Review Article

Skeletal effects of androgen withdrawal

R.G. Erben

Institute of Physiology, Physiological Chemistry and Animal Nutrition, Ludwig Maximilians University, Munich, Germany

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[1,2], it has been recognized in the past decade that osteoporosis in men is also a major public health issue[3]. 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[3,4]. 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[5]. Aromatase activity is found in several tissues, including adipose tissue[6] and osteo-blastic cells[5,7,8]. Bone marrow stromal cells and osteoblasts of males and females express androgen receptors[9,10] as well as estrogen receptors α[11] and β[12]. Androgen and estrogen receptors are also found in osteocytes[13-15] and osteoclasts[15-18]. 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[19]. The associated disease is called complete androgen insensitivity or testicular feminization[20,21]. The patients suffering from complete androgen insensitivity

Corresponding author: R.G. Erben, MD, DVM. Institute of Animal Physiology, University of Munich, Veterinärstrasse 13, 80539 Munich Germany. E-mail: R.Erben@lrz.uni-muenchen.de

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 women[21]. 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 rodents[22,23].

Males have bigger bones than females and an increased amount of cortical bone[3]. 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 axis[24-27]. On the other hand, estrogen suppresses periosteal bone expansion and longitudinal bone growth[28-30]. 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 dihydrotestosterone[29,31]. 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 animals[32]. Similarly, pre-pubertal hypogonadism in adolescent boys results in the failure to aquire normal peak bone mass with emphasis on a deficit in cortical bone accretion[33,34], and supplementation of androgens stimulates bone growth and increases especially cortical bone mass in male hypogonadal adolescents[35,36]. Taken together, there is good evidence that androgens are important for the determination of bone size and a male skeletal phenotype, and that these effects on 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-workers[38] showed that men treated for prostate carcinoma with combined androgen blockade, i.e. the gonadotropin releasing hormone (GnRH) agonist goserelin plus the
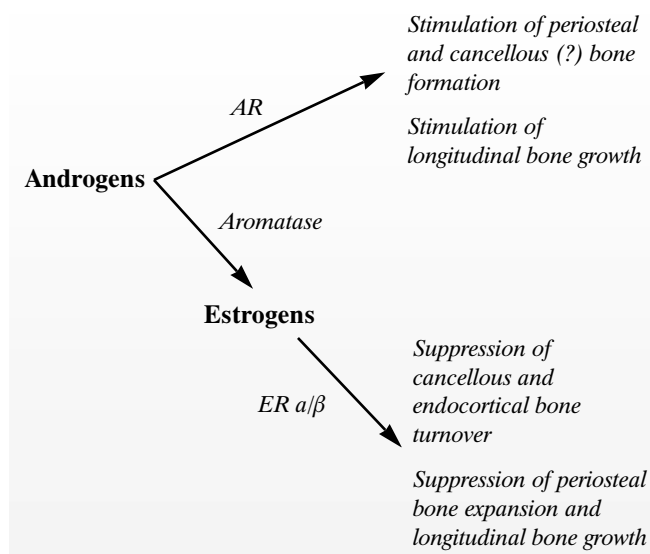

Figure 1. Skeletal effects of androgens in males. Androgens can act directly on bone cells and other organs through the androgen receptor (*AR*). Androgens stimulate periosteal bone apposition, longitudinal bone growth, and possibly also cancellous bone formation through this pathway. Therefore, androgens 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*) *a* 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.

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 men[39]. In the long term, orchidectomy for prostate cancer is frequently followed by severe osteoporosis[40]. 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 bone[3,4,42].

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 hypogonadism[42] and in osteoporotic eugonadal men[43].

The situation appears to be more complicated in osteo-

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 long-standing hypogonadism[3]. In line with these results, some small studies have suggested a stimulating effect of testosterone treatment on bone formation in hypogonadal male osteoporotic patients[44,45]. 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[46], rabbits[32], or guinea pigs[47] 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[46]. 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[29]. Orchidectomy of growing rats is also associated with cancellous bone osteopenia in appendicular bones[46]. 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[31]. However, this phase of increased cancellous bone turnover after orchidectomy appears to be short-lived, and has not been found in all studies[46,48]. In conclusion, the osteopenia found in growing ORX animals is mainly caused by a failure to adaequately 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[49-54]. 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[54]. The changes in bone turnover following orchidectomy did not differ between the first lumbar vertebral body and the proximal tibial metaphysis[54]. 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[55,56].

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[54]. 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[57], 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[54]. 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[58]. 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[54] relative to other strains (for example Wistar rats[50]), 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[59]. 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[60-64]. 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 post-surgery[65]. However, these changes were not paralleled by similar changes in bone histomorphometry in iliac cancellous bone. Iliac cancellous bone volume was reduced 1 year post-surgery 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[66].

A major progression in bone biology during recent years has been the elucidation of the molecular pathways involved in osteoclast differentiation and activation[67]. Receptor activator of NF-kB 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 the cell membrane of cells of the stromal cell/osteoblastic lineage but is also produced, in a soluble form, by activated T cells[68]. Furthermore, RANKL-deficient mice show defects in early differentiation of B and T lymphocytes in addition to severe osteopetrosis[69]. In this context, it is very interesting that both estrogen and androgen withdrawal result in a parallel upregulation of bone turnover and B lymphopoiesis in mice[70,71], and also in rats[72,73]. Orchidectomy of aged rats augments B lymphopoiesis in bone marrow over several months[73]. Moreover, male testicular feminization mice have substantially increased numbers of B cell precursors in bone marrow as compared with wild-type mice[12].

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[11,12,70]. 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[54]. Therefore, the question is whether the high turnover osteopenia following orchiectomy 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[74,75].

During recent years, there has been accumulating evidence that estrogen may play an important role in male skeletal homeostasis[4,76,77]. Young adult male patients with a non-functioning estrogen receptor[78] or with a defective aromatase[79] 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[80,81]. Furthermore, male estrogen receptor alpha knock-out mice[82] and aromatase knock-out mice[83] 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 maintainance 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[43]. Furthermore, in a large cross-sectional study bioavailable estradiol (among total and bioavailable testosterone, total and bioavailable estradiol, and dehydro-epiandrosterone) was most strongly associated with BMD[84].

Although cancellous bone loss in aged ORX rats can be prevented by the administration of aromatizable and nonaromatizable androgens as well as by estradiol[50,85], 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.[86,87] 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[54]. 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[73]. Total and free testosterone were not correlated with B lymphopoiesis in SHAM animals[73]. Therefore, although B lymphopoiesis and bone turnover in males can be suppressed by both estrogens and androgens[12,50,85], 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 lasofoxifene has been shown to prevent the rise in bone turnover and the bone loss induced by orchidectomy in aged rats[88]. In a similar fashion, tamoxifen prevents bone loss in male castrated mice[64]. However, the pure antiestrogen ICI 182,780 has no effect on bone growth in young male rats[89]. 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 and cortical bone mass (Fig. 1). This hypothesis may also explain the different effects of androgen withdrawal in young and aged rats.

The most compelling evidence in favor of an important role of estrogen in the regulation of skeletal metabolism in males comes from the elegant study by Falahati-Nini and co-workers[90]. In this study, healthy elderly men were treated with a long-acting GnRH agonist for suppression of endogenous sex steroid secretion. In addition, the men received supplements of testosterone and estradiol, alone or in combination, in the presence of an aromatase inhibitor. The changes in biochemical markers of bone turnover induced by the different treatment regimens clearly demonstrated that estradiol was the dominating hormone suppressing bone resorption. Interestingly, serum osteocalcin declined after the induction of hypogonadism in this study, and was maintained at control levels by both testosterone and estradiol. At present it is unclear why surgical orchidectomy[37], GnRH agonist treatment[39], or combined androgen blockade[38] induced a rise in bone formation and bone resorption in adult men in other studies, while administration of the GnRH agonist in the study by Falahati-Nini et al. caused an increase in bone resorption but a decline in bone formation. Nevertheless, this study unambiguously showed that estradiol has a major physiological function in male bone metabolism.

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.

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[91]. 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.

References

1. Riggs BL and Melton LJ. Evidence for two distinct syndromes of involutional osteoporosis. Am J Med 1983; 75:899-901.
2. Riggs BL and Melton LJ, III. Involutional osteoporosis. N Engl J Med 1986; 314:1676-1686.
3. Orwoll ES and Klein RF. Osteoporosis in men. Endocr Rev 1995; 16:87-116.
4. Anderson FH, Francis RM, Selby PL and Cooper C. Sex hormones and osteoporosis in men. Calcif Tissue Int 1998; 62:185-188.
5. Shozu M and Simpson ER. Aromatase expression of human osteoblast-like cells. Mol Cell Endocrinol 1998; 139:117-129.
6. Simpson ER, Bulun SE, Nichols JE and Zhao Y. Estrogen biosynthesis in adipose tissue: Regulation by paracrine and autocrine mechanisms. J Endocrinol 1996; 150 Suppl.:S51-S57.

7. Tanaka S, Haji M, Nishi Y, Yanase T, Takayanagi R and Nawata H. Aromatase activity in human osteoblast-like osteosarcoma cell. Calcif Tissue Int 1993; 52:107-109.

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

9. Viselli SM, Reese KR, Fan J, Kovacs WJ and Olsen NJ. Androgens alter B cell development in normal male mice. Cellular Immunology 1997; 182:99-104.

10. Colvard DS, Eriksen EF, Keeting PE, Wilson EM, Lubahn DB, French FS, Riggs BL and Spelsberg TC. Identification of androgen receptors in normal human osteoblast-like cells. Proc Natl Acad Sci U S A 1989; 86: 854-857.

11. Smithson G, Medina K, Ponting I and Kincade PW. Estrogen suppresses stromal cell-dependent lympho-poiesis in culture. J Immunol 1995; 155:3409-3417.

12. Smithson G, Couse JF, Lubahn DB, Korach KS and Kincade PW. The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. J Immunol 1998; 161:27-34.

13. Noble B, Routledge J, Stevens H, Hughes I, Jacobson W. Androgen receptors in bone-forming tissue. Horm Res 1999; 51:31-36.

14. Abu EO, Horner A, Kusec V, Triffitt JT and Compston JE. The localization of androgen receptors in human bone. J Clin Endocrinol Metab 1997; 82:3493-3497.

15. Vidal O, Kindblom LG and Ohlsson C. Expression and localization of estrogen receptor-b in murine and human bone. J Bone Miner Res 1999; 14:923-929.

16. Mano H, Yuasa T, Kameda T, Miyazawa K, Nakamaru Y, Shiokawa M, Mori Y, Yamada T, Miyata K, Shindo H, Azuma H, Hakeda Y and Kumegawa M. Mammalian mature osteoclasts as estrogen target cells. Biochem Biophys Res Commun 1996; 223:637-642.

17. Oursler MJ, Pederson L, Fitzpatrick L, Riggs BL and Spelsberg T. Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. Proc Natl Acad Sci U S A 1994; 91:5227-5231.

18. Pederson L, Kremer M, Judd J, Pascoe D, Spelsberg TC, Riggs BL and Oursler MJ. Androgens regulate bone resorption activity of isolated osteoclasts in vitro. Proc Natl Acad Sci U S A 1999; 96:505-510.

19. Brinkmann A, Jenster G, Ris-Stalpers C, van der KH, Bruggenwirth H, Boehmer A and Trapman J. Molecular basis of androgen insensitivity. Steroids 1996; 61:172-175.

20. Shah R, Woolley MM and Costin G. Testicular feminization: the androgen insensitivity syndrome. J Pediatr Surg 1992; 27:757-760.

21. Munoz-Torres M, Jódar E, Quesada M and Escobar-Jiménez F. Bone mass in androgen-insensitivity syndrome: Response to hormonal replacement therapy. Calcif Tissue Int 1995; 57:94-96.

22. Vanderschueren D, Van Herck E, Suiker AM, Visser WJ, Schot LP, Chung K, Lucas RS, Einhorn TA and Bouillon R. Bone and mineral metabolism in the androgen-resistant (testicular feminized) male rat. J Bone Miner Res 1993; 8:801-809.

23. Uesugi Y, Taguchi O, Noumura T and Iguchi T. Effects of sex steroids on the development of sexual dimorphism in mouse innominate bone. Anat Rec 1992; 234:541-548.

24. Soliman AT, Khadir MM and Asfour M. Testosterone treatment in adolescent boys with constitutional delay of growth and development. Metabolism 1995; 44:1013-1015.

25. Fukuda R, Usuki S, Mukai N, Amagai H, Hayashi K and Takamatsu K. Serum insulin-like growth factor-I, insulin-like growth factor binding protein-3, sex steroids, osteocalcin and bone mineral density in male and female rats. Gynecol Endocrinol 1998; 12:297-305.

26. Clark PA and Rogol AD. Growth hormones and sex steroid interactions at puberty. Endocrinol Metab Clin North Am 1996; 25:665-681.

27. Blizzard RM, Martha PM, Kerrigan JR, Mauras N and Rogol AD. Changes in growth hormone (GH) secretion and in growth during puberty. J Endocrinol Invest 1989; 12:65-68.

28. Turner RT, Colvard DS and Spelsberg TC. Estrogen inhibition of periosteal bone formation in rat long bones: down-regulation of gene expression for bone matrix proteins. Endocrinology 1990; 127:1346-1351.

29. Turner RT, Wakley GK and Hannon KS. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. J Orthop Res 1990; 8:612-617.

30. Ornoy A, Giron S, Aner R, Goldstein M, Boyan BD and Schwartz Z. Gender dependent effects of testosterone and 17b-estradiol on bone growth and modeling in young mice. Bone Miner 1994; 24:43-58.

31. Gunness M and Orwoll E. Early induction of alterations in cancellous and cortical bone histology after orchidectomy in mature rats. J Bone Miner Res 1995; 10:1735-1744.

32. Gilsanz V, Roe TF, Gibbens DT, Schulz EE, Carlson ME, Gonzalez O and Boechat MI. Effect of sex steroids on peak bone density of growing rabbits. Am J Physiol 1988; 255:E416-E421.

33. Finkelstein JS, Klibanski A, Neer RM, Greenspan SL, Rosenthal DI and Crowley WF, Jr. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. Ann Intern Med 1987; 106:354-361.

34. Finkelstein JS, Neer RM, Biller BM, Crawford JD and Klibanski A. Osteopenia in men with a history of delayed puberty. N Engl J Med 1992; 326:600-604.

35. Arisaka O, Arisaka M, Nakayama Y, Fujiwara S and Yabuta K. Effect of testosterone on bone density and bone metabolism in adolescent male hypogonadism. Metabolism 1995; 44:419-423.

36. Finkelstein JS, Klibanski A, Neer RM, Doppelt SH, Rosenthal DI, Segre GV and Crowley WFJ. Increases in bone density during treatment of men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 1989; 69:776-783.

37. Stepan JJ, Lachman M, Zverina J, Pacovsky V and Baylink DJ. Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab 1989; 69:523-527.

38. Diamond T, Campbell J, Bryant C and Lynch W. The effect of combined androgen blockade on bone turnover and bone mineral densities in men treated for prostate carcinoma-Longitudinal evaluation and response to intermittent cyclic etidronate therapy. Cancer 1998; 83:1561-1566.

39. Goldray D, Weisman Y, Jaccard N, Merdler C, Chen J and Matzkin H. Decreased bone density in elderly men treated with the gonadotropin-releasing hormone agonist decapeptyl (D-Trp6-GnRH). J Clin Endocrinol Metab 1993; 76:288-290.

40. Daniell HW. Osteoporosis after orchidectomy for prostate cancer. J Urol 1997; 157:439-444.

41. Daniell HW, Dunn SR, Ferguson DW, Lomas G, Niazi Z and Stratte PT. Progressive osteoporosis during androgen deprivation therapy for prostate cancer. J Urol 2000; 163:181-186.

42. Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ and Klibanski A. Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab 1996; 81:4358-4365.

43. Anderson FH, Francis RM, Peaston RT and Wastell HJ. Androgen supplementation in eugonadal men with osteoporosis: Effects of six months' treatment on markers of bone formation and resorption. J Bone Miner Res 1997; 12:472-478.

44. Baran DT, Bergfeld MA, Teitelbaum SL and Avioli LV. Effect of testosterone therapy on bone formation in an osteoporotic hypogonadal male. Calci Tiss Res 1978; 26:103-106.

45. Francis RM, Peacock M, Aaron JE, Selby PL, Taylor GA, Thompson J, Marshall DH and Horsman A. Osteoporosis in hypogonadal men: role of decreased plasma 1,25- dihydroxyvitamin D, calcium malabsorption, and low bone formation. Bone 1986; 7:261-268.

46. Ke HZ, Crawford DT, Qi H, Chidsey-Frink KL, Li M, Jee WSS and Thompson DD. Long-Term Effects of Aging and Orchidectomy on Bone and Body Composition in Rapidly Growing Male Rats. J Musculoskel Neuron Interact 2001; 3:215-224.

47. Vanderschueren D, Van Herck E, Suiker AM, Allewaert K, Visser WJ, Geusens P and Bouillon R. Bone and mineral metabolism in the adult guinea pig: long-term effects of estrogen and androgen deficiency. J Bone Miner Res 1992; 7:1407-1415.

48. Turner RT, Hannon KS, Demers LM, Buchanan J and Bell NH. Differential effects of gonadal function on bone histomorphometry in male and female rats. J Bone Miner Res 1989; 4:557-563.

49. Wink CS and Felts WJ. Effects of castration on the bone structure of male rats: a model of osteoporosis. Calcif Tissue Int 1980; 32:77-82.

50. Vanderschueren D, Van Herck E, Suiker AM, Visser WJ, Schot LP and Bouillon R. Bone and mineral metabolism in aged male rats: short and long term effects of androgen deficiency. Endocrinology 1992; 130:2906-2916.

51. Vanderschueren D, Jans I, Van Herck E, Moermans K, Verhaeghe J and Bouillon R. Time-related increase of biochemical markers of bone turnover in androgen-deficient male rats. Bone Miner 1994; 26:123-131.

52. Vanderschueren D, Van Herck E, Schot P, Rush E, Einhorn T, Geusens P and Bouillon R. The aged male rat as a model for human osteoporosis: evaluation by non-destructive measurements and biomechanical testing. Calcif Tissue Int 1993; 53:342-347.

53. Vanderschueren D, Vandenput L, Boonen S, Van Herck E, Swinnen JV and Bouillon R. An aged rat model of partial androgen deficiency: prevention of both loss of bone and lean body mass by low-dose androgen replacement. Endocrinology 2000; 141:1642-1647.

54. Erben RG, Eberle J, Stahr K and Goldberg M. Androgen deficiency induces high turnover osteopenia in aged male rats: a sequential histomorphometric study. J Bone Miner Res 2000; 15:1085-1098.

55. Yao W, Jee WS, Chen J, Tam CS, Setterberg RB and Frost HM. Erect bipedal stance exercise partially prevents orchidectomy-induced bone loss in the lumbar vertebrae of rats. Bone 2000; 27:667-675.

56. Yao W, Jee WS, Chen J, Liu H, Tam CS, Cui L, Zhou H, Setterberg RB and Frost HM. Making rats rise to erect bipedal stance for feeding partially prevented orchidectomy-induced bone loss and added bone to intact rats. J Bone Miner Res 2000; 15:1158-1168.

57. Parfitt AM. Age-related structural changes in trabecular and cortical bone: cellular mechanisms and biomechanical consequences. Calcif Tissue Int 1984; 36 Suppl 1:S123-S128.

58. Danielsen CC, Mosekilde L and Andreassen TT. Long-term effect of orchidectomy on cortical bone from rat femur: bone mass and mechanical properties. Calcif Tissue Int 1992; 50:169-174.

59. Erben RG. Trabecular and endocortical bone surfaces in the rat: Modeling or remodeling? Anat Rec 1996; 246:39-46.

60. Bellido T, Jilka RL, Boyce BF, Girasole G, Broxmeyer H, Dalrymple SA, Murray R and Manolagas SC. Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. J Clin Invest 1995; 95:2886-2895.

61. Weinstein RS, Jilka RL, Parfitt AM and Manolagas SC. The effects of androgen deficiency on murine bone remodeling and bone mineral density are mediated via cells of the osteoblastic lineage. Endocrinology 1997; 138:4013-4021.

62. Most W, Van der Wee-Pals L, Ederveen A, Papapoulos S and Löwik C. Ovariectomy and orchidectomy induce a transient increase in the osteoclastogenic potential of bone marrow cells in the mouse. Bone 1997; 20:27-30.

63. Broulik PD and St_rka L. Effect of antiandrogens casodex and epitestosterone on bone composition in mice. Bone 1997; 20:473-475.

64. Broulik PD. Tamoxifen prevents bone loss in castrated male mice. Horm Metab Res 2000; 32:181-184.

65. Fukuda S and Iida H. Effects of orchidectomy on bone metabolism in beagle dogs. J Vet Med Sci 2000; 62:69-73.

66. Boyce RW, Franks AF, Jankowsky ML, Orcutt CM, Piacquadio AM, White JM and Bevan JA. Sequential histomorphometric changes in cancellous bone from ovariohysterectomized dogs. J Bone Miner Res 1990; 5:947-953.

67. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT and Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 1999; 20:345-357.

68. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ and Penninger JM. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteo-protegerin ligand. Nature 1999; 402:304-309.

69. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ and Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999; 397:315-323.

70. Masuzawa T, Miyaura C, Onoe Y, Kusano K, Ohta H, Nozawa S and Suda T. Estrogen deficiency stimulates B lymphopoiesis in mouse bone marrow. J Clin Invest 1994; 94:1090-1097.

71. Onoe Y, Miyaura C, Ito M, Ohta H, Nozawa S and Suda T. Comparative effects of estrogen and raloxifene on B lymphopoiesis and bone loss induced by sex steroid deficiency in mice. J Bone Miner Res 2000; 15:541-549.

72. Erben RG, Raith S, Eberle J and Stangassinger M. Ovariectomy augments B lymphopoiesis and generation of monocyte-macrophage precursors in rat bone marrow. Am J Physiol Endocrinol Metab 1998; 274:E476-E483.

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).

74. Stepan JJ, Pospichal J, Presl J and Pacovsky V. Bone loss and biochemical indices of bone remodeling in surgically induced postmenopausal women. Bone 1987; 8:279-284.

75. Wronski TJ, Cintron M and Dann LM. Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. Calcif Tissue Int 1988; 43:179-183.

76. Riggs BL, Khosla S and Melton LJ, III. A unitary model for involutional osteoporosis: Estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 1998; 13:763-773.

77. Vanderschueren D, Boonen S and Bouillon R. Action of androgens versus estrogens in male skeletal homeostasis. Bone 1998; 23:391-394.

78. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB and Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994; 331:1056-1061.

79. Morishima A, Grumbach MM, Simpson ER, Fisher C and Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 1995; 80:3689-3698.

80. Bilezikian JP, Morishima A, Bell J and Grumbach MM. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 1998; 339:599-603.

81. Carani C, Qin K, Simoni M, Faustini Fustini M, Serpente S, Boyd J, Korach KS and Simpson ER. Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 1997; 337:91-95.

82. Korach KS, Couse JF, Curtis SW, Washburn TF, Lindzey J, Kimbro KS, Eddy EM, Migliaccio S, Snedeker SM, Lubahn DB, Schomberg DW and Smith EP. Estrogen receptor gene disruption: molecular characterization and experimental and clinical phenotypes. Recent Prog Horm Res 1996; 51:159-186.

83. Oz OK, Zerwekh JE, Fisher C, Graves K, Nanu L, Millsaps R and Simpson ER. Bone has a sexually dimorphic response to aromatase deficiency. J Bone Miner Res 2000; 15:507-514.

84. Greendale GA, Edelstein S and Barrett-Connor E. Endogenous sex steroids and bone mineral density in older women and men: The Rancho Bernardo study. J Bone Miner Res 1997; 12:1833-1843.

85. Wakley GK, Schutte HDJ, Hannon KS and Turner RT. Androgen treatment prevents loss of cancellous bone in the orchidectomized rat. J Bone Miner Res 1991; 6:325-330.

86. Vanderschueren D, Van Herck E, De Coster R and Bouillon R. Aromatization of androgens is important for skeletal maintenance of aged male rats. Calcif Tissue Int 1996; 59:179-183.

87. Vanderschueren D, Boonen S, Ederveen AG, De Coster R, Van Herck E, Moermans K, Vandenput L, Verstuyf A and Bouillon R. Skeletal effects of estrogen deficiency as induced by an aromatase inhibitor in an aged male rat model. Bone 2000; 27:611-617.

88. Ke HZ, Qi H, Crawford DT, Chidsey-Frink KL, Simmons HA and Thompson DD. Lasofoxifene (CP-336,156), a selective estrogen receptor modulator, prevents bone loss induced by aging and orchidectomy in the adult rat. Endocrinology 2000; 141:1338-1344.

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

estrogen receptor antagonist ICI 182,780 has no effect on bone growth in young male rats. Calcif Tissue Int 2000; 66:461-464.

90. Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R and Khosla S. Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest 2000; 106:1553-1560.

91. Frost HM. Intermediary Organization of the Skeleton. CRC Press, Boca Raton, FL, 1986.