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Estrogen Deficiency and Osteoporosis

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<http://dx.doi.org/10.5772/59407>

1. Introduction

Estrogen plays a principal role in skeletal growth and bone homeostasis. In women, estrogen deficiency after menopause accelerates frequently osteoclastic bone resorption. It was believed that the accelerated phase in women is most apparent during the first 3 to 5 years after menopause, involved unproportional loss of trabecular bone [1].

We know that the mechanisms of how estrogen deficiency causes bone losses are complex. Researches during the last decade have demonstrated that estrogen regulates bone homeostasis through unexpected regulatory effects on the immune system and on oxidative stress and direct effects on bone cells [2].

We review how the differential effects of estrogen on cortical and trabecular bone might occur and how estrogen might interact with other age-related processes. Estrogen has three fundamental effects on bone metabolism:

1. It inhibits the activation of bone remodeling and the initiation of new basic multicellular units (BMUs);
2. It inhibits differentiation and promotes apoptosis of osteoclasts, therefore bone resorption reduces and
3. While estrogen suppresses self-regeneration of early mesenchymal progenitors, it organizes the commitment and differentiation and prevents apoptosis of osteoblastic cells, therefore bone formation is maintaining at the cellular level [1].

In this review, we summarized each of these actions of estrogen Figure 1,2.

2. The effects of estrogen on the bone cells

At the tissue level, estrogen reduces bone turnover [4]. It would appear that osteocytes may regulate the activation of bone remodeling via connections with bone lining cells [5]. It is likely that the antiremodeling effects of estrogen are mediated via the osteocyte. Osteocytes are cells embedded within the bone matrix, derived from the osteoblast that helps to bone remodeling. The bone lining cells comprise a subpopulation of the osteoblast family. These cells are related with the bone surface at sites where a thin no mineralized collagen layer is present. The bone lining cells migrate to form a canopy over the remodeling area, especially at sites adjacent to osteoclasts [6,7]. Indeed, withdrawal of estrogen is associated with increased apoptosis of osteocytes in humans [8]. Recent studies have reported that serum estradiol levels are inversely associated with serum levels of the key inhibitor of Wnt signaling produced by osteocytes, sclerostin, and estrogen treatment of postmenopausal women reduces circulating sclerostin levels [9,10]. Moreover, it has been suggested that Wnt/ β -catenin signaling is important to respond to mechanical strain of the osteocyte, and this response also depends on estrogen receptor α (ER α). Wnt signaling is interrupted in the mesenchyme, osteoblast differentiation is reduced and the skeletal development is affected [11]. Thus there is a likely important argument between estrogen and Wnt signaling pathways mediated by the osteocyte [1] Figure 1.

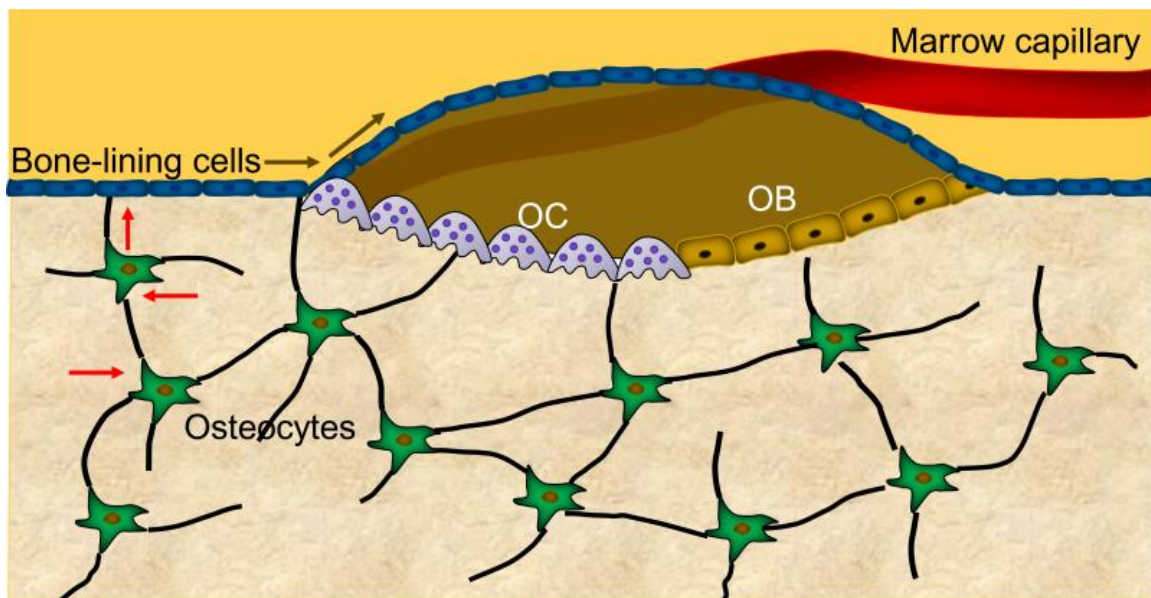


Figure 1. Schematic of the bone remodeling compartment [3] The bone remodeling compartment (BRC) consists of the cells comprising the basic multicellular unit (BMU)-osteoclasts (OCs), osteoblasts (OBs), and osteocytes-as well as the cover bone-lining cells and capillary. These cells are connected with gap junctions. Note also the potential direct physical contact between OCs and OBs, which would allow for signaling between these cells [3].

Estrogen suppresses both directly and indirectly bone resorption [1]. The dominant acute effect of estrogen is blocking the new osteoclast formation. In addition, estrogen also modulates RANK (Receptor activator of nuclear factor κ B) signaling in osteoclastic cells [12,13] and

induces apoptosis of osteoclasts [14,15]. Osteoclasts are cells of hematopoietic origin responsible for resorbing bone. These are originated from proliferation that occurs by cytokines and differentiation of the monocyte precursors. They are located on endosteal surfaces within the Haversian system and on the periosteal surface [7].

Osteoclast differentiation is supported by cells of the osteoblast lineage. Osteoblast lineage cells consist of osteoblasts, osteocytes and bone lining cells [7]. Osteoclast differentiation process is facilitated by stromal cells of bone marrow that provides physical support to immature OCs. The basic cytokines, need to OC formation under basal conditions are RANKL (Receptor activator of nuclear factor kappa-B ligand) and M-CSF (Macrophage colony-stimulating factor). These factors are produced by major stromal cells of bone marrow, OBs and active T cells. RANKL is a TNF superfamily member. RANKL links transmembrane receptor RANK expressed from OC surface and OC precursors. RANKL also links OPG (Osteoprotegerin), a soluble fake receptor produced by several hemapoietic cells. Therefore OPG functions as an power antiosteoclastogenic cytokine, secreting RANKL and blocking its binding to RANK. RANKL promotes differentiation of OC precursors from early stage of maturation to full maturation (multinucleated OCs) [16] Figure 2.

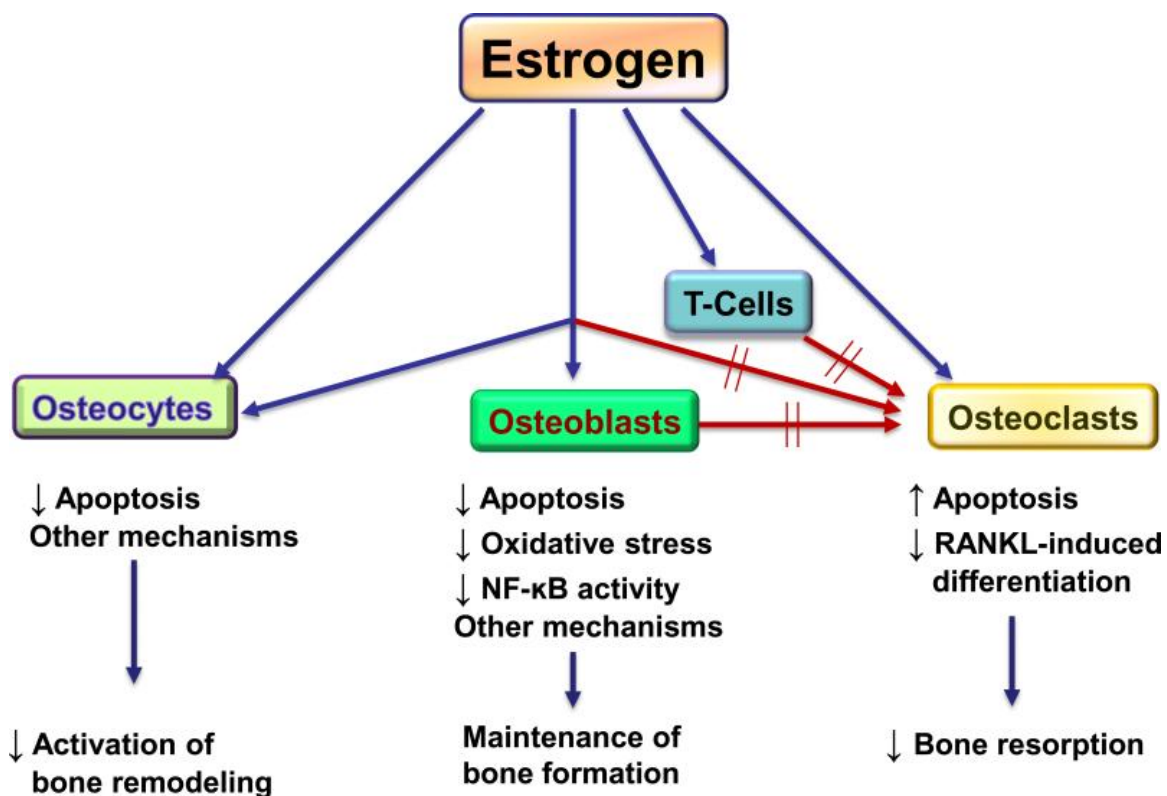


Figure 2. Working model for estrogen regulation of bone turnover via effects on osteocytes, osteoblasts, osteoclasts, and T-cells [3] The main effect of estrogen is inhibition of bone remodeling, likely via the osteocyte. Estrogen also inhibits bone resorption, by direct effects on osteoclasts, although effects of estrogen on osteoblast/osteocyte and T cell regulation of osteoclasts likely also play a role. Estrogen deficiency associated with a gap between bone resorption and formation, likely due to the loss of the effects of estrogen on decreasing osteoblast apoptosis, oxidative stress, osteoblastic NF-κB activity, and perhaps other, as yet undefined mechanisms [3].

Detection of molecular mechanisms that regulate the osteoclast formation and activation won a big acceleration by survey of RANK/RANKL signal system. M-CSF expression that is secreted by osteoblastic stromal cells need to differentiation to osteoclasts of progenitor cells but, M-CSF expression is not itself sufficient to differentiation of osteoclast. RANKL expression by osteoblastic stromal cells and RANK expression by osteoclast precursors need to be completed osteoclast differentiation [17] Figure 3.

Osteoblasts are cell of mesenchymal origin responsible for forming bone. These cells are found along the bone surface at sites of active bone formation. The principal function of the osteoblasts is bone formation [17].

Finally, estrogen is important for the maintenance of bone formation. Human studies show that acute (3 weeks) estrogen deficiency [18] is associated with a fall in bone-formation markers. Chronically estrogen deficient increased both bone-resorption and bone-formation markers [19]. It also appears that the effects of estrogen on progenitor and osteoblastic cells may be stage-specific. Thus, estrogen reduces the self-innovation of early mesenchymal progenitors [20].

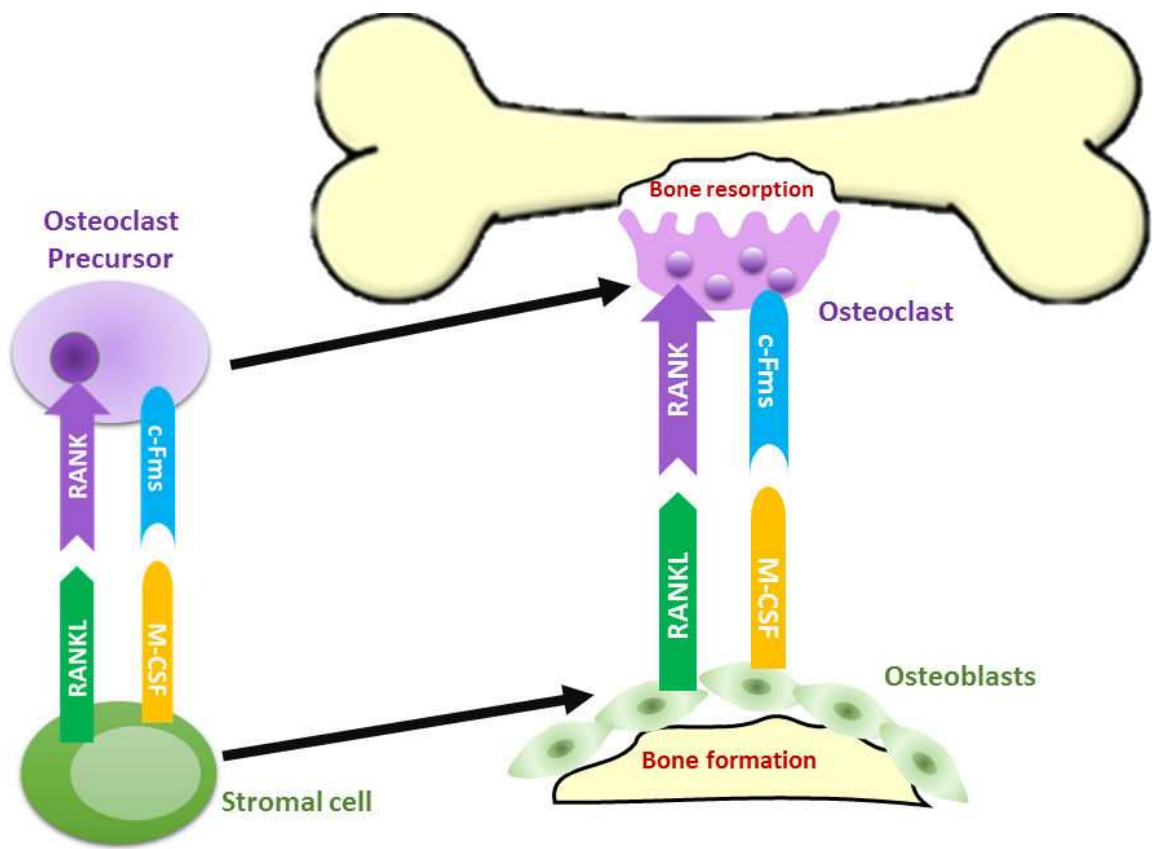


Figure 3. Cells and cytokines responsible for physiological OC renewal. OC precursors may differentiate from the population of monocytes/macrophages. When RANKL binds to receptor RANK in the presence of the trophic factor M-CSF, which in turn binds to its receptor, colony-stimulating factor receptor 1 (c-Fms), OC precursors differentiate and fuse together to multinucleated bone-resorbing OCs. Under physiological conditions the dominant source of RANKL and M-CSF in the bone marrow microenvironment is from the bone-forming cells, the OBs, and their stromal cell (SC) precursors.

It was reported that osteoblasts, osteocytes and osteoclasts express functional estrogen receptors (ERs). These receptors are also expressed in bone marrow stromal cells (SCs), the precursors of osteoblasts [21-24]. Estrogen signals through two receptors, ERalpha and beta [21]. Bone cells contain both receptors, but their distributions within bone are not homogeneous. In humans, ER alpha is the predominant in cortical bone, but ER beta is predominant in trabecular bone. Although many estrogenic effects are mediated by nuclear ERs, some effects originate in the plasma membrane. Estrogen produces rapid effects in various cell types such as bone cells. These nongenomic effects are due to signaling by a membrane receptor. The induce OC apoptosis and inhibit OB apoptosis of estrogen is linked to its ability to increase ERK1 and ERK2 phosphorylation and repression c-jun N-terminal kinase (JNK) activity [25,26].

It is well recognized that c-jun N-terminal kinases (JNKs) play important roles in cellular functions such as proliferation, differentiation, and cell death in a variety of cell types [24]. JNK activity is essential for the late-stage differentiation of osteoblasts [22].

Extracellular signal-regulated kinase 1 (ERK1) and ERK2 play essential roles in the lineage specification of mesenchymal cells. Inactivation of ERK1 and ERK2 significantly reduced RANKL expression, accounting for a delay in osteoclast formation. ERK1 and ERK2 not only play essential roles in the lineage specification of osteo-chondroprogenitor cells but also support osteoclast formation in vivo [27]. The phosphorylation of these cytoplasmic kinases and their transport to the nucleus modulates the activity transcription factors required for antiapoptotic actions of estrogen [16,26].

3. Estrogen loss and immune system

The immune system plays an important role in the pathophysiology of postmenopausal osteoporosis Figure 4. Estrogen deficiency induces bone loss and increases in production of cytokines, such as IL-1, IL-6, and TNF α . These cytokines are well known regulators of the immune system and T cell function [1,6]. The most important cytokine in the context of estrogen deficiency-induced bone loss has been shown to be TNF α produced by bone marrow T lymphocytes. T lymphocytes are stimulated by a complex mechanism involving several cytokines, such as IL-7, IFN-gamma, and TGF-beta [28].

Estrogen deficiency up-regulates TNF-alpha production by T cells through a complex pathway relative to the thymus and bone marrow. TNF-alpha increases OC formation by up-regulating stromal cell production of RANKL and M-CSF [28,29].

Another cytokine involving OC formation is interleukin-7 (IL-7). It has been reported that IL-7 promotes osteoclastogenesis by up-regulating T cell-derived osteoclastogenic cytokines including RANKL [28,30,31].

INFgamma influences OC formation both via direct and indirect effects [32]. It directly blocks OC formation and provides T cell activation inducing antigen presentation [28,33].

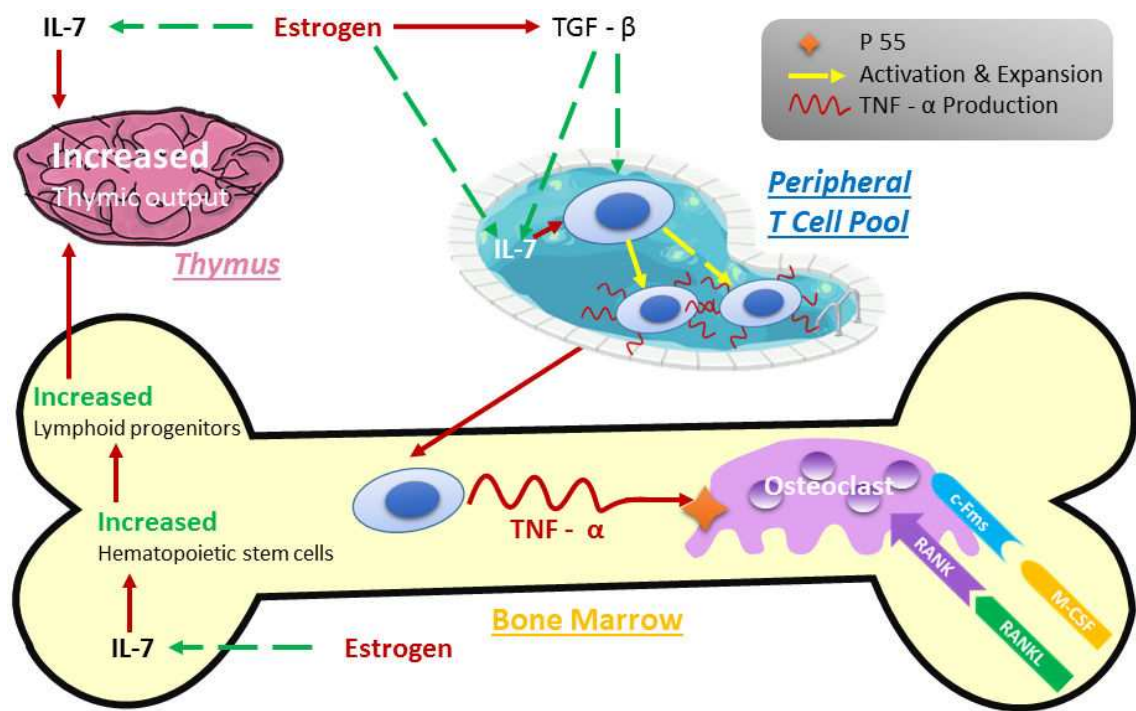


Figure 4. Estrogen suppresses T cell TNF production by regulating T cell differentiation and activity in the bone marrow, thymus, and peripheral lymphoid organs. In the bone marrow, estrogen downregulates the proliferation of hematopoietic stem cells through an IL-7 dependent mechanism. Estrogen prevents T cell activation both directly blunting antigen presentation and via repression of IL-7 and IFN- γ production. This effect is extended by the upregulation of the IL-7 suppressor TGF- β . The net result of them is a decrease in the number of TNF producing T cells. The blunted levels of TNF diminish RANKL-induced OC formation.

Estrogen deficiency induces the imbalance between RANKL and OPG. This situation is important in the occurrence of postmenopausal bone loss [34,35].

Recent studies show that postmenopausal women have a significantly higher concentration of circulating sclerostin than premenopausal women [28,36]. In vivo and in vitro studies report that TNF- α may stimulate the expression of sclerostin. Therefore, the increase of sclerostin mediated by TNF- α may contribute to the pathogenesis of postmenopausal women [28,37].

Sclerostin is a protein encoded by the SOST gene in osteocytes. It is a modulator of osteoblast function. Sclerostin antagonizes Wnt signaling and inhibits osteoblastic bone formation [38, 39].

Tyagi et al suggest that Th17 cells may be refer to the pathogenesis of bone loss. Th17 cells produce IL-17. Therefore, IL-17 plays a critical role in Ovx-induced bone loss and may be considered as a potential therapeutic target in pathogenesis of postmenopausal osteoporosis [40].

The thymus undergoes progressive structural and functional decline with age [41]. By middle age most parenchymal tissue is replaced by fat, and fewer T cells are produced, but the thymus progresses to generate new T cells even into old age [16,42,43]. The mechanism of thymic

rebound is not completely understood, but may be involving IL-7 [44]. IL-7 alone is not sufficient to enhance thymopoiesis in young mice [45].

Estrogen have suppressive effect on thymic function. Estrogen deficiency induces a rebound in thymic function. Stimulated thymic T cell output accounts for approximately 50% of the increase in the number of T cells in the periphery. Thymectomy also reduces by approximately 50% the bone loss induced by ovariectomy (ovx). This finding shows that estrogen deficiency induced thymic rebound may be responsible for exaggerated bone loss after surgical menopause or in the first 5-7 years after natural menopause [16].

4. Estrogen loss and oxidative stress

In fact, estrogen deficiency accelerates the effects of aging on bone by decreasing defense against oxidative stress (OS). Estrogen protects the adult skeleton against bone loss by slowing the rate of bone remodeling and by maintaining a focal balance between bone formation and resorption. The acute loss of sex steroids shows an increase in the rate of bone remodeling, resulting from an increase in both osteoclastogenesis and osteoblastogenesis [16].

Recent studies report that Reactive oxygen species (ROS) may play a role in postmenopausal bone loss by creating a more oxidized bone microenvironment [46,47] and increase the intracellular concentration of the antioxidant glutathione in bone prevents bone loss during estrogen deficiency in mice [48]. Glutathione peroxidase is responsible for intracellular degradation of hydrogen peroxide. It is the predominant antioxidant enzyme expressed by OCs. Its overexpression abolishes OC formation [49].

Though the mechanisms of ROS action on bone during estrogen deficiency are not well-known, it is reported that immune cells are biological targets of ROS. ROS are important stimulators of antigen presentation by dendritic cell (DC) as well as DC-induced T cell activation [50,51]. ROS are also raised upon dendritic cell interaction with T cells [52]. ROS can decrease T cell lifespan by stimulating T cell apoptosis [53].

It has been shown that NO donor nitroglycerin significantly prevents osteoporotic fractures in postmenopausal women [54] and N-acetyl-cysteine (NAC) treatment prevents against ovx-induced bone loss [48]. NAC treatment blunts ovx-induced dendritic cell activation in the bone marrow, and prevents T cell activation and TNF-alpha production [16].

5. Conclusion

Bone tissue is continually being renewed by osteoclast and osteoblast cells during normal physiology, but excessive resorption occurs without adequate new bone formation during postmenopausal osteoporosis. It has been suggested that such cellular changes are a result of postmenopausal estrogen deficiency. Recent studies report that estrogen deficiency exacer-

bates bone loss due to aging by diminishing the cells resistance to oxidative stress (OS). Moreover, a relationship between the immune system and bone has been speculated.

Remarkable progression has been registered for our understanding to the mechanisms of bone destruction during estrogen deficiency in the last 2 decades, but additional animal models and long-term human studies are needed.

Future studies will be guide to a new therapeutic advance in the treatment of osteoporosis with a novel mechanism of action that leads to the decrease of bone resorption and fracture risk.

Acknowledgements

The author thanks to Ajlan Okman, for assistance to design of figures.

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