Skeletal effects of androgen withdrawal

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Abstract

Hypogonadism is considered to be one of the major risk factors for osteoporosis in men. Therefore, it is an important goal for skeletal research to improve our understanding of the skeletal effects of androgens. Androgen deficiency during growth is associated with a failure to acquire normal peak bone mass, and there is good evidence that the effects of androgens on skeletal growth and the development of a male skeletal phenotype are mediated through the androgen receptor. In adult men, acute withdrawal of androgens by surgical or chemical castration induces high turnover bone loss. Similarly, orchidectomy of aged, non-growing male rats is associated with a pronounced and sustained increase in bone turnover and with true loss of cancellous and cortical bone. Interestingly, the changes in bone turnover induced by orchidectomy are paralleled by a concomitant increase in B lymphopoiesis in bone marrow of rats and mice. Although there is firm evidence that male bone metabolism can be influenced by androgens and estrogen, a variety of clinical and animal experimental data have strongly suggested that, under physiological circumstances, the maintenance of cancellous bone mass in males involves the skeletal action of estrogen derived from aromatization of androgens. Aged male rats appear to closely mimic the conditions induced by androgen withdrawal in adult humans, and this animal model may be used 1) to elucidate further the role of muscle as a mediator of the actions of androgens on bone, 2) to explore the regulatory functions of androgens and estrogens in the male skeleton and the immune system, and 3) to find new treatment strategies for the prevention and treatment of osteoporosis in men.

Keywords: Orchidectomy, Bone turnover, Androgens, Estrogen, B lymphocytes

Introduction

Although osteoporotic fractures are less common in men compared with women, it has been recognized in the past decade that osteoporosis in men is also a major public health issue. Therefore, the scientific interest in the pathophysiology and treatment of this bone disease has been growing during recent years. Hypogonadism, i.e., a reduction in circulating androgen levels, is considered to be one of the major risk factors for osteoporosis in men. However, the mechanisms of bone loss due to androgen deficiency are still unclear.

The elucidation of the skeletal actions of androgens is complicated by the fact that androgens can be metabolized into estrogens by the enzyme aromatase (Fig. 1). The major source for estrogen in males is extraglandular aromatization of gonadal or adrenal androgens. Aromatase activity is found in several tissues, including adipose tissue and osteoblastic cells. Bone marrow stromal cells and osteoblasts of males and females express androgen receptors as well as estrogen receptors α and β. Androgen and estrogen receptors are also found in osteocytes and osteoclasts. Therefore, some of the skeletal effects of androgens may be mediated by estrogen.

Androgens control the development of a male skeletal phenotype

Some experiments of nature have shed light on the importance of signaling through the androgen receptor for the skeleton. Mutations that result in a functional inactivation of the androgen receptor have been described in humans, but also in rats and mice. The gene coding for the androgen receptor is located on the X chromosome, so that males have only one allele of the androgen receptor gene. There are several known mutations in the human androgen receptor resulting in the functional inactivation of the protein, for example mutations in the hormone binding domain of the receptor. The associated disease is called complete androgen insensitivity or testicular feminization. The patients suffering from complete androgen insensitivity...
are genetic males (karyotype 46, XY) but are phenotypically female with abdominal testes in most cases and an absent uterus. The circulating levels of testosterone and estradiol are in the normal or high range for males. However, due to the defective receptor, all tissues are resistant to the actions of androgens in complete androgen insensitivity. The skeleton in these patients is female in size, and bone mineral density (BMD) at the lumbar spine and the femoral neck is low compared with age-matched women31. Similarly, studies in testicular feminized male (tfm) rats and tfm mice, which are characterized by a defective androgen receptor, have indicated that the presence of a functioning androgen receptor is essential for the development of a male skeletal phenotype also in rodents22,23.

Males have bigger bones than females and an increased amount of cortical bone3. The difference in size between bones from males and females is primarily due to the stimulatory effects of androgens on periosteal modeling drifts and on longitudinal bone growth. In part, these effects may be mediated indirectly through the stimulatory effects of androgens on the growth hormone/insulin-like growth factor axis24-27. On the other hand, estrogen suppresses periosteal bone expansion and longitudinal bone growth26-30. In agreement with the findings in tfm rats and tfm mice, orchidectomy of male growing rats reduces periosteal appositional growth in cortical bone, an effect that can be reversed by administration of testosterone or the nonaromatizable androgen dihydrotestosterone29,31. Moreover, testosterone treatment of growing, castrated New Zealand White rabbits from 6 weeks of age until the time of skeletal maturity increased vertebral BMD significantly over vehicle-treated animals32. Similarly, pre-pubertal hypogonadism in adolescent boys results in the failure to acquire normal peak bone mass with emphasis on a deficit in cortical bone accretion33,34, and supplementation of androgens stimulates periosteal bone expansion and longitudinal bone growth and on cortical bone accumulation are mediated via the androgen receptor (Fig. 1).

Skeletal effects of androgen withdrawal in adult men

It is clear from a number of clinical studies that androgens are not only important during the growth period but also have a role in the maintenance of the male skeleton. In a longitudinal study in a small group of orchidectomized (ORX) adult men, Stepan et al.37 reported an increase in bone turnover and a rapid decrease in lumbar spine BMD in response to orchidectomy. Diamond and co-workers38 showed that men treated for prostate carcinoma with combined androgen blockade, i.e. the gonadotropin releasing hormone (GnRH) agonist goserelin plus the androgen receptor antagonist flutamide, lost 6-7% of BMD at the lumbar spine and the femoral neck within 6 months of combined androgen blockade. The bone loss was accompanied by elevated bone turnover as assessed by increased serum osteocalcin and urinary deoxypyridinoline. Similar findings were reported by Goldray et al. in GnRH-treated elderly men39. In the long term, orchidectomy for prostate cancer is frequently followed by severe osteoporosis40. Daniell et al.41 showed that men treated with chemical or surgical castration for prostate carcinoma continue to lose bone at greatly accelerated rates for many years. In contrast to the effects of androgen deficiency during growth, bone loss due to androgen deficiency in adult male humans is most pronounced in cancellous bone3,4,42.

Taken together, the available clinical data suggest that acute androgen withdrawal due to surgical or chemical castration in adult men is associated with a profound increase in bone turnover, and with subsequent rapid loss of cancellous bone mass. In accordance with these findings, it has been shown that testosterone administration suppresses bone turnover in adult men with acquired hypogonadism42 and in osteoporotic eugonadal men43.

The situation appears to be more complicated in osteo-

![Figure 1. Skeletal effects of androgens in males. Androgens can act directly on bone cells and other organs through the androgen receptor (AR). Androgens stimulate periosteal bone apposition, longitudinal bone growth, and possibly also cancellous bone formation through this pathway. Therefore, androgens and a functioning androgen receptor are necessary for the accumulation of normal peak bone mass, especially for the accumulation of an adequate cortical bone mass, during growth. With the help of the enzyme aromatase, androgens can be metabolized into estrogens. Aromatase is present in many tissues, including bone. Estrogens in turn suppress cancellous and endocortical bone turnover as well as periosteal bone expansion and longitudinal bone growth through the estrogen receptors (ER) α and/or β. The latter pathway is probably the prevailing pathway for the maintenance of cancellous and cortical bone mass in a non-growing male skeleton.](image-url)
porotic patients with relative hypogonadism of slow onset. The changes in bone turnover in these patients are inadequately described but appear to be very heterogeneous, and bone formation may be reduced in patients with longstanding hypogonadism. In line with these results, some small studies have suggested a stimulating effect of testosterone treatment on bone formation in hypogonadal male osteoporotic patients. Therefore, testosterone may stimulate cancellous bone formation in some hypogonadal men.

**Animals models of androgen deficiency**

In order to elucidate further the pathogenesis of androgen deficiency-induced bone loss and to explore new treatment strategies for this disease, appropriate animal models are needed. A reduction in bone mass in response to orchidectomy has been shown in a variety of animal species, including rats, mice, rabbits, guinea pigs, and dogs. However, the great majority of work has been performed in the rat. When interpreting findings from studies in growing animals, it is very important to keep in mind that androgens have the abovementioned pronounced effects on bone size and cortical bone accretion during the growth period. Therefore, it is essential for the development and interpretation of animal models to clearly separate the skeletal effects of androgens during growth from the role of androgens in the maintenance of a mature male skeleton.

Orchidectomy of growing rats, rabbits, or guinea pigs results in bones of smaller size, and in the failure to adequately gain bone mass, especially cortical bone mass. Male rats reach their peak bone mass with approximately 12 months of age. The main mechanism for the reduction in cortical bone mass in growing ORX animals lies in the fact that androgen deficiency during the growth period reduces the androgen-driven periosteal bone modeling drifts. Orchidectomy of growing rats is also associated with cancellous bone osteopenia in appendicular bones. The mechanism for the reduction in cancellous bone in growing ORX rats is not entirely clear. It is possible that there is a transient phase of increased cancellous bone turnover immediately after orchidectomy of young rats. However, this phase of increased cancellous bone turnover after orchidectomy appears to be short-lived, and has not been found in all studies. In conclusion, the osteopenia found in growing ORX animals is mainly caused by a failure to adequately accumulate bone mass during growth.

In contrast, all studies performed in aged, 12–13-month-old rats have clearly demonstrated true loss of cancellous and cortical bone mass associated with a pronounced and sustained increase in bone turnover. Therefore, the skeletal effects of androgen withdrawal may differ in a growing, compared with a non-growing, male, skeleton. In a sequential study in 13-month-old Fischer 344 rats performed in our laboratory, ORX rats showed elevated biochemical and histomorphometric indices of osteoclastic bone resorption from 2 weeks until 9 months post-surgery. The increase in bone resorption in ORX rats was followed by a sustained increase in biochemical and histomorphometric indices of bone formation and a pronounced rise in activation frequency from 1 month post-ORX until 9 months post-ORX. The changes in bone turnover following orchidectomy did not differ between the first lumbar vertebral body and the proximal tibial metaphysis. Cancellous bone loss in the proximal tibia and the lumbar vertebra together with increased bone turnover has also been shown in 6-month-old male Sprague-Dawley rats.

The microanatomical mechanisms responsible for cancellous bone loss in aged ORX rats are a reduction in trabecular number as well as trabecular thinning in the vertebrae, and a decrease in trabecular number without concomitant trabecular thinning relative to sham-operated (SHAM) controls in the proximal tibial metaphysis. The reduction in trabecular number in a non-growing bone site is thought to be caused by trabecular plate perforations due to excessive osteoclast activity, and subsequent removal of whole structural bone elements, while trabecular thinning is probably the result of a negative remodeling balance, i.e., the amount of bone resorbed exceeds the quantum of newly formed bone in each remodeling site. Osteoblast insufficiency or reduced osteoblast team performance has not been reported in aged ORX rats. Therefore, excessive osteoclast activity causing trabecular plate perforations and a negative remodeling balance is probably of crucial importance in the pathogenesis of the cancellous bone osteopenia induced by androgen deficiency in aged rats. A study in 9-month-old rats has shown that the loss of cortical bone in ORX animals is due to a combination of increased endocortical bone resorption and decreased periosteal bone apposition. It is likely that in older rats (e.g., 12- or 13-month-old rats), when periosteal bone apposition subsides, the loss of cortical bone after orchidectomy is predominantly caused by increased endocortical bone resorption.

Taken together, there is good experimental evidence that androgen withdrawal induces high turnover osteopenia in cancellous and cortical bone of aged, 12- or 13-month-old rats. Although the magnitude of the rise in bone turnover after androgen withdrawal may be accentuated in inbred Fischer 344 rats relative to other strains (for example Wistar rats), it is important to note that the skeletal response to orchidectomy appears to be generally similar in different, inbred and outbred, strains of aged rats. Similar to human cancellous bone, the predominant turnover activity in rat cancellous bone is remodeling in aged rats. Therefore, aged male rats appear to be a very good model of the osteopenia induced by androgen withdrawal in adult humans.

In agreement with the findings in aged rats, mice appear to respond to orchidectomy with an increase in bone turnover and subsequent cancellous and cortical bone loss. However, more work has to be done to characterize further the murine model of post-ORX bone loss. This task may be even more important for future skeletal research since mice are the animal of choice for all transgenic and knock-out models.
Recently, a sequential study in a small number of 2-year-old ORX dogs reported a transient increase in biochemical markers of bone formation within the first 6 months postsurgery. However, these changes were not paralleled by similar changes in bone histomorphometry in iliac cancellous bone. Iliac cancellous bone volume was reduced 1 year postsurgery compared with baseline values, and the reduction in bone volume was accompanied by trabecular thinning and lowered values for mineralizing surface. Therefore, although dogs appear to lose cancellous bone after orchidectomy, they seem to lack the clear increase in bone turnover seen in aged rodents and adult humans following androgen withdrawal. These findings suggest that dogs may not be a good model for androgen deficiency-induced bone loss in humans. In a similar fashion, ovariectomized dogs have proved to be an unreliable model for estrogen deficiency-induced bone loss.

A major progression in bone biology during recent years has been the elucidation of the molecular pathways involved in osteoclast differentiation and activation. Receptor activator of NF-κB ligand (RANKL), a member of the tumor necrosis factor ligand superfamily, is an essential molecule for osteoclast formation and function. The discovery of RANKL has linked bone metabolism to the immune system, because RANKL is expressed not only in cells of the stromal cell lineage but is also produced, in a soluble form, by activated T cells. Furthermore, RANKL-deficient mice show defects in early differentiation of B and T lymphocytes in addition to severe osteopetrosis. In this context, it is very interesting to note that estrogen and androgen withdrawal result in a parallel upregulation of bone turnover and B lymphopoiesis in mice, and also in rats. Orchidectomy of aged rats augments B lymphopoiesis in bone marrow over several months. Moreover, male testicular feminization mice have substantially increased numbers of B cell precursors in bone marrow as compared with wild-type mice.

Although B lineage cells may express estrogen and androgen receptors, experiments in cell culture systems have strongly suggested that the suppressive effects of estrogen and androgens on B lymphopoiesis are mediated through stromal cells. Therefore, cells of the stromal cell lineage may regulate both lymphopoiesis and bone remodeling in response to changes in circulating levels of sex steroids, and it is an intriguing task for future research to elucidate the molecular links between bone metabolism and B lymphopoiesis.

Are the skeletal effects of androgen withdrawal mediated by estrogen deficiency?

Because testosterone synthesized by the testicles is the major source of circulating estradiol in males, orchidectomy not only reduces testosterone but also estradiol serum levels. Therefore, the question is whether the high turnover osteopenia following orchidectomy is induced by androgen deficiency or, in essence, by estrogen deficiency. In fact, the changes in male bone metabolism after orchidectomy are highly reminiscent of the changes induced by ovariectomy in females.

During recent years, there has been accumulating evidence that estrogen may play an important role in male skeletal homeostasis. Young adult male patients with a non-functioning estrogen receptor or with a defective aromatase show reduced bone mass and retarded skeletal maturation, despite normal or elevated testosterone serum levels. Treatment with estrogen increases bone mass in aromatase-deficient male patients. Furthermore, male estrogen receptor alpha knock-out mice and aromatase knock-out mice also have decreased bone mass. These findings suggest an important role for estrogen in male bone metabolism. However, one has to keep in mind that all these genetic defects are complicated by the fact that they are present throughout life, and affect the male skeleton during both growth and maintenance. Thus, at present, these findings do not allow us to establish a definitive role of estrogen in the maintenance of the male skeleton.

The results of several clinical and epidemiological studies have provided additional evidence in favor of a function of estrogen in the regulation of bone metabolism and bone mass in males. For example, six-months' treatment of eugonadal osteoporotic men with testosterone increased BMD and serum estradiol, and suppressed urinary excretion of deoxypyridinoline. Interestingly, the increase in spinal BMD due to testosterone treatment was correlated with the change in serum estradiol, but not with the change in serum testosterone. Furthermore, in a large cross-sectional study, bioavailable estradiol (among total and bioavailable testosterone, total and bioavailable estradiol, and dehydroepiandrosterone) was most strongly associated with BMD.

Although cancellous bone loss in aged ORX rats can be prevented by the administration of aromatizable and nonaromatizable androgens as well as by estradiol, some animal experimental data also have suggested that estradiol may be important for the maintenance of the male skeleton under physiological circumstances. Vanderschueren et al. reported that orchidectomy or treatment of intact aged male rats with the aromatase inhibitor vorozole induced similar reductions in BMD of the axial and appendicular skeleton. However, the aromatase inhibitor did not upregulate cancellous bone turnover in a similar fashion compared with orchidectomy. A possible confounding factor in these studies is that aromatase inhibition in intact animals is associated with a rise in circulating androgen levels through increased gonadotropin secretion as a result of interference with the hormonal negative feedback mechanisms. Therefore, it is not entirely clear how the aromatase inhibitor induced bone loss in aged rats.

Using multiple regression analysis we could show in a recent study that among estradiol, free and total testosterone, estradiol was the only significant predictor of histomorphometric indices of bone turnover in aged SHAM and ORX rats. When the multiple regression analysis was
performed on data from the SHAM animals only, circulating estradiol, but not testosterone, showed a strong negative association with histomorphometric indices of bone formation and activation frequency, suggesting that estradiol may suppress bone formation in aged male rats also under physiological circumstances. Interestingly, multiple regression analysis of the data from the same study revealed that, in analogy to the effects on bone turnover, circulating estradiol also was the major predictor of the percentage of B lineage cells in bone marrow of aged SHAM and ORX rats. Total and free testosterone were not correlated with B lymphopoiesis in SHAM animals. Therefore, although B lymphopoiesis and bone turnover in males can be suppressed by both estrogens and androgens, circulating estradiol may be a more important suppressor of bone turnover and B cell proliferation in bone marrow than testosterone under physiological conditions in aged rats.

In line with the notion that estrogen is an important regulator of bone remodeling in males, the selective estrogen receptor modulator tamoxifen has been shown to prevent the rise in bone turnover and the bone loss induced by orchidectomy in aged rats. In a similar fashion, tamoxifen prevents bone loss in male castrated mice. However, the pure antiestrogen ICI 182,780 has no effect on bone growth in young male rats. Therefore, in a growing male skeleton androgens may act mainly through the androgen receptor. This pathway is necessary for the accumulation of normal peak bone mass. However, in a non-growing male skeleton, aromatization of androgens into estrogens and signaling through the estrogen receptor may become the prevailing pathway for the maintenance of cancellous bone mass in males involves the skeletal action of estrogen derived from aromatization of androgens. Aged male rats appear to closely mimic the conditions induced by androgen withdrawal in adult humans, and this animal model may be used to further elucidate the regulatory functions of androgens and estrogens for the male skeleton, and to explore new treatment strategies for the prevention and treatment of osteoporosis in men.

An aspect that has not been adequately addressed in all clinical and animal experimental studies to date is that androgens may influence bone indirectly through their effects on muscle mass and strength. Muscle contractions result in mechanical loading of bones, and bones adapt to altered mechanical usage by structural changes. It is quite clear that androgens have pronounced effects on muscle mass and strength. Therefore, androgen deficiency may induce bone loss indirectly through a detrimental effect on muscle strength, and it will be important for future studies to quantify changes in muscle strength in response to changes in circulating sex steroid levels, in addition to the analysis of the bone effects.

**Conclusions**

Androgen deficiency during growth is associated with the failure to acquire normal peak bone mass, and there is very good evidence that the effects of androgens on skeletal growth and the development of a male skeletal phenotype are mediated through the androgen receptor. In adult men, acute withdrawal of androgens by surgical orchidectomy or chemical combined androgen blockade induces a high turnover osteopenia. Although it is clear that male bone metabolism can be influenced both via the androgen and via the estrogen receptors, a variety of clinical and animal experimental data have strongly suggested that, under physiological circumstances, the maintenance of cancellous bone mass in males involves the skeletal action of estrogen derived from aromatization of androgens. Aged male rats appear to closely mimic the conditions induced by androgen withdrawal in adult humans, and this animal model may be used to further elucidate the regulatory functions of androgens and estrogens for the male skeleton, and to explore new treatment strategies for the prevention and treatment of osteoporosis in men.

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Purohit A, Flanagan AM and Reed MJ. Estrogen synthesis by osteoblast cell lines. Endocrinology 1992; 131:2027-2029.


73. Erben RG and Eberle J. B lymphopoiesis is upregulated after orchidectomy and is correlated with estradiol but not testosterone serum levels in aged male rats. Horm Metab Res 2001; (in press).


89. Turner RT, Evans GL and Dobni H. The high-affinity...

