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Androgen prevents hypogonadal bone loss via inhibition of resorption mediated by mature osteoblasts/osteocytes

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ABSTRACT

Androgen receptor (AR) is expressed throughout the osteoblast lineage. Two different AR transgenic families (AR2.3-tg and AR3.6-tg mice) demonstrating overlapping and distinct expression profiles were employed to assess the effects of enhanced androgen sensitivity to ameliorate hypogonadal loss. Two different paradigms of steroid replacement following orchidectomy (ORX) were used as either preventative or therapeutic therapy. ORX was performed in male wild-type (WT), AR2.3-tg and AR3.6-tg mice at 5 months with immediate DHT replacement (prevention, higher turnover) or at 3 months with DHT treatment delayed for 2 months (therapeutic, lower turnover), both with treatment for the last 6 weeks. Dual energy X-ray absorptiometry (DXA), micro-computed tomography (μCT), and histomorphometry were performed. In the prevention model, ORX significantly reduced BMD and BMC in all genotypes compared to sham and DHT was effective at prevention of osteopenia. In the therapeutic model, all genotypes became osteopenic compared to sham, but after a prolonged hypogonadal period, delayed DHT treatment provided little benefit. μCT analysis of mid-shaft total bone in all genotypes generally showed reductions after ORX. Delayed DHT was ineffective at restoring bone volume in any genotype whereas immediate treatment prevented loss only in AR transgenic mice. Cortical thickness also decreased with ORX but immediate DHT treatment was effective to increase thickness only in WT mice, likely due to expansion of marrow volume in both AR-tg lines. In metabolically highly active cancellous bone, ORX resulted in lower bone volume/tissue volume (BV/TV) in all genotypes, consistent among 3 sites measured. Again with delayed treatment, there was little effect of DHT to restore BV/TV, but when administered at the time of ORX, DHT completely prevented the decrease in cancellous bone in all genotypes. Improvement in cancellous bone architecture was seen with immediate DHT replacement that was enhanced in AR transgenic lines compared to WT. In contrast, there were only modest changes in all genotypes using the delayed treatment paradigm. With ORX in both paradigms, trabecular number was decreased while spacing increased. Thus, androgen therapy is effective for the prevention of endosteal and cancellous osteopenia primarily through its anti-resorptive properties, but shows little anabolic action as a therapeutic strategy to restore bone. Given the similarity in response to androgen treatment in both AR transgenic lines, overlapping expression profiles suggest that the target cells mediating androgen action in vivo are mature osteoblast/osteocytes. Combined, these results demonstrate that in the adult mouse, androgen treatment can reduce bone resorption but has little overall anabolic activity.

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Introduction

Androgens have sometimes been characterized as anabolic agents that can increase bone mass by stimulation of bone formation [1–3], and may represent an important therapeutic class with the potential to rebuild lost bone. Bone loss is associated with a hypogonadal state in both women and men [4], and leads to the development of bone fragility and osteoporosis. Osteoporosis is generally characterized by a relative increase in bone resorption (mediated by osteoclasts) compared to bone formation (mediated by osteoblasts). Repletion of androgen-deficient males or androgen supplementation of osteoporotic females has been shown to reduce bone loss [5]. These observations have been

Abbreviations: AR2.3-tg, AR2.3-transgenic; AR3.6-tg, AR3.6-transgenic; BMC, bone mineral content; BMD, bone mineral density; BV/TV, bone volume/tissue volume; DXA, dual-energy X-ray absorptiometry; DHT, 5α-dihydrotestosterone; LABC, combined levator ani bulbocavernosus muscle; ORX, orchidectomy; SV, seminal vesicle; Tb.N., trabecular number; Tb.Sp., trabecular spacing; μCT, microComputed tomography; WT, wild-type.

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reproduced in genetic models of androgen receptor dysfunction or with androgen ablation [for review, see 6], indicating an important role for androgen signaling in skeletal homeostasis.

While there is a clear role for androgen signaling during development in males that is important for sexually-dimorphic skeletal growth [7], androgen action in adult bone is less well understood. For example, androgen supplementation has little positive effects on bone formation in non-hypogonadal adults [8]. Clinical trials that have evaluated the effectiveness of androgen therapy to improve bone mineral density have been disappointing, with only the most hypogonadal patients benefiting [9,10]. Additional confusion regarding the specific effects of androgen in skeletal homeostasis results from the use of testosterone as monotherapy. Testosterone, the major circulating androgen, can also serve as the substrate for the production of estradiol via aromatase activity. As a consequence, some testosterone action may result from estrogen receptor-dependent activation after conversion to 17-ß estradiol. Experimental strategies that employ GnRH agonists followed by treatment with testosterone coupled with an aromatase inhibitor have been used to determine the extent to which specific androgen signaling in the hypogonadal adult is anti-resorptive (i.e., inhibits bone resorptive activity) or anabolic (i.e., enhances bone formation). Conflicting results have been reported using such an approach and thus the extent of anabolic vs. anti-resorptive responses remains open to debate [11–13]. These results underscore the lack of understanding of the specific role for androgen in bone biology, and the distinct contribution of androgen to the maintenance of a healthy skeleton in the adult remains unclear.

Androgen action is mediated by androgen receptor (AR) and AR expression (gene and protein) is highest during the mineralization stage in the osteocyte population, determined during differentiation using a well characterized primary culture model [14]. In order to evaluate the cell-autonomous direct effects of androgen signaling, we have constructed transgenic animal models with skeletally-targeted overexpression of AR to increase sensitivity to endogenous androgens. Importantly, enhanced androgen action occurs as a consequence of enhanced AR signaling only in those cells with elevated levels of AR, without changes in circulating steroid levels and without systemic androgen administration [15,16]. In both transgenic families, AR overexpression is controlled using fragments of the type I collagen promoter, with both distinct and overlapping expression profiles. With overexpression driven from the 2.3 kb fragment, AR2.3-tg mice demonstrate AR overexpression targeted to mature osteoblasts and osteocytes for enhanced responsiveness in the bone compartment with the highest endogenous AR concentrations [14]. Characterization of AR2.3-tg males shows AR overexpression in mature osteoblasts is associated with the development of a low turnover bone phenotype, with compromised bone quality and strength [16] and cell autonomous inhibition of osteoblast differentiation mediated by inhibition of BMP signaling on the endocortical surface [17]. With transgene expression driven from the 3.6 kb fragment, AR3.6-tg mice show overexpression throughout the osteoblast lineage, including stem cells, immature osteoblasts and mature mineralizing osteoblasts and osteocytes [17–19]. Similar to AR2.3-tg mice, male AR3.6-tg mice also demonstrate a low turnover bone phenotype with compromised bone quality and strength [15]. Since both transgenic lines have enhanced androgen responsiveness in mature osteoblasts and osteocytes via AR overexpression, a comparison between the two families suggests that the predominant action of androgen in the skeleton produces a low turnover phenotype mediated by AR expression in that shared compartment (i.e., mature osteoblasts/osteocytes). Notably, the novel aspects of the consequences of unique AR overexpression in stem cells and immature osteoblasts is a body composition phenotype with reduced fat and increased muscle mass [19,20], not observed in AR2.3-tg mice.

The goal of these studies was to evaluate the anabolic vs. anti-resorptive effects of dihydrotestosterone (DHT), a nonaromatizable androgen in hypogonadal male adult mice, using littermate control WT mice and both AR transgenic families with enhanced androgen responsiveness in bone. To optimize detection of anabolic effects vs. anti-resorptive effects of androgen treatment in the adult, two experimental paradigms were employed. In the first model, DHT treatment was initiated immediately after ORX during a period of higher turnover [21]. In the second model, ORX animals were allowed to develop bone loss first before DHT treatment was initiated, in a lower turnover setting [21–23]. By comparing results observed between these paradigms in all genotypes, we sought to test the effectiveness of DHT replacement to either prevent osteopenia during high turnover or to restore bone after hypogonadal loss as an anabolic strategy and the specific role of AR in the skeleton.

Materials and methods

Animals

The generation of AR-transgenic mice employing the 2.3 kb $\alpha_1$1 collagen promoter fragment to drive expression has been described previously [16]. AR2.3-tg and AR3.6-tg mice were bred to WT B6D2F1 mice (Jackson Labs, Bar Harbor, ME). Mice were maintained under a 12 h light–dark cycle, had free access to tap water and were fed ad libitum a standard rodent chow containing 4.5% fat and 23% protein (LabDiet 5001, PMI Nutrition Int., St. Louis, MO). All animal studies were performed according to institutional, local, state, federal and NIH guidelines for the use of animals in research under an Institutional Animal Use and Care Committee (IACUC)-approved protocol.

Experimental protocols

The effectiveness of androgen therapy to ameliorate hypogonadal loss was tested following ORX using male WT, AR2.3-tg and AR3.6-tg mice. Two experimental approaches were taken to emphasize potential anti-resorptive vs. anabolic actions of androgen replacement (see Fig. 1). In the first paradigm, protracted hormone ablation after ORX allowed the development of a hypogonadal phenotype that was then followed by androgen treatment. WT littermate control (B6D2F2) and AR transgenic mice were sham operated or orchidectomized at 3 months of age, and after a 2 month delay the effect of 6 weeks of treatment with nonaromatizable dihydrotestosterone (DHT) or placebo was determined. DHT treatment for 6 weeks is sufficient time to observe response to therapy, as major effects of androgen depletion and/or replacement on bone metabolism have been observed in rodents in just 3 weeks [24]. High turnover with rapid bone loss and low turnover with slow bone loss are established states of bone turnover that occur immediately or several months after gonadectomy in both humans and rodents [21–23,25,26]. Thus, after the two month delay, the high bone turnover typically observed after ORX has stabilized to a lower turnover state (LT). Treatments during LT will enhance the ability to detect anabolic signaling rather than anti-resorptive responses. In the LT hypogonadal state, hormone administration is considered a therapeutic intervention to restore lost bone. In the second paradigm, gonadectomy was performed at 5 months of age, followed immediately by DHT treatment again for 6 weeks. In this second approach, animals are in a high turnover (HT) state and treatment is characterized as a preventative intervention. Both LT and HT groups were evaluated at the end of the study at 6.5 months of age following 6 weeks of DHT treatment.

Gonadectomy and steroid treatment

Anesthesia was induced with isoflurane (5% in air) and maintained with ~2% in air. For ORX, an incision was made through the skin at the tip of the scrotum on the left side and the testicular fat pad was localized and pulled through the incision using blunt forceps. A hemostat was placed across the testicular cord and the testes, epididymis and fat