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Bone metabolism regulation through RANK-RANKL-OPG system

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Summary

Osteoporosis is a disorder in which loss of bone strength leads to fragility fractures. The discovery of osteoprotegerin (OPG) and the receptor activator of nuclear factor-κβ ligand (RANKL) as final effector in osteoporosis pathogenesis have lead to a better understanding of bone remodelling. When RANKL binds to its natural receptor (RANK), osteoclastic differentiation and activation is initiated. OPG is a decoy receptor that binds to RANKL and prevents its osteoclastogenic effect.

Key words: *postmenopausal osteoporosis, osteoprotegerine, RANKL.*

Introduction

According to the definition proposed in 2001 by the National Institutes of Health (NIH) of the US, osteoporosis (OP) is a skeletal disorder characterised by a reduction in bone resistance which predisposes it to a high risk of fractures[1]. In Spain it has been estimated that approximately 13% of the female population has OP in the lumbar spine or in the femoral neck, these figures increasing to 26% in women over 50 years of age[2]. The prevalence in women is three times that in men[3].

The most significant clinical problems of OP are fractures and their complications. The fractures may develop towards a complete recuperation, or, on the contrary, be the cause of chronic pain, disability or even to an increase in mortality[4]. For hip fractures, this mortality has been estimated at 10-20% in one year[5]. In addition to this impact on the individual, osteoporotic fractures have significant repercussions on society, since they result in high health costs related to an increase in medical appointments, hospital admissions or in admissions into older peoples' residential homes[5].

Given the importance and the implications of this disease in the modern world, knowledge of its pathogenesis would support the development of its prevention and treatment. For this reason, this article will review this pathogenesis at some

length, starting with the process of bone remodelling and how this overlaps with mechanical processes and endocrine and local factors until it arrives at the final effector most recently described: the RANK/RANKL/OPG system.

Classical theory of bone remodelling

Bone fragility may be the result of a failure in the formation of bone, of an excess resorption or of inadequate formation of bone in response to an increase in resorption[6]. Bone remodelling is the physiological mechanism by which adult bone renews itself constantly by the activation of the basic multicellular units (BSUs), with the aim of repairing the microfissures caused by fatigue, and to maintain intact its structure and function[7]. The BSUs make up, morphologically and functionally, the bone tissue. They contain all the elements necessary for its remodelling, and act in an integrated and sequential way with the participation of the osteoclasts (derived from haematopoietic cells of monocyte-macrophage lineage) and the osteoblasts (of mesenquimal origin). The osteocyte, on its part, is a cell which is capable of activating osteoclasts or osteoblasts in response to antagonistic stimuli as a function of the local load. There are various theories about what the stimulus is which activates the osteocytes. It seems that the most commonly accepted theory would be that proposed by Parfitt[8], which suggests that the stimulus is produced by the movement of the fluid in the fibres which anchor the osteocyte to the bone walls in the canaliculi[9].

Each BSU, of which there can be a million functioning at any moment, starts its work at a set time and place, directing itself towards the area of bone which needs to be replaced[7]. To do this, it advances across the trabecular surface excavating and replacing the tissue. The cellular components of the BSUs, osteoclasts and osteoblasts act in an orchestrated fashion, completing each remodelling cycle[10]. The cycle (Figure 1) starts with the activation, by unknown mechanisms, of the bone surface in repose, which attracts from the bloodstream the pre-osteoclasts, precursors of the osteoclasts. The resorptive phase is initiated with the formation of the so-called Howship lacunae or resorption pits, and end with the apoptosis of the osteoclasts. The process of destruction is more rapid than that of regeneration, which means that any increase in the rhythm of remodelling will result in a loss of bone mass. Similarly, an excess of resorption may produce loss of trabecular structures, leaving the bone without a pattern for the new formation of bone[6].

As a consequence of bone remodelling, a number of biochemical markers for bone remodelling (MBR) are released, which show us in an indirect way the state of this formation/resorption process. An increase in products of resorption will indicate an accelerated bone turnover, showing a negative balance in the remodelling with an increase in bone loss. Thanks to these indirect markers it is possible to diagnose clinically and non-invasively the metabolic status of bone. Of these, those most used are the C and N telopeptides, which are terminal sections of the triple helix of type 1 collagen: one of these ends with carbon (C-telopeptide –CTx-) and the other two with nitrogen (N-telopeptide –NTx-). Due to their low molecular weight they are eliminated in the urine, from which we can determine their levels. In spite of this, nowadays most clinical studies determine the levels of the markers in the peripheral blood.

Initial role of bone mechanostat

The term mechanostat refers to the model proposed by Harold Frost[11] to explain the pathogenic mechanism by which the growth and loss of bone is regulated as a function of the mechanical stimulus which deforms it locally (force, pressure, torsion). According to this model, an increase in muscular force exerted on the bone (during growth or in response to an increase in load) or a reduction in load (through inactivity or immobilisation) will affect its mass, size and resistance in a positive or negative way, respectively. Thus through mechanical stimulus a system of feedback is put into action, which would determine when the bone needs more resistance or when it is not necessary[12].

Hence, the theory of mechanostat would explain how the mechanical load applied to the bone acts by setting in motion a complex process of bone remodelling, in which the osteocyte plays a fundamental role as mechanosensor, which transforms the mechanical signal in to a chemical one, which, in turn, produces the osteoblast/osteoclast response.

However, bones are essentially biomechanical. Remodelling is regulated by factors which are local and systemic: the process of adaptation of bone to load is not explained solely by a mechanical effect, but also depends on genetic-familial factors (most of the bone mass of an individual depends on their genetics) and on the normal state of the cells involved, on local-regional neurovascular factors, the endocrine-metabolic environment of the organism and the local environment of the bone[7,13]. Hence, the bone remodelling mechanism is modulated by both mechanical and non-mechanical factors (Table 1); among the latter, the most important are local factors, autocrines and paracrines, as well as endocrine-metabolic factors.

Hormonal regulation of bone metabolism

Among the non-mechanical factors involved in the metabolism of bone, the hormones play a primordial role. Hormonal regulation of the phosphorus-calcium metabolism is carried out through three principal hormones: parathyroid hormone (PTH), 1,25 colecacliferol (active metabolite of vitamin D_3) (Figure 2) and, to a lesser extent, calcitonin and four more hormones: growth hormone (GH), thyroxine, glucocorticoids and sexual steroids. Below, we briefly review the role of each of these.

1. PTH (parathyroid hormone), produced in the parathyroid glands, it is the hypercalcemic

hormone par excellence. It carries out its action at 3 levels: directly on the bone, stimulating the osteoclasts and favouring bone resorption, an action linked to the presence of vitamin D; in the kidney, increasing the distal tubular resorption of calcium; and indirectly on the intestine, stimulating the synthesis of 1,25 OH calciferol which, in turn, increases the absorption of calcium.

2. Calcitonin, produced in the C cells of the thyroid, acts directly on the osteoclast receptors. Its physiological role is controversial. It has been shown that in situations in which there is an increase in its secretion (carcinoma of the thyroid medullar) or in which there is an absence of C cells (total thryoidectomy) calcemia remains at normal levels and there are no changes in the bone[14]. However, at pharmacological doses, calcitonin possesses an inhibitory effect on bone resorption by reducing the number and activity of the osteoclasts, which means that it may be considered to be a hormone which is protective of bone tissue[15].

3. Vitamin D_3 is provided very scarcely through food, or is synthesised in the skin thanks to the action of ultraviolet solar radiation. It is transformed into 25 (OH) colecalciferol (calcidiol) in the liver and into 1,25 (OH) colecacliferol (calcitriol) in the kidney, biologically active forms. The principal action of vitamin D occurs in the small intestine, favouring the absorption of dietary calcium. In the bone it acts, in the presence of PTH, to stimulate the differentiation of the osteoclasts, and therefore, bone resorption, making possible adequate mineralisation. In the kidney it increases the proximal tubular resorption of calcium. A deficit or insufficiency of vitamin D_3, such as occurs in postmenopausal women, carries an increased risk of secondary hyperparathyroidism with the object of maintaining normocalcemia and an associated loss of bone mass.

4. Today, it is considered that GH (growth hormone) is synthesised, in addition to in the hypophysis, in other cells of the organism, including in the osteoblasts. Thus it is considered to have an endocrine, as well as a paracrine, effect[16]. GH acts directly on the osteoblast receptors, stimulating their activity, which produces an increase in the synthesis of collagen, osteocalcin, and alkaline phosphatase. It also acts indirectly by increasing the synthesis of insulin-like growth factors I and II (IGF-I and IGF-II) by the osteoblasts, which favours their proliferation and differentiation.

Figure 1. Phases of bone remodelling

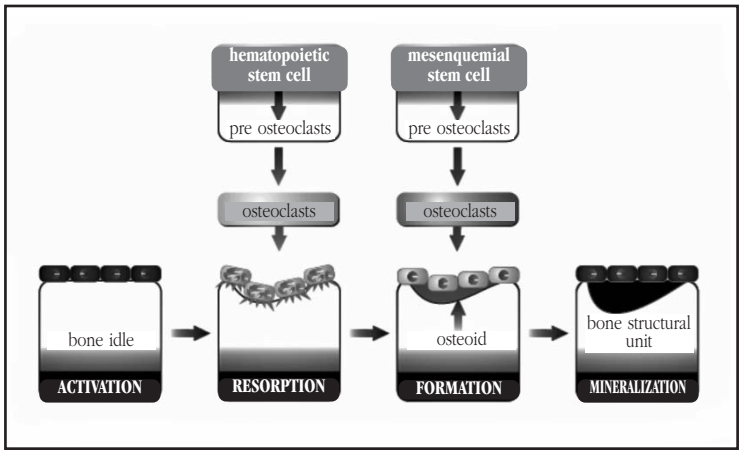
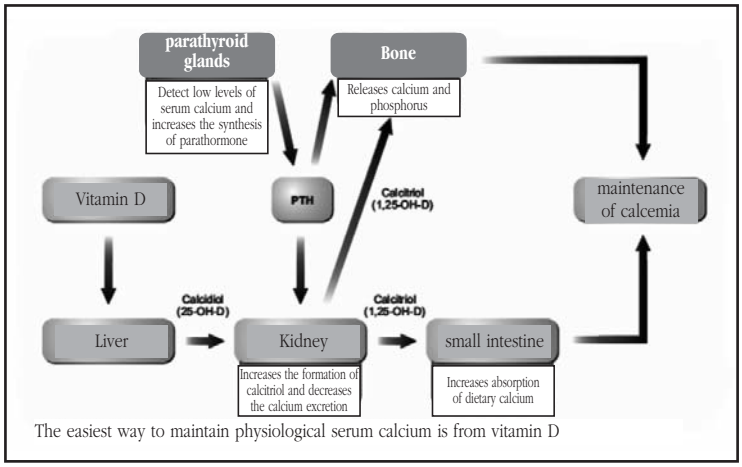


Figure 2. Regulation of phosphorus-calcium metabolism by PTH and vitamin D



5. The thyroid hormones are essentially hypercalcemics. In the bone, they act to stimulate the osteoclasts which accelerates the speed of bone turnover. Thus hyperparathyroidism brings with it the risk of OP.

6. The glucocorticoids act physiologically as modulators of bone remodelling. At pharmaceutical doses they diminish the activity of the osteoblasts and induce bone loss, leading to secondary OP, an action mediated in part by PTH and 1,25 OH colecalciferol.

7. The sex hormones play a significant role in bone. Until a few years ago it was thought that the fundamental role of the oestrogens in the maintenance of bone mass was the consequence of their interaction at all levels through the interleukine loop; generally, they inhibit resorption and stimulate the formation of bone. The oestrogens act on the osteoblasts directly, modulating their proliferation and differentiation, and increasing the secretion of the cytokines which, in turn, would act as a paracrine, stimulating or inhibiting the activity of the osteoclasts. On the other hand, they also have a direct effect on the osteoclasts in modulating

Table 1. Mechanical and non-mechanical factors related to the process of bone remodelling

Mechanical	Non-mechanical
• Gravitational force	• Hormones
• Weight of an individual	• Local factors (autocrines/paracrines)
• Physical activity-sedentariness	• Age/sex/genetics
• Muscular contractility	• Diet (calcium, vitamins, minerals...)
• Effort	• Work occupation/ergonomics
• Prolonged weightlessness-bedrest	• Some diseases (e.g. rheumatoid arthritis)

their activity (Figure 3)[17]. More recently it has been confirmed that the oestrogens can increase levels of osteoprotegerin (OPG), a protein which inhibits resorption produced by the osteoblasts, and because of which, could play a significant role in the regulation of osteoclastogenesis[15]. This activity would explain the loss of bone linked to hypoestrogenism after the menopause. For their part, the androgens have a fundamentally anabolic effect, in way which facilitates osteoblast action and inhibits bone resorption by diminishing the secretion of cytokines such as IL-6[18].

Local regulatory factors for bone remodelling

One of the most significant conceptual advances in the 1980s was the recognition of the regulatory role of many cytokines in the physiopathological regulation of bone resorption[19]. Currently it is known that bone contains cells which can produce prostaglandins, nitric oxide, growth factors, as well as cytokines, whose interactions are complex[20]. These substances, synthesized by the bone cells or coming from the medullar microenvironment, act in an autocrine or paracrine fashion, modulating bone remodelling[12]. Table 2 schematises the principal local factors and their principal action on bone formation or resorption.

Cytokines and growth factors

The cytokines are polypeptides synthesised in lymphocyte or monocyte cells whose role includes various cellular functions, such as the immunological response, inflammation and haematopoiesis. The growth factors are polypeptides originating in cells inside or outside the bone which act essentially on cell growth, differentiation and proliferation.

In the bone, those polypeptides act to increase the proliferation and differentiation of the preosteoclasts (resorptive action), in some cases (IL-1, GM-CSF, etc). This would immediately lead to the formation of active osteoclasts which favour bone resorption. In other cases, the growth factors act to stimulate the formation of bone, since they stimulate the differentiation of the osteoblasts (for example, TGB-β) or increase the number and function of these cells (IGF-I and II) and their consequent reparative action.

Convergence theory: the RANK-RANKL-OPG system

The remodelling of bone is responsible for the combined action of the osteoclasts and the osteoblasts in a sequential and antagonistic though independent action, both stimulatory and modulatory due to different factors (mechanical, hormonal, local). It has only been since the end of the 1990s that the final effects of the whole of this process has been known: the receptor activator of nuclear factor kappa β ligand (RANKL), its natural receptor (RANK) and osteoprotegerin (OPG), all belonging to the family of tumour necrosis factors (TNF). RANKL is a protein expressed by the osteoblasts and their precursors under the control of hormones, cytokines and pro-resorptive growth factors. The bonding of RANKL to its natural receptor on the cell surface of the osteoclasts and their precursors, RANK, stimulates the fusion of the pre-osteoclasts, promoting the adherence of the osteoclasts to the bone, activating their function and increasing their survival by avoiding apoptosis[21,22]. OPG is, in turn, a protein synthesised by the osteoblasts and the stromal cells, which acts as a dummy receptor, blocking the bonding of RANKL with its natural receptor RANK. In this way, OPG blocks every one of the actions of RANKL, producing a reduction in the number of osteoclasts and thus increasing their apoptosis[22,23] (Figure 4).

Taking into account the antagonistic effects of the RANKL and OPG proteins, it is easy to understand that bone remodelling depends ultimately on the balance between them, which, in turn, is influenced by the many factors which we have reviewed up to this point. Thus the “convergence

theory" considers that the RANK/RANKL/OPG system is the final effector of most of the regulatory factors for bone remodelling (Figure 5)[19].

The osteoclasts, as has already been said, are derived from mononuclear precursors from the monocyte-macrophage line. Their differentiation into mature osteoclasts requires the expression of the macrophage colony-stimulating factor (M-CSF) or the stromal osteoblasts, in a synergistic action. In addition, the completion of the process of differentiation requires the expression of RANKL by the osteoblasts, and of RANK by the osteoclast precursors. The osteocytes would regulate the recruitment and function of the osteoclasts, inducing the expression of RANKL by the osteoblast cells. The osteoclasts, in turn, are capable of regulating, positively or negatively the functions of the osteoblasts[23].

On their part, the hormones, the cytokines and the growth factors would act on receptors in the osteoblasts and other cells to induce the production of RANKL. Some of these factors also suppress the production of OPG by the osteoblasts, increasing the RANKL/OPG ratio. In this situation, the free RANKL acts on the osteoclast precursors (M-CFU), increasing their function and maturation, and also on the mature osteoclasts, increasing their activity and protecting them from apoptosis.

In addition to PTH, other proresorptive agents which regulate the expression of RANKL or OPG include the interleukins IL-1, IL-7 and IL-17; TNFα, glucocorticoids and vitamin D. On the other hand many molecules which inhibit bone resorption, such as the oestrogens, IFNγ, TGFβ and the interleukins IL-4 and IL-13, have also been shown to regulated the RANKL/OPG balance (Table 3).

The main physiological role of the RANK/RANKL/OPG system is the regulation of bone remodelling, involved in a wide range of bone diseases in which an imbalance occurs between formation and resorption[24]. In addition, other functions outside the skeleton related to vascular calcification, the immune system and the development of the mammary glands have been confirmed in animal models[25]. Mutations

Table 2. Principal local regulatory factors of bone remodelling (adapted from[16,20])

		Stimulus of resorption	Inhibition of resorption	Increase in formation
Cytokines				
Interleukins	IL-1	+		
	IL-4		+	
	IL-6	+		
	IL-11	+		
Tumour necrosis factors	TNF-α	+		
Colony stimulating factor	GM-CSF	+		
	M-CSF	+		
Interferón γ	IFN-γ		+	
Prostaglandins	PGE	+		
Growth factors				
Insulin-type	IGF-I y II			+
Transformer	TGF-β			+
Fibroblastic	FGF	+		
Derivatives of platelets	PDGF	+		
Bone morphogenic proteins	BMPs			+
Leukotrienes		+		
Nitric oxide	NO			+

Figure 3. Molecular and cellular actions of the oestrogens in the regulation of bone remodelling (adapted from Riggs[18])

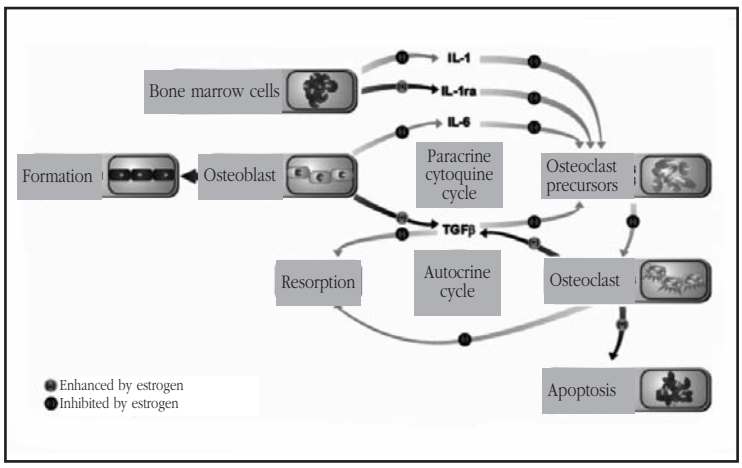


Figure 4. Regulation of osteoclastogenesis by the RANKL/RANK system: the RANK ligand bonds with its RANK receptor provoking the maturation of the pre-fusion osteoclasts into multinuclear osteoclasts and, finally, into activated osteoclasts

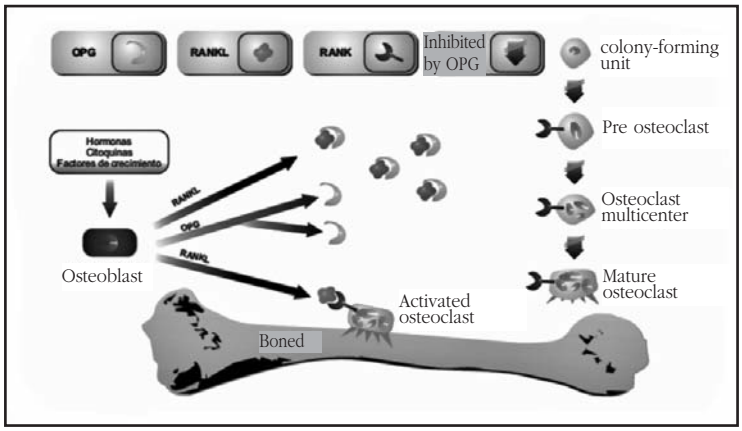
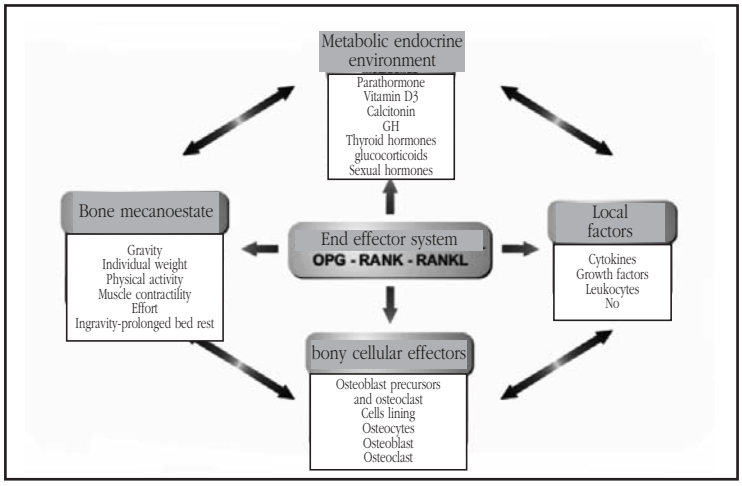


Figure 5. Convergence theory: confluence of modulatory factors of bone remodelling in the final effector system (adapted from Fiter[19])



have been identified in the genes for RANKL and OPG (and for their intracellular transporters) in diseases characterised by local alterations in bone remodelling, such as Paget's disease[26]. In animal models for rheumatoid arthritis, an early activation of RANKL and a suppression of OPG has been observed in inflamed joints[27]. It has also been seen that the cancerous cells in bone metastasis are capable of increasing the RANKL/OPG *ratio*, which stimulates bone resorption and makes available to the cancerous cells the space to grow within the bone. The same thing occurs with multiple myeloma, whose cells are even more destructive for bone[25]. The participation of the RANK/RANKL/OPG system in all these processes could have different clinical implications[25]:

- In prostate cancer OPG could be a new indicator for the diagnosis and early progression of the disease. In addition, given that those patients with prostate cancer who respond to antiandrogenic therapy have significantly lower levels of OPG, it could become a useful marker in the treatment of these patients. In addition, denosumab (AMG 162) (DMAB), an anti-RANKL monoclonal antibody, has been shown to increase bone mineral density and to reduce the incidence of new vertebral fractures in patients with prostate cancer who are receiving antiandrogenic therapy[28].

- Different studies have found that patients with multiple myeloma have lower concentrations of OPG than controls. Denosumab has shown persistent antiresorptive effects in patients with multiple myeloma and with bone metastasis from breast cancer[29].

- It is also possible that OPG participates in the pathogenesis of bone loss associated with chronic renal disease, and that which occurs after a solid organ transplant. However, as far as we know, the therapeutic possibilities of this participation by the RANK/RANKL/OPG system have not yet been explored.

- As has been commented on earlier, the RANK/RANKL/OPG system also plays a significant role in rheumatoid arthritis. Various randomised clinical trials with denosumab have shown the therapeutic usefulness of this anti-RANKL antibody in patients with rheumatoid arthritis which is not just limited to a reduction in bone loss, but also protection against bone erosion and structural damage[30,31,32].

- Finally, the aspect most investigated has been the aforementioned role of the RANK/RANKL/OPG system in postmenopausal osteoporosis. Thus, from the therapeutic point of view, it should be mentioned that denosumab has been authorized both by the America and European health authorities for this indication. The clinical data which endorse the utility of denosumab in the treatment of postmenopausal osteoporosis are reviewed in another article.

Final comments

Bone metabolism involves a series of phenomena much more complex than the simple interaction between a cell destructive of bone and one which forms new material as turnover, as was thought some decades ago. Thus, bone remodelling depends on many processes, not only cellular, but also endocrines of various kinds (oestrogens, vitamin D, PTH, calcitonin, even corticoids...), as well as auto- and paracrines with various growth factors, interleukins and leukotrienes, among others, which together with a true bone mechanostat, all come together in a final common effector system

which regulates the equilibrium between formation and resorption, which is the RANK-RANKL-OPG system.

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Table 3. Regulators of the expression of OPG, RANKL and RANK (adapted from Hofbauer[23])

	OPG	RANKL	RANK
Calcitriol	↑↓	↑	↑
Oestrogens	↑	↓ / —	
Testosterone	↑↓	—	
Glucocorticoids	↓	↑	
PTH	↓	↑	
IL1		↑	↑
IL4			↓ / —
IL7		↑	
IL-13	↑	↑	—
IL-17	↓	↑	—
TNF-α		↑	
Interferon γ	↑	↑	↑
PGE_2	↓	↑	
TGF-β	↑	↑↓	—
BMP_2	↑		—

↑ increase the expression; ↓ diminish the expression; — no changes were observed

Glossary

BMPs	Bone morphogenic proteins
M-CFU	Macrophage colony-forming units
M-CSF	Macrophage colony-stimulating factor
CTx	C-terminal telopeptide of type 1 collagen
FGF	Fibroblastic growth factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IFN-γ	Interferon γ
IGF	Insulin-like growth factor
IL	Interleukin
MBR	Markers for bone remodelling
NO	Nitric oxide
NTx	TN-terminal telopeptide of type 1 collagen
OP	Osteoporosis
OPG	Osteoprotegerin
PDGF	Platelet derived growth factor
PGE	Prostaglandin E
PTH	Parathyroid hormone
RANK	Receptor activator for nuclear factor κβ
RANKL	Receptor activator for nuclear factor κβ ligand
TGF-β	Transforming growth factor
TNF-α	Tumour necrosis factor
BMU	Basic multicellular unit

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