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PERSPECTIVES

Androgens Versus Estrogens: Different Theories About Opposing Actions on Periosteal Bone Expansion

Katrien Venken, Roger Bouillon, and Dirk Vanderschueren

Bone Research Unit, Laboratory for Experimental Medicine and Endocrinology, Katholieke Universiteit Leuven, Leuven, Belgium

Abstract

Sexual dimorphism in bone structure has been considered to result from divergent actions of sex steroid hormones, *i.e.,* androgens being stimulatory and estrogens being inhibitory for periosteal bone expansion. This traditional concept has been challenged by observations in male mice and men unable to produce or respond to estrogens, which demonstrate that both androgens and estrogens are necessary for maximal periosteal expansion. Besides this complex hormonal regulation, mechanical loading is another important determinant of radial bone expansion. However, neither hormonal nor mechanical stimuli alone are able to explain periosteal bone expansion and the interaction between both stimuli may be of particular importance for the acquisition of an optimal bone size. *IBMS BoneKEy*. 2008 April;5(4):130-136.

Introduction

Puberty has a fundamental role in skeletal growth and maturation. The cumulative (or total) bone mass acquired during puberty is more pronounced in males than females and the periosteum appears to be the major site in this process, whether this is due to a higher bone formation rate (per bone surface unit) and/or to a longer period of bone accrual in males (1;2). Periosteal bone formation and the resulting increase in bone size contribute to a great extent to bone strength as the resistance of bone to bending is related exponentially to its diameter (3). Moreover, bone size has been linked to fracture risk (4) and so the sexual dimorphism in bone structure can explain the sex differences in fracture occurrence. Several determinants have been considered to affect periosteal apposition, *i.e.,* sex, race, genetic and environmental factors, hormones and mechanical loading. In this *Perspective* we will focus on the different theories or mechanisms that may underlie the more pronounced periosteal bone expansion in men (Table 1). In particular, hormonal (mainly sex steroids) and mechanical stimuli of bone will be considered as well as the interaction between both stimuli.

Hormonal Theory of Sex Steroid Action on Periosteal Bone Expansion

In light of the sex differences in bone size and consequently bone strength, sex steroids have been proposed as key regulators in bone mineral accumulation during puberty. In this respect, a study in growing male and female rats, published in 1990 by Turner *et al.* (5), showed for the first time that periosteal bone formation was decreased in orchidectomized male rats, but was increased in ovariectomized female rats. This study led to the traditional concept that androgens in males are stimulatory and estrogens in females are inhibitory for periosteal bone expansion. In addition, the orchidectomy-induced bone loss in males could be reversed by androgens (6), and thus androgens were supposed to be exclusively responsible for the typical male bone phenotype. Further evidence for direct androgen receptor (AR)-mediated androgen action was provided by the decreased

Theory	Original observations	Recent observations
Hormonal theory	Periosteal bone expansion is regulated by the stimulatory action of androgens and the inhibitory action of estrogens as shown by studies in castrated male and female rats (R. Turner)	Estrogens are also required in the establishment of the typical male bone phenotype as shown by studies in mice and men unable to produce or respond to estrogens (D. Vanderschueren, R. Bouillon, C. Ohlsson). Estrogen action may be modulated by interactions with the GH/IGF-I axis (D. Vanderschueren, C. Ohlsson).
Mechanostat theory	Periosteal bone expansion is regulated by the mechanostat that senses mechanical strain (setpoint) and responds to it by adapting bone geometry (H. Frost)	
Interaction theory	Sex steroids, in particular estrogen, modulate the response of periosteal bone to loading as shown by exercise studies in humans and rodents.	According to some, estrogens lower the mechanical setpoint and thus increase the responsiveness of the periosteum to load (H. Frost, L. Lanyon), whereas according to others estrogens inhibit the responsiveness of the periosteum to load (L. Saxon, C. Turner, T. Jarvinen)
Receptor theory	ER is involved in the adaptive response of bone to mechanical loading as shown *in vitro* and *in vivo*.	ERα is involved in the stimulatory action of mechanical loading on female bone (L. Lanyon), whereas ERβ inhibits the response to mechanical loading (L. Saxon, C. Turner)

Table 1. Overview of the different theories on sex steroids and periosteal bone expansion.

cortical bone size in rats and mice with either a natural mutation or a genetic manipulation of the gene encoding the AR (6). Also, patients affected by the complete androgen insensitivity syndrome (cAIS) have low bone mass and thus provide arguments for a direct role of androgens on male bone growth (7).

However, the role of sex steroids in periosteal bone growth, and in particular in periosteal bone growth in males, appeared to be much more complex than originally anticipated. The observation that men unable to produce or respond to estrogens were osteopenic suggested that estrogens may be critically involved in male skeletal maturation as well (8-10). Likewise, a reduced bone size in growing male mice and rats treated with an aromatase inhibitor and in estrogen receptor α (ERα)-disrupted male mice further supported a role for estrogens in male periosteal growth (6). Along the same line, in an adolescent boy with aromatase deficiency and slightly supranormal endogenous testosterone (T) production, pubertal periosteal bone growth started only when low dose estrogen replacement therapy was initiated (10). In addition, administration of T plus an aromatase inhibitor diminished radial bone expansion in growing male mice, indicating

that androgen action on periosteal growth is partly dependent on aromatization into estrogens (11). Recently, a combined treatment of T plus 17β-estradiol (E_2) in an aromatase-deficient patient with mild hypogonadism was reported to result in an optimal gain in BMD and cortical thickness than either treatment alone (12).

Effects of estrogens on bone size, at least in rodents, are very often associated with changes in systemic insulin-like growth factor-I (IGF-I) (13;14), which suggests that estrogen-related changes in systemic IGF-I may mediate estrogen action on periosteal bone. In this respect, not only sex steroids but also other regulatory hormones such as growth hormone (GH) and IGF-I are essential for periosteal bone expansion as demonstrated by the fact that GH receptor (GHR)- and IGF-I gene-disrupted mice have extremely short and thin bones (15). IGF-I levels in aromatase deficient men have not been reported thus far and so it is presently unclear whether the estrogen stimulatory effects on bone in these men may be explained by changes in GH/IGF-I.

The study of sex steroid action on periosteal bone provides evidence for a major contribution of direct AR-mediated androgen action in the regulation of male periosteal bone expansion. However, observations that optimal periosteal bone expansion is only observed in the presence of both AR and ERα activation indicate that E_2 is necessary as a permissive factor for the anabolic action of androgens on male bone. The remaining question, however, is how AR and ER activation generate this periosteal bone expansion. Are the effects direct or indirect and what are the molecular mechanisms of action?

Mechanostat Theory of Sex Steroid Action on Periosteal Bone Expansion

The hormonal theory is challenged by the mechanostat theory. According to the mechanostat theory originally elaborated by Harold Frost (16), skeletal growth is fully regulated by a dominant feedback system (the 'mechanostat') that senses mechanical strain generated by muscle contraction and skeletal loading and thereby adapts bone modeling and remodeling. In healthy bone, the feedback system adjusts bone mass to keep peak mechanical strains in bone within an acceptable range. Thus, when mechanical strain exceeds a certain limit (set point), osteocytes might sense this and send out signals that lead to adaptations in bone mass and architecture (17). Body weight and in particular muscle mass continuously generate mechanical strain in bone and thus greater body weight and more muscle mass is supposed to translate into more bone. In this respect, men not only have higher body weight and muscle mass but also have a higher growth rate than women during puberty (18); as a result, mechanical load is supposed to be greater in males. Sex differences in bone size may therefore be predictable and largely explained by the mechanostat theory. This theory also implies that sex steroids only indirectly affect bone size by stimulation of muscle mass and subsequent mechanical loading of the skeleton or by changing the responsiveness of bone to mechanical loading. Detailed analysis of the bone phenotype of genetically modified mice with low or high muscle mass could help to clarify this hypothesis.

Interaction Theory of Sex Steroids and Mechanical Loading in Their Effects on Periosteal Bone Expansion

If there would be absolutely no interaction of sex with the mechanostat, bone mineral content (BMC) relative to body weight should be the same in males and females. However, studies in rodents and humans showed that this is not the case and so this challenged Frost's original mechanostat theory. Indeed, female rats have more bone than male rats relative to body weight over a wide range of body weights (19;20). In a study of Argentine boys and girls, a similar evolution is observed during puberty: bone mass increases along with lean body mass during growth, but the increase in bone mass relative to muscle mass is significantly greater in girls than boys during puberty (21; 22). Frost subsequently assumed that estrogens lower the mechanical setpoint in females and so less mechanical strain would be required to initiate bone formation in females (23). As a result, estrogens would

stimulate more bone than is needed mechanically. The extra bone, however, is accumulated at the inner bone surfaces, namely endocortical and trabecular bone, and does not confer additional advantage towards bending strength (24). This led others to conclude that the mechanostat theory fails to explain the interaction between loading (by means of exercise) and estrogen on periosteal bone formation (24; 25). According to the group of Saxon and Turner, the responsiveness of periosteal bone to load is rather decreased by estrogens and not increased as was proposed by Frost. Support for this concept comes from exercise studies that show a more beneficial effect on periosteal bone in prepubertal girls (low estrogen) than in postpubertal girls (high estrogen) (26;27). Also, Järvinen *et al.* showed that the skeletal response of estrogen-depleted female rats to exercise is higher compared with their estrogen-repleted counterparts (28). Moreover, the periosteal bone of intact female rats does not respond to exercise in contrast to intact male rats that show a significant periosteal response to load (28). Although the theories of Frost versus Saxon and Turner on the role of estrogens in periosteal bone expansion seem contradictory, one may speculate that they reflect different exposure to endogenous estrogens. Low levels of estrogen may increase the mechanical sensitivity of the periosteum and/or affect systemic IGF-I levels. Alternatively, higher concentrations of estrogen may inhibit periosteal bone expansion and its interaction with mechanical loading. In order to further clarify the interaction between estrogens and mechanical loading, one has to consider not only a potential role of different exposure to estrogen concentrations, but also the role of the ER subtypes, ERα and ERβ, and their differential actions on the male and female skeleton during loading.

Receptor Theory of the Interaction Between Sex Steroids and Mechanical Loading in Their Effects on Periosteal Bone Expansion

It becomes increasingly clear that the skeletal response to mechanical loading is sex-specific and that neither the hormonal theory nor the mechanostat theory alone is able to explain the regulation of periosteal bone expansion. Thus, somehow the mechanostat theory (mechanical loading) interferes with the hormonal theory (sex steroids and/or their receptors) in the response of periosteal bone to load. Indeed, recent *in vitro* and *in vivo* observations support the notion that sex steroids and their receptors and mechanical strain share common elements in their respective signaling mechanisms. These observations particularly focused on the role of the ER in the mediation of mechanical loading responses. The group of Lanyon expanded on Frost's mechanostat theory and questioned whether the ER influenced bone cells' adaptive response to mechanical strain. Strain stimulates the *in vitro* proliferation of osteoblast-like cells, derived from female and male bone, by a mechanism involving the ER as this response could be blocked by the ER modulators ICI 182,780 and tamoxifen but not by the AR antagonist hydroxyflutamide (29-31). In addition, periosteal bone formation *in vivo* is significantly reduced in female ERα KO mice, indicating that in the absence of ERα the adaptive response of bone to loading is less effective (32). In the context of osteoporosis, this implies that low estrogen levels in association with the down-regulation of the ERα result in a reduced ability of bone cells to adapt to loading and to maintain structural strength (33).

The group of Saxon and Turner proposed further refinement of the interactions between loading and estrogen and hypothesized that ERα stimulated bone formation on the endocortical surface via ERα but inhibited bone formation on the periosteum via ERβ. Disruption of ERβ indeed increased periosteal bone formation following loading in female mice but not male mice (34), indicating that estrogen signaling through ERβ suppresses the loading response of the female skeleton. The increased bone size in female, but not male, ERβ KO mice (13;35) further supports the notion that ERβ may act as an anti-mechanostat (25). Moreover, enhanced proliferation of ERβ null osteoblasts following loading has also been demonstrated *in vitro* (36). The idea of ERβ

acting as an anti-mechanostat would also fit with the hypothesis of a concentration-dependent effect of estrogens; *i.e.,* high estrogen levels in females activate ERβ, whereas low estrogen levels in males do not. This idea would also fit with the presence of a cortical phenotype in female, but not male, ERβ KO mice. Whether the cortical phenotype in response to load in these ER-disrupted mice may be affected by changes in systemic IGF-I is unknown as IGF-I levels were reported to be increased in females by some (14) but not all reports (34). Whether ERα plays a similar role in the loading response in males as in females is not known. Also, a potential role of androgens and the AR, GH/IGF-I and receptors, muscle and fat mass and fat hormones has not yet been fully explored.

Summary

The regulation of periosteal bone expansion during puberty is more complex than originally anticipated. Significant progress was made by intriguing observations concerning bone size in men and mice with an inactivating mutation of the ERα gene or aromatase gene and by using loading techniques in transgenic mice. These observations showed that AR and ER activation are both needed for male periosteal bone formation. This finding can be reconciled with the traditional concept by Turner *et al*. when a dose-dependent effect of estrogen is being considered, *i.e.,* low dose may be stimulatory (prepubertal, males, postmenopausal), whereas high dose may be inhibitory, potentially via activation of specific ER subtypes. Moreover, periosteal bone apposition is not dependent on either different hormonal or mechanical stimuli alone but rather on their mutual interactions, *i.e.,* mechanical loading is clearly stimulatory for bone but appears sex steroid hormone-dependent. As the difference in fracture rates between men and women to a great extent depend on bone geometry and bone strength, better insight into the regulation of periosteal bone formation will provide new therapeutic potential.

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References

1. Seeman E. From density to structure: growing up and growing old on the surfaces of bone. *J Bone Miner Res*. 1997 Apr;12(4):509-21.

2. Seeman E. Clinical review 137: Sexual dimorphism in skeletal size, density, and strength. *J Clin Endocrinol Metab*. 2001 Oct;86(10):4576-84.

3. Orwoll ES. Toward an expanded understanding of the role of the periosteum in skeletal health. *J Bone Miner Res*. 2003 Jun;18(6):949-54.

4. Seeman E, Duan Y, Fong C, Edmonds J. Fracture site-specific deficits in bone size and volumetric density in men with spine or hip fractures. *J Bone Miner Res*. 2001 Jan;16(1):120-7.

5. Turner RT, Wakley GK, Hannon KS. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. *J Orthop Res*. 1990 Jul;8(4):612-7.

6. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. *Endocr Rev*. 2004 Jun;25(3):389-425.

7. Marcus R, Leary D, Schneider DL, Shane E, Favus M, Quigley CA. The contribution of testosterone to skeletal development and maintenance: lessons from the androgen insensitivity syndrome. *J Clin Endocrinol Metab*. 2000 Mar;85(3):1032-7.

8. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*. 1994 Oct 20;331(16):1056-61.

9. Gennari L, Nuti R, Bilezikian JP. Aromatase activity and bone

homeostasis in men. *J Clin Endocrinol Metab*. 2004 Dec;89(12):5898-907.

10. Bouillon R, Bex M, Vanderschueren D, Boonen S. Estrogens are essential for male pubertal periosteal bone expansion. *J Clin Endocrinol Metab*. 2004 Dec;89(12):6025-9.

11. Venken K, De Gendt K, Boonen S, Ophoff J, Bouillon R, Swinnen JV, Verhoeven G, Vanderschueren D. Relative impact of androgen and estrogen receptor activation in the effects of androgens on trabecular and cortical bone in growing male mice: a study in the androgen receptor knock-out mouse model. *J Bone Miner Res*. 2006;21(4):576-585.

12. Rochira V, Zirilli L, Madeo B, Aranda C, Caffagni G, Fabre B, Montangero VE, Roldan EJ, Maffei L, Carani C. Skeletal effects of long-term estrogen and testosterone replacement treatment in a man with congenital aromatase deficiency: evidences of a priming effect of estrogen for sex steroids action on bone. *Bone*. 2007 Jun;40(6):1662-8.

13. Vidal O, Lindberg MK, Hollberg K, Baylink DJ, Andersson G, Lubahn DB, Mohan S, Gustafsson JA, Ohlsson C. Estrogen receptor specificity in the regulation of skeletal growth and maturation in male mice. *Proc Natl Acad Sci U S A*. 2000 May 9;97(10):5474-9.

14. Lindberg MK, Alatalo SL, Halleen JM, Mohan S, Gustafsson JA, Ohlsson C. Estrogen receptor specificity in the regulation of the skeleton in female mice. *J Endocrinol*. 2001 Nov;171(2):229-36.

15. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol*. 2001 Jan 1;229(1):141-62.

16. Frost HM. Bone "mass" and the "mechanostat": a proposal. *Anat Rec*. 1987 Sep;219(1):1-9.

17. Frost HM. A determinant of bone architecture. The minimum effective strain. *Clin Orthop Relat Res*. 1983 May;(175):286-92.

18. Bailey DA, Martin AD, McKay HA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: a longitudinal analysis. *J Bone Miner Res*. 2000 Nov;15(11):2245-50.

19. Saville PD. Changes in skeletal mass and fragility with castration in the rat; a model of osteoporosis. *J Am Geriatr Soc*. 1969 Feb;17(2):155-66.

20. Wang L, McMahan CA, Banu J, Okafor MC, Kalu DN. Rodent model for investigating the effects of estrogen on bone and muscle relationship during growth. *Calcif Tissue Int*. 2003 Feb;72(2):151-5.

21. Zanchetta JR, Plotkin H, Alvarez Filgueira ML. Bone mass in children: normative values for the 2-20-year-old population. *Bone*. 1995 Apr;16(4 Suppl):393S-399S.

22. Schiessl H, Frost HM, Jee WS. Estrogen and bone-muscle strength and mass relationships. *Bone*. 1998 Jan;22(1):1-6.

23. Frost HM. On the estrogen-bone relationship and postmenopausal bone loss: A new model. *J Bone Miner Res*. 1999 Sep;14(9):1473-7.

24. Jarvinen TL, Kannus P, Sievanen H. Estrogen and bone--a reproductive and locomotive perspective *J Bone Miner Res*. 2003 Nov;18(11):1921-31.

25. Saxon LK, Turner CH. Estrogen receptor beta: the antimechanostat? *Bone*. 2005 Feb;36(2):185-92.

26. Bass SL, Saxon L, Daly RM, Turner CH, Robling AG, Seeman E, Stuckey S. The effect of mechanical loading on the size and shape of bone in pre-, peri-, and postpubertal girls: a study in tennis players. *J Bone Miner Res*. 2002 Dec;17 (12):2274-80.

27. Daly RM, Saxon L, Turner CH, Robling AG, Bass SL. The relationship between muscle size and bone geometry during growth and in response to exercise. *Bone*. 2004 Feb;34(2):281-7.

28. Jarvinen TL, Kannus P, Pajamaki I, Vuohelainen T, Tuukkanen J, Jarvinen M, Sievanen H. Estrogen deposits extra mineral into bones of female rats in puberty, but simultaneously seems to suppress the responsiveness of female skeleton to mechanical loading. *Bone*. 2003 Jun;32(6):642-51.

29. Damien E, Price JS, Lanyon LE. The estrogen receptor's involvement in osteoblasts' adaptive response to mechanical strain. *J Bone Miner Res*. 1998 Aug;13(8):1275-82.

30. Damien E, Price JS, Lanyon LE. Mechanical strain stimulates osteoblast proliferation through the estrogen receptor in males as well as females. *J Bone Miner Res*. 2000 Nov;15(11):2169-77.

31. Cheng MZ, Rawlinson SC, Pitsillides AA, Zaman G, Mohan S, Baylink DJ, Lanyon LE. Human osteoblasts' proliferative responses to strain and 17beta-estradiol are mediated by the estrogen receptor and the receptor for insulin-like growth factor I. *J Bone Miner Res*. 2002 Apr;17(4):593-602.

32. Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L. Endocrinology: bone adaptation requires oestrogen receptor-alpha. *Nature*. 2003 Jul 24; 424(6947):389.

33. Lanyon L, Armstrong V, Ong D, Zaman G, Price J. Is estrogen receptor alpha key to controlling bones' resistance to fracture? *J Endocrinol*. 2004 Aug;182(2):183-91.

34. Saxon LK, Robling AG, Castillo AB, Mohan S, Turner CH. The skeletal responsiveness to mechanical loading is enhanced in mice with a null mutation in estrogen receptor-beta. *Am J Physiol Endocrinol Metab*. 2007 Aug;293(2):E484-91.

35. Windahl SH, Vidal O, Andersson G, Gustafsson JA, Ohlsson C. Increased cortical bone mineral content but unchanged trabecular bone mineral density in female ERbeta(-/-) mice. *J Clin Invest*. 1999 Oct;104(7):895-901.

36. Jessop HL, Suswillo RF, Rawlinson SC, Zaman G, Lee K, Das-Gupta V, Pitsillides AA, Lanyon LE. Osteoblast-like cells from estrogen receptor alpha knockout mice have deficient responses to mechanical strain. *J Bone Miner Res*. 2004 Jun;19(6):938-46.