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The bone remodelling cycle

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Abstract

The bone remodelling cycle replaces old and damaged bone and is a highly regulated, lifelong process essential for preserving bone integrity and maintaining mineral homeostasis. During the bone remodelling cycle osteoclastic resorption is tightly coupled to osteoblastic bone formation. The remodelling cycle occurs within the Basic Multicellular Unit and comprises five co-ordinated steps; activation, resorption, reversal, formation and termination. These steps occur simultaneously but asynchronously at multiple different locations within the skeleton. Study of rare human bone disease and animal models have helped to elucidate the cellular and molecular mechanisms that regulate the bone remodelling cycle. The key signalling pathways controlling osteoclastic bone resorption and osteoblastic bone formation are Receptor Activator of Nuclear factor-κB (RANK)/RANK ligand (RANKL)/ Osteoprotegerin (OPG) and canonical Wnt signalling. Cytokines, growth factors and prostaglandins act as paracrine regulators of the cycle whereas endocrine regulators include parathyroid hormone (PTH), vitamin D, calcitonin, growth hormone (GH), glucocorticoids, sex hormones, androgens and thyroid hormone. Disruption of the bone remodelling cycle and any resulting imbalance between bone resorption and formation leads to metabolic bone disease, most commonly osteoporosis. The advances in understanding the cellular and molecular mechanisms underlying bone remodelling have also provided targets for pharmacological interventions which include antiresorptive and anabolic therapies. This review will describe the remodelling process and its regulation, discuss osteoporosis and summarize the commonest pharmacological interventions used in its management.

Keywords: Bone disorders remodelling, osteoblast, osteoclast, osteocyte, Wnt signalling, RANK/RANKL/OPG signalling, osteoporosis.

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Introduction

The skeleton, although perhaps not ordinarily thought of as such, is a dynamic, metabolically-active and functionally diverse organ. It provides levers for muscle to allow locomotion, supports and protects vital organs and is the site of haematopoietic marrow. Metabolically it has roles in both mineral metabolism, via calcium and phosphate homeostasis, and in acid-base balance via its buffering hydrogen ions.[1] Recent studies have also suggested that bone may have additional important endocrine roles in fertility, glucose metabolism, appetite regulation and muscle function.[2-5]

Throughout life the dynamic skeleton is 'constructed' and 'reconstructed' by two processes: bone modelling and remodelling.[6] Both processes involve osteoclastic bone resorption and osteoblastic bone formation. In modelling, resorption and formation occur independently at distinct skeletal sites to bring about major changes in bone architecture. By contrast, in remodelling, resorption and formation are tightly coupled both spatially and temporally so that the overall bone volume and structure remains unchanged.

Bone remodelling occurs continuously to repair skeletal damage, prevent accumulation of brittle hyper-mineralized bone, and maintain mineral homeostasis by liberating stores of calcium and phosphorus. Small regions of bone are resorbed by osteoclasts and replaced by osteoblasts; this close coordination between resorption and formation ensures that structural integrity is maintained whilst allowing up to 10 % of the skeleton to be replaced each year.[7] Remodelling is regulated by both systemic and local factors and the key signalling pathways have been identified by the study of families with rare bone diseases and in animal models.

This review highlights recent advances in understanding skeletal maintenance and repair and discusses the cellular and molecular mechanisms that underlie the bone remodelling cycle. It emphasizes the central role of the osteocyte in orchestrating both osteoclastic bone resorption and osteoblastic bone formation and describes the key regulatory pathways and drug targets including RANK/RANKL/OPG and Wnt signalling.

Bone cells

Within bone there are four major skeletal cell types

Cartilage-forming chondrocytes
Bone-forming osteoblasts
Bone-resorbing osteoclasts
Mechanotransducing and regulatory osteocytes

The cellular origin of the skeletal cell types is illustrated in Figure 1 and Table 1 details their structure, function and regulation. Bone lining cells are mature osteoblasts that cover quiescent bone surfaces; however, their role is incompletely understood and they will not be discussed further.

INSERT FIGURE 1 HERE

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Cell type	Description	Major roles	Key signalling pathways
Chondrocyte	Derived from pluripotent mesenchymal stem cells. Contain a round or oval nucleus and prominent rough endoplasmic reticulum containing secretory material. Cytoplasmic extensions allow the chondrocyte to interact with surrounding matrix.[8]	Proliferating chondrocytes secrete a type II collagen rich cartilage template upon which the endochondral skeleton is formed. Subsequently chondrocytes undergo hypertrophic differentiation, secrete a mineralizing type X collagen matrix and finally apoptose. The mineralized cartilage forms the template for bone formation. During growth, this process continues at the proximal and distal ends of long bones with linear growth occurring at the epiphyseal growth plate.[9] Surprisingly, recent data suggests that hypertrophic chondrocytes may also trans-differentiate into osteoblasts.[10]	Chondrocyte differentiation is controlled by an Indian hedgehog (IHH)/PTH-related Protein (PTHrP) negative feedback loop. Prehypertrophic chondrocytes secrete IHH which promotes chondrocyte proliferation directly and induces osteoblast formation and ossification of the surrounding periosteum. Furthermore, IHH induces PTHrP expression in the perichondral region which then acts via the PTHrP/PTH receptor, in the chondrocyte, to maintain proliferation and inhibit further differentiation thus reducing IHH secretion.[11] Proliferation and differentiation is also controlled by fibroblast growth factor (FGF) signalling. FGF actions are opposed by bone morphogenic proteins (BMPs).[11] Key transcription factors include SOX9 and Runx2. SOX9 is required for all stages of chondrocyte differentiation whereas Runx2 is required for hypertrophic differentiation.[11] During linear growth chondrocytes also express RANKL that regulates the resorption of the mineralized cartilage.[12]

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Osteoblast	Differentiate from mesenchymal stem cells but may also derived from bone lining cells and potentially chondrocytes.[10, 13] When active they have a large Golgi apparatus and endoplasmic reticulum essential form rapid osteoid synthesis.[14] Osteoblasts have three possible fates: they can become a bone lining cell, an osteocyte or undergo apoptosis.[7]	Secrete type I collagen rich bone matrix and regulate matrix mineralization.[15]	Transcription factor, SOX9, is present in all osteoblast progenitor cells.[16] The Runx2 transcription factor is required to initiate differentiation.[17] Transition from osteoprogenitors to preosteoblasts is regulated by the zinc finger transcription factor, OSX, which lies downstream of Runx2.[18] Osteoblastogenesis is controlled by the canonical Wnt signalling pathway. Wnt binds its receptor, Frizzled, and co-receptors, LDL receptor related protein 5 or 6, to increase nuclear β-catenin, which is essential for the specification of osteoblasts from mesenchymal precursors. Wnt signalling is antagonized by the secreted proteins Sclerostin (SOST) and members of the Dickkopf (DKK) family synthesized by osteocytes.[19-22] Hedgehog protein signalling, NOTCH, FGF and BMP signalling are also involved in the regulation of osteoblastogenesis.[16]
Osteoclast	Multinucleated cell formed by fusion of precursors derived from the monocytes/macrophage lineage. Podosomes facilitate adhesion to the bone surface and formation of a sealing zone provides an	Bone mineral is dissolved by secretion of hydrochloric acid and bone matrix is broken down by secretion of proteolytic enzymes including cathepsin K.[24]	Differentiation is initiated by macrophage colony stimulating factor (M-CSF) and promoted by RANKL acting on its cognate receptor RANK on precursor cells.[23]

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	isolated acidic microenvironment within which the osteoclast can dissolve mineral and digest the bone matrix.[23]		Osteoclastogenesis is negatively regulated by osteoblast-derived decoy receptor OPG which binds RANKL to block its binding to RANK.[25] Osteoclastogenesis may also be induced by immune cells in inflammatory diseases such as rheumatoid arthritis.[26]
Osteocyte	Long-lived terminally differentiated osteoblasts, entombed within bone and comprising >90% of all adult bone cells.[27] Exhibit long dendritic processes that ramify in canaliculae, throughout the bone matrix interconnecting osteocytes, and connecting osteocytes to bone lining cells and bone marrow cells, in a complex intercellular network.[28]	Mechanosensors that transduce bone-loading signals to orchestrate bone modelling and remodelling by regulating the action of osteoclasts and osteoblasts.[29, 30] Osteocytes are also involved in mineral homeostasis and secrete the phosphate regulator Fibroblast Growth Factor 23 (FGF23). FGF23 reduces serum phosphate levels by inhibiting renal phosphate resorption and inhibiting the activation of vitamin D thus reducing intestinal phosphate absorption.[31-33]	Major source of RANKL required for osteoclastogenesis during bone remodelling.[12, 34] Secrete SOST and Dickkopf-related protein 1 (DKK-1) the negative regulators of Wnt signalling that limit osteoblastic bone formation. Osteocyte secretion of SOST and DKK-1 is inhibited by mechanical loading, thus increased loading results in a local increase in bone formation.[35]

Table 1. Specialized bone cells involved in the bone remodelling process.

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Bone structure

Bone is a combination of osteoid matrix and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystal but bone also contains water, non-collagenous proteins, lipids and specialized bone cells.[1, 36] The type 1 collagen bone matrix gives bone elasticity, flexibility and tensile strength. The collagen fibres are made up of three helical chains and combine together to form fibrils. Fibrils are then interwoven and bound by crosslinks.[37] Non-collagenous proteins, adsorbed from the serum, also make up the matrix. The role of such proteins is becoming increasingly clear and their major functions include strengthening the collagen structure and regulating its mineralization. Bone mineral, in the form of hydroxyapatite crystals, is an essential store of calcium and phosphate required for mineral homeostasis and provides the skeleton with mechanical rigidity and compressive strength. Recently, NMR spectroscopy has given new insights into the detailed composition of bone matrix and mineral.[38]

Bones fulfil a protective and supportive role but are also essential for locomotion; they are therefore required to be strong yet light. Consequently, bones are made up of two, structurally distinct, types of bone - cortical and trabecular (cancellous). Cortical bone is solid with penetrating vascular canals and makes up the outer dense shell. It has an outer periosteal surface containing blood vessels, nerve endings, osteoblasts and osteoclasts and an inner, endosteal surface adjacent to the marrow.[39] On the endosteal surface of cortical bone is the honeycomb-like trabecular bone, which is made up of a fine network of connecting plates and rods.[8]

The structural differences between cortical and trabecular bone underlie their diverse functions. The majority of the mature skeleton (~80%), is dense cortical bone that has a lower rate of turnover and a high torsional resistance. Nevertheless, it can release mineral in response to a significant or long-lasting deficiency. By contrast, trabecular bone, which is less dense, more elastic, has a higher turnover rate, and high resistance to compression makes up the rest of the skeleton. It serves to provide mechanical support, helping to maintain skeletal strength and integrity with its rods and plates aligned in a pattern that provides maximal strength. Trabecular bone has a large surface area for mineral exchange and is more metabolically active than cortical bone, rapidly liberating minerals in acute insufficiency.[40] Consequently, trabecular bone is also preferentially affected by osteoporosis.[41]

The proportions of cortical and trabecular bone present are dependent on the individual bone's function. In vertebrae, trabecular bone predominates to resist compressive forces. By contrast, long bones, which principally act as levers, are mostly composed of cortical bone to allow them to resist both compressive and torsional forces.[41, 42]

Bone development

The skeleton is formed in two distinct processes. Flat bones such as skull vault are formed by intramembranous ossification where mesenchymal cells differentiate into osteoblasts which secrete and mineralize osteoid directly to form plate-like bones (Figure 2).

INSERT FIGURE 2 HERE

The multistep process of endochondral bone formation is illustrated in Figure 3. Endochondral ossification forms the majority of the axial and appendicular skeleton. In this

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process skeletal elements are initially formed as a cartilage template that is subsequently replaced by bone. Endochondral ossification begins when chondrocytes, differentiated from embryonic mesenchymal stem cells and secrete a collagen II rich matrix. The chondrocytes proliferate and then subsequently undergo hypertrophic differentiation, secreting a type X collagen rich matrix which then mineralizes. Chondrocyte apoptosis results in vascularization and formation of the primary ossification centre. The mineralized cartilage acts a template for subsequent trabecular bone formation mediated by osteoclasts and osteoblasts. Secondary ossification centres also form in the epiphysis at the proximal and distal end of long bones. The chondrocytes that remain between the primary and secondary ossification centres form the growth plate where linear growth occurs until quiescence or fusion at puberty.[11, 43]

INSERT FIGURE 3 HERE

Bone modelling

Bone modelling, which begins early in skeletal development, modifies the size and shape of a bone. In this process bone resorption and formation must be uncoupled; bone is removed from one anatomical site and new bone is formed at another. One important example of modelling is to preserve skeletal shape during linear growth. In the metaphysis, below the growth plate, there is osteoclastic resorption on the periosteal surface whilst there is new bone formation on the inner endosteal surface thus converting the shape of the epiphysis into the diaphysis.[44, 45]When these processes are disrupted, for example following antiresorptive (bisphosphonate) treatment of childhood osteogenesis imperfecta, a dramatic inhibition of normal metaphyseal modelling "Metaphyseal inwaisting" is seen.[46] Modelling is also responsible for radial growth of the diaphysis of long bones. Here osteoclastic resorption occurs on the endosteal surface whilst osteoblast bone formation occurs at the periosteal surface thus increasing the overall diameter with age.

The majority of bone modelling is completed by skeletal maturity but modelling can still occur, even in adulthood such as in an adaptive response to a mechanical loading and exercise and in renal bone disease.[47-50]

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Adult bone maintenance

The bone remodelling cycle

The skeleton regulates its own maintenance and repair by remodelling and this process also provides a mechanism for rapid access to calcium and phosphate to maintain mineral homeostasis.[51, 52] First defined by Frost, the bone remodelling cycle is a tightly regulated process that replaces old and damaged bone with new.[53] Anatomically the cycle takes place within a Basic Multicellular Unit (BMU), which is composed of osteoclasts, osteoblasts and a capillary blood supply.[54] The BMU lasts longer than the lifespan of the osteoblasts and osteoclasts within it and so requires constant replenishment of these cells which is critically controlled by the osteocyte. The structure and composition of the BMU varies depending on whether it is located within trabecular or cortical bone. In trabecular bone the BMU is located on the surface such that a 'trench' of bone, called Howship's lacunae, is resorbed then refilled. By contrast, in cortical bone the osteoclasts within the BMU form a cutting cone that 'tunnels' into the cortex, removing damaged bone. Behind the cutting cone new bone is then laid down concentrically on the tunnel walls by differentiated osteoblasts to leave a vascular supply within the Haversian canal of the new osteon.[55] In both instances the BMU is covered by a canopy of cells which delineate the bone remodelling compartment (BRC). The BRC provides a defined area of remodelling with close anatomical coupling of osteoclasts and osteoblasts.[56, 57]

Key steps in the remodelling cycle – cellular and molecular mechanisms

The remodelling cycle occurs in a highly regulated and stereotyped fashion with five overlapping steps of activation, resorption, reversal, formation and termination occurring over the course of 120 - 200 days in cortical and trabecular bone respectively.[58] Osteocytes orchestrate the bone remodelling by regulating osteoclast and osteoblast differentiation and thus bone resorption and formation as per Figure 4.

INSERT FIGURE 4 HERE

Activation

Osteoclast precursor cells are recruited from the circulation and activated; the bone surface is exposed as the lining cells separate from underlying bone and form a raised canopy over the site to be resorbed.[56] Multiple mononuclear cells fuse to form multinucleated preosteoclasts which bind to the bone matrix to form sealing zones around bone-resorbing compartments, thus isolating the resorption pit from surrounding bone.

Initiation of bone remodelling is the first important step ensuring that, in health, remodelling only takes place when it is required. In targeted remodelling, which refers to removal of a specific area of damaged or old bone, the initiating signal originates from the osteocytes that use their extensive network of dendritic processes to signal to other cells.[51, 59-62] Osteocyte apoptosis, induced for example by the disruption of osteocyte canaliculi caused by bone matrix microdamage, leads to release of paracrine factors that increase local angiogenesis and

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recruitment of osteoclast and osteoblast precursors. [30, 31, 60, 63] By contrast, non-targeted remodelling refers to remodelling in response to systemic changes in hormones such as PTH, thus allowing access to bone calcium stores and is not directed towards a specific site.

Resorption (Approximately two weeks in duration)

Osteoclast differentiation and activation is also regulated by osteocytes. Rearrangement of the osteoclast cytoskeleton results in adherence to the bone surface, formation of a sealing zone and generation of a ruffled border that provides a greatly enhanced secretory surface area. Initially osteoclasts pump protons, generated by Carbonic Anhydrase II, into the resorbing compartment to dissolve the bone mineral. Specifically, the H^+-ATPase pumps H^+ into lacunae; this is coupled to Cl^- transport via a chloride channel thus maintaining electroneutrality.[64] Subsequently, the collagen rich bone matrix is degraded by proteases such as cathepsin K and matrix metalloproteinases.[65, 66] The resorption phase is terminated by osteoclasts programmed cell death, ensuring that excess resorption does not occur.[67]

Reversal (Approximately four - five weeks in duration)[68]

The reversal phase, where bone resorption switches to formation, is still not well understood. However, there are thought to be two key events occurring. Firstly the freshly resorbed bone surface is prepared for deposition of new bone matrix and further signalling occurs that couples resorption to formation, ensuring that there is no net bone loss.[69, 70] Preparation of the bone surface is carried out by cells of an osteoblastic lineage which remove unmineralized collagen matrix, and a non-collagenous mineralized matrix 'cement-line' is then deposited to enhance osteoblastic adherence.[71]

The exact signal that couples bone resorption to subsequent formation is not yet fully understood. However, it is likely that the cells of the reversal phase are involved in sending or receiving these signals.[72-74]

It has been postulated that osteoclasts may be the source of the coupling factor, either secreting cytokines such as interleukin 6 (IL-6), or via a regulatory receptor on their surface such as the Ephrin receptor family and their membrane bound ligand, Ephrins, present on osteoblasts.[75] Other signalling pathways may include matrix derived factors such as BMP-2, transforming growth factor β and insulin-like growth factor.[76, 77]

Formation (Approximately four months in duration)[78]

New bone formation can be divided into two parts. Firstly, osteoblasts synthesize and secrete a type 1 collagen rich osteoid matrix. Secondly, osteoblasts play a part in regulating osteoid mineralization.[60]

The process of bone mineralization, whereby hydroxyapatite crystals are deposited amongst collagen fibrils, is complex and its regulation is incompletely understood. Control is exerted by systemic regulation of calcium and phosphate concentrations, local concentration of calcium and phosphate within extracellular matrix vesicles and by local inhibitors of mineralization, including pyrpophosphate and non-collagenous proteins such as osteopontin. The ratio of inorganic pyrophosphate to phosphate is a critical regulator of mineralization and

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the relative activities of tissue-nonspecific alkaline phosphatase and ectonucleotide pyrophosphatase are the key determinants of this ratio.[79-81]

Termination

Once mineralization is complete, osteoblasts undergo apoptosis, change into bone-lining cells or become entombed within the bone matrix and terminally differentiate into osteocytes. Osteocytes play a key role in signalling the end of remodelling via secretion of antagonists to osteogenesis, specifically antagonists of the Wnt signalling pathway such as SOST.[28]

Major signalling pathways

The remodelling cycle is tightly regulated to achieve balanced resorption and formation. Whilst systemically-released factors play a regulatory role, the fact that remodelling occurs at multiple, anatomically distinct sites at the same time indicates that local regulation is critical to achieving this fine balance. Accordingly, two key pathways, RANKL/RANK/OPG and Wnt transduce systemically and locally produced signals. Their regulatory role in determining the balance and timing of bone resorption and formation within the remodelling cycle makes them potentially important targets for pharmacological interventions in disease states such as osteoporosis.

RANKL/RANK/OPG Signalling Pathway

Identification of the RANKL/RANK/OPG Signalling Pathway in the 1990s was a crucial breakthrough in understanding the regulation of osteoclastogenesis in the remodelling cycle and provided the pharmacological target for the novel antiresoprtive denosumab.[82]

A permissive concentration of M-CSF, which is expressed by osteocytes and osteoblasts and stimulates RANK expression, is required prior to the action of RANKL.[83, 84]

RANKL binding to its receptor, RANK, on osteoclastic precursor cells, drives further osteoclast differentiation and facilitates fusion, activation and survival.[85, 86] RANKL/RANK binding induces downstream signalling molecules including mitogen-activated protein kinase, TNF-receptor associated factor 6, NF-kB and c-fos and ultimately activation of key transcription factors, including NFATc1, that regulate the expression of osteoclast genes.[23, 83, 84, 87, 88]

Whilst RANKL can be produced by osteoblasts, osteocytes and chondrocytes it is the osteocytes, within the bone matrix, that sense changes in load and microdamage that are thought to stimulate osteoclastogenesis via production of RANKL at the initiation of the bone remodelling cycle.[34, 89]

OPG, a decoy receptor for RANKL, was identified prior to the discovery of RANK/RANKL. It is secreted by osteoblasts and osteocytes and is able to inhibit osteoclastic bone resorption by binding to RANKL and preventing its binding to RANK.[12, 34, 90] Thus, the RANKL: OPG ratio is key in the regulation of bone resorption, bone mass and skeletal integrity and is modulated by a number of systemic factors (Figure 5).

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Wnt signaling

Study of rare human diseases with extreme bone mass phenotypes identified the canonical, β catenin-dependent, Wnt signalling pathway as a major regulator of osteoblastic bone formation (Figure 6).

INSERT FIGURE 6 HERE

In the absence of Wnt, a secreted glycoprotein, cytoplasmic β-catenin is targeted for proteosomal degradation by a multi-subunit destruction complex which phosphorylates and ubiquitinates β-catenin. Wnt target gene expression is therefore inhibited. When Wnt is present it binds to a dual receptor complex comprising Frizzled, a seven transmembrane domain receptor, and a co-receptor either lipoprotein related protein (LPL) 5 or 6. This blocks the action of the destruction complex leading to accumulation of cytoplasmic β-catenin. The β-catenin then translocates to the nucleus to activate target-gene transcription, leading to osteoblast proliferation and differentiation.[91]

In patients with osteoporosis-pseudoglioma syndrome, loss of function mutation of the LPL 5 co-receptor results in impaired Wnt signalling and osteoblastic bone formation, resulting in a low bone mass phenotype.[92] The secreted Wnt inhibitor, SOST, was identified by the study of the rare high bone mass disorders, sclerosteosis and Van Buchem disease. These inherited conditions are associated with loss of function mutations of SOST.

SOST is secreted by osteocytes and negatively regulates Wnt signalling by binding the co-receptors LPL 5/6. In quiescent bone, osteocyte expression of the Wnt inhibitors SOST, and DKK-1/2 prevents further bone formation.[91, 93] However, during the bone remodelling cycle osteocyte expression of the Wnt-inhibitors declines permitting osteoblast bone formation to occur after bone resorption. During the termination phase newly formed osteocytes become entombed within the bone matrix, re-express Wnt inhibitors, resulting in cessation of bone formation.[28]

Endocrine regulation of the bone remodelling cycle

Parathyroid hormone

PTH can have directly opposing effects on bone remodelling, depending on duration of exposure. Continuous PTH stimulates bone resorption, and is a key physiological mechanism in calcium homeostasis. Furthermore, the prolonged exposure to excess PTH that occurs in primary hyperparathyroidism, due to parathyroid adenoma or parathyroid hyperplasia, results in hypercalcaemia, bone loss and increased fracture risk.[94] Continuous PTH induces both cortical and trabecular bone loss but cortical bone is more severely affected. These catabolic effects are due to PTH's modulation of the OPG-RANKL-RANK signalling system. Via action in osteocytes and osteoblasts continuous PTH increases RANKL and inhibits OPG to stimulate osteoclastogenesis.[95] Monocyte chemoattractant protein 1, which is involved in the recruitment and differentiation of osteoclasts precursors, is also increased in response to excess PTH and is thought to play a role in patients with primary hyperparathyroidism.[96]

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By contrast, intermittently administered PTH is used as an anabolic agent in the treatment of osteoporosis. Intermittent PTH receptor stimulation enhances bone formation via modulation of Wnt signalling. Intermittent PTH signalling reduces expression of osteocyte-derived Wnt inhibitors SOST and DKK-1, whilst also increasing the Wnt ligand Wnt10b. The increase in canonical Wnt signalling results in increased osteoblastogenesis, target-gene expression and enhanced bone formation.[95, 97-99]

Vitamin D

$1,25(OH)_2$Vitamin D regulates intestinal calcium and phosphate absorption providing the substrates for bone mineralization. However, the physiological actions of $1,25(OH)_2$Vitamin D in the bone remodelling cycle remain uncertain.

Several studies have reported expression of the Vitamin D Receptor (VDR) in osteoclast and osteoblast precursors, and in osteocytes, suggesting that vitamin D may also mediate direct effects in bone. VDR expression has been shown in human osteoclast precursors but studies in the mature osteoclast have been contradictory.[100-102] Similarly, osteoblast precursors express the VDR whereas only low levels are detectable in mature osteoblasts.[103, 104] Despite this, studies in osteocytes have demonstrated VDR expression.[105] Furthermore, *in vitro* studies have shown activity of the vitamin D activating enzyme 1α hydroxylase in human osteoblast, osteoclast and mRNA expression in osteocytes suggesting possible local regulation of vitamin D activity in skeletal cells.[105-107]

By contrast, initial studies in global VDR deficient mice showed that their abnormal skeletal phenotype could be rescued by dietary calcium supplementation alone, suggesting any direct actions of vitamin D in skeletal cells are likely be limited.[108, 109] Consistent with this, cell specific deletion of the VDR in the late osteoblast/osteocyte lineage, using *Dmp1-Cre*, resulted in no significant skeletal phenotype when animals were fed a normal diet. Nevertheless, these mice were partially resistant to hypercalcaemia and hypomineralization induced by high dose $1,25(OH)_2$Vitamin D indicating a potential role for the osteoblast VDR in regulating mineralization.[110] Furthermore, osteoblast specific VDR deletion, using the *Col1a1-Cre*, resulted in a small increase in trabecular bone volume in older animals [111] whilst transgenic osteoblast specific VDR over-expression increased bone mass and strength due to increased osteoblastic bone formation and reduced osteoclastic resorption.[112, 113]

Taken together, these data confirm a primary role for the intestinal VDR in regulating the calcium supply for skeletal mineralization, but suggest that vitamin D may also have direct actions in skeletal cells.

Calcitonin

Calcitonin is synthesized in the parafollicular C-cells of the thyroid, but its physiological role remains uncertain. At pharmacological concentrations calcitonin inhibits bone resorption, acting via the calcitonin receptor in osteoclasts, to reduce osteoclast number, secretory activity and ruffled border formation.[114, 115] By contrast, calcitonin deficient mice show increased bone formation and at physiological concentrations calcitonin inhibits the actions of sphingosine-1-phosphate, a coupling factor that links bone formation to resorption.[116, 117]

Thyroid hormone

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Thyrotoxicosis is an established cause of secondary osteoporosis and is associated with both increased osteoblastic bone formation and increased osteoclastic bone resorption. Thyroid hormones directly stimulate osteoblast differentiation and mineralization but it remains uncertain if thyroid hormones have direct action in osteoclasts.

Thyroid hormone deficiency leads to a lengthening of the bone remodelling cycle with low bone turnover and increased bone mass. Conversely, hyperthyroidism increases bone turnover, decreases the duration of the bone remodelling cycle and leads to uncoupling of osteoblastic and osteoclastic activity, resulting in a 10% loss of bone per remodelling cycle.[118]

Growth hormone and Insulin-like growth factor 1

GH induces Insulin-like growth factor 1 expression, increasing bone turnover by stimulating both osteoblast proliferation activity and osteoclastic bone resorption. Nevertheless, osteoblastic bone formation predominates, leading to a small net increase in bone mass.[119, 120] By contrast, in GH deficiency, bone resorption outweighs bone formation, ultimately leading to osteoporosis.

Glucocorticoids

At supra-physiological doses glucocorticoids cause osteoporosis (Table 3). Glucocorticoids inhibit osteoblast differentiation and function, and increase osteoblast apoptosis.[121] By contrast, glucocorticoids increase in osteoclastic bone resorption by reducing OPG and increasing RANKL expression by osteoblasts and increasing RANK expression in osteoclasts. However, the enhanced bone resorption is only transient and prolonged glucocorticoid treatment results in reduced osteoclast numbers and resorption.[122-124] At physiological concentrations, however, glucocorticoids have been shown to have an anabolic effect on bone turnover.[125]

Sex hormones

Postmenopausal osteoporosis is characterized by uncoupling of the bone remodelling cycle with increased osteoclastic bone resorption relative to osteoblastic bone formation, resulting in net bone loss. Accordingly, oestrogen, acting via the oestrogen receptor-α, inhibits bone resorption by reducing osteoclast number and activity and increasing osteoclast apoptosis. [126] Oestrogens also inhibit osteoblast and osteocyte apoptosis to maintain bone formation and limit bone remodelling.[127, 128]

Aromatase converts androgens to oestrogens and in postmenopausal women adrenal steroids are the only source of oestrogens.[129] Thus, women on aromatase inhibitors or with reduced aromatase activity are at an increased risk of osteoporosis. Similarly, aromatase plays an important role in bone mass in men. It has been shown that oestrogen, rather than androgen levels, determine bone mass in the aging male population.[130]

Androgens, like oestrogens, favour net bone formation by stimulating bone formation and inhibiting resorption.[131] Low levels in men lead to an increased rate of remodelling, which is also due to less oestrogen being aromatized from testosterone.

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Oestrogen or androgen deficiency leads to an increase in bone remodelling. Whilst both osteoblastic bone formation and osteoclastic bone resorption are increased, uncoupling results in resorption outweighing formation.[132]

Paracrine regulation of the bone remodelling cycle

Growth factors

Transforming growth factor β (TGF β) and BMPs are both members of the TGF β superfamily, and are present in the bone matrix. They signal through canonical (Smad) and non-canonical (Smad-independent) pathways. They induce expression of the master osteoblast transcription factor, Runx 2, which is required for initiation of osteoblast differentiation.[133] TGF β1 has also been implicated in coupling of resorption to bone formation by inducing migration of mesenchymal stem cells to resorptive sites.[134]

Prostaglandins

Prostaglandins act locally via multiple G-protein coupled receptors to regulate bone resorption and formation. Nevertheless, the exact role of prostaglandins in the bone remodelling cycle remains unclear. For example, Prostaglandin E_2 (PGE_2) is a potent stimulator of bone resorption and is thought to act by increasing the RANKL/OPG ratio to enhance osteoclastogenesis. However, PGE_2 also stimulates osteoblast proliferation and differentiation to increase bone formation. It is thought the divergent actions result from PGE_2 acting via different G-protein receptors and secondary messenger pathways.[135, 136]

Cytokines

Cytokines, such as IL-1 and IL-6, and TNFα can stimulate osteoclastogenesis whereas others, such as IL-4 and gamma interferon, inhibit osteoclast formation.[137, 138]

In post-menopausal women these cytokines play an important role in the pathophysiology of osteoporosis. Oestrogen deficiency results in an increase in IL-1, IL-6 and TNFα, leading to an increased RANKL expression and increased osteoclastogenesis and bone resorption.[139]

Abnormalities of the bone remodelling cycle

Osteoporosis

In healthy adults bone the remodeling cycle displays tight coupling between bone resorption and bone formation. Accordingly, several metabolic bone diseases including osteoporosis, hyperparathyroidism, Paget's disease and osteopetrosis are characterized by loss of such coupling. This field has been previously extensively reviewed by Feng and McDonald and therefore this review will focus specifically on osteoporosis.[140]

Osteoporosis is the most common metabolic bone disorder and resultant fragility fractures are associated with increased morbidity and mortality; its European prevalence is 27.6 million

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and 1 in 3 women and 1 in 5 men over 50 will sustain osteoporotic fractures.[141-143] Osteoporosis may be diagnosed following a fragility fracture or by Dual Energy X-ray Absorptiometry (DEXA) T-score ≤-2.5 (T-score represents the number of standard deviations from the mean of an appropriate young reference population). It may also be suggested by the results of plain radiographs or computed tomography scans. Alternatively, osteoporosis may be defined qualitatively as a decrease in bone mass and strength, leading to increased fracture risk.[144, 145] Osteoporosis may be a consequence of (i) a failure to reach normal peak bone mass during growth (ii) a relative increase in bone resorption during adulthood or (iii) a relative reduction in bone formation during adulthood.

Primary osteoporosis is the most common form of osteoporosis and includes both post-menopausal and age-related osteoporosis. By contrast, secondary osteoporosis is a consequence of systemic disease or pharmacological intervention and its aetiology includes:

i) Endocrine disorders (acromegaly, adrenal insufficiency, Cushing's syndrome, diabetes, hyperthyroidism, hyperparathyroidism, hyperprolactinaemia, hypogonadism, eating disorders and endometriosis).
ii) Connective tissue disease e.g. rheumatoid arthritis and ankylosing spondylitis.
iii) Genetic diseases, including osteogenesis imperfecta, homocystinura, hypophosphatasia
iv) Drugs, including glucocorticoids, antiepileptics, anticoagulants, chemotherapy, gonadotrophic-releasing hormone agonists/antagonists and immunosuppressants.
v) Metabolic disorders, including renal and liver disease.
vi) Gastrointestinal and nutritional disorders e.g. parenteral nutrition, gastrectomy or post-gastric bypass, malabsorption, pancreatic insufficiency, inflammatory bowel disease, coeliac, chronic cholestatic disease, primary biliary cholangitis.
vii) Disorders of the bone marrow e.g. myeloma, pernicious anaemia.
viii) Multiple sclerosis, congenital porphyria, chronic obstructive pulmonary disease, idiopathic hypercalciuria, idiopathic scoliosis, calcium deficiency.

The most common causes of secondary osteoporosis are glucocorticoid treatment and immobilization.[146]

Whilst osteoporosis has many and diverse causes, uncoupling of the bone remodelling cycle and increased bone resorption relative to formation is a common underlying pathophysiological mechanism. The excess skeletal resorption results in structural deterioration and increased fragility. Microscopically sites of osteoclastic bone resorption are incompletely repaired by newly formed bone, resulting in progressive bone loss and increasing cortical porosity.[41, 147]

Initially, osteoporosis may predominantly affect trabecular bone due to its greater surface area. Nevertheless, cortical bone is also affected and its increasing porosity is associated with fracture risk.[148, 149]

The underlying pathophysiology associated with the commonest forms of osteoporosis are detailed in Table 3.

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Osteoporosis type	Description	Cellular and molecular mechanism
Postmenopausal osteoporosis (Primary) [147]	The menopause is characterized by reduced oestrogen levels. This results in accelerated bone remodelling; both resorption and formation are increased but the rate of resorption exceeds formation.[150]	Oestrogen deficiency results in increased cytokines including IL-1, IL-6 and TNFα. Increased RANKL and reduced OPG result in enhanced osteoclastogenesis and decreased apoptosis.[151, 152]
Age-related osteoporosis (Primary) [140]	Due to a combination of age-related and postmenopausal factors in women and age-related factors in men. Multifactorial aetiology with bone loss being dependent upon genetic and lifestyle factors.	Osteoblastogenesis and bone formation are reduced by decreased GH, increased PTH and increased reactive oxygen species. Sex steroid deficiency in men leads to decreased levels of oestrogen in bone (conversion by aromatase) and thus increased osteoclastogenesis and bone resorption.
Glucocorticoid-induced osteoporosis (Secondary) [153]	An initial and transient increase in osteoclastic bone resorption is followed by a prolonged reduction in both osteoblastic bone formation and osteoclastic bone resorption. The largest reduction in bone mineral density (BMD) occurs in the first year of glucocorticoid therapy. Glucocorticoid treatment is associated with both a quantitative bone loss and a reduction in bone quality.[154]	Suppression of Wnt signalling leading to inhibition of osteoblast differentiation.[155] Mesenchymal precursors preferentially differentiate to adipocytes rather than osteoblasts following induction of transcription factors such as peroxisome proliferator-activated receptor gamma. Increase in osteoblast and osteocyte apoptosis.[121] Whilst glucocorticoids lead to reduced numbers of osteoclast progenitors, in the initial phase of glucocorticoid-induced bone loss, the lifespan of osteoclasts is prolonged.[154, 156]
Immobilization-induced osteoporosis (Secondary) [157]	Physiological response to reduced mechanical loading. Examples include paralysis following spinal cord injury, prolonged bed rest and space flight. Bone resorption is increased and formation reduced resulting in a deterioration in bone structure and a marked decrease in bone mass.[158]	Still incompletely understood. Osteocytes detect reduced load and the RANKL: OPG ratio increases leading to greater osteoclastic resorption.[63] SOST levels also increase inhibiting bone formation.[159-161]

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Table 3. Pathophysiology of commonest causes of osteoporosis.

Pharmacological interventions

Current osteoporosis treatments can be divided into; (i) those that inhibit osteoclastic bone resorption, such as bisphosphonates, Selective oEstrogen Receptor Modulators (SERMs) and anti-RANKL antibodies and, (ii) those that increase bone formation including strontium ranelate and human PTH (1-34). (Table 4).

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Therapy	Mechanism of action	Efficacy	Primary prevention guidelines for osteoporosis (The National Institute for Health and Care Excellence (NICE)/Scottish Medicines Consortium (SMC))	Secondary prevention guidelines for osteoporosis (NICE/SMC)	Important side effects
Bisphosphonates **Examples (route of administration):** **Nitrogen-containing bisphosphonates:** **-Alendronic Acid (oral)** **-Risedronate Sodium (oral)** **-Ibandronic acid (oral or IV)** **-Zoledronic acid (IV)** **-Pamidronate disodium (IV)** **Simple bisphosphonates:** **Etidronate**	Bisphosphonates selectively bind to the bone mineral surface and inhibit osteoclastic bone resorption. Nitrogen-containing bisphosphonates inhibit farnesyl pyrophosphate synthase (FPPS) in osteoclasts. FPPS is a rate limiting enzyme in the HMG CoA reductase pathway. Its inhibition results in impaired action of key regulatory GTP-binding proteins leading to inhibition of osteoclast function and increased osteoclast apoptosis. Bisphosphonates may also	Overall, bisphosphonates decrease vertebral and non-vertebral fracture risk by approximately 40%.[167]	NICE: Alendronic acid is first line oral treatment (risedronate/etidronate as alternatives) for all women aged 65 years and over and all men aged 75 years and over with ≥1% osteoporotic fracture risk over 10-years. Zoledronic acid or ibandronic acid if 10-year fracture risk >10% or patient intolerant of oral bisphosphonates.[168]	NICE: In those with a 10-year probability of osteoporotic fragility fracture of at least 1%. Alendronic acid first line treatment. (risedronate/etidronate as alternatives) Zoledronic acid or ibandronic acid if 10-year fracture risk >10% or patient intolerant of oral bisphosphonates. SMC specific advice: Zoledronic acid for the treatment of osteoporosis in those for whom oral treatment options for osteoporosis are inappropriate and when	GI side effects (oral). Nephrotoxicity Bisphosphonates not recommended in those with a creatinine clearance of <30-35ml/min.[169] Atypical fractures (38.9-107.5 cases per 100,000 patient-treatment years).[170] Osteonecrosis of the jaw (1-10 cases per 100,000 patient-treatment years).[171] Osteonecrosis of the external auditory canal – to date only 29 cases reported

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	have a beneficial effect on osteoblasts and osteocytes by limiting apoptosis[162-166].			initiated by a specialist.	worldwide.[172] **IV specific effects** Acute phase response. Affects 1 in 3 patients on the first infusion, rates decrease steeply thereafter.[173] Hypocalcaemia, usually transient and more common with IV bisphosphonates.[174]
Selective oEstrogen Receptor Modulators (SERMs)[175] **Example: Raloxifene**	Acts as an oestrogen receptor agonist in bone but as an antagonist in breast and uterine tissues.	Reduces vertebral fracture risk by 30-50% in postmenopausal women.[176] No significant reduction in risk of non-vertebral fractures.[177]	NICE: not recommended for primary prevention.	NICE: Treatment of vertebral fractures in postmenopausal women for whom alendronic acid, etidronate or risedronate are unsuitable and with appropriate disease severity, as determined by a combination of BMD and clinical risk factors such as age.	Vasomotor symptoms; influenza-like symptoms; leg cramps; peripheral oedema. Increased risk of venous thromboembolism (3.22 cases per 1000 patient years), increased risk of death due to stroke (0.7 excess fatal strokes per 1000 women treated per year).[178]

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Anti-RANKL antibodies **Example: Denosumab**	A fully humanised monoclonal antibody to RANKL which inhibits RANKL binding to its cognate receptor RANK on osteoclasts precursors, thus, inhibiting osteoclastogenesis, activation and survival [179].	Reduces vertebral fractures risk by 68%, hip fracture risk by 40% and non-vertebral fracture risk by 20% in women with postmenopausal osteoporosis. [180]	NICE: Primary prevention in postmenopausal women where alendronic acid, etidronate and risedronate are unsuitable and where disease severity is sufficient determined by BMD and clinical risk factors. SMC: For the treatment of osteoporosis in postmenopausal women at increased risk of fractures who have a bone mineral density T-score <–2.5 and ≥–4.0 and for women in whom bisphosphonates are unsuitable.	NICE: Secondary prevention of osteoporotic fractures in postmenopausal women if alendronic acid, etidronate and risedronate are unsuitable and where disease severity is sufficient determined by BMD and clinical risk factors. SMC: For the treatment of osteoporosis in postmenopausal women at increased risk of fractures who have a bone mineral density T-score <–2.5 and ≥–4.0 and for women in whom bisphosphonates are unsuitable.	Atypical femoral fractures (1-10 patients per 10,000 treated [180]. Osteonecrosis of the jaw and external auditory canal reported – rare although currently there are insufficient long term studies to draw firm conclusion.[181] Cellulitis – 1% increased risk. Hypocalcaemia – rare cases reported in post marketing surveillance. Increased risk of hypocalcaemia in those with impaired renal function (Creatinine clearance <30ml/min).[182]
Strontium ranelate	Uncertain mechanism of action. Putative dual role inhibiting osteoclastic bone resorption whilst also	Reduces risk of vertebral by approximately 40 % at 3 years, hip	European Medicines Agency concluded that should only be used in those where there are no	European Medicines Agency concluded that should only be used in those where there are no	Cardiovascular events (5.7 per 1000 patient-years versus 3.6 per 1000

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	having an anabolic effect on bone formation.[183-186]	fractures by 36% and non-vertebral fractures by 16 – 19 %.[187]	other treatments for osteoporosis and no history of heart or circulatory problems.[188]	other treatments for osteoporosis and no history of heart or circulatory problems.[188]	patient-years with placebo).[189, 190] Severe allergic reactions (Drug Reaction with Eosinophilia and Systemic Symptoms - DRESS) in rare cases(<1 in 10,000 cases).[191] DEXA results are abnormal as a result of incorporation of strontium within bone and need to be interpreted with caution.[192]
hPTH 1-34 [193] **Example: Teriparatide**	Recombinant human PTH 1-34 is an amino terminal fragment of PTH. This anabolic agent increases bone formation by promoting osteoblastogenesis and the differentiation of bone lining cells into osteoblasts whilst also reducing osteoblast apoptosis. The underlying mechanism is thought to include a	Reduces risk of vertebral fracture by 65% and non-vertebral fracture by 50%.[194]	Not currently recommended for primary prevention.	NICE: Recommended as an alternative for women in whom alendronic acid or risedronate or strontium ranelate are contra-indicated or not tolerated or where treatment with alendronic acid or risedronate has been unsatisfactory and with appropriate disease severity as determined by	Hypercalcaemia Transient in 6-11%, persistent in 1-3%.[195] Hypercalciuria. Nausea. Myalgia. Increased risk of osteosarcoma in rat studies therefore

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	reduction in the Wnt inhibitor SOST and an increase in the Wnt ligand Wnt10b.[99]			a combination of BMD and clinical risk factors. SMC: Established severe osteoporosis and initiated by specialist.	limited to 2 years duration. Should be followed by antiresorptive treatment or benefit is rapidly lost.

Table 4. Current pharmacological interventions for osteoporosis and guidelines for their use in primary and secondary prevention of osteoporotic fractures. IV (intravenous administration).

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New osteoporosis treatments

The molecular mechanisms underlying the regulation of the bone remodelling cycle are becoming increasingly well-defined and have provided a number of potential therapeutic targets to advance the management of osteoporosis.

Cathepsin K inhibitors (osteoclastic bone resorption)

In an effort to specifically inhibit the resorptive action of osteoclasts, inhibitors of cathepsin K have been developed. Cathepsin K inhibitors impair osteoclastic bone resorption by inhibiting the major protease responsible for Type 1 collagen degradation, the expression of which is restricted predominantly to osteoclasts. However, whilst several cathepsin K inhibitors have been clinically evaluated, they have not been pursued due to safety concerns. The most promising agent, odanacatib, proved effective, leading to a 72% relative risk reduction in clinical vertebral fractures and a substantial increase in bone mineral density.[196] However, due to an increased risk of stroke, identified in the phase 3 trial in postmenopausal women, its development was subsequently terminated.[197] Nevertheless, one cathepsin K inhibitor, MIV-711, is still being evaluated in an osteoarthritis clinical trial.

PTH analogues (osteoblastic bone formation)

Abaloparatide is highly selective and high affinity PTHrP analogue which binds to the PTH1 Receptor and can be administered subcutaneously or transdermally. In a cohort of 2,463 women at high risk of postmenopausal fractures, abaloparatide resulted in an 86% reduction in vertebral and a 43% reduction in non-vertebral fracture. In comparison, daily subcutaneous PTH 1-34 (teriparatide) resulted in an 80% reduction in vertebral and a 30% reduction in non-vertebral fracture. Furthermore, after 18 months of abaloparatide treatment total hip BMD increased by 3.4% and lumbar spine BMD by 9.2%.[198] The subcutaneous preparation of abaloparatide has now been approved by the USA's Food and Drug Administration for specified high risk groups of patients with postmenopausal osteoporosis.

Teriparatide is currently licensed for daily subcutaneous administration. However, a phase 3 trial of once weekly subcutaneous teriparatide at a dose of 56.5 µg in 578 healthy male patients and postmenopausal women with a prevalent vertebral fracture was as effective as daily treatment at preventing new vertebral fractures. Patient acceptability may be enhanced by the less frequent - once weekly - subcutaneous administration of teriparatide.[199]

Anti-sclerostin antibodies (osteoblastic bone formation)

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One of the most promising groups of anabolic agents targets the Wnt signalling pathway. Anti-SOST antibodies are currently in preclinical trials of which three are known to be in development: romosozumab, blosozumab and BPS804. Their mode of action is to prevent the inhibitory effects of osteocyte-derived SOST on osteoblastic Wnt signalling and thus to increase osteoblastic bone formation.[200] Targeting SOST is particularly attractive as its expression is predominantly limited to skeletal tissues whereas alternative Wnt antagonists such as DKK-1 or Secreted Frizzled Related Protein 1 are more widely expressed. A Phase II trial in 492 postmenopausal women with low bone mineral density compared monthly romosozumab to placebo, alendronic acid or teriparatide. After 12 months treatment lumbar spine BMD increased 11.3 % with romosozumab, 4.1 % with alendronic acid and 7.1% with teriparatide but fell by 0.1% in the placebo group.[201] Furthermore, vertebral fracture risk was reduced by 73% in the romosozumab group in comparison to placebo.[202] Despite these promising results, a recent phase 3 trial reported an increased rate of cardiovascular events in those taking romosozumab in comparison to alendronic acid; therefore further safety information will be required before it can be considered again for approval.[203, 204] Interestingly, a recent proteomic analysis in human aortic tissues demonstrated extra-skeletal SOST expression.[205]

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Summary and conclusions

To preserve its essential load bearing, protective and homeostatic functions the skeleton must undergo continual remodelling and repair. The bone remodelling cycle ensures that old or damaged bone is replaced and that mineral homeostasis is maintained. Bone remodelling is a highly regulated and stereotyped process characterized by osteoclastic bone resorption followed by osteoblastic bone formation. These two processes are tightly coupled to ensure that bone mass is ultimately preserved.

The osteocyte is the key orchestrator of the bone remodelling cycle. These long-lived, terminally-differentiated osteoblasts are entombed within the bone matrix, connected by an extensive dendritic network and act as the skeletal mechanosensor. They respond to micro-damage and changes in loading by initiating bone remodelling and, once the repair is complete, they inhibit further bone resorption and formation to maintain bone mass. Furthermore, osteocytes also secrete FGF23, respond to hormones such as PTH to initiate bone resorption and thus maintain mineral homeostasis.

Key osteocyte signalling pathways, including RANK/RANKL/OPG and Wnt, regulate osteoclast and osteoblast differentiation and function and are also the mechanism by which several hormones ultimately exert their actions. Skeletal diseases are frequently associated with dysregulation of the bone remodelling cycle, and the study of rare, inherited metabolic bone diseases has greatly enhanced our understanding of the cellular and molecular mechanisms underlying its regulation. Importantly, these studies have also identified novel therapeutic targets for the prevention and treatment of osteoporosis and other metabolic bone diseases.

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References

1. Clarke B. Normal bone anatomy and physiology. *Clinical journal of the American Society of Nephrology : CJASN*. 2008; 3 Suppl 3: S131-9.
2. Oldknow KJ, MacRae VE and Farquharson C. Endocrine role of bone: recent and emerging perspectives beyond osteocalcin. *Journal of Endocrinology*. 2015; 225: R1-R19.
3. DiGirolamo DJ, Clemens TL and Kousteni S. The skeleton as an endocrine organ. *Nat Rev Rheumatol*. 2012; 8: 674-83.
4. Mera P, Laue K, Ferron M, et al. Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. *Cell metabolism*. 2017; 25: 218.
5. Mosialou I, Shikhel S, Liu JM, et al. MC4R-dependent suppression of appetite by bone-derived lipocalin 2. *Nature*. 2017; 543: 385-90.
6. Seeman E and Delmas PD. Bone Quality — The Material and Structural Basis of Bone Strength and Fragility. *New England Journal of Medicine*. 2006; 354: 2250-61.
7. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev*. 2000; 21: 115-37.
8. Young B. *Wheater's functional histology : A text and colour atlas*. 5th ed. Edinburgh: Churchill Livingstone, 2006.
9. Mackie EJ, Tatarczuch L and Mirams M. The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. *The Journal of endocrinology*. 2011; 211: 109-21.
10. Yang G, Zhu L, Hou N, et al. Osteogenic fate of hypertrophic chondrocytes. *Cell Res*. 2014; 24: 1266-9.
11. Kronenberg HM. Developmental regulation of the growth plate. *Nature*. 2003; 423: 332-6.
12. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC and O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nature medicine*. 2011; 17: 1235-41.
13. Matic I, Matthews BG, Wang X, et al. Quiescent Bone Lining Cells Are a Major Source of Osteoblasts During Adulthood. *Stem cells (Dayton, Ohio)*. 2016.
14. Liu F, Malaval L and Aubin JE. The mature osteoblast phenotype is characterized by extensive plasticity. *Experimental cell research*. 1997; 232: 97-105.
15. Murshed M, Harmey D, Millan JL, McKee MD and Karsenty G. Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. *Genes & development*. 2005; 19: 1093-104.
16. Long F. Building strong bones: molecular regulation of the osteoblast lineage. *Nat Rev Mol Cell Biol*. 2011; 13: 27-38.
17. Ducy P, Zhang R, Geoffroy V, Ridall AL and Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell*. 1997; 89: 747-54.
18. Nakashima K, Zhou X, Kunkel G, et al. The Novel Zinc Finger-Containing Transcription Factor Osterix Is Required for Osteoblast Differentiation and Bone Formation. *Cell*. 2002; 108: 17-29.
19. Daoussis D and Andonopoulos AP. The emerging role of Dickkopf-1 in bone biology: is it the main switch controlling bone and joint remodeling? *Seminars in arthritis and rheumatism*. 2011; 41: 170-7.
20. Marie PJ. Transcription factors controlling osteoblastogenesis. *Archives of biochemistry and biophysics*. 2008; 473: 98-105.
21. Caetano-Lopes J, Canhao H and Fonseca JE. Osteoblasts and bone formation. *Acta reumatologica portuguesa*. 2007; 32: 103-10.

DOI: 10.1177/0004563218759371

22. Hartmann C. A Wnt canon orchestrating osteoblastogenesis. *Trends in cell biology*. 2006; 16: 151-8.
23. Boyle WJ, Simonet WS and Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003; 423: 337-42.
24. Ross FP. Osteoclast Biology and Bone Resorption. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. John Wiley & Sons, Inc., 2013, p. 25-33.
25. Udagawa N, Takahashi N, Yasuda H, et al. Osteoprotegerin produced by osteoblasts is an important regulator in osteoclast development and function. *Endocrinology*. 2000; 141: 3478-84.
26. Takayanagi H. New developments in osteoimmunology. *Nat Rev Rheumatol*. 2012; 8: 684-9.
27. Franz-Odendaal TA, Hall BK and Witten PE. Buried alive: how osteoblasts become osteocytes. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2006; 235: 176-90.
28. Bonewald LF. The amazing osteocyte. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2011; 26: 229-38.
29. Bonewald LF and Johnson ML. Osteocytes, mechanosensing and Wnt signaling. *Bone*. 2008; 42: 606-15.
30. Dallas SL, Prideaux M and Bonewald LF. The osteocyte: an endocrine cell ... and more. *Endocr Rev*. 2013; 34: 658-90.
31. Chen H, Senda T and Kubo KY. The osteocyte plays multiple roles in bone remodeling and mineral homeostasis. *Medical molecular morphology*. 2015; 48: 61-8.
32. Feng JQ, Ward LM, Liu S, et al. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nature genetics*. 2006; 38: 1310-5.
33. Quarles LD. Role of FGF23 in Vitamin D and Phosphate Metabolism: Implications in Chronic Kidney Disease. *Experimental cell research*. 2012; 318: 1040-8.
34. Nakashima T, Hayashi M, Fukunaga T, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nature medicine*. 2011; 17: 1231-4.
35. Moester MJC, Papapoulos SE, Löwik CWGM and van Bezooijen RL. Sclerostin: Current Knowledge and Future Perspectives. *Calcified tissue international*. 2010; 87: 99-107.
36. Boskey AL and Robey PG. The Composition of Bone. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. John Wiley & Sons, Inc., 2013, p. 49-58.
37. Viguet-Carrin S, Garnero P and Delmas PD. The role of collagen in bone strength. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2006; 17: 319-36.
38. Duer MJ. The contribution of solid-state NMR spectroscopy to understanding biomineralization: atomic and molecular structure of bone. *Journal of magnetic resonance (San Diego, Calif : 1997)*. 2015; 253: 98-110.
39. Augat P and Schorlemmer S. The role of cortical bone and its microstructure in bone strength. *Age and ageing*. 2006; 35 Suppl 2: ii27-ii31.
40. Fazzalari IHPaNL. Characterisation of Trabecular Bone Structure. *Skeletal Aging and Osteoporosis*. 2012: 31-51.
41. Seeman E. Invited Review: Pathogenesis of osteoporosis. *Journal of applied physiology (Bethesda, Md : 1985)*. 2003; 95: 2142-51.
42. Amling M, Herden S, Posl M, Hahn M, Ritzel H and Delling G. Heterogeneity of the skeleton: comparison of the trabecular microarchitecture of the spine, the iliac crest, the femur,

DOI: 10.1177/0004563218759371

and the calcaneus. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1996; 11: 36-45.

43. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS and Mirams M. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. *Int J Biochem Cell Biol*. 2008; 40: 46-62.

44. Seeman E. The structural and biomechanical basis of the gain and loss of bone strength in women and men. *Endocrinology and metabolism clinics of North America*. 2003; 32: 25-38.

45. Allen MR and Burr DB. Chapter 4 - Bone Modeling and Remodeling. *Basic and Applied Bone Biology*. San Diego: Academic Press, 2014, p. 75-90.

46. Grissom LE and Harcke HT. Radiographic features of bisphosphonate therapy in pediatric patients. *Pediatric Radiology*. 2003; 33: 226-9.

47. Ubara Y, Fushimi T, Tagami T, et al. Histomorphometric features of bone in patients with primary and secondary hypoparathyroidism. *Kidney international*. 63: 1809-16.

48. Ubara Y, Tagami T, Nakanishi S, et al. Significance of minimodeling in dialysis patients with adynamic bone disease. *Kidney international*. 68: 833-9.

49. Burr DB, Schaffler MB, Yang KH, et al. The effects of altered strain environments on bone tissue kinetics. *Bone*. 1989; 10: 215-21.

50. Krahl H, Michaelis U, Pieper HG, Quack G and Montag M. Stimulation of bone growth through sports. A radiologic investigation of the upper extremities in professional tennis players. *The American journal of sports medicine*. 1994; 22: 751-7.

51. Mori S and Burr DB. Increased intracortical remodeling following fatigue damage. *Bone*. 1993; 14: 103-9.

52. Bentolila V, Boyce TM, Fyhrie DP, Drumb R, Skerry TM and Schaffler MB. Intracortical remodeling in adult rat long bones after fatigue loading. *Bone*. 1998; 23: 275-81.

53. Frost HM. Dynamics of bone remodeling. *Bone biodynamics*. 1964; 315.

54. Frost HM. Skeletal structural adaptations to mechanical usage (SATMU): 2. Redefining Wolff's law: the remodeling problem. *The Anatomical record*. 1990; 226: 414-22.

55. Manolagas SC. Normal skeletal development and regulation of bone formation and resorption. *UpToDate*. 2016.

56. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L and Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2001; 16: 1575-82.

57. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord*. 2010; 11: 219-27.

58. Agerbaek MO, Eriksen EF, Kragstrup J, Mosekilde L and Melsen F. A reconstruction of the remodelling cycle in normal human cortical iliac bone. *Bone and mineral*. 1991; 12: 101-12.

59. Goldring SR. The osteocyte: key player in regulating bone turnover. *RMD open*. 2015; 1.

60. Atkins GJ and Findlay DM. Osteocyte regulation of bone mineral: a little give and take. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2012; 23: 2067-79.

61. Burr DB. Targeted and nontargeted remodeling. *Bone*. 2002; 30: 2-4.

62. Parfitt AM. Targeted and nontargeted bone remodeling: relationship to basic multicellular unit origination and progression. *Bone*. 2002; 30: 5-7.

63. Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell metabolism*. 2007; 5: 464-75.

DOI: 10.1177/0004563218759371

64. Tolar J, Teitelbaum SL and Orchard PJ. Osteopetrosis. *New England Journal of Medicine*. 2004; 351: 2839-49.
65. Silver IA, Murrills RJ and Etherington DJ. Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. *Experimental cell research*. 1988; 175: 266-76.
66. Delaisse JM, Andersen TL, Engsig MT, Henriksen K, Troen T and Blavier L. Matrix metalloproteinases (MMP) and cathepsin K contribute differently to osteoclastic activities. *Microsc Res Tech*. 2003; 61: 504-13.
67. Xing L and Boyce BF. Regulation of apoptosis in osteoclasts and osteoblastic cells. *Biochemical and biophysical research communications*. 2005; 328: 709-20.
68. Eriksen EF, Melsen F and Mosekilde L. Reconstruction of the resorptive site in iliac trabecular bone: a kinetic model for bone resorption in 20 normal individuals. *Metabolic bone disease & related research*. 1984; 5: 235-42.
69. Howard GA, Bottemiller BL, Turner RT, Rader JI and Baylink DJ. Parathyroid hormone stimulates bone formation and resorption in organ culture: evidence for a coupling mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 1981; 78: 3204-8.
70. Sims NA and Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *BoneKEy Rep*. 2014; 3.
71. Zhou H, Chernecky R and Davies JE. Deposition of cement at reversal lines in rat femoral bone. *Journal of Bone and Mineral Research*. 1994; 9: 367-74.
72. Everts V, Delaisse JM, Korper W, et al. The bone lining cell: its role in cleaning Howship's lacunae and initiating bone formation. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2002; 17: 77-90.
73. Raggatt LJ and Partridge NC. Cellular and Molecular Mechanisms of Bone Remodeling. *The Journal of biological chemistry*. 2010; 285: 25103-8.
74. Delaisse J-M. The reversal phase of the bone-remodeling cycle: cellular prerequisites for coupling resorption and formation. *BoneKEy Reports*. 2014; 3: 561.
75. Zhao C, Irie N, Takada Y, et al. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell metabolism*. 2006; 4: 111-21.
76. Sims NA and Martin TJ. Coupling Signals between the Osteoclast and Osteoblast: How are Messages Transmitted between These Temporary Visitors to the Bone Surface? *Frontiers in endocrinology*. 2015; 6: 41.
77. Matsuo K and Otaki N. Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. *Cell adhesion & migration*. 2012; 6: 148-56.
78. Eriksen EF, Gundersen HJ, Melsen F and Mosekilde L. Reconstruction of the formative site in iliac trabecular bone in 20 normal individuals employing a kinetic model for matrix and mineral apposition. *Metabolic bone disease & related research*. 1984; 5: 243-52.
79. Anderson HC. Matrix vesicles and calcification. *Current rheumatology reports*. 2003; 5: 222-6.
80. Anderson HC, Garimella R and Tague SE. The role of matrix vesicles in growth plate development and biomineralization. *Frontiers in bioscience : a journal and virtual library*. 2005; 10: 822-37.
81. Cui L, Houston DA, Farquharson C and MacRae VE. Characterisation of matrix vesicles in skeletal and soft tissue mineralisation. *Bone*. 2016; 87: 147-58.
82. Boyce BF and Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis research & therapy*. 2007; 9 Suppl 1: S1.

DOI: 10.1177/0004563218759371

83. Arai F, Miyamoto T, Ohneda O, et al. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. *J Exp Med*. 1999; 190: 1741-54.
84. Yoshida H, Hayashi S-I, Kunisada T, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature*. 1990; 345: 442-4.
85. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. 1999; 397: 315-23.
86. Yasuda H, Shima N, Nakagawa N, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95: 3597-602.
87. Takayanagi H, Kim S, Koga T, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Developmental cell*. 2002; 3: 889-901.
88. Kearns AE, Khosla S and Kostenuik PJ. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr Rev*. 2008; 29: 155-92.
89. Xiong J, Piemontese M, Onal M, et al. Osteocytes, not Osteoblasts or Lining Cells, are the Main Source of the RANKL Required for Osteoclast Formation in Remodeling Bone. *PloS one*. 2015; 10: e0138189.
90. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*. 1997; 89: 309-19.
91. Clevers H and Nusse R. Wnt/beta-catenin signaling and disease. *Cell*. 2012; 149: 1192-205.
92. Baron R and Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. *Nature medicine*. 2013; 19: 179-92.
93. Williams BO. Insights Into the Mechanisms of Sclerostin Action in Regulating Bone Mass Accrual. *Journal of Bone and Mineral Research*. 2014; 29: 24-8.
94. Stein EM, Silva BC, Boutroy S, et al. Primary Hyperparathyroidism is Associated with Abnormal Cortical and Trabecular Microstructure and Reduced Bone Stiffness in Postmenopausal Women. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2013; 28: 1029-40.
95. Silva BC and Bilezikian JP. Parathyroid hormone: anabolic and catabolic actions on the skeleton. *Current opinion in pharmacology*. 2015; 22: 41-50.
96. Siddiqui JA and Partridge NC. CCL2/Monocyte Chemoattractant Protein 1 and Parathyroid Hormone Action on Bone. *Frontiers in endocrinology*. 2017; 8: 49.
97. O'Brien CA, Plotkin LI, Galli C, et al. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PloS one*. 2008; 3.
98. Bellido T, Ali AA, Gubrij I, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology*. 2005; 146: 4577-83.
99. Li JY, Walker LD, Tyagi AM, Adams J, Weitzmann MN and Pacifici R. The sclerostin-independent bone anabolic activity of intermittent PTH treatment is mediated by T-cell-produced Wnt10b. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2014; 29: 43-54.
100. Menaa C, Barsony J, Reddy SV, Cornish J, Cundy T and Roodman GD. 1, 25-Dihydroxyvitamin D3 Hypersensitivity of Osteoclast Precursors from Patients with Paget's Disease. *Journal of Bone and Mineral Research*. 2000; 15: 228-36.

DOI: 10.1177/0004563218759371

101. Zarei A, Morovat A, Javaid K and Brown CP. Vitamin D receptor expression in human bone tissue and dose-dependent activation in resorbing osteoclasts. *Bone research*. 2016; 4: 16030.
102. Langub MC, Reinhardt TA, Horst RL, Malluche HH and Koszewski NJ. Characterization of vitamin D receptor immunoreactivity in human bone cells. *Bone*. 2000; 27: 383-7.
103. Wang Y, Zhu J and DeLuca HF. Identification of the Vitamin D Receptor in Osteoblasts and Chondrocytes But Not Osteoclasts in Mouse Bone. *Journal of Bone and Mineral Research*. 2014; 29: 685-92.
104. van Driel M and van Leeuwen JPTM. Vitamin D endocrine system and osteoblasts. *BoneKEy Rep*. 2014; 3.
105. Lanske B, Densmore MJ and Erben RG. Vitamin D endocrine system and osteocytes. *BoneKEy Rep*. 2014; 3.
106. van Driel M, Koedam M, Buurman CJ, et al. Evidence for auto/paracrine actions of vitamin D in bone: 1alpha-hydroxylase expression and activity in human bone cells. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2006; 20: 2417-9.
107. Yang D, Anderson PH, Turner AG, Morris HA and Atkins GJ. Comparison of the biological effects of exogenous and endogenous 1,25-dihydroxyvitamin D3 on the mature osteoblast cell line MLO-A5. *The Journal of steroid biochemistry and molecular biology*. 2016; 164: 374-8.
108. Amling M, Priemel M, Holzmann T, et al. Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. *Endocrinology*. 1999; 140: 4982-7.
109. Panda DK, Miao D, Bolivar I, et al. Inactivation of the 25-hydroxyvitamin D 1alpha-hydroxylase and vitamin D receptor demonstrates independent and interdependent effects of calcium and vitamin D on skeletal and mineral homeostasis. *The Journal of biological chemistry*. 2004; 279: 16754-66.
110. Lieben L, Masuyama R, Torrekens S, et al. Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D–induced inhibition of bone mineralization. *The Journal of clinical investigation*. 2012; 122: 1803-15.
111. Yamamoto Y, Yoshizawa T, Fukuda T, et al. Vitamin D receptor in osteoblasts is a negative regulator of bone mass control. *Endocrinology*. 2013; 154: 1008-20.
112. Gardiner EM, Baldock PA, Thomas GP, et al. Increased formation and decreased resorption of bone in mice with elevated vitamin D receptor in mature cells of the osteoblastic lineage. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2000; 14: 1908-16.
113. Eisman JA and Bouillon R. Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. *BoneKEy Rep*. 2014; 3.
114. Carter PH and Schipani E. The roles of parathyroid hormone and calcitonin in bone remodeling: prospects for novel therapeutics. *Endocrine, metabolic & immune disorders drug targets*. 2006; 6: 59-76.
115. Zaidi M, Inzerillo AM, Moonga BS, Bevis PJ and Huang CL. Forty years of calcitonin--where are we now? A tribute to the work of Iain Macintyre, FRS. *Bone*. 2002; 30: 655-63.
116. Pederson L, Ruan M, Westendorf JJ, Khosla S and Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105: 20764-9.
117. Keller J, Catala-Lehnen P, Huebner AK, et al. Calcitonin controls bone formation by inhibiting the release of sphingosine 1-phosphate from osteoclasts. *Nat Commun*. 2014; 5.

DOI: 10.1177/0004563218759371

118. Bassett JH and Williams GR. Role of Thyroid Hormones in Skeletal Development and Bone Maintenance. *Endocr Rev*. 2016; 37: 135-87.
119. Olney RC. Regulation of bone mass by growth hormone. *Medical and pediatric oncology*. 2003; 41: 228-34.
120. Iglesias L, Yeh JK, Castro-Magana M and Aloia JF. Effects of growth hormone on bone modeling and remodeling in hypophysectomized young female rats: a bone histomorphometric study. *Journal of bone and mineral metabolism*. 2011; 29: 159-67.
121. Weinstein RS, Jilka RL, Parfitt AM and Manolagas SC. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *Journal of Clinical Investigation*. 1998; 102: 274-82.
122. Henneicke H, Gasparini SJ, Brennan-Speranza TC, Zhou H and Seibel MJ. Glucocorticoids and bone: local effects and systemic implications. *Trends in Endocrinology & Metabolism*. 25: 197-211.
123. Mitra R. Adverse effects of corticosteroids on bone metabolism: a review. *PM & R : the journal of injury, function, and rehabilitation*. 2011; 3: 466-71; quiz 71.
124. Canalis E and Delany AM. Mechanisms of Glucocorticoid Action in Bone. *Annals of the New York Academy of Sciences*. 2002; 966: 73-81.
125. Sher LB, Woitge HW, Adams DJ, et al. Transgenic expression of 11beta-hydroxysteroid dehydrogenase type 2 in osteoblasts reveals an anabolic role for endogenous glucocorticoids in bone. *Endocrinology*. 2004; 145: 922-9.
126. Nakamura T, Imai Y, Matsumoto T, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell*. 2007; 130: 811-23.
127. Krassas GE and Papadopoulou P. Oestrogen action on bone cells. *Journal of musculoskeletal & neuronal interactions*. 2001; 2: 143-51.
128. Khosla S, Oursler MJ and Monroe DG. Estrogen and the skeleton. *Trends in endocrinology and metabolism: TEM*. 2012; 23: 576-81.
129. Ribot C, Trémollieres F and Pouillés J-M. Aromatase and regulation of bone remodeling. *Joint, bone, spine : revue du rhumatisme*. 2006; 73: 37-42.
130. Santen RJ, Brodie H, Simpson ER, Siiteri PK and Brodie A. History of aromatase: Saga of an important biological mediator and therapeutic target. *Endocrine Reviews*. 2009; 30: 343-75.
131. Vanderschueren D, Gaytant J, Boonen S and Venken K. Androgens and bone. *Current opinion in endocrinology, diabetes, and obesity*. 2008; 15: 250-4.
132. Manolagas SC, O'Brien CA and Almeida M. The role of estrogen and androgen receptors in bone health and disease. *Nature reviews Endocrinology*. 2013; 9: 699-712.
133. Bruderer M, Richards RG, Alini M and Stoddart MJ. Role and regulation of RUNX2 in osteogenesis. *European cells & materials*. 2014; 28: 269-86.
134. Tang Y, Wu X, Lei W, et al. TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nature medicine*. 2009; 15: 757-65.
135. Raisz LG. Prostaglandins and bone: physiology and pathophysiology. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 1999; 7: 419-21.
136. Blackwell KA, Raisz LG and Pilbeam CC. Prostaglandins in bone: bad cop, good cop? *Trends in Endocrinology & Metabolism*. 2010; 21: 294-301.
137. Roodman GD. Role of cytokines in the regulation of bone resorption. *Calcified tissue international*. 1993; 53 Suppl 1: S94-8.
138. Bertolini DR, Nedwin GE, Bringman TS, Smith DD and Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature*. 1986; 319: 516-8.

DOI: 10.1177/0004563218759371

139. Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1996; 11: 1043-51.
140. Feng X and McDonald JM. Disorders of bone remodeling. *Annual review of pathology*. 2011; 6: 121-45.
141. Hernlund E, Svedbom A, Ivergård M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden: A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Archives of Osteoporosis*. 2013; 8: 136.
142. Brown C. Osteoporosis: Staying strong. *Nature*. 2017; 550: S15-S7.
143. Holroyd C, Cooper C and Dennison E. Epidemiology of osteoporosis. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2008; 22: 671-85.
144. Barnett E and Nordin BEC. The radiological diagnosis of osteoporosis: A new approach. *Clinical Radiology*. 1960; 11: 166-74.
145. Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC and Khaltaev N. The diagnosis of osteoporosis. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1994; 9: 1137-41.
146. Reid IR. Overview of Pathogenesis. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. John Wiley & Sons, Inc., 2013, p. 357-60.
147. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. *The Journal of clinical investigation*. 115: 3318-25.
148. Zebaze RM, Ghasem-Zadeh A, Bohte A, et al. Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. *Lancet*. 2010; 375: 1729-36.
149. Bjornerem A. The clinical contribution of cortical porosity to fragility fractures. *Bonekey Rep*. 2016; 5: 846.
150. Eriksen EF, Hodgson SF, Eastell R, Cedel SL, O'Fallon WM and Riggs BL. Cancellous bone remodeling in type I (postmenopausal) osteoporosis: quantitative assessment of rates of formation, resorption, and bone loss at tissue and cellular levels. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1990; 5: 311-9.
151. Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL and Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *The Journal of clinical investigation*. 111: 1221-30.
152. Saika M, Inoue D, Kido S and Matsumoto T. 17beta-estradiol stimulates expression of osteoprotegerin by a mouse stromal cell line, ST-2, via estrogen receptor-alpha. *Endocrinology*. 2001; 142: 2205-12.
153. Briot K and Roux C. Glucocorticoid-induced osteoporosis. *RMD open*. 2015; 1: e000014.
154. Weinstein RS. Glucocorticoid-Induced Bone Disease. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. John Wiley & Sons, Inc., 2013, p. 473-81.
155. Ohnaka K, Tanabe M, Kawate H, Nawata H and Takayanagi R. Glucocorticoid suppresses the canonical Wnt signal in cultured human osteoblasts. *Biochemical and biophysical research communications*. 2005; 329: 177-81.
156. Weinstein RS, Chen J-R, Powers CC, et al. Promotion of osteoclast survival and antagonism of bisphosphonate-induced osteoclast apoptosis by glucocorticoids. *The Journal of clinical investigation*. 2002; 109: 1041-8.
157. Alexandre C and Vico L. Pathophysiology of bone loss in disuse osteoporosis. *Joint, bone, spine : revue du rhumatisme*. 2011; 78: 572-6.

DOI: 10.1177/0004563218759371

158. Sievänen H. Immobilization and bone structure in humans. *Archives of biochemistry and biophysics*. 2010; 503: 146-52.
159. Spatz JM, Wein MN, Gooi JH, et al. The Wnt-inhibitor Sclerostin is Up-regulated by Mechanical Unloading in Osteocytes in-vitro. *Journal of Biological Chemistry*. 2015.
160. Collet P, Uebelhart D, Vico L, et al. Effects of 1- and 6-month spaceflight on bone mass and biochemistry in two humans. *Bone*. 1997; 20: 547-51.
161. Bauman WA and Cardozo CP. Spinal Cord Injury: Skeletal Pathophysiology and Clinical Issues. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. John Wiley & Sons, Inc., 2013, p. 1018-27.
162. Maraka S and Kennel KA. Bisphosphonates for the prevention and treatment of osteoporosis. *Bmj*. 2015; 351: h3783.
163. Baron R, Ferrari S and Russell RGG. Denosumab and bisphosphonates: Different mechanisms of action and effects. *Bone*. 2011; 48: 677-92.
164. Russell RG. Bisphosphonates: mode of action and pharmacology. *Pediatrics*. 2007; 119 Suppl 2: S150-62.
165. Drake MT, Clarke BL and Khosla S. Bisphosphonates: Mechanism of Action and Role in Clinical Practice. *Mayo Clinic proceedings Mayo Clinic*. 2008; 83: 1032-45.
166. Russell RG. Bisphosphonates: the first 40 years. *Bone*. 2011; 49: 2-19.
167. Byun JH, Jang S, Lee S, et al. The Efficacy of Bisphosphonates for Prevention of Osteoporotic Fracture: An Update Meta-analysis. *J Bone Metab*. 2017; 24: 37-49.
168. NICE. Bisphosphonates for treating osteoporosis (TA464): (2017).
169. Miller PD, Jamal SA, Evenepoel P, Eastell R and Boonen S. Renal safety in patients treated with bisphosphonates for osteoporosis: a review. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2013; 28: 2049-59.
170. Gedmintas L, Solomon DH and Kim SC. Bisphosphonates and Risk of Subtrochanteric, Femoral Shaft, and Atypical Femur Fracture: A Systematic Review and Meta-analysis. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2013; 28: 1729-37.
171. Khosla S, Burr D, Cauley J, et al. Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2007; 22: 1479-91.
172. MHRA. Drug Safety Update. Bisphosphonates: very rare reports of osteonecrosis of the external auditory canal. *Drug Safety Update*. Crown, 2015.
173. Black DM, Delmas PD, Eastell R, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *The New England journal of medicine*. 2007; 356: 1809-22.
174. Rosen CJ and Brown S. Severe hypocalcemia after intravenous bisphosphonate therapy in occult vitamin D deficiency. *The New England journal of medicine*. 2003; 348: 1503-4.
175. Maximov PY, Lee TM and Jordan VC. The Discovery and Development of Selective Estrogen Receptor Modulators (SERMs) for Clinical Practice. *Current Clinical Pharmacology*. 2013; 8: 135-55.
176. Ettinger B, Black DM, Mitlak BH, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. *JAMA : the journal of the American Medical Association*. 1999; 282: 637-45.
177. Ensrud KE, Stock JL, Barrett-Connor E, et al. Effects of raloxifene on fracture risk in postmenopausal women: the Raloxifene Use for the Heart Trial. *Journal of bone and mineral*

DOI: 10.1177/0004563218759371

research : the official journal of the American Society for Bone and Mineral Research. 2008; 23: 112-20.
178. Stefanick ML. Risk–Benefit Profiles of Raloxifene for Women. *New England Journal of Medicine*. 2006; 355: 190-2.
179. Dempster DW, Lambing CL, Kostenuik PJ and Grauer A. Role of RANK ligand and denosumab, a targeted RANK ligand inhibitor, in bone health and osteoporosis: a review of preclinical and clinical data. *Clinical therapeutics*. 2012; 34: 521-36.
180. Cummings SR, Martin JS, McClung MR, et al. Denosumab for Prevention of Fractures in Postmenopausal Women with Osteoporosis. *New England Journal of Medicine*. 2009; 361: 756-65.
181. MHRA. Drug Safety Update. Denosumab (Prolia, Xgeva▼): reports of osteonecrosis of the external auditory canal In: MHRA, (ed.). Crown, 2017, p. 2-3.
182. Dave V, Chiang CY, Booth J and Mount PF. Hypocalcemia post denosumab in patients with chronic kidney disease stage 4-5. *American journal of nephrology*. 2015; 41: 129-37.
183. Fonseca JE and Brandi ML. Mechanism of action of strontium ranelate: what are the facts? *Clinical Cases in Mineral and Bone Metabolism*. 2010; 7: 17-8.
184. Stepan J. Strontium ranelate: in search for the mechanism of action. *Journal of bone and mineral metabolism*. 2013; 31: 606-12.
185. Russell RG. Pharmacological diversity among drugs that inhibit bone resorption. *Current opinion in pharmacology*. 2015; 22: 115-30.
186. Stepan JJ. Strontium ranelate: in search for the mechanism of action. *Journal of bone and mineral metabolism*. 2013; 31: 606-12.
187. Rizzoli R. Strontium Ranelate in the Prevention of Osteoporotic Fractures. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. John Wiley & Sons, Inc., 2013, p. 437-43.
188. Agency EM. Protelos/Osseor to remain available but with further restrictions. European Medicines Agency, 2014, p. 1-3.
189. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R and Reginster JY. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2013; 24: 23-57.
190. Reginster J-Y. Cardiac concerns associated with strontium ranelate. *Expert Opinion on Drug Safety*. 2014; 13: 1209-13.
191. Musette P, Kaufman J-M, Rizzoli R, Cacoub P, Brandi ML and Reginster J-Y. Cutaneous Side Effects of Antiosteoporosis Treatments. *Therapeutic Advances in Musculoskeletal Disease*. 2011; 3: 31-41.
192. Blake GM and Fogelman I. Effect of bone strontium on BMD measurements. *J Clin Densitom*. 2007; 10: 34-8.
193. Ebeling PR and Russell RG. Teriparatide (rhPTH 1-34) for the treatment of osteoporosis. *International journal of clinical practice*. 2003; 57: 710-8.
194. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *The New England journal of medicine*. 2001; 344: 1434-41.
195. Sikon A and Batur P. Profile of teriparatide in the management of postmenopausal osteoporosis. *International journal of women's health*. 2010; 2: 37-44.
196. Chapurlat RD. Odanacatib: a review of its potential in the management of osteoporosis in postmenopausal women. *Therapeutic Advances in Musculoskeletal Disease*. 2015; 7: 103-9.

DOI: 10.1177/0004563218759371

197. Mullard A. Merck & Co. drops osteoporosis drug odanacatib. *Nature reviews Drug discovery*. 2016; 15: 669-.
198. Miller PD, Hattersley G, Riis BJ, et al. Effect of Abaloparatide vs Placebo on New Vertebral Fractures in Postmenopausal Women With Osteoporosis: A Randomized Clinical Trial. *JAMA : the journal of the American Medical Association*. 2016; 316: 722-33.
199. Nakamura T, Sugimoto T, Nakano T, et al. Randomized Teriparatide [human parathyroid hormone (PTH) 1-34] Once-Weekly Efficacy Research (TOWER) trial for examining the reduction in new vertebral fractures in subjects with primary osteoporosis and high fracture risk. *The Journal of clinical endocrinology and metabolism*. 2012; 97: 3097-106.
200. Plotkin LI and Bellido T. Osteocytic signalling pathways as therapeutic targets for bone fragility. *Nature reviews Endocrinology*. 2016; advance online publication.
201. McClung MR, Grauer A, Boonen S, et al. Romosozumab in Postmenopausal Women with Low Bone Mineral Density. *New England Journal of Medicine*. 2014; 370: 412-20.
202. Cosman F, Crittenden DB, Adachi JD, et al. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. *New England Journal of Medicine*. 2016; 375: 1532-43.
203. Medscape. Heart Problems Hit Hopes for Experimental Amgen, UCB Bone Drug. Medscape2017.
204. Saag KG, Petersen J, Brandi ML, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. *New England Journal of Medicine*. 0: null.
205. Didangelos A, Yin X, Mandal K, Baumert M, Jahangiri M and Mayr M. Proteomics characterization of extracellular space components in the human aorta. *Molecular & cellular proteomics : MCP*. 2010; 9: 2048-62.
206. Long CL and Humphrey MB. Osteoimmunology: the expanding role of immunoreceptors in osteoclasts and bone remodeling. *BoneKEy Rep*. 2012; 1.
207. Boyce BF, Rosenberg E, de Papp AE and Duong LT. The osteoclast, bone remodelling and treatment of metabolic bone disease. *European journal of clinical investigation*. 2012; 42: 1332-41.

DOI: 10.1177/0004563218759371

DOI: 10.1177/0004563218759371

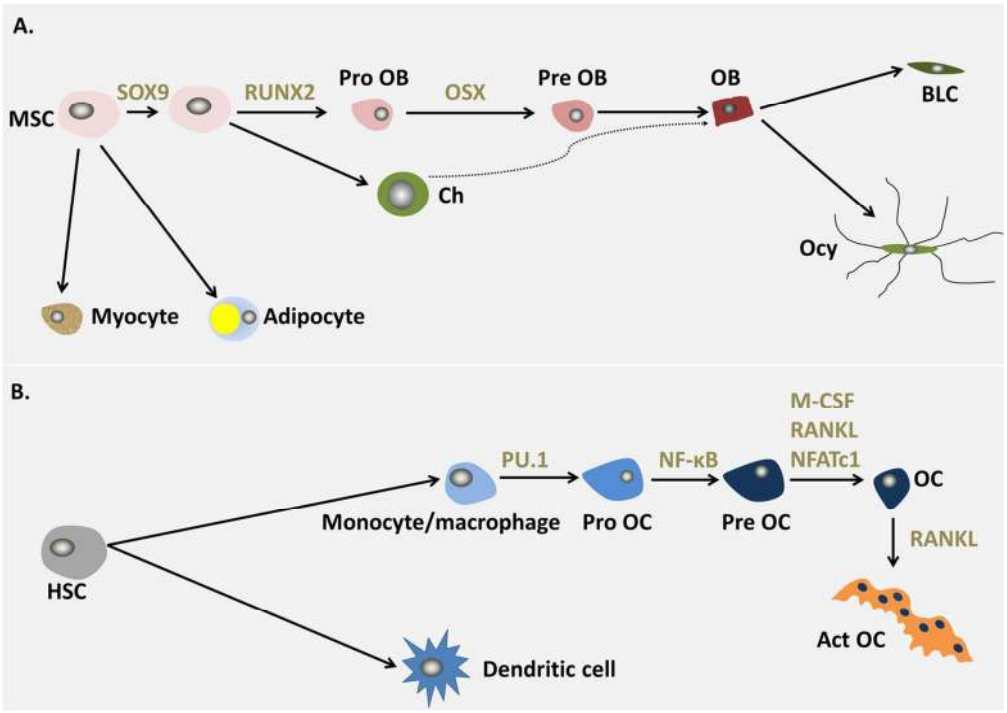

Figure 1. Derivation of bone cells.
A. Mesenchymal stem cells (MSCs) can form adipocytes, chondrocytes (Ch), myocytes or osteoblast precursors (Pro OB), pre-osteoblasts (Pre OB) then osteoblasts (OB). Mature osteoblasts can differentiate into bone lining cells (BLC) or osteocytes (Ocy). Recent evidence suggests that hypertrophic chondrocytes may also differentiate into OBs 10. The key transcriptional regulators in osteoblast differentiation are indicated. Sry-box 9 (SOX9), runt-related transcription factor 2 (Runx2), Osterix (OSX).
B. Haemopoietic stem cells (HSCs), specifically myeloid-committed precursors, differentiate into monocytes/macrophages or dendritic cells. Monocytes/macrophages then differentiate into osteoclast progenitors (Pro OC), pre-osteoclasts (Pre OC) then osteoclasts (OC). Active OC (Act OC) formation is stimulated by RANK Ligand 7, 20, 23, 206. The most important cytokines and transcriptional regulators of this pathway are indicated. PU box-binding-1 (PU.1), nuclear factor-κB (NF-κB), macrophage colony stimulating factor (M-CSF), nuclear factor of activated T cells 1 (NFATc1) and RANKL.

113x79mm (300 x 300 DPI)

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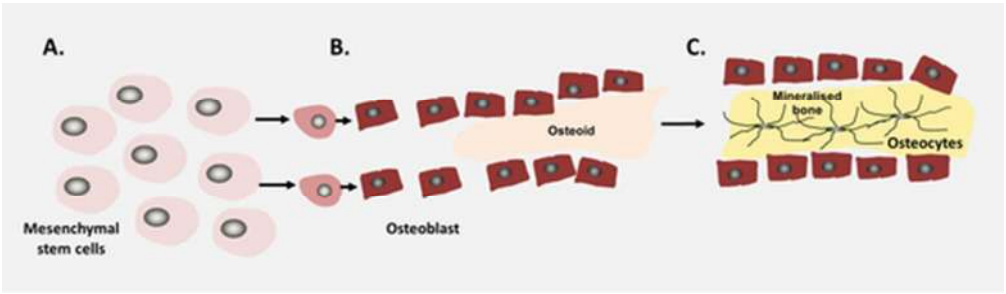

Figure 2. Schematic diagram illustrating intramembranous bone formation. Mesenchymal stem cells differentiate into osteoblasts and form bone directly.
A. Mesenchymal stem cells in connective tissue for a condensation and differentiate in osteoblasts.
B. Mature osteoblasts secrete a type I collagen rich matrix called osteoid.
C. The osteoid mineralizes to form an ossification centre from which mineralization spreads. Osteoblasts terminally differentiate into osteocytes and become entombed within the newly formed bone matrix.

46x13mm (300 x 300 DPI)

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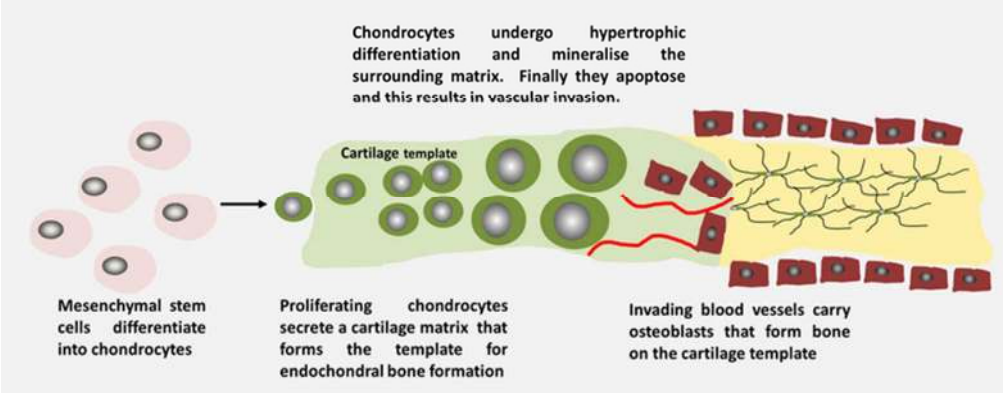

Figure 3. Schematic illustrating endochondral bone formation.

62x24mm (300 x 300 DPI)

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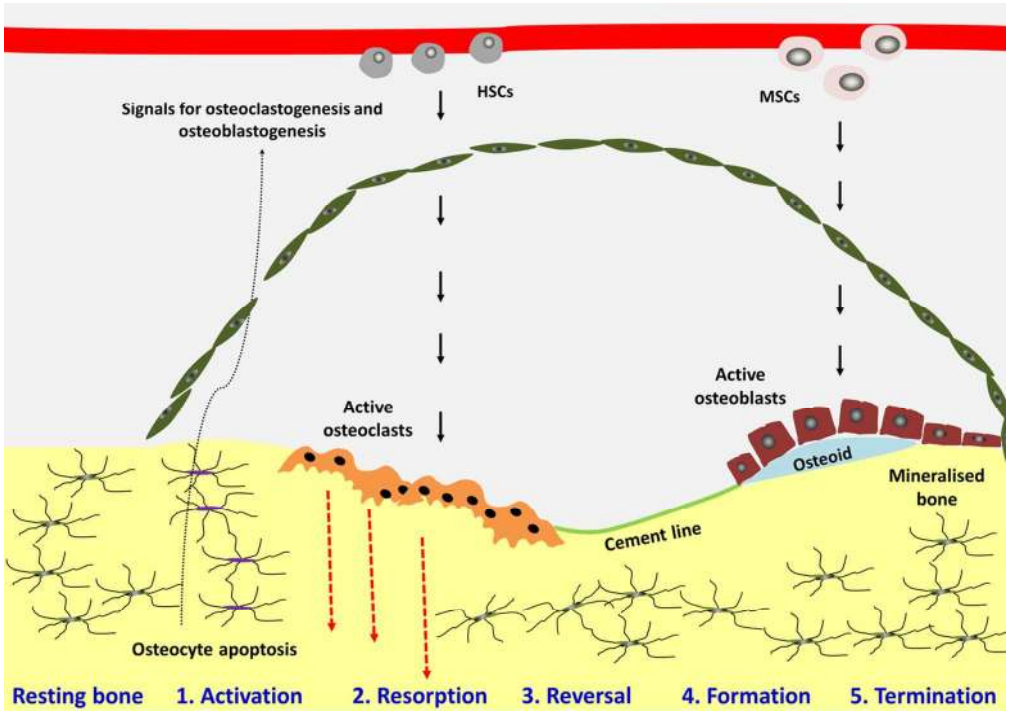

Figure 4. BMU at different phases of the bone remodelling cycle.
Schematic diagram of the bone remodeling cycle illustrating the phases of; Activation, Resorption, Reversal, Formation and Termination. Haemopoietic stem cells (HSCs), Mesenchymal stem cells (MSCs).

113x79mm (300 x 300 DPI)

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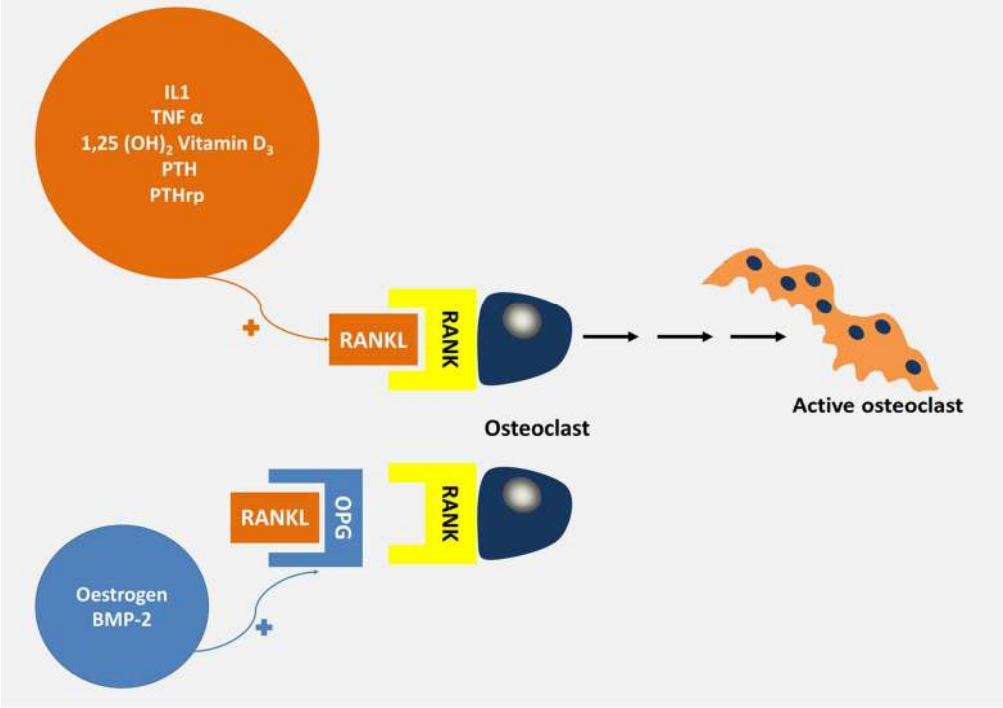

Figure 5. Factors affecting the RANK/RANKL/OPG signalling pathway 207. Oestrogen and Bone morphogenic Protein-2 (BMP-2) induce osteoprotegerin (OPG) expression whereas 1,25(OH)2 Vitamin D3, PTH, PTHrP, IL-1 and tumour necrosis factor α (TNFα) induce RANKL. OPG is a decoy receptor for RANKL blocking its binding to RANK. Thus, it is the RANKL: OPG ratio that determines the rate of osteoclastogenesis.

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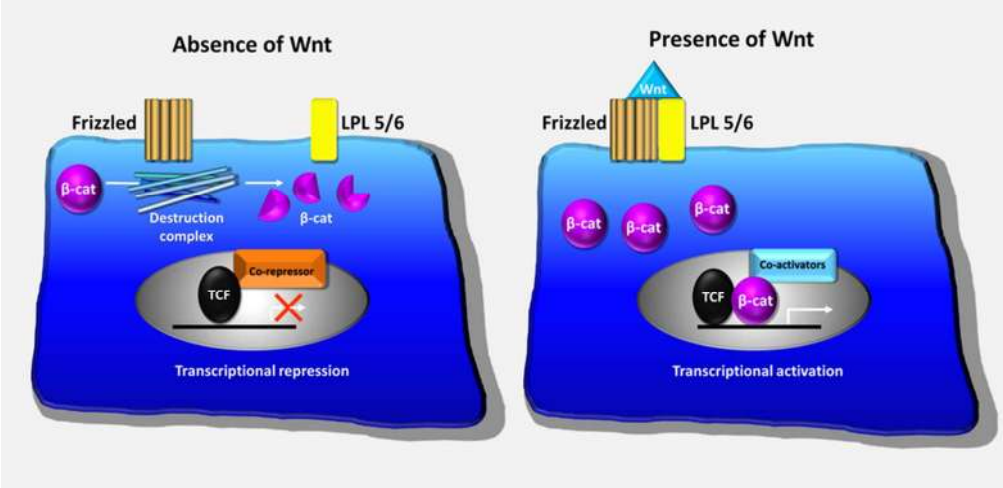

Figure 6. Schematic illustration of canonical Wnt signalling. In the absence of Wnt, Frizzled and its co-receptors LPL5/6 do not interact. The destruction complex, present in the cytoplasm, degrades β-catenin and target gene expression is repressed. In the presence of Wnt, Frizzled binds it co-receptors and blocks the action of the destruction complex. β-catenin accumulates in the cytoplasm, translocates to the nucleus displacing transcriptional co-repressors and recruiting co-activators leading to an increased expression of key target genes involved in osteoblast differentiation.

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