone earlier in life (midlife) leading to thinning of trabeculae and reduced remodeling surfaces with time. During aging, remodeling of the trabecular bone compartment in females leads to loss of trabeculae connectivity [3,4].

Elucidation of SSD requires understanding of the hormonal and tissue factors network that operates during growth and aging. There are some experimental evidence from humans and animal models suggesting that in addition to the differences in sex steroid actions on bone, sex-specific effects of the growth hormone (GH) and its downstream effector, insulin-like growth factor-1 (IGF-1), play roles in SSD. Both humans [8,9] and rodents [10] show sexual dimorphism in the GH secretory patterns, which consequently result in pulsatile activation of the signal transducer and activator of transcription (STAT) 5b, a mediator of the GHR. Loss of STAT5b in male mice results in a feminized liver gene expression profile, while females are not affected [11]. Likewise, studies of the molecular basis for GH-mediated sexual dimorphism show that female livers are less responsive to GH than male livers [12–14]. Similarly, in adults, it has been established that women who are taking oral estrogens require higher GH dose to achieve equivalent serum IGF-1 levels [15–20]. Understanding how GH/IGF-1 axis affects SSD is significant because GH is approved for the treatment of normal children with short stature, children who are born small for gestational age, children with idiopathic short stature, children with congenital GH deficiency, Noonan syndrome, Turner syndrome, and Prader Willi syndrome [21]. Additionally, it was lately suggested that using individually tailored doses of GH for treatment of short stature provides a more optimal skeletal outcomes [22]. Therefore, should the GH/IGF-1 axis determine SSD, GH treatment may also be tailored according to sex. This review will summarize briefly the contribution of sex steroids to SSD and focus specifically on the roles of GH/IGF-1 in determining SSD based on studies done in mice.

1.1. The contribution of sex steroids to SSD

Ovaries, in females, and testis, in males, are the main source of sex steroids, while the adrenals contribute about 5% to circulating sex steroids. In men, most of the circulating sex steroids are bound to the sex hormone binding globulins (SHBGs), while in rodents, which lack SHBGs, sex steroids circulate in a free form. In males the predominant sex steroid, testosterone (T), is produced from C19 by the testis and from dehydroepiandrosterone (DHEA) by the adrenals. DHEA can be further converted in tissues to dehydrotestosterone (DHT), a more potent androgen, by the 5α-reductase. Testosterone can also be converted to 17β-estradiol (E2) by aromatase (CYP19A1), thus leading to E2-like effects. Testosterone and E2 bind to the androgen receptor (AR) or the estrogen receptor (ER), respectively, which are found in almost all tissues, including bone. Bone tissue not only expresses the ER and AR but also the 5α-reductase and aromatase enzymes, suggesting that sex steroids also provoke autocrine/paracrine actions in bone.

Understanding the contributions of sex steroids to SSD was extensively studied using gonadectomized animals (Table 1A). In general, ovariectomy (OVX) or orchidectomy (ORX) resulted in decreases in bone mass, largely via decreases in trabecular bone, but also via decreases in bone mineral content (BMC) in the cortical bone envelope. Notably, however, gonadectomy has temporal effects. Therefore, the age at which removal of gonads is performed determines the skeletal outcomes. Growth rate accelerates during early puberty (in rodents at 3–5 weeks of age) and decreases later on (at 5–8 weeks of age). ORX results in increased body weight and *decreased* cross-sectional bone area only if done at the end of pubertal growth [23]. ORX done in aged animals does not affect total cross-sectional bone area but results in significant thinning of the bone cortices, suggesting an enhanced endosteal bone resorption [24]. On the other hand, OVX done during prepuberty results in significant *increases* in bone length and bone cross-sectional area [23,25], and associates with increases in serum IGF-1 levels in one study [25]. When performed in adults, OVX results in significant increases in body adiposity and significant reductions in BMC in both cortical and trabecular bone compartments due to increased bone resorption. In this context, it is important to note that gonadectomy not only removes estrogen and androgen, but also removes other ovarian and testicular hormones. Nonetheless, gonadectomy studies support the notion that in female mice, estrogen inhibits periosteal bone expansion during early puberty, while in male mice androgen stimulates periosteal bone apposition during late puberty. This concept was also supported by studies with mice overexpressing aromatase globally (aromatase-Tg) via the human ubiquitin C promoter (Table 1B) [26,27]. As expected, aromatase-Tg male mice exhibited increases in serum E2, which associated with decreased total cross-sectional area and increased endocortical bone apposition. Female aromatase-Tg mice also showed decreases in total cross-sectional area without alterations in endocortical bone apposition. When aromatase was overexpressed specifically in osteoblasts (OB-aromatase-Tg), serum E2 levels were normal and thus, total cross-sectional area in male mice remained normal (Table 1B) [28]. In both sexes of OB-Aromatase-Tg endocortical bone apposition increased, and in females it resulted in increased cortical bone thickness.

1.1.1. The estrogen receptor

To better understand the roles of sex steroids in skeletal acquisition and growth, animal models of global or tissue-specific ablation of the estrogen and androgen *receptors* were generated. There are two known E2 receptors; ERα and ERβ, which are expressed in bone (including the growth plate) and regulate skeletal homeostasis. ERα and ERβ have different actions on bone. ERα null females show decreased bone length, while ERβ null females exhibit increases in bone length [29]. In female mice, ablation of ERα results in increased body adiposity and increase in trabecular bone volume, while ablation of the ERβ does not affect body composition but results also in increased trabecular bone traits (Table 1C). With respect to the cortical bone compartment, both ERα and ERβ female null mice showed increases in total cross-sectional bone area, which associated with increased cortical bone thickness, suggesting that ERα/β inhibit radial bone growth. In males, ablation of ERα did not affect bone length, however resulted in significantly decreased total cross-sectional area, decreased cortical bone thickness, and thus decreased cortical BMC, suggesting that ERα plays essential roles in establishing bone size in males. Interestingly, total ablation of both ERα and ERβ in mice resulted in a bone phenotype similar to that observed for the ERα single gene ablation in both sexes. In males, double knockout of ERα/ERβ resulted in decreased linear growth, decreased cortical bone area and thickness, which associated with decreases in serum IGF-1 levels [29–31]. Trabecular bone, however, increased in the ERα/ERβ double knockout male mice. In contrast, in females double knockout of ERα/ERβ resulted in normal periosteal circumference but increased cortical thickness, while trabecular bone volume and BMD decreased [29–31]. It should be noted that ablation of both AR and the ERα in male mice resulted in decreased cortical bone traits and diminished trabecular bone, which associated with decreased levels of serum IGF-1 [32]. Overall these studies suggest that estrogen plays a role in skeletal growth and integrity in both sexes and that the predominant sex steroid receptor affecting bone morphology and BMD is the ERα.

Notwithstanding the valuable data from animal models with global ER inactivation, interpretation of the data is complicated by the fact that these mice show elevated serum sex steroid levels. To overcome this caveat a strategy of cell-specific ER ablation was developed, in which systemic regulation of sex steroid is normal (Table 1D). Ablation of the ERα in osteoblast-progenitors (using the paired related homeobox 1 (Prx) or osterix (Osx) promoter-driven Cre) resulted in decreased