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<R/Heads>BASIC SCIENCE

Normal bone physiology, remodelling and its hormonal regulation

Jennifer S Walsh PhD FRCP FHEA is Senior Clinical Lecturer in the Academic Unit of Bone Metabolism at the University of Sheffield, UK. Conflicts of interest: none declared.

Abstract

The skeleton has structural and locomotor functions, and is a mineral reservoir. Bone turnover by osteoclasts and osteoblasts is a lifelong process, incorporating growth, modelling and remodelling to repair microdamage and access the mineral reservoir.

Signalling between bone cells is essential for the co-ordination of these processes. Osteoblasts regulate osteoclast activity through the RANK/RANK ligand/OPG system, and osteocytes regulate osteoblast activity through sclerostin secretion.

If resorption and formation are balanced there is no net change in bone mass after each remodelling cycle, but with ageing and some disease states resorption exceeds formation, leading to negative bone balance, decreased bone mass and loss of microstructural integrity.

The rate of remodelling is determined by factors including mechanical loading and endocrine influences. The most important endocrine regulator of bone turnover is oestrogen, but other hormones regulating bone metabolism include IGF-1, PTH and gut and adipocyte hormones.

KEYWORDS: bone, bone turnover, osteoblast, osteoclast, osteocyte, hormones, oestrogen

Normal bone physiology

The skeleton has structural, protective and locomotor functions and is a reservoir for calcium.

Cortical bone is heavily calcified and fulfils a mainly structural and protective role. Trabecular bone is less heavily calcified, and has a greater surface area which allows it to be metabolically active. Overall the adult skeleton is about 80% cortical bone and 20% trabecular bone. The proportion of trabecular and cortical bone varies by skeletal site; for example vertebrae are rich in trabecular bone but have very little cortex, but long bones have much thicker cortices and relatively less trabecular bone.

Growth is the process through which bones increase in size and become mineralised during childhood and adolescence. Bone mass increases from approximately 80g at birth to 3000g at peak bone mass (at about age 25). Flat bones (such as the skull) grow by intramembranous ossification and long bones (such as the femur and humerus) grow in length by endochondral ossification and in width by periosteal apposition.

Modelling is the process through which bones are shaped and adapt to loading or other influences. Modelling can result in changes in bone mass, size and geometry. Cortical modelling at the periosteal or endosteal surfaces changes bone diameter and cortical thickness.

Remodelling is the continuous process of bone renewal and repair which continues throughout adult life.

Bone turnover is high during bone acquisition and modelling in growth and puberty, then decreases to a nadir at about age 40. There is a rapid increase in turnover in menopausal women, and a more gradual age-related increase in men. These age-related changes in bone turnover are reflected in bone mass.

Bone matrix and mineralisation

Bone matrix is composed of type I collagen fibres, with glycoproteins, proteoglycans, γ-carboxylated (gla) proteins and water. Many of the non-collagenous proteins have physiological roles in the regulation of bone cell activity or mineralisation.

Type I collagen is a triple-helical molecule containing two identical α1 chains and one α2 chain. The collagen fibres in mature bone are orientated in alternating layers which confers maximum strength on the structure (lamellar bone). Bone matrix laid down acutely after fracture healing, or in high turnover states such as Paget's disease is disorganised, without lamellar configuration (woven bone) and is weaker than lamellar bone.

The mineral component of bone tissue is calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The hydroxyapatite crystals lie along the collagen fibres and within the ground substance. The mineral strengthens the bone by increasing the mechanical resistance of the bone material.

The matrix and mineral components both contribute to the material properties of bone. The collagen matrix provides toughness (the maximum amount of energy bone can absorb before fracture) and the mineral provides stiffness (the extent to which bone resists deformation in response to an applied force). This is demonstrated in osteogenesis imperfecta (brittle bone disease), where quantitative or qualitative abnormalities of collagen lead to fracture due to reduced toughness. In osteomalacia (most commonly due to vitamin D deficiency), under-mineralisation can lead to bone deformity and fracture due to loss of stiffness.

Bone formation and resorption

Bone formation and resorption are the basis of growth, modelling and remodelling.

The bone remodelling cycle is an ongoing process that renews bone to repair microdamage and maintain strength. It also maintains serum calcium in the normal physiological range by release of mineral from the bone matrix as required. About 5 to 10% of the adult skeleton is replaced by remodelling each year.

On trabecular bone and at the endocortical surface, remodelling takes place on the surface of bone, but within cortical bone the osteoclasts form a cutting cone through the bone matrix.

The signal to initiate remodelling may be endocrine (such as increased PTH in response to hypocalcaemia), which leads to generalised increases in osteoclast activation. Localised remodelling is initiated in response to microdamage, probably through osteocytes signalling.

During a remodelling cycle, osteoclasts on the bone surface become activated and resorb bone matrix, creating a defect which is filled in by osteoblasts. The cycle usually takes about 200 days to complete. The bone remodelling cycle is highly regulated, and resorption and formation are closely coupled, so that in health and under normal conditions bone formation will equal resorption, and the amount of bone tissue will be the same at the beginning and end of the cycle (Figure 1).

Osteoclasts are giant multinucleated cells of monocyte lineage. They attach to bone with integrins, and their ruffled border forms a sealed compartment over the bone surface. They secrete hydrogen ions and enzymes such as cathepsin K and matrix metalloproteases (MMPs) into the sealed compartment. The acidification dissolves the bone mineral and the enzymes break down the matrix. Osteoclast differentiation, activation and apoptosis are subject to multiple local and endocrine influences. The critical factors in osteoclast differentiation are M-CSF and RANK ligand. Anti-resorptive treatments for osteoporosis (including bisphosphonates, hormone replacement, selective oestrogen receptor modulators and denosumab) decrease osteoclast activity and so slow remodelling. Bisphosphonates attach to the bone surface and are internalised by the osteoclast during bone resorption, disabling the osteoclast or inducing apoptosis. Denosumab is a monoclonal antibody against RANK ligand.

Osteoblasts generate bone matrix and facilitate mineralisation. They are derived from mesenchymal stem cells, and share a common lineage with chondrocytes, myoblasts, fibroblasts and adipocytes. Quiescent osteoblasts are seen as lining cells along the bone surfaces, and active osteoblasts are seen as a single layer of cuboidal cells on the surface of newly formed osteoid. Osteoblast differentiation is dependent on Runx2, and bone morphogenic proteins (BMPs) are important in the regulation of osteoblastogenesis and stimulation of bone matrix formation. In recent years Wnt signalling has been recognised as a fundamental regulator of osteoblast function. Wnt is a co-receptor with LRP-5, and Wnt signalling is transduced through β catenin to induce gene expression and drive bone formation. New bone anabolic agents for osteoporosis that target Wnt signalling are in development, such as inhibitors of DKK and sclerostin.

Primary mineralisation by deposition of hydroxyapatite crystals begins about two weeks after unmineralised matrix (osteoid) is laid down by the osteoblast and continues for about six months. Secondary mineralisation progresses over two to three years, with incorporation of more mineral and development of crystal structures.

When remodelling and bone formation is complete, some osteoblasts undergo apoptosis, some become lining cells and some become trapped in the mineralised bone where they remain as osteocytes.

Bone remodelling has a circadian rhythm, which is more exaggerated for resorption than formation. Bone turnover rises at night and decreases during the day. The rhythm is driven by a combination of endocrine factors (such as cortisol, oxytocin and melatonin), local factors (such as peroxisome proliferator-activated receptor γ) and clock genes.

Biochemical markers of bone turnover

Appreciation of the fundamental importance of the rate and balance of bone remodelling in bone health and disease led to the development of biochemical markers of bone turnover. These markers are products of or factors in bone resorption or formation than can be measured in blood or urine to give an indication of cellular activity. For example, CTX and NTX are commonly used resorption markers derived from components of collagen which are released when bone matrix is broken down. PINP is a component of pro-collagen which is cleaved when type I collagen is laid down by osteoblasts, and so is used as a formation marker.

Confirmation of high bone turnover is useful in the diagnosis of metabolic bone diseases, and change in markers can be used to monitor response to treatment.

Coupling of resorption and formation

The balance and rate of bone remodelling is critical for bone mass and bone quality. Resorption and formation are coupled by local factors, and one of the key regulators is the RANK/RANK ligand/OPG system. RANK (receptor activator of nuclear factor-κB) is a receptor expressed on the cell membrane of osteoclast precursors and mature osteoclasts and its activation stimulates osteoclast differentiation and activity. RANK ligand is secreted by stromal cells or osteoblasts and is the major paracrine factor in activating the bone remodelling unit. It requires the presence of M-CSF to activate the RANK system in the osteoclast or osteoclast precursor. OPG (osteoprotegerin) is also secreted by osteoblasts and is a soluble decoy receptor that neutralises RANK ligand and so decreases osteoclast differentiation and activity (Figure 2). The secretion of RANK ligand and OPG are regulated by hormones and cytokines, including sex steroids, IL-1 and PGE2. Many of the important regulators of bone resorption may act through the alteration of the relative amounts of RANK ligand and OPG secreted by osteoblasts.

The mechanisms that couple bone formation to resorption (to ensure that the resorption space is refilled) are less well understood. It is a locally regulated process and there may be contributions from factors released from the bone matrix during resorption and factors secreted by osteoclasts and other local cells such as macrophages.

<u>Osteocytes</u>

Osteocytes are the most numerous cells in bone; more than 90% of bone cells are osteocytes. Osteocytes develop from osteoblasts that have completed their role in bone formation. The osteocytes are embedded in lacunae within the bone matrix, and have long connecting processes running through canaliculi that allow them to act as a switchboard for intercellular communication.

Osteocytes play an important role in the response of bone to microdamage and mechanical stimuli, and regulate phosphate metabolism through secretion of FGF-23.

When microdamage develops in bone, local osteocytes undergo apoptosis, and signal to osteoclasts to initiate the remodelling cycle and begin bone resorption. In the absence of viable osteocytes, osteoclast activity is greatly increased, suggesting that osteocytes may exert a tonic inhibitory action on resorption.

It has been proposed that osteocytes are the sensors of loading and mechanical stimuli; as strain is applied to bone, fluid shear in the canaliculi deforms the osteocyte dendrite cell membrane, and initiates intracellular signalling.

One of the crucial factors produced by osteocytes is sclerostin (SOST). SOST is produced by osteocytes in response to off-loading and hormonal changes such oestrogen deficiency. SOST inhibits osteoblast differentiation and bone formation through inhibition of Wnt signalling (Figure 3). The gene was identified through genetic loss-of-function disorders which result in high bone formation (van Buchem's disease and sclerosteosis). Loading decreases SOST formation by osteocytes and so increases bone formation. SOST inhibition is a therapeutic target for osteoporosis and anti-SOST monoclonal antibodies are now in clinical trials.

Fracture healing

Fracture healing begins with an inflammatory phase, with haematoma formation, production of collagen from fibroblasts and the release of cytokines that attract stem cells to the site. Hypoxia due to blood vessel damage induces stem cells to differentiate into chondrocytes and osteoblasts and bone formation begins. Initial repair with woven bone is then remodelled into lamellar bone through stimulation of osteoclast activity by RANK ligand secretion from osteoblasts in the repair callus.

Loading and bone remodelling

Loading is an important stimulus for the maintenance of bone mass. Loss of the strain signal leads to decreased bone formation and increased resorption. In astronauts working on Skylab, urinary NTX increased by 25% after one week and by 150% at eight weeks. On return to loading, NTX returned nearly to baseline values within a few weeks.

Strain does not have to be induced by weight-bearing to have an effect on bone mass. For example, tennis players' radii are larger and more dense on their dominant side.

In clinical practice the increase in resorption with offloading can cause hypercalcaemia, but usually only in situations where there is pre-existing high turnover such as young men or patients with Paget's disease.

Skeletal ageing

The rate of bone remodelling increases with age, due to decreases in sex steroids and other endocrine changes, including decreased IGF-1 and increased PTH. Reduced muscle mass and loading may also contribute to increased turnover with ageing. Activation frequency of bone remodelling units is increased, so the remodelling space is increased and the amount of fully mature secondarily mineralised bone is decreased. Remodelling imbalance develops, so that formation is less than resorption and each remodelling cycle results in net bone loss. This leads to loss of cortical and trabecular bone. Trabeculae are perforated, leading to loss of connectivity and structural integrity. The endocortex becomes porous and 'trabecularised' and porosity increases throughout the cortex. Trabecular mass and connectivity and cortical mineralisation are important determinants of bone strength, and compromise of these properties reduces strength in compression and bending.

Periosteal perimeter increases with age, through periosteal apposition and endosteal resorption. This is likely to be an adaptive response to maintain bone strength, attempting to compensate for the loss of bone mass with increased bone size.

Hormonal regulation of bone

The major endocrine regulator of bone remodelling in men and women is oestrogen. The other important endocrine regulators include other sex hormones, IGF-1, cortisol and parathyroid hormone (PTH) (Figure 4), as well as more recently described roles for leptin and gut hormones.

Oestrogen

In premenopausal women 95% of circulating oestrogen is secreted by the ovaries, and the remainder is synthesised by extra-gonadal conversion of other sex steroids. In postmenopausal women nearly all the circulating oestrogen is derived from extra-gonadal conversion of adrenal steroids (by aromatase in fat and other tissue). Oestrogen in men is almost entirely derived from extra-gonadal synthesis.

The oestrogen receptor is expressed on growth plate chondrocytes, and has roles in bone growth and development. In normal puberty, the early rise in oestrogen may drive increasing IGF-1 and growth, and in the later stages oestrogen is the cause of epiphyseal fusion and the cessation of longitudinal growth. In the absence of oestrogen (for example in men with loss of function mutations of the aromatase gene) there is continuing longitudinal growth with tall stature and low bone mineral density. This suggests that oestrogen is not essential for bone growth, but is required for epiphyseal fusion. Oestrogen may also inhibit periosteal apposition during growth, contributing to gender differences in bone size.

Oestrogen inhibits osteoclast activity and increases osteoclast apoptosis through direct signalling, via osteoblast secretion of OPG and RANK ligand, and by decreasing secretion of pro-resorptive cytokines such as IL-1 and TNFα from bone marrow cells. It also increases osteoblast differentiation and bone formation, at least partly through inhibition of sclerostin secretion by osteocytes.

The menopausal loss of oestrogen is the primary cause of increased bone turnover and remodelling imbalance in older women.

Testosterone

Testosterone is the main gonadal androgen in males, and more than 95% is derived from testicular synthesis. In premenopausal women, 25% of circulating testosterone is ovarian, 25% adrenal, and 50% from peripheral conversion. In postmenopausal women the ovarian secretion of testosterone decreases.

The androgen receptor is expressed in bone cells and chondrocytes. Testosterone decreases bone resorption and increases bone formation through similar mechanisms to oestrogen.

During growth and modelling, testosterone increases periosteal apposition, which combines with the inhibition of apposition by oestrogen to cause the gender differences at bone size after puberty. It is likely that a major part of the anti-resorptive action of testosterone in older men is mediated through aromatisation to oestrogen.

Growth hormone and IGF-1

Growth hormone has some direct actions on target tissues, but the majority of its growth - promoting effect is through the action of IGF-1. Most circulating IGF-1 is synthesised in the liver, but it is also synthesised by bone cellsand in other tissues where it has paracrine or intracrine action.

IGF-1 increases osteoblast activity and bone formation. IGF-1 is necessary for longitudinal growth, but also acts on periosteal osteoblasts to increase periosteal bone formation.

Growth hormone and IGF-1 drive bone growth in childhood through stimulation of growth plate chondrocytes, and serum levels rise rapidly at puberty in response to rising oestradiol. Growth hormone and IGF-1 also play a role in the maintenance of bone geometry and bone mineral density in adults.

Cortisol

Cortisol regulates bone turnover and contributes to the diurnal variation in bone turnover. It increases resorption and decreases formation by increasing osteoclast lifespan, inducing osteoblast and osteocyte apoptosis and inhibiting osteoblast differentiation and function through suppression of RUNX-2, BMPs and Wnt signalling. Cortisol excess due to endogenous hypersecretion or glucocorticoid treatment leads to a 'double hit' bone disease of increased resorption and decreased formation with decreased BMD and increased fracture risk.

Adipocyte hormones

Leptin is secreted by adipocytes in direct proportion to fat mass. Its first identified function was as a satiety signal, but in recent years it has been found to have a role in bone metabolism.

It acts directly on osteoblasts to increased bone formation, which could contribute to the positive correlation between body weight and bone mass. However, experimental evidence suggests that it can also act through a hypothalamic relay and the sympathetic nervous system to increase bone resorption.

Other adipocyte hormones such as adiponectin have also been associated with BMD and bone turnover, but the mechanism of action and significance of these hormones in bone metabolism are not yet well understood.

Gut and pancreatic hormones

There is a bone turnover response to feeding; bone resorption decreases within about 20 minutes of a meal, and remains suppressed for a few hours. Glucagon-like peptide 2 (GLP-2) is secreted by the gut in response to feeding, and is likely to be one of the regulators of the bone turnover response to feeding. Administration of GLP-2 to postmenopausal women decreases bone turnover and increases BMD, demonstrating that this pathway is a potential therapeutic target for the treatment of osteoporosis.

Other gut hormones (such as the incretin GLP-1 which is used in the treatment of type 2 diabetes) and pancreatic hormones (including insulin and amylin) may also have favourable actions on bone metabolism.

Parathyroid Hormone

Parathyroid hormone is an 84 amino acid peptide secreted in response to hypocalcaemia detected by the calcium-sensing receptor. It maintains serum calcium concentrations in a narrow range by increasing bone resorption, renal reabsorption of calcium and renal 1α-hydroxylation of vitamin D (which increases gut calcium absorption).

PTH increases osteoclast activity via increased osteoblast expression of RANK ligand and decreased expression of OPG.

Continuously elevated PTH (for example in primary hyperparathyroidism or vitamin D deficiency) increases bone resorption and decreases BMD. However, pulsatile administration of PTH has anabolic effects on trabecular bone through osteocyte signalling, increased osteoblast differentiation and increased local production of IGF-1. Daily subcutaneous PTH is the only currently licensed anabolic treatment for osteoporosis (as PTH 1-34 or 1-84).

With ageing, renal function declines and gut fractional calcium absorption decreases. This leads to a rise in PTH, contributing to increased resorption and remodelling imbalance.

Summary

Bone turnover by osteoclasts and osteoblasts is essential for skeletal development and maintenance of bone strength in adulthood. Ageing and disease result in remodelling imbalance with loss of bone mass and structural strength. Understanding the intercellular signals and endocrine influences that regulate bone turnover has led to the development of several different therapeutic targets for the treatment of osteoporosis.

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