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The role of estrogen and androgen receptors in bone health and disease

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Abstract

Mouse models with cell-specific deletion of the estrogen receptor (ER) α, the androgen receptor (AR) or the receptor activator of nuclear factor κB ligand (RANKL), as well as cascade-selective estrogenic compounds have provided novel insights into the function and signalling of ERα and AR. The studies reveal that the effects of estrogens on trabecular versus cortical bone mass are mediated by direct effects on osteoclasts and osteoblasts, respectively. The protection of cortical bone mass by estrogens is mediated via ERα, using a non-nucleus-initiated mechanism. By contrast, the AR of mature osteoblasts is indispensable for the maintenance of trabecular bone mass in male mammals, but not required for the anabolic effects of androgens on cortical bone. Most unexpectedly, and independently of estrogens, ERα in osteoblast progenitors stimulates Wnt signalling and periosteal bone accrual in response to mechanical strain. RANKL expression in B lymphocytes, but not T lymphocytes, contributes to the loss of trabecular bone caused by estrogen deficiency. In this Review, we summarize this evidence and discuss its implications for understanding the regulation of trabecular and cortical bone mass; the integration of hormonal and mechanical signals; the relative importance of estrogens versus androgens in the male skeleton; and, finally, the pathogenesis and treatment of osteoporosis.

Introduction

Estrogens and androgens exert potent influences on the size and shape of the skeleton during growth. In addition, these hormones contribute to skeletal homeostasis during adulthood. The decline in estrogen levels associated with menopause causes bone loss in women,[1,2] which occurs in both the trabecular (also known as cancellous) and the cortical bone compartments. Post-menopausal bone loss is associated with a high bone remodelling rate, as indicated by increased numbers of both osteoclasts and osteoblasts (Box 1, Figure 1).[3] Although osteoblast numbers are also elevated, in part owing to the coupling of bone formation to bone resorption, the increase in bone formation is not sufficient to replace the bone matrix removed by osteoclasts, which results in net bone loss. Low androgen levels cause bone loss in men and are associated with increased bone remodelling.[4,5] However, some of the bone loss caused by androgen deficiency is due to reduced levels of estrogen, which is derived from aromatization of testos terone.[6] Receptor activator of nuclear factor

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κB ligand (RANKL, also known as tumour necrosis factor ligand superfamily member 11) is essential for the generation, survival and function of osteoclasts, and thereby for bone resorption. Thus, RANKL is also implicated in the increased bone resorption associated with deficiency of sex steroid hormones.[7,8] Importantly, all major characteristics of bone loss associated with sex-steroid deficiency in humans can be mimicked in mice by gonadectomy.[9-11]

Sex steroid hormones act on their target cells by binding to members of the nuclear hormone receptor superfamily: estrogens bind to estrogen receptor (ER) α or ERβ, and androgens bind to the androgen receptor (AR).[12] Members of this superfamily of receptors are zinc-finger-containing transcription factors characterized by an N-terminal domain, a central DNA-binding domain and a C-terminal, ligand-binding domain.[13] In addition, these receptors have two regions with transcription-activating functions, located in the N-terminal domain (AF-1) and in the ligand-binding domain (AF-2). Of note, the phenotypes of *Ar* or *Esr* (encoding AR and ER, respectively) gene deletion models, whether germ-line or cell-type-specific, do not necessarily mimic those of sex-steroid deficiency; ERα, ERβ and AR not only act as receptors for their respective ligands, but also have important functions in the absence of ligands and can regulate the production of their ligands. Estrogen-related receptor (ERR) α, another member of this superfamily, has no known ligand and might act constitutively to influence osteoblast differentiation. However, this subject has been reviewed recently and will not be discussed further here.[14]

Binding of estrogens or androgens to their receptors in the nucleus stimulates transcription of target genes resulting from direct interactions of the receptor proteins with DNA or from interactions with other transcription factors (Figure 2a,b).[15] In addition, estrogens and androgens bind to their cognate receptors outside the nucleus. In particular, through binding to sex-steroid receptors in the plasma membrane, estrogens and androgens can initiate signal transduction by triggering the production of cyclic nucleotides, calcium flux and activation of cytoplasmic kinases. Activation of these kinases, in turn, leads to the phosphorylation of substrate proteins and transcription factors, which mediate some of the gene-regulatory effects of estrogens (Figure 2c,d).[16,17] Notably, more genes are regulated by ERα through this indirect mode of action than are regulated via the direct as sociation of estrogens with DNA.[18]

In this Review, we highlight the results of studies in mice with cell-specific deletion or mutation of ERα and AR, as well as results from studies of treatment with signalling-cascade-selective estrogenic compounds. We also summarize studies with lymphocyte-specific deletion of RANKL. In addition, we discuss the implications of the insights so obtained for understanding the regulation of trabecular versus cortical bone mass; the integration of hormonal and mechanical changes for bone growth and homeostasis; the relative importance of estrogens versus androgens in the male skeleton; and, finally, the pathogenesis and treatment of osteoporosis.

Cell-specific receptor deletion models

ERα, ERβ and AR are expressed in bone and bone marrow cells. However, until about 10 years ago the specific receptors, cell type(s) or signalling pathways that mediate the effects of sex steroid hormones on skeletal homeostasis remained unclear. Male mice with germline deletion of the AR have low bone mass associated with high bone turnover—mimicking, to some extent, the effects of androgen deficiency in humans.[19,20] By contrast, mice with germline deletion of ERα have a complex skeletal phenotype that is difficult to interpret owing to the presence of high circulating levels of estrogens and androgens, and mice with germline deletion of ERβ have minimal skeletal effects.[21,22] However, mice with targeted

deletion of the ERα or the AR in individual cell types of bone and bone marrow do not have the high circulating levels of estrogens and androgens associated with germline deletions of these receptors, and these models have provided functional evidence for the role of these receptors in specifi c cell types (Table 1 and Figure 3).

Chondrocyte ERα deletion

After birth, long bones increase in length. Longitudinal bone growth occurs at the growth plate, through endochondral ossification, a process in which cartilage is formed and then replaced by bone.[23] Sex steroid hormones, growth hormone, insulin-like growth factor 1 (IGF-1), thyroid hormones (tri-iodothyronine and tetraiodothyronine) and glucocorticoids are all indispensable for normal longitudinal bone growth.[24] At the beginning of puberty, both boys and girls experience a growth spurt. At the end of puberty, cell replication in the proliferative zone declines, leading to a reduction in cartilage synthesis at the distal end of the growth plate, as well as diminished replacement of the cartilage by bone at the proximal end. These events lead to closure of the epiphyses and cessation of longitudinal growth.[24,25]

The initial rise in estrogen levels at the beginning of puberty triggers the pubertal growth spurt, whereas high levels of estrogens in the late stages of puberty are critical for closure of the growth plate in women as well as men. Indeed, a man with estrogen resistance resulting from an inactivating mutation in ERα exhibited continued longitudinal growth, owing to the lack of closure of growth plates.[26] Similar features are found in men and women with inactivating mutations in *CYP19A1* (which encodes aromatase, the enzyme that converts testos terone to estradiol).[27] Moreover, administration of estrogen to women or men with aromatase deficiency promotes the closure of growth plates.[28,29]

In contrast to humans, the growth plates of rodents do not fuse as a result of sexual maturation. However, ovariectomy increases the number of chondroblasts in the proliferative zone of growing rats[30] and promotes longitudinal bone growth in mice.[31] By contrast, high-dose estradiol administration results in growth plate closure in both rodent species, as well as in humans.[24] Importantly (and similarly to the man described above with an inactivat ing mutation of ERα), mice with global ERα deletion exhibit increased longitudinal bone growth associated with wider than normal growth plates.[32]

Mice with chondrocyte-specific deletion of *Esr1* (which encodes ERα) have longer bones than do wild-type control mice at 1 year of age, indicating that the effects of estrogens on the growth plate are mediated by ERα in chondrocytes.[33] Consistent with these observations, mice with targeted deletion of ERα in the entire mesenchymal lineage (including chondrocytes), failed to show an ovariectomy-induced increase in bone length.[31] Moreover, treatment with a cell-membrane-impermeable form of estradiol showed that closure of the epiphyseal growth plate results from nuclear actions of ERα.[34] In fact, non-nuclear ERα signalling might have an effect opposite to that of estradiol on growth plate chondrocytes, perhaps by stimulating the production and/or action of growth factors.[35,36]

Osteoclast ERα deletion

Deletion of *Esr1* from mature osteoclasts is sufficient to increase osteoclast number in trabecular bone and reduce bone mass in female mice.[37] The increase in osteoclast number in these mice is due to increased osteoclast lifespan, caused by reduced expression of Fas ligand.[37] Notably, deletion of ERα in mature osteoclasts does not alter bone mass in male mice.[37] From these results, the researchers concluded that estrogens protect bone in female individuals by stimulating expression of the Fas ligand and, thereby, promoting osteoclast apoptosis.

When ERα is deleted in the entire monocyte–macrophage cell lineage, which includes osteoclasts, mice exhibit an increase in osteoclast numbers and decrease in osteoclast apoptosis similar to that seen in mice with deletion of ERα only in mature osteoclasts.[38] However, in the monocyte–macrophage ERα-deletion model, estrogens did not stimulate expression of the Fas ligand in osteoclasts. Thus, disagreement remains regarding the molecular mechanism through which ERα promote osteoclast apoptosis. Nevertheless, both studies provide strong evidence that estrogens restrain both osteoclastogenesis and osteoclast survival via direct actions on osteoclasts and their progenitors. However, deletion of ERα from the osteoclast lineage has no effect on cortical bone mass, which represents 80% of the entire skeleton.[38] This result indicates that estrogen action on other cell types is responsible for the suppression of resorption in cortical bone and that ERα is not involved in the suppression of bone resorption in male mice.[38]

ERα deletion in osteoblast progenitors

Our research group generated several additional mouse models, in which ERα was deleted: from pluripotent mesenchymal osteoblast progenitors; from bipotential osteoblast precursors; or from mature osteoblasts and osteocytes only.[31] Deletion of ERα at any of these stages along the osteoblast differentiation lineage has no effect on trabecular bone mass in either estrogen-sufficient or estrogen-deficient female mice, which is consistent with the idea that ERα action in osteoclasts is the main mechanism by which estrogens protect against resorption of trabecular bone.[38] On the other hand, female mice lacking ERα in osteoblast progenitors do not show the expected increase in osteoclast numbers in the endocortical (inner) bone surface and, therefore, do not lose cortical bone mass following ovariectomy. Along with the evidence that mice with deletion of ERα in mature osteoblasts have no cortical bone phenotype, this finding shows that estrogens attenuate osteoclastic resorption in the endocortical surface through actions mediated by ERα in osteoblast progenitors.[38]

Mice with deletion of ERα in mesenchymal progenitors expressing the transcription factors encoded by *Prrx1* or *Sp7* have low cortical bone mass owing to decreased periosteal bone formation.[31] Furthermore, canonical Wnt signalling is decreased in these mice, which leads to decreased proliferation and differentiation of periosteal cells.[31] The Wnt pathway is indispensable for osteoblast formation and bone mass accrual.[39,40] Canonical Wnt signalling stabilizes β-catenin, which binds to and activates members of the T cell factor–lymphoid-enhancer factor family.[41] Through this mechanism, canonical Wnt signalling promotes the maturation of osteoblast progenitors expressing *Sp7* into bone-producing osteoblasts.[42]

In the absence of estrogens, Wnt-induced proliferation and differentiation of osteoprogenitors is attenuated in periosteal cells from mice with ERα deletion as well as in an osteoblastic cell line in which ERα is silenced.[31] By contrast, estradiol has either no effect or inhibits canonical Wnt-induced proliferation and differentiation of osteoprogenitors, in cell cultures from mice with intact ERα. Moreover, in agreement with previous results from our research group,[43,44] the addition of estradiol to periosteal or bone-marrow-derived cell cultures from mice with intact ERα leads to attenuation of osteoblastogenesis.[31] These results demonstrate that the effects of estrogen-activated ERα are opposite to those of ligand-free ERα. Estradiol also attenuates adipogenesis in the bone marrow of humans and rodents, and estrogen deficiency increases differentiation of bone marrow adipose tissue.[45,46] In line with these findings, mice with deletion of ERα in cells expressing *Prrx1* have increased numbers of adipocyte progenitors in the bone marrow.[31] These findings indicate that estrogens attenuate the differentiation of both osteoblasts and adipocytes from mesenchymal progenitor cells.

ERα deletion in osteoblasts and osteocytes

During the past 20 years, work with osteoblastic cell lines and primary osteoblast preparations as well as estrogen-deficient rats and mice has suggested that cells of the osteoblast lineage mediate an indirect effect of estrogens on osteoclast formation and bone resorption.[1,47,48] Specifically, this work has provided compelling evidence that estrogens act on cells of the osteoblast lineage to restrain the production of osteoclastogenic cytokines, and the mechanism behind this effect is in hibition of NF-κB.[1,47]

A series of studies in rodents and humans also revealed that estrogen deficiency causes an increase in osteoblast and osteocyte apoptosis in both the trabecular and cortical compartment.[11,49-51] Consistent with this evidence, osteoblast apoptosis is increased in mice with deletion of ERα in mature osteoblast and osteocytes.[31] However, bone mass is not altered in this model, which suggests that an increase in osteoblast apoptosis alone cannot cause bone loss. Lack of an effect of ERα deletion in mature osteoblast and osteocytes on trabecular bone was confirmed in two other mouse models generated by our research group, which used different marker genes to drive ERα deletion (Table 1).[31] The evidence for lack of an effect of ERα deletion from mature osteoblasts and osteocytes on trabecular bone in female mice is corroborated independently by a mouse model in which ERα was deleted in *Dmp-1*-expressing osteoblasts and osteocytes.[52] However, whereas our group found no effect of ERα deletion on trabecular bone in male mice in three different models (all of which deleted ERα from osteoblasts and osteocytes), ERα deletion in *Dmp-1* expressing cells resulted in a 20% decrease in trabecular bone mass in male mice.[52] In contrast to the lack of a phenotype associated with ERα deletion in mature osteoblast and osteocytes in these studies,[50,52] a third group reported that ERα deletion in osteocalcin-expressing cells reduces trabecular and cortical bone mass in the tibiae of female mice.[53] However, the most probable explanation for this incongruent result is that the control mice used were of a different genetic background than the experimental mice.[53]

Deletion of AR in male mice

An important role of the AR in the homeostasis of the male skeleton is well-established by the observations that men with idiopathic hypogonadotropic hypogonadism or complete androgen insensitivity syndrome because of a loss-of-function mutation in AR have low bone mass.[54,55] In agreement with the evidence in men, global deletion of AR in male mice results in high bone turnover and increased resorption, as well as decreased trabecular and cortical bone volume.[19] In addition, male mice lacking AR have decreased periosteal bone formation, which is in line with the evidence that androgens stimulate periosteal bone formation in both men and male rodents.[56,57] Male mice lacking 3-oxo-5-α-steroid 4-dehydrogenase 1 (which converts testosterone to nonaromatizable dihydrotestosterone) exhibit reduced cortical thickness, indicating that dihydrotestosterone rather than testosterone is the androgen responsible for periosteal expansion.[58]

Mice with deletion of AR in osteoblasts and osteocytes[59-61] or in osteoblast progenitors have normal cortical bone (S. C. Manolagas, unpublished work). These findings rule out the possibility that the effects of androgens on periosteal bone formation result from direct actions on cells of the osteoblast lineage. Instead, these effects might result from the well-known stimulatory effects of androgens on production of growth factors and/or muscle size, which in turn increase mechanical strain on bone.

By contrast, deletion of AR in mature osteoblasts and osteocytes decreases trabecular bone mass in male mice.[59-61] The cellular and molecular mechanisms responsible for this positive effect of AR on trabecular bone mass remain unknown. Nonetheless, the absence of AR from osteoblasts and osteocytes does not alter the stimulatory effect of mechanical strain on

trabecular bone formation.[59] However, activation of AR by dihydro testosterone does prevent osteoblast apoptosis in orchidectomized mice.[11] An increase in osteoblast apoptosis could, therefore, be responsible for the low trabecular bone mass in mice lacking AR in mature osteoblasts and osteocytes.

In sharp contrast to the evidence that dihydrotestosterone suppresses osteoclastogenesis and protects against orchidectomy-induced loss of bone,[11,38] and that mice with global deletion of AR have increased osteoclast numbers in trabelcular bone,[19] osteoclast numbers are unaffected in mice with deletion of AR in osteoblasts and osteocytes.[59,61] These findings raise the possibility that the AR in osteoclasts also contributes to the protective effects of androgens on bone mass.

Receptor deletion in lymphocytes

Both B and T lymphocytes are thought to contribute to the bone loss caused by sex-steroid deficiency.[62,63] Several different T-cell-deficient mouse strains are protected from the bone loss caused by ovariectomy;[63] moreover, ovariectomy in these mice increases the number of T cells that produce tumour necrosis factor and might also upregulate RANKL production by T cells.[65] Consistent with the latter possibility, greater production of RANKL by T cells has been observed in postmenopausal women than in premenopausal controls.[66,67] By contrast, another research group reported that various mouse strains lacking T cells lose cortical bone after ovariectomy.[68] Thus, there might be a differential requirement of T cells for trabecular versus cortical bone loss caused by estrogen deficiency.

The number of B lymphocytes increases in the bone marrow of rodents after ovariectomy or orchidectomy,[69-75] raising the possibility that this cell type has an important role in the bone loss caused by sex-steroid deficiency.[69] This view is supported by studies demonstrating that an elevation in B-cell numbers, induced by administration of interleukin (IL) 7, is sufficient to increase osteoclast numbers and cause bone loss in mice.[69] Mature B cells, however, do not seem to be required for the bone loss induced by ovariectomy, as ovariectomy causes similar bone loss in mice lacking mature B cells and in their B-cell-replete littermates.[76]

Deletion of RANKL in the entire B-cell lineage, from early precursors to fully mature cells, prevents the increase in osteoclast number and loss of trabecular bone caused by ovariectomy in mice.[77] The same study showed that T-cell production of RANKL is not required for ovariectomy-induced bone loss.[77] Importantly, the amount of RANKL mRNA or protein expressed by B cells in wild-type mice does not increase after ovariectomy, suggesting that an increase in the total number of B cells that express RANKL, rather than an increase in the expression level per cell, stimulates osteoclastogenesis after loss of estrogens. Consistent with this finding, the number of bone marrow cells that express RANKL is elevated in estrogen-deficient women, compared with women treated with estrogen for 3 weeks or premenopausal women.[78] By contrast, a second study in women suggests that estrogens suppress the expression of RANKL on the surface of B cells, as well as on T cells and bone marrow stromal cells, with no change in total cell number.[66] Although this study did not address the functional significance of the changes in cell-surface expression of RANKL, these observations nonetheless suggest that important differences exist in the response to estrogen loss between mice and humans. Alternatively, such differences might simply reflect differences in the timing of B-cell enumeration after estrogen loss. Ovariectomy in mice induces a transient increase in B-cell number, which peaks about 2 weeks after the operation.[70] Whether a similar transient increase in the number of RANKL-expressing B cells happens in humans after acute estrogen loss remains unclear.

B and T lymphocytes each express the AR, ERα and ERβ.[79,80] However, studies in which ERα or the AR has been deleted specifically in lymphocytes are limited, and none has measured the effects of such targeted deletion on the skeleton.[81,82] Nonetheless, deletion of the AR from B cells is sufficient to increase B-cell numbers, although not to the level observed after orchidectomy or germline deletion of AR.[82] These studies suggest that androgens control lymphocyte numbers via both direct and indirect mechanisms.

Molecular mechanisms of ERα action

Nucleus-initiated actions

The classic mode of action of ERα is ligand-stimulated binding of this protein to estrogen response elements (EREs) in target genes (Figure 2a). Notably, in many tissues, such as breast, uterus, liver and pituitary, expression of the mammalian *ESR1* gene is autoinduced via EREs located upstream of the transcription start site and/or via stabilization of ERα mRNA and thereby ERα protein levels.[83] However, this mechanism has not been confirmed to occur in mouse bone cells, where only a single target gene (*Fasl*, encoding Fas ligand) has been shown to be regulated via an ERE.[84] Specifically, estrogen stimulates expression of *Fasl* in osteoblasts, but not in osteoclasts.[84] Furthermore, estrogen controls *Fasl* expression via an ERE-containing transcriptional enhancer located downstream from this gene, which is responsive to ERα but not ERβ. As discussed above, nuclear ERα can also control the expression of target genes via interactions with other transcription factors. For example, ERα in cells of the osteoblast lineage binds to components of the NF-κB complex and thereby inhibits transcription of IL-6,[85] which is required for the bone loss induced by estrogen deficiency (Figure 2b).[86]

Non-nucleus-initiated actions

Selective activation of non-nucleus-initiated actions of the ERα using synthetic ligands (termed activators of nongenomic estrogen-like signalling, or ANGELS) can dissociate the beneficial effects of estrogens on bone from their effects on the breast and the uterus.[11,50,87,88] The use of selective ANGELS might, therefore, avoid the adverse effects of estrogen-replacement therapy on reproductive organs. However, the prototypic compound used to test this hypothesis, 4-estren-3α-17β-diol (estren), was subsequently found to also bind to AR (albeit with 30-fold lower affinity than dihydrotestosterone does) and to affect reproductive organs under certain experimental conditions.[89,90] Nevertheless, treatment with an isomer of estren prevents the loss of bone and muscle in orchidectomized rats but has minimal, if any, effects on the prostate and seminal vesicles.[91] Similarly, synthetic estrogen-like compounds, which selectively activate kinases but have weak genomic effects, have far more potent effects on bone than on the uterus and breast, and also reproduce the vasodilatory effect of estradiol.[92,93]

An estrogen–dendrimer conjugate (EDC) has been synthesized, in which an estradiol derivative is attached to a large, positively charged, nondegradable poly(amido) amine dendrimer via a hydrolytically stable linkage.[94] EDC binds to ERα with an affinity that is indistinguishable from that of the estradiol derivative component of the molecule alone.[95] Although EDC cannot enter the cell nucleus, it very effectively stimulates non-nuclear ER signalling. EDC is, however, ineffective in stimulating nuclear ER target-gene expression.[94] Thus, EDC provides an ideal compound for dissecting which of the effects of estrogens are regulated by non-nucleus-initiated pathways rather than by nucleus-initiated pathways. Studies using this compound in mice have shown that selective activation of non-nucleus-initiated ERα signalling replicates the protective effects of estrogens on the cardio vascular system without promoting uterine or breast cancer growth.[96] In addition, work from our

laboratory has revealed that EDC can replicate the effects of es trogens on osteoblasts and osteoclast apoptosis *in vitro*.[38,97]

To obtain definitive proof that at least some of the effects of estrogens on bone are mediated via non-nuclear signalling, our research group compared the effects of EDC and estradiol in ovariectomized mice.[34] EDC was as effective as estradiol for preventing the loss of cortical bone mass caused by estrogen deficiency, but, unlike estradiol, EDC did not prevent the loss of trabecular bone mass. In addition, EDC attenuated the loss of bone strength as well as the increased osteoclastogenesis, osteoblastogenesis and oxidative stress caused by ovariectomy. Moreover, EDC had no effect on the uterus, whereas estradiol restored the ovariectomy-induced loss of uterine weight. These findings demonstrate that the protection of cortical bone mass by estrogens results from a non-nucleus-initiated mechanism of action of the ERα that is distinct from the classic, nucleus-initiated actions of estrogen-activated ERα on reproductive organs. This evidence, and the effects of targeted deletion of ERα from osteoclasts and osteoblast progenitors, strongly suggests that the mechanism of EDC's protective effect on cortical bone mass involves non-nuclear ERα signalling in osteoblast progenitors. In addition, these observations imply that the effects of estrogens on the trabecular compartment are the results of nucleus-initiated actions of the ERα in osteoclasts.

In agreement with these conclusions, genetic manipulations that preclude nucleus-initiated actions of the ERα abrogate the protective effect of estrogens on trabecular bone but do not affect the cortical compartment. These manipulations include deletion of the ERα co-activator *Ncoa1* and inactivation of AF-1 in mice.[98,99]

Ligand-independent ERα functions

The mass, shape and microarchitecture of bone adapts to changes in mechanical strain. Osteocytes are thought to be responsible for both detection of strain and orchestration of the adaptive response.[100-102] However, mice lacking ERα have a defective response of bone to mechanical loading,[103] and ERα signalling potentiates activation of the canonical Wnt signalling pathway in response to mechanical stimulation *in vitro*.[104] In addition, other *in vitro* observations suggest that ERα or ERβ can transduce mechanical forces into prosurvival signals in osteocytes and osteoblasts and that this function is independent of estrogens.[105] Specifically, localization of ERα on the cell membrane and its interaction with caveolin-1 is required for stretch-induced activation of mitogen-activated protein kinases (MAPK) and attenuation of apoptosis. These results led to the suggestion that, in addition to their role as ligand-dependent mediators of the effects of estrogens, ERs might participate in the transduction of mechanical forces, albeit in a ligand-independent manner.

The contention that ERα in cells of the osteoblast lineage participates in the transduction of mechanical signals is supported by the effects of targeted deletion of ERα from osteoblast progenitors (which express transcription factor Sp7). This mouse model shows that ERα potentiates canonical Wnt signalling and periosteal cell proliferation without requiring activation of ERα by estrogens, and that these actions are required for optimal cortical bone accrual at the periosteum.[31] By contrast, the actions of ERα in mature osteoblasts and osteocytes do not alter bone mass or the response to mechanical loading. Even more tellingly, osteocyte depletion has no effect on the response of cortical bone to mechanical loading.[106] Taken together, these findings strongly suggest that ERα in osteoblast progenitors is responsible for transducing the effects of loading on cortical bone. In contrast to these effects of ERα, estradiol has either no effect or inhibits proliferation (induced by canonical Wnt signalling) and differentiation of osteoprogenitors in cultures of periosteal or bone-marrow-derived cells from mice with intact ERα, confirming other evidence for a suppressive effect of estrogens on osteoblastogenesis.[43,44]

Identical conclusions—that the ERα is required for the osteogenic response to mechanical loading in a ligand-independent manner—have been derived from axial loading experiments conducted in ovariectomized mice.[107,108] Loading increases cortical bone area as a result of increased periosteal bone formation in both estrogen-sufficient and estrogen-deficient mice. Similarly to mice with complete ERα inactivation, the effect of loading is severely reduced in mice with inactivation of AF-2, but not in those with inactivation of AF-1.[108] Unlike in female mice, ERα is not required for the adaptive response to loading in male mice, suggesting that other signals induced by loading in male mice override the anabolic actions of ERα on the periosteum.[109]

In line with the above evidence from targeted deletion of ERα in osteoblast progenitors, activation of the low-density lipoprotein receptor-related protein 5 (LRP-5)–Wnt–β-catenin signalling pathway is required for the physiological response of bone to mechanical loading, and activation of this pathway enhances the sensitivity of osteoblastic cells to mechanical loading.[110-112]

A pictorial summary of the effects of estrogens and their receptors on bone is provided in Figure 4.

Trabecular versus cortical bone regulation

The skeleton has two essential functions: to support mobility, by providing leverage against gravity, and to protect soft organs (including the haematopoietic marrow) by its toughness, while remaining light enough for optimal mobility. In addition, in female mammals, the skeleton needs to supply mineral for building the fetal skeleton and for lactation. To achieve these functions, bones adapt to changes in levels of growth factors and hormones, as well as mechanical load. Nonetheless, the response of bone to these changes differs greatly in the trabecular versus the cortical compartment,[113,114] as well as the periosteal versus the endocortical surfaces of long bones. For example, during growth, bone is simultaneously deposited at the periosteum to expand the cross-sectional area and removed from the endocortical surface to enlarge the medullary cavity. Furthermore, age-related loss of trabecular and cortical bone mass is associated with decreased remodelling in the trabecular bone compartment but increased remodelling in the cortical compartment, which results in increased cortical porosity.[51,115-117]

The evidence that estrogens regulate cortical and trabecular bone mass via different cell types (osteoblast progenitors and osteoclasts, respectively) strongly supports the notion that these two compartments respond in fundamentally different ways to external cues, such as hormonal and mechanical stimuli. Moreover, the evidence that ERα in osteoblast progenitors is required for mechanosensing at the periosteum, but not at the endosteum or in trabecular bone, raises the possibility that osteoblast progenitors residing in these different compartments respond to different cues. Thus, the proliferation, differentiation and lifespan of osteoblast progenitors residing in the periosteum might be controlled by different stimuli to those that regulate progenitors residing on the endocortical surface or in the bone marrow. In support of this concept, osteocyte apoptosis induced by estrogen deficiency (and the osteoclast formation stimulated by such apoptosis) is not uniform but restricted to distinct anatomical areas of cortical bone.[118] Similarly, osteoblast turnover, which is a function of the formation of these cells by replication of progenitors and death by apoptosis, is lower in periosteal than in trabecular bone in mice.[119] Likewise, the proliferation of osteoblast progenitors is slower at the periosteum than at the endosteum.[119] In the future, lineage-tracing studies should eventually distinguish whether the matrix-synthesizing mature osteoblasts in various bone compartments are derived from the same progenitors, but

experience different microenvironmental stimuli, or whether they are the progeny of distinct progenitor subpopulations.

Regulation of osteoclast number and thereby bone resorption could also differ in the trabecular and cortical compartments. This idea is strongly suggested by the evidence that the protective effects of estrogens against trabecular and cortical bone are mediated by the ERα in osteoclasts and osteoblast progenitors, respectively, and that B-cell-derived RANKL affects trabecular but not cortical bone in ovariectomized mice.[77] Shortening osteoclast lifespan might be the dominant mechanism by which estrogens control osteoclast numbers in the trabecular compartment. However, suppressing osteoclastogenesis by decreasing the levels of cytokines other than B-cell-derived RANKL could be the dominant mechanism by which estrogens control osteoclast numbers at the endocortical surface. Once again, future work based on targeted deletion of critical osteoclastogenic cytokines, such as RANKL, should d istinguish between these alternative scenarios.

Integration of hormonal and mechanical signals

As eloquently stated by our colleague Michael Parfitt, “bone growth is necessary for the health, not just of the present child, but of the future adult.”[113] Indeed, the changes in size and shape of children’s bones as they grow must result in sufficient strength to support their current physical activity. However, in addition to meet the immediate needs, after the completion of growth and the achievement of peak bone mass, the bones must be strong enough to meet all load-bearing demands throughout the rest of the individual’s lifetime. Thus, the accrual of bone mass and size during growth is a critical determinant of the risk of development of osteoporosis later in life.[120-122]

The amount of bone mass accumulated during growth is determined to a large extent by body size and by the mechanical load generated as a result of skeletal muscle forces actuating bony levers, such that limb muscle mass is a reasonable measure of mechanical load. Adaptation of bone to mechanical loading is much greater during growth than after the attainment of peak bone mass. However, the pattern of changes in muscle size, levels of growth factors and sex steroid hormones during life (Figure 5) makes it all but inevitable that these three external factors not only influence building of the skeleton from birth to the attainment of peak bone mass, but also affect its involution thereafter. Consequently, mechanical load is critically important for the maintenance of bone mass during adulthood, as well as for the accumulation of bone mass during growth. This dependency is readily manifested by the rapid and dramatic loss of bone that occurs in conjunction with reduced physical activity in old age, long-term bed rest or space flight.[123-126]

That the skeleton adapts to meet mechanical needs was first recognized in 1892,[127] but the cellular and molecular mechanisms responsible for changes in bone mass in response to alterations in mechanical forces remain poorly understood. The unique properties and distribution of osteocytes has made these cells the most logical candidates for sensing and responding to mechanical strains.[101,128] Consistent with the hypothesis that osteocytes have an important role in mechanical sensing and adaptation of the skeleton to changing strain, the age-related decline in osteocyte numbers is accompanied by reduced bone strength, both in patients with vertebral fractures[129] and in healthy mice.[51] Moreover, ablation of osteocytes in young mice recapitulates at least some of the effects of old age on bone and rapidly leads to decreased bone strength, microfractures and osteoporosis.[106]

Crosstalk between estrogen and growth factor signalling is extensively documented in breast development.[130,131] In 2013, *in vitro* experiments showed that estradiol amplifies growth hormone signalling in human osteoblastic cells by accelerating the degradation of suppressor

of cytokine signalling 2 (SOCS2), which is a negative regulator of growth hormone signalling. This effect does not require *de novo* gene transcription, suggesting that it involves a non-nucleus-initiated mechanism of action of estrogen.[132] This intriguing finding raises the possibility that ERα in osteoblastic cells might also serve as the nexus of the integration of estrogen and growth factor signalling, but that needs to be confirmed *in vivo*. In line with the idea that hormonal and mechanical signals on bone might be integrated, the rate of postnatal growth varies by several-fold from one compartment to the other within a single bone.[113,133,134] Indeed, in the ilium, the rate of bone formation is almost eightfold higher in the outer than in the inner periosteum, and more than fourfold higher on the inner than on the outer endocortical surface.[134] In addition, whereas little or no evidence exists for endocortical apposition in the metacarpal during adolescence, endocortical apposition in the ilium can be stimulated by vigorous physical exercise. Moreover, the compelling evidence that ERα is required for optimal bone accrual at the periosteum, independently of estrogens, suggests the distinct possibility that in addition to osteocytes, osteoblast progenitors that express *Sp7* might themselves be mechanosensing cells. The evidence that ERα in osteoblast progenitors stimulates Wnt signalling and periosteal bone accrual in response to mechanical strain raises the possibility that mechanical forces and estrogens act on bone in a coordinated fashion, perhaps partly via signals converging on ERα. Consistent with this hypothesis, osteocyte apoptosis following either estrogen loss or diminished mechanical forces in rodents occurs in distinct regions of the bone cortex, rather than uniformly throughout the cortex.[118,135,136]

ERα and AR in females versus males

Periosteal bone formation is restrained by estrogens and stimulated by androgens. Differences in levels of these hormones between boys and girls contribute to sexual dimorphism and the greater bone mass in men than in women.[137] Indeed, during puberty, boys acquire more bone mass at the periosteum than girls do. In both male and female mice, radial bone expansion peaks between 3 weeks and 5 weeks of age. However, periosteal expansion during this time is 40% higher in male mice than in female mice. The rate of periosteal bone formation is 70% higher in 5-week-old male mice than in female mice of the same age, owing to their greater levels of androgens.[138] Nonetheless, administration of estrogens to men with aromatase deficiency increases bone size, which suggests that estrogens might stimulate (rather than restrain) periosteal apposition in men.[57,139] Moreover, treatment of a man with aromatase deficiency with a combination of estradiol and testosterone caused a greater increase in bone size than did estradiol alone, supporting the notion that both sex steroid hormones promote periosteal apposition in men.[140]

In line with the notion that both androgens and estrogens are needed for optimal periosteal expansion in men, the stimulatory effect of testosterone on periosteal expansion in mice, was attenuated by blocking the conversion of androgens to estrogens.[141,56] In addition, the decrease of periosteal bone expansion caused by deletion of ERα or AR is exacerbated when both receptors were deleted simultaneously.[142-144] Male mice lacking ERα in osteoblast progenitors expressing *Prrx1* have decreased cortical bone mass, similarly to their female counterparts.[31] However, in contrast to female mice, the cortical defect in male mice is transient, probably because androgens can act via AR to stimulate periosteal bone expansion.[58,139,143,144] Nonetheless, deletion of AR from all cells of the osteoblast lineage has no effect on cortical bone accrual, indicating that (unlike those of estrogens) the effects of androgens are not exerted via direct actions on periosteal osteoblasts.

Studies in mice with global deletion of ERα or AR have suggested that, in contrast to their effects on cortical bone, ERα-mediated actions of aromatizable androgens have little or no role in the protective effects of androgens on trabecular bone.[36] Moreover, trabecular

number is preserved in men who are homozygous for a loss-of-function mutation of ERα.[26] In agreement with this result, targeted deletion of ERα in the osteoclast or osteoblast lineage has no effect on trabecular bone in male mice.[31,37,38] By contrast, nonaromatizable androgens prevent the orchidectomy-induced loss of trabecular bone, and deletion or overexpression of AR in mature osteoblasts and osteocytes decreases or increases trabecu lar bone volume, respectively.[60,145] Taken together, these findings indicate that AR is responsible for the preservation of trabecular bone in male mice.

The idea that estrogens might have both stimulatory and inhibitory effects on the sensitivity of the periosteum to mechanical forces, according to their levels, has been proposed by two different research groups.[146,147] These groups suggested that the differential effects of low and high estrogen levels on the periosteum were mediated via ERα to stimulate and via ERβ to restrain periosteal bone apposition. On the basis of the latest finding that, independently of estrogens, ERα stimulates cortical bone accrual,[31] we speculate that the low estrogen levels at the beginning of puberty induce the growth spurt by upregulating the expression of ERα, which is a well-documented phenomenon in many cell types.[148-150] This increased expression of ERα, in turn, would amplify the responsiveness of bone cells to mechanical forces, via ERα-mediated signals that are the predominant cue for ERα signalling while estrogen levels remain low. As much as half of peak adult bone mass is accumulated during the adolescent growth spurt, which occurs 2 years earlier in girls than in boys, and is driven by the interactions of sex steroid hormones and growth factors.[134,146] Later in puberty, the dramatic rise in estrogen levels that occurs in women restrains periosteal bone apposition, preventing bones from becoming excessively large and heavy as a result of the decreased resorption and increased formation at the endocortical surface. In women, this restraining effect of high estrogen levels on periosteal bone remains the predominant cue for ERα signalling until estrogen levels start to decline at menopause.

Pathogenesis and therapy of osteoporosis

With advancing age, the balance between bone formation and resorption progressively shifts to favour resorption, leading to loss of bone mass and strength. For over 60 years, the involution of the human skeleton with advancing age was thought to be the result of the decline in estrogen levels associated with menopause in women, and a later and smaller decline in estrogen levels that occurs in elderly men.[151,152] Nonetheless, evidence accumulated over the past 15 years has made it clear that, in both animals and humans, age-related bone loss in either sex begins immediately after peak bone mass is achieved—long before and independently of the decline in sex steroid levels.[51,153] Indeed, a significant proportion of trabecular bone loss throughout life is age-related and estrogen-independent.[151,152,154]

At menopause, the age-dependent loss of trabecular bone in the spine accelerates,[152] which leads to trabecular perforation and loss of connectivity. Evidence from the study of mice with osteoclast-specific deletion of ERα suggests that the acceleration of trabecular bone loss following menopause results from the loss of direct effects of estrogens on osteoclasts.[31,37] Not surprisingly, therefore, postmenopausal bone loss responds as effectively to estrogen replacement as it does to treatment with antiresorptive agents, such as bisphosphonates and the RANKL-neutralizing antibody denosumab.

Within <10 years following menopause, the rate of bone loss in women slows and parallels that in eugonadal men of the same age. This phase of slow bone loss, which affects both women and men equally, involves primarily cortical bone and has been attributed to skeletal ageing *per se*. Men, nonetheless, have a lower probability than women to suffer fragility fractures, possibly because men gain more bone during puberty and lose less bone in later

life, as they do not experience the abrupt loss of estrogens that women do. Histologically, the age-associated loss of bone is characterized by decreased wall width, the hallmark of decreased osteoblast number and/or function, which is consistent with the idea that ageing compromises the supply of osteoblasts.[3,155] High-resolution peripheral quantitative CT of the radius and femurs (the latter measured postmortem) of women aged 50–80 years has revealed that most bone loss occurs after the age of 65 years and that a substantial portion of the loss of cortical bone in old age is the result of increased intracortical porosity.[117,154] In fact, most fractures in individuals aged >65 years are nonvertebral and occur predominantly at cortical sites.[117] In agreement with the evidence in humans, studies in mice have revealed that age-related mechanisms intrinsic to bone (such as increased oxidative stress) are protagonists of the decline in bone mass, whereas age-related changes in other organs and tissues, such as the ovaries, have contributory effects.[151,152,156] In support of this notion, the seminal mechanism leading to increased osteoblast and osteocyte apoptosis is oxidative stress in both elderly mice and in those with acute sex steroid deficiency; moreover, osteoblast and osteocyte apoptosis can be prevented with antioxidants.[51,157]

Beginning at menopause, cortical bones becomes thinner, partly as a result of an increase in the medullary diameter.[158] Studies involving cell-specific deletion of ERα from osteoblast progenitors suggest that this change results from the loss of the effects of estrogens on osteoblast progenitors, which indirectly increases osteoclastogenesis. Periosteal apposition also increases following menopause, consistent with the evidence that estrogens (acting via ERα in osteoblast progenitors) attenuate periosteal expansion.[158] This outward expansion of the bone might partially preserve its strength, despite the overall loss of bone mass.

The evidence that treatment with EDC can protect against the loss of cortical bone mass in the ovariectomized mouse model strongly suggests that the increased endocortical resorption that ensues upon menopause results from the loss of nongenomic effects of estrogens on osteoblast progenitors.[34] Furthermore, the results of the EDC study raise the possibility that, because of its lack of effects on reproductive organs, this agent might represent a safer alternative to classic estrogens in the prevention of postmenopausal osteoporosis. This possibility, nonetheless, needs to be tempered by the serious adverse effects associated with estrogen-based therapies and their decreased efficacy in elderly women who are long past the menopause.[159,160]

Ageing increases the risk of fractures, independently of bone mass. Indeed, for the same BMD, a 20-year increase in age is accompanied by a fourfold increase in fracture risk.[161] Considering the major role of growth factors and mechanical forces in the building of the skeleton during puberty, the decline in physical activity, muscle mass and growth factor production with old age very probably contributes to the involution of the skeleton (Figure 5).[162-164] One possible mechanism is increased cortical osteocyte apoptosis, which in turn might increase the release of RANKL by healthy osteocytes in the vicinity of the apoptotic ones, leading to increased cortical porosity.[102,156] The osteocyte apoptosis that occurs with advancing age can also result in a decline in bone vascularity and hydration, which reduces bone strength by mechanisms that are not well-understood but probably include changes in the crystal structure of hydroxyapatite and promotion of micropetrosis (a phenomenon in which the canaliculae and the osteocyte lacunae are filled with mineralized tissue).[165,166]

The evidence that ERα in osteoblast progenitors potentiates Wnt signalling in a ligand-independent manner raises the possibility that, in addition to the loss of direct effects of estrogens on osteoclasts and osteoblasts, loss of estrogen at menopause leads to downregulation of ERα expression, and thereby to a decrease in the responsiveness of osteoblastic cells to mechanical stimulation. Decreased mechanical signals, in turn, could further potentiate the effect of estrogen loss on osteocyte apoptosis. Combined with the

decline of physical activity and skeletal mass in the late postmenopausal years,[162-164] such a decrease in ERα expression and mechanical responsiveness could exaggerate not only the acute effects of estrogen deficiency but also the estrogen-independent adverse effects of old age.

Conclusions

Elucidation of the function of ERα and AR in different cell types of the bone and the bone marrow (Figure 3) offers critical new insights for the function of these receptors (and, thereby, the roles of their ligands) in bone health and disease (Figure 4). These new insights indicate that bone mass in the trabecular and cortical bone compartments is regulated by different cell types and external cues. Hormonal, mechanical and growth factor signals are integrated to obtain both optimal building of the skeletal structure during growth and its maintenance during adulthood to match the organism's need at any time and also influence the pathogenesis of skeletal involution late in life. Moreover, these findings re-emphasize the concept that sex steroid hormones (and the deficiency of these hormones late in life) are only one of many critical factors that control bone homeostasis. Specifically, mouse models of cell-specific deletion of ERα, AR and RANKL, as well as studies of hormone replacement with cascade-selective estrogenic compounds, reveal that osteoblast progenitors, osteoclasts and B lymphocytes are all involved in the bone-protecting effects of estrogens. ERα-mediated direct effects on osteoclasts and direct or indirect effects on B lymphocytes attenuate the resorption of trabecular bone. ERα-mediated effects on osteoblast progenitors indirectly attenuate resorption at the endocortical surface via a non-nucleus-initiated mechanism. The AR in male mice exerts a protective effect on trabecular bone, but (unlike in female mice) this effect results from actions on osteoblasts and osteocytes and not osteoclasts. This difference between sexes could reflect the difference in body size and the need of female mammals to store easily resorbed bone on the medullary surface to enable formation of the fetal skeleton and for lactation.

The different role of sex-steroid receptors in trabecular versus cortical bone further suggests that hormonal signals are modified and integrated with different environmental cues in each compartment, including mechanical strain resulting from the forces exerted by muscles and the local concentration of paracrine cytokines and growth factors. The different role of these receptors in trabecular versus cortical bone might also reflect different lifespans between cortical and trabecular osteoblasts, and/or that adaptive changes in trabecular bone are best accommodated by changes in remodelling, whereas adaptive changes in cortical bone are best accommodated by changes in modelling. Perhaps more importantly, these studies alert us to the possibility that the progenitors of periosteal cells might be different from the progenitors of osteoblasts in the trabecular compartment; in addition to osteocytes, periosteal cells can indeed sense and respond to mechanical signals converging on ERα. Future work with lineage-tracing studies, combined with targeted gene deletion models, should be able to explore these intriguing possibilities.

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Box 1

The effect of sex steroid hormones on bone remodelling

Bone is periodically remodelled by basic multicellular units (BMUs), which consist of osteoclasts at the leading edge, osteoblasts at the trailing edge, a central capillary, nerve supply and associated connective tissue.[1] As the BMU advances, old or damaged bone matrix is removed by osteoclasts and replaced with new bone made by osteoblasts. In cortical bone, the BMU excavates and replaces a tunnel, whereas in trabecular bone it moves across the surface, excavating and refilling a trench. The lifespan of individual osteoclasts and osteoblasts is much shorter (2 weeks and 3 months, respectively, in humans) than the lifespan of the BMU (6–9 months).[1] A constant supply of new osteoclasts and osteoblasts is, therefore, essential for the function of the BMU. As a consequence, the balance between the supply of new cells and their lifespan determines the number of either cell type in the BMU and the amount of bone removed and replaced. Osteocytes, former osteoblasts entombed within the mineralized matrix during refilling of resorption lacunae, are long-lived and 10-fold and 1,000-fold more common than osteoblasts and osteoclasts, respectively. Osteocytes sense the need for bone regeneration and direct this process by controlling factors that are critical for the generation of both osteoclasts and osteoblasts. Estrogen or androgen deficiency increases the rate of bone remodelling, osteoclastogenesis, osteoblastogenesis, osteoclast and osteoblast numbers, as well as bone resorption and formation—albeit, in an unbalanced manner, with resorption exceeding formation.[1,169] Conversely, estrogens or androgens restrain the rate of remodelling and maintain a focal balance between bone formation and resorption. The attenuation of the remodelling rate is due to restrained effects of sex steroid hormones on the birth of osteoclast and osteoblast progenitors, as well as a proapoptotic effect on osteoclasts and an antiapoptotic effect on osteoblasts and osteocytes.[9,11,43,44,50,170-173]

Key points

- Estrogen receptor α (ERα)-mediated direct effects of estrogens on osteoclasts, and direct or indirect effects on B lymphocytes, attenuate the resorption of trabecular bone
- ERα-mediated effects of estrogens on osteoblast progenitors indirectly attenuate resorption at the endocortical surface, via a mechanism initiated outside of the nucleus
- The ERα of osteoblast progenitors stimulates Wnt signalling and periosteal bone accrual in response to mechanical strain, independently of estrogens
- The androgen receptor exerts a protective effect on trabecular bone in male mammals, but (unlike estrogen's effects in female mammals), this effect involves actions on osteoblasts and osteocytes
- Hormonal signals are modified and integrated with various environmental cues in different bone compartments, including mechanical strain, paracrine cytokines and growth factors

Review criteria

We searched the literature for original and review articles from 1990 until 2013 examining the role of estrogen and androgen receptors in bone using mouse genetics. Searches were performed in MEDLINE and PubMed using the terms “estrogen receptor”, “androgen receptor”, “osteoblast”, “osteoclast”, “osteocyte”, and “lymphocyte”, alone and in combination. All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers.

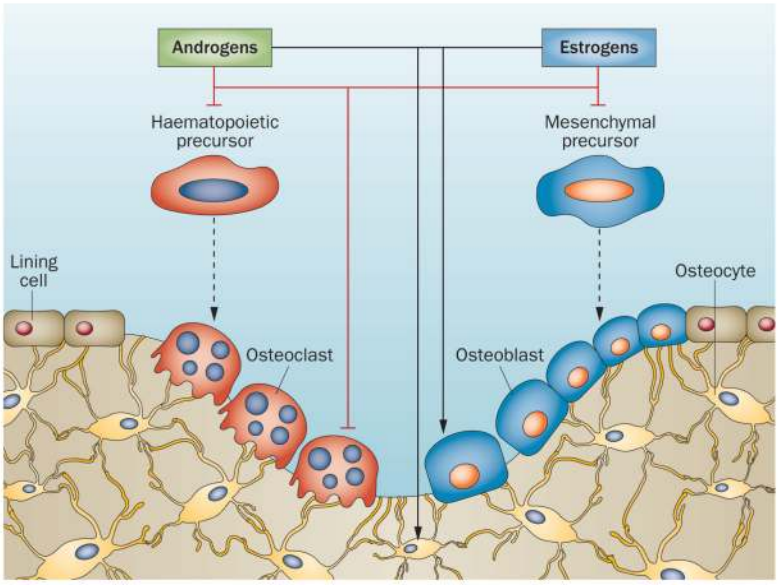

Figure 1.

Effects of estrogens and androgens on bone remodelling. Osteoclasts and osteoblasts are derived from haematopoietic and mesenchymal precursors, respectively. During the process of bone remodelling, bone matrix excavated by osteoclasts is replaced with new matrix produced by osteoblasts. Both estrogens and androgens influence the differentiation of osteoclast and osteoblast precursors and the lifespan of mature osteoclasts and osteoblasts, as well as the lifespan of osteocytes. Positive (black arrows) and negative (red bars) effects on the cells are depicted as well as differentiation of cells (dashed arrows).

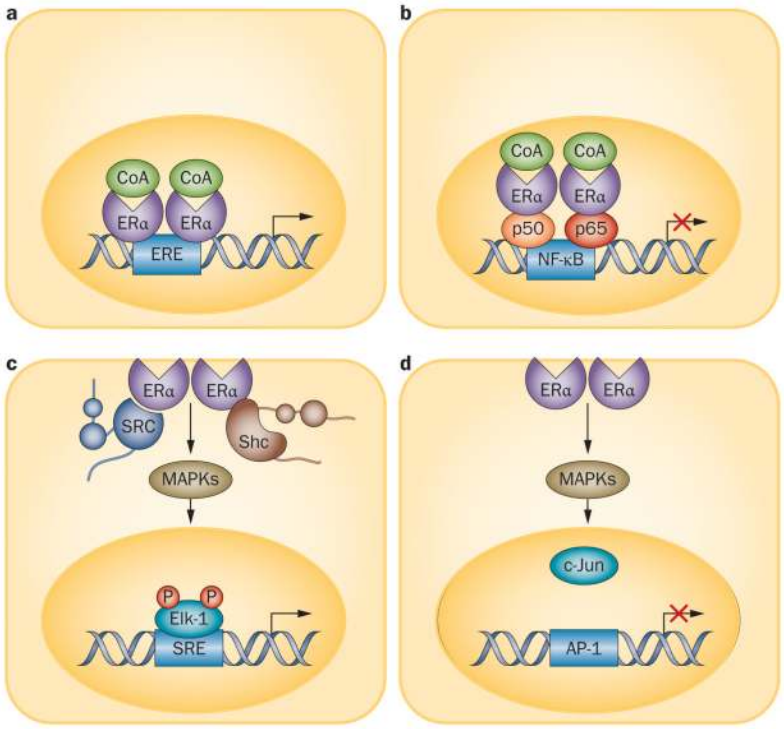

Figure 2.

Molecular mechanisms of action of ERα. **a** | Classic genomic signalling, in which ligand-activated ERα dimers attach to EREs on DNA and activate or repress transcription. **b** | ERE-independent genomic signalling, in which ligand-activated ERα binds to other transcription factors (such as the p50 and p65 subunits of NF-κB), which prevent them from binding to their response elements. **c**,**d** | Nongenotropic mode of action, in which ligand-activated ERα (in the plasma membrane) activates cytoplasmic kinases which, in turn, induce the phosphorylation of substrate proteins and transcription factors (such as Elk-1 and AP-1) that (c) positively or (d) negatively regulate transcription. Abbreviations: AP-1, transcription factor AP-1; CoA, coenzyme A; Elk-1, ETS domain-containing protein Elk-1; ERα, estrogen receptor α; ERE, estrogen response element; Shc, Shc-transforming protein; SRE, serum response element.

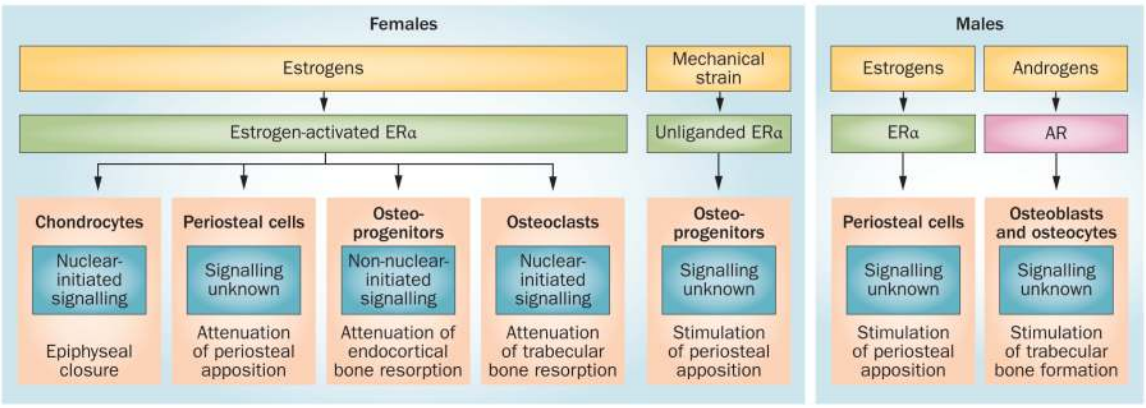

Figure 3.

Function and signalling mechanisms of ERα and AR in female and male mammals. Effects on different cell types were determined using mouse models of cell-specific deletions. Non-nuclear-initiated signalling mechanisms were elucidated using cascade-selective estrogenic compounds.

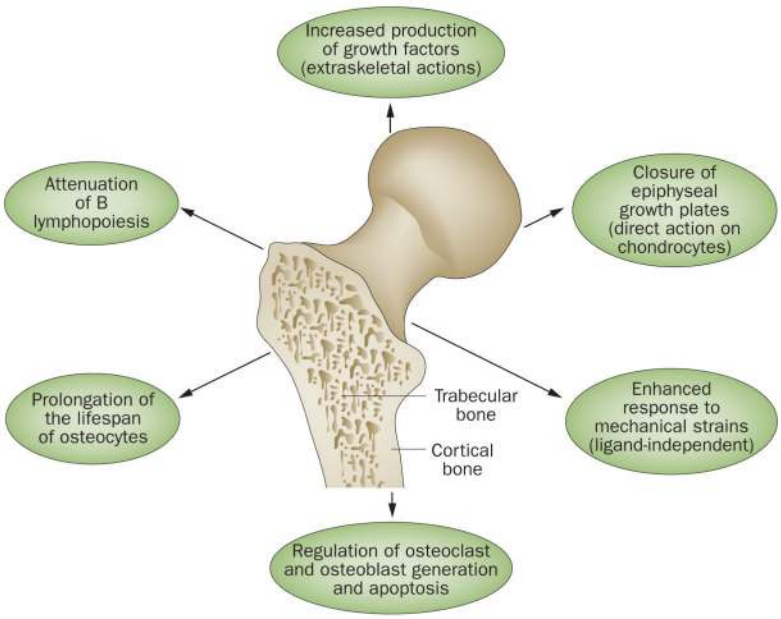

Figure 4.
Effects of sex steroid hormones and their receptors on bone.

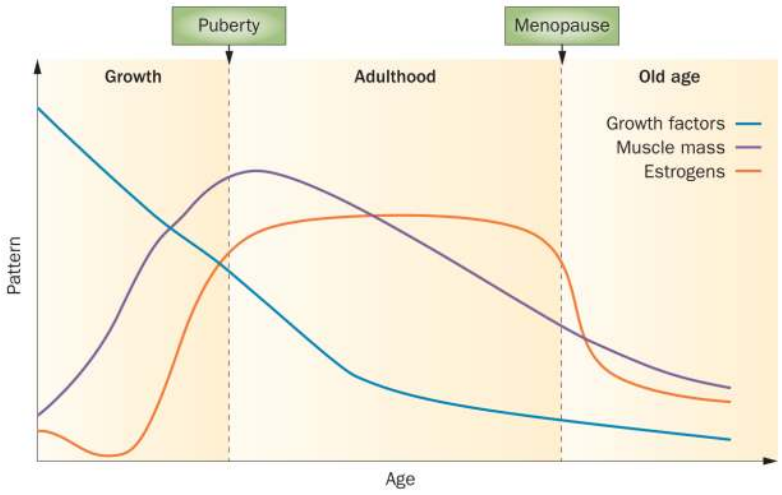

Figure 5.
Age-related changes in the levels of estrogens, muscle mass and growth factors. The Figure is based on data from two studies.[167,168]

Table 1

Bone mass changes in mouse models of targeted ERα or AR deletion

Target receptor	Genes used as drivers of Cre-mediated receptor deletion						
	LysM [38]	***Ctsk*** [37]	***Prrx1*** [31]	***Sp7*** [31]	***Bglap*** [53,60]	***Col1a1*** [31,61]	***Dmp1*** [52,59]
Female ERα							
Cortical	↔	↔	↓	↓	↓	↔	↔
Cancellous	↓	↓	→	→	↓*	↔	↔
Male ERα							
Cortical	ND	↔	↓*	↔	↔	↔	↔
Trabecular	ND	↔	↔	↔	↓*	↔	↓
Male AR							
Cortical	ND	ND	↔‡	ND	↓*	↔	↔
Trabecular	ND	ND	↓‡	ND	↓	↓	↓

*Transient changes.

‡S. C. Manolagas, unpublished work. Lysozyme M (*LysM*) Cre is expressed in myeloid monocytic precursors in several tissues. Cathepsin-k (*Ctsk*) Cre is highly expressed in cells committed to the osteoclast lineage. Paired related homeobox 1 (*Prrxl*) Cre is expressed in mesenchymal cells in the limbs. Sp7-Cre is expressed in cells committed to the osteoblast lineage and some chondrocytes. Osteocalcin (*Bglap*) Cre is expressed in osteoblast lineage cells and some chondrocytes. Collagen α-1(I) chain (*Col1a1*) Cre is expressed in osteoblast lineage cells. Dentin matrix acidic phosphoprotein 1 (*Dmp1*) Cre is expressed in mature osteoblasts and osteocytes. Abbreviations: ↔ no change; ↓ decreased bone mass; AR, androgen receptor; ER, estrogen receptor; ND, not determined.