

Physiology of Bone Formation, Remodeling, and Metabolism

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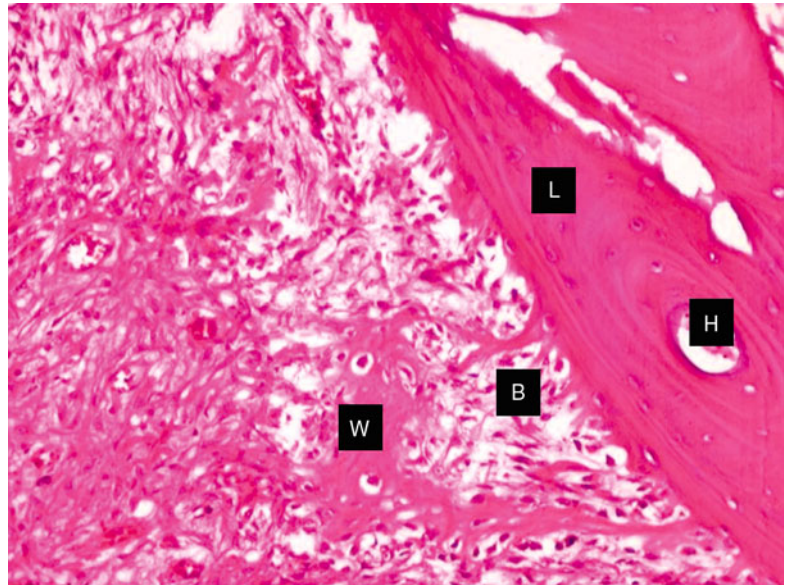
2.1 Introduction

Bone is a highly specialized supporting framework of the body, characterized by its rigidity, hardness, and power of regeneration and repair. It protects the vital organs, provides an environment for marrow (both blood forming and fat storage), acts as a mineral reservoir for calcium homeostasis and a reservoir of growth factors and cytokines, and also takes part in acid–base balance (Taichman 2005). Bone constantly undergoes modeling (reshaping) during life to help it adapt to changing biomechanical forces, as well as remodeling to remove old, microdamaged bone and replace it with new, mechanically stronger bone to help preserve bone strength.

The bones have two components – the *cortical bone* which is dense, solid, and surrounds the marrow space and the *trabecular bone* which is composed of a honeycomb-like network of trabecular plates and rods interspersed in the bone marrow compartment. Cortical bone has an outer periosteal surface and inner endosteal surface. The periosteum is a fibrous connective tissue sheath that surrounds the outer cortical surface of bone, except at joints where bone is lined by articular cartilage. It contains blood vessels, nerve fibers, osteoblasts, and osteoclasts. It protects, nourishes, and aides in bone formation. It plays an important role in appositional growth and fracture repair. The endosteum is a membranous structure covering the inner surface of cortical and cancellous bone and the blood vessel canals (Volkmann’s canals) present in bone.

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Fig. 2.1 Woven bone/
immature bone (*W*) rimmed
by osteoblasts (*B*) with
peripheral lamellar bone (*L*)
showing a Haversian
canal (*H*)



Further, based on the pattern of collagen forming the osteoid, two types of bone are identified: *woven bone*, which is characterized by a haphazard organization of collagen fibers (Eriksen et al. 1994), and *lamellar bone*, which is characterized by a regular parallel alignment of collagen into sheets (lamellae) (Fig. 2.1). Lamellar bone, as a result of the alternating orientations of collagen fibrils, has a significant mechanical strength similar to plywood. This normal lamellar pattern is absent in woven bone, in which the collagen fibrils are laid down in a disorganized manner. Hence, the woven bone is weaker than lamellar bone. Woven bone is produced when osteoblasts produce osteoid rapidly. This occurs initially in all fetal bones and in fracture healing, but the resulting woven bone is replaced by a process called *remodeling* by the deposition of more resilient lamellar bone. Virtually all the bone in the healthy mature adult is lamellar bone.

2.2 Physiology of Bone Formation

Bone is composed of support cells, namely, *osteoblasts and osteocytes*; remodeling cells, namely, *osteoclasts*; and non-mineral matrix of *collagen* and noncollagenous proteins called *osteoid*, with inorganic mineral salts deposited

within the matrix. During life, the bones undergo processes of longitudinal and radial growth, modeling (reshaping), and remodeling (Clarke 2008). Longitudinal growth occurs at the growth plates, where cartilage proliferates in the epiphyseal and metaphyseal areas of long bones, before subsequently undergoing mineralization to form primary new bone.

2.2.1 Bone Formation

Ossification (or osteogenesis) is the process of formation of new bone by cells called osteoblasts.

These cells and the bone matrix are the two most crucial elements involved in the formation of bone. This process of formation of normal healthy bone is carried out by two important processes, namely:

1. Intramembranous ossification characterized by laying down of bone into the primitive connective tissue (mesenchyme) resulting in the formation of bones (skull, clavicle, mandible). It is also seen in the healing process of fractures (compound fractures) treated by open reduction and stabilization by metal plate and screws.
2. Endochondral ossification where a cartilage model acts as a precursor (e.g., femur, tibia,

humerus, radius). This is the most important process occurring during fracture healing when treated by cast immobilization.

If the process of formation of bone tissue occurs at an extraskeletal location, it is termed as *heterotopic ossification*.

Three basic steps involved in osteogenesis are:

- (a) Synthesis of extracellular organic matrix (osteoid)
- (b) Matrix mineralization leading to the formation of bone
- (c) Remodeling of bone by the process of resorption and reformation

2.2.2 Osteoblasts

Osteoblasts originate from mesenchymal stem cells (osteoprogenitor cells) (Fig. 2.2) of the bone marrow stroma and are responsible for bone matrix synthesis and its subsequent mineralization. Commitment of mesenchymal stem cells to the osteoblast lineage requires the canonical Wnt/ β -catenin pathway and associated proteins (Logan and Nusse 2004).

Osteoblasts are mononucleated, and their shape varies from flat to plump, reflecting their level of cellular activity, and, in later stages of maturity, lines up along bone-forming surfaces (Fig. 2.1). Osteoblasts are responsible for regulation of osteoclasts and deposition of bone matrix (Mackie 2003). As they differentiate, they acquire the ability to secrete bone matrix. Ultimately, some osteoblasts become trapped in their own bone matrix, giving rise to osteocytes which, gradually, stop secreting osteoid. Osteocytes are the most abundant cells in bone; these cells communicate with each other and with the surrounding medium through extensions of their plasma membrane. Therefore, osteocytes are thought to act as mechanosensors, instructing osteoclasts where and when to resorb bone and osteoblasts where and when to form it (Boulpaep and Boron 2005; Manolagas 2000). The osteoblasts, rich in alkaline phosphatase, an organic phosphate-splitting enzyme, possess receptors for parathyroid hormone and estrogen. Also, hormones, growth fac-

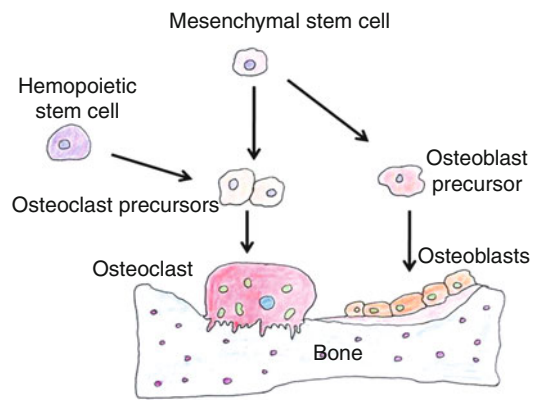


Fig. 2.2 Diagram to show evolution of osteoblasts and osteoclasts in the formation of bone

tors, physical activity, and other stimuli act mainly through osteoblasts to bring about their effects on bone (Harada and Rodan 2003).

2.2.2.1 Wnt Pathway on Osteoblastogenesis

Wnts are secreted glycoproteins that regulate a variety of cellular activities, such as cell fate, determination, proliferation, migration, survival, polarity and gene expression (Cadigan and Liu 2006; Caetano-Lopes et al. 2007). This pathway is essential for the differentiation of mature osteoblasts and consequently for bone formation. Reduced Wnt signaling has been associated with osteoporosis (Krishnan et al. 2006). The Wnt system is also important in chondrogenesis and hematopoiesis and may be stimulatory or inhibitory at different stages of osteoblast differentiation.

2.2.3 Bone Matrix

The structure of bone is constituted by:

- (a) Inorganic (69 %) component, consisting of hydroxyapatite (99 %)
- (b) Organic (22 %), constituted by collagen (90 %) and noncollagen structural proteins which include proteoglycans, sialoproteins, glycosaminoglycans, and 2HS-glycoprotein

The functional component of the bone includes growth factors and cytokines. The hardness and rigidity of bone is due to the presence of mineral

salt in the osteoid matrix, which is a crystalline complex of calcium and phosphate (hydroxyapatite). Calcified bone contains about 25 % organic matrix, 5 % water, and 70 % inorganic mineral (hydroxyapatite). Collagen 1 constitutes 90–95 % of the organic matrix of bone. Osteoblasts synthesize and lay down precursors of collagen 1 (Brodsky and Persikov 2005). They also produce osteocalcin, which is the most abundant noncollagenous protein of bone matrix, and the proteoglycans of ground substance. The collagen 1 formed by osteoblasts is deposited in parallel or concentric layers to produce mature (lamellar) bone. When bone is rapidly formed, as in the fetus or certain pathological conditions (e.g., fracture callus, fibrous dysplasia, hyperparathyroidism), the collagen is not deposited in a parallel array but in a basket-like weave resulting in woven, immature, or primitive bone (Fig. 2.1).

Osteoblasts also synthesize and secrete noncollagenous protein, such as proteoglycans, glycosylated proteins, glycosylated proteins with potential cell-attachment activities, and γ -carboxylated (gla) proteins. The main glycosylated protein present in bone is alkaline phosphatase, which plays an as-yet-undefined role in mineralization of bone (Whyte 1994).

2.2.4 Bone Minerals

Crystalline hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ is the chief mineral component of bone, constituting approximately about a quarter of the volume and half of the mass of normal adult bone. These mineral crystals (according to electron microscopy) are deposited along, and in close relation to, the bone collagen fibrils. The calcium and phosphorus (inorganic phosphate) components of these crystals are derived from the blood plasma and which in turn is from nutritional sources. Amorphous calcium phosphate matures through several intermediate stages to form hydroxyapatite. The end result is a highly organized amalgam of protein, primarily collagen, and mineral, primarily hydroxyapatite, that has sufficient structural integrity to serve the mechanical functions of the skeleton. Vitamin D metabolites and parathyroid hormone (PTH) are

important mediators of calcium regulation, and vitamin D deficiency or hyperparathyroidism will lead to depletion of the bone minerals.

2.2.5 Osteocytes

Osteocytes represent terminally differentiated osteoblasts and function within syncytial networks to support bone structure and metabolism. Osteocytes maintain connection with each other and the bone surface via their multiple filipodial cellular processes. Osteocytes are linked metabolically and electrically through gap junctions composed primarily of connexin (Bonewald 1999; Plotkin et al. 2002). The presence of empty lacunae in aging bone suggests that osteocytes may undergo apoptosis, probably caused by disruption of their intercellular gap junctions or cell-matrix interactions. Osteocyte apoptosis in response to estrogen deficiency or glucocorticoid treatment is harmful to bone structure. Estrogen and bisphosphonate therapy and physiologic loading of bone may help prevent osteoblast and osteocyte apoptosis (Plotkin et al. 2005; Xing and Boyce 2005).

2.2.6 Intramembranous (Mesenchymal) Ossification

Intramembranous ossification is one of the two essential processes during fetal development of the mammalian skeletal system resulting in the formation of bone tissue. Intramembranous ossification mainly occurs during formation of the flat bones of the skull but also the mandible, maxilla, and clavicles; it is also an essential process during the natural healing of bone fractures and the rudimentary formation of bones of the head. The bone is formed from connective tissue such as mesenchyme tissue rather than from cartilage. The steps in intramembranous ossification (Fig. 2.3) are:

1. Formation of ossification center
2. Calcification
3. Formation of trabeculae
4. Development of periosteum

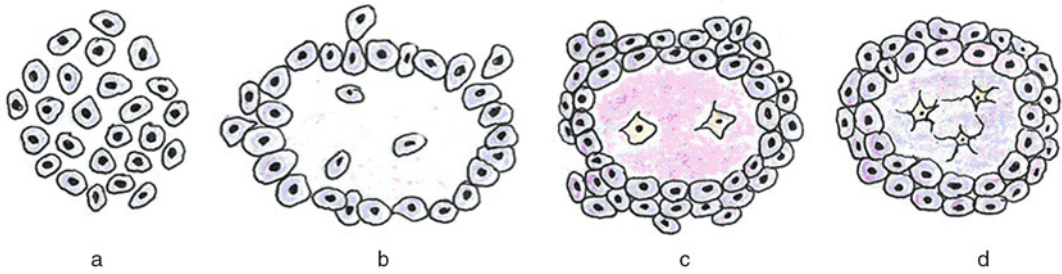


Fig. 2.3 Intramembranous ossification showing: (a) Aggregates of osteoprogenitor cells. (b) Amorphous ground substance and collagen meshwork formed in the center and in between the cells. (c) The mesenchymal

stem cell transform to osteoblasts which synthesize osteoid in the center of the aggregate. (d) A rudimentary bone tissue formed by the osteoblasts and some of these get incorporated within the osteoid to become osteocytes

The important cell in the creation of bone tissue by membrane ossification is a mesenchymal stem cell. Mesenchymal stem cells (MSCs) within human mesenchyme or the medullary cavity of a bone fracture initiate the process of intramembranous ossification. An MSC is an unspecialized cell whose morphology undergoes characteristic changes as it develops into an osteoblast. The process of membranous ossification, which is essentially the direct mineralization of a highly vascular connective tissue, commences at certain constant points known as centers of ossification. At such a center, the mesenchymal cells (osteoprogenitor cells) proliferate and condense around a profuse capillary network. Between the cells and around the vessels is amorphous ground substance (Fig. 2.3) with a fine meshwork of collagen fibers.

The osteoprogenitor cells differentiate (specialize) into osteoblasts, which create osteoid in the center of the aggregate. The osteoblasts produce bone matrix and get surrounded by collagen fibers and become osteocytes. At this point, the osteoid becomes mineralized, resulting in a nidus consisting of mineralized osteoid that contains osteocytes and is lined by active osteoblasts. The nidus that began as a diffuse collection of MSCs has become rudimentary bone tissue. This process of entrapping of osteoblasts proceeds, the trabeculae gradually thicken, and the intervening vascular spaces (spongy layer) become progressively narrowed. Where the bone persists as cancellous bone, however, the process slows down, and the spaces later become occupied by hemopoietic tissue (Netter 1987; Brighton and Hunt 1991).

As these changes are proceeding in the ossification center, the surrounding mesenchyme condenses as a fibrovascular periosteum around its edges and surfaces. The periosteum is thus formed, and bone growth continues at the surface of trabeculae. Much like spicules, the increasing growth of trabeculae results in interconnection, and this network is called woven bone. Eventually, woven bone is replaced by lamellar bone. Extension of the ossification process occurs through the agency of stem cells derived from the deeper layers of the periosteum.

2.2.7 Intracartilaginous (Endochondral) Ossification

Endochondral ossification (Greek: *endon*, “within,” *chondros*, “cartilage”) occurs in long bones and most of the rest of the bones in the body; it involves an initial hyaline cartilage which continues to grow. It is also an essential process during the growth of the length of long bones and the natural healing of bone fractures (Brighton and Hunt 1986; Netter 1987; Brighton et al. 1973).

The steps in endochondral ossification (Figs. 2.4, 2.5, and 2.6) are:

1. Development of cartilage model
2. Growth of cartilage model
3. Development of the primary ossification center
4. Development of the secondary ossification center
5. Formation of articular cartilage and epiphyseal plate

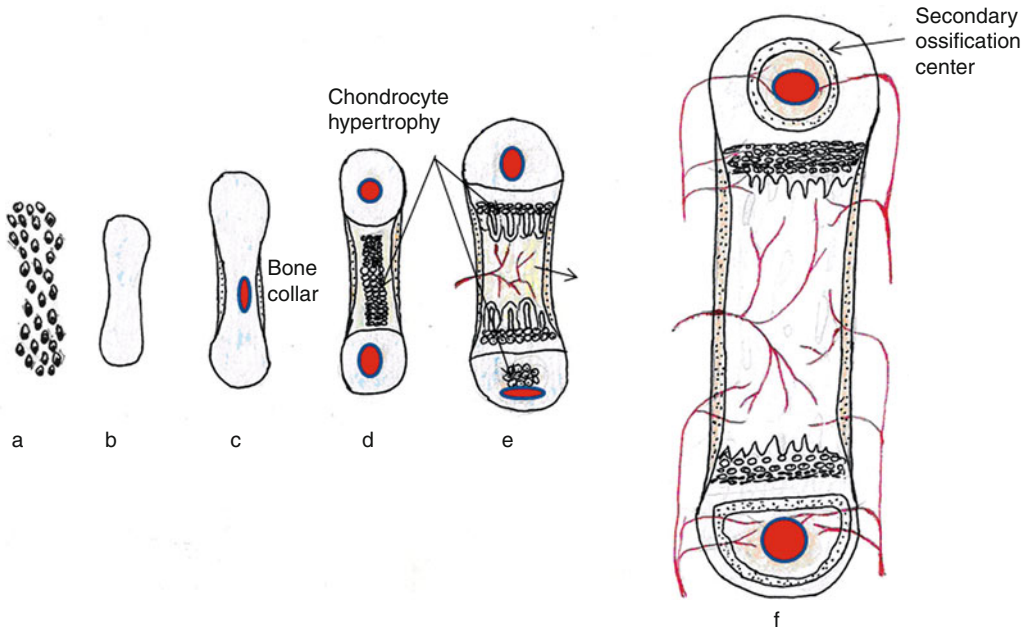


Fig. 2.4 Intracartilaginous ossification showing: (a) aggregates of osteoprogenitor cells (b) model of hyaline cartilage (c) primary center of ossification (d) secondary

center of ossification (e) bone with medullary cavity and epiphyseal ends (f) highlighting feeding blood vessels

Endochondral ossification begins with points in the cartilage called “primary ossification centers.” They mostly appear during fetal development, though a few short bones begin their primary ossification after birth. They are responsible for the formation of the diaphyses of long bones, short bones, and certain parts of irregular bones. Secondary ossification occurs after birth and forms the epiphyses of long bones and the extremities of irregular and flat bones. The diaphysis and both epiphyses of a long bone are separated by a growing zone of cartilage (the epiphyseal plate). When the child reaches skeletal maturity (18–25 years of age), all of the cartilage is replaced by bone, fusing the diaphysis and both epiphyses together (epiphyseal closure).

2.2.7.1 Development of Cartilage Model

Each long bone is represented in early fetal life by a rod of hyaline cartilage which replaces a rod of condensed mesenchyme, its shape

foreshadowing that of the early bone (e.g., carpal bones are preceded by appropriately shaped cartilaginous “models”). The cartilaginous model is surrounded by a highly vascular condensed mesenchyme or perichondrium, similar in every way to that which precedes and surrounds intramembranous ossification centers with its deeper layers containing osteoprogenitor cells.

2.2.7.2 Growth of the Cartilage Model

The cartilage model will grow in length by continuous cell division of chondrocytes, which is accompanied by further secretion of extracellular matrix. This is called interstitial growth. The process of appositional growth occurs when the cartilage model would also grow in thickness which is due to the addition of more extracellular matrix on the periphery cartilage surface, which is accompanied by new chondroblasts that develop from the perichondrium.

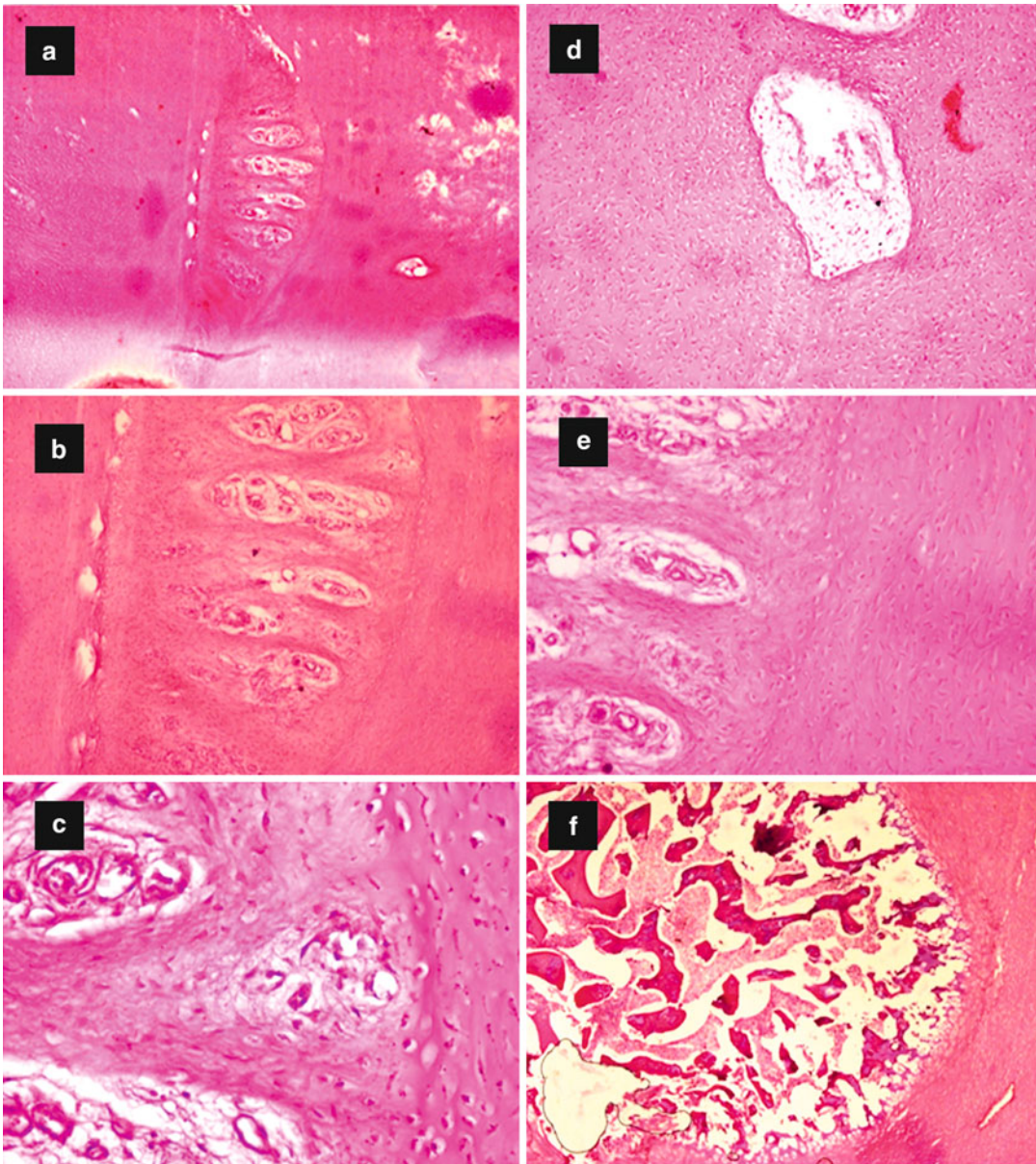


Fig. 2.5 Microphotographs to show primary centers of ossification: (a) first stage where the chondrocytes at the center of ossification undergo apoptosis/death (b–e) showing invasion of this ossification center by vascular

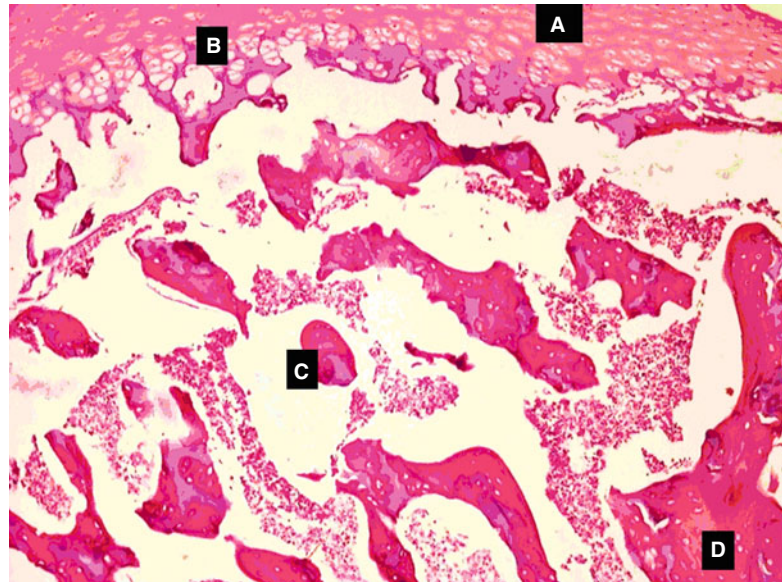
mesenchyme which carries with it hemopoietic cells and osteoprogenitor cells (f) showing trabeculae formation by osteoblasts differentiated from the osteoprogenitor cells and the hemopoietic cells forming the bone marrow

2.2.7.3 Primary Center of Ossification

The first site of ossification occurs in the primary center of ossification, which is in the middle of diaphysis (shaft) and is followed by the following events:

- *Formation of Periosteum.* Once vascularized, the perichondrium becomes the periosteum. The periosteum contains a layer of undifferentiated cells (osteoprogenitor cells) which later become osteoblasts.

Fig. 2.6 Endochondral ossification as seen at microscopy: *A* – showing resting hyaline cartilage, *B* – chondrocytes undergoing hyperplasia with rapid proliferation laid down as distinctive-looking stacks, *C* – calcification of the matrix occurs, *D* – chondrocytes are either dying or dead, leaving cavities that will later become invaded by bone-forming cells



- *Formation of Bone Collar.* The osteoblasts secrete osteoid against the shaft of the cartilage model (appositional growth). This serves as support for the new bone.
- *Calcification of Matrix.* Chondrocytes in the primary center of ossification begin to grow (hyperplasia) (Fig. 2.6). They stop secreting collagen and other proteoglycans and begin secreting alkaline phosphatase, an enzyme essential for mineral deposition. Then, calcification of the matrix occurs, and apoptosis of the hypertrophic chondrocytes occurs. This creates cavities within the bone.
- *Invasion of Periosteal Bud.* The hypertrophic chondrocytes (before apoptosis) secrete vascular endothelial cell growth factor that induces the sprouting of blood vessels from the perichondrium. Blood vessels forming the periosteal bud invade the cavity left by the chondrocytes and branch in opposite directions along the length of the shaft. The blood vessels carry hemopoietic cells, osteoprogenitor cells, and other cells inside the cavity. The hemopoietic cells will later form the bone marrow.
- *Formation of Trabeculae.* Osteoblasts, differentiated from the osteoprogenitor cells that entered the cavity via the periosteal bud, use the calcified matrix as a scaffold and begin to secrete osteoid, which forms the bone trabecula.

Osteoclasts, formed from macrophages, break down spongy bone to form the medullary (bone marrow) cavity.

2.2.7.4 Secondary Center of Ossification

About the time of birth, a secondary ossification center appears in each end (epiphysis) of long bones. Periosteal buds carry mesenchyme and blood vessels in, and the process is similar to that occurring in a primary ossification center. The cartilage between the primary and secondary ossification centers is called the epiphyseal plate, and it continues to form new cartilage, which is replaced by bone, a process that results in an increase in length of the bone. Growth continues until the individual is about 21 years old or until the cartilage in the plate is replaced by bone. The point of union of the primary and secondary ossification centers is called the epiphyseal line.

Appositional Bone Growth. The growth in diameter of bones around the diaphysis occurs by deposition of bone beneath the periosteum. Osteoclasts in the interior cavity continue to degrade bone until its ultimate thickness is achieved, at which point the rate of formation on the outside and degradation from the inside is constant. The cartilaginous extremity (where an epiphysis usually forms) continues to grow in pace with the rest of the bone by appositional and interstitial mechanisms.

When the whole bone is reaching maturity, epiphyseal and metaphyseal ossification gradually encroach upon this growth plate, and final bony fusion occurs with cessation of growth.

2.2.8 Biological Factors Involved in Normal Bone Formation and Its Regulation

There is a rapid formation of bone mass in the fetus and infant. This slows somewhat during childhood until age 11 in females and a year or so later in boys. During the growth spurt that accompanies adolescence, tremendous bone formation occurs. The vast majority of adult levels of bone mass are achieved by age 18 or so, with only a small amount added until about 28 years old.

2.2.8.1 Environmental Factors Influencing Normal Bone Formation

Physical activity and good nutrition are the most important of these environmental factors. People who are affected by any of these factors will likely have a lower bone mineral density (BMD) than their healthier peers. Poor activity levels and nutrition during the years of bone formation may prevent the normal growth of bones, which may cause them to be less dense. Smoking during these years may also decrease the amount of bone formed. A significant illness during the teenage years that causes prolonged bed rest and lack of exercise will also prevent the complete acquisition of bone density.

2.2.8.2 Hormones

There are a number of hormones that are important to this rapid formation of bone during the first two decades of life. These hormones include estrogen in females, testosterone in males, growth hormone, and others. They are discussed in detail in Sect. 2.3.2.

2.2.9 Bone Modeling

Modeling (reshaping) is the process by which bones change their overall shape in response to physiologic influences or mechanical forces, leading to

gradual adjustment of the skeleton to the forces that it encounters. Bones may widen or change axis by removal or addition of bone to the appropriate surfaces by independent action of osteoblasts and osteoclasts in response to biomechanical forces. Bones normally widen with aging in response to periosteal apposition of new bone and endosteal resorption of old bone. Wolff's law describes the observation that long bones change shape to accommodate stresses placed on them. Bone modeling is less frequent than remodeling in adults (Kobayashi et al. 2003). Modeling may be increased in hypoparathyroidism (Ubara et al. 2005), renal osteodystrophy (Ubara et al. 2003), or treatment with anabolic agents (Lindsay et al. 2006).

2.2.10 Determinants of Bone Strength

Bone strength depends on bone mass, geometry and composition, material properties, and microstructure. Bone mass accounts for 50–70 % of bone strength (Pocock et al. 1987). Bone geometry and composition are important, however, because larger bones are stronger than smaller bones, even with equivalent bone mineral density. As bone diameter expands radially, the strength of bone increases by the radius of the involved bone raised to the fourth power. The amount and proportion of trabecular and cortical bone at a given skeletal site affect bone strength independently. Bone material properties are important for bone strength. Some patients with osteoporosis have abnormal bone matrix. Mutations in certain proteins may cause bone weakness (e.g., collagen defects cause decreased bone strength in osteogenesis imperfecta, impaired γ -carboxylation of gla proteins). Bone strength can be affected by osteomalacia, fluoride therapy, or hypermineralization states. Bone microstructure affects bone strength also. In some pathological conditions, the thickness and extent of the osteoid layer may be increased (hyperosteoidosis) or decreased. Hyperosteoidosis may be caused by conditions of delayed bone mineralization (as in osteomalacia/rickets, vitamin D deficiency) or of increased bone formation (as in fracture callus, Paget's disease of bone, etc.).

2.3 Physiology of Bone Metabolism

Bone carries out some of the important metabolic functions; the important ones being:

- (a) *Mineral Reservoir.* Bones act as homeostatic reservoir of minerals important for the body, most important ones being calcium and phosphorus (Deftos 1998, 2001; Deftos and Gagel 2000). These bone minerals can be mobilized to maintain systemic mineral homeostasis. This metabolic function of bone prevails over its structural function in that calcium and other minerals are removed from and replaced in bone to serve systemic homeostatic needs irrespective of loss of skeletal structural integrity.
- (b) *Growth Factor and Cytokine Depository.* Mineralized bone matrix is also a depository for certain cytokines and growth factors that can be released upon its resorption and can exert their effects locally and systemically; notable among these are insulin-like growth factors (IGF), transforming growth factor- β (TGF- β) and bone morphogenetic proteins (BMP) (see Sect. 2.4.3.1).
- (c) *Fat Repository.* The yellow bone marrow acts as a storage reserve of fatty acids.
- (d) *Acid-base Equilibrium.* Bone buffers the blood against excessive pH changes by absorbing or releasing alkaline salts.
- (e) *Detoxification.* Bone tissues are capable of storing heavy metals and other extraneous elements, thus removing them from the circulation and helping in reducing their effects on other tissues.
- (f) *Endocrine Function.* Bone controls metabolism of phosphate by releasing fibroblast growth factor (FGF-23), which acts on kidneys to reduce phosphate reabsorption. A hormone called *osteocalcin* is also released by bone, which contributes to the regulation of blood glucose and fat deposition. Osteocalcin enhances both the insulin secretion and sensitivity, in addition to boosting up the number of insulin-producing β cells and reducing stores of fat.

2.3.1 Cellular and Intracellular Calcium and Phosphorus Metabolism

Both calcium and phosphorus, as well as magnesium, are transported to blood from bone, renal, and gastrointestinal cells, and vice versa. Mineral homeostasis requires the transport of calcium, magnesium, and phosphate across their target cells in bone, intestine, and kidney. Ninety-nine percent of body calcium, 85 % of phosphorus, and 65 % percent of total body magnesium are contained within the bones.

The regulation of bone and bone mineral metabolism results from the interactions among three important hormones – parathyroid hormone (PTH), calcitonin, and vitamin D – at three target organs, bone, kidney, and GI tract, to regulate the bone minerals (calcium and phosphorus).

2.3.2 Regulation of Skeletal Metabolism (Fig. 2.7)

Skeletal metabolism is regulated by bone cells and their progenitors. Among the population of bone cells are osteoblasts, osteocytes, and osteoclasts. Monocytes, macrophages, and mast cells may also mediate certain aspects of skeletal metabolism.

Osteoblasts express receptors to many bone-active agents such as PTH, parathyroid hormone-related protein (PTHrP), vitamin D metabolites, gonadal and adrenal steroids, and certain cytokines and growth factors. The major product of osteoblasts is type 1 collagen, which, along with other proteins, forms the organic osteoid matrix that is mineralized to hydroxyapatite. These osteoblasts become encased in bone during its formation and mineralization and reside in the resulting lacuna as *osteocytes*. While their synthetic activity decreases, the cells develop processes that communicate as canaliculi with other osteocytes, osteoblasts, and vasculature. These osteocytes, thus, present acres of cellular syncytium that permit translocation of bone mineral during times of metabolic activity

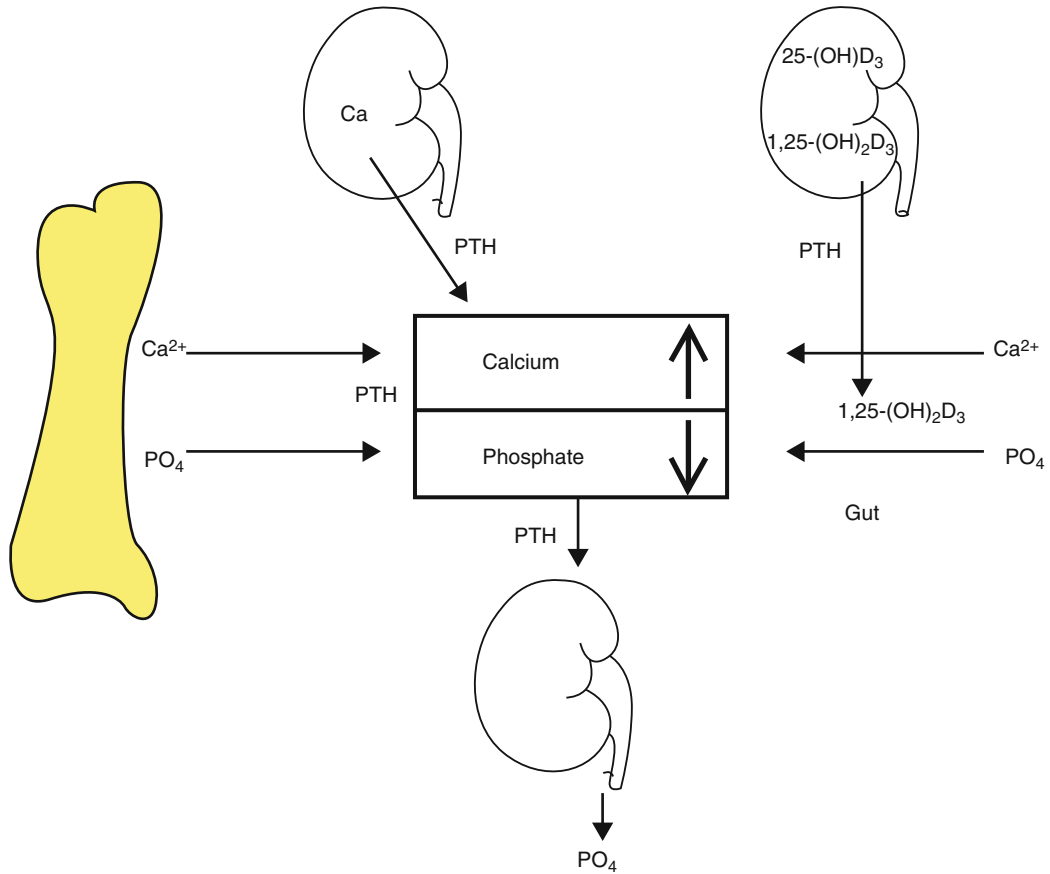


Fig. 2.7 Figure to show a close interlink between calcium, phosphate, and parathyroid hormone with vitamin D in bone metabolism

and can provide minute-to-minute exchanges of minerals from bone matrix.

Skeletal calcium is controlled through the regulatory pathways of the gastrointestinal (GI) tract and the kidney, and this regulation is mediated in bone by osteoblast and osteoclast. Calcium reaches the skeleton by being absorbed from the diet in the GI tract. Unabsorbed calcium passes into the feces, which also contains the small amount of calcium secreted into the GI tract. Minor losses occur through perspiration and cell sloughing. In pregnancy, substantial losses can occur across the placenta to the developing fetus and through breast milk. Absorbed dietary calcium then enters the extracellular fluid (ECF) space and becomes incorporated

into the skeleton through the process of mineralization of the organic matrix of bone, osteoid. ECF calcium is also filtered by the kidney at a rate of about 6 g/day, where up to 98 % of it is reabsorbed.

The various regulators playing an important role in skeletal metabolism are:

- Calcitropic hormones
- Parathyroid hormone (PTH)
- Calcitonin (CT)
- Vitamin D [1,25(OH)₂D]
- PTHrP
- Other hormones
- Gonadal and adrenal steroids
- Thyroid hormones
- Growth factors and cytokines

2.3.2.1 Parathyroid Hormone

Parathyroid hormone (PTH) is an 84-amino acid peptide secreted by two pairs of parathyroid glands located posterior to thyroid gland. The mature PTH is packaged into dense secretory granules for regulated secretion. The major regulatory signal for PTH secretion is serum calcium. Serum calcium inversely affects PTH secretion, with the steep portion of the sigmoidal response curve corresponding to the normal range of both.

The parathyroid gland senses the concentration of extracellular ionized calcium through a cell-surface calcium-sensing receptor (CSR) for which calcium is an agonist. The same sensor also regulates the responses to calcium of thyroid C cells, which secrete calcitonin in direct relationship to extracellular calcium; the distal nephron of the kidney, where calcium excretion is regulated; the placenta, where fetal-maternal calcium fluxes occur; and the brain and GI tract, where its function is unknown; and the bone cells. Serum phosphate has an inverse effect on calcium concentration, and ambient phosphate directly increases 1,25-D production. Thus, serum phosphate may directly and indirectly regulate PTH expression.

Metabolism and Clearance of Parathyroid Hormone

Parathyroid hormone, with a circulating half-life of less than 5 min, is metabolized in the liver, kidney, and blood. The carboxyl-terminal fragments are cleared by glomerular filtration, so they accumulate in renal failure. As a result of the biosynthesis, secretion, and metabolism of PTH, the circulation contains several forms of the molecule. Overall, 10–20 % of circulating PTH immunoreactivity comprises the intact hormone, with the remainder being a heterogeneous collection of peptide fragments corresponding to the middle and carboxy regions of the molecule.

Biologic Effects of Parathyroid Hormone (Fig. 2.7)

Parathyroid hormone regulates serum calcium and phosphorus concentrations through its receptor-mediated, combined actions on bone, intestine, and kidney. High levels of PTH, as seen in

primary and secondary hyperparathyroidism, increase osteoclastic bone resorption. Low levels, especially if delivered episodically, seem to increase osteoblastic bone formation. The skeletal effects of PTH are mediated through the osteoblast, since they are the major expressor of the PTH receptor. However, osteoblasts communicate with osteoclasts to mediate PTH effects. This communication seems mediated through the RANK-OPG pathway (see Sect. 2.4.1.3).

PTH increases the reabsorption of calcium in the kidney, predominantly in the distal convoluted tubule, and inhibits the reabsorption of phosphate in the renal proximal tubule, causing hypercalcemia and hypophosphatemia.

PTH mediates these effects through the PTH receptor which is an 80,000-MW membrane glycoprotein of the G protein receptor superfamily, while the parathyroid hormone-related protein (PTHrP) is the major humoral mediator of the hypercalcemia of malignancy and is secreted by many types of malignant tumors, notably by breast and lung cancer. Both PTH and PTHrP generate cyclic adenosine monophosphate (cAMP) as a cellular second messenger by activating protein kinase A (PKA), and the phospholipase C effector system increasing cellular IP₃ and calcium and activating protein kinase C (PKC).

2.3.2.2 Calcitonin

Calcitonin (CT) is a 32-amino acid peptide whose main effect is to inhibit osteoclast-mediated bone resorption. CT is secreted by parafollicular C cells of the thyroid and other neuroendocrine cells. In contrast to PTH, hypercalcemia increases secretion of hypocalcemia-inducing CT, while hypocalcemia inhibits secretion. It inhibits resorption of bone, increases calcium and phosphorus excretion, and decreases the blood levels of calcium and phosphorus (Deftos 1998).

2.3.2.3 Vitamin D

Vitamin D is a secosterol hormone that is present in humans in an endogenous (vitamin D₃) and exogenous (vitamin D₂) form. The endogenous form of vitamin D, cholecalciferol (vitamin D₃), is synthesized in the skin from the

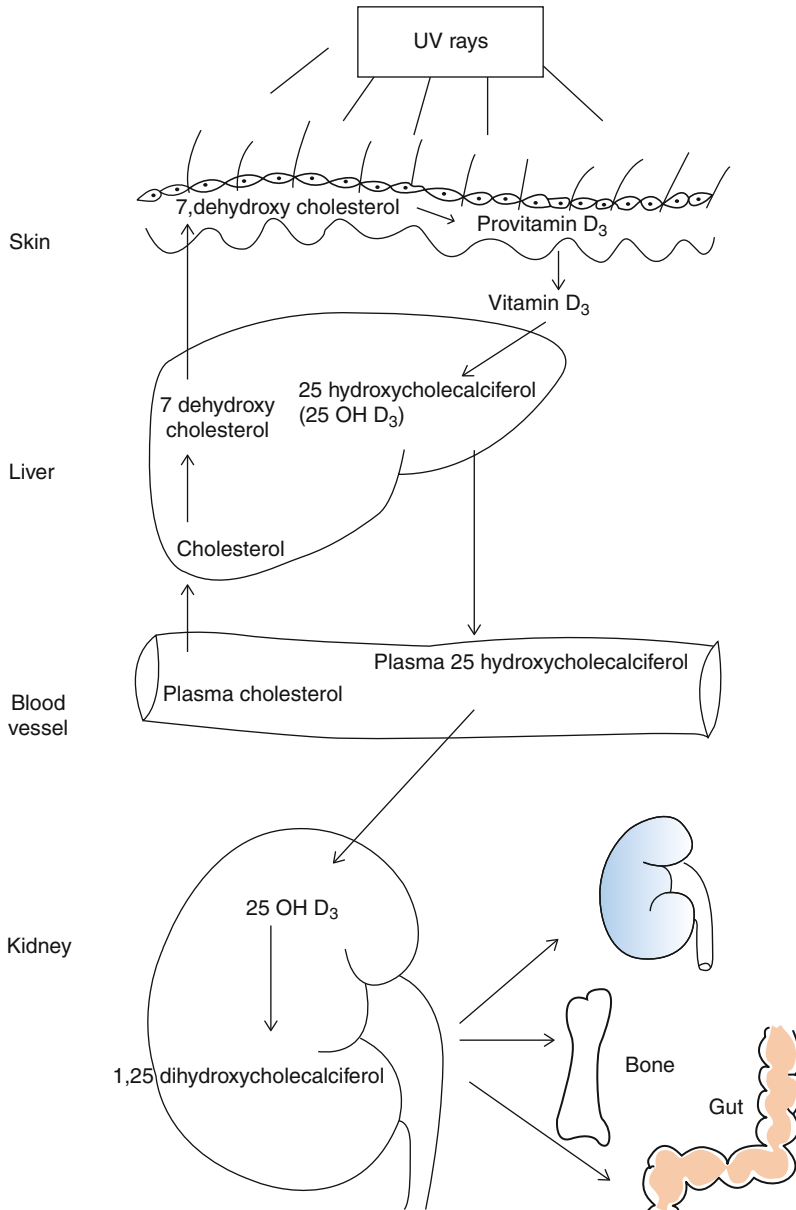


Fig. 2.8 Synthesis and sources of vitamin D₃ and its hydroxylation to form the hormone 1,25-dihydroxycholecalciferol with its main sites of action being *highlighted*

cholesterol metabolite 7-dehydrocholesterol under the influence of ultraviolet radiation (Deftos 1998) (Fig. 2.8). The exogenous form of vitamin D₂ (ergocalciferol) is produced by ultraviolet irradiation of the plant sterol ergosterol and is available through the diet. Both forms of vitamin D require further metabolism to be activated.

Effects of 1,25-Dihydroxyvitamin D on Mineral Metabolism

(a) *Intestinal Calcium Absorption.* Vitamin D increases intestinal calcium absorption, primarily in the jejunum and ileum, by increasing calcium uptake through the brush border membrane of the enterocyte. In a vitamin D-deficient state, only 10–15 % of dietary

calcium is absorbed by the gastrointestinal tract, but with adequate vitamin D, adults absorb approximately 30 % of dietary calcium (Deftos 1998). During pregnancy, lactation, and growth, increased circulating concentrations of 1,25-D promote the efficiency of intestinal calcium absorption by as much as 50–80 %. Vitamin D also regulates skeletal metabolism through the RANK pathway. 1,25-D also increases the efficiency of dietary phosphorus absorption by about 15–20 %.

- (b) *Bone*. Vitamin D promotes the mineralization of osteoid and causes bone resorption by mature osteoclasts, but this effect is indirect, requiring cell recruitment and interaction with osteoblasts and the fusion of monocytic precursors to osteoclasts. Vitamin D also regulates the expression of several bone proteins, notably osteocalcin. It promotes the transcription of osteocalcin and has bidirectional effects on type I collagen and alkaline phosphatase gene transcription.
- (c) *Kidney*. The kidney decreases calcium and phosphorus excretion.
- (d) *Blood*. Blood increases calcium and phosphorus levels.

2.3.2.4 Other Hormones

In addition to the primary calcemic hormones, other hormones play an important role in calcium and skeletal metabolism. Gonadal steroids maintain skeletal mass. Glucocorticoids are deleterious to all skeletal functions. Insulin, growth hormone, androgens, and thyroid hormones promote skeletal growth and maturation. Excess production of the latter can cause hypercalcemia (Deftos 1998; Kawaguchi et al. 1994). While in adults bone metabolism is basically limited to bone maintenance, the most obvious feature of bone metabolism in children and adolescents is increase in bone size in all three dimensions.

2.4 Bone Remodeling

Bone remodeling is a lifelong process wherein old bone is removed from the skeleton (a sub-process called bone resorption), and new bone is

added (a sub-process called ossification or bone formation). Remodeling involves continuous removal of discrete packets of old bone, replacement of these packets with newly synthesized proteinaceous matrix, and subsequent mineralization of the matrix to form new bone (Fernández-Tresguerres-Hernández-Gil et al. 2006; Fraher 1993). These processes also control the reshaping or replacement of bone during growth and following injuries like fractures but also microdamage (prevents accumulation of bone microdamage through replacement of old bone with the new one) (Turner 1998) which occurs during normal activity. Remodeling responds also to functional demands of the mechanical loading. As a result, bone is added where needed and removed where it is not required. This process is essential in the maintenance of bone strength and mineral homeostasis. The skeleton is a metabolically active organ that undergoes continuous remodeling throughout life. This remodeling is necessary both to maintain the structural integrity of the skeleton and to subservise its metabolic functions as a storehouse of calcium and phosphorus.

Normal bone remodeling cycle requires that the process of bone resorption and bone formation take place in a coordinated fashion, which in turn depends on the orderly development and activation of osteoclasts and osteoblasts, respectively. This property of bone, which constantly resorbs the old bone and forms new bone, makes the bone a very dynamic tissue that permits the maintenance of bone tissue, the repair of damaged tissue, and the homeostasis of the phosphocalcemic metabolism. The bone remodeling cycle involves a series of highly regulated steps that depend on the interactions of two cell lineages, the mesenchymal osteoblastic lineage and the hematopoietic osteoclastic lineage (Fraher 1993). The balance between bone resorption and bone deposition is determined by the activities of these two principle cell types, namely, osteoclasts and osteoblasts. Osteoblasts and osteoclasts, coupled together via paracrine cell signaling, are referred to as bone remodeling units.

In the young skeleton, the amount of resorbed bone is proportional to the newly formed. For this reason, it is referred to as a balanced process, linked in both space and time under normal

conditions. The average lifespan of each remodeled unit in humans is 2–8 months, the greater part of this time being taken up by bone formation.

Bone remodeling occurs throughout life, but only up to the third decade is the balance positive. It is precisely in the third decade when the bone mass is at its maximum, and this is maintained with small variations until the age of 50. From then on, resorption predominates and the bone mass begins to decrease. Bone remodeling increases in perimenopausal and early postmenopausal women and then slows with further aging but continues at a faster rate than in premenopausal women.

Although cortical bone makes up 75 % of the total volume, the metabolic rate is ten times higher in trabecular bone, since the surface area-to-volume ratio is much greater (trabecular bone surface representing 60 % of the total). Therefore, approximately 5–10 % of total bone is renewed per year.

Osteoclasts are endowed with highly active ion channels in the cell membrane that pump protons into the extracellular space, thus lowering the pH in their own microenvironment. This drop in pH dissolves the bone mineral (Blair et al. 1989).

The bone remodeling cycle involves a complex series of sequential steps (coupling of bone formation and bone resorption). Bone balance is the difference between the old bone resorbed and new bone formed. Periosteal bone balance is mildly positive, whereas endosteal and trabecular bone balances are mildly negative, leading to cortical and trabecular thinning with aging. These relative changes occur with endosteal resorption outstripping periosteal formation.

The main recognized functions of bone remodeling include preservation of bone mechanical strength by replacing older, microdamaged bone with newer, healthier bone and calcium and phosphate homeostasis. The relatively low adult cortical bone turnover rate of 2–3 %/year is adequate to maintain biomechanical strength of bone. The rate of trabecular bone turnover is higher, more than required for maintenance of mechanical strength, indicating that trabecular bone turnover is more important for mineral metabolism. Increased demand for calcium or phosphorus may require increased bone remodeling units.

2.4.1 Mediators of Remodeling

2.4.1.1 Osteoclasts

Osteoclasts are the only cells that are known to be capable of resorbing bone. They are typically multinucleated. Osteoclasts are derived from mononuclear precursor cells of the monocyte-macrophage lineage (hematopoietic stem cells that give rise to monocytes and macrophages) (Boyle et al. 2003). Mononuclear monocyte-macrophage precursor cells have been identified in various tissues, but bone marrow monocyte-macrophage precursor cells are thought to give rise to most osteoclasts.

2.4.1.2 Osteoblasts

Osteoblasts can be stimulated to increase bone mass through increased secretion of osteoid and by inhibiting the ability of osteoclasts to break down osseous tissue. Bone building through increased formation of osteoid is stimulated by the secretion of growth hormone by the pituitary, the thyroid hormone and the sex hormones (estrogens and androgens). The functional aspects of osteoblasts have been discussed in detail in the earlier section (Sect. 2.2.1) on bone formation.

2.4.1.3 RANK

The cell surface receptor called RANK (for receptor activator of NF κ B) prods osteoclast precursor cells to develop into fully differentiated osteoclasts when RANK is activated by its cognate partner RANK ligand (RANKL). RANKL belongs to the TNF superfamily and is critical for osteoclast formation. It is one of the key signaling molecules that facilitate cross talk between the osteoblasts and osteoclasts and help coordinate bone remodeling. RANKL and macrophage CSF (M-CSF) are two cytokines that are critical for osteoclast formation. Both RANKL and M-CSF are produced mainly by marrow stromal cells and osteoblasts in membrane-bound and soluble forms, and osteoclastogenesis requires the presence of stromal cells and osteoblasts in bone marrow (Teitelbaum and Ross 2003; Cohen 2006). Osteoprotegerin is another protein released by osteoblasts that acts as a decoy to prevent RANK and RANKL from coming in contact (Asagiri and Takayanagi 2007; Boyle et al. 2003; Gori et al. 2000; Lacey et al.

1998; Suda et al. 1999; Theill et al. 2002; Yasuda et al. 1998) (see Sect. 2.4.1.4).

Osteoblast precursors express a molecule called TRANCE, or osteoclast differentiation factor, which can activate cells of the osteoclast lineage by interacting with a receptor called RANK (Horwood et al. 1998; Yasuda et al. 1998).

2.4.1.4 Osteoprotegerin

Osteoprotegerin (OPG), also known as osteoclast inhibiting factor (OCIF) or osteoclast binding factor (OBF), is a key factor inhibiting the differentiation and activation of osteoclasts, and is, therefore, essential for bone resorption. Osteoprotegerin is a dimeric glycoprotein belonging to the TNF receptor family. Osteoprotegerin inhibits the binding of RANK to RANKL and thus inhibits the recruitment, proliferation, and activation of osteoclasts. Abnormalities in the balance of OPGL/RANK/OPG system lead to the increased bone resorption that underlies the bone damage of postmenopausal osteoporosis, Paget's disease, bone loss in metastatic cancers, and rheumatoid arthritis. Bone resorption depends on osteoclast secretion of hydrogen ions and cathepsin K enzyme. H^+ ions acidify the resorption compartment beneath osteoclasts to dissolve the mineral component of bone matrix, whereas cathepsin K digests the proteinaceous matrix, which is mostly composed of type I collagen (Boyle et al. 2003). Osteoclasts bind to bone matrix via integrin receptors in the osteoclast membrane linking to bone matrix peptides. They digest the organic matrix, resulting in formation of saucer-shaped Howship's lacunae on the surface of trabecular bone and Haversian canals in cortical bone. The resorption is completed by mononuclear cells after the multinucleated osteoclasts undergo apoptosis (Eriksen 1986; Reddy 2004; Teitelbaum et al. 1995; Vaananen et al. 2000).

The boundary between the old and new bone is distinguished in an hematoxylin and eosin section by a blue (basophilic) line called a cement line or reversal line.

2.4.1.5 Paracrine Cell Signaling

At various stages throughout this process of remodeling, the precursors, osteoclasts, and

osteoblasts communicate with each other through the release of various "signaling" molecules.

Osteoclasts are apparently activated by "signals" from osteoblasts. For example, osteoblasts have receptors for PTH, whereas osteoclasts do not, and PTH-induced osteoclastic bone resorption is said not to occur in the absence of osteoblasts. The action of osteoblasts and osteoclasts is controlled by a number of chemical factors which either promote or inhibit the activity of the bone remodeling cells, controlling the rate at which bone is made, destroyed, or changed in shape. The cells also use paracrine signaling to control the activity of each other, described in Sect. 2.4.3.

2.4.2 Remodeling Phases

Bone remodeling can be divided into the following six phases (Fig. 2.9), namely, quiescent, activation, resorption, reversal, formation, and mineralization. Activation precedes resorption which precedes reversal, with mineralization as the last step. These occur at remodeling sites which are distributed randomly but also are targeted to areas that require repair (Burr 2002; Fernández-Tresguerres-Hernández-Gil et al. 2006; Parfitt 2002).

1. *Quiescent Phase.* It is the state/phase of the bone when at rest. The factors that initiate the remodeling process remain unknown.
2. *Activation Phase.* The first phenomenon that occurs is the activation of the bone surface prior to resorption, through the retraction of the bone lining cells (elongated mature osteoblasts existing on the endosteal surface) and the digestion of the endosteal membrane by collagenase action. The initial "activation" stage involves recruitment and activation of mononuclear monocyte-macrophage osteoclast precursors from the circulation (Bruzzaniti and Baron 2007; Roodman et al. 1992), resulting in interaction of osteoclast and osteoblast precursor cells. This leads to the differentiation, migration, and fusion of the large multinucleated osteoclasts. These cells attach to the mineralized bone surface and initiate resorption by the secretion of

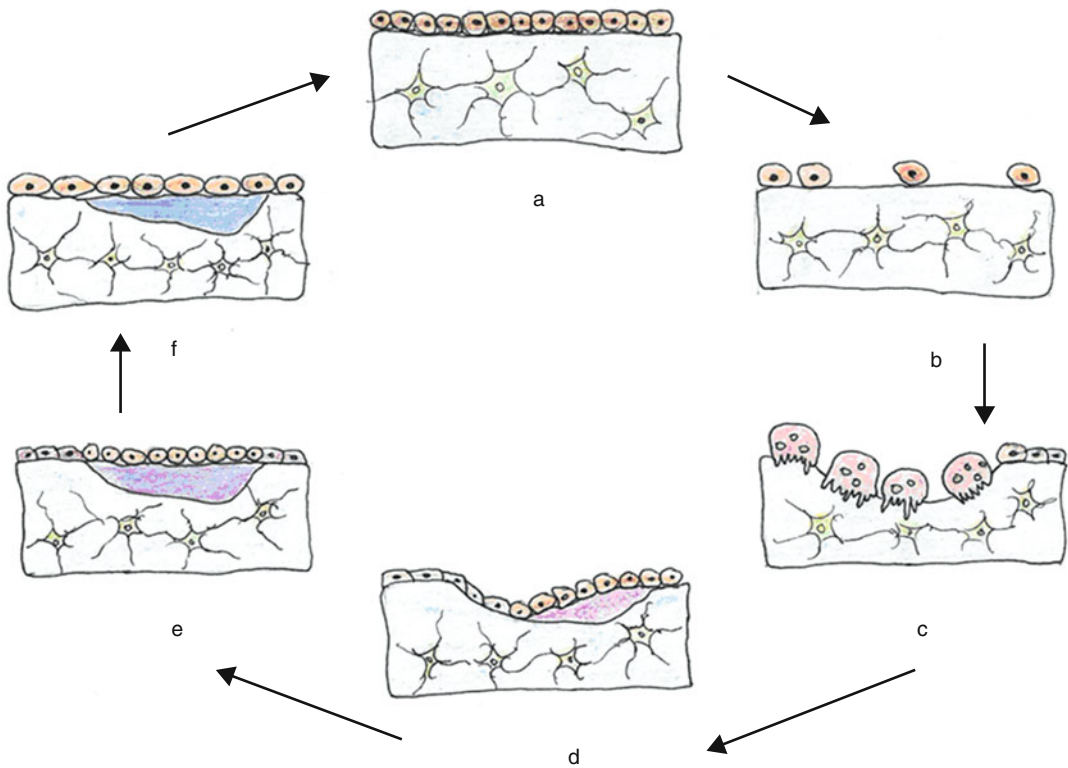


Fig. 2.9 Phases of bone remodeling: (a) quiescent phase where flat bone lining cells are seen lining the endosteal membrane (b) showing activation phase characterized by cell retraction with resultant membrane resorption (c) shows formation of bone structure unit with progression to quiescent phase

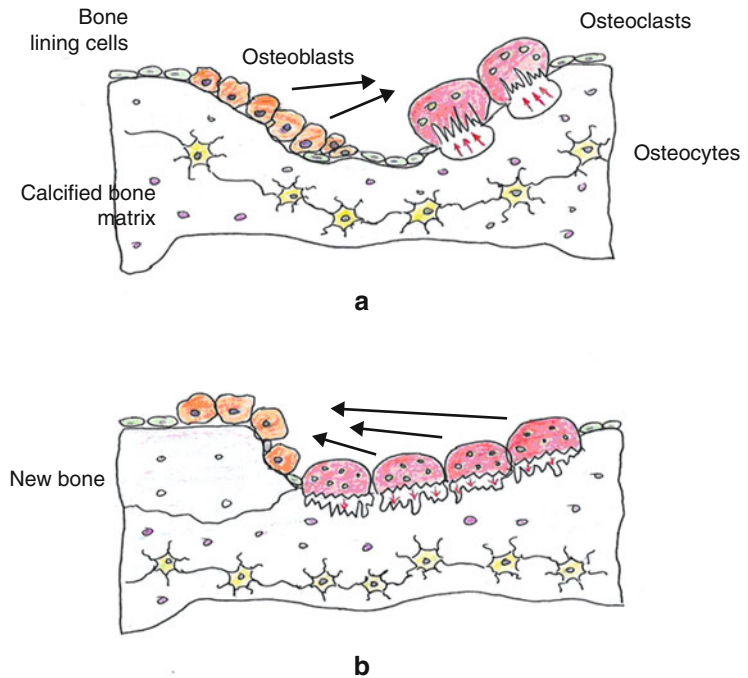
(d) shows formation phase where the osteoclasts are replaced by osteoblasts with underlying new osteoid matrix (e) shows mineralization of osteoid matrix (f) shows formation of bone structure unit with progression to quiescent phase

hydrogen ions and lysosomal enzymes, particularly cathepsin K, which can degrade all the components of bone matrix, including collagen, at low pH.

3. **Resorption Phase** (Fig. 2.10). The osteoclasts then begin to dissolve the mineral matrix and decompose the osteoid matrix. This process is completed by the macrophages and permits the release of the growth factors contained within the matrix, fundamentally transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), and insulin-like growth factor I and II (IGF-I and II). Osteoclastic resorption produces irregular scalloped cavities on the trabecular bone surface, called Howship's lacunae, or cylindrical Haversian canals in cortical bone. Osteoclast-mediated bone resorption takes only approximately 2–4 weeks during each remodeling cycle.

4. **Reversal Phase.** During the reversal phase, bone resorption transitions to bone formation. At the completion of bone resorption, resorption cavities contain a variety of mononuclear cells, including monocytes, osteocytes released from bone matrix, and preosteoblasts, recruited to begin new bone formation. The coupling signals linking the end of bone resorption to the beginning of bone formation are as yet unknown, but proposed coupling signal candidates include bone matrix-derived factors such as TGF- β , IGF-1, IGF-2, bone morphogenetic proteins, PDGF, or fibroblast growth factor (Bonewald and Mundy 1990; Hock et al. 2004; Locklin et al. 1999).
5. **Formation Phase.** Once osteoclasts have resorbed a cavity of bone, they detach from the bone surface and are replaced by cells of the osteoblast lineage which in turn initiate

Fig. 2.10 Figure to show normal bone remodeling (a) vs. defective resorption (b) seen in disease states



bone formation. The *preosteoblast* grouping phenomenon is produced and attracted by the growth factors liberated from the matrix which act as chemotactics and in addition stimulate their proliferation (Lind et al. 1995). The preosteoblasts synthesize a cementing substance upon which the new tissue is attached and express *bone morphogenic proteins* (BMP) responsible for differentiation (refer Sect 2.4.3.2). A few days later, the already differentiated osteoblasts synthesize the *osteoid matrix* which fills the (resorption cavity) perforated areas (Lind et al. 1995). The remaining osteoblasts continue to synthesize bone until they eventually stop and transform to quiescent lining cells that completely cover the newly formed bone surface and connect with the osteocytes in the bone matrix through a network of canaliculi.

6. *Mineralization Phase*. The process begins 30 days after deposition of the osteoid, ending at 90 days in the trabecular and at 130 days in the cortical bone. The quiescent or “at rest” phase then begins again.

When the cycle is completed, the amount of bone formed should equal the amount of bone resorbed.

2.4.3 Regulatory Factors in Bone Remodeling

The balance between bone resorption and formation is influenced by such interrelated factors as genetic, mechanical, vascular, nutritional, hormonal, and local.

2.4.3.1 Systemic Regulation of Bone Remodeling

1. *Genetic Factors*

These are important in determining the maximum bone mass, since between 60 and 80 % of this bone mass is genetically determined. Thus, Negroes have a greater bone mass than Whites, who in turn have a higher mass than Asians. Bone mass is a characteristic transmitted from parents to children, which is why daughters of mothers with osteoporosis are more predisposed

to having this condition themselves (Grant and Ralston 1997; Pocock et al. 1987).

2. *Mechanical Factors*

Remodeling is regulated by mechanical loading, allowing bone to adapt its structure in response to the mechanical demands. Physical activity is essential for the correct development of bone. It is believed that muscular action transmits tension to the bone, which is detected by the osteocyte network within the osseous fluid. On the other hand, the absence of muscular activity, rest, or weightlessness has an adverse effect on bone, accelerating resorption. It is well-known that trabeculae tend to align with maximum stresses in many bones. Mechanical stress improves bone strength by influencing collagen alignment as new bone is being formed. Cortical bone tissue located in regions subject to predominantly tensile stresses has a higher percentage of collagen fibers aligned along the bone long axis. In regions of predominant compressive stresses, fibers are more likely to be aligned transverse to the long axis.

3. *Vascular/Nerve Factors*

Vascularization is fundamental for normal bone development, supplying blood cells, oxygen, minerals, ions, glucose, hormones, and growth factors. Vascularization constitutes the first phase in ossification: the blood vessels invade the cartilage and later produce resorption via the osteoclasts originating from the nearby vessels. In the same way, vascular neof ormation is the first event in the repair of fractures or bone regeneration (Trueta 1963). Innervation is necessary for normal bone physiology. The bone is innervated by the autonomous nervous system and by sensorial nerve fibers. Autonomous fibers have been found in periosteum, endosteum, and cortical bone and associated with the blood vessels of the Volkmann conduit, and likewise neuropeptides and their receptors in bone (Wheless 2011). Examples of the importance of innervation in bone physiology are found in osteopenia and the bone fragility present in patients with neurological disorders, and also

in the decreased bone density in de-nerved mandibles.

4. *Nutritional Factors*

A minimum amount of calcium is needed for mineralization, which the majority of authors put at 1,200 mg/day to the age of 25, not less than 1 g/day from 25 to 45, and following menopause should be at least 1,500 mg/day. Likewise, it is known that toxic habits such as smoking, caffeine, alcohol, and excess salt constitute risk factors for osteopenia.

5. *Hormonal Factors*

Normal development of the skeleton is conditioned by the correct functioning of the endocrine system. The most important hormones in bone remodeling are:

- (a) *Thyroid Hormones*. Thyroid hormones can also stimulate bone resorption and formation (possess two opposing actions on bone) and are critical for maintenance of normal bone remodeling (Kawaguchi et al. 1994). In the first place, they stimulate the synthesis of the osteoid matrix by the osteoblasts and its mineralization, favoring the synthesis of IGF-I. For this reason, in congenital hypothyroidism (cretinism), short stature is produced by the alteration in bone formation. In the second place, a contrary effect is produced, stimulating resorption with the increase in number and function of the osteoclasts. The clinical manifestation of this effect is the appearance of bone loss in hyperthyroidism.
- (b) *Parathyroid Hormone (PTH)*. It controls the homeostasis of calcium by direct action on the bone and the kidneys and indirectly on the intestine. It is produced by the parathyroid glands in response to hypocalcemia. Continual supply of PTH would stimulate bone resorption through the synthesis of a factor favoring osteoclastogenesis (RANKL) on the part of the osteoblastic cells, while at intermittent doses it would stimulate the formation of bone, associated with an increase of the above-mentioned growth factors and with a decrease in the apoptosis of the osteoblasts. PTH regulates serum calcium concentration. It is a potent stimulator of bone resorption and

has biphasic effects on bone formation. There is an acute inhibition of collagen synthesis with high concentrations of PTH, but prolonged intermittent administration of this hormone produces increased bone formation, a property for which it is being explored clinically as an anabolic agent (Dempster et al. 1993). Plasma PTH tends to increase with age, and this may produce an increase in bone turnover and a loss of bone mass, particularly of cortical bone (see Sect. 2.3.2.1).

- (c) *Calcitonin*. Produced by the parafollicular C cells of the thyroid, it is an inhibitor of bone resorption, reducing the number and activity of the osteoclasts (see Sect. 2.3.2.1). However, this is a transitory action, since the osteoclasts seem to become “impermeable” to calcitonin within a few days.
- (d) *1,25(OH)₂ Vitamin D₃ or Calcitriol*. A steroid hormone, by favoring the intestinal absorption of calcium and phosphate, favors bone mineralization. It is necessary for normal growth of the skeleton. Some authors believe it may be produced by lymphocytic or monocytic bone cells, playing an important role as a local regulator of osteoclast differentiation (Raisz 1993).
- (e) *Androgens*. Androgens have an anabolic effect on bone through the stimulation of the osteoblast receptors. Likewise, they act as mediators of the growth hormone in puberty. While androgen deficiency is associated with lower bone density, the administration of testosterone in young people before the closure of the epiphyses increases bone mass. In the same way, women with an excess of androgens present higher bone densities. Androgens increase cortical bone size via stimulation of both longitudinal and radial growth. First, androgens, like estrogens, have a biphasic effect on endochondral bone formation: at the start of puberty, sex steroids stimulate endochondral bone formation, whereas they induce epiphyseal closure at the end of puberty. This effect of androgens may be important because bone strength in males seems to be determined by relatively higher periosteal bone formation and, therefore, greater bone dimensions, relative to muscle mass at older age. Androgens protect men against osteoporosis via maintenance of cancellous bone mass and expansion of cortical bone.
- (f) *Estrogens*. Estrogens are essential for the closure of the growth plates and have an important role in the development of the skeleton. Estrogens have a dual effect on bone metabolism: on the one hand, they favor bone formation, increasing the number and function of the osteoblasts, and on the other, they reduce resorption. Estrogen receptors have been described in human osteoblasts, osteocytes, and osteoclasts. Recent investigations have found that estrogens can increase the levels of osteoprotegerin (OPG), a protein produced by osteoblasts that inhibits resorption, so they may play an important role in the regulation of osteoclastogenesis (Hofbauer et al. 1999). Alternatively, estrogen may inhibit local factors that impair bone formation or enhance local factors that stimulate bone formation. For this reason, estrogen deficiency during menopause constitutes the most important pathogenic factor in bone loss associated with osteoporosis. Loss of estrogens or androgens increases the rate of bone remodeling by removing restraining effects on osteoblastogenesis and osteoclastogenesis and also causes a focal imbalance between resorption and formation by prolonging the lifespan of osteoclasts and shortening the lifespan of osteoblasts.
- (g) *Progesterone*. Progesterone also has an anabolic effect on bone, either directly, through the osteoblasts which possess hormone receptors, or indirectly, through competition for the osteoblastic receptors of the glucocorticoids.
- (h) *Insulin*. Insulin stimulates matrix synthesis both directly and indirectly, increasing the hepatic synthesis of IGF-I (insulin-like growth factor) (see Sect. 2.4.3.2).
- (i) *Glucocorticoids*. Glucocorticoids are necessary for bone cell differentiation during development, but their greatest postnatal effect is to inhibit bone formation (at high doses, they have a catabolic effect on bone), since they inhibit the synthesis of IGF-I by the osteoblasts

and directly suppress BMP-2, critical factors in osteoblastogenesis. This is the major pathogenetic mechanism in glucocorticoid-induced osteoporosis. Indirect effects of glucocorticoids on calcium absorption and sex hormone production may, however, increase bone resorption (Lukert and Kream 1996; Manolagas 2000).

- (j) **Growth Hormone.** Growth hormone acts both directly and indirectly on bone. Growth hormone acts directly on the osteoblasts with hormone receptors, stimulating their activity, thus increasing the synthesis of collagen, osteocalcin, and alkaline phosphate. The indirect action is produced through an increase in synthesis of IGF-I and II by the osteoblasts. These factors stimulate the proliferation and differentiation of the osteoblasts, increasing their number and function (Harvey and Hull 1998; Rosen and Donahue 1998).

Thus, the hormones that regulate bone metabolism are as follows:

- Decrease bone resorption
 - Calcitonin
 - Estrogens
- Increase bone resorption
 - PTH/PTHrP
 - Glucocorticoids
 - Thyroid hormones
 - High-dose vitamin D
- Increase bone formation
 - Growth hormone
 - Vitamin D metabolites
 - Androgens
 - Insulin
 - Low-dose PTH/PTHrP
 - Progestogens
- Decrease bone formation
 - Glucocorticoids

2.4.3.2 Local Regulators of Bone Remodeling

Bone remodeling is also regulated by local factors, among which principally growth factors and cytokines, and recently, the bone matrix proteins have been implicated as modulators of other local factors (Raisz 1999; Fernández-Tresguerres-Hernández-Gil et al. 2006). Bone cells also play

an important role in the production of prostaglandins and nitric oxide, as well as cytokines and growth factors.

The important local factors acting on the skeleton are as follows and are tabulated in Table 2.1:

Growth Factors

Bone contains a large number of growth factors. These are polypeptides produced by the bone cells themselves or in extra-osseous tissue and act as modulators of the cellular functions, fundamentally growth, differentiation, and proliferation. Among the most abundant are the IGFs, which, with their associated binding proteins, may be important modulators of local bone remodeling (Fraher 1993; Hakeda et al. 1996; Rosen and Donahue 1998). Transforming growth factor- β and the related family of bone morphogenetic proteins are present in the skeleton and have important functions not only in remodeling but also in skeletal development. Other growth factors, such as platelet-derived growth factor, PTH-related protein, and fibroblast growth factor, may play an important role in physiologic remodeling and an even more important role in the remodeling associated with skeletal repair.

- (a) *IGF-I and II (Insulin-Like Growth Factor I and II).* These are polypeptides similar to insulin; they are synthesized by the liver and osteoblasts and found in high concentrations in the osteoid matrix (Cohick and Clemmons 1993). They increase the number and function of the osteoblasts, stimulating collagen synthesis. They circulate linked to IGF-binding proteins (*IGFBP*), which in turn can exercise stimulatory or inhibitory effects on bone (Conover 2008). IGF synthesis is regulated by local growth factors and hormones; thus, growth hormone, estrogens, and progesterone increase their production, while the glucocorticoids inhibit it. They also mediate in the osteoblast-osteoclast interaction and actively participate in bone remodeling (Hill et al. 1995). IGF-II is the most abundant factor in the bone matrix and is important during embryogenesis (Canalis et al. 1989; Mohan and Baylink 1991).

Table 2.1 Local factors in bone remodeling

	Stimulate bone formation	Stimulate bone resorption	Inhibit bone resorption
Growth factors	BMP-2, BMP-4, BMP-6, BMP-7, IGF-I, IGF-II TGF- β , FGF, and PDGF	TNF, EGF, PDGF, FGF, M-CSF, and GM-CSF	
Cytokines	IL-4, IL-13, IFN, and OPG	IL-1, IL-6, IL-8, IL-11, PGE ₂ , PGE1, PGG2, PGI2, and PGH2	IFN- γ IL-4

- (b) *Transforming growth factor- β (TGF- β)* is a superfamily of proteins highly abundant in bone tissue (second after IGF). They are latently present in the matrix and activate during osteoclastic resorption. TGF- β is a potent stimulator of bone formation, promoting osteoblastic differentiation and the synthesis of the osteoid matrix and inhibiting the synthesis of the proteases, especially the matrix metalloproteinase (*MMP*), an enzyme which degrades it (Bonewald and Dallas 1994). TGF- β inhibits resorption on reducing the formation and differentiation of the osteoclasts, as well as mature osteoclast activity, and stimulating their apoptosis (Baylink et al. 1993).
- (c) *Bone morphogenetic proteins (BMP)* are included in the TGF- β family. They form a group of 15 proteins able to achieve the transformation of connective tissue into bone tissue, for which they are considered osteoinductive (Sakou 1998). They stimulate the differentiation of the stem cells toward different cell lines (adipose tissue, cartilage, and bone). They are highly abundant in bone tissue and during embryogenesis participate in the formation of bone and cartilage (Yamaguchi et al. 2000). They strongly promote osteoblastic differentiation and are believed to inhibit osteoclastogenesis in addition to stimulating osteogenesis (Canalis et al. 2003). Another cell surface receptor called the low-density lipoprotein (LDL)-related protein 5 receptor (LRP5) is also important for bone formation.
- (d) *Platelet-derived growth factor (PDGF)*, on the one hand, stimulates protein synthesis brought about the osteoblasts and, on the other, favors bone resorption. Other effects are the proliferation of fibroblasts and smooth muscle cells, neovascularization, and collagen synthesis, therefore favoring scarring (Nash et al. 1994).
- (e) *Fibroblastic growth factor (FGF)* has an anabolic effect on bone, as it is a mitogen of osteoblasts, vascular endothelial cells, and fibroblasts. As a practical example of the effect of FGF, it is known that mutations in its receptors produce alterations in the craniofacial skeleton, such as achondroplasia, Apert's syndrome, and Crouzon's syndrome, among others (Marie 2003).
- (f) *Epidermal growth factor (EGF)* is a powerful mitogen of cells of mesodermic or ectodermic origin. It has dual formative and destructive action, although the latter is the most well known.
- (g) *Vascular endothelial growth factor (VEGF)* induces angiogenesis and vascular endothelial proliferation. It produces vasodilation and an increase in vascular permeability. It is produced in hypoxia and is currently considered one of the key factors in the first phases of fracture repair and bone regeneration, as well as in tumor growth.
- (h) *Granulocyte/macrophage-colony stimulating factor (GM-CSF)* is important in osteoclastogenesis and may play a role in the pathogenesis of osteopetrosis.
- (i) *Macrophage-colony stimulating factor (M-CSF)* is produced by osteoblasts and medullar stromal cells. It is an essential factor in the first phases of osteoclastogenesis and is required for the formation of giant multinucleate cells but have no effect on osteoclastic activity.
- (j) *Tumor Necrosis Factor (TNF)*. Tumor necrosis factor in vitro stimulates resorption and has been related with bone loss in arthritis and periodontal disease.

Matrix Proteins

The matrix proteins have recently been discovered to act as growth factor modulators (Young 2003). Matrix proteins are found in concentrations a 1,000 times higher than growth factors and could therefore play a more important role in the regulation of the different cell functions (Horowitz 2003). Matrix proteins also participate in regulation of the differentiation of the cells contained within the matrix. For example, collagen I is one of the earliest markers which regulates the osteoprogenitor cells, and alkaline phosphatase is a surface protein that could participate in the regulation of the proliferation, migration, and differentiation of the osteoblastic cells. Osteonectin, fibronectin, and osteocalcin promote cell attachment, facilitate cell migration, and activate cells.

Cytokines

These are polypeptides synthesized in the lymphocytic and monocytic cells and play an important role in multiple cellular functions, such as the immunological response, inflammation, and hematopoiesis, having both an autocrine and paracrine effect. The following are important in bone:

- (a) *Interleukin 1 (IL-1)* directly stimulates osteoclastic resorption, increasing the proliferation and differentiation of the preosteoblasts, as well as the osteoclastic activity, and inhibiting the apoptosis of the osteoclasts (Compston 2001). In reality, they are three different related molecules: IL-1 α , IL-1 β , and the IL-1 receptor antagonist, the last being the inhibitor of the first two. They act both directly and indirectly on resorption through the synthesis of prostaglandins.
- (b) *Interleukin 6 (IL-6)* stimulates bone resorption and appears to be implicated in the pathogenesis of Paget's disease (Roodman 1999). It is believed to play an important role in the initial stages of osteoclastogenesis and is produced in response to PTH, IL-1, and $1,25(\text{OH})_2\text{D}_3$.
- (c) *Interleukin 11 (IL-11)*. Recently discovered, it is produced in bone marrow and induces osteoclastogenesis.
- (d) *Prostaglandins (PG)*. Prostaglandins, particularly prostaglandin E2 (PGE2), stimulate

bone resorption (Kawaguchi et al. 1995). This could also be important in inflammatory bone loss. The first step on PGE2 synthesis is carried out by an enzyme called cyclooxygenase 2 (COX2), and inhibitors of this enzyme can prevent bone formation in response to mechanical stress in animals. PGE2 may be required for exercise-induced bone formation.

- (e) *Leukotrienes*. Leukotrienes are another set of lipid molecules that appear to regulate bone remodeling.

Thus, the growth factors that regulate bone remodeling are:

- Insulin-like growth factors (IGF) I and II
- Transforming growth factor- β (TGF- β) superfamily, including the bone morphogenetic proteins (BMPs)
- Fibroblast growth factors (FGFs)
- Platelet-derived growth factors (PDGFs)
- Cytokines

2.4.4 Markers of Bone Metabolism

Biochemical markers of bone metabolism provide dynamic information about the turnover of osseous tissue (Schneider et al. 1998). They can be broadly classified as reflecting either bone formation or bone resorption.

2.4.4.1 Markers of Bone Formation

Alkaline Phosphatase

Alkaline phosphatase in serum has been used for more than 50 years to monitor bone metabolism and is still the most frequently used marker. Alkaline phosphatase is an ectoenzyme anchored to the cell surfaces of osteoblasts and other cells (Delmas 1995). However, alkaline phosphatase is not specific to bone, and ideally selective measurement of the bone isoenzyme should be used as a marker of bone formation. The clearance from the circulation is relatively slow, with a half-life in the order of 1–3 days for the bone isoenzyme. Its values in plasma and serum are raised in conditions such as Paget's disease, osteomalacia, and after fractures or ectopic bone formation.

In clinical practice, the major problem for diagnostic purposes is to distinguish between the isoenzymes derived from liver and bone, although the intestinal enzyme may be raised after meals and the placental isoenzyme during pregnancy. The bone isoenzyme can be distinguished based on sialic acid residues. In normal individuals, about half of the total alkaline phosphatase is derived from bone and the rest from liver. In conditions such as Paget's disease, the changes in alkaline phosphatase are often very substantial.

Osteocalcin

Osteocalcin, also known as bone gla protein (BGP), is a bone specific protein, which has proven to be a sensitive and specific marker of osteoblast activity in a variety of metabolic bone diseases. Its synthesis is dependent upon the presence of active metabolites of vitamin D, especially 1,25-dihydroxyvitamin D and requires vitamin K for the conversion by carboxylation of three glutamate residues to gamma-carboxyglutamate (gla) (Delmas 1995). The post-translational modifications confer calcium-binding properties on osteocalcin. This can be used to differentiate fully carboxylated from partially carboxylated osteocalcin in the circulation, and it has been shown that a significant proportion of osteocalcin in osteoporotic elderly patients is incompletely carboxylated.

Measurements of serum osteocalcin by immunoassays show increased values in conditions associated with increased bone formation, for example, hyperparathyroidism, hyperthyroidism, and bone metastases. In Paget's disease, however, the rises are less than expected, perhaps reflecting differential incorporation into bone matrix or altered synthesis by osteoblasts (Gallagher 1997). Reduced levels of osteocalcin may reflect lower rates of bone formation, as seen, for example, in myeloma. Osteocalcin values may be substantially reduced during treatment with glucocorticosteroids, although in this case, it should be remembered that glucocorticoids specifically suppress osteocalcin synthesis by osteoblasts, while not necessarily similarly depressing collagen synthesis

or production of alkaline phosphatase to an equivalent degree. Serum osteocalcin values can reflect the age-related increase in bone turnover, and the values rise after the menopause and fall after treatment with estrogens. Serum osteocalcin correlates with skeletal growth at the time of puberty and is increased in a variety of conditions characterized by increased bone turnover, such as primary and secondary hyperparathyroidism, hyperthyroidism, Paget's disease, and acromegaly (Fraher 1993).

Procollagen Peptides

Collagen is the major structural protein of bone and comprises about 90 % of the organic material. Collagen clearly contributes to the integrity and strength of bone matrix, and defects in its production, for example, in osteogenesis imperfecta, leads to bone of poor quality, susceptible to fracture. Attempts to measure collagen synthesis is, therefore, a more logical approach than measuring other less abundant matrix constituents in the assessment of bone formation. During collagen synthesis, pro-peptides are released both from the amino-terminal ("N-terminal") and carboxyterminal ("C-terminal") ends of the procollagen molecule, after the three individual alpha chains have formed the triple helix, which will become part of the collagen fibril. Assays for both the N- and C-terminal pro-peptides exist. The values are increased during growth and in situations of increased bone formation, such as occur in Paget's disease, and in response to growth hormone.

2.4.4.2 Markers of Bone Resorption

Biochemical markers used to monitor bone resorption include urinary measurements of:

- (a) *Hydroxyproline-Containing Peptides*. Studies in relation to osteoporosis show that, when measurements are made carefully, urinary hydroxyproline values rise after the menopause and fall again when antiresorptive drugs such as estrogens, calcitonins, and bisphosphonates are given. Pyridinoline crosslinks – pyridinoline (Pyr) and deoxypyridinoline (DPyr) – also called hydroxylysyl pyridinoline (HL) and lysyl pyridinoline (LP), respectively,

are currently receiving considerable attention as the most promising markers of bone resorption. Both are non-reducible crosslinks which stabilize the collagen chains within the extracellular matrix and are formed by the condensation of three lysine and/or hydroxylysine residues in adjacent alpha chains.

- (b) *Hydroxylysine glycosides* and pyridinoline crosslinks (Delmas 1995) are derived from type I collagen. Hydroxylysine, like hydroxyproline derived from proline, is produced by a post-translational hydroxylation. The subsequent glycosylation of hydroxylysine differs in collagens in different tissues. The monoglycosylated galactosyl hydroxylysine is enriched in bone compared with the diglycosylated form, glucosyl galactosyl hydroxylysine, which is the major form in skin. This may be a useful marker of bone resorption, for example, in osteoporosis.
- (c) *Acid Phosphatase*. Acid phosphatase is a lysosomal enzyme which exists in several forms in different tissues. The type 5 isoenzyme is the one found in osteoclasts, which appear to be released during bone resorption. Assays of total tartrate-resistant acid phosphatase (TRAP) in the circulation are moderately raised in disorders associated with increased bone resorption, but the assays are difficult to perform because of the instability of the enzyme and the relatively small changes observed in pathological states.
- (d) *Other Assays*. In some circumstances, fasting urine calcium can give an indirect measure of bone resorption rates and may be useful in Paget's disease and in patients with metastatic bone disease for following responses to treatment. Other measurements include assays of tartrate-resistant acid phosphatase and free gamma-carboxyglutamic acid. Many cytokines and growth factors influence bone metabolism (e.g., interleukins 1 & 6, tumor necrosis factors [TNFs], insulin-like growth factors I & II and their binding proteins). These assays have been of help with the diagnosis and management of patients with florid disorders of bone metabolism, such as Paget's disease or vitamin D-deficient osteomalacia.

2.4.5 Pathophysiology of Bone Remodeling (Fig. 2.11)

Abnormalities of bone remodeling can produce a variety of skeletal disorders (Raisz 1999). Inflammatory bone loss in periodontal disease and arthritis is probably the combined result of stimulation of resorption and inhibition of formation by cytokines and prostaglandins. Interleukin 1 (IL-1), IL-6, and tumor necrosis factor, as well as growth factors, have been implicated in pathologic responses in the skeleton, particularly in osteoporosis associated with estrogen deficiency, hyperparathyroidism, and Paget's disease (Lorenzo 1992; Mills and Frausto 1997; Raisz 1999; Papanicolaou et al. 1998). A few of the pathological entities wherein there is deranged skeletal metabolism/remodeling are addressed below, and their abnormalities are shown in Table 2.2.

2.4.5.1 Osteoporosis

Osteoporosis is a very common metabolic disorder of the skeleton, where in the bone mineral density (BMD) is reduced, the bone microarchitecture is disrupted (perforation of trabecular plates), and the amount and variety of noncollagenous proteins in bone is altered, leading to increased risk of fracture. Osteoporosis may be:

- Primary (postmenopausal/senile)
- Secondary cause (nutrition, endocrine, drug, malignancy, chronic disease, idiopathic) (Raisz 1997)

The loss of bone mass and strength can be contributed by:

- (a) Failure to reach an optimal peak bone mass as a young adult
- (b) Excessive resorption of bone after peak mass has been achieved
- (c) An impaired bone formation response during remodeling

There is a defect in osteoblast function or because of loss of template from excessive resorption with perforation of trabecular plates and removal of endosteal cortical bone. The defect in osteoblast function could be the consequence of cellular senescence but also may be the result of a decrease in the synthesis or activity of systemic

Fig. 2.11 Microphotograph showing osteoclasts (*star*) causing endosteal surface resorption of bone

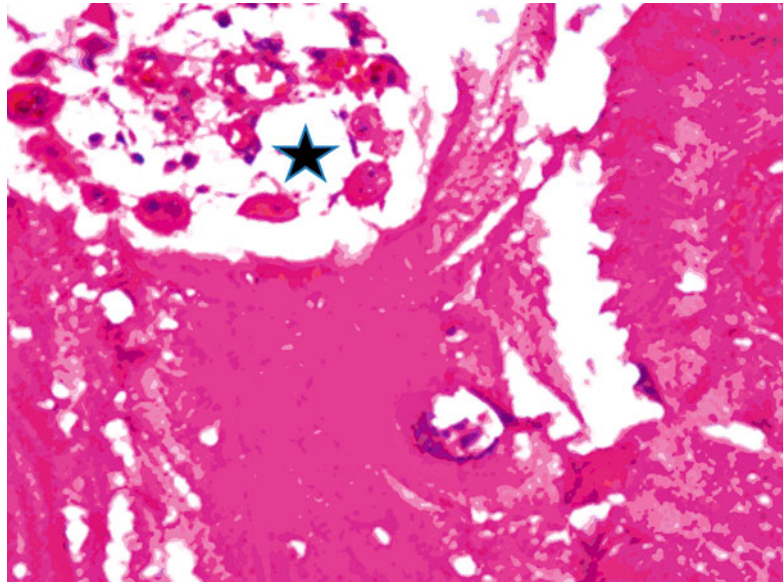


Table 2.2 Abnormalities of remodeling in disease conditions

	Bone resorption	Bone formation
Osteoporosis	↑↑	±↑
Glucocorticoid osteoporosis	↑	↓↓
Hyperparathyroidism	↑↑	↑↑
Hyperthyroidism	↑↑	↑↑
Paget disease	↑↑	↑↑
Inflammation	↑↑	±↓
Immobilization	↓	↓↓

and local growth factors. A combination of biochemical assays, including total alkaline phosphatase and osteocalcin in plasma and hydroxyproline and calcium in fasting urine, have strong predictive power in relation to rates of bone loss subsequently measured by bone densitometry techniques.

The two major needs for the use of markers in osteoporosis are:

1. To identify the patients at greatest risk, for example, those with rapid rates of bone loss compared with bone formation
2. To monitor the effects of specific treatment (e.g., with estrogens, calcitonins and bisphosphonates, or with bone-forming agents) in individual patients

2.4.5.2 Hyperparathyroidism and Hyperthyroidism

In these disorders, bone turnover may be markedly increased with or without decreased bone mass. Both parathyroid hormones and thyroid hormones can stimulate bone formation as well as resorption, and if the cells of the osteoblastic lineage are sufficiently responsive, then bone loss will not occur. Increased IL-6 has been reported in hyperparathyroidism.

2.4.5.3 Paget's Disease

Paget's disease is the best example of a disease in which biochemical markers of bone metabolism have been extensively used in clinical practice. A remarkable disorder of bone remodeling is Paget disease (Siris 1998). In this disorder, the osteoclasts become abnormally activated, possibly by viral infection, and produce a bizarre and irregular pattern of resorption, to which there is usually an intense osteoblastic response with irregular new bone formation often in the form of woven bone. Thus, in Paget's disease there may be increased bone density, but because of the irregular architecture, bone strength is decreased and pathologic fractures may occur.

Bone markers in Paget's disease are used to:

1. Assess and to monitor disease activity in individual patients

2. Evaluate dose–response relationships to existing and new drugs in therapeutic trials
3. Evaluate the value of novel biochemical markers of bone metabolism, compared with established markers (Mills and Frausto 1997)

2.4.5.4 Osteomalacia/Rickets

The growth plates affecting children is seen in rickets, while in osteomalacia affecting adults, there is incomplete mineralization of osteoid. There is decrease in Ca/PO_4 ratio, increase in alkaline phosphatase, and decrease in calcium excretion [$\text{Ca} \times \text{PO}_4 < 2.4$].

2.4.5.5 Osteopetrosis

Decreased bone turnover can also lead to skeletal abnormalities. There are several syndromes of osteopetrosis or osteosclerosis in which bone resorption is defective because of impaired formation of osteoclasts or loss of osteoclast function. In these disorders, bone modeling as well as remodeling is impaired, and the architecture of the skeleton can be quite abnormal (Charles and Key 1998; Schneider et al. 1998).

2.4.5.6 Other Orthopedic Disorders

Pathologic changes in the skeleton that occur in association with orthopedic disorders have also been found to involve local factors. For example, the heterotopic ossification that occurs after hip surgery may be mediated by prostaglandin because it can be diminished by giving inhibitors of prostaglandin synthesis, such as indomethacin. The loosening of prostheses has been shown to involve local cytokine and prostaglandin production by inflammatory cells (Knelles et al. 1997; Mohanty 1996).

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