Accepted Article

Journal of Research and Practice on the Musculoskeletal System JRPMS

Review Article

The effects of sex steroids on bone via extraskeletal actions

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Abstract

Sex steroids have direct effect on bone via their action on the estrogen and androgen receptors of bone cells. However, there is emerging evidence that their indirect actions on bone can play also important role in the pathogenesis of osteoporosis and bone loss in inflammatory diseases. The tissues that are involved in the extraskeletal actions of sex hormones and are better studied in the past few decades are adipose tissue, muscle tissue and the immune system. Key regulators on those complex pathways are molecules such as cytokines, adipokines and reactive oxygen species.

Keywords: Adipokines, Cytokines, Oxidative stress, Sarcopenia, Sex steroids

Introduction

The major role of sex steroids is the development of sexual characteristics. Testosterone, which is the main male sex steroid is produced by the Leydig cells in the testicle and is responsible for the differentiation of the male sex organs during the embryogenesis and the acquisition of secondary sexual characteristics, such as deep voice, sexual hair growth and penile enlargement during puberty. On the other hand, estradiol is the major female sex steroid and is synthetized mainly in the granulosa and theca cells in the follicles of the ovary, and plays a key role in the female sexual development and the growth of the female reproductive organs. Both estrogens and androgens are synthetized from cholesterol and their activation takes place within specific tissues such as bone. They are present in both sexes and are responsible for the differences of the male and female skeleton. Particularly estrogens are related with the epiphyseal maturation, the normal bone mineral accretion and the pubertal growth spurt.

The strong correlation between sex steroids and bone physiology became obvious during the study of osteoporosis, which is a very common metabolic disorder in older ages. Bone loss is a physiological characteristic of the ageing process but some people lose bone density faster and suffer greater risk of fractures. Women during menopause for example lose approximately 10% of bone mass and the decrease of estrogen levels plays a critical role in this condition. Sex steroid levels have direct effect on bone and they influence bone resorption and bone production via regulation of osteoclastic and osteoblastic activity. They directly reduce the number of osteoclasts on bone surfaces either by inhibiting the differentiation of osteoclasts or by promoting their apoptosis. Due to those antiosteoclastogenic properties, estrogens are used during menopause to prevent bone loss and hormone replacement therapy was a cornerstone in the treatment of osteoporosis in the past few decades.

Apart from the direct effect of sex steroids on osteoclasts, osteoblasts and osteocytes, estrogens and adrogens can also affect bone via extraskeletal actions. The aim of this article is to provide a contemporary summary of those actions. The key systems that will be analyzed are the immune system, the muscle cells, the adipose tissue and reactive oxygen species and how the actions of sex steroids on those tissues influence the bone.

The authors have no conflict of interest.

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Edited by: *Konstantinos Stathopoulos*

Accepted *22 June 2020*

Immune system

It has been noticed since many years that infections, inflammation and autoimmune diseases are accompanied by systemic or local bone loss. However, in the last few years researchers have expressed the hypothesis that lymphocytes mediate via diverse mechanisms in the bone loss. Recent findings from experimental models suggest that bone loss from lack of estrogens is tightly connected with pathologic activation of the adaptive immune system[1].

B and T lymphocytes are the 2 basic cell categories of the adaptive immune system, which is subcategorized in the humoral and the cell- mediated immune response. All lymphocytes are derived from multipotent hematopoietic stem cells in the red bone marrow. Later, they are differentiated to T or B lymphocytes in the primary lymphoid organs, more specifically in the thymus gland and the bone marrow, respectively. During the stages of their maturation they develop in their surface antigen receptors and they acquire the functional and phenotypic characteristics of mature lymphocytes.

T lymphocytes

It has recently been recognized that T cells and their products are key regulators of the formation and activity of osteoblasts and osteoclasts. Although it is known since many years that sex steroids and ageing regulate the immune system and the T cells, researchers have only in the last few years experimentally connected the lymphocytes with the genesis of postmenopausal osteoporosis, and with the bone lose which is related with endocrine diseases.

Experimental data

A characteristic example from a murine model is the fact that in mice which were T cell deficient, there wasn't noticed any bone loss after ovariectomy. One basic mechanism of bone loss in sex steroid deficiency is considered to be the increased production of TNF-a (tumor necrosis factor alpha) from activated T cells. Ovariectomy increases the number of TNF producing T cells in the bone marrow of mice[2]. TNF-α increases the formation of osteoclasts and bone resorption directly, but also indirectly via increasing the sensitivity of osteoclasts to RANKL, which is necessary for osteoclastogenesis. Besides it has been noticed increased production of RANKL by T lymphocytes of postmenopausal women compared to women before menopause. Therefore, the activation and the clonal expansion of the T cells that produce TNF-α and RANKL is a critical step in bone loss due to estrogen deficiency. In this phenomenon seems to be possible the participation of other proinflammatory cytokines such as TGF-β (transforming growth factor beta), IL-7 (interleukin 7), and IFN-γ (interferon gamma).

For example, TGF-β is a factor with controversial role in osteoclastogenesis and has three isoforms (TGF-β1, β2, β3), with TGF-β1 being dominant in lymphoid organs, while TGF- β2 and TGF- β3 are mainly expressed in mesenchymal tissues and bone[3]. Those factors activate or inhibit the proliferation of osteoblasts and osteoclasts depending on the cell lines and the in vitro culture conditions. Moreover TGF-β plays a role in the bone loss during estrogen deficiency. After local injection with TGF-β1 and β2, local bone loss was prevented in rats that had undergone ovariectomy[4]. Moreover, estrogens can increase the expression of TGF-β in murine osteoblasts, bone marrow cells and bone extracts. In humans persisting hormone replacement therapy with estrogens increases their serum levels of TGF-β1 and β2[5].

During the study of the rapid bone loss after menopause, researchers used many experimental models to evaluate the production of cytokines after the loss of estrogens. The majority of the experiments showed an increased production of TNF in monocyte cultures from postmenopausal women. The most remarkable finding was that this increase could be reversed after initiation of treatment with estrogens[6].

B lymphocytes

Apart from T cells, B cells are also important in the pathogenesis of bone loss in sex steroid deficiency. The number of B cells increases in the bone marrow of rats after the loss of either androgens or androgens. Furthermore, the administration of IL-7 to rats results in an increase in the number of B lymphocytes, as well as osteoclasts and promotes bone loss. Another interesting finding is that B cells or plasma cells derived from B lymphocytes from patients with multiple myeloma have the ability to enhance osteoclastogenesis possibly via overexpression of RANKL, or indirectly via IL-7 secretion, which is as mentioned above possible regulator of in vivo bone loss[7]. The increase of the number of B cells contributes to the fact that RANKL, which is expressed in their surface interacts with the RANK of osteoclast progenitors and promotes their differentiation to osteoclasts. Another possible scenario is that B cells themselves can act in some stage as osteoclast progenitors' pool, which are known to proliferate in the bone marrow after the loss of estrogens and adrogens.

The above mentioned scenario is supported by experiments that showed in vitro that B lymphocytes can function as osteoclast progenitors. More specifically, cells that express in their surface B220 or CD19 were exposed to RANKL and M-CSF in vitro. Under those circumstances the cells that were formed were multinuclear and had the ability to express TRAP and calcitonin receptor and could resorb bone[8]. As far as the correlation between B cells and sex steroids is concerned, it is remarkable that in male mice that the gene of androgen receptor was knocked out there was noticed increase in the number of B lymphocytes in their bone marrow.

Conclusively, on one hand sex steroid deficiency promotes B lymphopoiesis, on the other hand the treatment with estrogens suppresses B lymphopoiesis but stimulates the synthesis of immunoglobulins. In human in vitro

osteoclastogenesis models, B lymphocytes inhibit the formation of osteoclasts by the secretion of TGF-β, which enhances the osteoclast apoptosis and it has been noticed that stimulates the production of OPG.

Adipose tissue

Adipose tissue is a loose connective tissue and its basic components are the adipocytes. It is responsible for the storage of energy with the form of lipids. Apart from that it has been recently acknowledged the important endocrine role of fat, as it produces hormones such as leptin, estrogen, resistin, TNFα and plasminogen activator inhibitor-1, which are involved in energy homeostasis, the vascular homeostasis, the immune response and the fibrinolytic system. The main types of adipose tissue are white adipose tissue (WAT), which stores energy, and brown adipose tissue (BAT), which functions as body heat generator.

Testosterone and estradiol are key regulators of body fat distribution and it is known that they can generally be considered as promoters of adiposity. The gender dimorphism in body fat distribution is the following: women gather together fat in the gluteal and femoral regions, on the contrary men develop an android pattern with abdominal accumulation. Sex steroids are major determinants of body fat distribution via adipose tissue-specific expression of steroid receptors and local tissue steroid metabolism. ERα and ERβ are expressed in both subcutaneous and visceral adipose tissue, however ERβ is dominantly expressed in subcutaneous fat. Furthermore, enzymes that metabolize sex steroid hormones, such as 17α hydroxylase and aromatase are differentially expressed in subcutaneous and visceral adipose tissue and have the ability to locally convert steroids[9].

Menopausal estrogen deficiency leads to shifts in body fat distribution in postmenopausal women and it is well known that estrogen affects apart from the distribution, also the amount of fat mass. Also estrogen exerts a strong behavioral effect of spontaneous activity and low levels of estrogen are associated with low levels of physical activity. Those above mentioned increases in fat mass and the decrease of activity predispose women to lose muscle mass and strength secondary to inactivity[10].

Increased fat mass is considered to have positive effects on bone mineral density (BMD) and fracture risk due to greater mechanical forces that are applied on the skeleton and increased estrogen production through adipocyte aromatization. In premenopausal women, bone density is positively related to fat mass, lean mass and serum leptin and inversely related to adiponectin. On the contrary, researchers have observed that obese men suffer unaltered fracture risk despite higher BMD[11]. The factors that can affect the correlation between the adipose tissue and the bone mass are age, gender and menopausal status. Another parameter that must be noticed is the type of adipose depot, which can be subcutaneous or visceral[12]. Subcutaneous fat is positively related with bone mass and visceral fat is inversely associated with BMD. Testosterone induces a reduction in total body fat and promotes the accumulation of visceral fat in elderly men.

The relationship between fat and the skeleton has also been the subject of animal studies. Sex steroid receptors are expressed in adipocytes. In murine models the outcome of ERα and AR inactivation was obesity in both male and female mice[13]. Furthermore, in the recent study of McInnes[14] it was found that adipocyte-specific AR knock-out mice had greater risk of visceral obesity and impaired glucose homeostasis. The pathogenesis of adipogenesis during eradication of androgen receptor was studied by Huang[15] and his team as they examined the differentiation of bone marrow stromal cells (BMSCs) into bone or fat as an important stage at the development of osteoporosis. They found that the inactivation of AR enhanced adipogenesis of BMSCs via Akt activation through IGF signaling, and suppressed osteogenesis via inhibition of osteoblast differentiation from BMSCs.

Adipokines

The complex connection between adipose tissue and the skeleton arose during the study of factors that are secreted from the adipocyte, influence the fat mass and eventually have an impact on bone mass too. Those adipokines have been associated with circulating sex hormone levels in humans. Leptin, which is the most studied adipokine, as far as its relationship with the bone is concerned, is expressed in osteoblasts and chondrocytes and induces bone nodule formation via osteoblast differentiation. Moreover it promotes chondrocyte growth and is a regulator of osteoclastogenesis via changes in RANK, RANK-L and OPG secretion. Also leptin seems to influence the skeleton via actions on the central nervous system and more specifically the sympathetic nervous system. This can be supported by experimental data, that showed bone loss after the administration of leptin into the third ventricle of leptin-deficient mice[16]. This result was reversible after the β-adrenergic blockage. Central leptin administration leads to loss of appetite, weight reduction and bone loss which can also be connected with catabolic states and caloric restriction.

In animal models, systemic administration of leptin leads to a better bone formation, increased skeletal mass and strength[17]. Especially in leptin-deficient animals, leptin administration reverses the adipocyte phenotype and increases total body mineral content by >30%. On the other hand, high doses of leptin can act similarly to the central administration, leading to weight loss, bone loss and reduction of serum levels of IGF1. In humans systemic leptin administration seems to have beneficial actions on bone, as it was indicated by the study of Welt[18], who used leptin in 8 women with hypothalamic amenorrhea for 3 months, and noticed increases in estradiol, thyroid hormones, IGF1, bone alkaline phosphatase and osteocalcin.

Another important adipokine is adiponectin, which plays

important role in energy homeostasis, glucose and lipid metabolism and inflammation, and its low levels have been connected with coronary artery disease and type 2 diabetes mellitus. As far as bone is concerned, there are 2 receptors for adiponectin that have been isolated on osteoblasts and osteoclasts[19]. Adiponectin promotes osteoblast proliferation and inhibits osteoclastogenesis in vitro, but that could not be proved in vivo in studies with transgenic mice. Adiponectin seems to have more powerful indirect effects on bone through its influence on insulin resistance and growth factor binding. Finally, resistin increases osteoblast proliferation in both cell and organ culture systems as well as the formation and activity of osteoclasts[20].

As mentioned above the distribution of fat has different associations with bone mass. The production of leptin and adiponectin from visceral adipocytes is less. Also there is less aromatase activity and thus less estrogen production. On the contrary cytokines TNFα and IL6, which are related with bone resorption are found in greater concentrations in subjects with visceral adiposity.

Conclusivelly, adipose tissue and bone are connected with complex, dynamic and bidirectional pathways in order to achieve the goal of a strong skeleton to carry the essential for the homeostasis fat. Sex steroids and adipokines are key regulators of this relationship. Clinically and epidemiologically adiposity is positively related with bone density and is protective against osteoporosis, whereas sarcopenia and catabolic states are correlated with fracture risk.

Muscle tissue

Muscle tissue is a soft tissue that composes muscles in animals and is divided in three types: skeletal, smooth and cardiac. The basic component is the muscle cell (myocyte). Skeletal muscle is under voluntary control and is anchored by tendons to bone, in order to achieve skeletal movement and to maintain posture. Muscle is a tissue of great plasticity and thus can adapt to emerging functional demands. Increase mechanical forces applied on muscle lead to its hypertrophy, on the other hand reduced load or disuse results in atrophy. The hormonal profile and especially sex steroids play a central role in muscle growth, which is reflected by the differences in muscle mass between men and women. The mechanical forces that are applied on bone by muscles are very important for bone mass and strength, so the effects of sex hormones on muscle can indirectly affect bone mass. Both estrogens and androgens are crucial for the homeostasis and development of muscle and bone, but there is the perception that only testosterone has anabolic effects on muscle.

The anabolic effects of testosterone are widely known due to the androgen abuse by weightlifters and bodybuilders. In men with decreased endogenous sex steroids levels, the low levels of testosterone, but not estrogens results in lean mass, muscle size and strength loss. Testosterone augments muscle mass in young and older men. The same thing happens in postmenopausal women with low androgen levels that are treated with androgens. Despite the muscle mass increase, it is not proven beneficial effect on muscle strength and physical function.

Experimental data showed that male AR knockout mice had decreased muscle growth. Additionally, the eradication of ERα on the above mentioned mice diminished even more their muscle mass, giving proof of the role of estrogens on muscle mass development[21]. That doesn't seem to apply in humans, because testosterone alone is able to maintain muscle mass in older men. This was found since the inhibition of neither aromatase nor 5-a reductase could reduce the effectiveness of exogenous testosterone administration[22]. Furthermore, muscle-specific AR eradication in mice didn't reveal big changes in bone metabolism and there was a small decline in appendicular muscle mass, because only perineal muscles contain high levels of AR and are sensitive to androgens[23,24]. An interesting example is the maintenance of bone mass during estrogen replacement in male-to-female transsexuals, despite muscle loss due to androgen deficiency[25]. Despite the fact that the above observation is remarkable, it still cannot definitively prove that estrogens alone are enough for skeletal maintenance in older men.

Skeletal muscle and bone share common embryonic origin in humans and are highly responsive to sex steroids. In this above mentioned integration IGF-1 plays a key role. IGF-1 and IGF-II are mediators of anabolic effects on skeletal muscle and bone, whereas IGF binding protein-2 (IGFBP-2) is a negative regulator of BMD in both sexes. This is highlighted in the work of LeBrasseur[26] and his team, who observed that IGFBP-2, a protein whose circulating levels were in the past connected with low BMD and high bone -resorption markers, was now associated with low relative appendicular skeletal muscle mass in both women and men. The impressive finding of this study was that high IGFBP-2 levels were the stronger predictor of low BMD by DXA among men and postmenopausal women, independent of age and sex steroid levels. In older men they were also linked to osteoporotic fractures and poor physical performance and disability, providing new perception into possible biomarkers that evaluate the health of the muscle and bone.

The definition of sarcopenia includes the clinical features of loss of skeletal muscle mass and function. It is characterized by progressive and general decline of skeletal muscle mass and strength and is associated with physical disability, poor quality of life and death[27]. The study of Verschueren et al[28] achieved to investigate the correlation of sarcopenia and areal bone mineral density in middle-aged and older men in Europe. Men with muscle loss had lower areal BMD and suffered greater risk of osteoporosis compared to men without sarcopenia . It is still unclear if the maintenance or enhancement of muscle mass with physical exercise can reduce falls, bone loss and fractures. Exercise could be effective in states of hypogonadal bone

loss, as there is evidence for reversal of androgen deficiency on cancellous bone in AR knockout mice with increased physical activity[29].

Oxidative stress

Oxidative stress is the disturbance of balance between the production of reactive oxygen species (ROS) and antioxidants in favour of the oxidants leading to a disruption of redox signaling and tissue damage. ROS are naturally produced from the aerobic metabolism of cells. They are important for triggering essential signaling cascades. Moreover they can be products from the oxidation of fatty acids or be produced as a response to inflammation via inflammatory cytokines, or to other stimuli such as growth factors and UV light[30]. The research of oxidative stress raised interest to a wide spectrum of scientists from chemistry, cell biology and physiology to medicine and was associated with the complex ageing mechanisms. Increased levels of ROS were linked to age- related damage in many tissues. Bone couldn't be an exception and its interactions with ROS and sex hormones will be examined below.

The equilibrium between ROS generation and protection against ROS is necessary for bone homeostasis at any age. Loss of sex steroids enhances the aging of the bone partially by decreasing the defense against oxidative stress. The antioxidant properties of sex steroids seem to be reflected by their beneficial role on bone. More particularly, in the work of Almeida et al[31] it was noticed that estrogens and androgens reduced oxidative stress in bone and bone marrow and weakened the mature osteoblast apoptosis. In a mouse model it was shown that H2O2 triggers a PKCβ/p66^shc/ NF-κB cascade in cells of osteoblastic lineage and that P66^shc is an important mediator of the effects of H2O2 on the apoptosis of osteoblasts, and also H2O2 is capable to activate NF-κB. Both estradiol and dihydrotestosterone abolish the effects of ROS on p66^shc and NF-κB by weakening the phosphorylation of the redox- sensitive kinase PKCβ[32]. Moreover both of the above mentioned sex steroids protect from the H2O2- derived apoptosis via decreased phosphorylation of PKCβ. These information suggest that estrogens and androgens antagonize oxidative stress on osteoblastic cells.

There is also a strong correlation between ROS, estrogens and the osteoclasts. Osteoclasts are cells with high energy demands and contain large quantities of mitochondria. Mitochondria and ROS, especially H2O2 are important for osteoclast differentiation and function. Formation of ROS occurs in the mitochondria from the escape of electrons passing through the electron transport chain during aerobic metabolism. Free electrons are added to molecular oxygen and syperoxide, hydrogen peroxide (H2O2) and the hydroxyl radical are formed. H2O2 is the most important stimuli for apoptosis and changes to gene expression that lead to aging. ROS increase osteoclast number in vitro and in vivo by activating RANKL and TNFa expression through ERK and NF-κB activation[33]. On the other hand osteoclastogenesis leads to an increase of ROS through a cascade involving TNFα, TRAF6, Rac 1 and NADPH. NAC which is an antioxidant inhibits osteoclast formation and NF-κB stimulation in vitro, while H2O2 has the opposite effect which is indicative that a level of ROS generation is important for osteoclast formation[34]. Estradiol and dihydrotestosterone protect from bone loss via antioxidant effects in vivo. They diminish osteoclastogenesis and generate osteoclast apoptosis via a mechanism in which a crucial role is played by the upregulation of GSR[31].

Conclusion

On this review we got a glimpse of the broad spectrum of the extraskeletal indirect actions of sex steroids on bone. There are still many complex pathways and interactions of sex steroids with other tissues such as the above mentioned that could affect the bone. The research on this field and the better understanding of such mechanisms could be the next step on the development of new therapeutic targets on the battle against osteoporosis.

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