

BONE METABOLISM

[Arno Helmborg](#)

These lecture notes accompany my lectures on pathophysiology in the study module "Musculoskeletal System" at the Medical University of Innsbruck. The English version serves two purposes: as a learning aid for international students and to encourage German-speaking students to familiarize themselves with medical English; the lectures are delivered in German. The translation from the original [German version](#) is my own; I am afraid it will occasionally sound appalling to native English speakers, but it should at least be intelligible.

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1. BONE FORMATION AND BONE RESORPTION

Living bone is constantly being remodeled. The state of our bones is always close to an equilibrium between bone formation and bone resorption. In childhood and during the teens, bone formation is slightly ahead. We reach peak bone mass in the twenties, and from then onwards, resorption has the upper hand. There are two reasons for the constant remodeling process. Firstly, it allows our bones to adapt to changes in load. For example, consider how easily skilled orthodontists maneuver teeth in the jaw bone by applying modest targeted strain. Secondly, continuous remodeling is necessary to repair the damage caused by recurrent microtraumas. At a typical remodeling site, termed basic multicellular unit, specialized osteoclasts first remove bone over a period of approximately three weeks. The resulting resorption lacuna is subsequently filled by osteoblasts, a process lasting about three months. Bone tissue is found in two forms: *substantia compacta* and *substantia spongiosa*. As much weight is saved as possible: only the outer contour, the cortical compact bone, is massive. The inner part is made up of trabecular, or cancellous bone, a three-dimensional scaffold of pillars and beams that is constantly modified to accommodate load. Prominent examples of cancellous bone are found in vertebral bodies or at the ends of long bones.

The fundamental unit of compact bone is the osteon or Havers system. A central vascular canal is surrounded by massive concentric lamellae of mineralized fibers. In consecutive lamellae, matrix fibers are arranged in spirals with alternating sense of rotation, contributing to mechanical strength. Encased bone cells, osteocytes, are interspersed between lamellae.

In essence, bone metabolism is due to only two types of cells: osteoblasts and osteoclasts. Osteocytes are simply osteoblasts that have encased themselves in bone. Individual osteocytes remain connected by long cellular processes, forming a network connected by gap junctions. Osteocytes are able to sense mechanical strain, which they report to the bone construction units via this network.

Osteoblasts differentiate from stromal marrow cells. They produce the organic part of the bone matrix, an array of proteins collectively termed osteoid. In the following, a look at three out of a much larger number of these proteins:

1. Collagen type I represents the bulk of osteoid. It consists of triple helix units containing two $\alpha 1$ -chains and one $\alpha 2$ chain, which already form in the endoplasmic reticulum of the osteoblast after the individual chains have been posttranslationally hydroxylated on lysines and prolines. This procollagen unit is secreted, followed by proteolytic removal of C- and N-terminal peptides. The resulting collagen monomers spontaneously aggregate in a staggered fashion, forming long fibrils that are subsequently covalently cross linked via their hydroxylated lysines. A cofactor required for lysine and proline hydroxylation is vitamin C. Lack of vitamin C results in scurvy, characterized by collagen that is unstable due to insufficient cross linking.
2. Osteocalcin is a small protein that is carboxylated on glutamic acid residues with the help of vitamin K. As glutamate already contains one COO^- -group, carboxylation of the γ -C-Atom creates a second one right next to the first. The two adjacent negative charges are ideal docking sites for double positive Ca^{2+} ions. Osteocalcin binds hydroxyapatite $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, but is not required for its formation, as osteocalcin null mice have increased bone mineralization. The hydroxyapatite nucleation process, which makes sure that Ca^{2+} and phosphate precipitate in the bone and not in other tissues of the body, therefore must rely on other osteoblast products. But fracture toughness in these mice is substantially reduced: osteocalcin seems to prevent crack growth by stretching and dissipating energy. Osteocalcin may thus function as a shock-absorber between organic and inorganic matrix components. Vitamin K is also necessary to carboxylate clotting factors II, VII, IX, X, providing them with functionally essential Ca^{2+} binding sites. Therefore, deficiency of vitamin K results in bleeding disorder long before effects on bone might cause problems. A second vitamin is important for osteocalcin: the transcription of its gene is induced by activated vitamin D receptor. Osteocalcin itself has a second function, too. A proportion of non-carboxylated osteocalcin enters the blood stream and functions as a metabolic hormone enhancing insulin activity. Via a G protein-coupled receptor, it stimulates proliferation of pancreatic β -cells, and sensitizes fat cells to insulin by stimulating them to secrete adiponectin. Via this mechanism, bone metabolism influences energy metabolism.
3. Osteonectin is an osteoid component that makes contact to collagen type I as well as to hydroxyapatite, forming a link between organic and inorganic bone matrix.

In addition, osteoblasts engage in targeted export of Ca^{2+} and phosphate, inducing local super saturation conditions to mineralize the freshly produced osteoid. For this process, alkaline phosphatase tethered to the outside of the osteoblast plasma membrane seems to be important, although the enzyme's role remains insufficiently understood. It may increase extracellular phosphate concentration by dephosphorylating organic molecules or cleaving pyrophosphate.

Bone statics may be compared to the statics of reinforced concrete. Hydroxyapatite is highly resistant to compressive stresses, while the built-in collagen fibers provide the combined matrix with high strength in tension. The comparison with armored concrete illustrates why bone formation and bone resorption have to go hand in hand. Mechanical strain constantly results in micro fissures in the bone matrix. There, collagen ("steel") fibers are torn. Repair involves a large resorption lacuna, allowing to embed new fissure-spanning fibers in fresh mineral matrix ("concrete"). Pure mineralization of ("plastering over") the fissure would not

restore the structure to original strength. This is convincingly demonstrated in rare genetic diseases with defective bone resorption (osteopetrosis). The result is bone tissue that is extremely dense but at the same time fragile, as it is permeated by insufficiently repaired micro fissures that have been merely plastered over.

Once osteoblasts have encased themselves in bone, they change their expression pattern, becoming **osteocytes**. Osteocytes secrete sclerostin, which inhibits further bone formation in nearby osteoblasts by binding and antagonizing LRP5/6 receptors, thereby inhibiting Wnt-signaling. Thus, sclerostin promotes "sclerosis", rigidification of bone. Factors promoting remodeling, e.g., mechanical loading, parathyroid hormone or prostaglandin E inhibit production of sclerostin.

Pharmacology cross reference: Romosozumab, which has been submitted for FDA-approval for treatment of osteoporosis, is a monoclonal antibody binding to and inhibiting sclerostin. If approved, it would be the first medication promoting formation of new bone mass, instead of merely inhibiting bone resorption.

Osteoclasts are giant, multinucleated cells that derive from hematopoietic stem cells in the bone marrow, branching from the lineage leading to macrophages and neutrophils. A series of [cytokines](#) induces precursor cells to differentiate to osteoclasts. The basic mix combines M-CSF (*macrophage colony stimulating factor*) with RANKL (explained in the following section on parathyroid hormone), two cytokines produced by osteoblasts. In addition, mediators produced by macrophages and other cells during inflammatory responses enhance osteoclast differentiation: IL-1, IL-6, [TNF \$\alpha\$](#) and prostaglandin E. Osteoclasts break down bone tissue much like macrophages break down phagocytosed material; only the process is shifted to the extracellular space. Employing normal lysosomal chemistry, it involves acidification and activation of acid hydrolases. Osteoclasts seal off a certain matrix area, which they acidify with the help of a proton pump. To maintain intracellular pH, they release HCO₃⁻ at their back side. Hydroxyapatite dissolves in the acidic environment, setting free Ca²⁺. Thus, on the scale of the entire body, an orchestrated activation of osteoclasts is a means to increase extracellular Ca²⁺-concentration. After the mineral has melted away, acid proteases like Cathepsin K hydrolyze the remaining matrix proteins.

Growth of long bones is not possible in bone tissue itself, but happens in epiphyseal cartilage, the growth plate. Three zones of chondrocytes at different stages of differentiation can be observed. In all three zones, chondrocytes secrete proteins and proteoglycans that form the cartilage extracellular matrix, like collagen and aggrecan. Closest to the epiphysis is the resting zone, containing chondrocytes that serve as progenitor cells. Next is the proliferative zone, where spatial arrangement of cell division leads to long columns of chondrocytes parallel to the long axis of the bone. These cells produce collagen type II, which is characteristic for hyaline cartilage. Near the metaphysis, chondrocytes in the hypertrophic zone undergo terminal differentiation, grow in volume and secrete collagen type X and VEGF (vascular endothelial growth factor). At the border zone, hypertrophic chondrocytes undergo cell death. Attracted by VEGF, new capillaries sprout into the zone. The cartilaginous tissue is first simply mineralized (enchondral ossification), but soon remodeled to osteon structure by immigrating osteoclasts and osteoblasts. So, growth in cartilage results in elongation of the bone. The regulation of this process is complex. Genome-wide association studies identified about 200 genetic loci that influence height. Proliferation of chondrocytes is regulated by a multitude of paracrine factors, such as a bone morphogenetic protein gradient or C-type

natriuretic factor, as well as endocrine factors, such as growth hormone, IGF-1, sex steroids and leptin. The endocrine factors link rapid growth to the availability of sufficient nutrients.

A second ossification mechanism, intramembranous ossification, is the direct transformation of fibrous mesenchymal tissue to bone. This type of ossification is found in the development of large parts of the skull, as well as in healing of bone fractures.

2. REGULATION OF BONE METABOLISM

2.1 Calcium and phosphate balance

We looked at calcium and phosphate [before](#), when we studied their [renal](#) handling. Here, our goal is to understand the interdependence between calcium/ phosphate balance and our bones.

2.1.1 Soluble Ca^{2+} , hydroxyapatite and calcitonin

As calcium (Ca^{2+}) is one of the main components of our bones, large amounts are present in our body. At the same time, comparatively low extracellular concentrations of Ca^{2+} are fine-tuned to regulate important functions, not to speak of even far lower intracellular concentrations. This dichotomy is possible due to the low solubility product of Ca^{2+} and phosphate (PO_4^{3-}): if one ion is added to a solution of the other, most of it precipitates as calcium phosphate.

In the bone, the two ions combine with hydroxide (OH^-) to form hydroxyapatite $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, a hard mineral forming hexagonal crystals. Up to 70% of the weight of bone is due to hydroxyapatite. Dental enamel consists almost exclusively of the mineral, accounting for its mechanical resistance. The disadvantage to this solution is that hydroxyapatite is sensitive to acidity. Low pH attacks enamel via the same mechanism that osteoclasts use to resorb bone. Citric acid from an orange, or lactic acid produced by bacteria metabolizing sugar in dental plaque make protons come into contact with the enamel surface. A proton H^+ pulls out the hydroxide ion OH^- from $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ to form a H_2O water molecule, with the rest disintegrating into 5 Ca^{2+} and 3 PO_4^{3-} ions. The hydroxyapatite complex dissolves, ultimately leading to caries. If OH^- is replaced by a fluoride ion F^- to form fluorapatite $\text{Ca}_5(\text{PO}_4)_3\text{F}$, the mineral is much more stable at low pH. Fluorapatite forms spontaneously if enough fluoride ions are present, a condition that can be promoted by addition of fluoride to toothpaste, salt or, in some countries, drinking water.

Plasma Ca^{2+} concentration is physiologically maintained in a small window between 2.2 and 2.7 mM. This measured Ca^{2+} is the sum of three forms: Ca^{2+} bound to plasma proteins (about 45%), Ca^{2+} complexed with small organic anions (10%) and free ionized Ca^{2+} (about 45%). Hence, total Ca^{2+} depends on plasma protein concentration. The biologically relevant, regulated parameter is free Ca^{2+} .

Ca^{2+} balance is basically maintained by two hormones: parathyroid hormone (PTH) and calcitriol (1,25-dihydroxyvitamin D). PTH regulates short-term plasma Ca^{2+} concentrations by dipping into bone reserves. Vitamin D strategically maintains the total Ca^{2+} pool of the

body. A third hormone, fibroblast growth factor 23 (FGF23), regulates elimination of phosphate via the kidneys, which directly impacts on the calcium balance.

A fourth Ca^{2+} regulating hormone, **calcitonin**, is of minor importance in humans. It is secreted by parafollicular C cells in the thyroid gland and lowers plasma Ca^{2+} levels for a short time by directly inhibiting osteoclast activity, with the system quickly swinging back to a neutral position. Neither a total loss of calcitonin-producing cells (e. g., by thyroidectomy), nor massive overproduction by rare tumors lastingly interfere with Ca^{2+} balance. Probably, calcitonin is a remnant from evolution. Animals such as salmon, which switch from fresh water to calcium-rich sea water, seem to rely on calcitonin to cope with massive differences in Ca^{2+} intake.

Pharmacology cross-reference: Salmon calcitonin is actually used to treat patients, although it is nowadays produced recombinantly or by peptide synthesis. Why don't we use the human version? On a molar basis, salmon calcitonin is about 10 times as potent as the human peptide. Although it differs from the human version in 14 of the 32 amino acids, immunological complications are surprisingly rare. Calcitonin is used to lower Ca^{2+} in acute hypercalcemic situations. In addition, it is used in diseases with high bone resorption to intermittently inhibit osteoclast activity, e. g., in osteoporosis, Paget's disease and bone metastasis, where it may have the added benefit of alleviating pain.

2.1.2 Parathyroid hormone

Parathyroid hormone (PTH) is named for the four parathyroid glands producing it, tiny epitheloid bodies located right behind the thyroid. An increase in the concentration of free Ca^{2+} activates the *calcium-sensing receptor* (CaSR) located at the membrane of their chief cells. The cells react by decreasing PTH production. A second means to lower PTH secretion is a high concentration of 1,25 dihydroxyvitamin D. The message of Vitamin D seems to be: "Stop cannibalizing our bones, I'll organize more Ca^{2+} from outside in a minute!" PTH is a small protein of 84 amino acids and has an extremely short half-life of about four minutes. PTH increases Ca^{2+} concentration via two main mechanisms: by liberating it from bone and by influencing the kidneys.

PTH's net effect in bone is an increase in resorption by activation of osteoclasts. This is achieved via a detour, as osteoclasts do not express PTH receptors. PTH is sensed by osteoblasts, which react by producing IL-1, IL-6 and other cytokines to activate osteoclasts. In addition, PTH increases osteoblast production of the two molecules that induce differentiation and proliferation of more osteoclasts: M-CSF (macrophage colony-stimulating factor) and RANKL.

RANK-ligand (RANKL) is a molecule from the TNF-superfamily. It acts as a trimer, either on the surface of osteoblasts, or, "cut off", as a soluble signaling molecule. In the bone marrow, M-CSF and RANKL encounter precursor cells of the hematopoietic lineage leading to macrophages and neutrophil granulocytes. These precursor cells express **RANK** (receptor-activator of $\text{NF}\kappa\text{B}$), a transmembrane protein of the TNF receptor superfamily. RANK functions as receptor for RANKL. As precursor cell RANK is trimerized by osteoblast-emitted RANKL, the precursor cells are activated to differentiate first to mononucleated osteoclast precursors that subsequently fuse to mature polynucleated osteoclasts. Osteoblasts

secrete a further protein, **osteoprotegerin (OPG)**, that looks like a soluble receptor for RANKL. This is called a decoy receptor; by neutralizing RANKL, it acts as its inhibitor. Thus, the formation rate of osteoclasts depends on the relative amounts of RANKL and OPG produced by osteoblasts. While PTH induces expression of M-CSF and RANKL, it inhibits production of OPG, cranking up the generation of osteoclasts.

If PTH just mobilized Ca^{2+} , not much would be gained: due to the low solubility product with phosphate, it would soon reprecipitate. Therefore, PTH simultaneously lowers phosphate levels by inhibiting renal reabsorption in both the proximal and distal tubule. This is achieved by removing the Na/phosphate cotransporter from the luminal membrane and parking it in vesicles below. Apart from inducing phosphaturia, PTH increases reabsorption of Ca^{2+} in the distal tubule, further reducing the already minimal loss of Ca^{2+} in the urine. The third renal function of PTH is to stimulate hydroxylation of carbon atom 1 of vitamin D: this is the last and rate-limiting step in its activation. From there, 1,25 dihydroxyvitamin D sets out to refill the Ca^{2+} pool.

Pharmacology cross-reference: Cinacalcet (Mimpara[®], Sensipar[®]) is a small molecule binding to another site of the calcium-sensing receptor (CaSR), allosterically sensitizing the receptor to free Ca^{2+} . Its main use is in treating secondary hyperparathyroidism in patients with chronic renal failure on dialysis. Failing kidneys excrete too little phosphate and activate too little vitamin D, resulting in high plasma phosphate and low Ca^{2+} , a dysequilibrium that is ultimately taken out on the bones via PTH.

2.1.3 Vitamin D

Lipid-soluble vitamin D3 can be taken up with animal foods, especially fatty fish species (cod liver oil, mackerel, salmon), or can be produced in our own skin from 7-dehydrocholesterol. This requires sunlight to open the second ring of the cholesterol backbone. This UV B-dependent synthesis is probably the cause of Caucasians' pale complexion. Until the first wave of *homo sapiens* left Africa about 60,000 years ago, all modern humans probably had dark skin. The further north the people migrated, the less ultraviolet light they absorbed. Those with lighter complexions obtained a selective advantage, as they were better able to synthesize vitamin D. Ethnic groups using the sea as their primary food source, like the Inuit, took up enough vitamin D3 with their food and were thus able to retain a higher level of pigmentation than people living off the land. The causal relationship between a lack of sunlight and rickets was only recognized in the late 19th century.

(The selective advantage of individuals with a lighter complexion in northern regions was probably not only due to better bone stability. UV B-generated Vitamin D also seems to play a role in infection defense. Low vitamin D levels during wintertime may contribute to susceptibility to infections of the respiratory tract. Unfortunately, the molecular mechanisms remain unclear. Largely for empirical knowledge, patients of the mountain-based tuberculosis clinics in Europe's 19th and early 20th century were seated outside in the sun at regular intervals. At that altitude, this treatment implied considerable UV exposure.)

As an alternative to synthesis or intake of cholecalciferol (vitamin D3), a very similar molecule, ergocalciferol (vitamin D2, with a slightly different side chain) is present in vegetables, but its concentration is usually too low to make up for the deficit.

Two successive hydroxylation steps are required to metabolize D3 and D2, which already contain one hydroxyl group, to their active form, calcitriol. The first hydroxyl group is added at position 25, the end of the side chain, in the liver. The second hydroxylation occurs in the kidney, at position 1 of the first ring of the erstwhile cholesterol structure. This decisive, last activation step is performed in the proximal tubule under tightly regulated conditions. PTH stimulates hydroxylation, while the end product calcitriol as well as increased levels of FGF23 and/or phosphate act inhibitory. 1,25-dihydroxyvitamin D (calcitriol) equilibrates over the entire body and binds to the vitamin D receptor (VDR), a member of the nuclear receptor superfamily. As a ligand-dependent transcription factor, one of its functions is the induction of genes that are necessary to maintain Ca^{2+} reserves.

The central target organ in this respect is the duodenum. Here, calcitriol induces several proteins that in concert enhance absorption of Ca^{2+} from food. While Ca^{2+} concentrations in the lumen of the gut and in blood are in the nanomolar range, they are much lower inside the cell; too much free Ca^{2+} in the cytosol would be dangerous. At the luminal side of the duodenal epithelial cell, vitamin D induces a channel, allowing Ca^{2+} to trickle in passively. In the cytosol, the Ca^{2+} -affine protein calbindin is increased to neutralize passaging Ca^{2+} . At the basolateral membrane, an ATP-driven Ca^{2+} - H^+ -antiporter as well as a Na^+ -driven Ca^{2+} - Na^+ -antiporter are induced to pump Ca^{2+} into the blood against a steep concentration gradient. Calcitriol also enhances phosphate absorption in the small intestine.

In the kidney, the action of vitamin D parallels that of PTH by increasing reabsorption of Ca^{2+} in the distal tubule, although its effect is much weaker. Contrary to PTH, vitamin D also enhances reabsorption of phosphate: both ions are required to promote bone mineralization.

Together, these effects of vitamin D raise Ca^{2+} and phosphate concentrations above their solubility product, inducing their precipitation in osteoid. This predominant, indirect effect outweighs an opposite direct, receptor-mediated activation of osteoblast and osteoclast precursors that would enhance bone turnover and Ca^{2+} mobilization. In addition, vitamin D-stimulated transcription of the osteocalcin gene in osteoblasts helps to build fracture resistance.

2.1.4 Fibroblast growth factor-23 (FGF23)

FGF23 is produced by osteocytes and osteoblasts in response to 1,25-dihydroxyvitamin D and dietary phosphate loading. It increases renal phosphate excretion by reducing the number of Na/phosphate cotransporters in the apical membrane of the proximal tubule. In this function, it acts similar to PTH. Yet, it counters PTH by inhibiting 1α -hydroxylation of vitamin D. It thus lowers active vitamin D, which in turn reduces uptake of Ca^{2+} via the intestines.

CKD-MBD (*chronic kidney disease- mineral and bone disorder*): We take up all available phosphate from the intestines and balance that by eliminating the surplus via the kidneys. In many old people, a problem arises from the decline in glomerular filtration rate. As filtrated volume comes down, a progressively higher percentage of filtrated phosphate needs to be excreted, so FGF23 levels steadily rise higher and higher. FGF23 keeps calcitriol down,

meaning plasma Ca^{2+} concentration can only be maintained by parathyroid hormone. Over time, secondary hyperparathyroidism results in bone disorder.

X-linked hypophosphatemia: The X-chromosomal PHEX gene (phosphate-regulating neutral endopeptidase, X-linked) encodes a peptidase which inhibits FGF23. Deficiency of this peptidase causes FGF23 hyperactivity resulting in renal phosphate losses. In affected children, the disease is reminiscent of rickets, yet does not respond well to vitamin D.

2.2 Growth hormone and IGF-1

Growth hormone (GH) is essential for longitudinal bone growth. It is a 191 amino acid protein produced by somatotrophs in the anterior pituitary under control of the hypothalamus: GH secretion is stimulated by GH releasing hormone (GHRH), inhibited by somatostatin. GH is secreted in short bursts of pulses only during sleep or during exercise (it makes no sense to assay GH levels at daytime in a child at rest). GH has a few fast, direct effects that are almost directly opposed to the more important slow, indirect effects via insulin-like growth factors. The receptor-mediated fast effects are antipodal to insulin action and include lipolysis in fat cells, gluconeogenesis in liver and an inhibition of glucose uptake by muscles. Many other effects, including those promoting growth in cartilage and bone, are mediated indirectly. GH stimulates hepatocytes to secrete insulin-like growth factor-1 (IGF-1) into the blood. Likewise, many other cells, including chondrocytes and osteoblasts, are induced to produce IGF-1 that acts in a paracrine fashion.

IGF-1 is closely related to insulin, with a little less than 50% identical amino acids. Like insulin, it binds to a heterotetrameric receptor consisting of two extracellular α chains and two transmembrane β chains containing a tyrosine kinase domain. "Mixed-chain" insulin/IGF-1 receptors exist that can be activated by either hormone. IGF-1 is protected from proteolysis by IGF-1-binding proteins and integrated into the organic cartilage and bone matrix. In bone, part of it is embedded by mineralization, together with other growth factors like TGF β and PDGF (transforming growth factor β and platelet-derived growth factor). This creates a reservoir of growth factors that is only activated in case of bone resorption (one reason why metastasizing tumor cells frequently find a fertile soil in bone). IGF-1 acts back on the cells in a paracrine fashion, stimulating chondrocytes in the epiphyseal plate and osteoblasts to divide. IGF-1 levels directly depend on GH, but their effect is much more stable due to these buffering mechanisms. Both a deficiency of GH and a deficiency of IGF-1 lead to stunted growth, while overproduction of GH results in gigantism.

Pharmacology cross-reference: Following insulin, GH was licensed for Genentech as the second protein drug produced by recombinant DNA technology in 1985. Before, human GH was purified from the pituitaries of deceased individuals. Indications are GH deficiency as well as small stature as a result of Turner's syndrome or renal insufficiency, where GH is applied symptomatically to increase height. In some countries, pediatricians are exposed to pressure from parents to administer GH to healthy children to increase height, as this is viewed as a social advantage. IGF-1 is used in patients who suffer from GH receptor defects, a condition known as Laron dwarfism.

[GH and IGF-1 have more functions than just promoting growth. In some countries, e. g. the USA, bovine GH is used to increase milk yield in cows. Unfortunately, this only works when cows are fed optimally, meaning it is of little help to the countries needing it most. The EU, after intense discussions, decided not to license bGH treatment of dairy cows.]

2.3 Thyroid hormone

GH and IGF-1 are necessary, but not sufficient for bone growth and maintenance of bone mass. Also necessary are thyroid hormone and, depending on gender, estrogens or androgens. Like IGF-1, thyroid hormone and sex steroids are under indirect control of the CNS. At present, the exact molecular mechanisms of their actions can be insufficiently described. Virtually all tissues express thyroid hormone receptors, and many tissues express receptors for estrogens and androgens. The three receptor types are related. Along with vitamin D receptor and glucocorticoid receptor, they are members of the superfamily of nuclear receptors. All nuclear receptors are ligand activated transcription factors, each regulating large numbers of genes. In the presence of ligand, many genes are transcriptionally activated and even more genes are silenced. At present, we know too little about which of these genes are important for bone growth and maintenance.

While other members of the nuclear receptor superfamily are primarily cytoplasmic, switching to the nucleus only following ligand binding, the thyroid hormone receptors (α and β) are bound to the DNA no matter whether ligand is present. In the absence of ligand, they frequently repress transcription from the respective genes. Triiodothyronine (T₃), or to a lesser extent thyroxine binding to the receptor causes it to actively contribute to transcription initiation. Thyroid hormone receptors are expressed in chondrocytes, bone marrow stromal cells, osteoblasts and osteoclast precursors. It is unclear whether T₃ has direct actions in osteoclasts. Apart from thyroid hormone itself, the superordinate controlling hormone thyrotropin (TSH) has been reported to have a direct inhibiting effect on osteoclasts, which were found to express TSH reporters.

Lack of thyroid hormone in children causes dwarfism. On the other hand, hyperthyroidism causes secondary osteoporosis.

2.4 Estrogens, progesterone and androgens

Likewise, the importance of sex steroids for bone metabolism became obvious from clinical observations. In various forms of hypogonadism, lack of these hormones results in osteoporosis. Overproduction of androgens or estrogens during childhood initially accelerates growth (as normally seen around puberty) but results in early epiphyseal closure with reduced final height. Postmenopausal osteoporosis starts with a decrease in estrogen concentrations.

Both estrogen and androgen receptors are expressed in either sex. While there is only one androgen receptor, two forms of DNA-binding estrogen receptors (ER) exist, ER α and ER β . ER α is expressed predominantly in ovary, uterus, and breast, while ER β is expressed in numerous additional tissues, but both types are expressed in bone cells. Specifically, ER α expression in osteoclasts is of functional importance. In addition to these receptors, which shuttle between nucleus and cytoplasm, a totally unrelated G protein coupled estrogen-

binding protein exists in the membrane of the endoplasmic reticulum, with no known involvement in bone metabolism. Numerous mechanisms have been proposed to account for the anabolic action of estradiol and related estrogens. Regarding bone resorption, estrogens reduce the number and activity of osteoclasts. Part of this effect is mediated via the RANK system. Activated estrogen receptors do not directly regulate the promoter of RANKL or related genes. Rather, they regulate the system indirectly via several different points of contact. For example, estrogens stimulate OPG production by osteoblasts and inhibit production of M-CSF, IL-1, IL-6 and TNF α . The result is a decrease in the rate of osteoclast generation. By the same and other mechanisms, activity levels and lifespan of existing osteoclasts are reduced. All in all, it is firmly established that estrogens reduce bone resorption. There are ample indications that they also directly stimulate bone formation, but there is no consensus regarding the involved mechanisms.

In contrast, progesterone drives RANKL expression and osteoclast formation. Progesterone peaks during pregnancy. This helps to release Calcium from the mother's bones to help calcify the bones of the fetus. In addition, progesterone-driven RANKL expression is important to spur mammary epithelial cell division, helping to develop new breast tissue for lactation.

Follicle stimulating hormone (FSH), the hormone stimulating estrogen production, has also been found to have a direct effect on bone. It was reported to stimulate osteoclast activity, an effect that would counter that of thyrotropin. Before menopause, this effect is more than compensated for by the anabolic actions of estrogen, but following menopause, it might be responsible for the phase of high-turnover bone loss.

In males, androgen levels come down only at a later age. As a result, male osteoporosis manifests itself at least ten years later than in females. Androgen-dependent mechanisms stimulating bone mass probably overlap to a large extent with those due to estrogens. Yet, there is a second possibility: also in males, androgens are being converted to estrogens by the enzyme aromatase, expressed in fatty tissue. Consequently, it is possible that some of the bone-protective effect of androgens might in fact be due to estrogens.

2.5 Cortisol and related glucocorticoids

Glucocorticoids affect bone formation as well as bone resorption. Glucocorticoids inhibit osteoblast function, e. g., by inhibiting transcription of collagen and osteocalcin genes (this also happens in other tissues: an impairment in collagen production can sometimes lead to visible *striae* in skin). Glucocorticoids also reduce the life span of osteoblasts. On the resorption front, glucocorticoids simultaneously induce RANKL and inhibit OPG production in osteoblasts. Combined, these two effects increase the number and activity of osteoclasts. All in all, glucocorticoid effects on both sides of the coin therefore strongly promote osteoporosis.

2.6 Mechanical strain

Being subject to mechanical load is essential to maintain bone mass. The trabeculae of cancellous bone are constantly being remodeled to adapt the bones to mechanical strain. Inactivity leads to a rapid loss of bone mass, as can be observed in bedridden patients. A

reduction of load due to conditions of zero gravity, as for astronauts manning the space station, has the same results. Mechanical strain is sensed by osteocytes, the bone cells positioned between osteon lamellae. Via their gap junction-connected network of long cellular processes, they are able to relay signals. Unfortunately, the molecular details of the osteocytes' load sensor remain insufficiently understood. One of the hypotheses under discussion holds that mechanical strain results in a fluid wave permeating porous bone and deforming osteocytes (like laundry on the line). This might open mechanosensitive ion channels, leading to a signal that may be relayed through gap junctions to sites near active bone construction units (basic multicellular units).

2.7 Food situation: leptin

[Leptin](#) was first identified in a mouse strain that had been inbred by selecting its obese phenotype. In Ob/Ob mice, a gene encoding an extracellular signaling molecule was found to be defective. Accordingly, the signaling molecule was termed "leptin" (the Greek word *leptos* means "thin"). In addition to being obese, homozygous mice were infertile. Remarkably, their bone mass was increased, while hypogonadism is otherwise associated with osteoporosis. For experimental reasons, the molecular mechanisms of leptin biology have been elucidated in the mouse. From what we know from data points verifiable in humans, these findings likely apply to humans as well.

The signal protein leptin is almost exclusively secreted by adipocytes (an *adipokine*). Its long term plasma level is proportional to the size of an individual's fat storage. Around this level, leptin levels oscillate diurnally dependent on food intake, with a minimum at breakfast and a maximum late in the evening. In addition, changes in food situation cause temporary divergence. Leptin levels decrease following a few hungry days, and increase after a period of feasting. Leptin acts on numerous tissues, but its main target is thought to be the brain. Leptin is able to cross the blood brain barrier and affects the autonomic nervous system via hypothalamic centers. A fall in leptin causes the sensation of hunger, an increase a feeling of satiety. For some time, leptin was hoped to be the answer to the obesity epidemic. However, obese individuals were found to be leptin resistant, much like type 2 diabetics are insulin resistant, meaning increased leptin does not reduce their appetite.

The leptin effect on bone metabolism, too, is mediated by the autonomic nervous system. Leptin-induced hypothalamic impulses are conveyed to the bone via sympathetic neurons, directly affecting osteoblasts by stimulating their β_2 -adrenergic receptors with norepinephrine. In the osteoblast, these signals are modulated by the cellular molecular clock mechanism. Depending on the phase of this clock, incoming signals either accelerate or delay osteoblast cell division and function. Osteoclast function, too, is affected by this adrenergic pathway. This mechanism explains a fact that has been known for a long time: markers of bone metabolism, like plasma osteocalcin, follow a circadian rhythm. It seems plausible, for example, that bone remodeling is easier to accomplish during nighttime.

From what we presently know, leptin acts as an input into the central nervous system reflecting the prevailing food situation. In response, the CNS reacts with meaningful adaptations of, e.g., eating behavior, reproductive functions and bone metabolism (*anorexia nervosa*, for example, combines decreased leptin levels, amenorrhea and osteoporosis). With respect to bone metabolism, computed output is additionally embedded in a useful circadian

rhythm. We are far from fully understanding leptin's bone effects: A complete lack of leptin in mice results in increased bone mass, while in anorectic women, low leptin is associated with decreased bone mass- but keep in mind these women suffer from severe malnutrition.

Pharmacological cross reference: If the net effect of leptin is decreased bone formation via a β -adrenergic mechanism, β -blockers should positively affect osteoporosis. Retrospective data analyses seem to strengthen this case. Conclusive prospective studies remain to be conducted.

3. DISORDERS OF BONE METABOLISM

3.1 Osteoporosis

Primary or idiopathic osteoporosis, by far the most common form of the disease, affects people in the second half of their lives. Though mechanisms in females and males are probably similar, the disease starts earlier in women, as estrogens in women decrease earlier than androgens in men. In women, the disease is termed postmenopausal osteoporosis.

The main symptoms of osteoporosis are bone fractures. Typically, these affect the femoral neck or the vertebral bodies (impression fractures). Of course, fractures occur at peak loads as happen in falls, but at comparable loads, the probability of fracture increases with decreasing bone mass. When a bone is weakened to an extent that it breaks from a minor stress, we speak of a pathological fracture. We reach our peak bone mass in our twenties. From then on, the net effect of the many factors affecting bone metabolism is slightly negative. In women, net resorption accelerates with menopause due to the fall-off in estrogens. During the first 5-10 years following menopause, accelerated, so-called high turnover bone loss is observed, characterized by increased osteoclast activity. Later, the system swings back to normalized osteoclast activity with a slight deficit in osteoblast function (low turnover bone loss).

In primary osteoporosis, several factors contribute to the negative net effect:

1. Decrease in estrogen and androgen concentrations
2. Reduced physical activity
3. Insufficient vitamin D and calcium intake
4. Reduced UV exposure, resulting in lower endogenous production of vitamin D
5. Reduced renal function secondary to diabetes, arteriosclerosis, or analgesics abuse, resulting in insufficient 1-hydroxylation of vitamin D

With bone resorption outbalancing bone formation, plasma Ca^{2+} levels increase. Lower PTH levels mean less renal Ca^{2+} reabsorption, or more urinary Ca^{2+} loss. In addition, PTH dependent 1-hydroxylation of vitamin D is decreased in the kidneys, lowering intestinal Ca^{2+} uptake. All in all, Ca^{2+} balance follows bone mass balance (anything else would make little sense: where to put all that Ca^{2+} ?) and both are negative.

Interestingly, an excess of weight to a certain degree protects from osteoporosis. Whether this is due to increased load, or to enhanced residual estrogen synthesis from androgens by fatty tissue aromatase remains to be elucidated.

Diagnostics

The most relevant property of bone would be its resistance against fractures. Of course, this cannot be tested. Measurable surrogates are bone density and biochemical markers of bone formation and bone resorption.

Bone density is usually determined by the DXA method (dual energy X-ray absorptiometry). DXA is based on the fact that the density of tissue affects its differential absorption properties for low energy versus high energy X rays. Put differently, from two images taken at different X ray energies, the density of the X-rayed tissue can be inferred; with a few assumptions and a lot of computing even the density of bone. The result is expressed as a multiple of the standard deviation from mean bone density of 30 year-olds of the same sex. This dimensionless value is termed T-score. Osteoporosis is defined by a T-score smaller than -2.5 (a bone density more than two and a half standard deviations below the mean bone density of individuals at peak bone mass). Another method to assess bone density is quantitative computed tomography (QCT). It is technically more complex and thus more expensive, but yields more information, e. g., allowing individual assessment of compact and cancellous bone.

Bone formation can be assessed by measuring plasma osteocalcin, as osteocalcin is produced exclusively by osteoblasts. In addition, peptides released during collagen polymerization can serve as proxies for bone formation, as most of collagen type I synthesis occurs in bone. Specifically, these are procollagen I C-terminal propeptide (PICP) and procollagen I N-terminal propeptide (PINP). An additional marker of osteoblast activity is bone specific alkaline phosphatase (ostase).

Bone resorption by osteoclasts in turn involves cleavage of cross-linked collagen. The C-terminal cross-linked ("x") fragments of collagen I (CTx-I, also designated "crosslaps") can be measured in plasma, serving as a marker for bone resorption. When these collagen fragments are further degraded in the body, what remains is the specific chemical structure formed by cross-linking hydroxylysines, pyridinoline. Pyridinoline (Pyr) and strictly bone specific deoxypyridinolin (Dpyr) can be measured in urine (sometimes, these are termed "crosslinks").

For the circadian rhythm of bone metabolism, it is important to take blood samples for each check-up at the same time of day.

Therapeutic options:

Estrogen replacement?

For many years, estrogen replacement therapy was very popular to mitigate unwelcome effects of menopause. People were unconcerned about potential long term side effects. When results of the necessary large scale randomized double blind studies finally came in (Women's Health Initiative Study in the USA, Million Women Study in the UK), they were sobering. There was a reduction in femoral neck fractures, but this was more than outweighed by increases in endometrial carcinoma, breast cancer, myocardial infarction, stroke and pulmonary embolism. Especially for myocardial infarction, the opposite had been assumed, as heart attacks are less frequent in premenopausal women than in men of the same age. With

these studies, general hormone replacement therapy is no longer an issue; newer studies propose beneficial effects of replacement for a few years immediately following menopause.

Raloxifen is an alternative. Raloxifen is a selective estrogen receptor modulator (SERM) like tamoxifen. These lipophilic ligands bind to the estrogen receptor and induce a change in conformation, leading to a complex that is slightly different from the original estradiol-receptor complex in three-dimensional structure. In some tissues, SERMs have estrogen-like effects, while in others, effects are estrogen-antagonistic. This depends on the individual cells' mix of transcription coactivators and corepressors, some of which bind the slightly modified complex better than the original, while others bind it less well. Raloxifen has estrogen-like effects in bone, slowing progression of osteoporosis. In the mammary gland, raloxifen acts antagonistically and actually reduces the risk of breast cancer. According to presently available data, Raloxifen seems to be neutral with respect to the risks of endometrial carcinoma and vascular disease, with exception of a slight increase in the risk of venous thrombosis.

Bisphosphonates

In phosphonate, the central phosphorus atom is surrounded by three oxygen atoms, instead of four as in phosphate. In bisphosphonate, two such groups are attached to a carbon atom: the phosphorus atoms are directly bound to the carbon, not via an oxygen as in a phosphate. While phosphates are easily removed by phosphatases hydrolytically cleaving the O-C bond, this is not the case for the P-C-P bonds of bisphosphonates; bisphosphonates are thus very stable in the body. Once settled in the bone, they have a half-life of several years. In many respects, they behave just like phosphate: they form insoluble complexes with Ca^{2+} , making it difficult to take them up from the intestines (they are frequently administered parenterally). They preferentially adhere to hydroxyapatite. From there, they are ingested by "nibbling" osteoclasts and interfere with their function via several mechanisms; over time, many osteoclasts enter apoptosis. In summary, bisphosphonates like Alendronate (Fosamax[®]) inhibit bone resorption, reestablishing some balance between resorption and formation in osteoporosis. There are also indications that bisphosphonates help to prevent establishment of bone metastases in cancer. In addition, some data suggest that bisphosphonates counteract "hibernation" of micrometastases in bone marrow (please see section on metastasis below). Problematic side effects result when bisphosphonate toxicity extends to other bone cell types, in rare cases causing the especially worrying osteonecrosis of the jaw.

Denosumab

As a natural RANKL-neutralizing protein, osteoprotegerin was a logical candidate to treat osteoporosis. It was introduced into early clinical trials by biotech company Amgen. However, a few worrying aspects instigated a search for better solutions. Apart from RANKL, OPG binds to additional members of the TNF superfamily. OPG is also expressed by endothelial cells and natural OPG levels correlate with coronary disease.

As an alternative solution with a presumably higher margin of safety, Amgen developed a monoclonal antibody, denosumab (Prolia[®]), mimicking the function of OPG. Advantages are an increased specificity for RANKL and a reduced risk of causing neutralizing antibodies against OPG. The human monoclonal IgG2 antibody (IgG2 is far less able to activate

complement than IgG1 or IgG3) is injected subcutaneously twice per year. In clinical studies, it proved to be effective against postmenopausal osteoporosis as well as against the osteolytic effects of breast cancer metastases. In May 2010, denosumab was approved for the treatment of osteoporosis in postmenopausal women at increased risk of fractures, and for the treatment of bone loss associated with hormone ablation in men with prostate cancer. As RANKL knockout mice also show immunological problems (although these are induced early in development), potential long-term side effects will have to be monitored carefully.

Vitamin D and Ca²⁺

To counteract the multiple potential causes for deficiency, it makes sense to orally substitute vitamin D. As it can only be effective with an adequate supply of nutritional Ca²⁺, Ca²⁺ is substituted as well.

Physical activity and sunlight

Weight bearing physical activity (this includes, e. g., walking, jogging, dancing, but not swimming) markedly counters progression of osteoporosis, not to speak of numerous additional beneficial effects on metabolism and mind. Intensity does not have to be high: going for a walk on a regular basis helps to maintain bone mass. As an additional benefit, concomitant UV exposure stimulates vitamin D synthesis.

Secondary osteoporosis

Apart from its primary or idiopathic occurrence, osteoporosis may be caused by a number of diseases, for example:

- Hypercortisolism
- Hyperparathyroidism
- Hyperthyreosis
- Anorexia
- some forms of neoplastic disease like multiple myeloma, which in many cases is diagnosed only following a pathologic fracture

3.2 Rickets and osteomalacia

Lack of vitamin D causes rickets in children, osteomalacia in adults. In both cases, organic matrix is produced in sufficient amounts, but is insufficiently mineralized. The difference in symptoms result from the fact that children's bones, which are still growing, are easily deformed if too soft. Consequently, affected children show deformities of the skull, thorax and legs (craniotabes, Harrison's groove caused by the pull of the diaphragm, bow legs) and hyperplastic epiphysial cartilage and costochondral joints (widened wrists and rachitic rosary). Logically, dental enamel is defective. Additional symptoms are caused by low serum calcium (agitation, sweating, muscle weakness, pot belly, constipation, tetany). In adults, osteomalacia causes bone pain and insidious pathological fractures. Rickets and osteomalacia are prevented or treated by supplementation of vitamin D and Ca²⁺. Infants who are breast-fed should be supplemented with vitamin D drops on a regular basis.

3.3 Bone resorption in the context of inflammatory disease

In chronic forms of arthritis, for example in rheumatoid arthritis, activated macrophages and other cells produce IL-1, IL-6, TNF α and prostaglandin E. In surrounding cells like synoviocytes, these induce matrix metalloproteases that break down the organic matrix of cartilage and bone. In addition, the inflammatory cytokines induce osteoclast differentiation in adjacent bone. This process is enhanced by RANKL, which is expressed by several cell types in inflamed tissue, including synoviocytes and T cells. Together, these mechanisms can lead to massive destruction of bone next to inflammatory joints.

The same mechanism is responsible for painful dental necks and ultimate loss of teeth due to periodontitis. Bacteria in plaque at the gingival margin cause chronic low-key inflammation of the gums. Inflammatory cytokines induce osteoclasts which progressively break down the thin layer of bone around the neck of teeth.

3.4 Paget's disease of bone (*osteitis deformans*)

Paget's disease remains an enigmatic disease characterized by focal areas of increased but disorganized bone turnover. Bone in affected areas may be dense, but unable to withstand normal load. In many cases, Paget's disease is only diagnosed by chance on an X-ray for unrelated causes, and remains limited to a single focus in a single bone. Yet, depending on location and intensity, it may cause pain, pathological fractures, scoliosis or neurological symptoms (e. g., hearing loss or radiculopathy) by nerve compression in case of skull or spine involvement. Altogether, it is quite frequent (percentage in the single digits), affecting mainly people in the second halves of their lives. Both genetic and environmental factors are thought to contribute to the disease. In an affected bone area, the process seems to start with hyperactive osteoclasts. Several disease-associated alleles have been identified that may explain this hyperactivity: polymorphisms in RANK, for example, or in Sequestosome 1 (SQSTM1), which is important for RANK-dependent activation of transcription factor NF κ B. In addition, environmental factors such as nutritional deficits in Ca²⁺ and paramyxoviral infections have been discussed. Unfortunately, neither of these hypotheses is able to explain the focal characteristics of Paget's disease, suggesting an important etiological factor remains out of sight. For screening and therapy monitoring, bone-specific alkaline phosphatase is determined. Diagnosis involves conventional radiology and bone scans, including scintigraphy. Bisphosphonates are the mainstay of therapy, combined with supplementation of Ca²⁺ and vitamin D, exercise and sunlight.

3.5 Bone metastasis

Certain types of cancer preferentially [metastasize](#) into bone, including mammary, prostate, lung and thyroid carcinoma. Obviously, conditions in the bone promote settling of metastasizing tumor cells.

Migrating cells are frequently directed by chemokine gradients. For example, breast cancer cells express CXCR4, the receptor for CXCL12, which is secreted in bone. Another relevant factor is RANKL, which is strongly expressed by osteoblasts. Mammary carcinoma, prostate

carcinoma and melanoma cells frequently express its receptor RANK. RANK, trimerized by RANKL, induces migration and other metastasis-promoting changes in these cells. Injections of OPG strongly inhibited bone metastasis formation in an experimental mouse model. Consequently, this might be another future application of the OPG-mimicking monoclonal antibody denosumab.

Bone tropism may have additional causes. The molecular changes at the root of the malignant tumor may activate a master switch in osteoblast differentiation, e. g., the transcription factor *Runx2*. Tumor cells originating from mammary or prostate tissue may thus secrete osteoblast-specific proteins like osteocalcin and osteonectin. In other words, they assume properties from bone cells, a behavior termed osteomimicry. These changes are likely to facilitate settling in bone.

Once established, metastases may grow osteolytically or osteoblastically (osteosclerotically). This depends on whether signaling by metastasizing cells preferentially activates osteoclasts or osteoblasts. Prostate carcinoma cells frequently express endothelin-1. Endothelin-1 stimulates osteoblasts and inhibits bone resorption, leading to osteoblastic metastases.

It is common for metastases of breast cancer to cause osteolysis. All molecules mentioned as stimulators of osteoclast differentiation (M-CSF, RANKL, TNF α , IL-1, IL-6) have been found overexpressed in some osteolytic metastases. In addition, some tumors such as mammary carcinoma, bronchial carcinoma, melanoma or hematologic neoplasms tend to overexpress PTHrP (parathyroid-hormone-related protein). PTHrP binds to the PTH receptor, causing bone resorption. Systemic action can lead to tumor hypercalcemia, a life-threatening condition for the effect of Ca²⁺ on membrane potential. Local action may contribute to osteolysis, making room for further tumor growth. Osteolysis induces a vicious circle: as a considerable amount of growth factors is embedded in bone matrix, these in turn stimulate tumor cell growth. Metastasizing cells break down bone, and resorption of bone stimulates growth of metastasizing cells.

Metastases sometimes appear years after removal of the primary tumor. Micrometastases of single or very few cells somehow are able to survive for a long time in some backwaters of the body. There are indications that bone marrow may be such an area of retreat. Hematopoietic stem cells at the highest hierarchical level spend most of their lives in a Rip Van Winkle-sleep (a state called dormancy or quiescence). From this G₀ phase, they are only woken up in case of a sudden requirement for a lot of hematopoietic cells. This may be indicated by a sudden surge of G-CSF (incidentally, recombinant G-CSF is indicated to mobilize stem cells in stem cells donors). Following a few rapid divisions, the stem cells sink back into their deep sleep. The rationale for this behavior is to minimize chances for mutations, e. g., by misincorporation. What keeps a very small number of stem cells in dormancy? There are indications that the surrounding cells somehow form a functional stem cell "bedroom", a niche promoting dormancy. These niches seem to be located at the border zone between bone and marrow, the so-called endosteum, and are made up of a specific form of osteoblasts, *spindle-shaped, N-cadherin positive osteoblasts (SNO)*. One hypothesis to explain the observed dormancy of micrometastases holds that single metastatic tumor cells may get trapped in one of these stem cell bedroom niches and be put to sleep. After a long Rip Van Winkle-sleep, they may still awaken years later.
