Oestrogen action on bone cells

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Abstract

Sex steroids have an important impact on bone physiology. Oestrogen (E) appears to be the most important sex steroid in preventing osteoporosis in women. Despite the overwhelming evidence that oestrogens modulate bone growth and turnover in vivo, oestrogen receptors (ER) were detected only recently. Two forms of ER have been discovered so far, ERα and ERβ. Both have been detected in osteoblasts and osteoclasts as well. A number of growth factors and cytokines appear to modulate bone resorption in vitro and in vivo. Among others, interleukin-1 and -6 and tumor necrosis factor alpha and beta were found to be extremely potent stimulators of bone resorption. Binding of different cytokines to their receptors in osteoblasts result in the release of soluble factors that act directly on osteoclasts to modulate their recruitment or activity. Thus, E, apart from the direct regulation of osteoclasts, which it achieves through its receptors, can inhibit the release of osteoclast stimulatory factors or enhance the release of osteoclast inhibitory factors. In general, E is an inhibitor of bone resorption that decreases both osteoclast numbers and activity. Recently, it has also been shown that it promotes apoptosis. Moreover, it also has anabolic effects on osteoblasts. However, E action on osteoclasts is superior in comparison with that on osteoblasts. Recent data have shown that transforming growth factor beta (TGFβ) mediates the actions of E in bone. Following the example of raloxifene it may be proved that the role of TGFβ in the actions of E in bone is central and has not only academic interest. More data are needed to elucidate this issue. Finally, recent data suggest the importance of E for bone maturation and development of peak bone mass in men. It seems likely that both E and androgens are required for the growth and maintenance of the adult male skeleton.

Keywords: Oestrogen, Osteoclasts, Osteoblasts, Oestrogen Receptors, Cytokines, Growth Factors

Bone differs from reproductive tissues in that many aspects of skeletal growth, turnover, and function occur in the absence of gonadal hormones. Nevertheless, sex steroids have an important impact on bone physiology: they participate in the sexual dimorphism of the skeleton, play a role in maintenance of mineral homeostasis during reproduction and are essential for maintaining bone balance in adults. Insufficient levels of certain sex steroids predispose the human skeleton to bone loss and to osteoporotic fractures. Oestrogen appears to be the most important sex steroid in preventing osteoporosis in women1.

Despite the overwhelming evidence that oestrogens (E) modulate bone growth and turnover in vivo, oestrogen receptors (ER) were not initially detected in cultured bone cells2 and E was not shown to affect bone cells in organ culture3. The early negative results led to the long-held belief that the effects of oestrogens on bone in vivo were entirely indirect and were mediated by calcitrophic hormones such as calcitonin, parathyroid hormone, and 1,25 dihydroxyvitamin D3 or by some unidentified humoral factors.

In 1988 Komm et al.4 and Eriksen et al.5 simultaneously reported the detection of high-affinity, competent estradiol binding, ERα mRNA, and the induction of E responsive genes in cultured rat and human osteoblast-like cells, the bone-forming cell. Oursler et al.6 have since demonstrated ERα and oestrogenic activity in cultured avian osteoclasts, the bone-resorbing cell type, including oestrogen induction of the genes for c-fos and c-jun. Using in situ RT-PCR analysis, Hoyland et al.7 demonstrated the presence of ERα mRNA in both osteoblasts and osteoclasts in bone grafts from human females. Immunocytochemical methods have also been used to demonstrate the presence of ERα in multiple bone cell lines8. Therefore, there is adequate experimental evidence to support the presence of a direct ERα mediated oestrogen-signaling pathway in bone.

Until recently, only one “classical” form of the ER was
known to exist. In 1996, Mosselman et al. discovered a new form of the receptor named ERβ. The latter has been shown to be expressed in the human thymus, spleen, ovary and testis and recently its expression in osteoblasts from rat bone has been described. Both ERα and ERβ have also been detected in human epiphyseal chondrocytes.

Several recent studies have reported the detection of ERβ in bone cells. Onoe et al. demonstrated the presence of both ERα and ERβ mRNA in immortalized as well as primary osteoblast cell cultures from the rat. Similar to reports of ERα, both Onoe et al. and Arts et al. reported significant increases in ERβ mRNA levels during in vitro dexamethasone – induced differentiation of osteoblasts derived from the rat and human, respectively. Therefore, the generation of mice lacking ERα or ERβ will once again prove invaluable in delineating the roles of the two receptors in bone physiology.

The majority of animal studies concerning the role of E in bone morphology and metabolism have been carried out in the rat. Ovariectomy in the rodent results in increased bone turnover similar to that seen in postmenopausal women; however, the mechanisms of action may differ between the species.

Oestrogens are thought to passively diffuse into all cells. In general, steroids are preferentially retained in target cells through the formation of complexes with specific intracellular receptor proteins that are both steroid- and tissue- specific. It is generally accepted now that both osteoclasts and osteoblasts do express ERα. Evidence for receptors in bone cells was reported in mice and humans using binding assays, immunohistochemistry and radioautography. The mRNA for ER was reported in rat bone, providing additional evidence for receptors. The latter are typically present at a concentration of 10,000 – 100,000 molecules per target cell in reproductive tissues in mammals, but much lower levels, i.e. 200-1000 molecules per cell, have been reported in other tissues of mammals including the skeleton and in tissues of birds and lower vertebrates. The low concentration of ER in osteoblasts relative to reproductive tissues is consistent with the hormone having a more limited number of direct actions on the skeleton.

**Oestrogens, cytokine production and growth factors**

The major effect of oestrogen on bone remodeling in adult humans may be to decrease bone resorption rather than to modulate bone formation. Despite the presence of ER in osteoclasts, it is possible that the binding of E to receptors in osteoblasts regulates osteoclast function indirectly, which would add a second layer of regulation to the direct regulation of osteoclasts by E and provide an opportunity for cell-cell interactions. New studies suggest that many factors regulating bone resorption do so indirectly through an osteoblast – mediated mechanism.

Binding of different cytokines to their receptors in osteoblasts is hypothesized to result in the release of soluble factors that act directly on osteoclasts to modulate their recruitment or activity. Thus, E could inhibit the release of osteoclast stimulatory factors or, alternatively, could enhance the release of osteoclast inhibitory factors (Fig. 1). Based on their action in experimental animals, in organ cultures, or in cell cultures, a number of cytokines and growth factors appear to modulate bone resorption and could play roles in the coupling of bone formation to bone resorption. Cytokines that have been reported to increase osteoclastic activity include colony stimulating factor (GM-CSF), macrophage-colong stimulating factor (M-CSF), tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1) and interleukin-6 (IL-6).

In the mid-to-late 1980s the proinflammatory cytokines IL-1 and TNFs alpha and beta were found to be extremely potent stimulators of bone resorption in vitro and in vivo. Spontaneous release of these cytokines along with IL-6 by peripheral blood mononuclear cells was found to be increased after menopause or ovariectomy. The following key data summarize the role of these cytokines in the pathogenesis of oestrogen-deficiency – related bone loss. First, blockade of IL-6 by infusion of a neutralizing antibody prevents postovariectomy bone loss in mice. Second, IL-6 knock out mice have been reported not to lose bone after ovariectomy. Third, simultaneous treatment of animals with IL-1 receptor antagonist and TNF binding protein prevents postovariectomy bone loss. Finally, the cytokines are enhancers of osteoclast function, IL-6 probably acting mainly at the levels of osteoclast generation and IL-1 and TNFs acting more (but not exclusively) at the level of the mature osteoclast, possibly via autocrine/paracrine signals and possibly by inhibition of apoptosis.

In several classical oestrogen-responsive tissues, such as the breast and uterus, insulin-like growth factor (IGF) -1 appears to mediate the E induction of cell proliferation and differentiation. A number of studies suggest that E has similar...
effects on the rodent osteoblastic system, e.g. in rat calvarial osteoblasts, E increases IGF-1 gene transcription and oestrogen-induced increases in osteoblast proliferation are blocked by neutralizing anti-IGF-1 antibodies. In contrast, E treatment did not affect gene transcription or protein production of IGF-2 in rat osteoblasts. Thus, the data obtained in rodent models suggest that some of the E effects on bone cells are mediated through the IGF-1. It is not known, however, whether a similar action of E on IGFs occurs in human osteoblasts. This is important to clarify because differences between rodents and humans have been reported in the IGF system in bone. Also, in contrast to rodents, E treatment did not affect gene transcription or protein production of IGF-2 in human osteoblasts. This is important to clarify because the data obtained in rodent models suggest that some of the E effects on bone cells are mediated through the IGF-1. It is not known, however, whether a similar action of E on IGFs occurs in human osteoblasts. This is important to clarify because differences between rodents and humans have been reported in the IGF system in bone. Also, in contrast to the findings in rodent osteoblasts, IGF-2, rather than IGF-1, is the main IGF produced by human osteoblasts. Whereas IGF-2 seems to be constitutively produced by rodent osteoblasts and is not regulated by oestrogenic hormones, little is known about the regulation of IGF-2 in human osteoblasts.

In addition to growth factors, bone cells produce proteins that modulate the activity of growth factors either by binding to them and thereby preventing interaction with their receptors, by competing with the same receptors, or by promoting the activity of a particular factor. For example, osteoblasts produce several IGF-binding proteins (IGFBPs). It has been described that IGFBP-4 binds to IGF and blocks its action, whereas IGFBP-5 promotes the stimulatory effects of IGF on osteoblasts.

**Mechanism of oestrogen action on bone**

**Osteoblastogenesis and osteoclastogenesis**

Both osteoblasts and osteoclasts are derived from precursors originating in the bone marrow. The precursors of osteoblasts are multipotent mesenchymal stem cells, which also give rise to bone marrow stromal cells, chondrocytes, muscle cells, and adipocytes, whereas the precursors of osteoclasts are hematopoietic cells of the monocyte/macrophage lineage.

It has been found that even though millions of small packets of bone are constantly remodeled, bone mass is preserved thanks to a remarkably tight balance between the amount of bone resorbed and formed during each cycle of remodeling. In any established basic multicellular unit (BMU), bone resorption and formation are happening at the same time; new osteoblasts assemble only at sites where osteoclasts have recently completed resorption, a phenomenon referred to as coupling, and formation begins to occur while resorption advances. The whole process ends with the formation of a new bone, either a cylindrical osteon or Haversian system, or a plate-like hemiosteon, that has replaced the older bone which was removed.

The distinction between the cross-sectional and longitudinal events during BMU progression could be explained on the basis of two models of osteoblast recruitment, a serial and a parallel (Fig. 2).

According to the serial model, factors released from resorbed bone stimulate osteoblast precursor, cell proliferation and differentiation. According to the parallel model, osteoblast and osteoclast precursor, cell proliferation and differentiation occur concurrently in response to whatever signal conveys the need for initiation of new BMUs, and whatever hormone prolongs their progression. At the end with either model, the new osteoblast must be directed to the right location.

The molecular mechanism of the dependency of osteoclastogenesis on cells of the mesenchymal lineage has been elucidated in recent years with the discovery of three proteins involved in the TNF-signaling pathway. Two of these proteins are membrane-bound cytokine-like molecules: the receptor activator of nuclear factor-κB (NF-κB) (RANK) and the RANK-ligand. RANK is expressed in hematopoietic osteoclast progenitors, while RANK-ligand is expressed in committed preosteoblastic cells and T lymphocytes. RANK-ligand binds to RANK with high affinity. This interaction is essential and sufficient for osteoclastogenesis. Osteoprotegerin (OPG), the third of the three proteins, unlike the other two, is a secreted disulfide-linked dimeric glycoprotein. Unlike other members of the TNF receptor family, OPG does not possess a transmembrane domain. OPG has very potent inhibitory effects on osteoclastogenesis and bone resorption in vitro and in vivo.

OPG transgenic mice develop osteopetrosis, whereas OPG knock out mice exhibit severe osteoporosis, a finding which is in accord with the important role of OPG in the regulation of osteoclast formation. The antosteoclastogenic property of OPG is due to its ability to act as a decoy by binding to RANK-ligand and blocking the RANK-ligand/RANK interaction. In addition to skeletal metabolism, the RANK/RANK-ligand/OPG circuit may regulate several other biological systems. Indeed, OPG, is produced by many tissues other than bone, including skin, liver, stomach, intestine, lung,
heart, kidney, and placenta as well as hematopoietic and immune organs. Mice deficient in RANK-ligand completely lacked lymph nodes as well as osteoclasts, a finding which supports the latter. Moreover, OPG is also a receptor for the cytotoxic ligand TRAIL (TNF-related apoptosis-inducing ligand) to which it binds with high affinity and inhibits TRAIL-mediated apoptosis in lymphocytes and also regulates antigen presentation and T cell activation.

Evidence from in vivo studies. Apoptosis

Oestrogen is an inhibitor of bone resorption that decreases both osteoclast numbers and activity. The osteoclast is a fairly short-lived cell that appears to die by apoptosis during the reversal phase of bone remodeling. Thus, the reduction of osteoclast numbers seen in vivo could be due simply to reduced osteoclast generation. There is evidence to suggest that E does reduce formation of osteoclasts from their precursors. Moreover, Liu and Howard demonstrated that E treatment caused osteoclasts to lose attachment to the bone surface and to undergo degenerative anatomical change, indicating that E treatment has effects on mature osteoclasts. More recently it has been shown that these morphological changes reflect induction of osteoclast apoptosis by E33.

The average lifespan of human osteoclasts is about 2 weeks, while the average lifespan of osteoblasts is 3 months. After osteoclasts have eroded to a particular distance, either from the central axis in cortical bone or to a particular depth from the surface in cancellous bone, they die and are quickly removed by phagocytes. The majority (65%) of the osteoblasts that originally assembled at the remodeling site also die. The remaining are converted to lining cells that cover quiescent bone surfaces or are entombed within the mineralized matrix as osteocytes. Both osteoclasts and osteoblasts die by apoptosis or programmed cell death, a process common to several regenerating tissues.

Eventually, the cell breaks apart to form so-called apoptotic bodies. Osteoblast apoptosis explains the fact that 50-70% of the osteoblasts initially present at the remodeling site of human bone cannot be accounted for after enumeration of lining cells and osteocytes. Moreover, the frequency of osteoblast apoptosis in vivo is such that changes in its timing and extent could have a significant impact on the number of osteoblasts present at the site of bone formation. Osteocytes are long-lived but not immortal cells; some die by apoptosis. Osteocyte apoptosis could be of importance to the origination and/or progression of the BMU.

Evidence from in vitro studies

Various effects of E on cells of the osteoclast lineage have been described in different species (calvariae, rodents, mice) which can be explained by recent evidence that E promotes apoptosis of both mature osteoclasts (thus reducing bone resorption) and their precursors (thus reducing osteoclast formation). As mentioned above, osteoblasts and osteoclasts do express ER. The latter have anabolic effects on osteoblasts in humans. There is a species difference, which makes it rather difficult to extrapolate data from animals to humans, at least at the level of bone formation.

The mechanism of action of the ER

The ER is a member of the steroid receptor (SR) superfamily of ligand-dependent transcription factors. Within this

Figure 3. The mechanism of action of the oestrogen receptor.
superfamily, the sex steroid receptors are the most conserved both in primary sequence and mechanism of action.

Consequently, a composite model of the mechanism of action of this class of proteins has emerged. Specifically, ER agonists mediate their effect on gene transcription via specific intracellular receptor proteins located within target cell nuclei. Upon interaction with each cognate ligand the latent receptor becomes “activated”. This event permits the displacement of heat-shock proteins (HSPs), facilitates receptor dimerization and promotes the interaction of the receptor with specific steroid response elements (SRE) located within the regulatory regions of target promoters. At this location, depending on the cellular and promoter context, the ligand – activated receptor can interact with the general transcription apparatus (GTA) directly or indirectly through adaptor proteins. Ultimately, these interactions stabilize the transcription preinitiation complex and enhance RNA polymerase activity. Although several rounds of phosphorylation of the receptor have been shown to occur, its ER signaling has yet to be determined71 (Fig. 3).

In general, E are “conditional” inhibitors of bone resorption, in contrast to other inhibitors of bone resorption, such as bisphosphonates and calcitonin, which have far more predictable and universal effects. Thus, E are potent inhibitors of bone resorption in the setting of oestrogen deficiency but are far less effective in the oestrogen – replete organism. In vitro, their action appears to be influenced by species, age and probably by the presence of other cell types. Taking these factors together, it would appear likely that E require the presence of co-factors, second messengers, or both and that the potency of their action depends on other stimuli to which the target cell is subjected62. The role of oestrogen on bone tissue in men

Recent data suggest the importance of E for bone maturation and development of peak bone mass in men.

Oestradiol is detectable in the serum of healthy men at levels comparable to those in postmenopausal women. This is a result of peripheral conversion of testosterone by the enzyme aromatase, a member of the microsomal cytochrome P450 group (Fig. 4)72.

Because these levels are rather low, they were not regarded as physiologically important until epidemiological research into heart disease risk suggested a protective effect of endogenous E in men73.

A role for E in skeletal maintenance in the human male is supported by evidence at the cellular level by animal experiments and clinical findings. Osteopenia was reported in an aromatase-deficient young man whose oestradiol levels were below 26 pmol/l, but whose testosterone levels were high74. It was also reported in another case with non-functioning ER75. Serum testosterone and androgen receptors were normal or elevated. In both cases, bone mineral density (BMD) values were similar to those seen in the converse syndrome of genetic males with androgen insensitivity (androgen receptor defect but normal testosterone and oestradiol levels)76. Perhaps the most convincing evidence is from another case report, in which a 28-year-old man with an inactivating mutation of his aromatase gene presented with infertility and was found to have a eunuchoid habitus with nonclosure of the epiphyses, below-average BMD, and a bone age of 15 years. Treatment with regular intramuscular testosterone produced no benefit, but treatment with transdermal oestradiol, 50 Ìg twice weekly, resulted in skeletal maturation, a rapid increase in lumbar spine BMD, and epiphyseal closure77.

Moreover, in a cross-sectional study in 37 healthy older men with no history of bone disease, Anderson et al.78 found that BMD at the lumbar spine and hip correlated more closely with serum oestradiol (r = 0.383, p<0.03) than with testosterone (r = 0.245, p>0.15). Also, in a prospective study of 93 healthy men aged over 65, serum oestradiol levels were positively associated with initial BMD values at all sites79 and were associated with significantly lower rates of bone loss at the radius and hip on twice-yearly BMD measurements over a mean of 2 years (p<0.05, test for trend), whereas testosterone levels were not predictive80. In men with vertebral osteoporosis, oestradiol levels were found to be positively correlated with BMD at the femoral neck (r = 0.41, p<0.02)81 and spine (r = 0.29, p<0.03)82 and negatively correlated with markers of bone resorption such as hydroxyproline (r = -0.57, p < 0.05)83.

It seems likely, on the basis of the somewhat limited evidence currently available, that both E and androgens are required for the growth and maintenance of the adult male skeleton.

**TGFβ and bone**

The concept that transforming growth factor beta (TGFβ) mediates the actions of E in bone is
supported by a growing body of literature. The term TGFβ refers to a family of closely related proteins with similar amino acid sequence and similar biological activities. TGFβ has biological effects on E responsive tissues, such as breast epithelium and endometrium and may have a role in the pathogenesis of breast and ovarian cancers.

In the mid-1980s, it was demonstrated that TGFβ is an abundant constituent of bone matrix where its concentration is higher than that of all other tissues, except platelets. TGFβ is produced by both osteoclasts and osteoblasts, the latter presumably being the source of the TGFβ present in bone matrix.

There are at least four main evidences that TGFβ mediates the action of E in bone. First, E increase TGFβ production in bone, second, TGFβ production in bone is reduced after E withdrawal, third TGFβ inhibits bone resorption in ovariectomized animals and finally, 17β E-mediated osteoclasts apoptosis is blocked by an anti-TGFβ antibody in vitro.

However, if there is real substance behind this theory, the evidence should fulfill a number of postulates such as: Oestrogens should increase TGFβ production and E deficiency should result in decreased TGFβ production. Blocking TGFβ should inhibit the action of E. TGFβ should be able to substitute for E in their absence. The in vivo and in vitro effects of TGFβ and E should be similar. Substances such as drugs that have oestrogen-like effects on bone are likely to have similar effects on TGFβ production and finally, factors mediating the effects of E deficiency should have opposite effects to TGFβ.

Most of these postulates are supported by some fairly strong evidence in vitro and in vivo, details of which are presented in references.

It has been suggested that the bone-selective effects of raloxifene may be due to its selective induction of TGFβ3. If this is true, influencing TGFβ3 production in bone may be a central therapeutic objective, thus facilitating selective drug development. With that in mind, the relative roles of the different TGF family members in mediating the action of E may prove to be of great importance and not only of academic interest. Moreover, a more precise understanding of the actions of different E metabolites in bone is required, along with knowledge of how other endocrine factors (e.g. other sex steroids, corticosteroids and others) cooperate with E in exerting their actions.

In summary, oestrogen affects the skeleton in all species studied. They act on osteoblast precursors to increase or decrease proliferation and differentiation to osteoblasts. A pronounced inhibition of the resorptive activity of osteoclasts have also been demonstrated. Moreover, ER, ERα and ERβ, have been identified in bone cells in cultures. However, there is no definite evidence that these cells represent the target cells for E in vivo. Potential target cells in addition to osteoblasts, and perhaps osteoclasts, include lymphocytes, macrophages, mast cells, and stromal cells. Second messengers implicated in coordinating the sequence of events initiated by E include interleukins (IL-1, IL-6, IL-11), prostaglandins (PGE-2), TNF-α, the insulin-like growth factor (IGF) system and TGFβ.

Specifically, TGFβ as outlined above mediates the actions of E in bone, at least to some extent. Oestrogens clearly can stimulate TGFβ gene transcription in bone in vivo, and E withdrawal has the opposite effect. However, there are a lot of observations, which still cannot be explained, as well as questions, which still cannot be answered. One of those is the observation that suggests that pituitary function is required for the effects of E on bone. In hypophysectomized rats, E fails to reduce bone turnover and protect skeletal mass. It is obvious that further research is needed until all such issues can be solved.

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