CALCIUM AND PHOSPHATE HOMEOSTASIS

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ABSTRACT

Calcium and phosphate are critical to human physiology (e.g., neuromuscular function) and are also needed for skeletal mineralization. An understanding of calcium and phosphate metabolism is required for the clinician to evaluate disorders of the levels of calcium and phosphorus as well as metabolic skeletal disorders. In this chapter, we review calcium and phosphate homeostasis including the critical organs involved (skeleton, parathyroids, GI tract, kidneys etc.) as well as the hormones (PTH, vitamin D, FGF23, calcitonin) that regulate calcium and phosphate.

INTRODUCTION

Understanding the physiology of calcium and phosphate homeostasis is needed to manage patients with abnormalities of this homeostatic system. Disorders of calcium, phosphate, and skeletal metabolism are among the most common group of diseases in endocrinology (1). They can involve abnormalities in the serum concentrations of the two minerals, especially calcium; abnormalities of bone; and abnormalities of the major regulating organ systems, especially the parathyroid gland, kidneys and gastrointestinal (GI) tract (Table 1). The serum calcium concentration can be abnormally high, as in malignancy and primary hyperparathyroidism, or abnormally low as it is in renal failure and hypoparathyroidism. The skeleton can have low bone density, as occurs in osteoporosis and osteomalacia, or high bone density as Paget's disease of bone, osteopetrosis, and other osteosclerotic disorders. The GI tract can exhibit low calcium absorption, as in malabsorptive states, or high calcium absorption, as in vitamin D intoxication and the milk-alkali syndrome. The kidneys can under-excrete calcium, as occurs in some hypercalcemic disorders; over-excrete calcium, as in some patients with nephrolithiasis; under-excrete phosphorus, as in renal failure and defects in fibroblast growth factor 23 (FGF23) action; and over-excrete phosphorus, as in some renal tubular disorders and renal phosphate wasting due to excess FGF23 and other phosphatonins. Corresponding events occur for magnesium, but they will not be discussed in this chapter. The goal of this chapter is to discuss the normal regulation of bone and mineral metabolism in order to provide the clinician a basis for diagnosis and management of patients with the common disorders that involve this homeostatic system.

Table 1. Regulation of Calcium and Skeletal Metabolism
Minerals
Calcium (Ca)
Phosphorus (P)
Magnesium (Mg)
Organ Systems
Skeleton
Kidney
GI tract
Skin
Other
Hormones
Calciotropic hormones
Parathyroid Hormone (PTH)
Calcitriol (1,25(OH2)D)
PTH-related Protein (PTHrP)
FGF23 and other phosphatonins
Calcitonin (CT
Other hormones
Gonadal and adrenal steroids
Thyroid hormones
Growth factor and cytokines

As detailed in other chapters, disorders of mineral and skeletal metabolism can be due to a primary disease of one of the involved organ systems, as in primary hyperparathyroidism due to a tumor of one or more parathyroid glands; secondary hyperparathyroidism, due to a compensatory response of the parathyroid glands to a low serum calcium, low vitamin D, calcium malabsorption, kidney disease, etc.; perturbations in serum calcium due to malignancy and bone metastases; and the complex mineral and skeletal complications of renal failure. A basis for understanding the pathogenesis of the primary and secondary diseases of bone and its minerals that are discussed in this text is an appreciation of the interplay among hormones, minerals, and organ systems that regulate normal bone and bone and mineral metabolism (Figure 1).

The skeleton is the reservoir of calcium for many physiological functions, and it serves a similar but not so unique role for phosphorus and magnesium (Table 2) (2,3). Skeletal calcium is controlled through the regulatory pathways of the gastrointestinal (GI) tract and the kidneys, and in bone by the osteoblast, the bone-forming cell, and the osteoclast, the bone-resorbing cell. Calcium reaches the skeleton by being absorbed from the diet in the GI tract. Unabsorbed calcium passes into the feces, which also contains the small amount of calcium secreted into the GI tract. Minor losses occur through perspiration and cell sloughing. In pregnancy, substantial losses can occur across the placenta to the developing fetus and in the postpartum period through lactation. Absorbed dietary calcium then enters the extracellular fluid (ECF) space and becomes incorporated into the skeleton through the process of mineralization of the organic matrix of bone, osteoid. ECF calcium is also filtered by the kidney at a rate of about 6 grams per day, where up to 98 percent of it is reabsorbed (Figure 1).

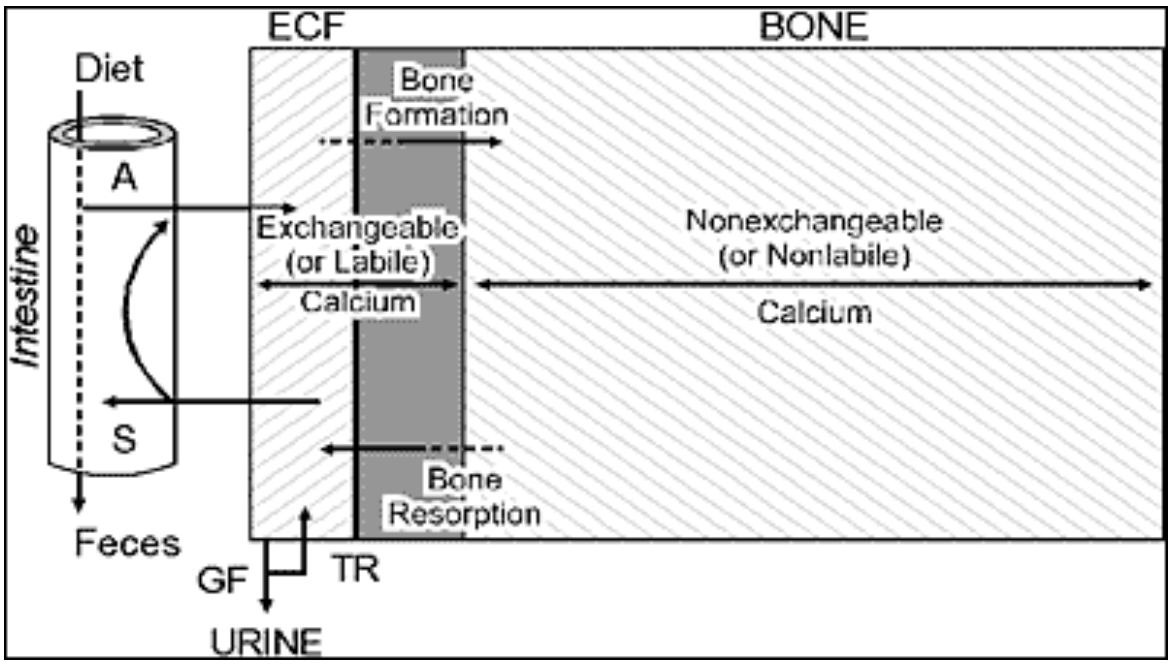

Figure 1. Schematic Representation of Calcium and Skeletal Metabolism. Abbreviations: A, absorption; S, secretion; ECF, extracellular fluid; GF, glomerular filtration; TR, tubular reabsorption. The dark vertical line between bone and ECF represents bone surface and bone-lining cells. Shaded area represents labile skeletal calcium. The various calcium compartments are not to scale. See text for discussion. (see Acknowledgements).

The major regulation of bone and bone mineral metabolism results from the interactions of four hormones – parathyroid hormone (PTH), vitamin D (VD), fibroblast growth factor 23 (FGF23) and to a much lesser extent calcitonin (CT) – at three target organs – bone, kidneys, and GI tract – to regulate three bone minerals – calcium, magnesium, and phosphorus. Other hormones also play a role, and skin is a participating organ system (Table 1). Understanding the normal regulatory mechanisms of this system will aid the clinician in evaluation and management of disorders of mineral metabolism (1-3).

CELLULAR AND INTRACELLULAR CALCIUM AND PHOSPHORUS METABOLISM

Physicians are most aware of the clinical status of calcium and skeletal metabolism in the patient as revealed by the concentrations of these minerals in biological fluids, especially blood and urine, and by the structural integrity of the skeleton (1). The actions of the calcemic hormones to regulate mineral concentrations in biological fluids are well understood at the target organ level. However, less well understood are the cellular and intracellular mechanisms that underlie the clinically important phenomena.

Both calcium and phosphorous, as well as magnesium, are transported to blood from bone, renal, and GI cells, and visa versa (4-6). These transport mechanisms can be through cells (transcellular) and around cells (paracellular). The cellular transport is mediated by the membrane structures illustrated in Figure 2 and by binding transport proteins (7,8). The paracellular transport is generally passive and mediated by mineral gradients. These mechanisms also involve corresponding co-transportation and exchange-transportation with other ions, notably sodium, potassium, chloride, hydrogen, and bicarbonate, some of which are powered by ATP hydrolysis. Similar mechanisms allow for the intracellular distribution of calcium, where it partitions primarily between the mitochondria and cytosol.

The details of the regulation of these cellular and intracellular mineral transports are not as well understood as are the whole organ mechanisms that they effectuate. However, some evidence along with inferences lead to the tentative clinical conclusion that changes in ambient concentrations of mineral in extracellular fluids are mirrored by corresponding intracellular changes and redistribution (Figure 2).

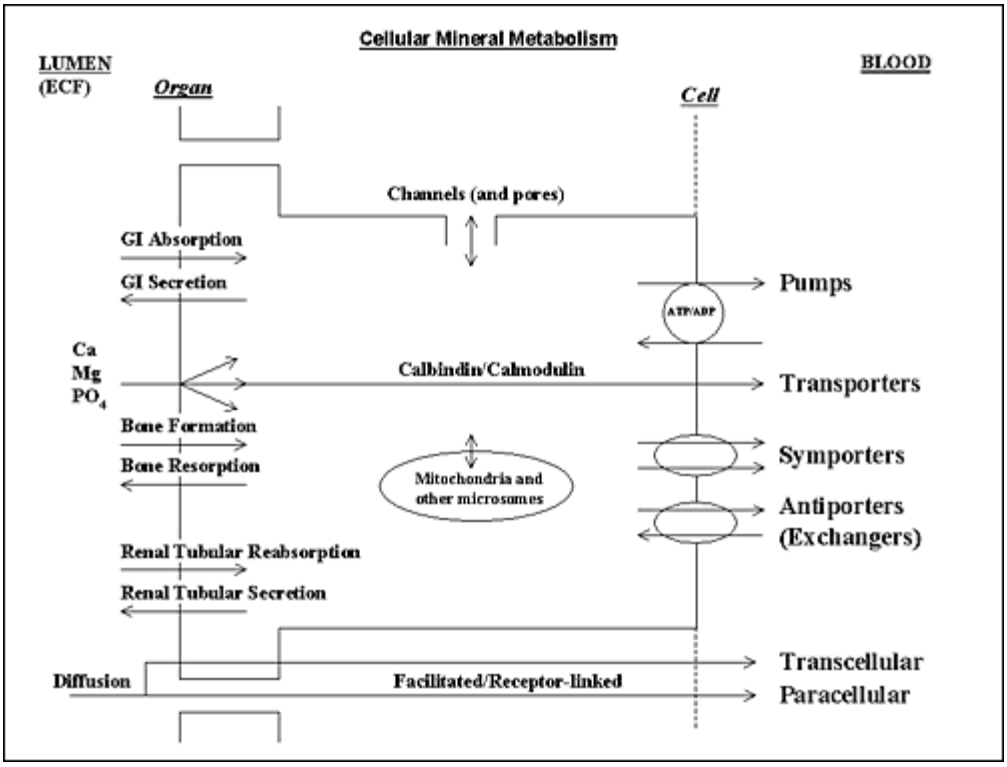

Figure 2. Schematic representation of cellular transport of bone minerals. The model can be applied to transport of calcium, magnesium, and phosphorus for cells of the renal tubules, gastrointestinal tract enterocytes, and bone cells. The mineral transport can be with (downhill) or against (uphill) a gradient. Lumen refers to GI and renal tracts; for bone, it can refer to bone marrow, blood, and/or matrix space. The site of the indicated membrane transport structures is schematic. Microsomes designate other intracellular organelles such as secretory vesicles and endoplasmic reticulum. See text for details.

Figure 2 provides a simplified version of the cellular regulation of bone minerals metabolism and transport. Mineral homeostasis requires the transport of calcium, magnesium, and phosphate across their target cells in bone, intestine, and kidney. This transport can be across cells (transcellular) and around cells (pericellular). The pericellular transport is usually diffusional, down a gradient ("downhill"), and not hormonally regulated. Diffusion can also occur through cell channels, which can be gated. Transport across cells is more complex and usually against a gradient ("uphill"). This active transport is energized by either ATP hydrolysis or electrochemical gradients and involves membrane structures that are generally termed porters, exchangers, or pumps. Three types of porters have been described, uniporters of a single substance; symporters for more than one substance in the same direction; and anti-porters for more than one substance in opposite directions (7,8).

Once through the luminal cell membrane, the bone minerals can cross the cell into the extracellular fluid compartment, blood for enterocytes and urine for renal epithelium cells (5,6). For bone cells, the

corresponding compartments are marrow and blood (1,2). For calcium, the transcellular transport is ferried by the interaction among a family of proteins that include calmodulin, calbindin, integral membrane protein, and alkaline phosphatase; the latter three are vitamin D dependent in their expression (6). Cytoskeletal interactions are likely important for transcellular transport as well. Exit from the cell is regulated by membrane structures similar to those that mediate entry. There do not appear to be any corresponding binding proteins for phosphorous, so diffusional gradients and cytoskeletal interactions seem to regulate its cellular transport.

The molecular details of the hormonal regulation of cellular bone mineral transport have not been fully elucidated. It is reasonable to hypothesize that PTH, vitamin D, FGF23, and CT, regulate these molecular mechanisms through their biological effects on the participating membrane structures and transport proteins. For the enterocyte, vitamin D enhances the movement of calcium into the cell through its stimulation of calbindin synthesis (6). For kidney tubules, PTH and FGF23 are the key regulators for the transport of calcium and phosphate (1,5,9). For bone, PTH and to a lesser extent CT are important regulators of cellular calcium and phosphate transport, while vitamin D provides appropriate concentrations of these minerals through it's GI and perhaps renal actions (1-3).

It is important to note that these mineral translocations not only mediate the mineral metabolism represented in Figure 2, but also the cellular effects summarized in Table 3.

Table 2. Distribution of Calcium, Phosphorus, and Magnesium

TOTAL BODY CONTENT, G		% IN SKELETON	% IN SOFT TISSUES
Calcium	1000	99	1
Phosphorus	600	85	15
Magnesium	25	65	35

CALCIUM METABOLISM

Serum and extracellular calcium concentrations in mammals are closely regulated within a narrow physiologic range that is optimal for the many cellular functions. (1,2). More specifically, it is the ionized component of serum calcium that is closely regulated, as it subserves the physiological functions of this divalent cation (Table 3). Ambient calcium is so close to its saturation point with respect to phosphates that deviations in concentrations of either can cause precipitation. Intracellular calcium, which serves as second messenger in many signal transduction pathways, is also tightly controlled, but at concentrations several orders of magnitude lower than extracellular calcium. Extraskeletal calcium accounts for only 1% of the total body calcium, as calcium is primarily sequestered in bone (Table 4-6). The average diet contains about 1 gm of calcium, but there are great variations. About 500 mg undergoes net absorption from the diet, and the unabsorbed and secreted components appear in the stool (Table 6-9). Approximately 10,000 mg/day is filtered at the glomerulus and most is reabsorbed by the renal tubules, with only a few hundred milligrams appearing in urine each day (Tables 10 and 11). The skeleton turns over about 250 mg/day of calcium, but there is wide variation. This turnover is attributed to a labile calcium pool near bone surfaces, but it is difficult to give anatomical assignment to either labile or non-labile calcium compartments. The turnover is mediated by bone-forming osteoblasts and bone-resorbing osteoclasts. In disease states, the turnover can be increased (e.g., hyperparathyroidism) or decreased (e.g., hypoparathyroidism) with corresponding changes in blood and urinary calcium. The primary calcium regulating hormones that control this homeostatic system are PTH and vitamin D, which

act at bone, kidney, and GI tract to increase serum calcium and to a lesser extent calcitonin, which decreases bone resorption, but does not appear to have a major effect on serum calcium under normal circumstances (10) (Figure 1).

Table 3. Multiple Biological Functions of Calcium
Cell signaling
Neural transmission
Muscle function
Blood coagulation
Enzymatic co-factor
Membrane and cytoskeletal functions
Secretion
Biomineralization

Table 4. Distribution of Calcium
Total body calcium- 1kg
99% in bone
1% in blood and body fluids Intracellular calcium
Cytosol
Mitochondria
Other microsomes
Regulated by “pumps”
Blood calcium – 10mgs (8.5-10.5)/100 mls
Non diffusible – 3.5 mgs
Diffusible – 6.5 mgs

Table 5. Bone Structure (cellular and non-cellular)
Inorganic (69%)
Hydroxyapatite – 99%
3 Ca10 (PO4)6 (OH)2
Organic (22%)
Collagen (90%)
Non-collagen structural proteins
proteoglycans
sialoproteins
gla-containing proteins
α2HS-glycoprotein
Functional components
growth factor
cytokines

Table 6. Blood Calcium – 10mgs/100 mls (2.5 mmoles/L)
Non diffusible – 3.5 mgs
Albumin bound – 2.8

Globulin bound – 0.7
Diffusible – 6.5 mgs
Ionized – 5.3
Complexed – 1.2 mgs
bicarbonate – 0.6 mgs
citrate – 0.3 mgs
phosphate – 0.2 mgs
other
Close to saturation point
tissue calcification
kidney stones

Table 7. Diet
Dietary calcium
Milk and dairy products (1qt ~ 1gm) Dietary supplements
Other foods
Other dietary factors regulating calcium absorption
Lactose
Phosphorus

Table 8. Calcium Absorption (0.4-1.5 g/d)
Fastest in duodenum
15-20% absorption
Adaptative changes
low dietary calcium
growth (150 mg/d)
pregnancy (100 mg/d)
lactation (300 mg/d)
Fecal excretion

Table 9. Mechanisms of GI Calcium Absorption
Vitamin D dependent
Duodenum > jejunum > ileum
Active transport across cells
calcium binding proteins (e.g., calbindins)
calcium regulating membranomes
Ion exchangers
Passive diffusion

Approximately 50% of the total calcium in serum is ionized, with the rest bound primarily to albumin or complexed with counter-ions, including phosphates (Table 6) (1,2). The ionized calcium concentration averages 1.25 + 0.07 mmol/L and the total serum calcium concentrations range from 8.5 to 10.5 mg/dL. Since ionized calcium has the primary regulatory role, it is in turn the regulated component that maintains homeostasis. This regulation takes place through the complex interactions at their target organs of the

primary calcium regulating hormones, parathyroid hormone (PTH) and vitamin D and its metabolites (Tables 4-11). Other hormones participate, most notably gonadal steroids.

Table 10. Urinary Calcium
Daily filtered load
10 m (diffusible)
99% reabsorbed
Two general mechanisms
Active – transcellular
Passive – paracellular
Proximal tubule and Loop of Henle reabsorption
Most of filtered load
Mostly passive
Inhibited by furosemide
Distal tubule reabsorption
10% of filtered load
Regulated (homeostatic)
stimulated by PTH
inhibited by CT
vitamin D has small stimulatory effect
stimulated by thiazides
Urinary excretion
50 – 250 mg/day
0.5 – 1% filtered load

Table 11. Regulation of Urinary Calcium
Hormonal – tubular reabsorption
PTH – decreases excretion (clearance)
CT – increases excretion (calciuretic)
1,25(OH)2D – decreases excretion
Diet
Little effect
Logarithmic
Other factors
Sodium – increases excretion
Phosphate – decreases excretion
Diuretics – thiazides vs loop
thiazides – inhibit excretion
furosemide – stimulate excretion

Table 12. Other Routes of Excretion
Perspiration
Lactation

PHOSPHORUS METABOLISM

Phosphorus is more widely distributed than calcium and also serves a variety of biological functions (Table 2) (3,4). While most of phosphorus is skeletal as hydroxyapatite, 15 % is distributed among extraskeletal sites like phosphoproteins, phospholipids, and nucleic acids (Table 13). In blood, phosphorus exists as the phosphates, H2PO4G and HPO4=, but its concentration is measured as phosphorus, with a normal range of 2.5 – 4.5 mg/100 ml. The regulation is not as tight as it is for calcium, with substantial perturbations due to diet and alimentation.

Table 13. Phosphorus Metabolism

General
- Widely distributed
- Multiple biological functions
- Distribution
- Skeletal – Hydroxyapatite:
  - $Ca(PO_4)_2$ o $Ca(OH)_2$
  - 15% extraskeletal
    - Phosphoproteins
    - Phospholipids
    - Nucleic acids

Blood Phosphate:
$H_2PO_4^-$ and $HPO_4^=$
Concentration measured as phosphorus: 2.5 – 4.5 mg/100 ml
Regulation
- Not as closely as calcium
- Diet
- Alimentation
- Growth
- Diurnal rhythm
- Hormones
- Other factors

Table 14. Dietary Phosphorus

Most foods
1 gm per day – variable
Absorption
- Site – distal to duodenum
- Mechanism
  - Calcium dependent
  - Calcium independent

Regulation Diet – 70% absorbed Calciotropic hormones Vitamin D – increases CT – decreases Other factors GH – increases Phosphate binders decrease Calcium – decreases Fecal – non-absorbed and secreted

Table 15. Urinary Phosphate
Major route of regulation Related to diet 90% filtered (? protein binding) Proximal tubule – 90% reabsorbed H2PO4- – active HPO4= – passive Distal tubule – 10% reabsorbed Regulation Diet Calciotropic hormones PTH – increases excretion CT – increases excretion Vitamin D – decreases excretion FGF23 and other phosphatonins increase excretion Proximal renal tubular NaPi2a, NaPi2c

Dietary phosphorus comes from most foods, averaging about 1 gm per day (Table 14), with the most important sources being dairy products, grains, meats, and food additives (3,4). Absorption takes place at a site distal to duodenum and utilizes both calcium dependent and calcium independent mechanisms that can be active or passive. The most significant quantitatively is post-prandial passive absorption. Approximately 60-80% is absorbed primarily by a diffusional process without a significant saturable component; however, there is regulation by the calciotropic hormones, especially vitamin D, whose active metabolites increases absorption, while PTH and CT have only minor direct effects (6) (Tables 13 and 14). Calcium- and aluminum-containing phosphate binders as well as newer phosphate binders such as sevelamer, lanthanum carbonate, ferric citrate, and sucroferric oxyhydroxide can inhibit absorption and are used to do so in the treatment of the hyperphosphatemia associated with chronic kidney disease (11). Fecal phosphate comprises non-absorbed and secreted components (Table 14).

Renal phosphate reabsorption controls the concentration of phosphate in serum, and it is usually quantified as the tubular reabsorption of phosphorus and expressed as the renal phosphate threshold (TmP/GFR), which closely mirrors the normal range of serum phosphorus (5). Although the TmP/GFR can be measured, it is usually estimated by a nomogram from fasting measurements of serum and urinary phosphorus and creatinine. The proximal convoluted tubule reabsorbs about 75 percent of filtered phosphate, and most of the remainder is reabsorbed

in the proximal straight tubule; the distal tubule segments may have a limited capacity for reabsorption, about 5 percent of filtered load (1,5).

An important role for FGF23 in phosphate metabolism has been elucidated (9). This glycoprotein product of osteocytes and osteoblasts promotes the renal excretion of phosphorus by decreasing expression of NaPi2a and NaPi2c resulting in decreased renal tubular reabsorption. The expression of FGF23 is up-regulated by serum phosphate and 1,25 dihydroxyvitamin D (9,12).

SKELETAL METABOLISM

The metabolic function of bone is to provide a homeostatic mineral reservoir, primarily for calcium, but also for other minerals, especially magnesium and phosphorus (1-3). These bone minerals can be mobilized to maintain systemic mineral homeostasis. This metabolic function of bone prevails over its structural function in that calcium and other minerals are removed from and replaced in bone to serve systemic homeostatic needs irrespective of loss of skeletal structural integrity. Bone is also a depository for certain cytokines and growth factors that can be released upon bone resorption and can exert their effects locally and systemically; notable among these is TFG beta.

Bone consists of a mineral phase and an organic phase (Table 5) (2). The major component of the mineral phase is hydroxyapatite crystal and the major component of the organic phase is type 1 collagen which, with other bone proteins, comprises the osteoid matrix of bone. The organic components of bone are products of the osteoblast. Bone mineral is present in two forms in the skeleton. Hydroxyapatite crystals, represented by the formula $Ca10(PO4)6(OH)2$, are the major forms and occur in mature bone. Amorphous calcium phosphate comprises the remainder; it occurs in areas of active bone formation and matures through several intermediate stages to hydroxyapatite. The end result is a highly organized amalgam of protein, primarily collagen, and mineral, primarily hydroxyapatite, that has sufficient structural integrity to serve the mechanical functions of the skeleton. Upon completion of this process, the osteoblast becomes encased in bone and become an osteocyte. Mineralization can occur if there is a functionally adequate local concentration of these ions, if nucleators are present to promote crystallization, and if local inhibitors of mineralization are removed. While vitamin D is key to providing sufficient ambient concentrations of calcium and other minerals to promote mineralization of osteoid, this hormone does not seem to exert a direct regulatory effect on mineralization.

Cortical bone comprises approximately 80% of the skeleton and trabecular bone 20% (1,3). However, the surface area of cortical bone is only one fifth that of trabecular bone, so trabecular bone is metabolically more active than cortical bone, with an annual turnover (remodeling) of approximately 20% to 30% for the former and 3% to 10% for the latter. A given skeletal site in the adult is remodeled approximately every 3 years. Bone mass is acquired up to the fourth decade, with a rapid phase during adolescent growth. Much of peak bone mass is genetically determined. Women have approximately 30% less peak bone mass than men and experience an accelerated loss after the menopause. Both genders experience age-related loss of bone mass.

A role for the central nervous system role in fat and skeletal metabolism has received much recent experimental support. The adipocyte-derived hormone leptin appears to inhibit bone mass accrual through a brain pathway, while having direct peripheral anabolic effects on bone (13). Furthermore, calcium metabolism has recently become linked to glucose metabolism through an appreciation of the biological effects of the osteoblast product, osteocalcin. When carboxylated, osteocalcin acts as a structural bone protein. However, in its undecarboxylated state, osteocalcin may act to regulate glucose metabolism by stimulating insulin secretion. Thus, two major metabolic pathways –

calcium/bone and glucose/insulin – seem to be linked (14).

Table 16. Skeletal Metabolism
Bone cells
Osteoblast
Osteoclast
Osteocyte
Other – marrow elements
Bone structure
Cortical bone
Trabecular bone
Mix

Bone Cells

Skeletal metabolism is regulated by bone cells and their progenitors (Figure 3). Among the population of bone cells are osteoblasts, osteocytes, osteoclasts, and lining cells (Table 16) (1-3). Monocytes, macrophages, and mast cells may also mediate certain aspects of skeletal metabolism. Marrow cells contribute to the population of bone cells. The osteoblast forms bone. Osteoblasts express receptors to many bone-active agents such as PTH, PTHrP, vitamin D metabolites, gonadal and adrenal steroids, and certain cytokines and growth factors. The major product of osteoblasts is type 1 collagen, which along with other proteins, forms the organic osteoid matrix that is mineralized to hydroxyapatite.

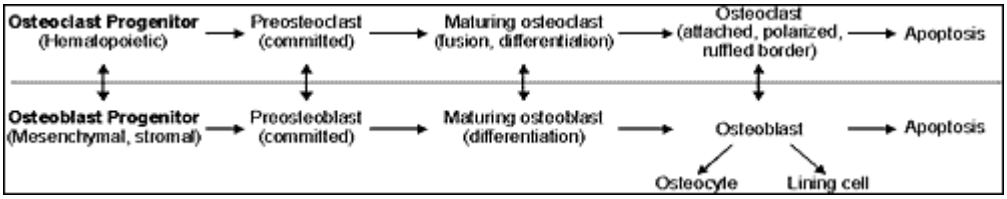

Figure 3. Schematic Representation of Osteoclast and Osteoblast Lineages. Schematic representation of the osteoclast (top) and osteoblast (bottom) lineages. The two lineages are distinct, but there is regulatory interaction among the cells (vertical arrows). Osteoclasts originate from a hematopoietic stem cell that can also differentiate into a macrophage, granulocyte, erythrocyte, megakaryocyte, mast cell, B-cell, or T-cell. Osteoblasts originate from a mesenchymal stem cell that can also differentiate into a chondrocyte, myocyte, fibroblast, or adipocyte. The terminology for these lineages is still evolving and is herein [over] simplified. Many intermediate steps and regulatory factors are involved in lineage development. (see Acknowledgements).

Osteocytes are osteoblasts that become encased in bone during its formation and mineralization and reside in the resulting lacuna (2,3). They comprise 90-95% of bone cells in the adult human skeleton (15). The cells develop processes that communicate as canaliculi with other osteocytes, osteoblasts, and the vasculature. Osteocytes thus present acres of cellular syncytium that permits translocation of bone mineral during times of metabolic activity and can provide minute-to-minute exchanges of minerals from bone matrix.

Osteocytes are extremely important in normal skeletal homeostasis. Their function is reviewed by Bonewald

(15). These cells are the likely transducers through their canaliculi of mechanical forces on bone and mediate the complex remodeling response to mechanical stimuli of the skeleton that causes appropriate changes in formation and resorption in response to skeletal loading. These cells produce sclerostin (*SOST* gene), which decreases bone formation and increases bone resorption (15). Defects in sclerostin function either by a mutation in *SOST* or a mutation downstream to sclerostin cause the high bone mass disorders sclerosteosis and van Buchem disease respectively (15). Osteocytes are also important endocrine cells that produce enzymes and hormones which affect bone mineralization and regulate phosphate such as Phosphate Regulating Endopeptidase X-Linked (PHEX), Dentin Matrix Acidic Phosphoprotein 1 (DMP1), Matrix Extracellular Phosphoglycoprotein (MEPE), and FGF23 (15). Sclerostin antagonism represents a therapeutic target for osteoporosis therapy (16,17). FGF-23 antagonism with a monoclonal antibody to FGF23, burosumab, is now used to treat FGF23-mediated disorders causing renal phosphate wasting (18).

The osteoclast resorbs bone. It is a terminally-differentiated, large, multinucleated giant cell that arises from hematopoietic marrow precursors under the influences of hormones, growth factors, and cytokines (3). The osteoclast resorbs bone by attachment with a ruffled border through adhesion molecules and by secretion of hydrogen and chloride ions that dissolve mineral and lytic proteases, notably lysosomal proteases active at low pH and metalloproteinases and cysteine proteinases that dissolve matrix. One enzyme involved in bone resorption, (cathepsin K), has been an investigational target for treatment of osteoporosis (19). In contrast to the receptor-rich osteoblast, the mature osteoclast has few receptors, but it robustly expresses the receptor for CT. After completing its function, the terminally-differentiated osteoclast undergoes apoptosis.

Bone-lining cells are flat, elongated cells that cover inactive bone surfaces. Their function is unknown, but they may be osteoblast precursors or function to clean up resorption and formation debris. Mast cells can be seen at sites of bone resorption and may also participate in this process. Cells of the immune system play a key role in bone metabolism, especially resorption, by their interactions with bone cells that are described later.

BONE GROWTH, MODELING AND REMODELING

Growth, modeling, and remodeling are important processes that allow the skeleton to play its many important roles (1). Bone grows and models under the influence of metabolic, mechanical, and gravitational forces during growth through adolescence, changing its size and shape in the process. Bone growth continues until approximately the third decade. Bone mass continues to increase until the fourth decade (Figure 4).

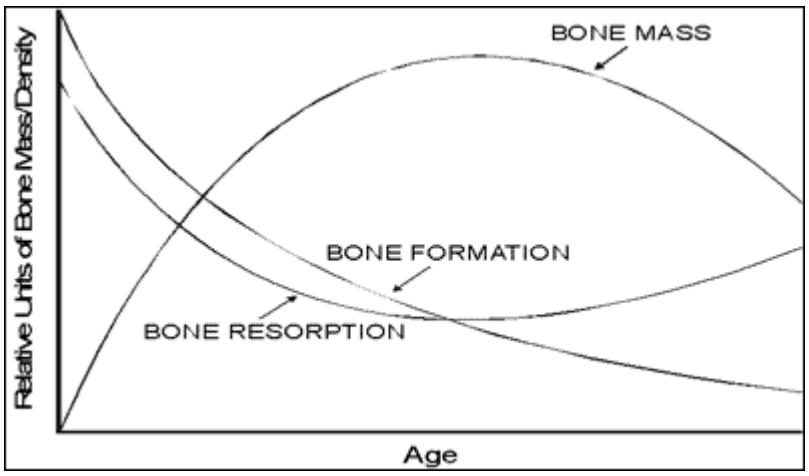

Figure 4. Peak Bone Mass. Schematic representation in relative units of normal skeletal development, demonstrating changes in bone resorption and formation. The crossover of formation/resorption occurs during the fourth decade. In osteoporosis, there is an accelerated loss of bone because of increased resorption and decreased formation. (see Acknowledgements).

Bone in adults renews itself by remodeling, a cycle in which old bone is first resorbed and new bone is then formed to replace it (1-3). Both cortical bone and trabecular bone remodel, but the latter is more metabolically active. Bone remodeling can be divided into several stages that include resorption by osteoclasts and formation by osteoblasts. Remodeling serves to repair skeletal microdamage and to improve skeletal strength in response to mechanical forces. Osteoclasts and osteoblasts communicate with each other during remodeling in a process that is referred to as coupling and mediated by local regulatory signals that are discussed subsequently. Coupling assures a balance of bone formation and bone resorption in the adult skeleton. The process of bone formation is thus balanced by the process of bone resorption.

Cortical bone is resorbed by “cutting cones” of osteoclasts that tunnel through it (2). Trabecular bone remodels on its surface. Most remodeling occurs in trabecular bone and on the endosteal surfaces of cortical bone, with little periosteal remodeling. However, in diseases like hyperparathyroidism, subperiosteal resorption is activated. With aging, periosteal remodeling and expansion seems to compensate (mechanically) for bone loss at other sites.

Bone resorption is mediated by the osteoclast, a large, multinucleated cell that is molecularly equipped to dissolve both the mineral and organic phases of bone (1,3). The processes of osteoblast-mediated bone formation and osteoclast-mediated bone resorption can be assessed by measurement in urine and blood of bone markers. The markers of bone formation include osteoblast products (e.g., alkaline phosphatase and osteocalcin) and by-products of collagen synthesis such as procollagen-1 N-terminal peptide (P1NP). Markers of bone resorption include osteoclasts products such as tartrate resistant acid phosphatase (TRAP) and by products of collagen breakdown such as such as N-terminal telopeptide (NTX) and C-terminal telopeptide (CTX) (20). Approximately 20% of adult bone surface is undergoing remodeling at any time. The homeostatic end-point of skeletal metabolism is to provide the appropriate amount of ambient calcium for the many biological functions that this ion serves, with the structural integrity of the skeleton taking second place. These metabolic activities of bone cells can release into blood and urine certain bone cell and matrix products that can serve as clinically useful markers of skeletal metabolism (Figure 5).

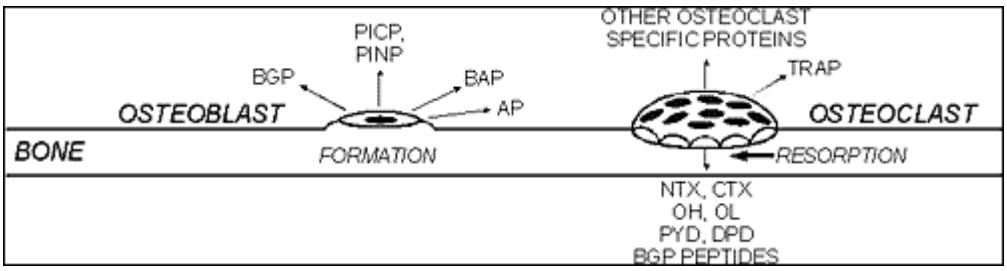

Figure 5. Schematic Representation of the Cellular and Skeletal Sources of Serum and/or Urinary Markers of Bone Formation and Bone Resorption. Abbreviations: BGP, bone gamma carboxyglutamic acid (GLA) protein (osteocalcin); PICP, C-terminal propeptide of type I procollagen; P1NP, N-terminal propeptide of ty pe I procollagen; BAP, bone-specific alkaline phosphatase; AP, alkaline phosphate; TRAP, tartrate-resistance acid phosphatase; NTX, N-terminal cross-linked telopeptide of type I collagen; CTX, C-terminal cross-linked telopeptide of type I collagen; OH, hydroxyproline glycoside; OL, hydroxylysine glycoside; PYD, pyridinoline (total, free); DPD, deoxypyridinoline (total, free). (see Acknowledgments).

RANKL, RANK, AND OPG

The elucidation of this pathway of molecular regulation has provided both a physiologic link among bone cell functions as well as a pathogenic link among cancer cells, the immune system, and bone cells in the regulation of the osteoclastic bone resorption that is the final cellular mediator of most cases of hypercalcemia (Figure 1) (21,22). The molecular participants in this pathway are the membrane-associated protein named RANKL (receptor activator of nuclear factor kappa B ligand,) a member of the tumor necrosis factor family of cytokines; its cognate receptor, RANK, and OPG (osteoprotegerin), a soluble “decoy” receptor for RANKL.

In the physiology of bone metabolism, RANKL is expressed on the surface of osteoblastic stromal cells (21). By binding to RANK, its receptor, on osteoclast precursors, RANKL enhances their recruitment into the osteoclastogenesis pathway in the physiology of bone metabolism. RANKL also activates mature osteoclasts to resorb bone. RANKL is considered to be a “coupling factor” through which osteoblasts regulate osteoclasts and bone formation is coupled to bone resorption. In the pathophysiology of hypercalcemia, many of the tumor cell types that are associated with cancer-stimulated bone resorption express a soluble form of RANKL, sRANKL. Furthermore, during the inflammation that can be associated with malignancy, activated T-lymphocytes also express increased amounts of RANKL, which can stimulate osteoclasts. The activated lymphocytes also express interferon gamma (INF), which opposes the effect of RANKL on osteoclast mediated bone resorption. The osteoclastic effects of RANKL can also be attenuated by its soluble decoy receptor, OPG, also produced by osteoblasts and tumor cells. Hypercalcemia results when these opposing regulatory interactions of RANKL, RANK, OPG, and INF allow osteoclastic activation to predominate (Figure 5).

These molecular participants in the interaction between bone cells, tumor cells, and the immune system are also regulated by several hormones, growth factors, and cytokines that mediate increased bone resorption, both physiologic and pathophysiologic. They include PTH, PTHrP, TNF, PGE2, vitamin D metabolites, IL-1, and TGF (22).

An antibody to RANKL (denosumab) decreases bone resorption, increases bone density, and decreases fractures and is FDA approved for treatment of osteoporosis (23).

Furthermore, defects in this system may cause bone diseases. Loss of function mutations of OPG are

responsible for the excess bone resorption in juvenile Paget's disease and gain of function mutations of RANK cause familial expansile osteolysis and expansile skeletal hyperphosphatasia (24,25).

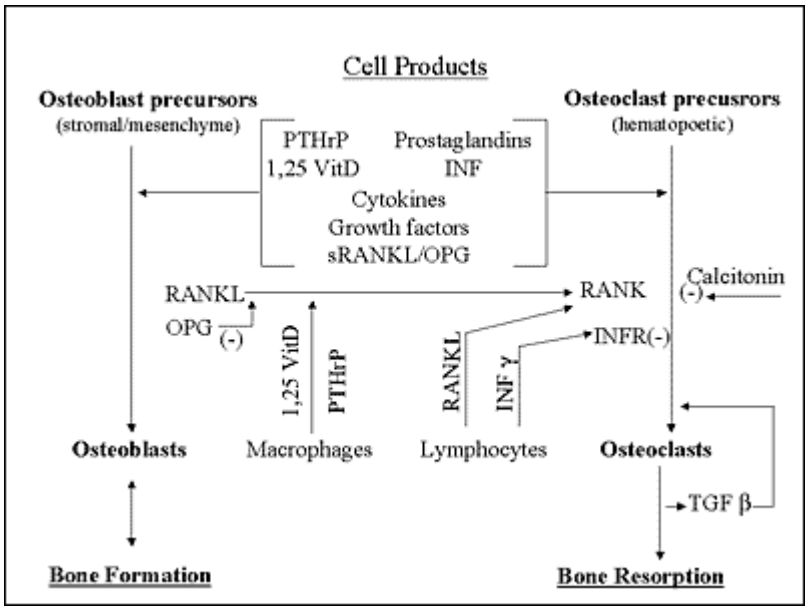

Figure 6. Schematic representation of the cellular and molecular mechanisms of the effects of OPG, RANK, and RANKL on skeletal metabolism. A variety of skeletal and non-skeletal cells can express several cell products [in brackets] that regulate the balance between osteoblastic bone formation (left) and osteoclastic bone resorption (right). They include PTHrP (parathyroid hormone related protein); 1, 25 Vit D (1, 25- dihydroxyvitamin D); prostaglandins, especially of the PGE2 series; cytokines, especially interleukin 1 (IL-1); growth factors, especially TGF beta; RANKL (receptor activator of nuclear factor kappa B ligand), a cell membrane-associated member of the tumor necrosis factor family of cytokines; soluble RANKL (sRANKL); and their cognate receptor, RANK; and OPG (osteoprotegerin), a soluble "decoy" receptor for RANKL. The latter group are also expressed by osteoblast precursors as they develop into osteoblasts in the osteoblastic cascade (left). In addition to OPG, the stimulation of osteoclastic bone resorption by RANKL is opposed by activation of the gamma interferon receptor (INFR) by gamma interferon (INF) production by activated lymphocytes and by the peptide hormone, calcitonin. The relative activity of the osteoclast stimulatory effects of RANKL and sRANKL and the inhibitory effects of OPG and INF determine the balance between bone resorption and formation. Arrows indicate a positive (stimulatory) effect except where indicated by the negative sign, (-). Several growth factors in addition to TGF beta reside in bone matrix and can be released upon resorption to exert their biological effects, often osteoclast stimulation. They include BMP (bone morphogenetic proteins, especially BMP-2); FGF (fibroblast growth factor); PDGF (platelet derived growth factor); and IGFs in (insulin like growth factors). Macrophages may fuse into giant cells and resorb bone. (see Acknowledgements).

LIPOPROTEIN RECEPTOR-RELATED PROTEIN (LRP)-5, WNT, BETA CATENIN SYSTEM

Activation of the LRP5/WNT system increases intracellular beta catenin which increases bone formation (26). Gain-of-function mutations of LRP-5 cause a high bone density phenotype and loss-of-function mutations cause the osteoporosis-glioma syndrome (26). Dkk1 and sclerostin inhibit this pathway and decrease bone formation and increase

bone resorption. Sclerostin production by osteocytes is increased with acute immobilization; resulting in decreased bone formation (27). Loss-of-function mutations of sclerostin cause the high bone density conditions sclerosteosis and van Buchem disease (15). A monoclonal antibody to sclerostin (romosozumab) increases bone formation and decreases bone resorption with resultant increased bone density and decreased fracture risk. This drug is approved for women with post-menopausal osteoporosis at high risk for fractures (16,17). Other monoclonal antibodies to sclerostin are being studied for treatment of osteogenesis imperfecta (OI) (28) and hypophosphatasia (29).

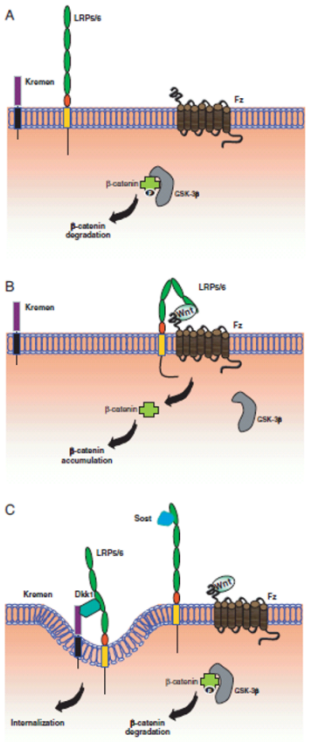

Figure 7. Wnt/β-catenin signaling pathway. A, In the absence of Wnt ligand, β-catenin is phosphorylated by GSK-3β leading to its degradation and pathway signaling inactivation. B, After Wnt binding to its LRP5/6 and Fz coreceptors, GSK-3β is inactivated. β-Catenin is then stabilized and accumulates in the cytoplasm. β-Catenin will consequently translocate into the nucleus where it affects gene expression. C, The secreted Dkk proteins bridge LRP5/6 and the transmembrane protein Krm. This results in the LRP5/6 membrane depletion by internalizing the receptors. As a consequence, Wnt signaling is inhibited. Sclerostin (Sost) also inhibits Wnt signaling through binding to LRP5/6, but its activity is independent of Krm proteins. Reprinted with permission from Baron, R and Rawadi G. Targeting the Wnt/β-Catenin Pathway to Regulate Bone Formation in the Adult Skeleton. Endocrinology 148: 2635-2643, 2007 ***Copyright (2007), The Endocrine Society.***

HORMONAL REGULATION OF SKELETAL AND MINERAL METABOLISM

PARATHYROID HORMONE

Parathyroid hormone is an 84-amino-acid peptide secreted by two pairs of parathyroid glands located adjacent to the back of the thyroid gland in the neck. There can also be ectopic parathyroid glands along their developmental route between the thyroid gland and mediastinum. The mature PTH is packaged into dense secretory granules for regulated secretion (1,2).

Secretory Regulation Of Parathyroid Hormone And The Calcium Sensor

PTH is synthesized as a 115 amino acid pre-pro-peptide, however, the 84 amino acid peptide is secreted by the parathyroid glands. The major regulatory signal for PTH secretion is serum calcium (Table 17) (30). Serum calcium inversely affects PTH secretion, with the steep portion of the sigmoidal response curve corresponding to the normal range of both. An increase in ionized calcium inhibits PTH secretion by increasing intracellular calcium through the release of calcium from intracellular stores and the influx of extracellular calcium through cell membranes and channels. This mechanism differs from most cells, where secretion of their product is stimulated by increased calcium. Intracellular magnesium may serve this secretory function in the parathyroids in that hypermagnesemia can inhibit PTH secretion and hypomagnesemia can stimulate PTH secretion. However, prolonged depletion of magnesium will inhibit PTH biosynthesis and secretion, as it will the function of many cells. Hypomagnesemia also attenuates the biological effect of PTH by interfering with its signal transduction. Serum calcium also inversely regulates transcription of the PTH gene, and increased levels of 1,25-dihydroxyvitamin D (1,25-D) inhibit PTH gene transcription. The parathyroid gland senses the concentration of extracellular ionized calcium through a cell-surface calcium-sensing receptor (CaSR) for which calcium is an agonist. The same sensor also regulates the responses to calcium of thyroid C cells, which secrete CT in direct relationship to extracellular calcium; the distal nephron of the kidney, where calcium excretion is regulated; the placenta, where fetal-maternal calcium fluxes occur; and the brain and gastrointestinal (GI) tract, where its function is unknown, and bone cells. Loss-of-function mutations of the CaSR cause familial hypocalciuric hypercalcemia (FHH) 1 (31). Two other mutations downstream in this pathway (GNA11 and AP2S1) have been identified that cause FHH2 and FHH3 respectively (31). Gain-of-function mutations of CaSR and GNA11 cause autosomal dominant hypocalcemia (ADH) type 1 and type 2 respectively (32).

Drugs have been identified that allosterically activate the CaSR (calcimimetics) and are useful treatment agents; they are available for treatment of the increased PTH secretion that occurs in secondary hyperparathyroidism of renal failure (oral cinacalcet, intravenous etelcalcetide) (33) severe primary hyperparathyroidism (oral cinacalcet), and parathyroid cancer (oral cinacalcet)(34). Calcilytic agents which antagonize the CaSR are being studied for treatment of ADH (35).

FGF23 may also inhibit PTH secretion, an action that requires binding to the FGF receptor and the co-receptor alphaKlotho (36). (See below)

Table 17. Regulation of PTH Biosynthesis and Secretion
Ambient calcium acting through the calcium sensing receptor (CaSR)
Vitamin D [1,25(OH)2D]
Ambient phosphorus
FGF23
Other

Some studies fail to demonstrate a direct effect of serum phosphate on PTH secretion, however, others show that high phosphate increases PTH biosynthesis and visa versa (4). However, serum phosphate has an inverse effect on calcium concentration and low ambient phosphate directly increases 1,25-D production. Thus, serum phosphate may directly and indirectly regulate PTH expression.

Metabolism And Clearance Of Parathyroid Hormone

Parathyroid hormone has a circulating half-life of less than 5 minutes (2,36). The hormone is metabolized to amino-terminal and carboxyl-terminal fragments primarily in the liver, also in the kidney, and perhaps in the parathyroid gland and blood. The carboxyl-terminal fragments are cleared by glomerular filtration (GF), so they accumulate in renal failure. All of the classic biological effects of PTH are mediated by the amino terminus, PTH1-34, and likely a subpeptide of this sequence, but other fragments may have their own biologic actions. For example, the carboxy terminus may regulate calcium channel flux.

As a result of the biosynthesis, secretion, and metabolism of PTH, the circulation contains several forms of the molecule (36). The forms that comprise this heterogenous collection of PTH species include primarily native PTH1-84 and amino terminal, mid-region and carboxy terminal PTH fragments. Overall, 10-20% of circulating PTH immunoreactivity comprises the intact hormone, with the remainder being a heterogeneous collection of peptide fragments corresponding to the middle and carboxy regions of the molecule. Recent studies have demonstrated a PTH 7- 84 fragment that accumulates in renal failure and may even be secreted by the normal as well as abnormal parathyroid gland. While only the amino terminus of PTH can bind to the PTH receptor at a site that mediates its classical biological effects, which result in hypercalcemia, PTH 7 – 84 may act as an antagonist and/or weak agonist to PTH at its receptor. Nevertheless, it should be kept in mind that each of the circulating forms of PTH, regardless of biological activity, contain within them peptide sequences that can be recognized by a variety of immunoassay systems and thus complicate clinical interpretation. The so-called intact PTH assays do not require the far amino-terminus of the molecule, a sequence need for full biological activity. The intact PTH assays recognize both PTH 1-84 and PTH 7-84. Newer assays, designated “bio-intact” or “whole” apparently do not recognize PTH 7-84, but there does not appear to be any clear clinical advantage of the “whole” compared to intact PTH assays (37).

Biologic Effects Of Parathyroid Hormone

Parathyroid hormone regulates serum calcium and phosphorus concentrations through its receptor-mediated, combined actions on bone, intestine, and kidney (3,38). The skeletal effects of PTH on bone are complex. High levels of PTH, as seen in primary and secondary hyperparathyroidism, increase osteoclastic bone resorption. Low levels, especially if delivered episodically, seem to increase osteoblastic bone formation, an effect that has been applicable to osteoporosis treatment by daily injections of teriparatide (PTH 1-34) (39) and the PHTrP analogue, abaloparatide (40). The skeletal effects of PTH are mediated through the osteoblast, since they are the major expressor of the PTH receptor. However, osteoblasts communicate with osteoclasts to mediate PTH effects. This communication seems mediated through the RANK-OPG pathway (21).

Any direct gastrointestinal (GI) effect of PTH on intestinal calcium or phosphate absorption is weak. However, PTH through its stimulating effects on the renal production of 1,25-D, discussed later, promotes the absorption of both. In the kidney, PTH increases the reabsorption of calcium, predominantly in the distal convoluted tubule, and inhibits the reabsorption of phosphate in the renal proximal tubule, causing hypercalcemia and hypophosphatemia. PTH also inhibits NA+/H+ antiporter activity and bicarbonate reabsorption, causing a mild hyperchloremic metabolic acidosis.

PTH mediates most of its effects through the PTH/PTHrP receptor (PTH1 receptor) (38). This receptor is an 80,000-MW membrane glycoprotein of the G protein receptor superfamily. The classic PTH receptor recognizes the amino-terminus of PTH and the homologous terminus of the parathyroid hormone-related protein (PTHrP) with indistinguishable affinity; it is therefore designated the PTH/PTHrP receptor. Both PTH and PTHrP generate cyclic adenosine

monophosphate (cAMP) as a cellular second messenger by activating protein kinase A (PKA), and the phospholipase C effector system increasing cellular IP3 and calcium and activating protein kinase C (PKC). There may be some tissue specificity as to which pathway dominates.

In addition to this shared receptor, there is accumulating evidence for the existence of receptors that are respectively specific for PTH and PTHrP and for some of their subpeptides. The PTH2 receptor is activated by PTH but not PTHrP and is expressed in brain and pancreas (41). For PTH, a carboxy-terminal peptide seems to mediate cellular calcium flux; for PTHrP, a nuclear localizing sequence (NLS) has been identified (38).

Table 18. Effects of Parathyroid Hormone on Calcium and Skeletal Metabolism
Bone
Increases resorption
Increases formation, especially at low and intermittent concentrations
Kidney
Decreases calcium excretion (clearance)
Increases phosphorus excretion
Gastrointestinal Tract
Increases calcium and phosphorus absorption
Indirect effect via 1,25-D production
Blood
Increases calcium
Decreases phosphorus

PARATHYROID HORMONE-RELATED PROTEIN (PTHrP)

PTHrP is a major humoral mediator of the hypercalcemia of malignancy (1,3,22). The polypeptide is a product of many normal and malignant tissues (22). PTHrP is secreted by many types of malignant tumors, notably by breast and lung cancer, and produces hypercalcemia by activating the PTH/PTHrP receptor. PTHrP is produced in many fetal tissues, but as development proceeds its expression becomes restricted. PTHrP expression reappears in adult tissues when injury or malignancy occurs (22).

The PTHrP gene expresses three native forms of the polypeptide through alternate mRNA splicing, PTHrP 1-141, a truncated 139 residue form, and a 173 residue form expressed primarily in humans (37). Whereas PTHrP 1-139 is quite similar to PTHrP 1-141, PTHRP 1-173 completely diverges from both at its own carboxy terminus. The amino-terminus of PTHrP reacts with the shared PTH/PTHrP receptor and has the potential to produce most of the biological effects of native PTH, including hypercalcemia. Other cell products, such as cytokines and growth factors, are also likely to play a casual role in the hypercalcemia because of their direct and indirect skeletal actions. As discussed later, these can be produced by the tumor cells or immune cells. TGF beta can also participate in pathogenesis by stimulating PTHrP production from tumors or immune cells as it is released from its skeletal reservoir upon resorption.

PTHrP is required for normal development as a regulator of the proliferation and mineralization of cartilage cells and as a regulator of local calcium transport. The amino terminus of PTHrP reacts with the PTH/PTHrP receptor and produces most of the

biological effects of native PTH, including hypercalcemia. The PTHrP gene expresses three forms of polypeptide through alternate messenger ribonucleic acid (mRNA) splicing. In addition to mRNA splicing, processing of PTHrP into peptides is an important regulatory mechanism. Distinct biological properties have been attributed to the different PTHrP peptides, and specific receptors and effects have been identified.

Although multiple, the functions of PTHrP in malignant and normal tissues seem to be growth- and proliferation-related (22). In most physiologic circumstances, PTHrP carries out local rather than systemic actions. When produced in excess by malignancy, PTHrP has systemic effects, especially hypercalcemia. Because of its protean and developmental effects, PTHrP can be considered an oncofetal protein.

Malignancy and PTHrP

The hypercalcemia of malignancy is usually due to increased bone resorption that is caused by skeletal metastases or the production by the tumor of a "humour" that stimulates osteoclasts (22). It is likely that the first mechanism also involves the second, since most tumor cells do not have the capacity to directly resorb bone and more likely stimulate the neighboring osteoclast to do so through their "humours." Many cell types and their products participate in and many tumor products have been implicated in the pathogenesis of the hypercalcemia of malignancy (Figure 5). The most common seems to be PTHrP, especially in solid tumors where abnormal PTHrP expression can be implicated in up to 80% of patients. Originally discovered as a product of malignant cells that produce hypercalcemia, PTHrP has been demonstrated to be a product of many normal and malignant tissues. The growing appreciation of the key role of PTHrP in the pathogenesis of the hypercalcemia of malignancy has revealed that ectopic PTH production by cancer cells is a rare event.

PTHrP expression was initially noted to be common in squamous cell cancers, but it has been subsequently shown that many other cancer types can overexpress PTHrP. PTHrP production and secretion by breast and prostate cancers is especially common, occurring in more than half of the cases, with even a higher incidence in breast when the patient is hypercalcemic. Breast tumors that produce PTHrP are more likely to metastasize to bone, and breast cancers that metastasize to bone are even more likely to produce PTHrP. PTHrP is commonly expressed in lung cancer, especially in those lung cancers that metastasize to bone. While breast and lung cancer are among the most common PTHrP producing tumors that cause hypercalcemia, this pathway has been described in many cancers. PTHrP production that often accompanies prostate cancer does not usually cause hypercalcemia, perhaps because this tumor processes the polypeptide to a non-hypercalcemic peptide. It is notable that some non-malignant PTHrP-producing tumors can also be associated with hypercalcemia (42).

While PTHrP is the most common humour produced by malignant cell to cause osteoclast-mediated hypercalcemia, increased 1,25-dihydroxy vitamin D is causal in lymphomas and some leukemias. Furthermore, certain cytokines, notably IL-1, and growth factors, notably TGF beta, can also produce hypercalcemia by stimulating osteoclastic bone resorption; but excess prostaglandin production is no longer considered an important hypercalcemic humour in malignancy.

VITAMIN D

Metabolism and Activation

Vitamin D is a secosterol hormone that is present in humans in an endogenous (vitamin D3) and exogenous (vitamin D2) form (43, 44). The endogenous form of vitamin D, cholecalciferol (vitamin D3), is synthesized in the skin from the cholesterol metabolite 7-dehydrocholesterol under the influence of ultraviolet radiation. Vitamin D3 is also available in

oral supplements. An exogenous form of vitamin D (vitamin D2) (ergocalciferol) is produced by ultraviolet irradiation of the plant sterol ergosterol and is available through the diet. Both forms of vitamin D require further metabolism to be activated, and their respective metabolism is indistinguishable. Vitamin D metabolites are solubilized for transport in blood by specific vitamin D-binding proteins.

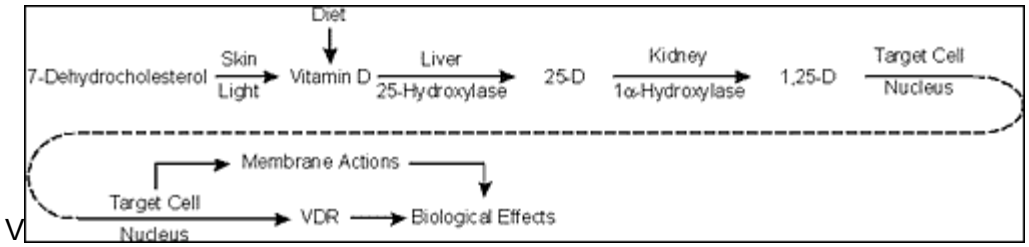

Figure 8. The Metabolic Activation of Vitamin D. Abbreviations: 25-D, 25-hydroxyvitamin D; 1,25-dihydroxyvitamin D; VDR, vitamin D receptor. Vitamin D from the diet or the conversion from precursors in skin through ultraviolet radiation (light) provides the substrate of the indicated steps in metabolic activation. The pathways apply to both the endogenous animal form of vitamin D (vitamin D3, cholecalciferol) and the exogenous plant form of vitamin D (vitamin D2, ergocalciferol), both of which are present in humans at a ratio of approximately 2:1. In the kidney, 25-D is also converted to 24-hydroxylated metabolites which seem generally inactive but may have unique effects on chondrogenesis and intramembranous ossification. The many effects (Table 8) of vitamin D metabolites are mediated through nuclear receptors or effects on target-cell membranes (see Acknowledgments).

In the liver, vitamin D is converted by a hydroxylase to 25-hydroxyvitamin D (25-D), the principal fat storage form of vitamin D (45). Thus, the serum level of 25-D is the best measure of overall vitamin D status. In the proximal tubule of the kidney, 25-D is 1alpha-hydroxylated to produce 1,25-D, the most active form of the hormone. The animal form is referred to as 1,25-dihydroxycholecalciferol. This hydroxylation step is up-regulated by several factors, the most important of which are PTH and low ambient concentrations of calcium, phosphorus, and 1,25-D itself. The 1alpha-hydroxylase that mediates this conversion in the kidney is also produced in the placenta and in keratinocytes. In certain disease states, macrophages (e.g., in sarcoidosis) and lymphocytes (e.g., in lymphoma) overexpress 1alpha-hydroxylase and produce hypercalcemia (46).

The normal serum concentration of 1,25-D is about 20-60 pg/ml. The kidney can also convert 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D. Although this metabolite circulates at 100-fold higher than the concentration of 1,25-D, its biologic role is unclear. Some studies suggest that it is a degradation product with no important biological effects; others suggest that it is important in chondrogenesis and bone formation, especially intramembranous. Vitamin D and its metabolites are inactivated in the liver by conjugation to glucuronides or sulfates and oxidation of their side chains. Mutations of the 24-hydroxylase enzyme (CYP24A1) have been shown to cause hypercalcemia and hypercalciuria in infants and adults (47). In this condition, 1,25(OH)vitamin D levels are elevated because of inadequate metabolism of 1,25(OH)2D (47). Studies also suggest the presence of the C-3 epimer of 25(OH)D in serum (48). The biologic importance of this epimer is unknown.

There is controversy about the optimal 25(OH) vitamin D level. The Institute of Medicine (IOM) has suggested that a 25(OH) vitamin D > 20 ng/ml is adequate (49), while The Endocrine Society suggests that > 30 ng/ml is optimal (50). The IOM suggests that supplements of 600-800 IU daily will produce adequate levels in most adults, with an upper safe dose of 4000 IU daily (49).

Biological Effects of Vitamin D and It's Mechanism of Action

Vitamin D mediates its biological effects through its own member of the nuclear hormone receptor superfamily, the vitamin D receptor (VDR) (43). The receptor binds many vitamin D metabolites with affinities that generally mirror their biological effects, and 1,25-D thus has the highest affinity. The VDR regulates gene transcription by homodimerization and by heterodimerization to a retinoic acid X receptor (RXR). The complex binds to target DNA sequences and regulates the transcription of several genes important in mediating vitamin D's effects on calcium and skeletal metabolism and its diverse biological effects. Vitamin D metabolites, as well as other steroid hormones, may also act through a membrane receptor to produce rapid changes in cellular calcium flux (Figure 7) (51).

There continues to be debate about the relative importance of Vitamin D2 and Vitamin D3 in human health and disease. Administration of vitamin D3 may result in more persistent elevation of 25(OH)D than administration of vitamin D2 (52-54).

Intestinal Calcium Absorption

Vitamin D increases intestinal calcium absorption, primarily in the jejunum and ileum, by increasing calcium uptake through the brush border membrane of the enterocyte (Tables 8, 9, and 19). For this action, vitamin D induces the calcium-binding calbindins, which participate in calcium transport across the cell, and through its action on calcium transporting membrane structures (Figure 2), it promotes the efflux of calcium from the basolateral side of the enterocyte into the circulation. The initial effects of vitamin D on intestinal calcium absorption occur within minutes, so the actions of vitamin D on intestinal calcium transport may be also mediated by a membranous nongenomic receptor. The net result is an increase in the efficiency of intestinal calcium transport. In a vitamin D-deficient state, only 10 to 15% of dietary calcium is absorbed by the gastrointestinal tract, but with adequate vitamin D adults absorb approximately 30% of dietary calcium. During pregnancy, lactation, and growth, increased circulating concentrations of 1,25-D promote the efficiency of intestinal calcium absorption by as much as 50% to 80%. Vitamin D also regulates skeletal metabolism through the RANK pathway (Figure 6). 1,25-D also increases the efficiency of dietary phosphorus absorption by about 15 to 20%.

Table 19. Mechanisms of GI Calcium Absorption
Vitamin D Dependent
Duodenum > jejunum > ileum
Active transport across cells
calcium binding proteins (calbindins)
calcium channels and pumps
Na exchanger
Passive diffusion

Bone

The effects of vitamin D metabolites on bone are complex (1). By providing sufficient ambient calcium and/or through some other unappreciated direct effect, vitamin D promotes the mineralization of osteoid. Vitamin D causes bone resorption by mature osteoclasts, but this effect is indirect, requiring cell recruitment and interaction with osteoblasts. Vitamin D also promotes the fusion of monocytic precursors to osteoclasts. Vitamin D regulates the expression several bone proteins, notable osteocalcin. It promotes the transcription of osteocalcin and has

bidirectional effects on type I collagen and alkaline phosphatase gene transcription

Kidney

The VDR is robustly expressed in the kidney, and acting through it, 1,25-D stimulates renal proximal phosphate reabsorption and maintenance of normal calcium reabsorption. However, compared to PTH, these effects are relatively weak (43).

Other Tissues

Vitamin D and its metabolites have protean effects on cell function and signaling (45). Although vitamin D has many in vitro effects on the immune system, no major immune defect is apparent in individuals who are deficient or who lack vitamin D or its receptor. Vitamin D also inhibits proliferation and stimulates maturation of epidermal keratinocytes, which robustly express the VDR. This antiproliferative effect is being used for the treatment of psoriasis, a hyperproliferative skin disorder. Since many persons who lack vitamin D receptors have lifelong alopecia totalis, vitamin D may play a role in the maturation of the hair follicle (55).

Many studies have suggested the association of low 25(OH)D levels with a variety of diseases including cardiovascular, metabolic, autoimmune, malignant, and neurologic disorders. Thus far, these largely observational findings have not been confirmed in randomized trials (56).

Table 20. Effects of 1,25-D (1,25-dihydroxyvitamin D) on Mineral Metabolism

Bone
Promotes mineralization of osteoid
Increases resorption at high doses
Kidney
Decreases calcium excretion
Decreases phosphorus excretion
Gastrointestinal Tract
Increases calcium absorption
Increases phosphorus absorption
Blood
Increases calcium
Increases phosphorus

FGF23

FGF23 is a 251 amino acid peptide hormone produced by osteoblasts, osteocytes and flattened bone-lining cells. *O*-glycosylation of FGF23 by UDP-N-acetyl-alpha-D-galactosmanine; polypeptide N- acetylgalactosaminyl transferase 3 (GALNT3) at specific sites is required to prevent intracellular degradation of the intact active molecule. The action of FGF23 is mediated by binding an FGF receptor (FGFR) with it's coreceptor alphaKlotho (9,12).

FGF23 decreases production of the sodium phosphate cotransporters, Npt2a and Npt2c. As these cotransporters increase phosphate reabsorption in the renal proximal tubule, FGF23 increases renal phosphate wasting. FGF23 also decreases 1,25(OH)2D levels probably by decreasing the expression of the 1 alphahydroxylase enzyme and increasing production of the 24-hydroxylase enzyme (9,12).).

FGF23 expression is regulated by phosphorus and by 1,25(OH)2D. Although regulation by 1,25(OH)2D is believed to be via the vitamin D receptor, the mechanism

of phosphate sensing is unknown. Iron deficiency also increases FGF23 transcription and translation. In normal subjects, however, increased processing of FGF23 prevents hypophosphatemia. Patients with autosomal dominant hypophosphatemic rickets (ADHR), however, who have an abnormal FGF23 which is resistant to degradation may not be able compensate particularly when iron deficiency is present (57).

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X-linked hypophosphatemic rickets (XLH) (*PHEX gene*), autosomal dominant hypophosphatemic rickets (ADHR) *(FGF23 gene),* autosomal recessive hypophosphatemic rickets (ARHR) *(DMP1, ENPP1, FAM20C genes),* and tumor-induced osteomalacia (TIO) are associated with excessive FGF23 (58). Interestingly, intravenous iron (especially iron carboxymaltose) may cause FGF23 mediated renal phosphate wasting, hypophosphatemia, and osteomalacia (59). Recently, a monoclonal antibody to FGF23 (burosumab) was approved for treatment of XLH and TIO (18). Loss-of-function mutations of GALNT3, FGF23, and alpha Klotho result in decreased intact FGF23 levels or decreased FGF23 action and result in hyperphosphatemia and tumoral calcinosis. (9, 58)

FGF23 is elevated in chronic kidney disease. Elevations of FGF23 may be associated with progression of renal disease, left ventricular hypertrophy, cardiovascular events, and mortality. It is not known whether these associations are due to FGF23 or are related to more severe underlying disease (9,12) FGF23 may be measured by a c-terminal assay which measures full-length FGF23 in addition to c-terminal fragments as well as by an intact assay. FGF23 in both assays is elevated or inappropriately normal in XLH, ADHR, ARHR, and TIO. In tumoral calcinosis due to FGF23 and GALNT3 mutations, these assays may be discordant with elevated C-terminal FGF23 and reduced intact (active) FGF23. FGF23 measured by both assays is elevated in TC caused by Klotho mutations (58) because of resistance to FGF23.

Table 21. FGF23 Secretion and Action
FGF23 Secretion
Increased by high phosphate
Increased by high 1,25(OH)2D
FGF23 Action
Mediated via FGF receptor and Klotho
Increases renal phosphate wasting
Decreases production of 1,25(OH)2D
Lowers serum phosphate

CALCITONIN

Calcitonin is a 32-amino acid peptide whose main effect is to inhibit osteoclast-mediated bone resorption (60). CT is secreted by parafollicular C cells of the thyroid and other neuroendocrine cells. Hypercalcemia increases secretion of hypocalcemia-inducing CT while hypocalcemia inhibits secretion (61). CT secretion is controlled by serum calcium through the same CaSR that regulates PTH secretion, but in an inverse manner and at higher concentrations of calcium. CT directly inhibits bone resorption by inactivating the CT-receptor rich osteoclast. CT also inhibits the renal reabsorption of phosphate, thus promoting renal phosphate excretion. CT also induces a mild natriuresis and calciuresis, the latter contributing to its hypocalcemic effect. However, calcitonin does not appear to have a major effect on human calcium metabolism as evidenced by normocalcemia in thyroidectomized patients as well as

patients with medullary thyroid cancer and very high calcitonin levels (10,60). Calcitonin in pharmacologic doses has been used to decrease bone resorption in osteoporosis, Paget's bone disease, and hypercalcemia of malignancy (10). It is unclear whether long-term use of calcitonin is associated with increased cancer risk (62).

Table 22. Regulation of Calcitonin Secretion
Calcium and related ions (CaSR)
Age and gender
Gastrointestinal factors

The CT receptor, like the PTH and calcium-sensing receptor, is a heptahelical G protein-coupled receptor coupled to the PKA, PKC, and Ca++ signal transduction pathways (63, 64).

The CT gene through alternative exon splicing and polypeptide processing ultimately encodes two peptide products, CT in thyroid C-cells which is processed from a 141-amino acid precursor, and a 37-amino peptide called gene-related peptide (CGRP) in neural tissues which is processed from a 128-amino acid precursor (1,65). CGRP is weakly recognized by the CT receptor and thereby has a CT-like effect on osteoclasts and osteoblasts. CGRP also acts through its own receptor to produce vasodilation and to act as a neurotransmitter. In addition to its role in calcium and skeletal metabolism, CT is important as a tumor marker in medullary thyroid carcinoma and other neuroendocrine tumors. The receptor that mediates the effects of the peptide products of the CT gene can be modulated by accessory proteins to alter binding characteristics (65).

Table 23. Effects of Calcitonin on Mineral Metabolism
Bone
Inhibits resorption
Kidney
Increases calcium excretion
Increases phosphorus excretion
Gastrointestinal Tract
? Inhibitory effect on calcium/phosphorus absorption
Blood
Decreases calcium
Decreases phosphorus

OTHER HORMONES

In addition to the primary calcemic hormones, other hormones play an important role in calcium and skeletal metabolism (1-3). Gonadal steroids maintain skeletal mass. Estrogen deficiency is a major factor in the development of postmenopausal osteoporosis by permitting increased bone resorption. There is controversy about whether the elevation in FSH that accompanies menopause also contributes to increased bone resorption (66). In an animal model, a blocking antibody to the beta subunit of FSH decreased bone resorption (67). Glucocorticoids have significant deleterious effects on the skeleton including decreased bone density, increased fracture risk, and increased risk of avascular necrosis (68). Glucocorticoids transiently increase bone resorption, chronically decrease bone formation and cause osteoblast and osteocyte apoptosis (68). Insulin, growth hormone, and thyroid hormones promote

skeletal growth and maturation. Excess production of the latter can cause hypercalcemia (Table 24).

Table 24. Effects of Calcitonin on Mineral Metabolism

Decrease Bone Resorption
Calcitonin
Estrogens
Increase Bone Resorption
PTH/PTHrP
Glucocorticoids (early)
Thyroid Hormones
High dose vitamin D
? FSH
Increase Bone Formation
Growth Hormone
Vitamin D Metabolites
Androgens
Insulin
Low-dose PTH/PTHrP
Decrease Bone Formation
Glucocorticoids (also increase osteocyte apoptosis)

SUMMARY

Through their actions and interactions on bone, kidney and the gastrointestinal (GI) tract, the calciotropic hormones, parathyroid hormone (PTH), FGF23, and vitamin D metabolites, especially 1,25-D, act to maintain serum (and extracellular fluid) calcium within a normal range, a range that optimally subserves many calcium-requiring physiological functions such as neural transmission and muscle contraction. Perturbations in serum calcium, which plays an important role in regulating the concentrations of the calciotropic hormones, will cause a homeostatically appropriate and reciprocal change in the secretion of PTH by the parathyroid glands. These responses are designed to return the serum calcium, and, to a lesser extent, the serum phosphorus and magnesium to normal, with the skeleton acting as a reservoir for these minerals that can be emptied or filled. During the last several years, a more physiologically integrated view of calcium metabolism has emerged. The metabolism of the skeleton has been linked to the metabolism of glucose in a manner that coordinates the regulation of bone mass with energy expenditure. And in addition to peripheral hormone regulation, the CNS exerts important regulatory effects on both systems, which encompass calcium and glucose metabolism, body and skeletal mass regulations, and energy expenditure and appetite.

The patient with hypoparathyroidism will have hypocalcemia with an inappropriately normal or low PTH and low 1,25(OH)2D.

The patient with nonparathyroid hypocalcemia will have an increased serum PTH and1,25-D (unless vitamin D stores are severely reduced). This will result in increased GI absorption of calcium, increased bone resorption, and decreased renal calcium excretion all acting to increase the serum calcium toward normal.

The patient with primary hyperparathyroidism will have hypercalcemia and inappropriately normal or elevated PTH. The patient with PTH-independent hypercalcemia (e.g., due to bone metastases) will have a decreased serum PTH and 1,25-D (unless the hypercalcemia is PTHrP-mediated or calcitriol-mediated). This will result in decreased GI absorption of calcium, decreased bone resorption, and increased renal calcium excretion all acting to decrease the serum calcium toward normal. Although these compensatory mechanisms act to restore serum calcium to normal, the homeostasis will not be complete until the primary abnormality has been corrected. In addition to these calciotropic hormones, other hormones, cytokines, and growth factors play an important role in calcium metabolism. Among the other important hormones are insulin, growth hormone, and the gonadal and adrenal steroids and thyroid hormone (Table 20). They are discussed in other chapters.

FGF23 is an important phosphate regulator with excess action causing renal phosphate wasting, hypophosphatemia, and low 1,25(OH)2D and decreased action causing renal phosphate retention, hyperphosphatemia, and inappropriately high 1,25(OH)2 D levels.

CLINICAL IMPLICATIONS

The clinician can consider a simplified scheme when confronted with a patient with a disorder of calcium and skeletal metabolism – the serum or urinary calcium can be abnormally high or low and bone density can be increased or decreased.

In practical terms, when the serum calcium is high, primary hyperparathyroidism, granulomatous and inflammatory conditions causing unregulated 1,25D production, and malignancy are at the top of the diagnostic list. When the serum calcium is low, hypoparathyroidism, malabsorption, vitamin D deficiency, and kidney disease should be considered. Chronically abnormal phosphate levels in the non-acutely ill patient may be caused by renal failure, renal tubular defects, and abnormalities of FGF23 action.
When bone density is decreased, it is usually due to osteoporosis or osteomalacia; when increased, osteopetrosis and other osteosclerotic disorders should be considered.
These diagnostic categories can be properly assigned when one considers the interaction among the calcium regulating hormones that have been described in this chapter and orders the appropriate diagnostic tests. In most cases, the correct diagnosis is readily made.

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