

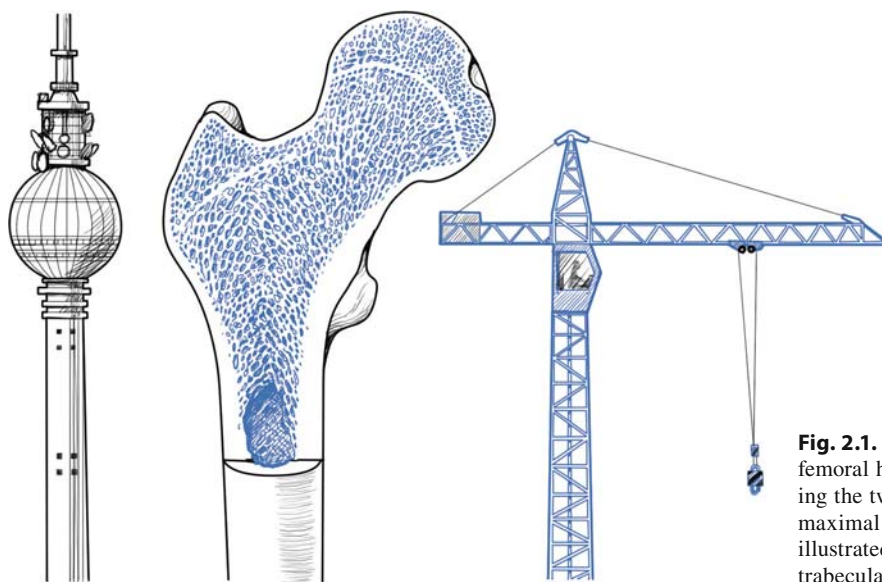
## 2.1 Bone: An Architectural Masterpiece

The structure, function, physiology, normal processes of preservation and maintenance of the skeleton, as well as the pathologic processes underlying osteodys-trophies are briefly outlined in this chapter.

The skeleton consists of about 220 bones and constitutes approximately 15 % of the total body weight.

Bone has *five main tasks* to fulfill:

- Support and locomotion: of the body as a whole and of its individual components, e.g. from the smallest (the toes) to the largest (the legs and spine).
- Protection: the skeleton protects internal organs from possibly harmful external effects. For example, the ribs shelter the heart and lungs, while the cranial bones protect the brain.
- Storehouse for minerals: The skeleton is the largest depot for minerals in the body. In total, 99 % of calcium, 85 % of phosphate and 50 % of magnesium are stored in the bones. Approximately 1–1.5 kg calcium is built into the skeleton in the form of hydroxyapatite.
- Storehouse for bone matrix proteins: The mineralized bone substance consists of about 50 % organic material: 25 % matrix (ground substance) and 25 % water. The matrix contains 90 % collagen type I and 10 % other proteins such as glycoprotein, osteocalcin, osteonectin, bone sialoprotein, osteopontin, fibronectin, as well as various proteoglycans. All these proteins are synthesized and secreted by osteoblasts and have a variety of functions, such as seeding crystal formation, binding calcium crystals and serving as sites for the attachment of bone cells. Collagen also has direct effects on important bone cell functions, including apoptosis, cell proliferation and differentiation, which are under complex control from the cell surface to the nucleus. Although collagen may have less effect on bone strength and stiffness than mineral does, it may still have a profound effect on bone fragility. Collagen changes that occur with age and reduce bone toughness or stiffness may be an important factor in the risk of fracture. Bone matrix also contains proteins such as bone morphogenic proteins (BMPs), thrombospondin-2 and metalloproteinases that stimulate or inhibit the actions of bone cells. Some studies have shown that bone also contains growth factors and cytokines, such as transforming growth factor beta 1 (TGF- $\beta$ 1).
- The skeleton participates in the endocrine regulation of energy by means of mechanisms involving leptin and osteocalcin, by which glucose levels in the serum as well as adiposity are both effected. In this context it is clear that the processes involved in energy balance influence many organs and tissues, while imbalance induces adverse effects in the liver, pancreas and skeletal muscle, which in turn affect the bones.



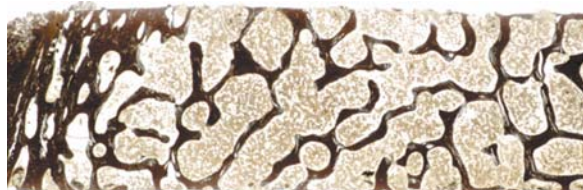
**Fig. 2.1.** Architectural organization of femoral head, neck and shaft, combining the two principles of construction for maximal weight-bearing: tubular structure illustrated by the television tower and trabecular structure by the crane

Bone has two mechanical functions to fulfill: weight-bearing and flexibility (Fig. 2.1). Specific *structural organizations*, from the macroscopic through the microscopic to the molecular, enable bone to perform these functions:

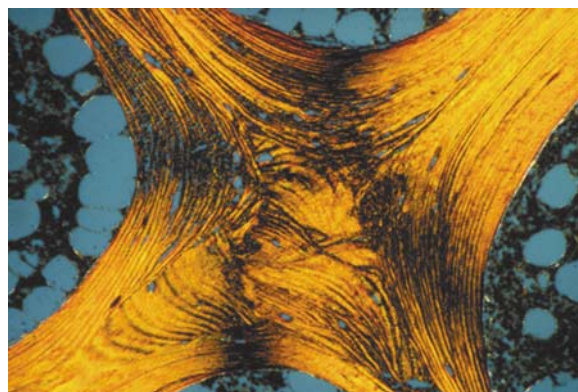
- Configuration and size of bones
- Proportion of compact (cortical) to cancellous (trabecular) bone; adapted to weight-bearing (Fig. 2.2)
- Trabecular bone structure with “nodes” to support weight (a “node” comprises the nodular junction of three or more trabeculae) (Fig. 2.3)
- Lamellar organization of osseous tissue
- Degree of mineralization of osseous tissue
- Arrangement of collagen fibres and filaments, together with non-collagenous matrix proteins (NCPs)
- Cable-like organization of collagen molecules and their “cross-linking”

The elasticity of bone is achieved mainly by a special mixture of its component parts, known as “two-phase

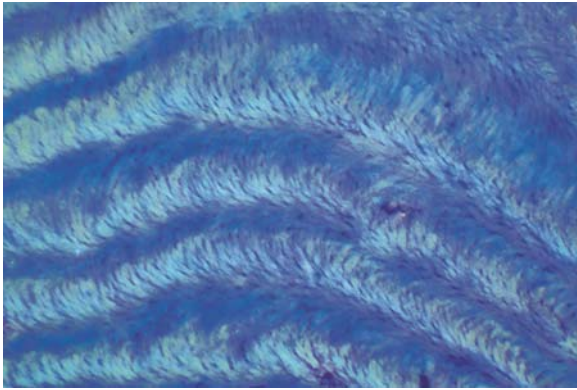
components” in the building industry. Bone consists of the matrix (the material laid down by the osteoblasts) made up of layers of collagen molecules between which crystalline calcium and phosphate are deposited (Fig. 2.4). This “passive gradual mineralization” increases density as the bone gets older. The new matrix begins to mineralize after about 5–10 days from the time of deposition (*primary mineralization*) (Fig. 2.5). On completion of the bone remodelling cycle, a phase of *secondary mineralization* begins. This process consists of a gradual maturation of the mineral component, including an increase in the amount of crystals and/or an augmentation of crystal size toward its maximum dimension. This secondary mineralization progressively increases the mineral content in



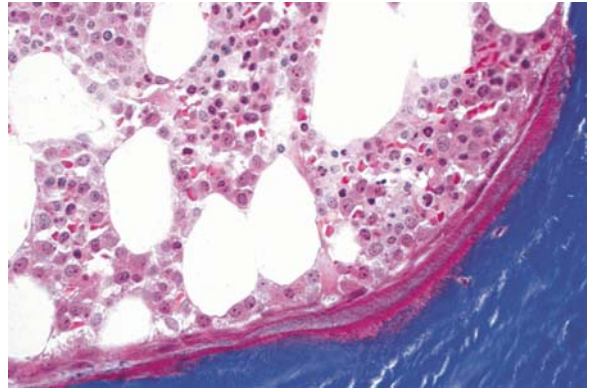
**Fig. 2.2.** Overview of a section of normal bone biopsy from a middle-aged man showing wide cortex and uniform, connected trabeculae. Gomori staining



**Fig. 2.3.** “Node” at the intersection of four trabeculae showing an osteon with concentric lamellae. Gomori staining, polarization



**Fig. 2.4.** Alternating light and dark “undulating” lamellae due to arrangement of collagen fibres. Giemsa staining, polarization



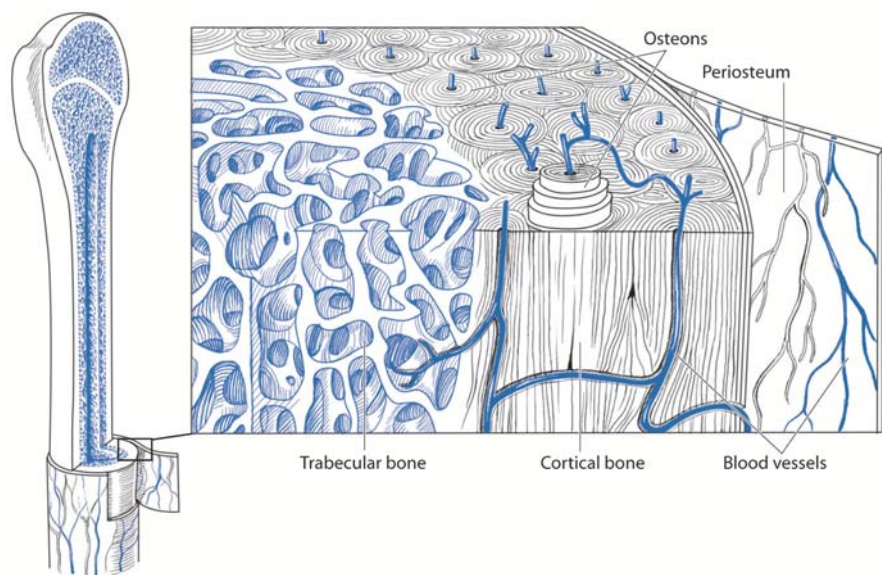
**Fig. 2.5.** Flat osteoblasts and layers of newly formed osteoid (various shades of red) on mineralized bone (blue). Ladewig

bone matrix. At the end of the primary mineralization, mineral content represents only about 50% of the maximum degree of mineralization obtained at the end of the secondary mineralization phase. Various trace elements, water and mucopolysaccharides serve as binding material (glue) which binds the proteins and minerals firmly together. Collagen is responsible for the elasticity (flexibility) of bone while the minerals provide strength and rigidity. The bundles of collagen fibres are arranged parallel to the layers of matrix and are connected by cement lines. In adult bone, the degree of mineralization depends on the rate of remodelling. That means that the biologic determinant of mineralization is the rate of bone turnover. These

correlations also demonstrate that “bone mass” and “bone mineral density” (BMD), although often used synonymously, are two different entities. Indeed, the term “bone mineral density” has been introduced in the interpretation of the positive effects of the bisphosphonates on fracture risk.

The external aspect of bone conceals its inner architecture (Fig. 2.6). The two main supporting structures of bone are only recognized in X-ray films or bone biopsy sections:

- **Compact, cortical bone:** This forms the outer layer of the long bones, is very densely packed and hard, and has a slow metabolic rate. Therefore, cortical bone is resorbed and replaced at a much slower rate



**Fig. 2.6.** Structure of bone: Cortical bone together with its vascular system surrounds the trabecular network



than trabecular bone. The layer of cortical bone of the long tubular bones (femur, humerus) consists of osteons also called Haversian systems, which are longitudinally oriented cylinders about 5 mm long and made up of 5–20 “rings”.

- *Spongy, cancellous, trabecular bone, sometimes also known as ossicles:* The axial skeleton (cranium, vertebral column, thorax and pelvis) has a specialized construction. At first glance the trabeculae appear to be randomly distributed, but closer inspection reveals that they are oriented precisely along the lines of stress and weight-bearing (“trajection lines”), producing sponge- and lattice-like structures (Fig. 2.7). The more closely the trabecular “nodes” are spaced, the greater the stability and strength of the bone, while the trabecular plates dominate the elastic properties of the trabecular bone.

*Cortical bone* has three surfaces and each has different anatomic features:

- The endosteal envelope faces the marrow cavity and comprises a high surface area and therefore supports a high bone turnover.
- The periosteal envelope, the outer surface of the bone to which the tendons, ligaments and muscles are attached, is capable of remodelling, as is the intracortical surface.
- The intracortical envelope, with bone surfaces inside the Haversian system, i.e. the osteons.

The *skeleton* can be divided into two main compartments:



**Fig. 2.7.** Surface of a trimmed plastic block showing a fairly uniform trabecular network

- *Axial skeleton:* This refers to the spine and proximal femur. The bone in this area is primarily trabecular with a high turnover.
- *Appendicular skeleton:* This refers to the long bones of the legs and arms. The bone in these areas is primarily cortical with a low turnover.

Approximately 80 % of bone is cortical and only 20 % is trabecular and they undergo *different rates of remodelling*:

- Cortical bone is dense, is 90 % calcified, has a low surface:volume ratio and therefore has a slow remodelling rate.
- In contrast, cancellous bone has a porous structure and a large surface area. Approximately 25 % of cancellous bone is remodelled annually compared to only 2.5 % of cortical bone. It therefore follows that any decrease in bone is first manifest in bones with a large proportion of trabeculae and therefore with a higher surface area.

The *proportion of trabecular bone* varies in different skeletal regions:

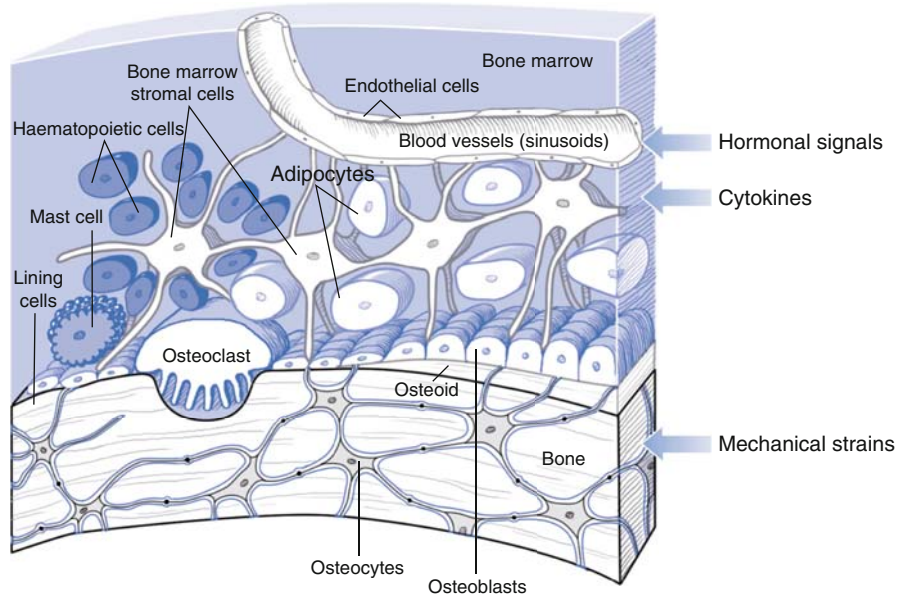
- Lumbar vertebrae 75 %
- Heels 70 %
- Proximal femur 50–75 %
- Distal radius 25 %
- Middle of the radius <5 %

## 2.2

### Bone: A Permanent Building and Rebuilding Site

Bone is a dynamic organ, highly vascularized and very active metabolically (Fig. 2.8). As bones are not completely developed at birth, they continue to be formed slowly out of cartilage or connective tissue, which are converted into the hard, lamellar components of the skeleton. Growth of bones (“*modelling*”) comes to an end at puberty with ossification of the “growth plates”. Modelling is of particular interest as bone is much more capable of reacting to external loads during growth than at any other time. About 90 % of adult bone is formed by the end of adolescence and subsequent gains during adulthood are very small.

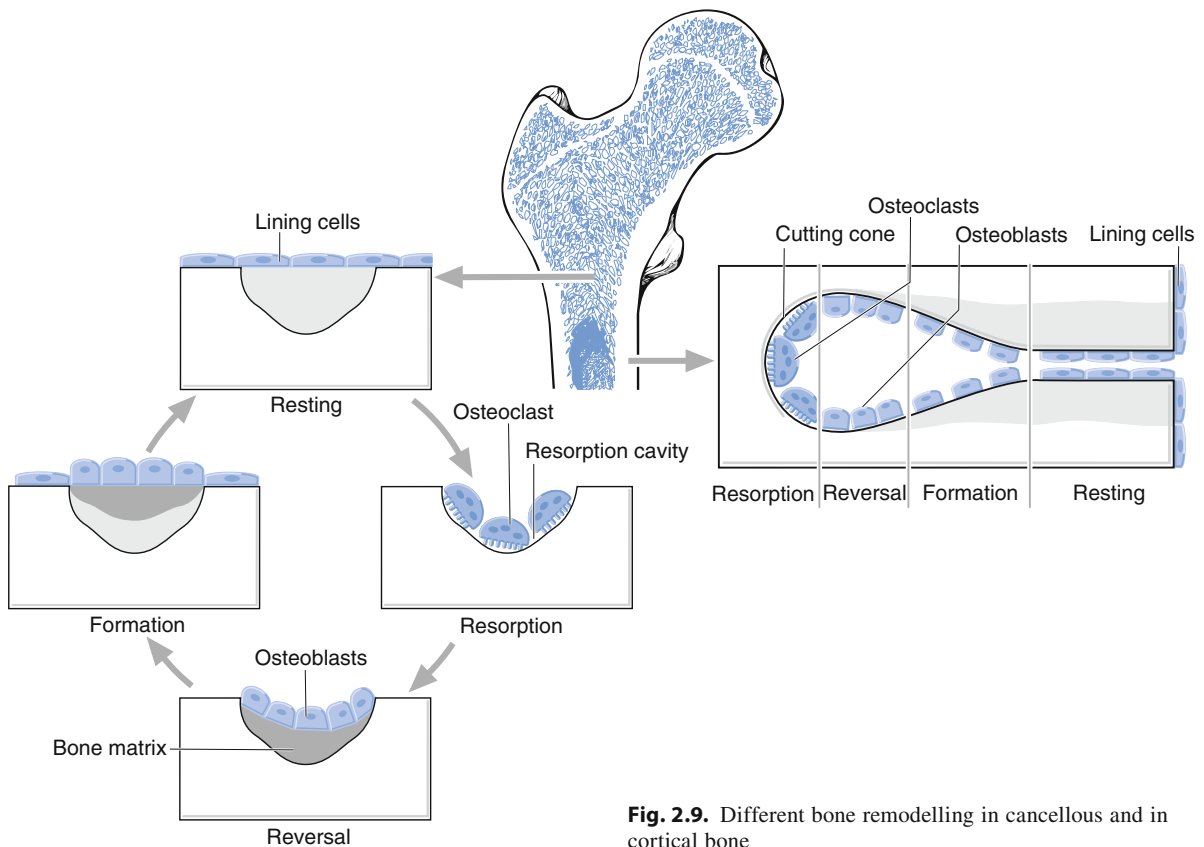
During adulthood, i.e. throughout life, there is a continuous process of remodelling which maintains



**Fig. 2.8.** Interdependence of bone and marrow: together they form a single structural and functional entity

the skeleton and adapts the bones to the changing external circumstances (Fig. 2.9). Nevertheless as the body ages, bone loses some of its strength and elas-

ticity and therefore breaks more easily. This is due to loss of mineral and changes in the bone matrix. Bones undergo a constant process of removal and replace-



**Fig. 2.9.** Different bone remodelling in cancellous and in cortical bone

**Table 2.1.** Quantitative parameter of bone remodelling in normal adults

Trabecular bone surface covered with	
Osteoblasts	2–7%
Osteoclasts	1%
Lifespan	
BMU	6–9 Months
Osteoclast	3 Weeks
Osteoblasts	3 Months
Number of active BMUs at any time	
BMUs initiated per year	3–4 Million/year
BMU size	1–2 mm long and 0.2–0.4 mm wide
Mean time for renewal of the skeleton	
Renewal per day	0.027% (1 BMU/7 s)

BMU, bone multicellular unit.

ment so that the components of bone are exchanged at regular intervals. This process is called remodelling and serves the following purposes (Table 2.1):

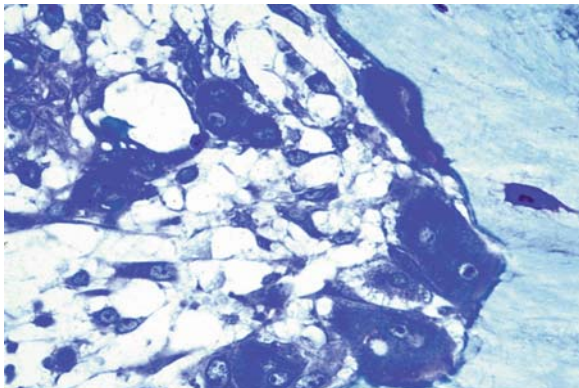
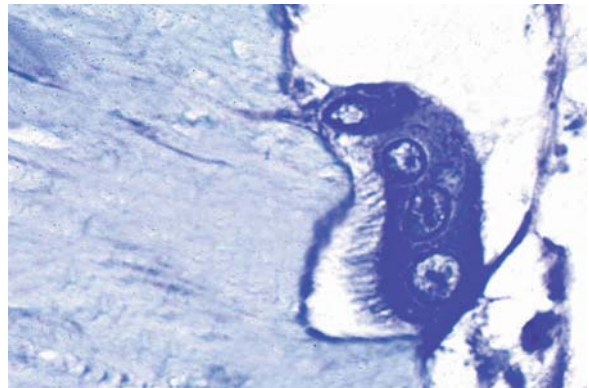
- Mobilization of calcium in the framework of calcium homeostasis
- Replacement of old osseous tissue
- Overall skeletal and individual local adaptation to different loads, weight-bearing and stress

- Repair of damaged bone, both microscopic and macroscopic

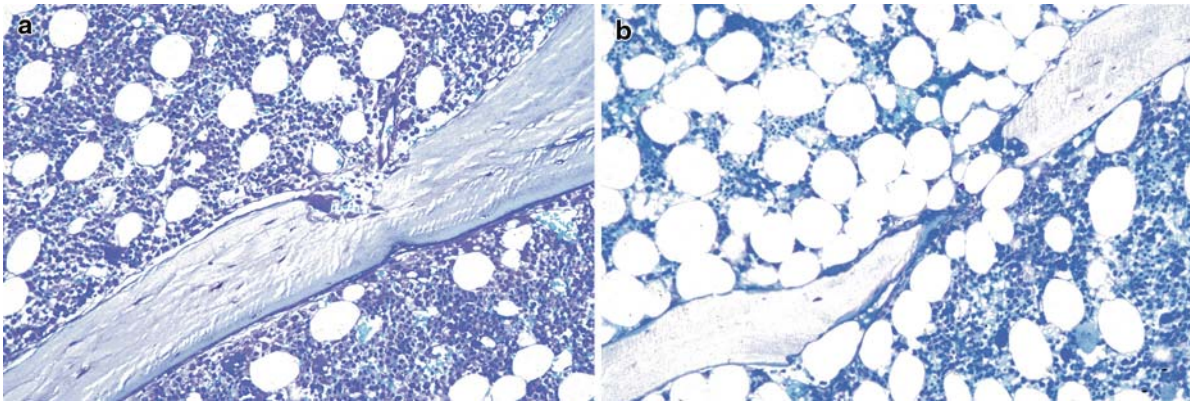
The last refers not only to repair or healing of fractures of whole bones, but also to the countless perforations, breaks or cracks of the trabeculae, the “microfractures”, “microdamage” or “fatigue damage” which occur constantly and which together with the thickness of the bones determine the fracture risk. As these tiny breaks, cracks or fractures accumulate they weaken older bones and contribute to fracture risk if not quickly and adequately repaired. This, in addition to a slightly negative bone balance over time, eventually leads to reduction in structural continuity of the trabecular network and thereby loss of strength. In cortical bone microcrack density is greater in older individuals and on average the microcracks are shorter in areas of the cortex with more resorption spaces, indicating a relationship with the rate of bone remodelling.

The *bone cells* constitute a specialized osseous cell system responsible for the repair, maintenance and adaptation of bone:

- *Osteoclasts* (“bone resorbers”, “bone breakers”, “bone carvers”) can resorb old, weak bone in a short period of time (Fig. 2.10). These multinucleated giant cells are derived from monocytes of the bone marrow, which is from a haematopoietic cell line. The cell membrane consists of numerous “folds” – the “ruffled border” which faces the surface of the bone (Fig. 2.11). The osteoclasts release quantities of proteolytic and other enzymes into the space between the ruffled membrane and the bone. These substances

**Fig. 2.10.** Active osteoclasts in deep resorption bay. Giemsa staining**Fig. 2.11.** Active osteoclast with ruffled border in lacuna with paratrabeular sinusoid. Giemsa staining



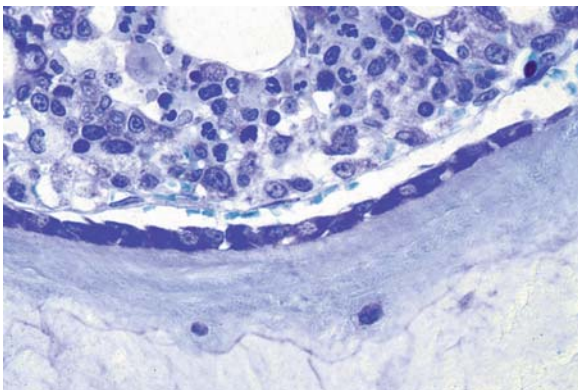


**Fig. 2.12a,b.** Osteoclasts perforate a trabecula (a) and transect it (b), thereby disconnecting it from the trabecular network which irreversibly and mechanically weakens that area of bone. Giemsa staining

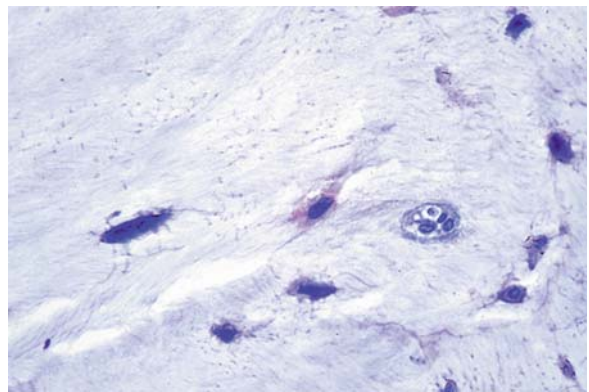
dissolve the minerals and some of the bone matrix, the rest is phagocytosed and metabolized in the cytoplasm of the osteoclasts. If the trabecula is thin enough, active osteoclasts may perforate and transect it, thereby disconnecting it from the trabecular network which irreversibly weakens that area of bone (Fig. 2.12a,b). Recruitment, differentiation and activation of osteoclasts are accomplished by numerous systemic hormones (such as parathyroid hormones, oestrogens, androgens, leptin and thyroid hormones) as well as cytokines. Various growth factors are also involved. Recent investigations of the RANK/RANKL signalling pathway in the osteoclast have clarified mechanisms of stimulation and activation of resorption. Osteoclasts possess oestrogen receptors, by means of which oestrogen inhibits their recruit-

ment. Androgens also act on osteoclasts. The actions of sex steroids on bone cells are discussed below.

- **Osteoblasts** (“bone builders”) are derived from the mesenchyme in the bone marrow. They produce new bone slowly, over several weeks to replace that resorbed by the osteoclasts (Fig. 2.13). Their main function is the synthesis of bone matrix, in particular collagen type I, but also osteocalcin, osteonectin and BMPs. Osteoblasts also possess receptors for oestrogen.
- **Osteocytes** (“bone maintainers”, “bone controllers”): The osteocytes are the most numerous of all the bone cells (Fig. 2.14). They develop from osteoblasts. Approximately every tenth osteoblast situated on the surface of the bone is entrapped by the newly formed bone matrix and thus becomes



**Fig. 2.13.** Active cuboidal osteoblasts on osteoid seam of variable width. Giemsa staining

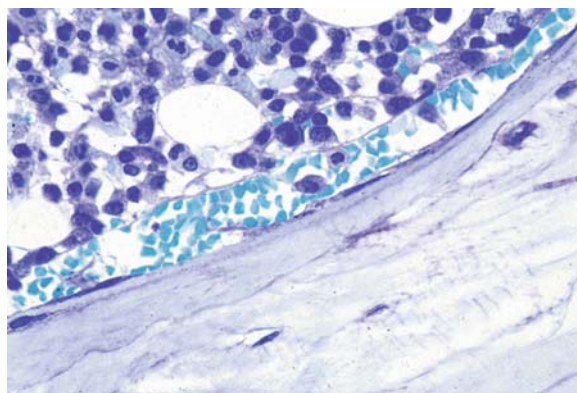


**Fig. 2.14.** Osteocytes cut at different angles in trabecular bone. Note a narrow trabecular canal containing a blood vessel. Giemsa staining

an osteocyte. It possesses receptors for various hormones including PTH and sex hormones. The osteocytes occupy spaces in the bone called “lacunae” and are connected to each other and to the endosteal cells on the surface of the bone by thin channels called “canaliculi” within which long cytoplasmic processes join osteocytes to each other and thus form a circulatory system. Osteocytes possess functional gap junctions enabling them to communicate with one another (like neurons) as well as with the surface lining cells – the endosteal cells. Therefore, osteocytes are in a position to transmit the load-induced signals to pre-osteoblasts which then differentiate and secrete osteoid. The total surface of combined lacunae and canaliculi has been estimated at 1200 m<sup>2</sup>. The function of osteocytes has not yet been fully elucidated, but they are known to play an important part in the transport of organic and inorganic materials within the bones. Furthermore, their strategic location enables them to function as mechanosensory cells and thus to detect the need for bone increase or decrease during functional adaptation of the skeleton, as well as the need for repair of microfractures. Osteocytes detect changes in flow of the fluid in the canaliculi and in the levels of circulating hormones such as oestrogen, glucocorticoids and raloxifene, which influence their activities and survival. Recent data suggest that mechanical loading decreases the osteocytes’ potential to regulate local osteoclastogenesis by direct cell–cell contact and/or via soluble signals. Quite possibly they also receive impulses from the muscles which they relay to the cells of the remodelling units at the surface of the bone. They also register the age of the bone and initiate its remodelling. Osteocytes also produce various factors, notably sclerostin, a molecule that regulates osteoblast activity, as well as DMP 1, and FGF 3, a factor also involved in the regulation of renal phosphate uptake. To summarize the function of the osteocytes: Osteocytes are actively involved in remodelling and in its control mechanisms. Osteocytes actively participate in ion exchange. Osteocytes are mechanosensory cells with a major part in the functional adaptation of bone. The number (density) of osteocytes determines bone mass both for cortical and trabecular bone. Disruption of the osteocyte network and decrease in osteocyte number with age

is inevitably accompanied by a decrease in bone mass, as well as by a decrease in bone quality by impairment of repair of microfractures. Although regulated by all the control mechanisms outlined above, in the final analysis it is the highly complex intercellular signalling between the osteoprogenitor cells and the mature osteoclasts, osteoblasts and osteocytes which balance their activities in growth and remodelling.

- **Endosteal lining cells** (bone “housekeepers”): these are flat cells that cover 80–95% of the internal surface of the bones. They are presumed to develop from inactive osteoblasts. They form a protective layer and constitute a surveillance system for the bones (Fig. 2.15). They are connected to a thin collagenous membrane covering the mineralized bone surface, the osteocytic lacunae and their canaliculi (Fig. 2.16). Recently, it has been shown that the endosteal lining cells may participate in activation of osteoclasts. Certain surface molecules expressed on lining cells and on osteoclast progenitors react with the receptor RANK (also found on osteoclast progenitors) and thereby set in motion a cycle of remodelling. Other important factors which participate in the remodelling cycle have also been analysed and these are: ODF (osteoclast differentiation factor), OPGL (osteoprotegerin ligand), TRANCE and RANKL (RANK ligand). PTH, PGE<sub>2</sub>, IL1 and 1,25 (OH) vitamin D exercise a negative influence on osteoprotegerin production and thereby stimulate resorption. Osteoblast precursors produce M-CSF which can activate osteoclasts. The endosteal lining



**Fig. 2.15.** Flat endosteal lining cells and paratrabecular sinusoid. Giemsa staining





**Fig. 2.16.** At higher magnification, collagen in transverse section (thin collagenous membrane) under the flat endosteal lining cells, and in longitudinal section (mineralized bone) in the lower part of the micrograph. Note processes of endosteal lining cells (arrows) extending between collagen fibres, presumably to connect with processes of osteocytes within the canaliculi. EM  $\times 11,300$

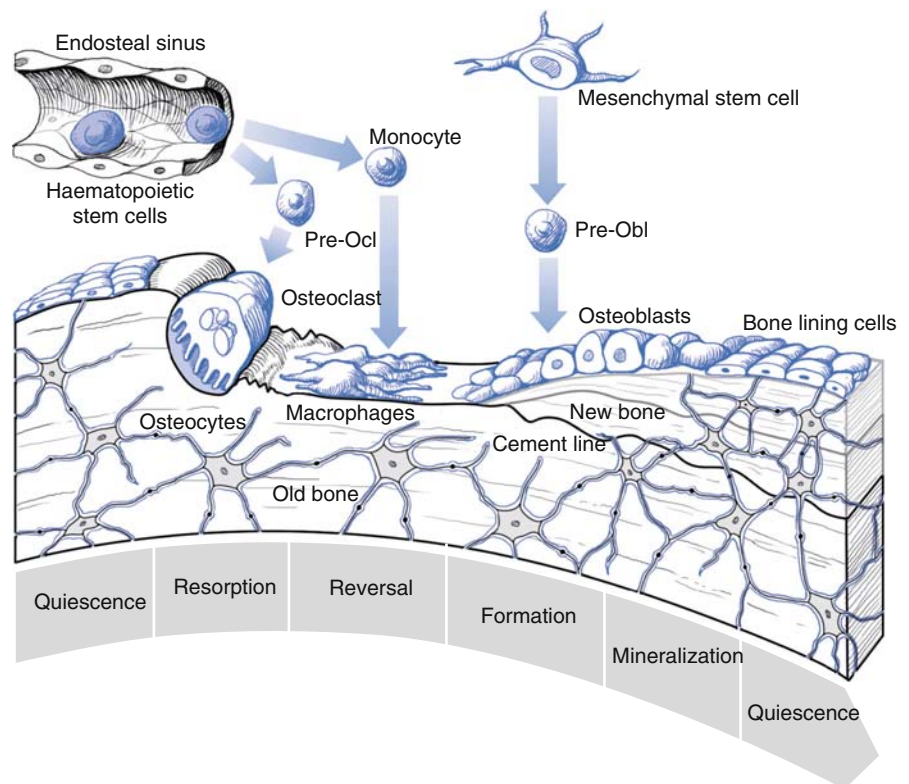
cells also participate in bone remodelling. They remove fragments of bone collagen left by osteoclasts, thereby cleaning up resorption pits and initiating formation of new bone.

## 2.3 Remodelling Units

There are 2–5 million bone remodelling units (BRUs) in the skeleton (Fig. 2.17). These units, required for the maintenance and integrity of the skeleton, are of crucial importance for the development of osteoporosis (Table 2.2). The total quantity of bone decreases if more bone is resorbed than is produced over the years. It has been estimated that osteoporosis develops when for every 30 units of bone resorbed only 29 are produced. This negative “bone balance” has three possible causes:

- Increased osteoclastic activity without increased osteoblastic activity (“high turnover”)
- Normal osteoclastic but decreased osteoblastic activity (“low turnover”)
- Decreased osteoclastic and osteoblastic activity (“atrophic” or “adynamic” bone)

Consequently, an overall decrease in bone correlates primarily with the number of BRUs and with the lack of coordination between the cells of the BRU. The



**Fig. 2.17.** Steps of bone remodelling in adult trabecular bone

**Table 2.2.** Bone remodelling and its clinical correlations

Phase of remodelling	Quiescence	Bone resorption by osteoclasts	Reversal	Bone formation by osteoblasts	Quiescence
Stimulating factors		Parathormone Vitamin D Thyroxine		Growth hormone Parathormone Oestrogen Testosterone Cytokines Prostaglandins Vitamin D	
Inhibiting factors		Oestrogen Calcitonin Testosterone Bisphosphonates Raloxifene		Corticosteroids Smoking Alcohol	
Bone markers		Pyridinoline NTX, CTX		Bone-specific alkaline phosphatase Osteocalcin	

level of excretion of calcium and collagen metabolites in the urine reflects the degree of resorption of bone. The process of bone remodelling is as yet incompletely understood. One remodelling cycle takes approximately 120 days, and it has been divided into six phases:

- Phase of quiescence: a layer of flat lining cells over a thin collagenous membrane covers the surface of the bone.
- Phase of activation: the quiescent bone surface is prepared for resorption. This involves retraction of the endosteal lining cells and removal of the thin collagenous membrane covering the bone surface. There is evidence that matrix metalloproteinases produced by osteoblasts are involved in this process. The site-specific activation may be achieved by mechanical stresses transmitted to the endosteal lining cells via the osteocytic-canalicular network.
- Phase of resorption: recruitment and fusion of osteoclastic precursors, preparation of osteoclasts for resorption, development of the ruffled membrane; osteoclasts resorb the bone, which leads to formation of lacunae or pits; osteoclasts migrate slowly or undergo apoptosis.
- Phase of reversal: osteoblast progenitors are attracted to the resorption pit, while monocytes and endosteal lining cells prepare the surface of the

resorption pit for new bone production by removal of the debris left by the osteoclasts.

- Phase of early formation: production of osteoid by active osteoblasts.
- Phase of late formation: mineralization of osteoid.
- Phase of quiescence: the osteoblasts turn into the flat endosteal lining cells or into osteocytes if trapped in the newly formed bone.

The phase of resorption is completed within 2 weeks, while that of mineralization may take months and depends on the presence of active metabolites of vitamin D. On completion of a remodelling cycle a “structural bone unit” is formed; about 35 million are present in the whole skeleton. In total, 8% of the skeleton is replaced annually by the activity of the BRUs.

The following four stages of osteoclast activity are involved:

- Formation and differentiation of osteoclasts from their precursors (RANKL)
- Migration and attachment of osteoclasts to the osseous surface ( $\beta 3$  integrins)
- Osteoclastic secretion of factors for acidification and solution of minerals (V-H + -ATPase, chloride channels)
- Dissolution of matrix (cathepsin K).

Currently, the majority of treatments for osteoporosis tested so far target inhibition of bone resorption.

## 2.4

### Some Biological Perspectives on the Mechanisms Involved in the Control and Regulation of Bone Remodelling

During the first few years of the 21st century, the complex mechanisms which characterize the control of bone remodelling were just beginning to be recognized and starting to be investigated. Results of the overwhelming amount of research subsequently published have illuminated the extreme complexity of the cellular and molecular interactions, as well as the network of interwoven highways and byways representing the numerous pathways that transport factors to stimulate or inhibit specific cellular activities. A highly significant aspect of the results of these investigations is the unequivocal demonstration of the participation of many of these pathways, not only in the control of bone remodelling, but also in numerous processes – both physiological and pathological – involving other organs and tissues, and as such are examples of systems biology.

One striking demonstration of this is *osteimmunology* in which the participation of lymphocytes and various immunological cytokines (among other factors) in the processes of bone remodelling has now been unequivocally established. Inflammatory reactions, acute and especially long-lasting chronic reactions, are major causes of local and even systemic bone loss. One such cause, for example, is the tumour necrosis factor (TNF) in inflammatory arthritis. Another example is the induction of bone destruction by the activation of T-cells by means of RANKL. In complete contrast, CD44 acts as an inhibitor of the deleterious consequences of TNF on bones and joints, similar to the effect of selectin-9 (a growth hormone) which stimulates the production of regulatory T-cells. Moreover, additional studies have pointed out that various signalling molecules, transcription factors and membrane receptors are also shared by the immune and skeletal systems. For example, NF- $\kappa$ B is a vital component in inflammatory responses and also in osteoclast differentiation and osteolysis. Unhealthy lifestyles, including poor nutrition as well as overweight and morbid obesity, result in imbalances in the oxidation/redox systems, leading to inflammatory reactions and disorders of many organs including the bones and joints.

On the other hand, the localization of the osteoclastic resorption may affect haematopoiesis, such as the mobilization of haematopoietic progenitors when osteoclasts resorb an endosteal region of bone, with secretion and release of proteolytic enzymes, adjacent to stem cell niches. It has been speculated that this constitutes a link between bone remodelling and regulation of haematopoiesis.

Towards the end of the 19th century (in the 1890s) researchers developed the hypothesis that mechanical loads affect the architecture of bone in living organisms. However, the processes by which this occurs were only investigated much later in the 20th century when the weight-bearing and the load-bearing bones (not always the same), as well as various systems including “feedback systems”, were identified and subsequently termed the “Mechanostat” and which was held responsible for the strength of the bones.

The Mechanostat hypothesis was then also applied in order to provide functional definitions of bone competence and bone quality in normal physiological conditions, as well as in pathological states, such as osteopenias and osteoporoses. Evidence was provided that:

- Muscle strength largely determines the strength of the load-bearing bones
- This in turn has implications for numerous aspects of bone physiology such as in Modelling and remodelling, and in interventions such as bone grafts, osteotomies, arthrodeses and possibly also in the actions of pharmaceutical agents.

Additional discoveries during the first half of the 20th century led to the formulation of the *Utah Paradigm of skeletal physiology*, according to which the skeletal effector cells (osteoblasts, osteoclasts, chondroblasts etc.) themselves determine the structure and function of bones, together with the fascia, ligaments and tendons. However, with the passage of time and a great deal of additional work, the results of studies on tissue-level mechanisms were added to the Utah Paradigm, which by then also encompassed determinants of skeletal architecture and strength. Moreover, with the accumulating information on skeletal physiology, the Utah Paradigm also came to include and integrate scientific evidence on anatomy and biochemistry – from histology to cellular and molecular biology – to pathologies of skeletal disorders and their clinical aspects.



Most significant for everyday medicine worldwide was, and still is, the bottom-line conclusion, which, simply expressed, states that strong muscles make and maintain strong bones throughout life!

The highly significant implications of this for diagnosis, the instigation of preventive measures and for therapeutic interventions are discussed later. Many studies have now been published on mechano-biology, i.e. the relationship between mechanical forces and biological processes, including the specific pathways involved in the transmission of signals elicited by mechanical loading and stress, also known as *mechano-transduction*, which is regulated by two structural combinations:

- Focal adhesions, linking cells to the extracellular matrix
- Junctional adhesions, linking adjacent cells to each other by means of adherins; and these, through the cellular circuits, co-ordinate tissue responses to mechanical loading; or, in simple terms, enable cells to translate the signals produced by mechanical loading into biochemical responses.

But these biomechanical stimuli are only a few of the numerous signals essential for an integrated osseous physiology, which are transported by the intra-osseous circulation formed by the dendritic extensions and gap junctions of the osteocytes, and possibly also the hemichannels which contain connexin 43 (CX 43). Immuno-reactive sites for CX 43 have already been identified in mature osteoclasts, as well as in marrow stromal cells.

It should also be remembered that the osteocytes, among their other responsibilities, function as sensors for mechanical stimuli and as regulators of mineralization in bone, which is part of their participation in overall mineral metabolism, particularly that of calcium. It is the gap junctions which enable adjacent cells to exchange second messengers, ions and cellular metabolites. The junctions also participate in development of bone cells, for example providing signalling pathways downstream of RANKL, for osteoclast differentiation.

To summarize with but one more example: as a response to mechanical stress, prostaglandins are released by osteocytes into the hemichannels. Among the actions of prostaglandin D2 is modulation of osteoprotegerin, RANKL and various receptors which, directly or indirectly, stimulate an anabolic response.

In addition, recent studies have shown that the prostaglandin D2 receptors expressed by osteoblasts participate in the control of osteoclastogenesis and in the activity of mature osteoclasts. Prostaglandin D2 itself has recently been shown to be chemotactic for mast cells, also by way of one of its receptors. The hemichannels (mentioned above) together with gap junctions constitute the intraosseous circulation for the transmission of signals required for maintenance and repair of the bones, and for implementation of the numerous skeletal functions including the effects of growth factors, hormones, cytokines and others, as demonstrated in the remodelling of bone by the BRUs. Clearly, these intercellular connections are crucial for the maintenance of the skeleton and therefore disruption of the communication between the bone cells themselves, and between bone cells and other cells (e.g. endosteal cells, endothelial and periosteal cells all active in osseous remodelling) is associated with many structural and functional disorders, localized and systemic, of the bones of the skeleton.

## 2.5 Minimodelling

“Minimodelling” is the term used to describe activity of bone cells, primarily of the osteoblasts, which is completely independent of the BRUs. First put forward about a decade ago, it was suggested that minimodelling is a mechanism for trabecular bone renewal that goes on throughout life. Minimodelling is accomplished by formation of bone on quiescent surfaces; it is not preceded by bone resorption and it leaves smooth cement lines, i.e. it is simply resumption of osteoblastic activity by the bone lining cells which results in increases in lamellar cancellous bone mass and possibly in trabecular connectivity.

An early study of bone biopsies from patients aged 38–81 years confirmed this hypothesis and the recommendation was made that the results of minimodelling should be taken into account when dealing with estimation of osteoid volume and mineralization. Shortly thereafter, an investigation was carried out on bone specimens taken from bone biopsies and from autopsy material of patients with adynamic bone disease. The results showed that in the absence of parathyroid hormone, and especially in bone from

relatively younger patients (about 60–64 years), minimodelling correlated significantly with total bone volume. Another recent study of patients with hyperparathyroidism demonstrated minimodelling at the endocortical and intracortical surfaces, particularly in those specimens with narrow cortices and high porosity. Finally, results of the most recent study published to date demonstrated conclusively that bone formation by minimodelling accounts, in part, for the increase in bone volume which occurs after parathyroidectomy in patients with hyperparathyroidism. The mechanisms and stimuli involved in minimodelling have not yet been elucidated.

Additional investigations in the future will undoubtedly clarify the significance of minimodelling in the maintenance and repair of the bones.

## 2.6 Stimuli, Triggers and Mechanisms of Activation of Bone Remodelling

The elucidation of the mechanisms of maintenance of the size, structure and quality of the bones throughout life by means of the BRUs stimulated extensive research into the question of what activated these units. Moreover, it has been known for about half a century that the bones contain *cracks* caused by the physiological loading activities of daily life, and numerous studies have now been published on how cracks are formed and grow and how they can be detected and repaired. A large part of this research was carried out on animals – from rats to horses, in vivo and in vitro. These studies clarified many aspects of osseous reactions, what provoked them and what conclusions could be drawn; examples of these are briefly summarized below, in more or less chronological order. In the early 1990s, it was shown that remodelling repairs fatigue damage and thereby prevents fractures and it was deduced that microdamage itself evokes local bone remodelling.

At the start of the new century, investigations of bovine, equine and human long bones which had been loaded in vitro demonstrated that microcracks initiated at osteocytic lacunae, indicating that these functioned as stress concentrating organelles, thereby providing a potential mechanism for detection of strain and damage by osteocytes. Shortly thereafter,

studies on the effects of mechanical loading in sheep encompassed the timing, location, density and length of microcracks, as well as the stimulation of reactive resorption cavities. The conclusion drawn from these studies was that microdamage itself is a stimulus for initiation of bone remodelling. Subsequently, investigations were made on large bones from race horses, and from human autopsy material as well as biopsies. One of the results observed in the race horses is of special interest: namely that in the more exercised race horses, additional bone was deposited in the spaces previously occupied by adipocytic bone marrow and this was not preceded by resorption and not limited by hyper-mineralized cement lines. The conclusion was drawn that bone subjected to mechanical overload exercise, within normal limits, does not loose bone but makes more bone! Likewise, additional studies on the ulnae of rats during accumulation and coalescence of microcracks demonstrated that small increases in bone size and density substantially increased the resistance of the whole bone to microdamage. Nevertheless, the results of other studies led to the conclusion that the number of disrupted intracanalicular processes determines the osteocytic response and influences targeted remodelling. Similar data were obtained from an in vivo study on rats, which focused on microcrack formation, propagation, accumulation and disruption of osteocytic canalicular processes during increased, as well as normal, loading. Here also it was concluded that the effect on osteocytes participates in initiation of bone remodelling. However, an extensive evaluation of highly trained race horses led to the opposite conclusion, namely that in established athleticism bone turnover is influenced by pathways not involving microcracks and disrupted osteocytic processes. Moreover, the effects of a single period of cyclic fatigue on blood flow and interstitial fluid flow in the bone of the unilateral effected ulnae of rats, as well as changes in the contra-lateral unaffected ulnae of the same rats, suggested that functional adaptation to the cyclic fatigue included a more generalized neuro-vascular reaction.

Other recent studies have compared the response to loading, particularly that of the trabecular bone, in younger and older animals. The results indicated that in older animals the ability to initiate and repair microdamage is definitely reduced with age. Finally, the effects of microdamage on bone in both control and ovariectomized sheep, as examples of normal and

osteoporotic bone, were investigated and the results demonstrated the differences between the two groups, as well as the unfavourable consequences for bone quality and bone fragility, of the osseous reactions in the ovariectomized group.

Many of the numerous investigations in animals have now been confirmed by studies in humans, both healthy and diseased. A brief summary follows. The mechanisms and pathways of bone remodelling were studied during the last decades of the previous century, and extensively at the beginning of the 21st century. Several reports dealt with hypotheses and results achieved so far have shown: that osteocyte apoptosis is induced by bone fatigue and is located in regions of bone containing microcracks as well as resorption cavities, indicating that the targeted removal and repair of microdamage is preceded by disruption of the osteocytic processes which then emit the appropriate signals to trigger repair. In addition, it was shown that the direction of crack growth, i.e. lengthening, is due to the local orientation of the fibres in the bone and also that the cracks were arrested by the vascular canals in the bone.

Various hypotheses were suggested and confirmed concerning remodelling in both compact as well as in trabecular bone. For example, in cortical bone the lamellar structure of osteons and the cement lines arrest the microcracks, changes are produced in the walls of the Haversian canals and repair is initiated, thus avoiding accumulation of microdamage and providing protection from fatigue fractures. However, some subsequent studies could not confirm the hypothesis that cracks always initiate resorption spaces, i.e. remodelling. Other investigations dealt with the density and length of microcracks and the number of resorption spaces as indicators of activated remodelling. Also demonstrated was a substantial increase in microcracks with age and this correlated with fracture incidence in the elderly.

Detailed investigation (of sections of bone from the ribs of women aged 50–60 years) demonstrated that both density and length of cracks were five times higher in interstitial than in cortical bone, while osteocytic lacunae were significantly fewer, indicating that accumulation of microdamage and osteocyte deficiency occurred in the same bone regions. Other investigations of age-related histologic changes, i.e. diffuse damage or linear microcracks, showed that the latter were longer in bones of older than younger

individuals, but that the opposite was true for microdamage, i.e. more in the younger bones. These results have recently been confirmed in the tibiae of humans (age 19–89 years) and the conclusion was drawn that age-related changes in bone microstructure play a key role in microcrack formation and repair. Recent reports have discussed the effects of microdamage as a stimulus for adaptation of bone as well as for bone biochemistry. It should be mentioned that targeted (activated) remodelling, as described above, also involves “steering”, i.e. attracting or steering pre-existing BMUs towards areas of microdamage.

Other recent investigations have come to the conclusion that the BRUs are mechanically regulated: strain-induced osteocytic signals inhibit osteoblastic activity and stimulate osteoclastic activity. Consequently, cortical BRUs are attracted by apoptotic osteocytes and create load aligned osteons, while cancellous BRUs work on the trabecular surfaces, without piercing them, thus retaining the network. How these processes act on osteoporotic bone is dealt with later.

## 2.7

### Control of Bone Remodelling: A Network of Complex Mechanisms

The skeleton possesses an efficient feedback-controlled system that continuously integrates both the signals and the responses which together maintain its function of delivering calcium into the circulation while preserving its own strength. The question arises: How do mesenchymal and haematopoietic cells, as well as the osteoclasts, osteoblasts and osteocytes cooperate to achieve such a perfect balance between resorption and formation of bone? This complex system is just starting to be unravelled (Table 2.3). There appear to be five groups of mechanisms regulating bone mass:

- *Systemic hormones*: The most important hormones are parathyroid hormone (PTH), calcitonin, thyroid hormone T<sub>3</sub>, insulin, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) which mediates many of the effects of GH on longitudinal growth and on bone mass: cortisone and sex hormones and, of these, oestrogens regulate mainly osteoclastic activity and thus bone resorption. PTH together with vitamin D is the principal regulator of calcium homeostasis (Fig. 2.18). PTH exerts its

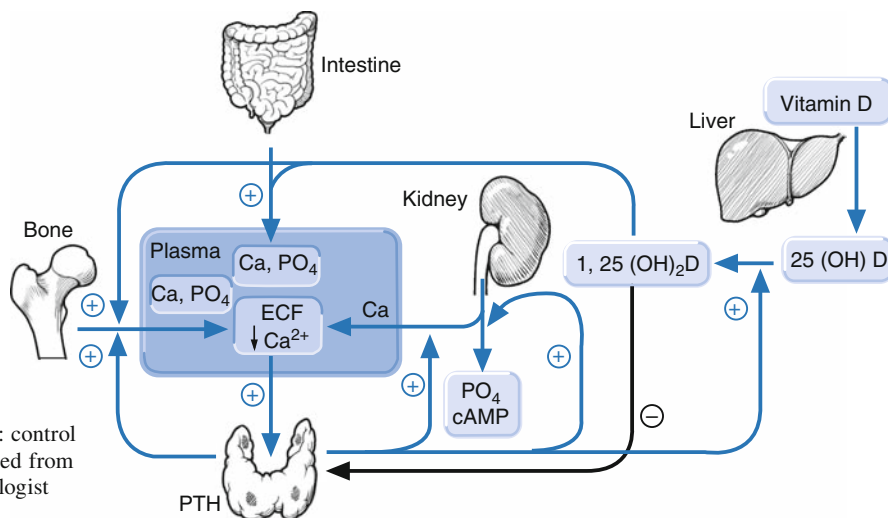


**Table 2.3.** Hormonal and local regulators of bone remodelling

<b>Hormones</b>	
Polypeptide hormones	
Parathyroid hormone (PTH)	
Calcitonin	
Insulin	
Growth hormone	
Steroid hormones	
1,25-Dihydroxyvitamin D3	
Glucocorticoids	
Sex steroids	
Thyroid hormones	
<b>Local factors</b>	
Synthesized by bone cells	
IGF-I and IGF-II	
Beta-2-microglobulin	
TGF- $\beta$	
BMPs	
FGFs	
PDGF	
Synthesized by bone-related tissue	
Cartilage-derived	
IGF-I	
FGFs	
TGF- $\beta$	
Blood cell derived	
G-CSF	
GM-CSF	
IL-1	
TNF	
Other factors	
Prostaglandins	
Binding proteins	

effects by way of actions on the bone cells as well as on other organs such as the kidney and gut. On bone, PTH exerts its influence mainly by participa-

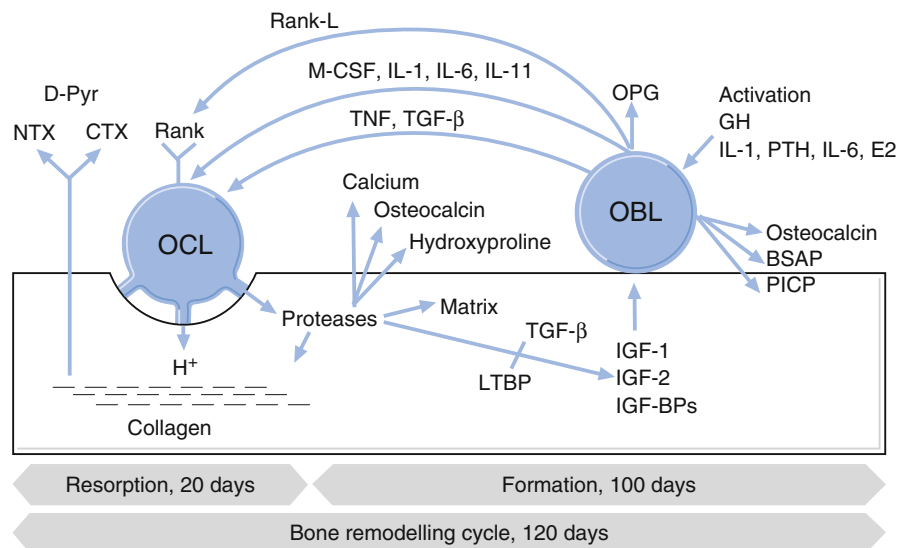
tion in the mechanisms controlling bone turnover. Androgens are also important in bone formation. Osteoblasts and osteocytes as well as mononuclear and endothelial cells in the bone marrow possess receptors for androgen; the pattern and expression of the receptors is similar in men and women. Fat cells, the adipocytes, also have receptors for sex hormones which they are able to metabolize by means of the enzymes called the aromatases. Sex steroids also influence lipid metabolism in pre-adipocytes. Significant levels of both oestrogens and androgens are present in the blood in men and women and both hormones play important, but not necessarily identical, roles in bone metabolism. For example androgens may act on osteoblasts during mineralization while oestrogens more likely affect osteoblasts at an earlier stage during matrix formation. Moreover, the sex hormones may also act at different sites on the bones – for example androgens are important in the control of periosteal bone formation which contributes to the greater width of the cortex in men. There are receptors for oestrogen and testosterone on osteoblasts, osteoclasts and osteocytes, but one or other of the sex hormones may dominate at different stages of the remodelling cycle. Androgens in particular exercise a strong influence on bone formation and resorption by way of local enzymes, cytokines, adhesion molecules and growth factors. Androgens increase BMD in women as well as in men, in normal as well as in some pathologic conditions. Moreover, when given together therapeutically the two hormones increase BMD more than



**Fig. 2.18.** PTH and vitamin D: control of calcium homeostasis (modified from Brown et al. [1994], *Endocrinologist* 4:419–426)

oestrogen given alone. Other influences such as muscular mass, strength, activity and mechanical strain may stimulate osteoanabolic activity – that is bone formation while inhibiting bone resorption. Put briefly, during growth the processes of modelling and remodelling optimize strength by deposition of bone where it is needed and decreasing bone mass where it is not. It is essential to stress that the highly complicated mechanisms controlling bone remodelling are only briefly outlined here and the elucidation of their pathways has already lead to disclosure of additional points of possible therapeutic intervention, and undoubtedly will continue to do so in the future.

- **Local cytokines and signals:** Also significant are local cytokines, electromagnetic potentials and, most importantly, signals transmitted over intercellular networks. Bone cells synthesize whole families of cytokines: for example IGF-I, IGF-II, Beta<sub>2</sub>-microglobulin, interleukin (IL)-1, IL-6, TGF- $\beta$ , BMPs, fibroblast growth factors (FGFs) and platelet derived growth factor (PDGF) (Fig. 2.19). Prostaglandins play a significant part in resorption of bone during immobilization. Osteoprotegerin (OPG), a member of the tumour necrosis factor receptor family produced by osteoblasts, blocks differentiation of osteoclasts from precursor cells and thereby prevents resorption. In fact, OPG could represent the long-sought-after molecular link between arterial calcification and bone resorption. This link underlies the clinical coincidence of vascular disease and osteoporosis, an association most frequent in postmenopausal women and in elderly people. OPG could represent a novel pathway for possible therapeutic manipulation of bone remodelling. Specific aspects of remodelling involve specific factors, as for example the impact of vascular endothelial growth factor (VEGF) in angiogenesis and endochondral bone formation, in ossification of mandibular condyles and in the growth of the long bones.
- **Vitamins and minerals:** The bone cells as well as the surrounding cell systems are also influenced by various vitamins, minerals and other factors. Vitamin D, K, C, B<sub>6</sub> and vitamin A are all required for the normal metabolism of collagen and for mineralization of osteoid.
- **Mechanical loading:** Exercise may improve bone mass and bone strength in children and adolescents. However, the osteogenic potential diminishes at the end of puberty and longitudinal growth of the bones. The adult skeleton is only moderately responsive to mechanical loading. A new way which might be use-



**Fig. 2.19.** Factors controlling bone resorption and formation. *OCL*, osteoclast; *OBL*, osteoblast. The osteoblast synthesizes cytokines and growth factors that activate osteoclasts. The two major ones essential for osteoclastogenesis are macrophage colony-stimulating factor (*M-CSF*) and osteoprote-

gerin ligand (OPGL), also called Rank-L. Rank-L activates its receptor Rank on the osteoclast. Osteoprotegerin (*OPG*) is a dummy receptor for Rank-L and can suppress osteoclastogenesis if it binds enough OPG (modified from Rosen and Bilezikian [2001])

ful to manipulate bone tissue is high-frequency, low-amplitude “vibration” exercise, combined with rest periods between loading events. Bone tissue cells must transduce an extracellular mechanical signal into an intracellular response. A mechanoreceptor is known to be a structure made up of extracellular and intracellular proteins linked to transmembrane channels. Touch sensation, proprioception and blood pressure regulation are mediated by ion channels. It has been proposed that osteocyte processes are tethered to the extracellular matrix and that these tethers amplify cell membrane strains. Presumably the extracellular fluid flow creates tension on the tethers which in turn stretches the cell membrane.

- **Transcriptional regulation and genes:** There are a number of transcriptional factors that control osteogenesis and differentiation of osteoblasts. These include runt-related transcription factor 2 (Runx2), Osterix (Osx) and sex determining region Y-box 9 (Sox9), “master” regulators of osteogenesis. New genes responsible for hereditary skeletal disorders could also provide new therapeutic opportunities. For example the identification of LRP5 as a key molecule in bone regulation was shown recently to promote osteoblastic differentiation. Finally, it should be noted that research is still ongoing concerning the cells involved, their origin and differentiation and the control mechanisms of remodelling, particularly at different sites, for example endosteal and periosteal, adjacent to haematopoietic (red) marrow, or to adipocytic (yellow) marrow, as well as the activities (if any) of the endothelial cells of the blood vessels connected to the active remodelling units. Results of these studies may reveal additional aspects of remodelling.

## 2.8

### Osteoimmunology: A Representative of Systems Biology

It is important to emphasize that elucidation of the mechanisms underlying metabolism of bone has revealed that these mechanisms include elements of the immune system. This in turn has led to the establishment of a new interdisciplinary field. *Osteoimmunology*, originally triggered by the observation that the increased bone resorption in inflammatory disorders

such as rheumatoid arthritis, is caused by increased expression of RANKL (receptor activator of nuclear factor kappaB ligand), which induced accelerated osteoclastic differentiation and activity. Moreover, such interplay/crosstalk also provides bridges for many factors – cytokines, signalling molecules, transduction factors, receptors etc. – which implement bone remodelling, are involved in the homeostasis of bone and are essential participants in the regulation of other organs and systems such as cardiology, nephrology, hepatology, gastroenterology as well as in cardiovascular, hormonal and endocrinological disorders. This multi-system participation provides a well-high irrefutable explanation for the fact that the skeleton is affected one way or another by disorders of practically all the other organs and systems in the body and why osteoporosis now constitutes a global epidemic.

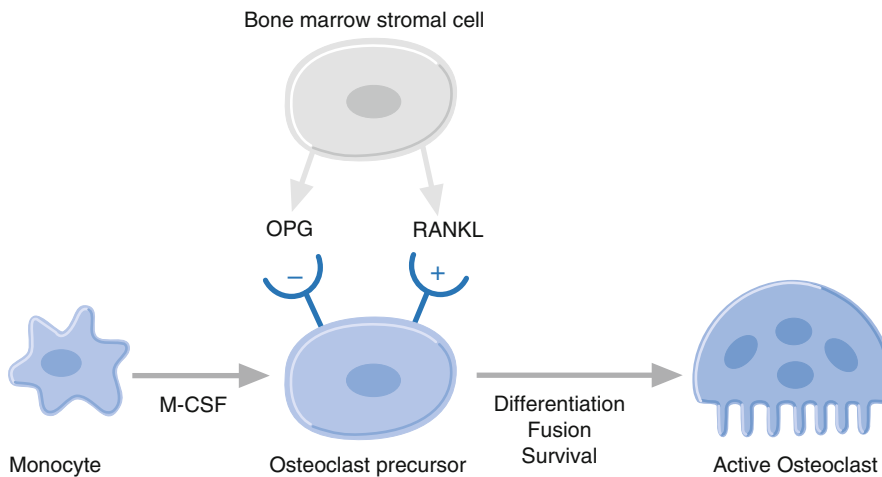
## 2.9

### The RANK/RANKL/Osteoprotegerin System

The *RANK/RANKL/Osteoprotegerin cytokine system* plays a key role in the regulation of and “coupling” within the processes of remodelling. The discovery of this cytokine system was a milestone for understanding osteoclastogenesis and the regulation of bone resorption as well as other processes involved in local bone remodelling. Osteoprotegerin is an important member of the TNF-receptor family which is produced by osteoblasts and which blocks the differentiation of osteoclasts from their precursor cells and thus inhibits bone resorption. RANKL (also known as osteoprotegerin ligand, OPGL) and its receptors RANK and osteoprotegerin (OPG) are the key components of the regulation of BRUs. RANKL, a member of the TNF family, is the main stimulus for osteoclast maturation and is essential for osteoclast survival. The processes of local remodelling are illustrated in Fig. 2.20.

An increase in the expression of RANKL leads directly to increased resorption and loss of bone. RANKL is produced by osteoblastic cells and by activated T-lymphocytes. Its specific receptor RANK is located on the surface membranes of osteoclasts, dendritic cells, smooth muscle cells and endothelial cells. The production of RANKL by T-lymphocytes and the consequent activation of dendritic cells represent





**Fig. 2.20.** The OPG/RANKL/RANK system and its control of osteoclastic resorption of bone

a connection between the immunoregulatory system and osseous tissues. The close collaboration between bone and haematopoiesis is reflected by the fact that M-CSF is required for osteoclastic differentiation.

The effect of RANKL is regulated by OPG, which is secreted in various organs including: bone, skin, liver, stomach, intestine, lungs, kidneys and placenta. It also acts as a soluble endogenous receptor antagonist. Numerous cytokines, hormones and drugs may stimulate or inhibit the effects of RANKL or of OPG and thereby sway the results to the advantage or detriment of either of these two cytokines as follows:

- TGF- $\beta$  increases production of OPG
- PTH increases RANKL/decreases OPG production
- Vitamin D3 increases production of RANKL
- Glucocorticoids increase RANKL/decrease OPG production
- Oestrogen increases production of OPG

Other stimulators of OPG production are vitamin K, leptin, genistein, raloxifene, statins, e.g. atorvastatin, bisphosphonates and mechanical forces. Moreover, new facets of these mechanisms are constantly being elucidated by ongoing research, for example the suppression of osteoclastogenesis by alpha-lipoic acid. In addition, it has become clear that the relationship between RANKL and OPG contributes to the preservation of the balance between resorption and formation in bone, i.e. “coupling” of these activities, and that the relative concentration of RANKL and OPG in bone is one of the main determinants of bone mass and strength.

Animal experiments have also demonstrated the important part played by OPG in the regulation of bone resorption. Genetically manipulated mice, which over-express OPG, develop osteopetrosis; while OPG knock-out mice develop severe osteoporosis. These experiments indicate that OPG functions as a “brake” for the effects triggered by RANKL. Quite possibly, in the not-so-distant future, OPG may well be introduced as a therapeutic agent in numerous disorders characterized by increased resorption, such as:

- Postmenopausal osteoporosis and osteoporosis of the elderly
- Disorders with locally increased resorption
- Paget’s disease of bone
- Periodontitis
- Rheumatoid arthritis
- Bone marrow oedema syndrome
- Osteoporosis in various immunologic disorders
- Haematological disorders, e.g. multiple myeloma
- Carcinomatosis of bone
- Hypercalcaemic syndrome

During the last few years the significance of the OPG/RANKL/RANK system has been elucidated, not only in primary disorders of bone but also in secondary skeletal-related and vascular conditions which include common diseases such as diabetes, atherosclerosis, rheumatoid arthritis and metastases (Table 2.4). This confirms that the RANKL/OPG system is a cytokine system with widespread, far-reaching systemic effects. In a recent study, a substantially increased risk of hip fracture in women was demonstrated after the onset of a cardiovascular disease (CVD), a finding compatible

**Table 2.4.** OPG/RANKL/RANK system in the pathogenesis of bone, immune and vascular diseases

<b>Metabolic bone diseases</b>
Postmenopausal osteoporosis
Glucocorticoid-induced osteoporosis
Hyperparathyroidism
Sporadic Paget disease of bone
<b>Immune-mediated bone diseases</b>
Rheumatic arthritis
Periodontal infection
<b>Malignant diseases</b>
Multiple myeloma
Bone metastases
Hypercalcaemia of malignancy
<b>Inherited skeletal diseases</b>
Familiar expansile osteolysis
Familial Paget disease of bone
Idiopathic hyperphosphatasia
<b>Cardiovascular diseases</b>
Atherosclerosis
Peripheral vascular disease
Coronary artery disease

with the concept of common pathologic pathways for osteoporotic fractures and CVD. The MINOS study, a long-term prospective trial, showed that aortic calcification is a considerable and independent risk factor for incident fractures in older men. Significantly, in all the conditions mentioned above, there are windows of opportunity for the inclusion of bisphosphonates in therapeutic interventions – some are already being explored and applied.

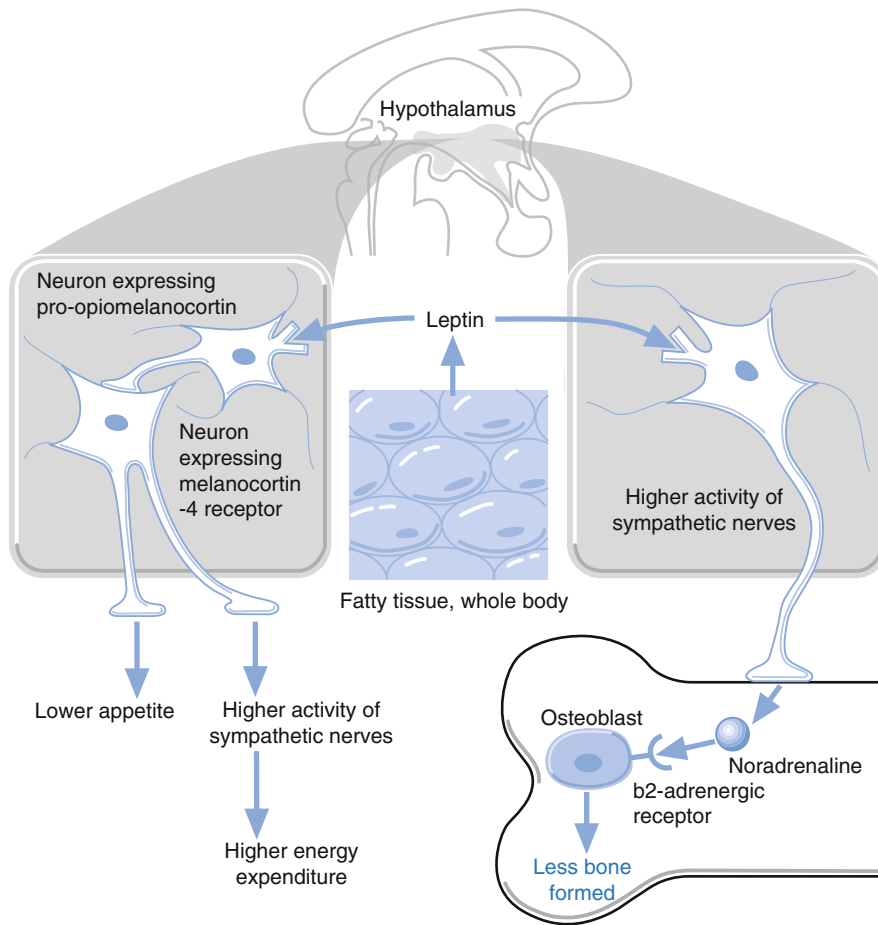
## 2.10

### Leptin: Role of the Central Nervous System in Regulation of Bone

The observation that overweight individuals are less susceptible to osteoporosis implies a possible connection between obesity and the skeleton. It was first suggested that the effects of increased weight-bearing might protect bone mass (Fig. 2.21). However, experimental studies have implicated leptin: a hormone produced by fat cells and which interacts with neurons in the brain and thereby influences weight. It was then discovered that in mice leptin is also anti-osteogenic and it was speculated that increased bone mass in obese people may result from resistance to leptin's anti-osteogenic activity. The amount of leptin released

into the bloodstream is proportional to the amount of body fat. Leptin regulates the body's energy balance as well as the bone mass by binding to certain receptor proteins of specific neurons in the hypothalamus, and these in turn activate sympathetic nerves. The nerves extend into the bones, where they stimulate release of the neurotransmitter noradrenaline, which then activates beta2-adrenergic receptors on osteoblasts, inhibiting osteoblastic activity. Leptin thus prevents bone formation through its action on already differentiated osteoblasts; it has no overt effect on osteoclast differentiation or function. These results seem to suggest that the millions of patients who have been treated with "beta blockers" such as propranolol for hypertension should have increased bone mass – an argument for re-assessing these clinical studies with respect to changes in bone density. Extreme changes in body weight and bone mass are also partly mediated by leptin, as well as the sex hormones. The identification of leptin as a powerful inhibitor of bone formation definitely has potential therapeutic implications in the future.

To summarize briefly: leptin has a circadian pattern of secretion with peak levels at midnight; it reflects total body adipose tissue mass. Many effects of leptin on energy metabolism are mediated by interaction with insulin. Leptin impacts skeletal metabolism and is also involved in pathological conditions such as obesity, atherosclerosis, oxidative stress and malignancies. Other neuropeptides such as neuromedin U are also involved in the control of bone remodelling, as are the endocannabinoids, synthesized by both osteoclasts and osteoblasts. Receptors for these substances are present in the sympathetic nerve terminals located near the bone cells and constitute a signalling pathway between brain and bone. The endocannabinoid receptors are also involved in the regulation of bone mass and osteoclast function such as, for example, enhancement of bone loss in ovariectomized animals. The endocannabinoids are involved in food intake and energy metabolism. These systems influence pathways from insulin signalling in the pancreas to oxidative processes in skeletal muscles. In summary, it should be stressed that the skeleton is equipped with numerous nerve fibres which participate in the regulation of skeletal metabolism by the CNS. More than 10 neuropeptides have already been identified in bone, including substance P (SP). Receptors for SP are located on osteoclasts and SP stimulates resorption.



**Fig. 2.21.** Central nervous participation via leptin in bone turnover (modified from Harada and Rodan [2003])

Additional factors in bone metabolism are still being identified in current research projects, for example, prostaglandin E<sub>2</sub>, a notable lipid mediator of bone remodelling. It has also been shown that patients with hypercholesterolemia/dyslipidemia have increased bone remodelling associated with osteoporosis, as well as with atherosclerosis. Animal studies have recently demonstrated that haematopoietic stem cells in the niches adjoining the endosteum also participate in the production of BMP-2 and BMP-6 and participate in bone remodelling.

## 2.11 Growth of the Embryo in the Uterus

Genetic and environmental factors are involved in the normal growth and development of the embryo in the uterus, as well as in growth retardation. In ad-

dition, recent studies that followed the trajectories of growth from birth to adulthood have demonstrated that size at birth and postnatal growth both effect the risk of, and are associated with, the development of a number of chronic diseases in adulthood. These include hypertension, coronary heart disease, stroke, diabetes and disturbances of renal function. Moreover, adverse maternal and other environmental factors are also involved. The most recent studies have shown that circumstances during the foetal period and early childhood may have life-long programming effects on different physical functions. This realization gave rise to the new concept of the developmental origins of health and disease (DOHaD). One characteristic example is the programming of the hypothalamic–pituitary–adrenal axis. Interestingly, not only physical, but also psychological aspects play a part, as shown by a Swedish study of 318,953 men, followed-up from date of birth (1973–1980) to date of attempted

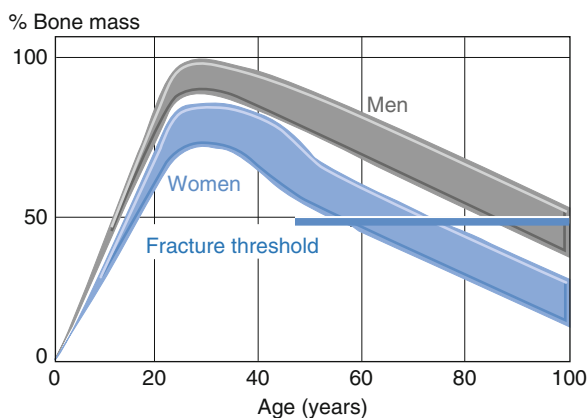
suicide, date of death, emigration or end of the study in 1999. The results showed that both short length at birth and short adult stature seemed to increase the risk of violent suicide attempts.

Intra-uterine growth restriction has now been positively associated with low bone mass in infancy and with increased risk of developing osteoporosis in adulthood. Importantly, in the context of this book, it is now believed that osteoporosis is, at least partly, programmed in utero and that nutritional and other environmental factors in the pre- and postnatal period exercise a profound influence on skeletal development, which, in turn, has considerable consequences in adulthood. In addition, there is no racial discrimination, since this applies equally to Blacks and Whites, as demonstrated in an investigation of bone size and bone mass in both black and white South African children. Moreover, not only organs such as the skeleton, but also cell systems may be modulated by the intrauterine metabolic environment as shown by intra-uterine epigenetic modification of beta pancreatic cells towards a pre-diabetic phenotype, but this could be corrected by early intervention, i.e. supplementation of the maternal diet. However, many other studies have now demonstrated that the adverse pre- and postnatal effects listed above can and should be modified by application of appropriate measures (nutrition, supplements, possibly medications, exercise and lifestyle) during childhood, adolescence and adulthood into old age.

## 2.12

### Peak Bone Mass: An Investment for a Healthier Life

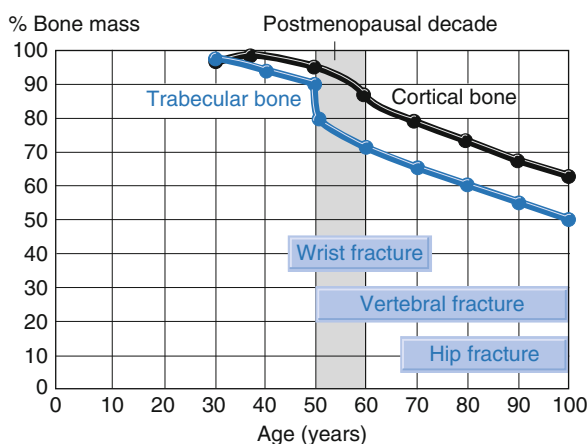
The skeleton acquires the maximal bone density – “peak bone mass” – at 25–30 years of age (Fig. 2.22). Consequently, the periods of growth before that age provide the maximal opportunities for building the peak bone mass, 60–80% of which is determined by genetic factors, the remaining 20–40% by other determinants such as nutrition and exercise. Thereafter, beginning at about 30 years, a negative bone balance sets in, so that on average 1% of bone is lost every year, independent of sex. Measurements of trabecular bone density between the ages of 20 and 80 years have shown reductions of approximately 50% in density (Fig. 2.23). This bone loss is apparently genetically



**Fig. 2.22.** Age-related changes in bone mass

programmed. Marked racial differences in peak bone mass occur, with higher values in American Blacks than in Caucasians, and the lowest values in Asians and Japanese. Some of the multiple pathogenic mechanisms and factors that contribute to subsequent loss of bone (“osteoporosis”), after the attainment of peak bone mass, include the following:

- Genetic factors
- Foetal and neonatal factors
- Factors during growth
- Inadequate peak bone density
- Nutritional and lifestyle factors
- Menopause and reduction of oestrogen in women
- Age and deficiency of testosterone in men
- Reduction of about 80% in adrenal steroids during ageing



**Fig. 2.23.** Correlation between predominant trabecular bone loss and incidence of types of fractures



- Co-morbidities
- Other effects of ageing on the body and on the activities of daily life

Race and diet interactions are also involved in the attainment, maintenance and loss of bone. Some of the genes that may influence bone mass and rates of bone loss include the genes responsible for:

- Vitamin D receptor
- Oestrogen receptor
- Parathyroid hormone receptor
- IL-1 receptor antagonist
- TGF- $\beta$
- Spl site in  $\alpha 1$  chain of type I collagen

The bones are like a bank savings account for calcium. If the calcium supply is adequate, savings deposits are made and the calcium bone bank account builds up. If the dietary calcium intake is too

low, then withdrawals of calcium are made from the “bone bank” itself, i.e. the skeleton. The peak bone mass attained in early life is a major determinant of subsequent bone mass and of fracture risk in later life. Peak bone mass is greater in men than in women, although these differences are reduced or even reversed if bone mass is expressed as volumetric bone density.

Calcium tends to be stored in the osseous tissue during the day and slowly released during the night. A bone biopsy study has shown that the loss of bone occurs fairly equally in all regions of the skeleton, perhaps slightly more in the vertebral bodies and the proximal femur. In postmenopausal women, the decline in oestrogen is accompanied by an increase in the loss of bone of up to 4% annually. This implies that women may lose 40% of their bone mass from 40 to 70 years. During the same period men lose only about 12%.

Osteoporosis

Diagnosis, Prevention, Therapy

Bartl, R.; Frisch, B.

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