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Hormonal Regulation of Calcium and Phosphate Metabolism

LEARNING OBJECTIVES

Upon completion of this chapter the student should be able to answer the following questions:

1. Describe the pool of serum calcium and phosphate, including ionized, complexed, and protein bound. Describe the normal concentration ranges of these ions and the major routes of influx and efflux.
2. Discuss the role of the parathyroid gland in the regulation of serum calcium and explain the role of the calcium-sensing receptor in the regulation of parathyroid hormone (PTH) secretion.
3. Describe the production of 1,25-dihydroxyvitamin D, including sources of vitamin D precursor, sites and key regulators of vitamin D hydroxylation, and transport of vitamin D metabolites in the blood.
4. List the target organs of PTH and describe its effects on calcium and phosphate mobilization or handling at each of these sites.
5. List the target organs and key actions of 1,25-dihydroxyvitamin D.
6. Discuss the regulation of phosphate metabolism by FGF23.
7. Predict the hormone responses that would be triggered by perturbations of serum calcium and phosphate or by vitamin D deficiency, and discuss the consequences of these compensatory hormone actions.

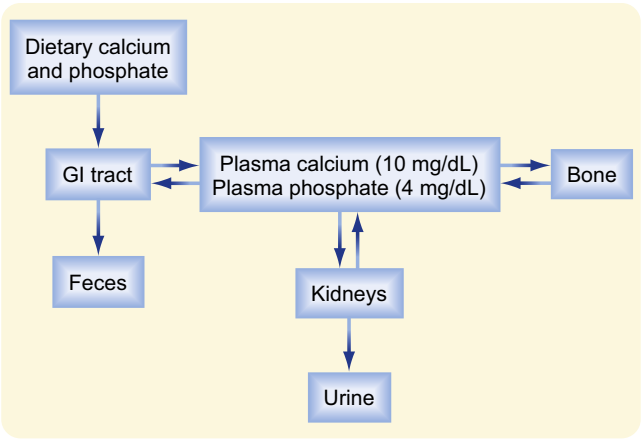
Calcium (Ca) and phosphate are essential to human life because they play important structural roles in hard tissues (i.e., bones and teeth) and important regulatory roles in metabolic and signaling pathways. In biological systems, **inorganic phosphate (P_i)** consists of a mixture of dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^-). The two primary sources of circulating Ca and P_i are the diet and the skeleton (Fig. 40.1). Two hormones, **1,25-dihydroxyvitamin D** (also called **calcitriol**) and **parathyroid hormone (PTH),** regulate intestinal absorption of Ca and P_i and release of Ca and P_i into the circulation after bone resorption. The primary processes for removal of Ca and P_i from blood are renal excretion and bone mineralization (see Fig. 40.1). 1,25-Dihydroxyvitamin D and PTH regulate both processes. Fibroblast Growth Factor-23 (FGF23) regulates serum P_i by inhibiting its renal reabsorption.

Crucial Roles of Calcium and Phosphate in Cellular Physiology

Ca is an essential dietary element. In addition to obtaining Ca from the diet, humans contain a vast store (i.e., >1 kg) of Ca in bone mineral, which can be called upon to maintain normal circulating levels of Ca in times of dietary restriction and during the increased demands of pregnancy and nursing. Circulating Ca exists in three forms (Table 40.1): free ionized Ca^{++}, protein-bound Ca, and Ca complexed with anions (e.g., phosphates, HCO_3^-, citrate). The ionized form represents about 50% of circulating Ca. Since it is critical to so many cellular functions, [Ca^{++}] in both the extracellular and intracellular compartments is tightly controlled. Circulating Ca^{++} is under direct hormonal control and normally maintained within a relatively narrow range. Either too little calcium (**hypocalcemia;** total serum calcium < 8.7 mg/dL [2.2 mM]) or too much Ca (**hypercalcemia;** total serum Ca > 10.4 mg/dL [2.6 mM]) in blood can lead to a broad range of pathophysiological changes, including neuromuscular dysfunction, central nervous system dysfunction, renal insufficiency, calcification of soft tissue, and skeletal pathology.

P_i is also an essential dietary element, and it is stored in large quantities in mineral. Most circulating P_i is in the free ionized form, but some P_i (<20%) circulates as a protein-bound form or complexed with cations (see Table 40.1). Because soft tissues contain 10-fold more P_i than Ca, tissue damage (e.g., crush injury with massive muscle cell death) can result in **hyperphosphatemia,** whereupon the increased P_i complexes with Ca^{++} to cause acute hypocalcemia.

P_i is a key intracellular component. Indeed, it forms the high-energy phosphate bonds of adenosine triphosphate (ATP) that maintain life. Phosphorylation and dephosphorylation of proteins, lipids, second messengers, and cofactors represent key regulatory steps in numerous metabolic and signaling pathways, and phosphate also serves as the backbone for nucleic acids.



• **Fig. 40.1** Daily Ca^{++} and P_i flux.

TABLE 40.1 Forms of Ca and P_i in Plasma

Ion	mg/dL	Ionized	Protein Bound	Complexed
Ca	8.5–10.2	50%	45%	5%
P_i	3–4.5	84%	10%	6%

Ca^{++} is bound (i.e., complexed) to various anions in plasma, including HCO_3^-, citrate, and SO_4^{2-}. P_i is complexed to various cations, including Na^+ and K^+.
From Koeppen BM, Stanton BA. *Renal Physiology.* 4th ed. Philadelphia: Mosby; 2007.

Hormonal Regulation of Calcium and Phosphate: PTH, Vitamin D, and FGF23

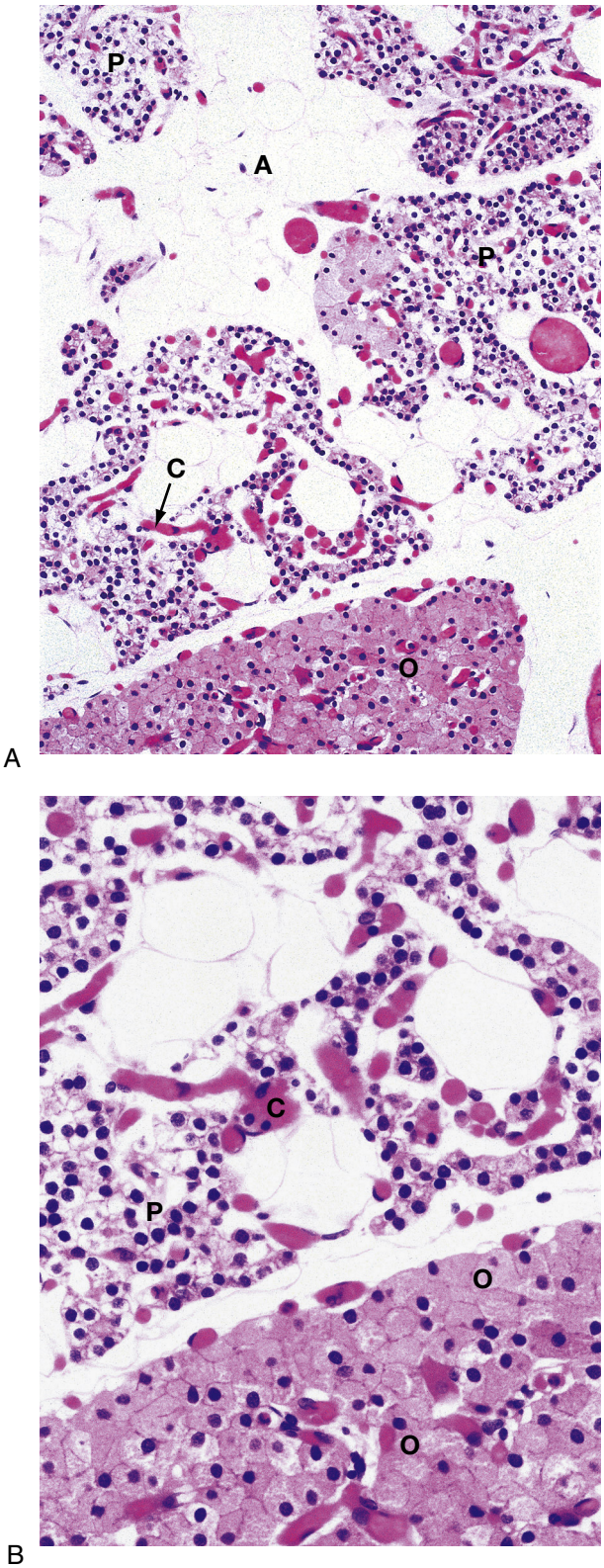
Classically, **PTH** and **1,25-dihydroxyvitamin D** are the most important hormones dedicated to the maintenance of normal blood Ca and P_i in humans. As such, they are referred to as **calciotropic hormones.** More recently, a role for fibroblast growth factor-23 (FGF23), produced by osteocytes in bone, has been elucidated in the regulation of serum P_i levels. The structure, synthesis, and secretion of these hormones and their receptors will be discussed first. In the following section, the detailed actions of PTH, 1,25-dihydroxyvitamin D, and FGF-23 on key target organs (i.e., gut, bone, and kidney) are discussed.

Parathyroid Hormone

The predominant parenchymal cell type in the parathyroid gland is the **principal** (also called **chief**) **cell** (Fig. 40.2). PTH produced and secreted by these cells is the primary hormone that protects against hypocalcemia. The direct targets of PTH are bone and the kidneys. PTH also functions in a positive feed-forward loop by stimulating the production of 1,25-dihydroxyvitamin D.

Structure, Synthesis, and Secretion

PTH is secreted as an 84–amino acid polypeptide and is synthesized as **prepro-PTH,** which is proteolytically



• **Fig. 40.2** **A** and **B**, Histology of parathyroid glands. *A,* Adipose tissue within parathyroid glands; *C,* capillaries; *O,* oxyphil cells; *P,* principal or chief cells. (From Young B et al. *Wheater's Functional Histology.* 5th ed. Philadelphia: Churchill Livingstone; 2006.)

processed to **pro-PTH** in the endoplasmic reticulum and then to PTH in the Golgi apparatus and secretory vesicles. PTH has a short half-life in the circulation (2 minutes), consistent with its role in minute-to-minute regulation of plasma calcium.

AT THE CELLULAR LEVEL

Extracellular [Ca^{++}] is sensed by the parathyroid chief cell through a plasma membrane **calcium-sensing receptor (CaSR).** The primary signal that stimulates PTH secretion is a decrease in circulating [Ca^{++}] (Fig. 40.3). Conversely, increasing amounts of extracellular Ca^{++} bind to the CaSR and stimulate signaling pathways that repress PTH secretion. Although the CaSR binds to extracellular Ca^{++} with relatively low affinity, the CaSR is extremely sensitive to minute changes in extracellular [Ca^{++}]. The relationship between [Ca^{++}] and the rate of PTH secretion is described by a steep inverse sigmoidal curve. A 0.2-mM difference in blood [Ca^{++}] spans the full range of the curve, altering PTH secretion from basal (5% of maximum) to maximal levels (Fig. 40.4). The steady-state "set point" will vary between individuals but typically resides below the midpoint of the curve (i.e., half-maximal PTH secretion). Thus the CaSR is a rapid, robust, and continuous regulator of PTH output in response to subtle [Ca^{++}] fluctuations.

In addition to inhibiting PTH secretion, activation of the CaSR also promotes degradation of stored PTH in the parathyroid chief cell. As a result, biologically inactive carboxy-terminal PTH fragments are secreted from the parathyroid gland and are also produced by peripheral metabolism of PTH by the liver and kidney. Therefore, current PTH assays use two antibodies that recognize epitopes from both ends of the molecule to accurately measure intact PTH(1-84).

Over a longer time frame, PTH production is also regulated at the level of mRNA stability and gene transcription (see Fig. 40.3). Decreased [Ca^{++}] leads to production of proteins that bind the 3-untranslated region of PTH mRNA and stabilize it, leading to increased PTH translation. *PTH* gene transcription is repressed by 1,25-dihydroxyvitamin D in a negative feedback loop (acting through vitamin D response elements—see later). The ability of 1,25-dihydroxyvitamin D to hold *PTH* gene expression in check is reinforced by the coordinated upregulation of *CASR* gene expression by positive vitamin D response elements in the promoter region of the *CASR* gene (see Fig. 40.3). It should be noted, however, that during a hypocalcemic challenge, the decrease in [Ca^{++}] overrides the inhibitory effect of 1,25-dihydroxyvitamin D on PTH transcription, allowing both of these hormones to be elevated simultaneously.

IN THE CLINIC

Patients with **benign familial hypocalciuric hypercalcemia (FHH)** are heterozygous for inactivating mutations of the CaSR. In these patients, because of complete or partial loss of one CaSR allele, higher levels of [Ca^{++}] are required to suppress PTH secretion. This results in an elevated [Ca^{++}] set point for PTH secretion, accounting for the hypercalcemia. The CaSR is also expressed in the thick ascending limb of the renal tubule, where it normally inhibits Ca^{++} reabsorption when blood Ca^{++} rises. The hypocalciuria in the face of hypercalcemia in FHH is due to the reduced ability of the CaSR in the kidney to sense and respond to elevated blood [Ca^{++}] by increasing Ca excretion.

AT THE CELLULAR LEVEL

Parathyroid hormone–related peptide (PTHrP) is a peptide paracrine hormone produced by several adult tissues (skin, hair, breast), where it may regulate proliferation and differentiation. It also plays a role in relaxation of smooth muscle in response to stretch in blood vessels, uterus, and bladder. During lactation, PTHrP promotes maternal bone resorption and the transport of calcium into milk. During development, PTHrP regulates calcium transport across the placenta and is a key regulator of chondrocyte proliferation and differentiation in the growth plate of long bones. The 30 amino acids at the N-terminus of PTHrP have significant structural homology with PTH. PTHrP is not regulated by circulating Ca^{++} and normally does not play a role in Ca/P_i homeostasis in adults. However, certain tumors secrete high levels of PTHrP, which causes **hypercalcemia of malignancy** and symptoms that resemble hyperparathyroidism.

Parathyroid Hormone Receptor

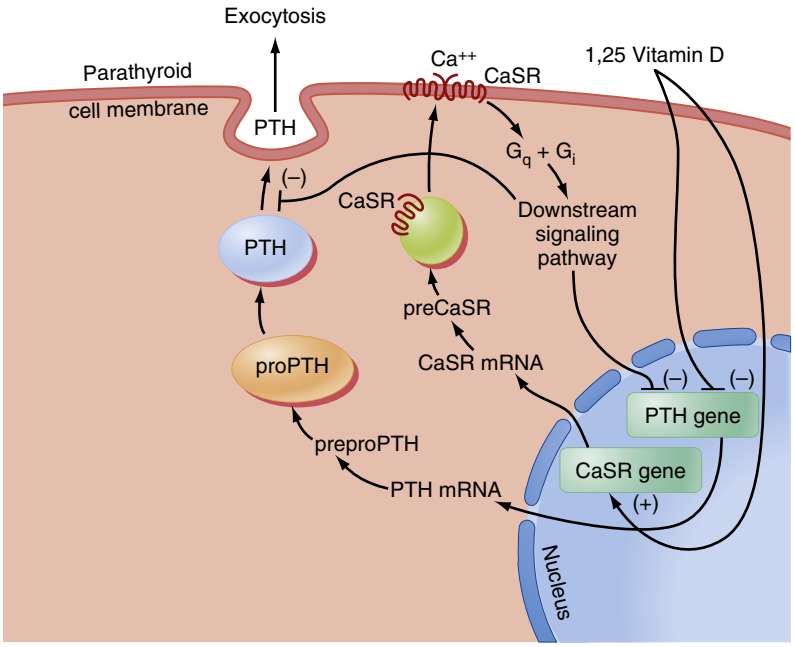
The PTH receptor, designated PTHR1, binds both PTH and PTH-related peptide (PTHrP). It is expressed on osteoblasts and osteocytes in bone and in the proximal and distal tubules of the kidney to mediate the systemic actions of PTH. However, PTHR1 is also expressed in many developing organs where PTHrP has important paracrine functions. One such example is regulation of chondrocyte proliferation in the growth plate during endochondral bone growth.

Vitamin D

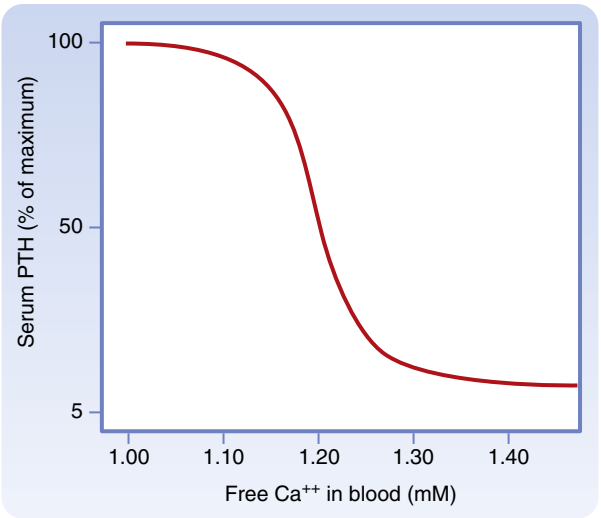
Vitamin D is a prohormone that must undergo two successive hydroxylation reactions to become the active form known as **1,25-dihydroxyvitamin D** or **calcitriol** (Fig. 40.5). This hormone plays a critical role in Ca absorption and, to a lesser extent, P_i absorption by the small intestine. It also regulates bone metabolism and renal reabsorption of Ca and P_i.

Structure, Synthesis, and Transport of Active Vitamin D Metabolites

Vitamin D_3 (also called **cholecalciferol**) is synthesized via conversion from 7-dehydrocholesterol by ultraviolet B (UVB) light in the basal layers of the skin (Fig. 40.6). Chemically, vitamin D_3 is a **secosteroid** in which one of the cholesterol rings is opened (see Fig. 40.5). **Vitamin D_2 (ergocalciferol)** is produced in plants. Vitamin D_3 and, to a lesser extent, vitamin D_2 are absorbed from the diet and are both effective after conversion to active hydroxylated forms. The balance between UVB-dependent endogenously synthesized vitamin D_3 and absorption of the dietary forms of vitamin D becomes important in certain situations. Individuals who have higher melanin content in skin and/or live at higher latitudes convert less 7-dehydrocholesterol to vitamin D_3 and thus are more dependent on vitamin supplements or dietary sources of vitamin D (natural or fortified, e.g. milk). Institutionalized elderly patients who stay



• **Fig. 40.3** Regulation of *PTH* gene expression and secretion. *CaSR,* Calcium-sensing receptor; *PTH,* parathyroid hormone. (Modified from Porterfield SP, White BA. *Endocrine Physiology.* 3rd ed. Philadelphia: Mosby; 2007.)



• **Fig. 40.4** Sigmoidal relationship between serum [Ca^{++}] and serum PTH, which reflects the rate of PTH secretion. *PTH,* Parathyroid hormone. (Modified from Porterfield SP, White BA. *Endocrine Physiology.* 3rd ed. Philadelphia: Mosby; 2007.)

indoors and avoid dairy products are particularly at risk for development of **vitamin D deficiency.**

Vitamin D is transported in blood from the skin to the liver. Dietary vitamin D reaches the liver directly via transport in the portal circulation and indirectly via chylomicrons (see Fig. 40.6). In the liver, vitamin D is hydroxylated at the 25-carbon position to yield **25-hydroxyvitamin D.** The hepatic 25-hydroxylase is constitutively expressed and unregulated, so circulating levels of 25-hydroxyvitamin D reflect the amount of precursor available for 25-hydroxylation. For this reason, and because of its relatively long half-life in the circulation (2–3 weeks), measurement of 25-hydroxyvitamin D levels is used to assess vitamin D status.

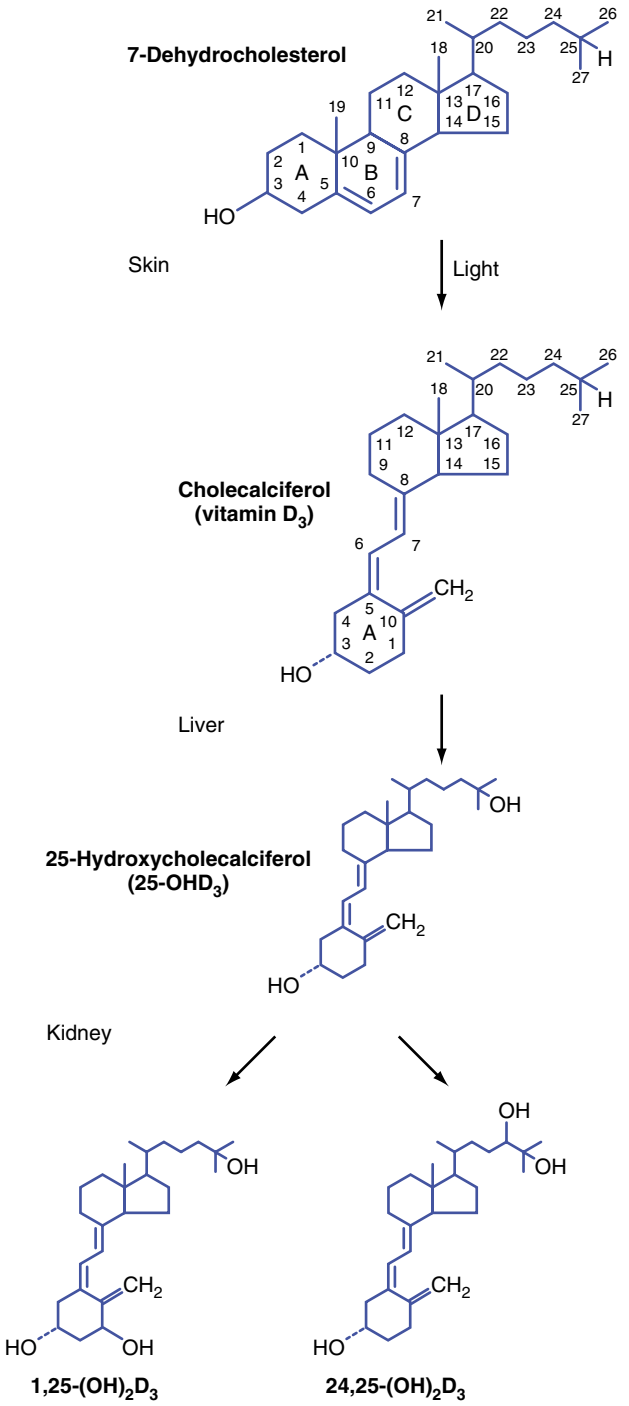
25-Hydroxyvitamin D undergoes further hydroxylation in the proximal tubule of the kidney (see Figs. 40.5 and 40.6). Hydroxylation at the 1α position generates **1,25-dihydroxyvitamin D,** the most active form of vitamin D. Hydroxylation at the 24 position generates **24,25-dihydroxyvitamin D,** which represents an inactivation pathway.

Renal 1α-hydroxylase is tightly regulated by a number of factors (Fig. 40.7). PTH and hypophosphatemia are the primary inducers of 1α-hydroxylase activity, resulting in increased levels of 1,25-dihydroxyvitamin D. Conversely, [Ca^{++}] and 1,25-dihydroxyvitamin D, the enzyme product, inhibit it. FGF-23, a major regulator of P_i metabolism (see later), also represses 1α-hydroxylase activity. A reduction of FGF23 levels likely mediates the effect of hypophosphatemia on 1,25-dihydroxyvitamin D production, at least in part.

Vitamin D and its metabolites circulate in blood primarily bound to **vitamin D–binding protein (DBP).** DBP is a serum glycoprotein that is synthesized by the liver. DBP binds more than 85% of circulating 1,25-hydroxyvitamin D. Because of binding to other proteins, only 0.4% of 1,25-dihydroxyvitamin D circulates as free hormone. DBP transports the highly lipophilic vitamin D in blood and provides a reservoir of vitamin D that protects against vitamin D deficiency.

1,25-Dihydroxyvitamin D Receptor

1,25-Dihydroxyvitamin D exerts its actions primarily through binding to the nuclear **vitamin D receptor (VDR),** which is a member of the nuclear hormone receptor family. The VDR is a ligand-dependent transcription factor that binds to cognate DNA sequences **(vitamin D response elements)** as a heterodimer with the **retinoid X receptor**. Thus, the primary



• **Fig. 40.5** Biosynthesis of 1,25-dihydroxyvitamin D. (Modified from Porterfield SP, White BA. *Endocrine Physiology.* 3rd ed. Philadelphia: Mosby; 2007.)

action of 1,25-dihydroxyvitamin D is to regulate gene expression in its target tissues, including the small intestine, bone, kidneys, and parathyroid gland.

The genomic actions of 1,25-dihydroxyvitamin D mediated by the VDR occur over a period of hours to days. 1,25-Dihydroxyvitamin D also has rapid effects (seconds to minutes). For example, 1,25-dihydroxyvitamin D rapidly induces absorption of Ca^{++} by the duodenum. The VDR is also expressed in the plasma membrane of cells and is linked to rapid signaling pathways (e.g., G proteins, phosphatidylinositol-3′-kinase). Current molecular modeling has led to development of vitamin D analogs that specifically bind to the nuclear- versus the membrane-localized VDR, paving the way for selective treatment of disorders related to the rapid versus genomic actions of 1,25-dihydroxyvitamin D.

Fibroblast Growth Factor-23

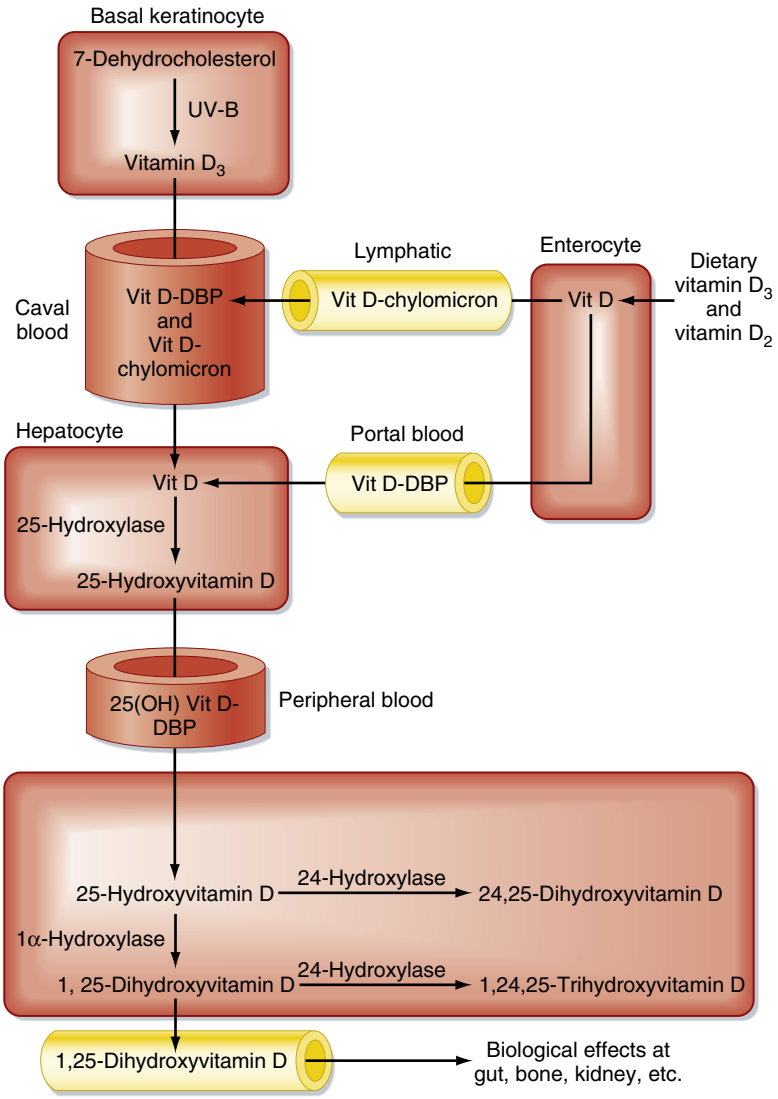
The discovery that FGF23, a peptide produced by osteocytes, is a negative regulator of serum P_i via its ability to inhibit Pi reabsorption in the kidney has led to the realization that bone can function as an endocrine organ. Several hypophosphatemic disorders are associated with excess production of FGF23, including rickets in children and tumor-induced osteomalacia in adults. In autosomal dominant hypophosphatemic rickets (ADHR), a mutation in FGF23 prevents its cleavage and inactivation. X-linked hypophosphatemic rickets is caused by excess FGF23 secondary to a mutation of the *PHEX* gene (**p**rotein with **h**omology to **e**ndopeptidases on the **X** chromosome), which produces a protein that downregulates FGF23 production. Finally, FGF23 is sometimes ectopically produced by slow-growing occult mesenchymal tumors, causing a hypophosphatemic paraneoplastic syndrome. The physiological role of the FGF23 pathway is not completely understood, and many questions remain, including how P_i is sensed. However, new evidence suggests that the FGFR1 receptor in osteocytes can bind to P_i independently of FGF and may function as a phosphate sensor. It should be noted that P_i is not as tightly regulated as calcium, either temporally or with respect to concentration range, but long-term elevation of P_i is associated with increased production of FGF23. In what appears to be a negative feedback loop, 1,25-dihydroxyvitamin D, which enhances intestinal P_i absorption, decreases production of FGF23 by osteocytes.

Hormonal Effects on Target Organs

An overview of the regulation of Ca and P_i by the actions of PTH, 1,25-dihydroxyvitamin D, and FGF23 on their various target organs is summarized in Table 40.2 and in the following sections.

Kidney

Renal handling of Ca^{++} and P_i has been discussed in detail in Chapter 36 and will not be repeated here. Summarized briefly, PTH promotes Ca reabsorption in the distal portion of the thick ascending limb of the Loop of Henle and in the distal tubule. At the same time, PTH inhibits P_i reabsorption in the proximal tubule by inhibition of NPT2 transporters on the luminal membrane, thereby favoring P_i excretion. This allows PTH to correct a hypocalcemic challenge without causing hyperphosphatemia. FGF23 binds to an FGR1/Klotho receptor complex in proximal tubule cells and, like PTH, inhibits NPT2 to promote P_i excretion. FGF23 also inhibits expression of 1α-hydroxylase in the proximal



• **Fig. 40.6** Vitamin D metabolism. *DBP*, Vitamin D–binding protein. (Modified from Porterfield SP, White BA. *Endocrine Physiology.* 3rd ed. Philadelphia: Mosby; 2007.)

tubule, thereby inhibiting production of 1,25-dihydroxyvitamin D to reduce intestinal P_i absorption. Vitamin D plays a supportive role in renal calcium reabsorption by stimulating production of calbindin-D_{28k}, which buffers and escorts intracellular Ca^{++} from the luminal to the basolateral membrane during transcellular transport.

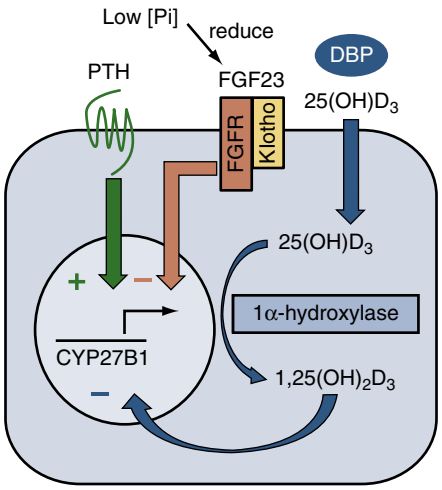
Small Intestine

Dietary intake of Ca can vary widely among individuals and from day to day. Assuming an intake of 1000 mg 350 mg would typically be absorbed, counterbalanced by 150 mg secreted by the intestine, for a net intake of 200 mg. Most Ca^{++} absorption takes place in the proximal small intestine. Importantly, absorption of Ca^{++} is stimulated by 1,25-dihydroxyvitamin D, so absorption is more efficient in the face of declining dietary Ca^{++}.

Ca^{++} is absorbed from the duodenum and jejunum by both a Ca^{++}-regulated and a hormonally regulated transcellular route and by a passive paracellular route. The transcellular route of Ca^{++} absorption is summarized in Fig. 40.8. Movement of Ca^{++}

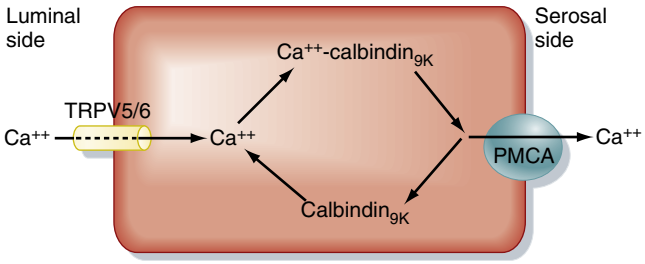
AT THE CELLULAR LEVEL

Calcitonin is a peptide hormone produced by the medullary cells, or C-cells, of the thyroid gland. Calcitonin secretion is positively regulated by serum [Ca^{++}] via the CaSR. The calcitonin receptor is expressed in osteoclasts, where calcitonin acts rapidly and directly to inhibit bone resorption. However, in humans, calcitonin does not appear to play a major role in regulating serum Ca. In support of this view, production of excess calcitonin or complete absence of calcitonin (e.g., following thyroidectomy) does not perturb serum Ca levels. More potent forms of the hormone (e.g., salmon calcitonin) have been used therapeutically as an antiresorptive in the treatment of **Paget's disease** (characterized by excessive osteoclastic bone resorption) and in osteoporosis. Calcitonin is also a useful histochemical marker of medullary thyroid cancer.



• **Fig. 40.7** Regulation of 1α-hydroxylase gene *(CYP27B1)* expression in the proximal tubule, showing stimulation by PTH and inhibition by FGF23 and 1,25-dihydroxyvitamin D. Hypophosphatemia probably stimulates 1α-hydroxylase by reducing FGF23 levels at least in part. *DBP,* Vitamin D–binding protein; *PTH,* parathyroid hormone.

from the gastrointestinal lumen into the enterocyte, which is driven by both chemical and electrical gradients, occurs via apical calcium channels called **TRPV5** and **TRPV6.** Once inside the cell, Ca^{++} ions bind to **calbindin-D_{9K},** which maintains a low cytoplasmic [Ca^{++}], preserving the favorable transluminal membrane Ca^{++} gradient. Calbindin-D_{9K} also plays a role in apical-to-basolateral shuttling of Ca^{++}, which is transported across the basolateral membrane against an electrochemical gradient by **plasma membrane calcium ATPase (PMCA).**



• **Fig. 40.8** Intestinal absorption of Ca^{++} via the transcellular route. *PMCA,* Plasma membrane calcium ATPase. (Modified from Porterfield SP, White BA. *Endocrine Physiology.* 3rd ed. Philadelphia: Mosby; 2007.)

The **Na^+/Ca^{++} exchanger (NCX)** also contributes to basolateral Ca^{++} transport. 1,25-Dihydroxyvitamin D stimulates expression of all the components involved in absorption of Ca^{++} by the small intestine.

The fraction of dietary P_i absorbed by the jejunum remains relatively constant at about 70% and is under minor hormonal control by 1,25-dihydroxyvitamin D. The limiting process in transcellular P_i absorption is transport across the apical brush border, which is mediated by the **Na^+/P_i cotransporter NPT2.**

Bone

Bone stores vast amounts of Ca and P_i. Once peak bone mass has been achieved in an adult, the skeleton is constantly remodeled through the concerted activities of bone cells. Bone remodeling involves removal of fatigued or microdamaged

TABLE 40.2 Actions of PTH, 1,25-Dihydroxyvitamin D, and FGF23 on Ca^{++}/P_i Homeostasis

	Small Intestine	Bone	Kidney	Parathyroid Gland
PTH	No direct action	Intermittent PTH promotes osteoblastic bone formation Regulates M-CSF, RANKL, OPG in osteoblasts Sustained high levels promote osteoclastic bone resorption, Ca^{++} and P_i release from bone	Stimulates 1α-hydroxylase activity Stimulates Ca^{++} reabsorption by thick ascending limb of Henle's loop and distal tubule Inhibits P_i reabsorption in proximal tubule (inhibits NPT2a)	No direct action
1,25-Dihydroxyvitamin D	Increases Ca^{++} absorption by increasing TRPV, calbindin, and PMCA expression Modestly increases P_i absorption	Regulates osteoclast differentiation via RANKL expression in osteoblasts Maintains [Ca^{++}] and [P_i] to support bone mineralization	Supports actions on Ca^{++} reabsorption through calbindin expression Promotes P_i reabsorption by proximal nephrons (stimulates NPT2a expression)	Directly inhibits *PTH* gene expression (negative feedback) Directly stimulates *CASR* gene expression
FGF23	None	Produced by osteocytes	Inhibits P_i reabsorption in proximal tubule (inhibits NPT2a)	Inhibits PTH synthesis and secretion

CASR, Calcium-sensing receptor; *FGF23,* fibroblast growth factor-23; *M-CSF,* macrophage colony-stimulating factor; *NPT2,* Na^+/P_i cotransporter; *OPG,* osteoprotegerin; *PTH,* parathyroid hormone; *RANKL,* receptor activator of nuclear factor κ-B.

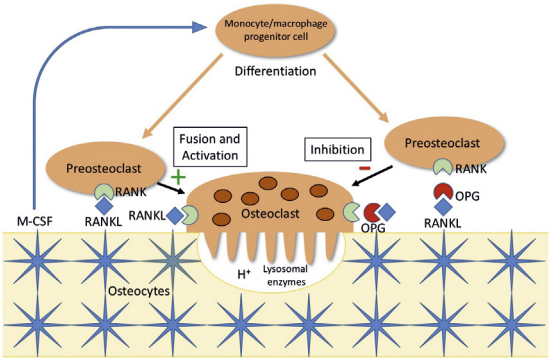
bone by bone-resorbing osteoclasts. This is followed by recruitment of bone-forming osteoblasts to replace bone at the same location. These cells synthesize **osteoid** (yet to be mineralized bone matrix) which then undergoes controlled mineralization with Ca^{++} and P_i to form new mature bone. Many of these processes are controlled by osteocytes, which are now thought to occupy a central role in the regulation of bone remodeling. Osteocytes are terminally differentiated osteoblast-lineage cells that have become surrounded by and entrapped within bone in small cavities called *lacunae.* They are interconnected through an extensive network of dendritic cell processes that run within canaliculi and form communicating junctions with adjacent osteocytes, reaching all the way to the surfaces of bone. The processes of bone formation and bone resorption are in balance in a healthy, physically active, and well-nourished individual. However, the process of bone remodeling can be modulated to provide a net gain or loss of Ca^{++} and P_i into blood and is responsive to physical activity (loading), diet, age, and hormonal regulation. Because the integrity of bone is absolutely dependent on Ca and P_i, chronic dysregulation of these ions or the hormones that regulate them leads to pathological changes in bone.

Regulation of Bone Formation

The process of bone remodeling is a highly coordinated process involving multiple cell types (Fig. 40.9). It has been known for some time that osteoblast-lineage cells express factors that promote the differentiation of osteoclasts from progenitors of the monocyte/macrophage lineage and maintain mature osteoclast function. Recent genetic evidence indicates that osteocytes are the primary cell type supporting osteoclast differentiation. Osteocytes produce macrophage **colony-stimulating factor (M-CSF)**, which expands and differentiates early hematopoietic progenitors into preosteoclasts that express a cell surface receptor called **RANK (receptor activator of NFκB)**. Osteocytes display **RANK ligand (RANKL)** on dendritic processes that reach the surface surfaces. RANKL binds to RANK on preosteoclasts to promote fusion of these precursors, giving rise to a large multinucleated osteoclast (Fig. 40.9). The perimeter of the osteoclast membrane facing mineralized bone adheres tightly to the bone and seals off the area of osteoclast-bone contact. The region within the sealed zone forms a highly invaginated membrane called the *ruffled border,* from which HCl and hydrolytic lysosomal enzymes are secreted. The acidic enzyme-rich microenvironment beneath the osteoclast dissolves the bone mineral, thereby releasing Ca^{++} and P_i into blood, and also degrades the bone matrix. There is an additional inhibitory component of the RANK/RANKL system. Osteocytes also produce a soluble factor called **osteoprotegerin (OPG),** which acts as a decoy receptor for RANKL and inhibits osteoclast differentiation and function (see Fig. 40.9). Therefore, the balance between RANKL and OPG expression by osteoblasts determines how much osteoclast differentiation and bone resorption will occur.

As a calciotropic hormone, PTH is a potent regulator of bone resorption in adults. The PTHR1 receptor is expressed on osteoblast-lineage cells but not on osteoclasts. PTH acts on osteocytes to increase expression of osteoblast paracrine factors (i.e., M-CSF, RANKL) that upregulate osteoclast differentiation and stimulate bone resorption. 1,25-Dihydroxyvitamin D also stimulates bone resorption by upregulating RANKL expression in osteocytes.

It is important to recognize that elevated PTH (in concert with 1,25-dihydroxyvitamin D) will defend plasma Ca levels by promoting bone resorption during a hypocalcemic challenge. When PTH levels are normal, however, bone remodeling is a locally controlled process by which old or damaged bone is replaced.



• **Fig. 40.9** Osteocytes are the primary regulators of osteoclast differentiation and function. Osteocytes express RANKL on their dendritic processes that extend to the surface of the bone. RANKL binds to RANK on preosteoclasts to promote fusion and differentiation to multinucleated osteoclasts *(left)*. Increased expression of OPG relative to RANKL *(right)* inhibits osteoclast differentiation and function. *M-CSF,* Macrophage colony-stimulating factor; *OPG,* osteoprotegerin; *RANK*, receptor activator of NFκB; *RANKL,* RANK ligand.

IN THE CLINIC

Discovery of molecular pathways regulating bone remodeling has presented new therapeutic opportunities for treating osteoporosis and other metabolic bone disease. A biological antiresorptive drug based on a humanized antibody directed against RANKL (denosumab) is available for treatment of postmenopausal osteoporosis. This has proven to be an effective antiresorptive treatment that improves bone density and reduces the risk of fracture.

It has been shown that intermittent administration of low-dose PTH promotes osteoblastic bone formation. This has led to development of anabolic treatments using teriparatide (PTH1-34) or abaloparatide, a 34–amino acid analog of PTHrP.

Emerging evidence indicates that osteocytes are able to sense mechanical strain in bone and signal that additional local bone formation is needed. Osteocytes produce a peptide paracrine factor called sclerostin (SOST) that inhibits Wnt signaling in osteoblast precursors, thereby acting as a brake on osteoblast differentiation. SOST expression by osteocytes is reduced in areas of bone undergoing mechanical strain, and is also reduced by intermittent PTH treatment. This has led to the development of a humanized monoclonal antibody directed against SOST (romosozumab) as a potential therapy to increase bone formation.

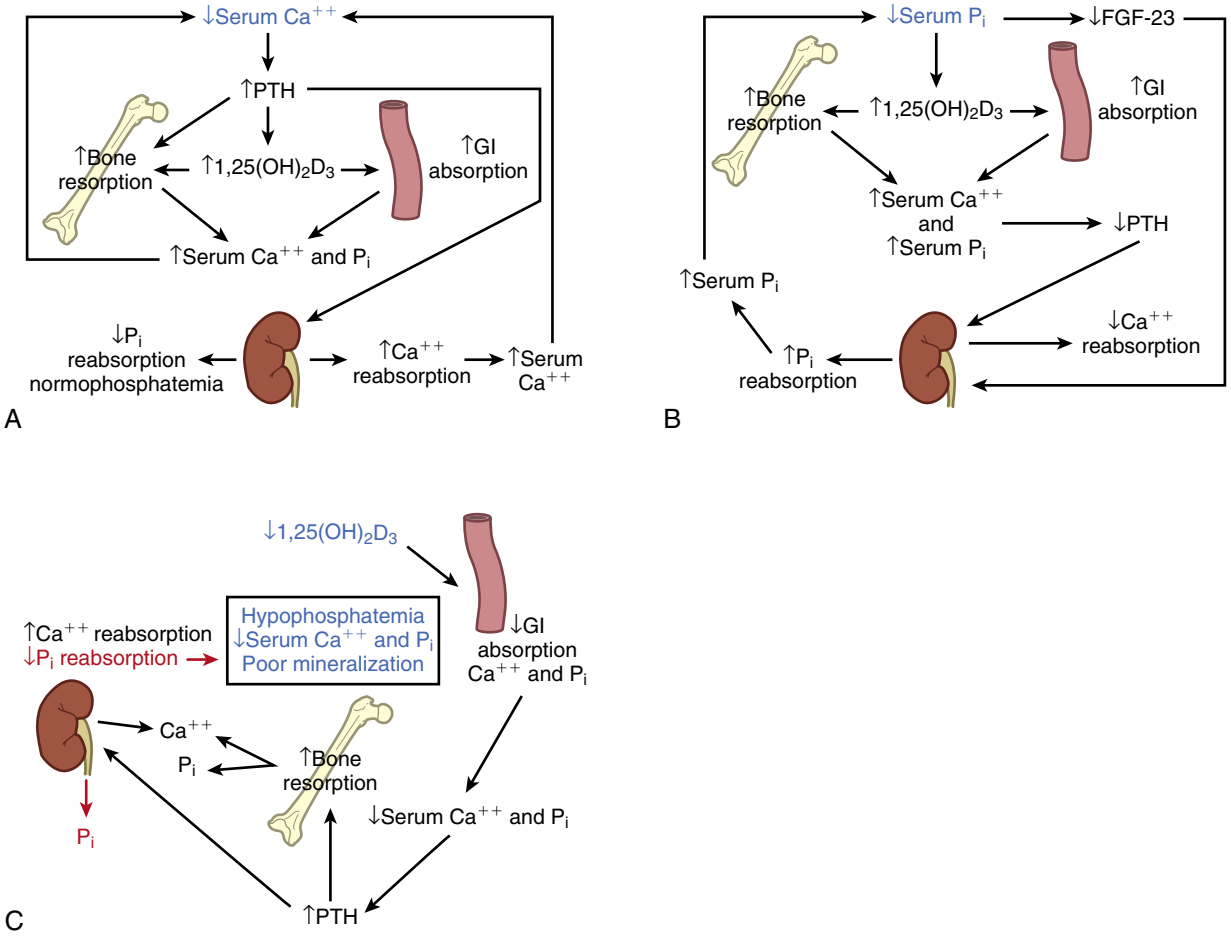
IN THE CLINIC

Vitamin D deficiency (Fig. 40.10C) produces a hypocalcemic challenge by decreasing gastrointestinal absorption of Ca^{++} and P_i. A drop in serum [Ca^{++}] increases compensatory *PTH* gene expression, PTH secretion, parathyroid cell proliferation, and PTH-mediated upregulation of renal 1-hydroxylase. In the absence of sufficient 25-hydroxyvitamin D precursor, however, 1,25-dihydroxyvitamin D levels fall. The secondary elevation of PTH mobilizes Ca^{++} from bone and kidney but promotes renal excretion of P_i, causing hypophosphatemia. Because the $Ca^{++} \times P_i$ product in serum is low, bone mineralization is impaired. In children this leads to **rickets,** in which the growth of long bones is abnormal and impaired. The rib cage, wrists, and ankles show characteristic bone deformities, and the impaired mineralization causes bowing of the legs. In adults, vitamin D deficiency leads to **osteomalacia,** which is characterized by poor mineralization of newly formed osteoid, visible on radiographs as pseudofractures. In severe cases, osteomalacia results in weakness, bone pain, and increased risk of fracture.

Regulation by Gonadal and Adrenal Steroid Hormones

Gonadal and **adrenal steroid hormones** have profound effects on bone. **17β-Estradiol** (E_2; see Chapter 44) has important anabolic effects on bone and is a potent regulator of osteoblast and osteoclast function. Estrogen promotes survival of osteoblasts and apoptosis of osteoclasts, thereby favoring bone formation over resorption. **Androgens** also have bone anabolic effects, although some of these effects are due to local conversion of testosterone to E_2 in men (see Chapter 44). The combined effects of testosterone and E_2 account for the higher peak bone mass observed in men. In postmenopausal women, estrogen deficiency results in an initial phase of rapid bone loss that lasts about 5 years, followed by a second phase of slower age-related bone loss that is similar in both sexes. For this reason, women are susceptible to **postmenopausal osteoporosis.**

Glucocorticoids at high therapeutic doses promote bone resorption and inhibit intestinal Ca absorption. However,



• **Fig. 40.10** Integrated hormone responses to perturbations of Ca^{++} (A), P_i (B), and vitamin D (C). *PTH,* Parathyroid hormone.

the most critical adverse effect is inhibition of osteoblast differentiation, which impairs bone formation. Therefore, patients treated with high levels of a glucocorticoid as an anti-inflammatory or immunosuppressive drug are at risk for **glucocorticoid-induced osteoporosis** and should be monitored carefully.

Integrated Physiological Regulation of Ca^{++}/P_i Metabolism

Hypocalcemic Challenge

The integrated response of PTH and 1,25-dihydroxyvitamin D to a hypocalcemic challenge is shown in Fig. 40.10A. A decrease in serum [Ca^{++}] detected by the CaSR on parathyroid chief cells stimulates secretion of PTH. In the kidney, PTH increases Ca^{++} reabsorption in the distal tubule and, to a lesser extent, in the distal thick ascending limb of the loop of Henle. In bone, elevated PTH stimulates osteocytes to express RANKL, which increases osteoclast activity and leads to increased bone resorption and release of Ca^{++} and P_i into blood. PTH stimulates 1α-hydroxylase expression in the proximal renal tubule, thereby increasing 1,25-dihydroxyvitamin D levels. 1,25-Dihydroxyvitamin D stimulates absorption of Ca and P_i in the small intestine and upregulates expression of RANKL, thereby amplifying the effect of PTH on bone resorption. In the kidney, PTH inhibits NPT2 in the proximal tubule to lower P_i reabsorption and increase P_i clearance, thereby counterbalancing P_i mobilized from the bone and gut.

Hypophosphatemic Challenge

Although not as tightly regulated as Ca^{++}, perturbations in serum P_i will also elicit hormonal responses (see Fig. 40.10B). Low serum P_i stimulates production of 1,25-dihydroxyvitamin D in the kidney, which in turn will mobilize Ca and P_i from the intestine. The rise in Ca^{++} will suppress PTH secretion to prevent hypercalcemia. This drop in PTH will enhance P_i reabsorption in the proximal tubule to help restore serum P_i. Over a longer time course, a decrease in serum P_i will inhibit FGF23 production, which will favor P_i reabsorption in the proximal tubule. These integrated responses will allow correction of hypophosphatemia while maintaining normocalcemia. For hormonal responses to vitamin D deficiency, see the In the Clinic box and Fig. 40.10C.

IN THE CLINIC

Primary hyperparathyroidism is caused by excessive production of PTH by the parathyroid glands. It is most frequently caused by a single **adenoma** confined to one of the parathyroids. Owing to elevated PTH, patients with primary hyperparathyroidism have high serum [Ca^{++}] and, in most cases, low serum [P_i]. **Hypercalcemia** is a result of bone resorption, increased gastrointestinal Ca absorption (mediated by 1,25-dihydroxyvitamin D), and increased renal Ca^{++} reabsorption. The major symptoms of the disorder are related to increased bone resorption, hypercalcemia, and **hypercalciuria.** These include radiographic manifestations of excessive bone resorption and, psychological disorders, particularly depression. Progressive neurological symptoms include fatigue, mental confusion, and, at very high levels, (>15 mg/dL), coma. Kidney stones **(nephrolithiasis)** composed of calcium phosphate are common because hypercalcemia leads to hypercalciuria, and increased P_i clearance causes **phosphaturia.** Fortunately, routine blood chemistry screening over the past several decades has resulted in earlier detection of primary hyperparathyroidism, precluding development of severe symptoms in most cases.

Key Concepts

1. Serum [Ca^{++}] is determined by the rate of Ca absorption by the gastrointestinal tract, bone formation and resorption, and renal excretion. Serum [Ca^{++}] is normally maintained within a very narrow range.
2. Serum [P_i] is determined by the rate of P_i absorption by the gastrointestinal tract, soft tissue influx and efflux, bone formation and resorption, and renal excretion. Serum [P_i] normally fluctuates over a relatively wider range.
3. The major physiological hormones regulating serum [Ca^{++}] and [P_i] are PTH, 1,25-dihydroxyvitamin D (calcitriol), and FGF23.
4. Vitamin D is synthesized from 7-dehydrocholesterol in skin in the presence of UVB light or acquired in the diet. It is hydroxylated to 25-hydroxycholecalciferol in the liver and activated by renal 1α-hydroxylase to 1,25-dihydroxyvitamin D.
5. 1,25-Dihydroxyvitamin D promotes intestinal Ca^{++} absorption and modestly increases P_i absorption.
6. The flux of Ca^{++} and P_i into and out of bone is determined by the relative rates of osteoblastic bone formation and osteoclastic bone resorption.
7. The PTH/PTHrP receptor is expressed on osteoblast-lineage cells, not on osteoclasts. PTH has both anabolic and catabolic actions in bone depending on the dose and timing of administration. PTH promotes bone resorption by upregulation of M-CSF and RANKL in osteocytes.
8. 1,25-Dihydroxyvitamin D binds to the VDR in osteoblasts to support osteoclast differentiation via RANKL and promotes bone mineralization by maintaining appropriate serum [Ca^{++}] and [P_i].