Overview of Biochemical Markers of Bone Metabolism

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Abstract

The bone has the function of supporting the body; the bone is a tissue characterized by its rigidity, hardness, and power of regeneration and repair. The bone has several functions including protection of the vital organs, environment for marrow, mineral reservoir for calcium homeostasis, reservoir of growth factors and cytokines, and taking part in acid–base balance. Bone metabolism is a dynamic and continuous remodeling process that is normally maintained in a tightly coupled balance between resorption of old or injured bone and formation of new bone. Several hormones and factors are involved in bone metabolism, which regulation depends from the complex interaction among them. Considering the various phases of the bone cycle, markers of bone metabolism may be classified either as markers of bone formation, markers of bone resorption, and markers of bone metabolism regulation. The aim of this chapter will be to examine biochemical markers in bone metabolism in order to give readers a guide about the normal physiological process to better understand the mechanisms underlying bone diseases.

Keywords

Biomarkers • Bone diseases • Bone metabolism • Calcium • Regulation

List of Abbreviations

D-Pyr	Deoxypyridinoline
FGF	Fibroblast growth factors
IGF	Insulin-like growth factors
IL-1	Interleukin-1
IL-6	Interleukin-6

PG	Prostaglandins
PTH	Parathyroid hormone
Pyr	Pyridinoline
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α

Key Facts of Bone Metabolism

- The bone has several functions including protection of the vital organs, environment for marrow, mineral reservoir for calcium homeostasis, reservoir of growth factors and cytokines, and role in acid–base balance.
- Bone metabolism is a dynamic and continuous remodeling process that is normally maintained in a tightly coupled balance between resorption of old or injured bone and formation of new bone.
- Biomarkers relevant to bone metabolism include markers of bone formation, markers of bone resorption, and markers of bone metabolism regulation.
- The knowledge of biomarkers linked to bone metabolism is very useful to promptly identify bone abnormalities and to guide physicians in the right direction to better understand the mechanisms underlying bone diseases.

Definition of Word and Terms

Biomarker	The term refers to a measurable indicator of some biological state or condition that can be used for diagnosis or follow-up a particular disease.
Cytokines	The term refers to proteins released by cells with specific effect on the interactions among cells. Cytokines include interleukins, lymphokines, and cell signal molecules.
Osteoblast	The term refers to a cell producing the organic part of the bone matrix, an array of proteins collectively called "osteoid."
Osteoclast	The term refers to a cell with the function of resorption of existing bone and active early in the bone remodeling cycle.
Transforming growth factor type-β	The term refers to molecules implicated in the regulation of a variety of cellular events involved in the regulation of bone growth and turnover.

Introduction

The bone has the function of supporting the body; the bone is a tissue characterized by its rigidity, hardness, and power of regeneration and repair. The bone has several functions (Taichman 2005) including:

- Protection of the vital organs
- Environment for marrow (both blood forming and fat storage)
- Mineral reservoir for calcium homeostasis
- Reservoir of growth factors and cytokines
- Part in acid–base balance

Bone metabolism is a dynamic and continuous remodeling process that is normally maintained in a tightly coupled balance between resorption of old or injured bone and formation of new bone. On a microscopic level, bone metabolism always occurs on the surface of the bone at focused sites, called "bone metabolism unit." Global bone metabolism represents the cumulative behavior of many bone metabolism units such that defects in the organization of bone formation or any imbalance to the side of bone resorption can result in substantial changes in functional integrity over time. Changes can occur rapidly when the rate of turnover is increased. The bone has two components, the cortical and the trabecular bone. Cortical bone is dense and solid and surrounds the marrow space; on the other hand, the trabecular bone is composed of a network of trabecular plates and rods interspersed in the bone marrow compartment. The bone is composed of support cells, including osteoblasts and osteocytes, remodeling cells including osteoclasts, non-mineral matrix of collagen, and noncollagenous proteins called osteoid, with inorganic mineral salts deposited within the matrix (Fig. 1). The main characters in bone metabolism are osteoclasts and osteoblasts; they carry out bone metabolism at the fundamental bone metabolism unit site. Osteoblasts are involved in bone formation and differentiation from stromal marrow cells, in particular from precursor blood cells, and histologically have one nucleus and an extensive network of rough endoplasmic reticulum, responsible for synthesis of bone matrix proteins. Osteoblasts produce the

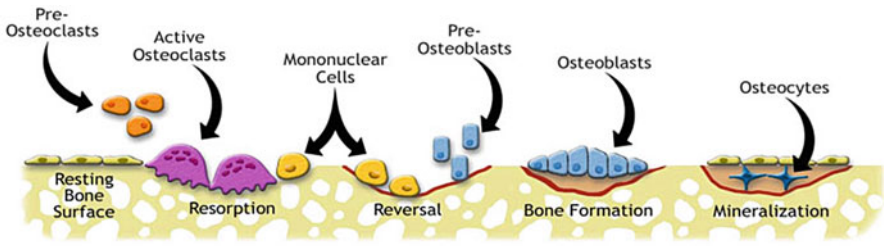

Fig. 1 Bone remodeling cycle

organic part of the bone matrix, an array of proteins collectively called "osteoid." Osteoid includes:

- **Collagen type I** which represents the bulk of osteoid. It consists of triple helix units containing two α1 chains and one α2 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 forming long fibrils that are subsequently covalently cross-linked via their hydroxylated lysines.
- **Osteocalcin** is a small protein that is carboxylated on glutamic acid residues with the help of vitamin K. Osteocalcin concentrated calcium in the bone, attracting Ca++ with the double-negative charges of glutamic acid residue after carboxylation. Osteocalcin, acting together with integrin-binding sialoprotein, nucleates crystals with phosphate ions to form hydroxyapatite $Ca_5(PO_4)_3(OH)$. Transcription of the gene of osteocalcin is induced by activated vitamin D. Osteocalcin itself, in non-carboxylated form, enters the bloodstream and enhances insulin activity. It stimulates proliferation of pancreatic β-cells and sensitizes fat cells to insulin by stimulating them to secrete adiponectin.
- **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.

On the other hand, osteoclasts are giant, multinucleated cells that derive from hematopoietic stem cells in the bone marrow, from the lineage leading to macrophages and neutrophils. A series of cytokines induces precursor cells to differentiate to osteoclasts, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and prostaglandin E. Osteoclasts reabsorb existing bone and are active early in the bone remodeling cycle. Osteoclasts use lysosomal chemistry, in addition to acidification and activation of acid hydrolases. Osteoclasts seal off a matrix area, which they acidify to dissolve hydroxyapatite, setting free Ca^{2+}. After the mineral has melted away, acid proteases like cathepsin K hydrolyze the remaining matrix proteins.

Several hormones and factors are involved in bone metabolism, which regulation depends from the complex interactions among them. Considering the various phases of the bone cycle, markers of bone metabolism may be classified either as:

- **Markers of bone formation**: osteoblasts, alkaline phosphatase, procollagen I extension peptides, and osteocalcin
- **Markers of bone resorption**: osteoclasts, urinary calcium, acid phosphatase, hydroxyproline, N-telopeptide, C-telopeptide, and pyridinoline (Pyr) and deoxypyridinoline (D-Pyr) cross-links
- **Markers of bone metabolism regulation**: parathyroid hormone (PTH) and calcitonin, thyroid hormones, estrogens, testosterone, vitamin D, cortisol, insulin, fibroblast growth factors (FGFs), insulin-like growth factors (IGFs, types I and II),

transforming growth factors (TGFs-β1 and TGFs-β2), prostaglandins, and interleukins.

Markers can be used in both generalized disorders of bone remodeling, such as osteoporosis or osteogenesis imperfecta, or in localized disorders of bone turnover, such as Paget's disease and cancer metastases. At this regard, the aim of this chapter will be to examine biochemical markers in bone metabolism in order to give readers a guide about the normal physiological process to better understand the mechanisms underlying bone diseases.

Markers of Bone Formation

Alkaline Phosphatase

Alkaline phosphatase (ALP) is present in mucosal epithelia of small intestine, proximal convoluted tubule of the kidney, bone, liver, and placenta. It is involved in lipid transportation in the intestine and calcification in the bone. Bone alkaline phosphatase (BAP) is the bone-specific isoform of alkaline phosphatase, a glycoprotein found on the surface of osteoblasts. Bone alkaline phosphatase is synthesized by the osteoblasts and is presumed to be involved in the calcification of bone matrix, though its precise role in the formation process is unknown; what is certain is that BAP reflects the biosynthetic activity of these bone-forming cells. Normal value of BAP ranges $\leq$20 mcg/L in males and $\leq$14 mcg/L in premenopausal women and $\leq$22 mcg/L in postmenopausal women (Nicoll 2007). Bone alkaline phosphatase has been shown to be a sensitive and reliable indicator of bone metabolism (Kress 1998). The highest amount of serum ALP activity is observed in Paget's disease, a metabolic bone disease caused by excessive rates of bone remodeling, resulting in local lesions of abnormal bone matrix. These lesions can result in fractures or neurological involvement. Under this condition, ALP activity is almost 10–25 times higher than the normal limit. The moderate increase in ALP activity is observed in osteomalacia, which is slowly decreased toward normal ranges in response to vitamin D therapy. The ALP activity rate is generally normal in osteoporosis, while in rickets disease, a 2–4 times increase is seen in the enzyme activity, which gradually moves toward the normal range following vitamin D therapy. Very high levels of ALP enzyme activity can be also found in patients with bone metastatic carcinoma and osteogenic sarcoma, while a transitory increase in the enzyme activity may be observed during the healing of bone fractures (Kubo et al. 2012; Hatayama et al. 2012; Corathers 2006; Simko 1991; Ross and Knowlton 1998).

Procollagen I Extension Peptides

Collagen is the major structural protein of the bone and comprises about 90% of the organic material. Collagen plays a central role in the integrity and strength of bone

matrix, and defects in its production lead to bone of poor quality, susceptible to fracture. Type 1 collagen is synthesized by osteoblasts as a larger precursor protein termed procollagen I (Risteli and Risteli 1993). The carboxy-terminal and amino-terminal ends of procollagen I are enzymatically "clipped" during extracellular processing and fibril formation prior to incorporation of type 1 collagen into the bone matrix. The amino-terminal ("N-terminal") and carboxy-terminal ("C-terminal") ends of the procollagen are released in the blood and can be quantified. Values of procollagen I extension peptides are increased during growth and in situations of increased bone formation, such as in Paget's disease, and in response to growth hormone.

Osteocalcin

Serum osteocalcin has been found to correlate with bone formation. Osteocalcin is a relatively small protein produced by osteoblasts during the matrix mineralization phase (Fottrell and Power 1991). Osteocalcin is both released into circulation and incorporated into the bone matrix, where it is the most abundant noncollagenous protein (Fottrell and Power 1991). In common with many factors involved in the coagulation cascade, osteocalcin synthesis is dependent on vitamin K, which posttranslationally modifies the gene product with gamma-carboxyglutamate (Gla) residues; due to this modification, osteocalcin is also known as bone γ-carboxyglutamate protein. The γ-carboxylated form binds hydroxyapatite and is abundant in bone extracellular matrix, while the undercarboxylated circulating form has been implicated as a novel hormone and positive regulator of glucose homeostasis. It is secreted into circulation and, in individuals having normal renal function, excreted in urine due to its low molecular weight (Kapustin and Shanahan 2011). Normal reference ranges for osteocalcin are 9–42 ng/mL for subjects of 18 years or older (Delmas et al. 2000). Elevated levels of osteocalcin can be found in conditions associated with increased bone formation, as happened in hyperparathyroidism, hyperthyroidism, and bone metastases. Reduced levels of osteocalcin can be found in condition with lower rates of bone formation, as seen in myeloma, or in patients taking glucocorticosteroids, or antiresorptive agents (bisphosphonates or hormone-replacement therapy), usually within 3–6 months after therapy begins. Decreasing osteocalcin levels indicate effective response to treatment. Within 3–6 months after surgical cure, osteocalcin levels in patients with primary hyperparathyroidism should return to the reference range (Harris et al. 2001).

Markers of Bone Resorption

Urinary Calcium

Calcium salts provide rigidity to the skeleton and calcium ions play a fundamental role. In the vertebrate skeleton, calcium is provided by a form of calcium phosphate, which approximates hydroxyapatite [$Ca_{10}(OH)_2(PO_4)_6$] and is embedded in

collagen fibrils. The average adult store of calcium is approximately 1–2 kg. The vast majority (99%) resides in the skeleton. Only a fraction of the stored calcium is present in extracellular fluid and available for the use in the form of ionized calcium. Ionized calcium is tightly regulated by PTH. Adult calcium plasma concentrations are normally between 8.5 and 10.5 mg/dL (2.2–2.6 mmol/L). Most of this circulating calcium is bound to albumin. Because of this, changes in serum protein concentrations can affect total blood calcium concentrations. Calcium enters the extracellular fluid through absorption from the gut and resorption from the bone. It is removed through secretion into the gastrointestinal tract and urine as well as losses in sweat and deposition in the bone. Urine calcium levels will reflect dietary intake. Normal values are collected on urine sample over 24 h, average between 100 and 250 mg of calcium (15–20 mmol). Moreover, calcium excretion is influenced by sodium excretion; patients following low-sodium diets tend to have a lower calcium excretion. High levels of urine calcium (>300 mg/24 h) are often a sign of an overactive parathyroid gland. Parathyroid hormone is produced in response to serum calcium levels: when serum calcium levels are low, parathyroid calcium-sensing receptors stimulate the PTH release. Parathyroid hormone works to increase serum calcium levels, increasing renal tubule resorption of calcium and simultaneously decreasing phosphorus resorption. Parathyroid hormone also causes resorption of calcium from the bone and increases synthesis of 1,25-dihydroxy vitamin D, which stimulates calcium absorption from the gut (Foley 2010). For the reasons reported above, hyperparathyroidism results in excessive uptake and increased concentrations of calcium in serum, leading to hypercalcemia, hypercalciuria, and hyperphosphaturia. Thus, urine calcium levels are often increased in the setting of hyperparathyroidism.

Acid Phosphatase

Acid phosphatase is an enzyme stored in lysosomes and localized in different organs. In particular, there are five isoenzymes of acid phosphatase in blood, localized in the bone, prostate, platelets, erythrocytes, and spleen (Moss and Henderson 1994). The bone isoenzyme is derived from osteoclasts, where it is present in high concentration and excreted into the microenvironment between the membrane sealing zone and the bone matrix. Acid phosphatase is a potent enzyme that plays an important role in the bone resorption process. It is released into circulation by "leakage," during resorption and after detachment of the osteoclast's sealing zone. Due to its molecular size, assays for acid phosphatase are serum or plasma based. Normal values of acid phosphatase range between 0 and 0.8 U/L. Abnormal levels of acid phosphatase in the blood may indicate Paget disease, hyperparathyroidism, and multiple myeloma.

Hydroxyproline

Proline and its metabolite hydroxyproline are unique amino acids, both chemically and biochemically (Hu et al. 2008; Kaul et al. 2008). Hydroxyproline is a modified

amino acid produced from the posttranslational hydroxylation of integral proline residues of type 1 collagen. It constitutes one-third of amino acids in the collagen proteins and has an essential role in collagen stability (Nelson and Cox 2005). It is also a major extracellular component in connective tissues (skin, tendon, cartilage, vessels of the vascular system, and bone). After collagen breakdown, hydroxyproline is not reutilized; 90% is degraded to the free amino acid form and passes through the glomerulus; moreover, hydroxyproline is almost completely resorbed and catabolized in the liver to urea and carbon dioxide. The remaining 10% of hydroxyproline is released in small polypeptide chains that pass through the glomerulus and are excreted in urine (Kivrikko 1983).

Testing hydroxyproline in the serum and in the urine is common. The reference range of hydroxyproline is different according to different age (Laitinen et al. 1966), in particular:

- Total hydroxyproline in the urine among subjects aged 18–21 years is 13–28 mg/24/m^2
- Total hydroxyproline in the urine among subjects aged 22–55 years is 8.5–23.5 mg/24/m^2
- Free hydroxyproline in the serum of males ranges between 0.7 and 1.55 μg/mL
- Free hydroxyproline in the serum of females ranges between 0.7 and 1.40 μg/mL

Elevated hydroxyproline levels can be found in Paget disease, hyperparathyroidism, osteomyelitis, hyperthyroidism, and skeletal metastases. However, also other diseases can give an increase of this marker, including rheumatoid arthritis, polyarteritis nodosa, Marfan syndrome, acromegaly, Turner syndrome, and pregnancy.

N-Telopeptide and C-Telopeptide

During bone resorption, amino- and carboxy-terminal fragments of collagen are released with cross-links attached. These fragments with attached cross-links are called telopeptides. N-Telopeptides and C-telopeptides are excreted in the urine. N-Telopeptides are measured by immunoassay using an antibody to the α2 chain of the N-telopeptide fragment. C-Telopeptides are measured by immunoassay (Watts 1999).

Pyridinoline (Pyr) and Deoxypyridinoline (D-Pyr) Cross-Links

Posttranslational modification of lysine and hydroxylysine produces the nonreducible pyridinium cross-links, pyridinoline (Pyr) and deoxypyridinoline (D-Pyr), that stabilize mature collagen. Both Pyr and D-Pyr are released from the bone in a ratio of approximately 3:1. Deoxypyridinoline is specific for the bone, while Pyr is also found in articular cartilage and in soft tissues such as ligaments and

tendons. Almost 60% of the cross-links released during resorption are bound to protein, with the remaining 40% being free. Pyridinium cross-links are not metabolized, and they can be measured in urine by HPLC or immunoassay either before or after hydrolysis.

Markers of Bone Metabolism Regulation

Parathyroid Hormone and Calcitonin

As already described above, PTH plays an important role in bone metabolism, regulating calcium levels in blood. When serum calcium levels are low, parathyroid calcium-sensing receptors stimulate the PTH release (Fig. 2). Parathyroid hormone works to increase serum calcium levels, increasing renal tubule resorption of calcium and simultaneously decreasing phosphorus resorption. Parathyroid hormone also causes resorption of calcium from the bone and increases synthesis of 1,25-dihydroxy vitamin D, which stimulates calcium absorption from the gut (Foley 2010). Parathyroid hormone is antagonized by calcitonin, a hormone produced by

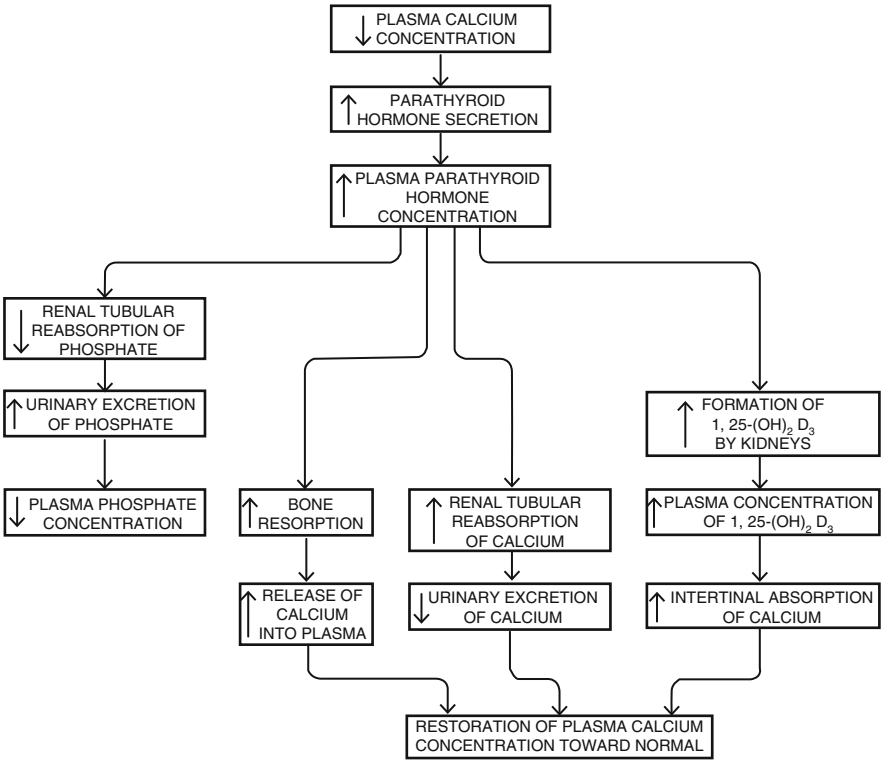

Fig. 2 Parathyroid, calcium, and phosphate metabolism

Table 1 Calcium and phosphate regulation

	Calcium deprivation	Calcium loading
Parathyroid hormone	Secretion stimulated	Secretion inhibited
Vitamin D	Production stimulated by increased parathyroid hormone secretion	Synthesis suppressed due to low parathyroid hormone secretion
Calcitonin	Very low-level secretion	Secretion stimulated by high blood calcium
Intestinal absorption of calcium	Enhanced due to activity of vitamin D on intestinal epithelial cells	Low basal uptake
Release of calcium and phosphate from the bone	Stimulated by increased parathyroid hormone and vitamin D	Decreased due to low parathyroid hormone and vitamin D
Renal excretion of calcium	Decreased due to enhanced tubular resorption stimulated by elevated parathyroid hormone and vitamin D; hypocalcemia also activates calcium sensors in loop of Henle to directly facilitate calcium resorption	Elevated due to decreased parathyroid hormone-stimulated resorption
Renal excretion of phosphate	Strongly stimulated by parathyroid hormone; this phosphaturic activity prevents adverse effects of elevated phosphate from bone resorption	Decreased due to hypoparathyroidism
General response	Typically see near-normal serum concentrations of calcium and phosphate due to compensatory mechanisms. Long-term deprivation leads to bone thinning (osteopenia)	Low intestinal absorption and enhanced renal excretion guard against development of hypercalcemia

C cells of the thyroid gland in response to high serum calcium levels. In order to decrease serum calcium levels, calcitonin prevents calcium loss from bones suppressing the activity of osteoclasts throughout receptors present on the surface of osteoclasts to stop them from breaking down the bone. Calcitonin also prevents the absorption of calcium from the intestine, maintaining normal blood levels of vitamin D. Calcitonin also regulates the level of calcium and other mineral levels in the kidneys. It reduces the kidney's reabsorption of calcium and magnesium, leading to increased calcium excretion via the urine (Stevenson 1982) (Table 1).

Thyroid Hormones

Thyroid diseases have systemic manifestations including effects on bone metabolism. Thyrotoxicosis is an important cause of secondary osteoporosis, while hypothyroidism has only a minimal effect on bone mineral metabolism (Donangelo and Braunstein 2011). Lower bone mass associated with hyperthyroidism may be caused by increased bone turnover as a result of imbalance between bone resorption and

formation. Thyroid hormones have direct catabolic effect on bone mineral homeostasis, leading to increased bone mineral resorption and calcium loss through kidneys. Histomorphometric studies demonstrate that thyroid hormones increase the activation of new remodeling cycles and stimulate osteoclastic and osteoblastic activity in trabecular and cortical bone, with an increase in number and activity of osteoclasts. The mechanisms of thyroid hormone-induced bone resorption include cAMP-mediated increased sensitivity of beta adrenergic receptors to catecholamines, increased sensitivity of bone cells to PTH, osteoclast activator factor, and IL-1-mediated increased bone resorption.

Estrogens

Estrogen actions on bone are complex. The major physiological effect of estrogen is to inhibit bone resorption. Bone cells have two kinds of intracellular steroid receptors for estrogen. When estrogen binds to the receptors, various genes become active. Estrogen also has effects that do not depend on activating the DNA. Estrogen effects may be mediated in part by growth factors and interleukins. For example, IL-6 is a potent stimulator of bone resorption, and estrogen blocks the osteoblast's synthesis of IL-6. Estrogen may also antagonize the IL-6 receptors (Väänänen and Härkönen 1996).

Testosterone

Men with hypogonadism are at increased risk of osteoporosis (Jackson et al. 1992). On the other hand, many observational studies have found an association between testosterone use in men and important gains in bone density, favorable changes in bone turnover biomarkers, and lower risk of osteoporotic fractures (Kenny et al. 2001). Androgen receptors have been identified in osteoblasts. Androgens likely stimulate longitudinal bone growth by their direct effects on growth plate chondrocytes. Androgen effects on the bone may also be indirectly mediated by regulation of cytokines and growth factors expressed locally in the bone. Androgens, in fact, upregulate TGF-β and IGFs, which stimulate bone formation, and downregulate IL-6, which stimulates osteoclastogenesis. Androgens inhibit PTH or IL-1-induced prostaglandin E2 (PGE2) production. Androgens stimulate IL-1β production and enhance the mitogenic effect of fibroblast growth factor (FGF) in cultured osteoblasts. Finally, dihydrotestosterone has been shown to reduce osteoprotegerin levels, which could potentially stimulate osteoclasts activity (Clarke and Khosla 2009).

Vitamin D

Vitamin D is important for normal development and maintenance of the skeleton. It is well known that vitamin D deficiency is related to rickets and osteomalacia.

Vitamin D is available either as ergocalciferol, or vitamin D2, derived from plants or as cholecalciferol, or vitamin D3, from animal sources. Both are converted by the liver to 25-hydroxyvitamin D, then by the kidneys to 1,25-dihydroxyvitamin D (Holick 2007). Ultraviolet B (UV-B) radiation (290–315 nm) converts 7-dehydrocholesterol in the deep epidermal layers to the provitamin cholecalciferol. Measurement of the active form 1,25-dihydroxyvitamin D is not useful in clinical practice. The serum 25-hydroxyvitamin D level, instead, reflects the vitamin D stores in the body. Normal serum 25-hydroxyvitamin D level values have been defined as >20 ng/mL (50 nmol/L). Deficiency is defined if serum values are <20 ng/mL (50 nmol/L); on the other hand, serum values of 25-hydroxyvitamin D level >200 ng/mL (500 nmol/L) are considered as toxic (von Domarus et al. 2011). As already said before, plasma calcium concentrations are maintained at a very constant level; if plasma becomes less than saturated with respect to calcium and phosphate, then mineralization fails, which results in rickets among children and osteomalacia among adults (Underwood and DeLuca 1984). The vitamin D hormone functions to increase serum calcium concentrations in three different ways:

1. Vitamin D is able to induce the proteins involved in active intestinal calcium absorption. Furthermore, it stimulates active intestinal absorption of phosphate.
2. Vitamin D is able to mobilize calcium from the bone when calcium is absent from the diet. Vitamin D, in fact, stimulates osteoblasts to produce receptor activator nuclear factor-kB ligand (RANKL). RANKL then stimulates osteoclastogenesis and activates resting osteoclasts for bone resorption (Suda et al. 2002). Both vitamin D and PTH are required for this mobilization event (Garabedian et al. 1974).
3. Vitamin D stimulates calcium absorption by the distal renal tubule, responsible for resorption of the last 1% of the filtered load of calcium (Yamamoto et al. 1984). Because 7 g of calcium are filtered every day, this represents a major contribution to the calcium pool. Again, both PTH and the vitamin D hormone are required.

Cortisol

The periosteum may affect bone formation by providing precursor cells, needed to achieve a normal osteoblastic cell population, or by providing bone growth factors, known to be released by cultured fibroblasts and intact bones. The long-term inhibitory effect of cortisol on bone collagen is secondary to a decrease in cell population, whereas the short-term stimulatory effect could be related to locally released growth factors (Canalis 1984). Data about cortisol effect on the bone come primarily from experience in patients with Cushing's syndrome, characterized by elevated levels of cortisol. In these patients, the prevalence of osteopenia and osteoporosis is usually estimated between 60–80% and 30–65%, respectively (Mancini et al. 2004). The end result of glucocorticoid excess is a loss of bone mineral content and increased bone fragility. The pathogenesis of glucocorticoid-induced

osteoporosis involves both skeletal and extraskeletal mechanisms. Glucocorticoids have extraskeletal effects including hypogonadotrophic hypogonadism, a decrease in intestinal calcium absorption and an increase in renal calcium excretion. Moreover, there is also a decreased secretion of adrenal androgens and estrogens and changes in the GH-IGF-1 axis and insulin. Glucocorticoids also have direct effects on skeleton, with a decrease in bone formation due to impaired osteoblastogenic differentiation, decreased osteoblast function, and increased osteoblastic apoptosis, resulting in decreased bone formation. The impairment of osteoblastic differentiation of bone marrow stromal cells parallels a shift toward the adipocytic lineage due to a decrease in bone morphogenetic protein-2, an increase in peroxisome proliferator-activated receptor-c-2 and CAAT enhancer-binding proteins, as well as inhibition of the Wnt/beta-catenin pathway (Tóth and Grossman 2013).

Insulin

The anabolic effects of insulin do not have to be confused with those of insulin-like growth factor (IGF-1), although the homology of molecular structure of both molecules may in fact account for some of the anabolic effects of insulin on the bone. A first difference between the two molecules is that insulin is produced in the pancreatic β-cells, while endocrine IGF-1 is synthesized in the liver. The release of insulin production is induced by glucose and osteocalcin, while IGF-1 is produced in response to growth hormone and the paracrine IGF-1 produced by bone cells, including pre-osteoblasts and osteoblasts, osteocytes, and osteoclasts (Klein 2014). Insulin proved to have an anabolic effect on the bone. Recent data suggest that, under normal conditions, insulin stimulates osteoblast differentiation to produce more osteocalcin, which would then stimulate more insulin production by the pancreas and greater insulin sensitivity of skeletal muscle. In insulin-resistant patients, such as in type 2 diabetes, osteocalcin levels are lower; moreover, insulin resistance is also caused by factors that cause bone resorption, such as IL-6-mediated chronic low-grade inflammation (Tarantino et al. 2010).

Fibroblast Growth Factors

Fibroblast growth factors (FGFs) are polypeptides originally isolated from the central nervous system but also found in a variety of tissues including the bone. Two forms of FGFs have been isolated: acidic (aFGF) and basic (bFGF); they have 55% homology in their amino acid sequence, have similar biological effects, and interact with the same cell receptors (Gimenex-Gallego et al. 1986). The skeletal tissue is a rich source of growth factors; both aFGF and bFGF are contained in bone matrix, suggesting that they are either trapped by the bone matrix or are synthesized by skeletal cells. They play a role in the local regulation of percent collagen synthesized. Fibroblast growth factors are important factors that promote osteoprogenitor cell proliferation and osteogenesis. They were found to exert

anabolic effects on bone formation in intact animals and to reduce bone loss in experimental models of osteoporosis (Fromigué et al. 2004).

Insulin-Like Growth Factors (IGFs, Types I and II)

IGF-I is the major mediator of growth hormone (GH) action, and it plays a central role in growth, development, and metabolism of skeletal tissue. GH stimulates skeletal growth indirectly by stimulating liver production of IGF-I to act in an endocrine manner to stimulate bone growth. However, subsequent studies showed that GH also has direct effects on the bone and that these effects are largely mediated via GH regulation of local IGF-I expression and its action in the bone. Osteoblasts contain GH receptors and GH treatment increases the production of IGF-I in these cell types (Mohan and Kesavan 2012). IGF-1 binds to IGF1-R, a type II tyrosine kinase, leading to autophosphorylation of Tyr residues 1131, 1135, and 1136 in the kinase domain, followed by phosphorylation of Tyr 950 in the juxtamembrane domain, which activates downstream substrates, insulin receptor substrate (IRS) proteins, and Shc by tyrosine phosphorylations. The IRS protein family consists of four isomers IRS1, 2, 3, and 4. Two of these proteins, IRS1 and IRS2, have been studied with respect to the bone; IRS1 is expressed in chondrocytes and osteoblasts; IRS2 is expressed in osteoblasts and osteoclasts but not in chondrocytes (Hernández-Sánchez et al. 1995).

Transforming Growth Factors (TGFs β1 and β2)

Transforming growth factors type-β (TGF-β1, TGF-β2, TGF-β3) have been implicated in the regulation of a variety of cellular events involved in the regulation of bone growth and turnover. They are produced by osteoblasts and chondrocytes and are highly concentrated in skeletal tissue. Autocrine and paracrine stimulation by TGF-β is important in the maintenance and expansion of the mesenchymal stem/progenitor cells, the progenitors of osteoblasts. The bone and cartilage contain large amounts of TGF-β and target cells for TGF-β activity. At earlier developmental stages, osteoblast-enriched populations from fetal bone are more sensitive to the mitogenic effect of TGF-β than similar populations from newborns. Furthermore, TGF-β signaling also promotes osteoprogenitor proliferation, early differentiation, and commitment to the osteoblastic lineage (Derynck and Akhurst 2007).

Prostaglandins

Prostaglandins are potent, multifunctional regulators of bone metabolism, which have both stimulatory and inhibitory effects. Prostaglandins stimulate bone resorption by increasing the number and activity of osteoclasts. Prostaglandin E2 is the most potent agonist, although other prostanoids, particularly prostacyclin (PGI2), are

potent stimulators. Most of the potent stimulators of bone resorption increased prostaglandin production in the bone by induction of COX-2, although they also stimulate resorption by prostaglandin-independent pathways. However, PG can stimulate bone formation by increasing replication and differentiation of osteoblasts. This effect is associated with an increase in the production of growth factors. There is indirect evidence that stimulation of bone formation is mediated by the EP2 receptor, which is expressed in osteoblast precursor cells.

Cytokines

Interleukin-1 is a prototypic pro-inflammatory cytokine that regulates a wide variety of cellular and tissue functions. There are two forms of IL-1, IL-1a and IL-1b, with similar biological activities but different functional roles. Both bind to IL-1 type I receptor (IL-1RI) with an equal affinity. In addition, IL-1 receptor antagonist (IL-1Ra) serves as a natural competitive inhibitor of IL-1a and IL-1b. The bone is very sensitive to IL-1, which regulates both bone formation and bone resorption. Interleukin-1 is an osteoclast-activating factor; it stimulates osteoclast formation indirectly by stimulating prostaglandin E2 synthesis in osteoblasts/stromal cells. Interleukin-1 also induces the fusion of mononuclear osteoclasts leading to multinucleation, it potentiates osteoclast function, and it is involved in the survival part of osteoclasts (Lee et al. 2010).

As already said above, IL-6 is an essential mediator of the bone loss. Interleukin-6 is a pleiotropic cytokine influencing many biological events in several organs including the bone marrow. In the bone, activation of the glycoprotein (gp)-130 signaling pathway by IL-6 and its soluble receptor (sIL-6R) is a key pathway for the regulation of osteoclastogenesis (Roodman 1992).

Another cytokine involved in bone resorption is tumor necrosis factor-α, a multifunctional cytokine mainly produced by activated macrophages, with numerous functions. Tumor necrosis factor-α is associated with several cell signaling systems via two types of cell surface receptors, namely, TNFR I and TNFR II. Both receptors are expressed on several cell types including bone marrow hematopoietic cells. Both TNFR I and II mediate biological properties of TNF-α. Osteoclast recruitment by TNF-α is probably essential in the pathogenesis of inflammatory osteolysis (Kwan Tat et al. 2004).

Potential Applications to Prognosis, Other Diseases, or Conditions

Bone metabolism is a dynamic and continuous remodeling process that is normally maintained in a tightly coupled balance between resorption of old or injured bone and formation of new bone. Several hormones and factors are involved in bone metabolism, which regulation depends from the complex interaction among them. The knowledge of biomarkers linked to bone metabolism is very useful to promptly

identify bone abnormalities and to guide physicians in the right direction to better understand the mechanisms underlying bone diseases.

Summary Points

- This chapter focuses on biomarkers relevant to bone metabolism.
- Biomarkers include measurable indicators of some biological state and are useful to diagnose or follow-up a specific condition or risk factor.
- Biomarkers relevant to bone metabolism include markers of bone formation, markers of bone resorption, and markers of bone metabolism regulation.
- The knowledge of biomarkers linked to bone metabolism is very useful to promptly identify bone abnormalities and to guide physicians in the right direction to better understand the mechanisms underlying bone diseases.

References

Canalis E. Effect of cortisol on periosteal and nonperiosteal collagen and DNA synthesis in cultured rat calvariae. Calcif Tissue Int. 1984;36(2):158–66.

Clarke BL, Khosla S. Androgens and bone. Steroids. 2009;74(3):296–305.

Corathers SD. Focus on diagnosis: the alkaline phosphatase level: nuances of a familiar test. Pediatr Rev. 2006;27:382–4.

Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. Osteoporos Int. 2000;11(6):S2–17.

Derynck R, Akhurst RJ. Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat Cell Biol. 2007;9:1000–4.

Donangelo I, Braunstein GD. Update on subclinical hyperthyroidism. Am Fam Physician. 2011;83:933–8.

Foley KF. Urine calcium: laboratory measurement and clinical utility. Lab Med. 2010;41:683–6.

Fottrell PF, Power MJ. Osteocalcin: diagnostic methods and clinical applications. Crit Rev Clin Lab Sci. 1991;28:287–335.

Fromigué O, Modrowski D, Marie PJ. Growth factors and bone formation in osteoporosis: roles for fibroblast growth factor and transforming growth factor beta. Curr Pharm Des. 2004;10(21): 2593–603.

Gimenex-Gallego G, Conn G, Hatcher VB, Thomas KA. Human brain-derived acidic and basic fibroblast growth factors: amino terminal sequences and specific mitogenic activities. Biochem Biophys Res Commun. 1986;135:541–8.

Garabedian M, Tanaka Y, Holick MF, DeLuca HF. Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D3 in thyroparathyroidectomized rats. Endocrinology. 1974;94:1022–7.

Harris SS, Soteriades E, Dawson-Hughes B. Secondary hyperparathyroidism and bone turnover in elderly blacks and whites. J Clin Endocrinol Metab. 2001;86(8):3801–4.

Hatayama K, Ichikawa Y, Nishihara Y, Goto K, Nakamura D, Wakita A, et al. Serum alkaline phosphatase isoenzymes in SD rats detected by polyacrylamide-gel disk electrophoresis. Toxicol Mech Methods. 2012;22:289–95.

Hernández-Sánchez C, Blakesley V, Kalebic T, Helman L, LeRoith D. The role of the tyrosine kinase domain of the insulin-like growth factor-I receptor in intracellular signaling, cellular proliferation, and tumorigenesis. J Biol Chem. 1995;270:29176–81.

Holick MF. Vitamin D, deficiency. N Engl J Med. 2007;357(3):266–8.
Hu CA, Khalil S, Zhaorigetu S, Liu Z, Tyler M, Wan G, et al. Human D1-pyrroline-5-carboxylate synthase: function and regulation. Amino Acids. 2008;35:665–72.
Jackson JA, Riggs MW, Spiekerman AM. Testosterone deficiency as a risk factor for hip fractures in men: a case–control study. Am J Med Sci. 1992;304:4–8.
Kapustin AN, Shanahan CM. Osteocalcin. A novel vascular metabolic and osteoinductive factor? Arterioscler Thromb Vasc Biol. 2011;31:2169–71.
Kaul S, Sharma SS, Mehta IK. Free radical scavenging potential of L-proline: evidence from in vitro assays. Amino Acids. 2008;34:315–20.
Kenny AM, Prestwood KM, Gruman CA, Marcello KM, Raisz LG. Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels. J Gerontol A Biol Sci Med Sci. 2001;56:M266–72.
Kivrikko K. Excretion of urinary hydroxyproline peptide in the assessment of bone collagen deposition and resorption. In: Frame B, Potts Jr JT, editors. Clinical disorders of bone and mineral metabolism. Amsterdam: Excerpta Medica; 1983. p. 105–7.
Klein GL. Insulin and bone: recent developments. World J Diab. 2014;5(1):14–6.
Kress BC. Bone alkaline phosphatase: methods of quantitation and clinical utility. J Clin Ligand Assay. 1998;21(2):139–48.
Kwan Tat S, Padrines M, Théoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004;15(1): 49–60.
Kubo K, Yuki K, Ikebukuro T. Changes in bone alkaline phosphatase and procollagen type-1 C-peptide after static and dynamic exercises. Res Q Exerc Sport. 2012;83:49–54.
Laitinen O, Nikkila EA, Kivirikko KI. Hydroxyproline in the serum and urine. Normal values and clinical significance. Acta Med Scand. 1966;179(3):275–84.
Lee YM, Fujikado N, Manaka H, Yasuda H, Iwakura Y. IL-1 plays an important role in the bone metabolism under physiological conditions. Int Immunol. 2010;22(10):805–16.
Mancini T, Doga M, Mazziotti G, Giustina A. Cushing's syndrome and bone. Pituitary. 2004;7:249–52.
Mohan S, Kesavan C. Role of insulin-like growth factor-1 in the regulation of skeletal growth. Curr Osteoporos Rep. 2012;10(2):178–86.
Moss DW, Henderson AR. Enzymes. In: Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry. 2nd ed. Philadelphia: W.B. Saunders Co; 1994. p. 882–90.
Nelson DL, Cox MM. Lehninger's principles of biochemistry. 4th ed. New York: W. H. Freeman and Company; 2005.
Nicoll DC. Appendix: therapeutic drug monitoring and laboratory reference ranges. In: Stephen JM, Maxine AP, editors. Current medical diagnosis and treatment. 46th ed. New York: Mc Graw Hill; 2007. p. 1767–75.
Risteli L, Risteli J. Biochemical markers of bone metabolism. Ann Med. 1993;25:385–93.
Roodman GD. Interleukin-6: an osteotropic factor? J Bone Miner Res. 1992;7:475–8.
Ross PD, Knowlton W. Rapid bone loss is associated with increased levels of biochemical markers. J Bone Miner Res. 1998;13:297–302.
Simko V. Alkaline phosphatase in biology and medicine. Dig Dis. 1991;9:189–209.
Stevenson JC. Regulation of calcitonin and parathyroid hormone secretion by oestrogens. Maturitas. 1982;4(1):1–7.
Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. J Cell Biochem. 2002;88:259–66.
Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem cell niche. Blood. 2005;105:2631–9.
Tarantino G, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance. World J Gastroenterol. 2010;16:4773–83.
Tóth M, Grossman A. Glucocorticoid-induced osteoporosis: lessons from Cushing's syndrome. Clin Endocrinol (Oxf). 2013;79(1):1–11.

Underwood JL, DeLuca HF. Vitamin D is not directly necessary for bone growth and mineralization. Am J Physiol. 1984;246:E493–8.
Väänänen HK, Härkönen PL. Estrogen and bone metabolism. Maturitas. 1996;23:S65–9.
Von Domarus C, Brown J, Barvencik F, Amling M, Pogoda P. How much vitamin D do we need for skeletal health? Clin Orthop Relat Res. 2011;469(11):3127–33.
Yamamoto M, Kawanobe Y, Takahashi H, Shimazawa E, Kimura S, Ogata E. Vitamin D deficiency and renal calcium transport in the rat. J Clin Invest. 1984;74:507–13.
Watts NB. Clinical utility of biochemical markers of bone remodeling. Clin Chem. 1999; 45(8 Pt 2):1359–68.