BONE METABOLISM AND ITS REGULATION. BIOCHEMICAL MARKERS OF BONE METABOLISM

Alexandra Crăciun
Department of Biochemistry,
University of Medicine and Pharmacy „Iuliu Hatieganu", Cluj-Napoca, Romania

Abstract

The author presents the main stages in the osseous metabolism in correlation with the process of bony remodeling, as well as the factors that modulate it. The clinical implications of these processes are approached from the point of view of the biochemical markers utilized in the clinical laboratory and of the main diseases with which the medical practitioner is confronted in this domain.

Rezumat

Reglarea metabolismului osos. Markeri biochimici ai metabolismului osos

Autoarea prezintă etapele-cheie ale metabolismului osos în raport cu procesul remodelării osoase, precum şi factorii care îl modulează. Implicaţiile clinice ale acestor procese sunt private din perspective markerilor biochimici utilizaţi în laboratorul clinic şi a principalelor boli cu care se confruntă la acest capitol al patologiei medicul practician.

BONE METABOLISM

99% of calcium is stored in the skeleton, in hydroxyapatite (Ca10(PO4)6 (OH)2). The mineral part is closely connected to the organic matrix, represented by collagen type I and osteocalcin.

There are two different types of bone – cortical bone and trabecular bone. The cortical bone is denser and more calcified than the trabecular bone and is found on the outside of bones and along the length of the long bones. The trabecular bone is spongier in appearance and is primarily located at the ends of the long bones and in the spine. The trabecular bone has a higher turnover rate of 9% in comparison to the cortical bone, which has an annual turnover of 2%. Therefore, bones with high amounts of trabecular bone will be more susceptible to fracture in pathological states associated with increased resorption. In figure 1 the structure of a healthy bone and a decreased bone density in osteoporosis may be observed.

The skeleton is an active tissue and throughout its life two processes are of importance in the formation and maintenance of the bone structure: bone modeling and bone remodeling.

Bone modeling is the process that defines the size and the shape of the bone; this process occurs in all growing vertebrates and ends in adulthood. Bone remodeling is essential in the maintenance of normal bone structure and this process occurs throughout one's life. Two types of bone cells are involved in bone metabolism – osteoblasts and osteoclasts. Osteoblasts (bone-forming cells) work at bone surfaces where they secrete osteoid (unmineralised collagen), modulate the crystallization of hydroxyapatite and influence the activity of osteoclasts.

Osteoclasts (bone-resorbing cells) are multinucleated cells, differentiated from haematopoetic stem cells precursors, responsible for bone resorption. Osteoclasts have receptors for vitamin D, calcitonin and nuclear receptors for estrogens, while receptors for PTH are found only in osteoblasts (1). PTH is supposed to act on osteoclasts indirectly, via the osteoblasts that produce a soluble factor with affinity for the osteoclasts. In this context, increases in PTH will raise bone formation as well as bone resorption, equally, by increasing the rate of bone turnover.

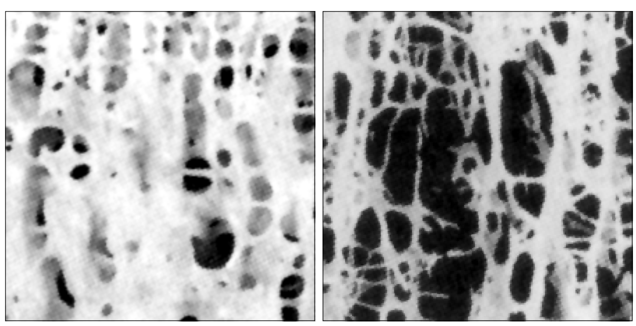

Figure 1
Bone structure in the healthy bone (left) and in the osteoporotic bone (right)

A bone remodeling cycle is a coordinated and sequential action initiated by osteoclasts. The process of bone resorption followed by an equal amount of formation has been termed coupling (Frost, 1964), and one of the most intriguing issues of bone cell biology is to learn how osteoblasts „know“ to replace the exact amount of bone which has been resorbed. This may be due to some local chemical signals. Figure 2 presents the bone remodeling cycle formed by bone resorption and formation stages.

THE BONE TURNOVER RATE IS INFLUENCED BY SEVERAL FACTORS

The bone turnover represents the rate at which the existing bone is replaced by an equal amount of newly formed bone. Formation and resorption are closely coupled processes that are in balance in healthy people. There are many factors capable of influencing the bone formation and resorption balance. Among these factors calcium and phosphate levels in the extracellular fluid, hormones and local factors (cytokines) are of significance. In addition to these factors, mechanical stress plays an important role in stimulating osteoblastic activity and formation of the organic matrix.

Young and old subjects have a high bone turnover in comparison with the adult population. This may be related to vitamin D metabolism, calcium absorption and parathyroid function. It seems that the responsiveness of the renal 21-hydroxylase decreases with age to the action of PTH, decreasing in the elderly calcium absorption at the intestinal level (2).

Apart from PTH, other hormones influencing the balance of bone turnover, and for which bone cells present receptors, are calcitonin (3, 4) and the sex hormones (estrogens and testosterone) (5, 6). Estrogens appear to stimulate bone formation, but this is rather indirectly, due to an inhibitory effect on the osteoclasts (7). Oral contraception is associated with a moderate, but significant decrease in bone turnover, but effective on bone mass only after prolonged use (8).

Apart from the sex hormones, glucocorticoids act as direct inhibitors on the proliferation and function of osteoblasts. Glucocorticoids directly inhibit vitamin D-mediated intestinal calcium absorption, bone mineral mobilization and enhance the sensitivity of bone cells to 1,25(OH)2 D by stabilizing the 1,25(OH)2 D receptor or by increasing the affinity or number of receptors (9). Also local factors may contribute to the modulation of bone metabolism, but their role needs further investigation.

Cytokines which stimulate osteoblasts are: tissular growth factor (TGF β), gamma interferon (IFN γ), while other factors such as TGF α, interleukin-1 (IL-1) activate osteoclasts, a process which is evident in some bone tumours by „malignant hypercalcemias“ (10).

Overweight (11) and regular sport (12) are beneficial for bone structure, while low weight and physical stress such as competitive sports 150 may influence bone turnover, inducing a change in the bone turnover rate. The mechanisms originate at various levels of the brain-hypothalamic-pituitary-gonadal axis (12).

The quality of bone and the peak bone mass are closely related to nutritional aspects, such as calcium intake (13), phosphate (14), vitamin D (15), vitamin K status (16), vitamin C, magnesium, to which risk factors including inheritance, parity, body weight, alcohol, tobacco and caffeine consumption are added.

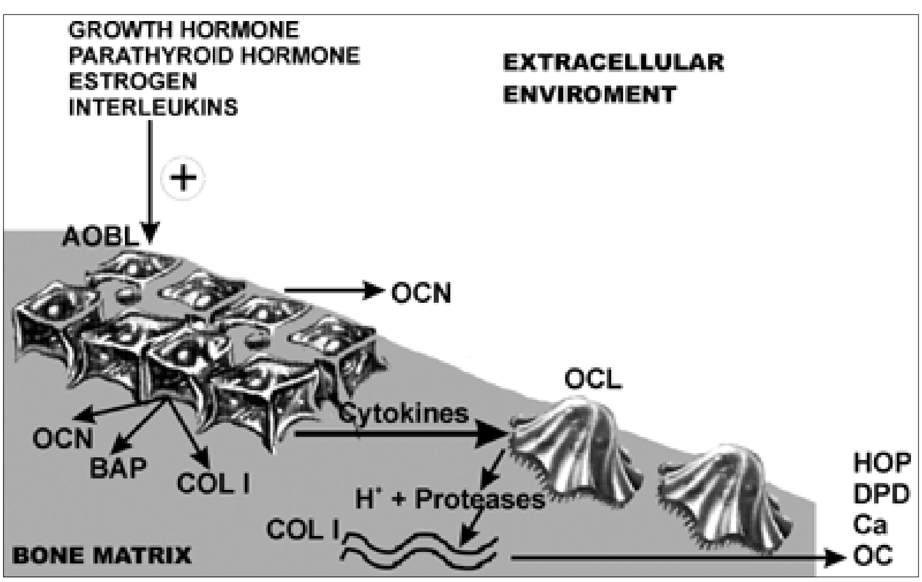

Figure 2
The remodeling cycle starts with the activation of osteoclasts by the growth hormone, parathyroid hormone (PTH), interleukins (IL-1, IL-6, and IL-11), and estrogen. The network of cytokines and growth factors is essential for the regulation of both osteoclastogenesis and osteoblast formation: these factors play a pivotal role in the paracrine regulatory control of bone turnover under physiological conditions. (AOBL = Activated Osteoblast; OCN = Osteocalcin "de novo"; BAP = Bone Alkaline Phosphatase; COL I = Collagen type I; OCL = Osteoclast; HOP = Hydroxyproline; DPD = Deoxypyridinoline; OC = Osteocalcin; Ca = Calcium)

BIOLOGICAL MARKERS FOR BONE METABOLISM

Bone formation and resorption markers are useful tools especially when they allow the clinician to predict the risk of a fracture or to evaluate a therapeutic effect.

Markers of bone formation reflect the activity of osteoblasts. One of the markers we measure is osteocalcin, a protein of which synthesis may be induced by vitamin D, while its function is vitamin K dependent. When the supply of vitamin K to bone is low, the osteocalcin molecules have fewer carboxyl Ted side-chains, resulting in a reduced ability to bind to calcium.

We measure carboxyl Ted and undercarboxylated osteocalcin because the degree of carboxylation is a useful index of vitamin K status, and a high ratio of undercarboxylated osteocalcin to carboxylated osteocalcin is a risk factor for osteoporosis and fracture (16). Other markers for bone formation are measuring newly formed collagen P1NP (N-propeptide of type I collagen) and of bone specific alkaline phosphatase. Each of them may reflect different phases of bone formation – proliferation and maturation of bone matrix (figure 3).

Bone resorption markers describe osteoclast activity and collagen breakdown. Breakdown products of collagen, which we measure, include deoxypyridinoline and pyridinoline and their associated peptides (NTX and CTX).

These two determinations in serum have been shown to better reflect bone resorption than hydroxyproline and calcium excretion assays, which in spite of their long-time use as the most frequently performed tests for the measurement of bone resorption, present the inconvenience of dietary interferences. Tartrate-resistant acid phosphatase (TRAP) also reflects osteoclast activity, but it is not used frequently because it is difficult to measure due to its instability.

Also, it seems that the bone turnover markers may vary substantially from country to country, suggesting systematic geographic and genetic differences that may potentially relate to osteoporotic fracture rates (17).

MAJOR DISEASES ASSOCIATED WITH BONE RESORPTION AND INFLAMMATION

High bone turnover is found in Paget disease, which affects only some parts of the skeleton, characterized by increased bone turnover. Bone metastases, especially those that disseminate on the osteoblast cells are associated with high bone turnover (breast, lung and kidney cancers). These changes in bone turnover are explained by increased PTH related peptide that appears in malignancy, acting as a hormone responsible for the malignant hypercalcemia (18).

Inflammatory bone loss appears in chronic rheumatoid diseases (CRD). The mediators of inflammation (cytokines) and oxygen radicals are involved in resorption of bone.

In rheumatoid arthritis (RA), an exemplary disease among CRD, osteoporosis was studied the most. These patients present, apart from a generalized osteoporosis, mostly caused by lack of movement, corticotherapy and methotrexate, a localized form of osteoporosis at juxta-articulation level, in relation to local inflammation, for example at the level of the hands. Recent studies speak about an "inflammatory osteoporosis", in which the role of the system osteoprotegerine (OPG) – RNAK – RANKL, which is under the inflammatory cytokines, is essential. Systemic lupus erithematosus (SLE) and ankylosant spondylitis (AS)

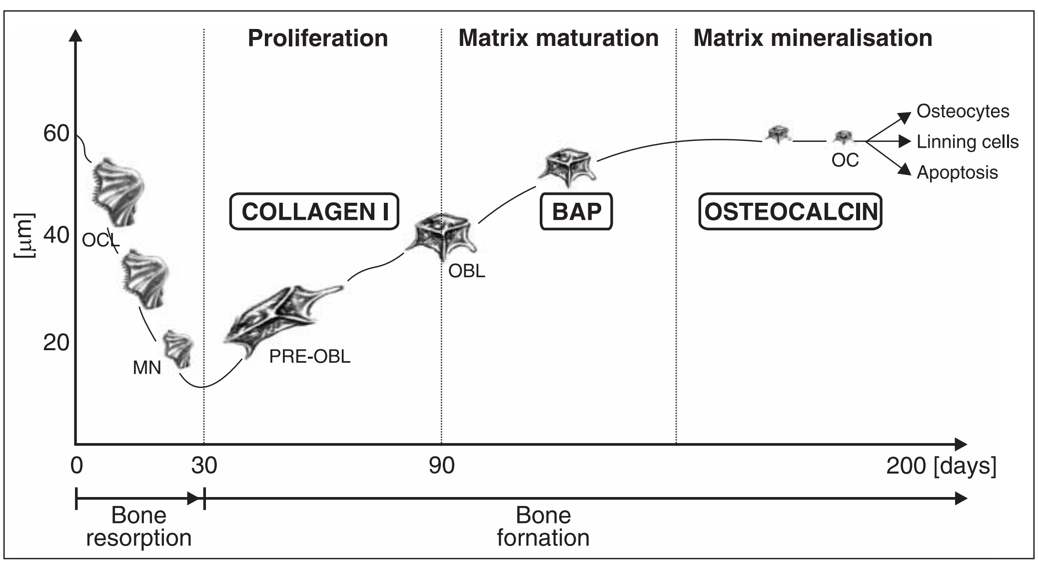

Figure 3
Total sequence of remodeling. The main stages of bone formation, reflecting the osteoblast proliferation and differentiation. OCL = Osteoclast; MN = mononucleated cells; PRE-OBL = Pre-Osteoblast; OBL = Osteoblast; OC = Osteocyte)

are two examples of chronic inflammatory diseases with increased risk for osteoporosis. Therapies with glucocorticosteroids accelerate osteoporosis, but some recent studies suggest that this risk exists independently regarding therapy with glucocorticosteroids. The mechanism of bone loss is different from that in rheumatoid arthritis, the cause being connected to physical inactivity, myopathy, renal and endocrine dysfunction.

Treatment with immunomodulators and non steroids anti-inflammatory drugs contributes as well to the development of osteoporosis.

From the point of view of laboratory markers, IL-1, IL-2, TNFa are increased, inducing osteoclastogenesis through osteoclast precursor's recruitment, proliferation, and activation of differentiated osteoclasts. In RA the pathogenesis is centralized on the granulous inflammation of the synovial membrane, which develops an invasive form, leading to erosions of the cartilage and juxta-cartilage bone. The inflammation process will extend to intra-articulation region and to structures around articulation, leading to dysfunctions and deformed articulation. Inflammatory cytokines IL-1, IL-6, TNFα, initiate a chain of reactions including vasculitis, interstitial oedema, and perivascular infiltration with mononuclear cells. If the process continues, the phenomenon will generalize, the stroma will be invaded by the mononuclear cells, producing cytokines, maintaining and continuing the process. Increased cytokines levels are correlated with disease activity. It is known that the main inflammatory cytokines IL-1 and TNFα are capable of inducing a series of pathogenetic processes including stimulation of metalloproteinases, prostaglandins, formation of oxidants (NO), inducing the expression of genes for adhesion of cellular molecules, increase of destruction and decrease of cartilage formation. The same mediators are responsible for the recruitment and activation of osteoclasts, leading to articular erosions, which are the morphological base of joint destruction as a functional structure.

In SLE the systemic inflammation is produced by lymphokines produced by B lymphocytes, leading to a systemic inflammation, contributing to the development of osteoporosis. Also T lymphocytes, fibroblasts, osteoblasts and mononuclear cells produce cytokines contributing to systemic inflammation, explaining the relation between the activity of the bone and osteopenia. Estrogen and testosterone deficiency in patients with SLE accelerates the development of osteoporosis, these hormones inhibiting, normally, the bone resorption activity through osteoclast's apoptosis (19).

Glucocorticosteroids used as therapeutic agents have the following effects on bone: inducing osteoblast's and osteocyte's apoptosis, suppressing the expression of mRNA of osteoprotegerine (OPG), stimulating the production of OPG ligand; decreasing the calcium intestinal and renal absorption. These effects appear at low amounts as well. If, to the effects of the corticosteroids, are added the effects of chronic anticoagulant therapy (heparins or coumarone) in the antiphospholipidic syndrome (associated frequently with SLE), this will contribute considerably to bone loss. Clinical trials (crossectional studies) reveal that bone loss in SLE is due mainly to the treatment and disease evolution. There are no studies in which bone markers, cytokines and bone mineral density are evaluated, most studies analyzing the therapeutic effects on bone mineral density, especially in SLE (20).

Osteoporosis affects 1 in 3 women at post-menopausal ages and 1 in 12 men in Caucasian populations of northern Europe, the USA and Australasia. In other populations, such as those in Africa and China, the incidence is much lower. However, independent of the geographical region or race, hip fracture incidence is increasing in parallel with the increase in life expectancy. The essential features are bone loss, particularly due to lack of estrogen.

REFERENCES

1. **McSheehy PM, Chambers TJ** – Osteoblastic cells mediate osteoclastic responsiveness to parathyroid hormone. *Endocrinology*, 1986, 118: 824-828.
2. **Rodan GA, Martin TJ** – Role of osteoblasts in hormonal control of bone resorption – a hypothesis. *Calcif Tissue Int*, 1981, 33: 349-351.
3. **Holtrop ME, Raisz LG, Simmons HA** – The effects of parathyroid hormone, colchicine, and calcitonin on the ultrastructure and the activity of osteoclasts in organ culture. *J Cell Biol*, 1974, 60: 346-355.
4. **Rodriguez M, Felsenfeld AJ, Torres A, Pederson L, Llach F** – Calcitonin, an important factor in the calcemic response to parathyroid hormone in the rat. Kidney Int, 1991, 40: 219-225.
5. **Oursler MJ, Landers JP, Riggs BL, Spelsberg TC** – Oestrogen effects on osteoblasts and osteoclasts. *Ann Med*, 1993, 25(4): 361-371.
6. **Gay CV, Kief NL, Bekker PJ** – Effect of estrogen on acidification in osteoclasts. Biochem Biophys Res Commun, 1993, 192(3): 1251-1259.
7. **Liel Y, Kraus S, Levy J, Shany S** – Evidence that estrogens modulate activity and increase the number of 1,25 dihydroxyvitamin D receprors in osteoblastlike cells. *Endocrinology*, 1992, 130 (5): 2597-2601.
8. **Garnero P, Sornay-Rendu E, Delmas D** – Decreased bone turnover in oral contraceptive users. *Bone*, 1995, 16: 499-503
9. **Reid IR, Veale A, France JT** – Glucocorticoid osteoporosis. *Journal of Asthma*, 1994, 31(1): 7-18.

10. **Mundy GR** – Calcium homeostasis, hypercalcemia and hypocalcemia. Martin Dunitz, London 1989, 64-91.
11. **Gossain VV, Rao DS, Carella MJ, Divine G, Rovner DR** – Bone mineral density (BMD) in obesity effect of weight loss. *J Med*, 1999, 30(5-6): 367-376.
12. **Bennell KL, Malcom SA, Brukner PD, Green RM, Hopper JL, Wark JD, Ebeling PR** – A 12-month prospective study of the relationship between stress fractures and bone turnover in athletes. *Calcif Tissue Int*, 1998, 63: 80-85.
13. **Lau EMC – Osteoporosis in Asians** – The role of calcium and other nutrients. In: Nutritional aspects of osteoporosis '94, Editors: Burkhardt P, Heaney RP. *Ares-Serono Symposia Publications*, 1994, 7:45-54.
14. **Faisant C, Lauque S, Stebenet Rouillon M, Vellas B, Albarcde JL** – Calcium and Phosphorus Intake in different groups of elderly persons with good or poor nutritional status. In: Nutritional aspects of osteoporosis '94, Editors: Burkhardt P, Heaney RP. *Ares-Serono Symposia Publications,* 1994, 7: 209-212.
15. **Lukert B, Higgins J, Stoskopf M** – Menopausal bone loss is partially regulated by dietary intake of vitamin D. *Calcif Tissue Int*, 1992, 51(3): 173-179.
16. **Feskanich D, Weber P, Willet WC, Rockett H, Booth S, Colditz GA** – Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr*, 1999, 69: 74-79.
17. **Anderson JJB, Tylavski FA, Lacey JM, Adeleke VM** – Ethnicity, Nutrition, and Bone Mass, Challenges of Modern Medicine, Nutritional aspects of osteoporosis '94, Editors: Burkhardt P, Heaney RP. *Ares-Serono Symposia Publications,* 1994, 7: 27-44.
18. **Correale P, Micheli L, Del Vecchio MT, Sabatino M, Petriol R and Francini G** – A parathyroid-hormone-related-protein (PTH-rP)-specific cytotoxic T cell response induced by in vitro stimulation of tumourinfiltrating lymphocytes derived from prostate cancer metastases, with epitope peptide-loaded autologous dendritic cells and low-dose IL-2. *British Journal of Cancer*, 2001, 85, 1722-1730.
19. **Lee C, Chadha AB, Dunlop D et al** – Presentation: Cumulative disease damage is associated with lower bone mineral density: An analysis of women with SLE for the Chicago Lupus Database ever and never receiving corticosteroid therapy. New York, NY: 7th International Congress on Systemic Lupus Erythematosus and Related Conditions: Long-term outcome: bone May 9-13, 2004, Abstract 40B.
20. **Nancy E Lane** – Therapy Insight: Osteoporosis and Osteonecrosis in Systemic Lupus Erythematosus. *Nat Clin Pract Rheumatol*, 2006, 2(10): 562-569

În actualitate

Metotrexat pentru pseudogută

Metotrexatul nu este numai un medicament imunodepresor, ci se recomandă, de asemenea, ca unul cu efect antiinflamator. În două spitale universitare din Elveţia, cinci cazuri severe de condrocalcinoză articulară cu atacuri severe de pseudogută au fost tratate cu metotrexat în doză de 5-20 mg/săptămână. Criteriile de apreciere au fost frecvenţa atacurilor, intensitatea durerii, numărul articulaţiilor sensibile şi tumefiate şi evoluţai indicatorilor biologici de inflamaţie. După, în medie, 50,4 luni (intervalul 6-81 luni), toţi pacienţii au răspuns excelent, iar efectul favorabil s-a instalat la, în medie, 7,4 săptămâni. Durerea simţită s-a redus semnificativ ($p < 0,0001$) faţă de valorile iniţiale. Metotrexatul a fost bine tolerat şi astfel se poate situa printre indicaţiile terapeutice în formele severe de pseudogută.

Sursa: Chollett-Sanin A, Finckh A, Dudle J, Guerne PA, Methotrexate as an alternative therapy for chronic calcium pyrophosphate deposition disease, Arthr Rheum 2007, 56: 688-692