

APPROACH TO HYPERCALCEMIA

David Goltzman, MD, Antoine Massabki Professor of Medicine and Physiology, McGill University and McGill University Health Centre, 1001 Decarie Blvd, Room EM1.3220, Montreal, QC, Canada.
david.goltzman@mcgill.ca

Updated April 16, 2023

ABSTRACT

A reduction in serum calcium can stimulate parathyroid hormone (PTH) release which may then increase bone resorption, enhance renal calcium reabsorption, and stimulate renal conversion of 25-hydroxyvitamin D, to the active moiety 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] which then will enhance intestinal calcium absorption. These mechanisms restore the serum calcium to normal and inhibit further production of PTH and $1,25(\text{OH})_2\text{D}$. Normal serum concentrations of total calcium generally range between 8.5 and 10.5 mg/dL (2.12 to 2.62 mM) and ionized calcium between 4.65-5.30 mg/dL (1.16-1.31 mM). Decreased PTH and decreased $1,25(\text{OH})_2\text{D}$ should accompany hypercalcemia unless PTH or $1,25(\text{OH})_2\text{D}$ is causal. Hypercalcemia may be caused by: Endocrine Disorders with Excess PTH including primary sporadic and familial hyperparathyroidism (syndromic and non-syndromic), and tertiary hyperparathyroidism; Endocrine Disorders Without Excess PTH including hyperthyroidism, pheochromocytoma, VIPoma, hypoadrenalism, and Jansen's Metaphyseal Chondrodysplasia; Malignancy-Associated Hypercalcemia, which can be caused by elevated PTH-related protein (PTHrP), or other factors (e.g. increased $1,25(\text{OH})_2\text{D}$ in lymphomas); Inflammatory Disorders including Granulomatous Diseases, where excess $1,25(\text{OH})_2\text{D}$ production may be causal, and viral syndromes (HIV); Pediatric Syndromes including

Williams Syndrome and Idiopathic Infantile Hypercalcemia, where inappropriate levels of $1,25(\text{OH})_2\text{D}$ may occur due to a mutation in the 25-hydroxyvitamin D-24-hydroxylase gene (CYP24A1); medication, including thiazide diuretics, lithium, vitamin D, vitamin A, antiestrogens, theophylline; and prolonged immobilization, particularly in states of high bone turnover. Treatment should be aimed at the underlying disorder, however, if serum calcium exceeds 12 to 14 mg/dL (3 to 3.5 mM), acute hydration and agents that inhibit bone resorption are required. Under selected conditions, calcimimetics, calciuresis, glucocorticoids, or dialysis may be needed.

DEFINITION OF HYPERCALCEMIA

Hypercalcemia can be defined as a serum calcium greater than 2 standard deviations above the normal mean in a reference laboratory. Calcium in the blood is normally transported partly bound to plasma proteins (about 45%), notably albumin, partly bound to small anions such as phosphate and citrate (about 10%) and partly in the free or ionized state (about 45%) (1). Although only the ionized calcium is metabolically active i.e., subject to transport into cells and capable of activating cellular processes, most laboratories report total serum calcium concentrations. Concentrations of total calcium in normal serum generally range between 8.5 and 10.5 mg/dL (2.12 to 2.62 mM) and levels above this are considered to be consistent with hypercalcemia. Nevertheless,

reference ranges may vary between laboratories. The normal range of ionized calcium is generally 4.65-5.25 mg/dL (1.16-1.31 mM), but again values may vary slightly between laboratories. When protein concentrations, and especially albumin concentrations, fluctuate substantially, total calcium levels may vary, whereas the ionized calcium may remain relatively stable. Thus, dehydration, or hemoconcentration during venipuncture may elevate serum albumin, and a falsely elevated total serum calcium may be reported ("pseudo-hypercalcemia"). Such elevations in total calcium, when albumin levels are increased, can be "corrected" by subtracting 0.8 mg/dL from the total calcium for every 1.0 g/dL by which the serum albumin concentration is >4 g/dL. Conversely when albumin levels are low, total calcium can be corrected by adding 0.8 mg/dL for every 1.0 g/dL by which the albumin is <4 g/dL. Thus, to correct for an abnormally high or low serum albumin the following formula can be used: Corrected calcium (mg/dL) = measured total serum calcium (mg/dL) + [4.0 - serum albumin (g/dL) X 0.8] or Corrected calcium (mM) = measured total Ca (mM) + [40 - serum albumin (g/L)] X 0.02. Nevertheless, although algorithms to adjust for albumin levels are widely used, their accuracy may be poor. Even in the presence of a normal serum albumin, changes in blood pH can alter the equilibrium constant of the albumin-Ca⁺⁺ complex, with acidosis reducing the binding and alkalosis enhancing it. Consequently, when major shifts in serum protein or pH are present it is most

prudent to directly measure the ionized calcium level in order to determine the presence of hypercalcemia.

PHYSIOLOGY OF CALCIUM HOMEOSTASIS

The extracellular fluid (ECF) concentration of calcium is tightly maintained within a rather narrow range because of the importance of the calcium ion to numerous cellular functions including cell division, cell adhesion and plasma membrane integrity, protein secretion, muscle contraction, neuronal excitability, glycogen metabolism, and coagulation.

The skeleton, the gut and the kidney play a major role in assuring calcium homeostasis. Overall, in a typical individual, if 1000 mg of calcium are ingested in the diet per day, approximately 200 mg will be absorbed. Approximately 10 g of calcium will be filtered daily through the kidney and most will be reabsorbed with about 200 mg being excreted in the urine. The normal 24-hour excretion of calcium may however vary between approximately 100 and 300 mg per day (2.5 to 7.5 mmoles per day). The skeleton, a storage site of about 1 kg of calcium, is the major calcium reservoir in the body and bone turnover (bone formation coupled with bone resorption) will determine the net entry of calcium into or egress of calcium out of the skeleton. When bone turnover is balanced, approximately 500 mg of calcium is released from bone per day and the equivalent amount is accreted per day (Fig. 1).

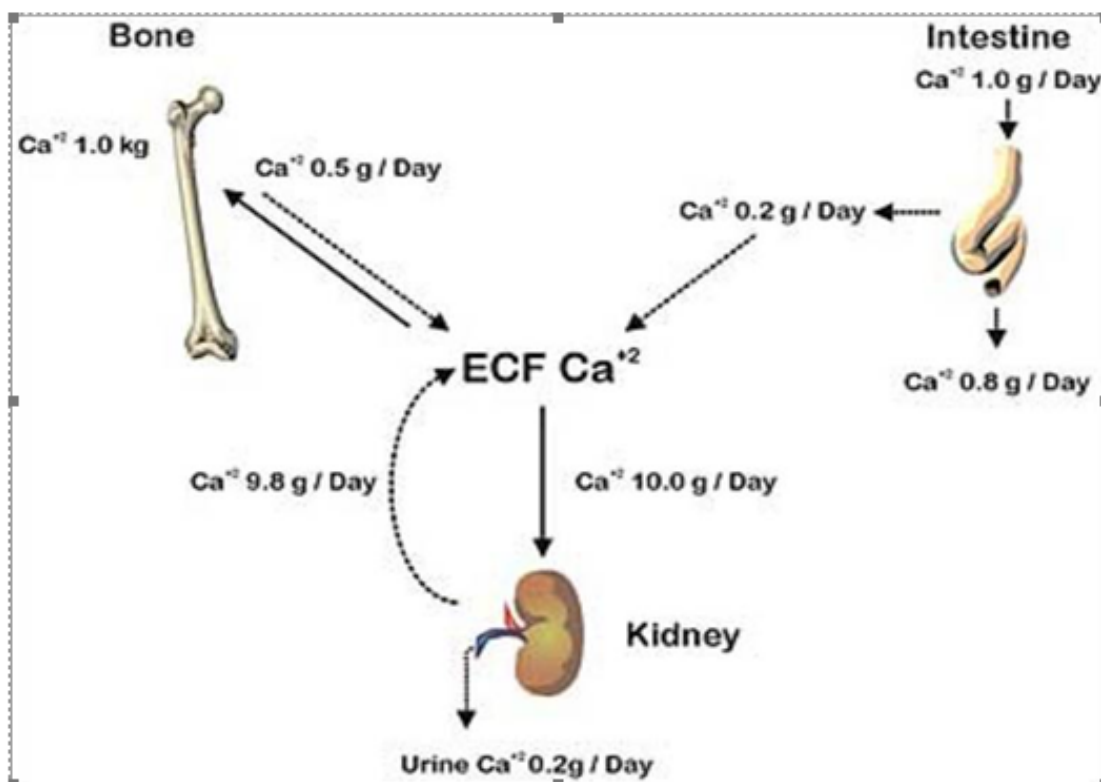


Figure 1. Calcium balance. On average, if, in a typical adult approximately 1g of elemental calcium (Ca^{+2}) is ingested per day, about 200mg/day will be absorbed and 800mg/day excreted. Approximately 1kg of Ca^{+2} is stored in bone and about 500mg/day is released by resorption or deposited during bone formation. Of the 10g of Ca^{+2} filtered through the kidney per day only about 200mg appears in the urine, the remainder being reabsorbed.

Tight regulation of the ECF calcium concentration is maintained through the action of calcium-sensitive cells which modulate the production of hormones (2-5). These hormones act on specific cells in bone, gut and kidney which can respond by altering fluxes of calcium to maintain ECF calcium. The parathyroid glands detect ECF calcium via a calcium sensing receptor (CaSR) (6). Thus, a reduction in ECF calcium can reduce stimulation of the parathyroid CaSR and facilitate release of parathyroid hormone (PTH) from the parathyroid glands in the neck. PTH can then act to enhance calcium reabsorption in the kidney while at the same time inhibit phosphate reabsorption producing phosphaturia. Reduced ECF calcium per se

can also act via a CaSR in the loop of Henle to allow renal calcium reabsorption.

PTH and hypocalcemia can both stimulate the conversion of the inert metabolite of vitamin D, 25-hydroxyvitamin D [$25(\text{OH})\text{D}$], to the active moiety 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] (7), which in turn will enhance intestinal calcium absorption, and to a lesser extent phosphate reabsorption. $1,25(\text{OH})_2\text{D}$ can stimulate the production of the hormone fibroblast growth factor 23 (FGF23) from osteocytes in bone and the released FGF23 can inhibit phosphate transport in the renal proximal tubule and therefore cause phosphaturia and hypophosphatemia. PTH can also increase bone resorption and liberate both calcium

and phosphate from the skeleton. The net effect of the increased reabsorption of renal calcium, the increased absorption of calcium from the gut, and the mobilization of calcium from bone, is to restore the ECF calcium to normal and to inhibit further production of PTH and 1,25(OH)₂D. FGF23 elevation will also reduce 1,25(OH)₂D production. The opposite sequence of events i.e., diminished PTH and 1,25(OH)₂D secretion should occur when the ECF calcium is raised above the normal range and the effect of suppressing the release of these hormones should diminish skeletal calcium release, intestinal calcium absorption, and renal calcium reabsorption and restore the elevated ECF calcium to normal. **Consequently, decreased levels of PTH and decreased levels of 1,25(OH)₂D should accompany hypercalcemia unless the PTH or 1,25(OH)₂D is the cause of the hypercalcemia.**

REGULATION OF THE PRODUCTION AND ACTION OF HUMORAL MEDIATORS OF CALCIUM HOMEOSTASIS

Regulation of Parathyroid Hormone Production

PTH is an 84 amino acid peptide whose known bioactivity resides within the NH₂-terminal 34 residues. Consequently, a synthetic peptide, PTH (1-34) (teriparatide), can mimic many of its actions. The major regulator of PTH secretion from the parathyroid glands is the ECF calcium acting via CaSR. The relationship between ECF calcium and PTH secretion is governed by a steep inverse sigmoidal curve which is characterized by a maximal secretory rate at low ECF calcium, a midpoint or "set point" which is the level of ECF calcium which half-maximally suppresses PTH, and a minimal secretory rate at high ECF calcium (8). The rate at which ECF calcium falls may also dictate the magnitude of the secretory response with a rapid fall in ECF calcium stimulating a more robust secretory response. As well higher levels of PTH are observed at the same ECF calcium when

calcium is falling rather than rising, producing a hysteresis response (9).

CaSR has a large NH₂-terminal extracellular domain which binds ECF calcium, seven plasma membrane-spanning helices and a cytoplasmic COOH-terminal domain. It is a member of the superfamily of G protein coupled receptors and in the parathyroid chief cells is linked to various intracellular second-messenger systems. Transduction of the ECF calcium signal via this molecule leads to alterations in PTH secretion.

A change in ECF calcium will also produce a change in PTH metabolism in the parathyroid cell however this response is somewhat slower than the secretory response. Thus, a rise in calcium will promote enhanced PTH degradation and the release of bioinert mid-region and COOH fragments and a fall in calcium will decrease intracellular degradation so that more intact bioactive PTH is secreted (10-12). Bioinactive PTH fragments, which can also be generated in the liver, are cleared by the kidney (13). With sustained low ECF calcium there is a change in PTH biosynthesis which represents an even slower response. Thus, low ECF calcium, acting via CaSR leads to increased transcription of the gene encoding PTH and enhanced stability of PTH mRNA (14,15). Finally, sustained hypocalcemia can eventually lead to parathyroid cell proliferation (16) and an increased total secretory capacity of the parathyroid gland. Although sustained hypercalcemia can conversely reduce parathyroid gland size, hypercalcemia appears less effective in diminishing parathyroid chief cells once a prolonged stimulus to hyperplasia has occurred.

Additional factors including catecholamines and other biogenic amines, prostaglandins (17), cations (e.g., lithium and magnesium), phosphate per se (5) and transforming growth factor alpha (TGFα) (18) have been implicated in the regulation of PTH secretion (5). The phosphaturic factor, FGF23, also suppresses

PTH gene expression and secretion (19). One of the most important regulators appears to be $1,25(\text{OH})_2\text{D}$ which may tonically reduce PTH release (20), decrease PTH gene expression (15) and inhibit parathyroid cell proliferation (16, 21).

PTH Actions

RENAL ACTIONS

The kidney is a central organ in ensuring calcium balance and PTH has a major role in fine-tuning this renal function (22-24). PTH has little effect on modulating calcium fluxes in the proximal tubule where 65% of the filtered calcium is reabsorbed, coupled to the bulk transport of solutes such as sodium and water (23). Nevertheless, in this region PTH binds (25) to its cognate receptor, the type I PTH/PTHrP receptor (PTHR1) a 7-transmembrane-spanning G protein-coupled protein which is linked to both the adenylate cyclase system and the phospholipase C system (26-28). Stimulation of adenylate cyclase is believed to be the major mechanism whereby PTH causes internalization of the type II Na^+/Pi (inorganic phosphate) co-transporters, NaPi-IIa and NaPi-IIc , in the proximal tubule, leading to decreased phosphate reabsorption and phosphaturia (29).

In this nephron region, PTH can, after binding to the PTHR1, also stimulate CYP27B1, the $25(\text{OH})\text{D}-1\alpha$ hydroxylase [$1\alpha(\text{OH})\text{ase}$], leading to increased conversion of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ (30). A reduction in ECF calcium can itself stimulate $1,25(\text{OH})_2\text{D}$ production. Finally, PTH can also inhibit Na^+ and HCO_3^- reabsorption in the proximal tubule by inhibiting the apical type 3 Na^+/H^+ exchanger (31), and the basolateral $\text{Na}^+/\text{K}^+-\text{ATPase}$ (32) as well as by inhibiting apical Na^+/Pi cotransport.

About 20% of filtered calcium is reabsorbed in the cortical thick ascending limb of the loop of Henle

(CTAL) and 15% in the distal convoluted tubule (DCT) and it is here that PTH also binds to PTHR1 (27) and again by a cyclic AMP-mediated mechanism (33), enhances calcium reabsorption. In the CTAL, at least, this appears to occur by increasing the activity of the $\text{Na}/\text{K}/2\text{Cl}$ cotransporter that drives NaCl reabsorption and also stimulates paracellular calcium and magnesium reabsorption (34). The CaSR is also resident in the CTAL (35) and can respond to an increased ECF calcium by activating phospholipase A_2 , reducing the activity of the $\text{Na}/\text{K}/2\text{Cl}$ cotransporter and of an apical K channel, and diminishing paracellular calcium and magnesium reabsorption. Consequently, a raised ECF calcium antagonizes the effect of PTH in this nephron segment and ECF calcium can in fact participate in this way in the regulation of its own homeostasis. Furthermore, the inhibition of NaCl reabsorption and loss of NaCl in the urine that results may contribute to the volume depletion observed in severe hypercalcemia.

In the DCT, PTH can also influence transcellular calcium transport (36). This is a multistep process involving transfer of luminal Ca^{2+} into the renal tubule cell via the transient receptor potential channel (TRPV5), translocation of Ca^{2+} across the cell from apical to basolateral surface a process involving proteins such as calbindin-D28K, and finally active extrusion of Ca^{2+} from the cell into the blood via a $\text{Na}^+/\text{Ca}^{2+}$ exchanger, designated NCX1. PTH markedly stimulates Ca^{2+} reabsorption in the DCT primarily by augmenting NCX1 activity via a cyclic AMP-mediated mechanism.

SKELETAL ACTIONS

In bone, the PTHR1 is localized on cells of the osteoblast lineage which are of mesenchymal origin (37) but not on osteoclasts which are of hematogenous origin. Nevertheless, in the postnatal

state the major physiologic role of PTH appears to be to maintain normal calcium homeostasis by enhancing osteoclastic bone resorption, notably cortical bone resorption, and liberating calcium into the ECF. This effect of PTH on increasing osteoclast stimulation is indirect, with PTH binding to the PTHR1 on pre-osteoblastic stromal cells (38) and other cells of the osteoblast lineage including osteocytes (39) and enhancing the production of the cytokine RANKL (receptor activator of NFkappaB ligand), a member of the tumor necrosis factor (TNF) family (40). Simultaneously, levels of a soluble decoy receptor for RANKL, termed osteoprotegerin, are diminished facilitating the capacity for increased cell-bound RANKL to interact with its cognate receptor, RANK, on cells of the osteoclast series. Multinucleated osteoclasts are derived from hematogenous precursors which commit to the monocyte/macrophage lineage, and then proliferate and differentiate as mononuclear precursors, eventually fusing to form multinucleated osteoclasts (41). These can then be activated to form bone-resorbing osteoclasts. RANKL can drive many of these proliferation/differentiation/fusion/activation steps although other cytokines, notably monocyte-colony stimulating factor (M-CSF) may participate in this process (41).

Endogenous PTH has also been shown to exert a physiologic anabolic effect, particularly on trabecular bone formation in both the fetus and neonate (42,43), and intermittent exogenous PTH administration can increase both cortical and trabecular compartments in adult mice. (44). PTH has been reported to increase growth factor production, notably insulin-like growth factor-1 (IGF-1) production, which may contribute to its anabolic effect (45). In addition, the anabolic effect of PTH in part lies via activation of the canonical Wnt growth factor signaling pathway, a critical pathway for bone formation. One mechanism of this activation is via inhibition of sclerostin (39), an osteocyte-derived antagonist of the Wnt pathway. PTH has been

suggested to elicit increases in production and activity of cells of the osteoblast pathway and to decrease osteoblast apoptosis (46). It is conceivable that different modes of anabolic action occur depending on the stage of development of the organism and environmental stimuli.

It has been noted that although increased PTH activity increases coupled bone turnover i.e., both osteoblastic bone formation and osteoclastic bone resorption, continuous exogenous administration of PTH in vivo can lead to net enhanced bone resorption and hypercalcemia whereas intermittent exogenous administration can lead to net increasing bone formation and therefore an anabolic effect (47).

Regulation of Vitamin D Production

Vitamin D₃ (cholecalciferol) is a biologically inert secosteroid that is made in the skin (48). After exposure to sunlight 7-dehydrocholesterol is transformed by UVB radiation to previtamin D₃ which undergoes isomerization into vitamin D₃. Vitamin D₃ is then translocated into the circulation where it is bound to the vitamin D-binding protein (DBP). There are no documented cases of vitamin D intoxication occurring due to excessive sunlight exposure most likely due to the fact that prolonged UVB exposure transforms both previtamin D₃ and vitamin D₃ to biologically inactive metabolites. Vitamin D₃ (and vitamin D₂ or ergocalciferol) can also enter the circulation after absorption from food in the gut notably fatty foods, fish oils, and foods fortified with vitamin D. In the liver, vitamin D can be converted to 25(OH)D by a cytochrome P450-vitamin D 25-hydroxylase (CYP2R1), which generally converts vitamin D to 25(OH)D almost constitutively (49).

Consequently serum 25(OH)D is the most abundant circulating metabolite of vitamin D, reflects the integrated levels of vitamin D from both cutaneous and dietary sources, and can be used as an index of

vitamin D deficiency, sufficiency, or intoxication. However, 25(OH)D is also biologically inert except when present in very high concentrations, and is transported, bound to DBP, to the kidney where it is converted by the cytochrome P450- monooxygenase, 25(OH)D-1 α hydroxylase (CYP27B1) to the active moiety, 1,25(OH) $_2$ D (50). Although the kidney is the major source of circulating hormonal 1,25(OH) $_2$ D, a variety of extra-renal cells have been reported to synthesize 1,25(OH) $_2$ D, notably skin cells, monocytes/macrophages, bone cells (51), and the placenta during pregnancy (52). The 1,25(OH) $_2$ D produced by many of these non-renal tissues may act in a paracrine/autocrine fashion to regulate cell growth, differentiation, and local function. The renal production of 1,25(OH) $_2$ D is stimulated by hypocalcemia, hypophosphatemia, and elevated PTH levels. The renal 1 α (OH)ase is potently inhibited by the phosphaturic hormone, fibroblast growth factor (FGF) 23 and also by 1,25(OH) $_2$ D per se in a negative feedback loop. As well, FGF23 and 1,25(OH) $_2$ D can both stimulate a 24-hydroxylase enzyme (CYP24A1). This cytochrome P450 monooxygenase produces 24,25(OH) $_2$ D and 1,24,25(OH) $_3$ D from 25(OH)D and 1,25(OH) $_2$ D respectively (53). These metabolites are generally believed to be biologically inert and represent the first step in biodegradation. After several further hydroxylations, cleavage of the secosteroid side chain occurs between carbons 23 and 24 leading to the production of the inert, water-soluble end product calcitroic acid. This metabolism may occur in kidney, liver and target tissues such as intestine and bone.

Vitamin D Actions

The unbound active form of vitamin D, 1,25(OH) $_2$ D can enter target cells and interact with the ligand-binding domain of a specific nuclear receptor (VDR) (54). The 1,25(OH) $_2$ D-VDR complex heterodimerizes with the retinoid X receptor (RXR) and then interacts with a vitamin D-responsive element (VDRE) on a target gene to enhance or inhibit the transcription of such

target genes. The activity of the VDR is enhanced by co-activator proteins that can also bind to discrete regions of the VDR and remodel chromatin, acetylate nucleosomal histones and contact the basal transcriptional machinery. Co-repressors can bind to the VDR in the absence of ligand and also modify its activity. Although ligand-independent VDR activation and non-genomic actions of 1,25(OH) $_2$ D have been reported their physiologic significance is currently unclear.

A major biologic function of circulating 1,25(OH) $_2$ D is to increase the efficiency of the small intestine to absorb dietary calcium. Intestinal absorption of calcium occurs by an active transcellular path and by a non-saturable paracellular path. Active calcium absorption accounts for 10-15% of a dietary load (55). Active transcellular intestinal absorption involves (as does Ca $^{+2}$ reabsorption in the kidney), three sequential cellular steps, a rate-limiting step involving transfer of luminal Ca $^{+2}$ into the intestinal cell via the epithelial Ca $^{+2}$ channel TRPV6, or via other calcium channels, intracellular diffusion, mediated by the Ca $^{+2}$ -binding protein, calbindin-D9K or by other calcium binding proteins such as calmodulin, and extrusion at the basolateral surface into the blood predominantly through the activity of the Ca $^{+2}$ -ATPase, PMCA 1b (56). 1,25(OH) $_2$ D, by interacting with the VDR (57) mainly, but not exclusively, in the duodenum, appears to increase all 3 steps by increasing gene expression of TRPV6, a channel-associated protein, annexin2 calbindin-D9K and to a lesser extent, the basolateral extrusion system PMCA1b (36,56). Calcium within the cell may also be sequestered by intracellular organelles such as the endoplasmic reticulum and mitochondria which could also contribute to the protection of the cell against excessively high calcium. Increasing evidence now supports regulation by 1,25(OH) $_2$ D of active transport of calcium by distal as well as proximal segments of the intestine as well as paracellular calcium transport (58), and by modulating additional intestinal targets (59). Reductions in dietary

intake of calcium can lead to increased PTH secretion and increased 1,25(OH)₂D production which can enhance fractional calcium absorption and compensate for the dietary reduction. Although 1,25(OH)₂D also increases phosphate absorption, mainly in the jejunum and ileum, nearly 50% of dietary phosphorus can be absorbed even in the absence of 1,25(OH)₂D.

Although vitamin D is known to be essential for normal mineralization of bone, its major role in this respect appears to be largely indirect i.e., by enhancing intestinal absorption of calcium and phosphate in the small intestine, maintaining these ions in the normal range and thereby facilitating hydroxyapatite deposition in bone matrix. The major direct function of 1,25(OH)₂D on bone appears to be to enhance mobilization of calcium stores when dietary calcium is insufficient to maintain a normal ECF calcium (60). As with PTH, 1,25(OH)₂D enhances osteoclastic bone resorption by binding to its receptors in cells of the osteoblast lineage and stimulating the RANK/RANKL system to enhance the proliferation, differentiation and activation of the osteoclastic system from its monocytic precursors (41), but high concentrations may also inhibit calcium deposition in bone (61). Endogenous 1,25(OH)₂D has also been reported to have an anabolic role in vivo (56,62).

Although effects of 1,25(OH)₂D on both calcium and phosphorus handling in the kidney have been reported, it remains uncertain whether 1,25(OH)₂D plays a major role in altering renal tubular reabsorption or excretion of these ions in humans.

Parathyroid Hormone Related Protein (PTHrP)

PTHrP was discovered as the mediator of the syndrome of "humoral hypercalcemia of malignancy" (HHM) (63). In this syndrome a variety of cancers, essentially in the absence of skeletal metastases, produce a PTH-like substance which can cause a

constellation of biochemical abnormalities including hypercalcemia, hypophosphatemia, and increased urinary cyclic AMP excretion. These mimic the biochemical effects of PTH but occur in the absence of detectable circulating levels of this hormone.

PTHrP is encoded by a single-copy gene, *PTHrP*, located on chromosome 12 whereas the gene encoding PTH is found on chromosome 11. Nevertheless, these two chromosomes encode many similar genes and are believed to have arisen by an ancient duplication event. Consequently, *PTHrP* and *PTH* may be members of a single gene family (64,65). The human *PTHrP* gene which is driven by at least three promoters, contains at least seven exons, shows several patterns of alternative splicing, and is considerably more complex than the *PTH* gene. Each gene encodes a leader or "pre" sequence, a "pro" sequence and a mature form. In the case of human PTH, the mature form is 84 amino acids. In the case of human PTHrP, 3 isoforms of 139, 141 and 173 residues can occur by alternate splicing, and sequences of these isoforms are identical through residue 139. Several common structural features of the *PTHrP* and *PTH* genes suggest that they are related. Thus, the major coding exon of both genes starts precisely at the same nucleotide, one base before the codons encoding the Lys-Arg residues of the prohormone sequences of each hormone. In the NH₂ terminus of both peptides, 8 of the first 13 amino acid residues are identical. These identities although limited are believed to be responsible for the similar bioactivities of the NH₂ terminal domains of these peptides (66), such that synthetic PTH (1-34) and synthetic PTHrP (1-34) interact with a common receptor (PTHR1) (26,27) and have similar effects on calcium and phosphate homeostasis. Thus, PTHrP is the second member of the PTH family to have been discovered. A hypothalamic peptide called tuberoinfundibular peptide of 39 residues (TIP 39) appears to represent a third member of the PTH gene family (67) and can interact at a second PTH receptor

termed the type II receptor (PTHR2) (68) (to which PTHrP does not bind (Fig. 2). The precise physiologic role of TIP 39 (also called PTH2) and of PTHR2 remain to be elucidated, however the TIP39/PTHR2 system has been implicated in the control of

nociception, fear and fear incubation, anxiety and depression-like behaviors, and maternal and social behaviors. It also appears to influence thermoregulation and potentially auditory responses (69).

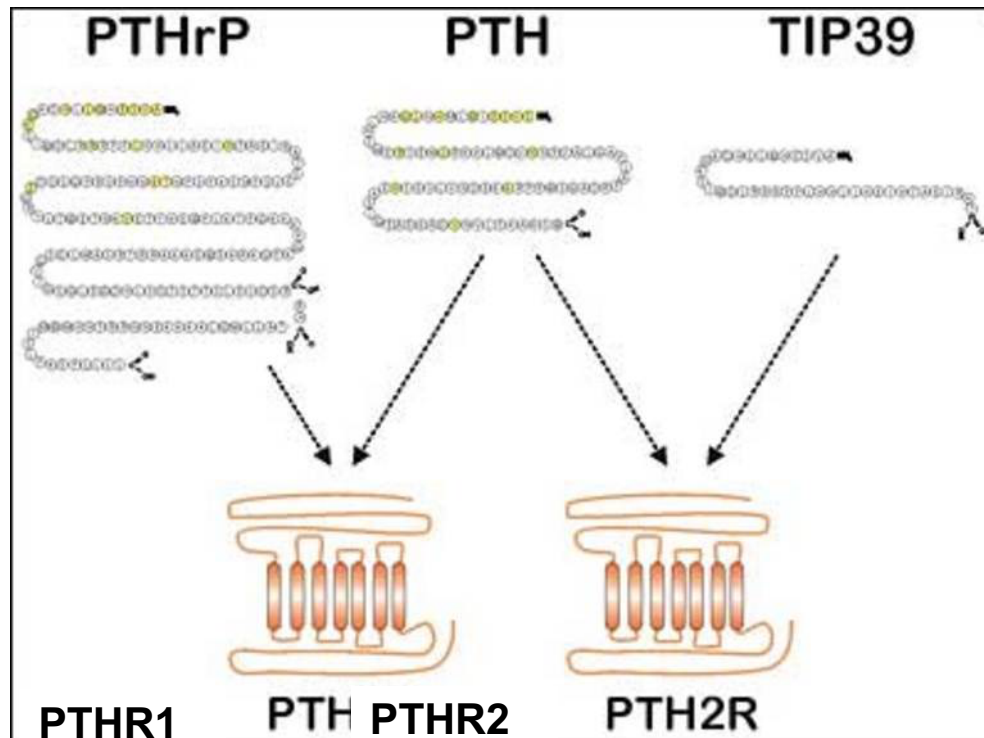


Figure 2. PTH and PTHR gene families: PTHrP, PTH and TIP39 appear to be products of a single gene family. Although only nine amino acids in the NH₂-terminal domains of these three peptides are conserved these are functionally important residues. The receptors for these peptides, PTHR1 and PTHR2, are both 7 transmembrane-spanning G protein-coupled receptors which seem to be products of a single gene family. PTHrP binds and activates PTHR1; it binds weakly to PTHR2 and does not activate it. PTH can bind and activate both PTHR1 and PTHR2. TIP39 can bind to and activate PTHR2 but not PTHR1.

REGULATION OF PTHrP PRODUCTION

In contrast to PTH, whose expression is limited mainly to parathyroid cells, PTHrP is widely expressed in many fetal and adult tissues (70). This is compatible with its primary role as a modulator of cell growth and differentiation. A major locus of regulation of PTHrP

production is at the level of gene transcription although both regulated and constitutive secretion of the hormone have been described in various cell types (71,72).

Key stimulators of gene transcription are a variety of growth factors and cytokines (73) including epidermal

growth factor (EGF) (74), IGF-1 (75), and transforming growth factor b (TGFb) (76). Inhibition of growth factor action, by employing a farnesyl transferase inhibitor to decrease ras-mediated cell signaling, has proved effective in inhibiting PTHrP production in vitro and in studies in vivo using an animal model of malignancy which overproduced PTHrP (77). Hypercalcemia associated with these tumors was also diminished.

Several steroidal hormones including 1,25(OH)₂D (78), glucocorticoids (79), and androgens (80) have been reported to be potent inhibitors of PTHrP gene expression. This prompted the use of 1,25(OH)₂D (81) and of low calcemic analogues of vitamin D (82) in studies with tumor cells, both in vitro and in animals in vivo, to determine if overproduction of PTHrP by these tumors could be inhibited. Indeed, PTHrP production was inhibited, the associated hypercalcemia was reduced, and survival of the animals was increased.

PTHrP is biosynthesized as a precursor form, proPTHrP and the propeptide must be cleaved to the mature peptide in order to achieve optimal bioactivity. This occurs by prohormone convertase activity (83). This processing locus was attacked using a furin antisense approach to block prohormone convertase activity in an animal tumor model which overproduces PTHrP (84). Bioactive PTHrP production was diminished with this intervention, and, in vivo, the hypercalcemia associated with the control tumor was not observed.

Serine proteases may also act on PTHrP internally, in various cell types, to cleave an NH₂ terminal fragment, a midregion fragment (85) and carboxyl terminal fragments (86) from the mature forms, each with apparently distinct bioactivities. The in vivo significance of this processing remains to be determined. Nevertheless, PTHrP has been described as a polyhormone.

PTHrP ACTIONS

The major effects of PTHrP appear to be mediated by binding of an NH₂ terminal domain, PTHrP (1-36), to the PTHR1 linked to adenylate cyclase, or phospholipase C. In some developing tissues, e.g., teeth. PTHrP is expressed in epithelial cells whereas the PTHR1 is in adjacent mesenchymal cells facilitating epithelial-mesenchymal interactions (87).

A mid-region domain of PTHrP (37-86) has been implicated in placental calcium transport (85) and a COOH terminal region (107-139) has been reported to inhibit osteoclasts (86). Nevertheless, distinct receptors for these putative bioactive regions have not been described.

A bipartite nuclear localization sequence (NLS) has been discovered in PTHrP at sequence positions 87 to 106 and has been shown to be capable, in vitro, of directing PTHrP to the nucleus and, in fact, to the nucleolus (88). Translocation from the cytoplasm to the nucleus is facilitated by binding to importin beta and seems cell cycle dependent. Although cyclin-dependent (cdc2) kinase can phosphorylate PTHrP this may not be the sole regulator of PTHrP nuclear import (89). Inasmuch as PTHrP contains a presequence or leader sequence which directs it to the secretory pathway, 3 pathways have been postulated which could lead it to access to the cytoplasm and thence the nucleus. Thus, PTHrP has been shown in some studies to be internalized after secretion and to access the cytoplasm by this route (90). Reverse transport of PTHrP from the endoplasmic reticulum to the cytoplasm has been reported in other studies (91). Finally, alternate initiation of translation at downstream non-AUG codons that allowed nascent PTHrP to bypass ER transit and localize to the nucleus and nucleolus has also been reported (92). In vitro studies have suggested that nuclear localization of PTHrP may be involved in its proliferative activity and/or in inhibition of apoptosis (87), and in vivo, PTHrP “knockin” mice have been reported which express truncated forms of PTHrP that lack the NLS and the

carboxyl -terminus but retain the amino terminus and the capacity to bind to PTHR1. The resulting mutants show growth retardation, defects in multiple organs and early lethality. Consequently, these studies indicate a functional *in vivo* role for the nuclear localization of this protein (93,94).

Overall, reported physiologic effects of PTHrP can be grouped into those relating to ion homeostasis; those relating to smooth muscle relaxation; and those associated with cell growth, differentiation and apoptosis. The majority of the physiological effects of PTHrP appear to occur by short-range *i.e.*, paracrine/autocrine and intracrine mechanisms rather than long-range *i.e.* endocrine mechanisms.

With respect to ion homeostasis PTHrP can modulate placental calcium transport and appears necessary for normal fetal calcium homeostasis (95). In the adult, however the major role in calcium and phosphorus homeostasis appears to be carried out by PTH rather than by PTHrP in view of the fact that PTHrP concentrations in normal adults are either very low or undetectable. This situation reverses when neoplasms constitutively hypersecrete PTHrP in which case PTHrP mimics the effects of PTH on bone and kidney and the resultant hypercalcemia suppresses endogenous PTH secretion.

PTHrP has been shown to cause smooth muscle relaxation in a variety of tissues including blood vessels (96) (leading to dilatation), uterus (97), and bladder (98). The physiologic significance of these effects however remains to be determined.

Finally, PTHrP has been shown to modify cell growth, differentiated function and programmed cell death in a variety of different fetal and adult tissues. Most notable have been breast (99), skin (100), nervous tissue (101) and pancreatic islets (102) where PTHrP appears to function to assure normal development. The most striking developmental effects of PTHrP

however have been in the skeleton. Targeted deletion of the PTHrP gene in mice produces a lethal chondrodysplasia (103,104), demonstrating the important and non-redundant role of PTHrP in endochondral bone formation. Animals die at birth, although the cause of death is uncertain. A major alteration appears to occur in the cartilaginous growth plate where, in the absence of PTHrP, chondrocyte proliferation is reduced and accelerated chondrocyte differentiation and apoptosis occurs. Increased bone formation occurs, apparently due to secondary hyperparathyroidism (42) and the overall effect is a severely deformed skeleton. Even more severe skeletal dysplasia occurs when either the gene encoding the PTHR1 itself (105) or the genes encoding both PTH and PTHrP are deleted (42). Both models produce similar phenotypes in mice. In the PTHrP knock-in mice that express PTHrP (1-84) but not the NLS or carboxyl terminus, the epiphyseal growth plate was markedly abnormal in this model, but the abnormality consisted of a reduced proliferative zone but normal hypertrophic zone architecture, suggesting that secreted and intracellular PTHrP may act synergistically to regulate the growth plate. In humans, an inactivating mutation of the PTHR1 produces a similar lethal chondro-osseous dysplasia termed Blomstrand's Syndrome (106,107). Consequently these *in vivo* observations demonstrate that PTHrP is essential, at least for normal development of the cartilaginous growth plate and endochondral bone formation. Interestingly mice that are heterozygous for PTHrP ablation appear normal at birth but develop reduced trabecular bone as they age demonstrating an osseous phenotype due to haploinsufficiency (37). This has been shown to be via a paracrine effect of PTHrP located in osteoblastic cells (108). Furthermore, hypoparathyroid mice that have PTHrP haploinsufficiency do not develop the increased trabecular bone mass that is a characteristic of hypoparathyroidism (109). PTHrP knock-in mice that express PTHrP (1-84) but lack the NLS and carboxyl terminus also appear to develop reductions

in osteoblastic activity again suggesting synergy between the extra-cellular and intracellular actions of PTHrP (110). In humans, variants of the *PTH1LH* gene have been associated with achievement of peak bone mass and in genome wide association studies have been associated with reduced bone mineral density. Overall, therefore the two ligands of PTHR1 i.e., PTH and PTHrP appear to have differing roles in utero and post-natally. In the fetus PTH appears to exert anabolic activity in trabecular bone whereas PTHrP regulates the orderly development of the growth plate. In contrast, in postnatal life, PTHrP acting as a paracrine/autocrine modulator assumes an anabolic role for bone whereas PTH predominantly defends against a decrease in extracellular fluid calcium by resorbing bone.

MEDIATORS OF BONE REMODELING

Normal adult bone is constantly undergoing "turnover" or remodeling (111). This is characterized by sequences of activation of osteoclasts followed by osteoclastic bone resorption followed by osteoblastic bone formation, which occur on the same bone surface. The process of bone modelling is the predominant event in the growing skeleton and can lead to changes in skeletal shape, but the process can also persist into adult bone; modelling is characterized by bone-forming osteoblasts and bone-resorbing osteoclasts acting on different bone surfaces and acting independent of each other, resulting in changes in bone size and shape. The sequential cellular activities in remodeling occur on the same bone surface in focal and discrete packets in both trabecular and cortical bone and are termed bone remodeling units or bone multicellular units (BMUs). This coupling of osteoblastic bone formation to bone resorption may occur via the action of growth factors released by resorbed bone e.g., TGF β , IGF-1 and fibroblast growth factor (FGF) which can induce osteoclast apoptosis and also induce chemotaxis of osteoblast precursors, including mesenchymal stem cells, and facilitate their

proliferation and differentiation at the site of repair. Homodimeric platelet-derived growth factor (PDGF) composed of two B units (PDGF-BB) may also be released from matrix and induce blood vessel formation that may also provide progenitor cells for later differentiation into osteoblasts and bone formation (112). In addition, direct activation of cells of the osteoblast phenotype by osteoclast family members appears to occur, and such "coupling" mechanisms include a number of secreted factors, as well as molecules involved in direct cell-cell communication between osteoclasts and the osteoblast lineage. Although a number of molecular signals regulating this direct osteoclast-osteoblast coupling process have been described (113), their precise in vivo role remains to be established. A number of additional systemic and local factors regulate the process of bone remodeling. In general, those factors which enhance bone resorption may do so by creating an imbalance between the depth of osteoclastic bone erosion and the extent of osteoblastic repair or by increasing the numbers of remodeling units which are active at any given time i.e., by increasing the activation frequency of bone remodeling. These latter processes can also result in thinning and ultimately in perforation of trabecular bone and in increased porosity of cortical bone. One predominant example in which osteoblastic activity does not completely repair and replace the defect left by previous resorption is in multiple myeloma; in this case it has been reported that myeloma cells may release inhibitors of the Wnt signaling pathway such as the protein Dickkopf (Dkk) which inhibit osteoblast production (114), while stimulation of osteoclastic resorption continues. Such an imbalance can occasionally also occur in association with some advanced solid malignancies.

Systemic hormones such as PTH, PTHrP and 1,25(OH) $_2$ D can all initiate osteoclastic bone resorption and increase the activation frequency of bone remodeling. Thyroid hormone receptors are

present in osteoblastic cells and triiodothyronine can stimulate osteoclastic bone resorption and produce a high turnover state in bone (115). Vitamin A has a direct stimulatory effect on osteoclasts and can induce bone resorption as well (116).

A variety of local factors are critical for physiologic bone resorption and regulation of the normal bone-remodeling sequence and can be produced by osteoblastic, osteoclastic and immune cells. Thus, for example, interleukin-1 (IL-1) and M-CSF can be produced by both osteoblastic cells and by cells of the osteoclastic lineage. TNF α is released by monocytic cells, TNF β (lymphotoxin) by activated T lymphocytes, and interleukin-6 (IL-6) by osteoclastic cells (117). All

can enhance osteoclastic bone resorption. Leukotrienes are eicosanoids that are produced from arachidonic acid via a 5-lipoxygenase enzyme and can also induce osteoclastic bone resorption. Prostaglandins, particularly of the E series, may also stimulate bone resorption in vitro but appear to predominantly increase formation in vivo (118). Consequently, a variety of cytokines, growth factors, and eicosanoids may be produced in the bone environment and act to regulate the bone remodeling sequence. The inappropriate production of these regulators in pathologic conditions such as cancer (Fig. 3) may therefore contribute to altered bone dynamics, altered calcium fluxes through bone, and ultimately in altered ECF calcium homeostasis.

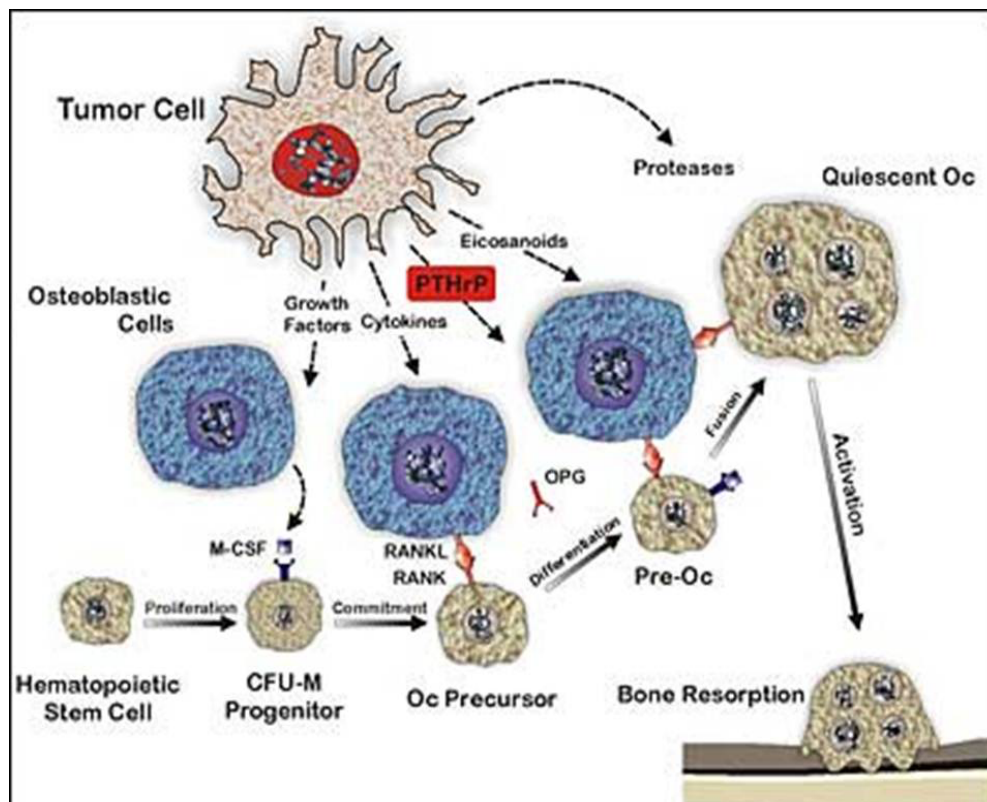


Figure 3. Production of bone resorbing substances by neoplasms. Tumor cells may release proteases which can facilitate tumor cell progression through unmineralized matrix. Tumors cells can also release PTHrP, cytokines, eicosanoids and growth factors (eg EGF) which can act on cells of the osteoblastic lineage to increase production of cytokines such as M-CSF and RANKL and to decrease production of

OPG. RANKL can bind to its cognate receptor RANK in osteoclastic cells, which are of hepatopoietic origin, and increase production and activation of multinucleated osteoclasts which can resorb mineralized bone.

HYPERCALCEMIC DISORDERS

Hypercalcemic disorders can be broadly grouped into Endocrine Disorders, Malignant Disorders, Inflammatory Disorders, Pediatric Syndromes,

Medication-Induced Hypercalcemia, and Immobilization (Table 1) (119). Approximately 90% of patients with hypercalcemia have primary hyperparathyroidism (PHPT) or malignancy-associated hypercalcemia (MAH).

Table 1. Hypercalcemic Disorders
1. Endocrine Disorders with Excess PTH Production A. Sporadic PHPT B. Familial Syndromic PHPT <i>a) Multiple Endocrine Neoplasia, Type 1 (MEN1) and 4 (MEN4)</i> <i>b) Multiple Endocrine Neoplasia, Type 2A (MEN2A)</i> <i>c) Hyperparathyroidism-Jaw Tumor Syndrome</i> C. Familial Non-Syndromic PHPT <i>a) Familial Isolated Hyperparathyroidism (FIH)</i> <i>b) Familial Hypocalciuric Hypercalcemia (FHH) 1-3 and Neonatal Severe Primary Hyperparathyroidism (NSHPT)</i> <i>c) Autoimmune Hypocalciuric Hypercalcemia</i> D. Tertiary Hyperparathyroidism
2. Endocrine Disorders without Excess PTH Production A. Hyperthyroidism B. Pheochromocytoma C. Vipoma D. Hypoadrenalism E. Jansen's Metaphyseal Chondrodysplasia
3. Malignancy-Associated Hypercalcemia (MAH) A. MAH with Elevated PTHrP <i>a) Humoral Hypercalcemia of Malignancy (HHM)</i> <i>b) Solid Tumors With Elevated PTHrP and Skeletal Metastases</i> <i>c) Hematologic Malignancies With Elevated PTHrP</i> B. MAH with Elevation of Other Systemic Factors <i>a) MAH With Elevated 1,25(OH)2D</i> <i>b) MAH With Elevated Cytokines</i> <i>c) Ectopic Hyperparathyroidism</i> <i>d) Multiple Myeloma</i>
4. Inflammatory Disorders Causing Hypercalcemia

A. Granulomatous Disorders	
B. Viral Syndromes (HIV)	
5. Pediatric Syndromes	
A. Williams Syndrome	
B. Idiopathic Infantile Hypercalcemia(<i>CYP 24A1</i> , <i>SLC34A1</i> mutations)	
C. Hypophosphatasia	
D. Congenital Lactase Deficiency	
E. Congenital Sucrase-Isomaltase Deficiency	
6. Medication-Induced	
A. Thiazides	H. Milk-Alkali Syndrome
B. Lithium	I. SGLT2 Inhibitors
C. Vitamin D	J. Immune Checkpoint Inhibitors
D. Vitamin A	K. Denosumab
E. Antiestrogens	L. Teriparatide, Abaloparatide
F. Theophylline	M. Foscarnet
G. Aluminum Intoxication	N. Ketogenic diet
7. Alterations in Muscle and Bone	
A. Immobilization	
B. Intense Exercise	
C. Rhabdomyolysis	

ENDOCRINE DISORDERS ASSOCIATED WITH HYPERCALCEMIA

Endocrine Disorders Associated with Excess PTH Production

A detailed discussion of primary hyperparathyroidism appears in an associated Endotext chapter. Consequently, only selected issues will be addressed here.

SPORADIC PRIMARY HYPERPARATHYROIDISM

Sporadic PHPT is generally (at least 85-90% of cases) associated with a single parathyroid adenoma which overproduces PTH. Although 10-15% of cases may be associated with multigland hyperplasia, it seems prudent to consider that at least some if not most of

these cases represent familial rather than sporadic disease (120). The presence of multiple adenomas should also suggest the possibility that all glands are involved as part of a familial syndrome. Malignant sporadic PHPT may occur as a consequence of parathyroid carcinoma, but is a relatively rare event (about 1% of cases).

To date, the genes that are the most strongly implicated in parathyroid adenomas underlying sporadic benign PHPT are an oncogene *CCND1*, that encodes a key regulator of the cell cycle (cyclin D1) and *MEN1*, a tumor suppressor gene, also implicated in familial multiple endocrine neoplasia type I (121). Mutations in *CDKN1B/p27* and other cyclin-dependent kinase inhibitor genes, including *EZH2*, *ZFX*, *CTNNB1/β-catenin*, have been seen in smaller subsets of sporadic adenomas (122). as have other

cyclin-dependent kinase inhibitor genes, including CDKN1A, CDKN2B, and CDKN2C which have each been implicated in familial forms of PHPT. Rare germline mutations in *CDC73* (*HRPT2*, a tumor suppressor gene associated with the Hyperparathyroidism-Jaw Tumor syndrome (123), and implicated in most sporadic parathyroid carcinomas (124). can occasionally be observed in sporadic adenomas. Additionally, mutations in *CASR*, which has also been implicated in a familial form of PHPT (Familial Hypocalciuric Hypercalcemia), has occasionally been observed in sporadic PHPT (125). The glial cells missing 2 (*GCM2*) gene (previously called GCMB) encodes the GCM2 transcription factor, which is essential to the development of the parathyroid glands and subsequent PTH expression. Activating variants of *GCM2*, which have been implicated in familial isolated hyperparathyroidism (FIHP) have also been reported as potential predisposition alleles in sporadic parathyroid tumors (126-129).

Other important parathyroid regulatory pathways that may play a role in the pathogenesis of hyperparathyroidism are those related to the principal regulators or parathyroid cell proliferation and PTH secretion i.e., 1,25(OH)₂D, Ca⁺² and phosphate. Rarely, sporadic hyperparathyroidism with hypocalciuria may occur, caused by inhibitory antibodies to the calcium-sensing receptor. This syndrome has been termed Autoimmune Hypocalciuric Hypercalcemia (130). The clinical manifestations of these disorders are caused by the overproduction of PTH and its effect on bone resorption, on its capacity to stimulate renal 1,25(OH)₂D production and renal calcium reabsorption, and on the resultant elevation of ECF calcium which can result in an increased filtered renal load of calcium. Increased ECF calcium can itself increase calcium excretion by stimulating the renal tubular CaSR and inhibiting tubular calcium reabsorption (Fig. 4).

Three major clinical subtypes of benign sporadic PHPT, the most common form of PHPT, have been described (131). In Symptomatic PHPT, the symptoms and signs of hypercalcemia are present and are determined by the rate of increase in serum calcium, and the severity of the hypercalcemia. Symptomatic PHPT may also be associated with overt skeletal and renal complications that may include fractures and/or osteitis fibrosa cystica, and/or, chronic kidney disease, nephrolithiasis and/or nephrocalcinosis.

In Asymptomatic PHPT, there are no overt symptoms or signs of hypercalcemia and the disorder is generally discovered by biochemical screening. After evaluation, target organ involvement may or may not be found. In the third subgroup, normocalcemic PHPT, skeletal or renal complications may or may not be found after clinical evaluation.

About 80% of cases of patients with benign sporadic PHPT present as mild or “asymptomatic” hyperparathyroidism in which hypercalcemia is generally less than 1mg/dL (0.25 mM) above the upper limit of normal and may be normal intermittently (132). However significant increases in serum calcium may occur even after 13 years of follow up. Excess PTH production can produce significant bone loss. Classically this is manifested by discrete lesions including subperiosteal bone resorption of the distal phalanges, osteitis fibrosa cystica characterized by bone cysts and "brown tumors" (i.e., collections of osteoclasts intermixed with poorly mineralized woven bone), and ultimately fractures. However, although these manifestations were commonly seen in the past, they are less frequently seen today (2% of cases) (133-136). Whether this severe bone disease reflects a delay in detecting primary hyperparathyroidism early, or as seems equally plausible, is a manifestation of excess PTH action in the face of marginal or deficient vitamin D and calcium intake (137), remains to be determined. The more common skeletal manifestation

of excess circulating PTH, reflects the "catabolic bone activity" of PTH, (138) and production of an osteoporosis clinical picture. Consequently, the severity of bone disease appears considerably diminished.

Possibly as a consequence of less severe bone disease, hypercalcemia is also less marked, the filtered load of renal calcium is lower and the incidence of kidney stones and particularly of nephrocalcinosis has declined as well. Nevertheless, hypercalciuria still occurs in 35-40% of patients with primary benign sporadic hyperparathyroidism and kidney stones occur in 15-20% (139). About 25% of patients with mild ("asymptomatic") sporadic PHPT have been reported to develop renal manifestations within 10 years, including renal concentrating defects or kidney stones. In regions of the globe, where relative or absolute

vitamin D deficiency may limit the severity of hypercalcemia and therefore the filtered load of calcium, the incidence of nephrolithiasis (10-40%) does not appear to be as different as is the incidence of bone disease.

The higher incidence of benign sporadic PHPT in women and in an older age group (140) also appears to distinguish the current presentation of this disorder in certain regions of the world. Overall, in countries where routine biochemical screening is common (with tests including serum calcium and albumin), although the incidence of PHPT rises, the presentation changes toward the asymptomatic and even the normocalcemic variants of PHPT. Healthcare system practices, as well as more routine biochemical screening of the population both appear to account for this (141,142).

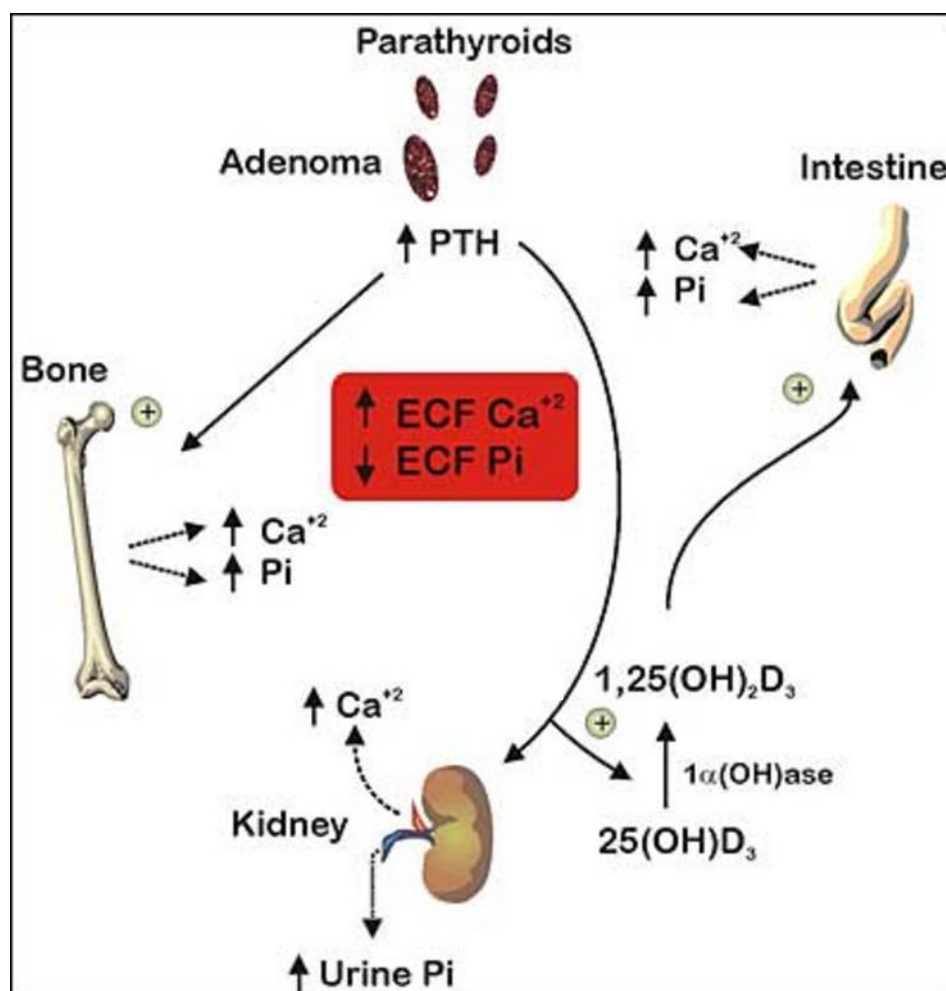


Figure 4. Disordered mineral homeostasis in hyperparathyroidism. In primary sporadic hyperparathyroidism PTH is generally overproduced by a single parathyroid adenoma. Increased PTH secretion leads to a net increase in skeletal resorption with release of Ca^{+2} and Pi (inorganic phosphate) from bone. PTH also increases renal $1\alpha(\text{OH})\text{ase}$ activity leading to increased production of $1,25(\text{OH})_2\text{D}_3$ from $25(\text{OH})\text{D}_3$ and increased Ca^{+2} and Pi absorption from the small intestine. PTH also enhances renal Ca^{+2} reabsorption and inhibits Pi reabsorption resulting in increased urine Pi excretion. The net result is an increase in ECF calcium and a decrease in ECF phosphate.

Abnormalities other than skeletal and renal have been associated with benign sporadic PHPT. These include gastrointestinal manifestations such as dyspepsia and acute pancreatitis. The incidence of peptic ulcer disease in sporadic PHPT is currently estimated to be about 10%, the same as in the general population but, the presence of multiple peptic ulcers may suggest the presence of multiple endocrine neoplasia type I

(MENI). Acute pancreatitis may be a manifestation of hypercalcemia per se but is estimated to occur in only 1.5% of those with sporadic PHPT. Neuromuscular abnormalities manifested by weakness and fatigue and accompanied by EMG changes may occur although the pathophysiology is uncertain. The relationship of hypertension and other cardiovascular manifestations as well as neuropsychiatric symptoms

to the hyperparathyroidism remains unclear inasmuch as the former is generally not reversible when the hyperparathyroidism is treated and the latter is quite common in the population at large and difficult to ascribe to hyperparathyroidism. Rarely, primary sporadic PHPT may present with severe acute hypercalcemia (parathyroid crisis) (143).

Surgical removal of the parathyroid adenoma currently remains the treatment of choice if the serum calcium is consistently greater than 1mg/dL (0.25mM) above normal; if there is evidence of bone disease [i.e. a BMD T-score of <-2.5 at the lumbar spine, total hip, femoral neck, or 33% radius (1/3 site), and/or a previous fracture fragility or a fracture diagnosed on imaging (densitometric vertebral fracture assessment [VFA] or vertebral X-ray)]; if there is evidence of renal involvement [i.e. if eGFR or creatinine clearance is reduced to <60 ml/min, the urinary calcium excretion is greater than 250mg/d (>6.2 mmol) for women or greater than 300mg/d (>7.48 mmol) for men, or if there is evidence of nephrocalcinosis or nephrolithiasis by ultrasound or x-ray or other imaging modality; (144). Surgery is also indicated if the patient is less than age 50. and in patients for whom medical surveillance is either not desired or not possible (145).

Parathyroid imaging is not recommended to establish or confirm the diagnosis of PHPT, but has become routine for preoperative localization of the abnormal parathyroid tissue. The most commonly employed preoperative parathyroid imaging techniques are radionuclide imaging (i.e., technetium-99 m-sestamibi subtraction scintigraphy), high resolution neck ultrasound and contrast-enhanced four dimensional (4D) computed tomography (CT). Magnetic resonance imaging, and positron emission tomography scanning, arteriography, and selective venous sampling for PTH are usually reserved for patients who have not been cured by previous explorations or for whom other localization techniques are not informative or are discordant.

The type of surgical procedure i.e., noninvasive or standard, and the use of operative adjuncts (e.g., rapid PTH assay) is institution specific and should be based on the expertise and resource availability of the surgeon and institution. Where more than one gland is enlarged it is reasonable to assume that this is multiple glandular disease and removal of 3½ glands is indicated. Severe, chronic hypercalcemia is more commonly associated with parathyroid carcinoma. Complete resection of the primary lesion is urgent in this case.

For those patients with PHPT who meet guidelines for surgery but are unable or unwilling to undergo parathyroidectomy, medical therapy may be considered. Calcium intake should be 800 mg/day for women <50 and men <70 years of age, 1000 mg/day for women >50 and men >70 years old (corresponding to the U.S. Institute of Medicine nutritional guidelines). If necessary, vitamin D should be supplemented to achieve levels of 25OHD which are >30 ng/mL (70nmol/L) but less than the upper limit of normal for the laboratory reference range. The calcimimetic agent cinacalcet (146) (that mimics or potentiates the action of calcium at the CaSR) may be used in patients with PHPT with severe chronic hypercalcemia who are not surgical candidates, in order to reduce the serum calcium concentration into the normal range. Bisphosphonates, e.g., alendronate (147), or denosumab, can be employed to increase bone density if there are no contraindications. Bisphosphonates or denosumab in combination with cinacalcet can be considered to lower the serum calcium and to increase BMD.

Although estrogen therapy has been advocated for the treatment of PHPT in postmenopausal women (148) potential adverse effects of estrogen therapy, including breast cancer and cardiovascular complications, make this option unattractive. The effect of conjugated estrogen on the reduction of

serum calcium is inconsistent (149,150). Although selective estrogen receptor modulators may be an alternative (149), and a very short-term report with raloxifene demonstrated a significant reduction in the serum calcium concentration, (151) few long-term studies have been done to assess this.

FAMILIAL SYNDROMIC PRIMARY HYPERPARATHYROIDISM

Although familial PHPT syndromes are dominantly inherited, approximately 10% may occur through de novo pathogenic variants. Genetic evaluation should be considered for patients <30 years old, those with multigland disease by history or imaging, and/or those with a family history of hypercalcemia and/or a syndromic disease. Family history was the strongest predictor of hereditary PHPT in a recently reported cohort (152).

Multiple Endocrine Neoplasia, Type 1 and 4 (MEN1 and 4)

MEN1 is a familial disorder with an autosomal dominant pattern of inheritance which is characterized by tumors in the anterior pituitary, parathyroid, and enteropancreatic endocrine cells (although tumors in several other endocrine and non-endocrine tissues may also be associated with the syndrome) (153) (Fig. 5). Patients exhibit loss-of-function germline mutations in the tumor suppressor gene, *MEN1*, which encodes the nuclear protein, menin (154).

Tumors in a proband in at least 2 MEN1 sites (pituitary, parathyroid, or enteropancreatic endocrine cells) and

in at least one of these sites in a first-degree relative confirms the clinical phenotype. The most common and the earliest endocrinopathy is PHPT (80-100% of cases) (155). In contrast to sporadic PHPT however MEN1 occurs in both sexes equally and patients are younger at the time of diagnosis. Furthermore, in contrast to the frequent occurrence of a single adenoma in sporadic disease, multigland involvement in an asymmetric fashion is the norm in MEN1. Enteropancreatic tumors are usually multiple and gastrinomas are the most common. These may produce the Zollinger-Ellison Syndrome, and occur in the duodenum as well as in the pancreatic islets. Gastrinomas can potentially produce considerable morbidity due to the potential for ulcers and the possibility of metastatic disease. Insulinomas, glucagonomas, VIPomas, and other islet tumors can occur as well. A variety of functioning anterior pituitary tumors can occur although prolactinomas are most frequent and anterior pituitary tumors may also be non-functioning. Finally, foregut carcinoids and other endocrine tumors have been described with lesser frequency and skin tumors such as facial angiofibromas and truncal collagenomas may occur and appear specific for MEN1.

Probands or kindreds with MEN1-like features with germline autosomal dominant mutation of the cyclin dependent kinase inhibitor *CDKN1B*, encoding P27 (Kip1) have been described and the syndrome named MEN4. Patients with MEN4 are characterized by the same combination of tumors as MEN1 but MEN4 is a rarer cause of hereditary PHPT; thus, the prevalence of MEN4 among all MEN1 probands plus MEN1-like probands is approximately 1%.

Familial Hyperparathyroid States

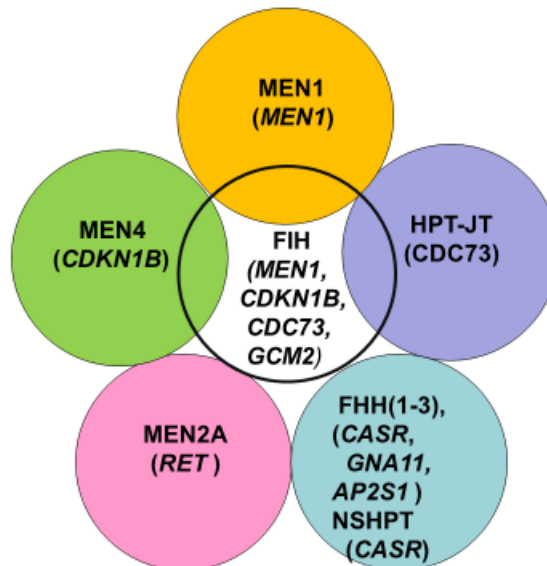


Figure 5. Familial hyperparathyroidism. Familial hyperparathyroidism (FHPT) can occur in the MEN1 Syndrome, in which *MEN1* is mutated; in MEN4 in which *CDKN1B* is mutated; in the MEN2A Syndrome, in which *RET* is mutated; in FHH and NSHPT in which *CaSR*, *GNA11* or *AP2S1* is mutated; and in the Hyperparathyroidism-Jaw Tumor (HPT-JT) Syndrome in which *CDC73* is mutated. Familial isolated hyperparathyroidism (FIH) refers to familial hyperparathyroidism in the absence of the specific features of the other documented syndromes and suggests that other genes relevant to parathyroid neoplasia await identification, although variants of several genes identified with syndromic FHPT have been found in some with this disorder.(eg *MEN1*, *CDKN1B*, *CDC73*, and *GCM2*).

Patients with hyperparathyroidism due to MEN1 have multiglandular disease and surgical resection of fewer than 3 glands lead to high rates of recurrence. Consequently, either subtotal parathyroidectomy with 3½ gland removal or total parathyroidectomy is recommended.

Multiple Endocrine Neoplasia, Type 2A (MEN2A)

MEN2A is an autosomal dominant familial syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytomas, and hyperparathyroidism (156,157). This syndrome results from activating germline mutations in the rearranged during

transfection (*RET*) protooncogene which is a receptor tyrosine kinase (158). Two variants of this disorder are MEN2B (also called MEN3) which includes familial MTC, familial pheochromocytomas, mucosal and intestinal neuromas, and a Marfanoid habitus, but no hyperparathyroidism, and familial MTC alone (159). Two other variants of MEN2 include MEN2 with cutaneous lichen amyloidosis, and MEN2 with Hirschsprung's disease. Analyses of *RET* mutations in these syndromes have provided good genotype-phenotype correlations (160).

The dominant feature of the MEN2A syndrome is MTC, a calcitonin-secreting neoplasm of thyroid C

cells (161). Genetic testing for mutations in the *RET* oncogene is of value in considering prophylactic thyroidectomy to prevent MTC. Another major feature is pheochromocytomas which are frequently bilateral but which generally have low malignant potential.

Hyperparathyroidism is milder and less frequent (5-20%) in MEN2A than in MEN1 but is also associated with multigland involvement in which gland enlargement may be asymmetric. The treatment of the hyperparathyroidism is as for MEN1.

Hyperparathyroidism-Jaw Tumor Syndrome

Hyperparathyroidism - Jaw Tumor Syndrome (HPT-JT) is an autosomal-dominant syndrome with incomplete penetrance and variable expression, caused by germline inactivating pathogenic variants in the tumor suppressor gene, Cell Division Cycle 73 (*CDC73*) (formerly called *HRPT2*), which encodes a protein termed parafibromin. Patients may present with early onset of single or multiple cystic parathyroid adenomas which may develop asynchronously, and ossifying fibromas of the mandible and maxilla. These jaw tumors lack osteoclasts and therefore differ from "brown tumors" (162,163). Affected individuals also have an increased risk (15–38%) of developing parathyroid carcinoma. Surgical removal of the affected parathyroid tissue is clearly indicated in this disorder. A variety of renal tumors have been described in some kindreds and other e.g., uterine tumors have been described in others. Mutations in *CDC73*, have been implicated in this syndrome (123), in sporadic parathyroid cancer (124), and in a minority of families with isolated hyperparathyroidism (164). Genetic testing in relatives can result in identification of individuals at risk for parathyroid carcinoma, enabling preventative or curative parathyroidectomy.

FAMILIAR NON-SYNDROMIC PRIMARY HYPERPARATHYROIDISM

Familial Isolated Hyperparathyroidism (FIH)

Familial Isolated Hyperparathyroidism (FIH) has been reported in which familial PHPT occurs in the absence of any other manifestation of the familial disorders described. The genetic etiology of FIHP is not fully understood but can arise due to pathogenic variants in genes associated with syndromic PHPT such as *MEN1*, *CDKN1B* and *CDC73*, which raises the possibility that FIHP is an incomplete manifestation of a syndromic form of PHPT. FIHP can also occur with activating pathogenic variants in *GCM2*. Such activating *GCM2* variants may contribute to facilitating more aggressive parathyroid disease (165). Undoubtedly additional research will uncover new genetic mutations which contribute to the pathogenesis of these cases.

Familial Hypocalciuric Hypercalcemia (FHH) and Neonatal Severe Primary Hyperparathyroidism (NSHPT)

Familial hypocalciuric hypercalcemia (FHH) (166), also called Familial Benign Hypercalcemia (FBH) (167) is an autosomal dominant inherited trait, causing a lifelong generally benign disorder of hypercalcemia. Inasmuch as the increased ECF calcium is inadequately sensed by altered CaSR function in the parathyroid gland, mild hyperplasia may also occur and "normal" levels or elevated levels of PTH are secreted despite the hypercalcemia. While most patients with FHH are asymptomatic, chondrocalcinosis and acute pancreatitis have occasionally been observed. Patients with FHH have often been misdiagnosed as having PHPT, since both disorders can have elevated or inappropriately normal PTH levels with elevated serum calcium. The hallmark feature of FHH is an inappropriately low urine calcium in relationship to the prevailing hypercalcemia i.e., a

calcium/ creatinine clearance ratio (CCCR), which is typically < 0.01; nevertheless, 20% of FHH patients may have a CCCR > 0.01, and therefore be indistinguishable from individuals with PHPT.

The molecular basis, in most cases, is a loss-of-function mutation in the calcium-sensing receptor (*CaSR*) gene (168) in which case the syndrome is now called FHH1. The protein product, CaSR, is a G-protein coupled receptor that predominantly signals via the G-protein subunit alpha-11 ($G\alpha_{11}$) to regulate calcium homeostasis. As a consequence, in heterozygotes, diminished ability of the CaSR in the parathyroid cell and in the CTAL of the kidney to detect ECF calcium occurs leading to increased PTH secretion and enhanced renal tubular reabsorption of calcium and magnesium respectively, leading to hypercalcemia, and often hypermagnesemia. Inactivating variants in the *GNA11* gene, encoding $G\alpha_{11}$ have also been reported to cause the syndrome, now termed FHH2. Hypercalcemia may be milder in FHH2 than in FHH1 (159). Adaptor protein-2 δ -subunit (*AP2 δ*) plays a pivotal role in clathrin-mediated endocytosis of CaSR. Missense variants of the *AP2S1* gene, encoding AP2 δ may also occur, causing the syndrome, FHH3, which is associated with the highest serum calcium levels. All FHH forms are inherited in an autosomal dominant fashion.

Genetic testing may be of particular value when hypocalciuric hypercalcemia occurs in an isolated patient without access to additional family members or familial isolated hyperparathyroidism (FIH) occurs in the absence of classical features of FHH.

In view of the fact that the increased renal reabsorption of calcium related to loss of CaSR function, and therefore hypercalcemia, persist after parathyroidectomy, and that the patients are generally asymptomatic, it is important to identify these patients to ensure that they are not subjected to parathyroidectomy.

Individuals who are homozygous for the mutated genes, or who are compound heterozygotes and therefore have little functional CaSR, can develop Neonatal Severe Hyperparathyroidism (NSHPT) (169). This disorder generally presents within a week of birth and is characterized by severe life-threatening hypercalcemia, hypermagnesemia, increased circulating PTH concentrations, massive hyperplasia of the parathyroid glands and relative hypocalciuria. Skeletal abnormalities include demineralization, widening of the metaphyses, osteitis fibrosa and fractures. This disorder may be lethal without urgent total parathyroidectomy.

Heterozygous inactivating variants in *CASR* can also present in the neonatal period with a less severe, intermediate form of PHPT.

Autoimmune Hypocalciuric Hypercalcemia

A biochemical phenotype of PTH-dependent hypercalcemia resembling that caused by heterozygous inactivating mutations of the CaSR in FHH1 can be observed in patients with antibodies to the extracellular domain of CaSR, which appear to inhibit activation of the CaSR by ECF Ca, and thereby stimulate PTH release. Autoimmune hypocalciuric hypercalcemic is an acquired disorder of extracellular calcium sensing that should be differentiated from that caused by inactivating mutations of the *CasR* (170).

TERTIARY HYPERPARATHYROIDISM

Tertiary hyperparathyroidism, is a clinical state due to the autonomous function of parathyroid tissue that develops in the face-of-long-standing secondary hyperparathyroidism (171).

Tertiary hyperparathyroidism may occur with monoclonal expansion of nodular areas of the parathyroid gland. These in turn can be associated with decreased VDR and decreased CaSR expression which may lead to an increased set point for PTH

secretion. The most common circumstance in which this occurs is in chronic renal failure where $1,25(\text{OH})_2\text{D}$ deficiency, hyperphosphatemia and hypocalcemia produce chronic stimulation of the parathyroid glands. However, tertiary hyperparathyroidism may occur in malabsorption syndromes (e.g., active celiac disease, extensive bowel resection, gastric bypass surgery), and has also been described in some cases of X-linked hypophosphatemic rickets, or other hypophosphatemic osteomalacias, after long-term treatment with supplemental phosphate which is believed to induce intermittent slight decreases in ECF calcium and stimulation of PTH secretion. Tertiary hyperparathyroidism should be readily identified by the clinical context in which the hypercalcemia presents. In symptomatic patients, the use of the calcimimetic agent, cinacalcet, which enhances the capacity of ECF calcium to stimulate the CaSR, may be tried or surgical treatment may be required, i.e., either sub-total removal of the parathyroid mass or total parathyroidectomy

Endocrine Disorders Without Excess PTH Production

HYPERTHYROIDISM

Hypercalcemia has been reported in as many as 50% of patients with thyrotoxicosis (172). Bone turnover and resorption are increased due to direct effects of increased triiodothyronine on bone (173,174). The liberated calcium appears to suppress PTH release so that $1,25(\text{OH})_2\text{D}$ levels are reduced and renal calcium reabsorption is diminished. Treatment with a beta-adrenergic antagonist may reduce the hypercalcemia and therapy of the hyperthyroidism reverses the hypercalcemia (175,176).

PHEOCHROMOCYTOMA

Hypercalcemia has been reported with pheochromocytomas and may be due to excess PTHrP production (177). Serum PTHrP concentrations may be reduced by alpha-adrenergic blockers in these patients (178).

VIPOMA

Hypercalcemia has frequently been reported in association with the neuroendocrine tumor VIPoma but whether the hypercalcemia is due to the overproduction of vasoactive intestinal polypeptide (VIP) per se causing bone resorption or to other co-secreted peptides such as PTHrP is uncertain (179).

HYPOADRENALISM

Although both primary and secondary hypoadrenalism have been associated with hypercalcemia (180,181), the underlying etiology is unclear. Ionized calcium appears to be elevated and PTH and $1,25(\text{OH})_2\text{D}$ are suppressed. The hypercalcemia is reversed by volume expansion and glucocorticoids.

JANSEN'S METAPHYSEAL CHONDRODYSPLASIA

Jansen's Syndrome is a rare autosomal dominant form of short-limbed dwarfism in which neonates presents with metaphyseal chondrodysplasia, hypercalcemia, and hypophosphatemia which is lifelong (182). PTH and PTHrP levels in serum are undetectable but renal tubular reabsorption of phosphate is decreased and urinary cyclic AMP is increased. An activating mutation of the PTHR1 has been described in such patients. A variety of skeletal abnormalities have been noted which reflect the overactivity of PTH and PTHrP during development, growth and in the adult skeleton.

MALIGNANCY-ASSOCIATED HYPERCALCEMIA

It has been estimated that hypercalcemia can occur in up to 10% of malignancies. Malignancy-associated hypercalcemia (MAH) can occur in the presence or the absence of elevated PTHrP production. Using two-site immunoradiometric assays for PTHrP several groups have confirmed that 50-90% of patients with solid tumors and hypercalcemia and 20-60% of patients with hematologic malignancies and hypercalcemia have elevated circulating PTHrP. MAH both with and without elevated serum PTHrP concentrations will therefore be discussed.

MAH With Elevated PTHrP

HISTORICAL CONSIDERATIONS

The association between hypercalcemia and neoplastic disease was first reported in the 1920's and the suggestion was made that the direct osteolytic action of malignant cells was responsible for the release of calcium from bone, resulting in hypercalcemia (183). An association between neoplasia and hypercalcemia was later reported in the English medical literature (184). In 1941 Fuller Albright, while discussing a case of a patient with a renal cell carcinoma, who in fact had a bone metastasis, noted that hypophosphatemia should not have accompanied the hypercalcemia if the tumor was simply producing hypercalcemia by causing osteolysis (185). He suggested that the tumor might be secreting a hypercalcemic substance which was also phosphaturic. Consequently, the concept of "ectopic" PTH production by tumors arose and led to the term "ectopic hyperparathyroidism" (186) or "pseudohyperparathyroidism". Nevertheless, immunoreactive PTH could not be detected in the circulation of these patients (187) and PTH mRNA could not be detected in their tumors (188). To circumvent these issues, bioassays sensitive to PTH were employed to identify PTH-like bioactivity in blood and tumor extracts (189,190) and eventually to identify

a novel protein (191), PTHrP. Despite the limited homology of PTH and PTHrP within the NH₂-terminal 13 amino acids, PTH (1-34) and PTHrP (1-34) exhibit similar effects on raising blood calcium and lowering blood phosphorus, reducing renal calcium clearance, and inhibiting the renal tubular reabsorption of phosphate. The molecular basis of these effects was shown to be cross-reactivity at the PTHR1. In animal models of MAH associated with high PTHrP secretion, passive immunization with PTHrP antiserum reduced hypercalcemia (192,193). Initially after passive immunization, urine calcium increased reflecting reduction in PTHrP induced renal calcium reabsorption; only subsequently did urine calcium decline as bone resorption was neutralized and the filtered load of calcium fell (193). Consequently, the hypercalcemia induced by PTHrP involved a renal mechanism as well as an osseous one. Other similarities were noted between PTHrP and PTH including similar capacities to raise 1,25(OH)₂D levels (194) and effects on bone characterized, for both PTHrP and PTH, by enhanced bone turnover and increased bone formation as well as resorption (195).

HUMORAL HYPERCALCEMIA OF MALIGNANCY

The classic neoplasms associated with hypercalcemia and excess PTHrP have few or no skeletal metastases, and are solid tumors (Fig. 6). This constellation has been termed humoral hypercalcemia of malignancy (HHM). The availability of assays to detect PTHrP demonstrated a broad spectrum of tumors that produce the peptide (196-200). Hypercalcemia is notably associated with squamous cell tumors arising in most sites including esophagus, cervix, vulva, skin, and head and neck, but especially in lung. Renal cell carcinomas are also commonly associated with the syndrome as are bladder and ovarian carcinomas. On the other hand, patients with colon, gastric, prostate, thyroid, and non-squamous cell lung cancers manifest hypercalcemia much less commonly.

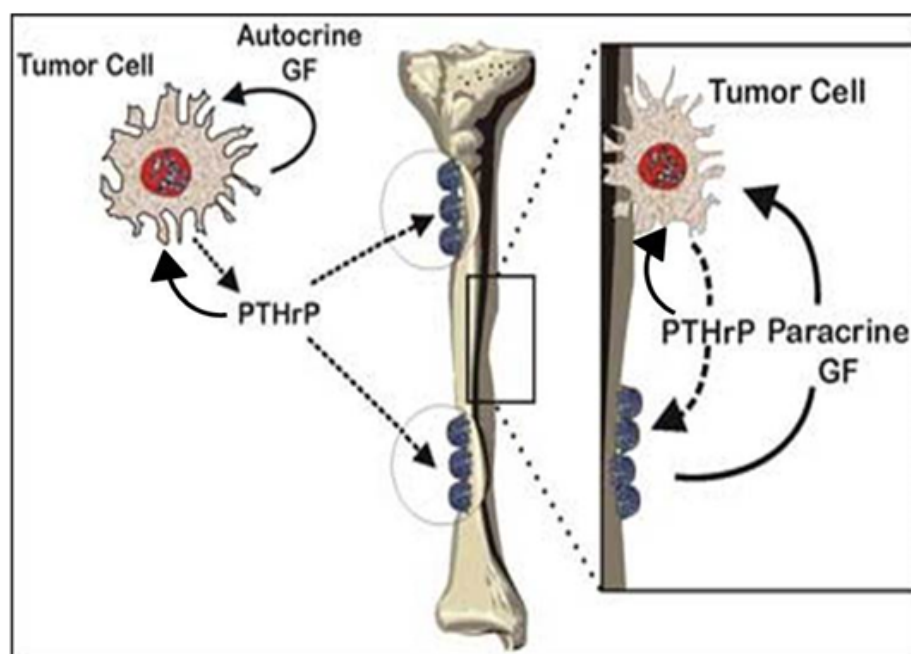


Figure 6. Growth factor-regulated PTHrP production in tumor states. Tumor cells at a distance from bone may be stimulated by autocrine growth factors (GF) to increase production of PTHrP which can then travel to bone via the circulation and enhance bone resorption. Tumor cells metastatic to bone (inset) may secrete PTHrP which can resorb bone and release growth factors which in turn can act in a paracrine manner to further enhance PTHrP production. PTHrP may itself promote tumor growth and progression.

Inasmuch as PTHrP is broadly distributed in normal tissues, PTHrP secretion by tumors likely represents eutopic overproduction rather than ectopic PTHrP production. Although demethylation of the PTHrP promoter (201) and gene amplification (202) have been implicated as mechanisms responsible for PTHrP overproduction by malignancies, it seems likely that in most cases overproduction of PTHrP is driven by enhanced gene transcription of tumor growth factors and by oncogenes which are signaling molecules in the growth factor pathways, e.g., TGF- β which can increase the Gli2 signaling molecule and stimulate PTHrP (203).

Patients who manifest hypercalcemia usually present with advanced disease. These tumors are generally obvious clinically when hypercalcemia occurs, and carry a poor prognosis. Elevated PTHrP per se may be an independent prognostic factor signaling a poor prognosis (204). This appears to be because in addition to its role in hypercalcemia, (205) PTHrP produced by tumor cells acts in an intracrine manner, increasing cell survival, apoptosis resistance, and anoikis evasion, and in autocrine manner via the PTHR1 to increase tumor cell proliferation, survival, apoptosis resistance. PTHrP is also a potential candidate for premetastatic niche formation in bone marrow, causing expansion of myeloid cells required for forming a conducive niche for metastatic growth in

bone. PTHrP can also upregulate tumor-surface expression of the GPCR, CXCR4 (C-X-C chemokine receptor type 4) (206). PTHrP and TGF β can also co-stimulate tumor cell production of IL-8, which can then further enhance CXCR4 expression. CXCL12, the natural ligand of CXCR4, is highly expressed in the bone microenvironment (as well as in other potential metastatic sites including lung and liver) and acts as a chemoattractant of circulating tumor cells, facilitating the homing of tumor cells to bone (207) and metastatic seeding.

Thus, PTHrP participates in all steps of the metastatic process, including tumor growth, progression, invasion, migration and survival in bone in order to skeletal support metastases (208).

Hypercalcemia in association with malignancy is generally more acute and severe than in association with primary hyperparathyroidism. (209). Nevertheless, as in primary hyperparathyroidism hypercalcemia is accompanied by hypophosphatemia, reduced tubular reabsorption of phosphorus, enhanced tubular reabsorption of calcium, and increased excretion of nephrogenous cyclic AMP, reflecting the PTH-like actions of PTHrP. Nevertheless, serum 1,25(OH) $_2$ D concentrations which are generally high or high normal in hyperparathyroidism are frequently low or low normal in HHM (210). This may reflect the higher levels of ambient calcium observed in HHM which may directly inhibit the renal 1 α (OH)ase enzyme. In hyperparathyroidism, bone resorption is increased but osteoblastic bone formation is also accelerated reflecting a relatively balanced increase in turnover. However, in HHM, osteoclastic bone resorption is markedly increased and osteoblastic bone formation may be reduced (211). The reasons for this uncoupling are unclear and could reflect the action of osteoblast inhibitory factors co-released from the tumor or in the bone microenvironment or perhaps the effect of the very high levels of calcium per se.

SOLID TUMORS WITH ELEVATED PTHrP AND SKELETAL METASTASES

Several studies have indicated that elevated PTHrP correlates better with hypercalcemia than does the presence or absence of skeletal metastases (196,198,200). This appears particularly relevant to certain neoplasms such as breast cancer which is commonly associated with hypercalcemia but is even more commonly associated with osteolytic skeletal metastases. Elevated circulating PTHrP concentrations (198,199) may contribute to the development of hypercalcemia in these cases in part through augmented bone resorption and in part through increased renal calcium reabsorption. PTHrP may also contribute to the pathogenesis of local osteolytic lesions (212,213). PTHrP per se may be a supportive factor for the growth and progression of cancers by acting in paracrine, autocrine and intracrine modes to modulate tumor cell proliferation, apoptosis, survival, and anoikis, and can therefore influence cell processes which enhance the capacity for tumor dissemination and metastasis (205). In addition, tumors, such as breast tumors that are metastatic to bone, may release PTHrP in the bone microenvironment which will bind to cells of the osteoblastic lineage (including stromal cells, osteoblasts and likely osteocytes), release RANKL ligand (RANKL) and decrease osteoprotegerin (OPG), stimulate osteoclasts to resorb bone and release, in addition to calcium, growth factors such as TGF β (214), IGF-1 FGF, PDGF and BMP; released growth factors can then stimulate further PTHrP release from the tumor, thus setting up a positive feedback loop (Fig. 6, see above). PTHrP released from cancers such as osteoblasts may also release the chemokine and angiogenic factor CCL2/MCP-1 from osteoblasts which may also increase osteoclastic activity and potentially angiogenesis, and further enhance tumor proliferation (215). Consequently, the presence of skeletal metastases in association with a malignancy is not mutually exclusive with high circulating PTHrP,

which can contribute to the hypercalcemia, through both osseous and renal mechanisms; at the same time, locally released PTHrP may contribute to the focal osteolysis. It is in fact uncertain whether local osteolysis per se ever effectively raises ECF calcium in the absence of some cause of reduced renal calcium excretion.

HEMATOLOGIC MALIGNANCIES WITH ELEVATED PTHrP

Hematologic malignancies that may cause hypercalcemia (216,217) include non-Hodgkin's lymphoma, chronic myeloid and lymphoblastic leukemia, adult T cell leukemia/lymphoma (ATL) and multiple myeloma.

ATL is an aggressive malignancy that develops after 20-30 years of latency in about 5% of individuals infected with human T-cell lymphotropic virus type I (HTLV-I). This tumor can be associated with hypercalcemia and increased PTHrP production (218). The mechanism of PTHrP production appears to be stimulation of the PTHrP promoter by the viral protein TAX in the infected lymphoid cells, causing increased PTHrP gene transcription.

Non-Hodgkin's lymphoma may also be associated with increased PTHrP secretion and hypercalcemia and this appears to occur predominantly in patients with late-stage disease and high-grade pathology (217). Multiple Myeloma, although frequently associated with hypercalcemia (about 30% of cases) is rarely associated with increased PTHrP production.

UTILITY OF PTHrP ASSAYS

PTHrP assays that recognize NH₂-terminal regions or mid-regions of the molecule, and two-site assays detecting two molecular epitopes have been developed. These assays have generally been quite sensitive and specific and successful in detecting

PTHrP in MAH where PTHrP overproduction occurs. Circulating levels in normal individuals are generally low or undetectable. Studies have also shown that PTHrP levels do not fall after treatment of the hypercalcemia of MAH but do fall after reducing the tumor burden (198,219). Consequently, the assays may prove useful to track PTHrP as a tumor marker to monitor the course of therapy. Detection of elevated serum PTHrP concentrations in malignancy may, however, predict a poor prognosis (220). Nevertheless, further work is necessary to understand the identity of circulating PTHrP fragments in order to produce even more useful assays. In most reported NH₂-terminal or mid-region assays, PTHrP levels may be elevated in some normocalcemic cancer patients. Whether this represents the detection of bioinert fragments which might be useful as tumor markers or the detection of bioactive PTHrP which presages the development of hypercalcemia and therefore also has predictive value needs to be clarified.

MAH with Elevation of Other Systemic Factors

Although PTHrP is the principal mediator of MAH, and elevated circulating PTHrP levels correlate strongly with hypercalcemia in patients with common tumors of widely diverse histological origin, other systemic factors have been described which may act with PTHrP or in the absence of PTHrP.

MAH WITH ELEVATED 1,25(OH)₂D

Concentrations of 1,25(OH)₂D are generally normal or low in most patients with MAH, however elevated serum concentrations have been reported in some cases of non-Hodgkin's and Hodgkin's lymphoma in association with hypercalcemia (149,150,221). In view of the fact that extra-renal production of 1,25(OH)₂D has been shown in various cell types and that renal impairment accompanied several of the reported lymphoma cases it seems likely that synthesis was occurring in the tumor tissue. This would be analogous

to expression of a 1 α (OH)ase enzyme in granulomatous tissue. Although it is likely that elevated 1,25(OH)₂D contributed to the hypercalcemia, co-production of PTHrP has also been reported in some cases (216). Consequently, production of 1,25(OH)₂D, lymphoid cytokines and PTHrP individually or in concert might all contribute to disordered skeletal and calcium homeostasis in these tumor states.

MAH WITH ELEVATED CYTOKINES

A variety of manifestations of malignancy including anorexia, cachexia, and dehydration may be due to tumor-produced circulating proinflammatory cytokines. Cytokines such as IL-1, IL-6, IL-8, IL-11, TNF, and RANKL which are produced in the bone microenvironment have been identified as regulators of bone turnover. PTHrP released from tumors may increase the local production of several of these cytokines however animal studies have reported that tumor activity can increase systemic levels of certain cytokines such as IL-6 and IL-1 which may contribute along with PTHrP to skeletal lysis and hypercalcemia. Some studies of tumor models have implicated a soluble form of RANKL as contributing to MAH (151). Overall, therefore it seems likely that other modulators of skeletal and calcium metabolism may be secreted by malignancies and, generally in the presence but occasionally in the absence of PTHrP, may contribute to the dysregulation of bone and mineral homeostasis occurring with MAH.

ECTOPIC HYPERPARATHYROIDISM

Inasmuch as PTH per se is expressed mainly in the parathyroid gland, the secretion of PTH by non-parathyroid tumors constitutes true ectopic hyperparathyroidism. A number of such cases of MAH with true PTH production have now been well documented by immunological and molecular biological techniques (222,223). These tumors include

ovarian, lung, thyroid, thymus and gastric malignancies (224). Consequently, true ectopic hyperparathyroidism may occur as a cause of MAH, but is rare.

MULTIPLE MYELOMA

Unlike other hematologic malignancies, multiple myeloma appears to have a special predilection to grow in bone (225). This may be related to production of growth factors, notably IL-6, by osteoblastic and osteoclastic cells, which facilitate its growth, and factors such as MIP-1 α which may promote its adherence to bone. The annexin AXII axis also appears to play a critical role in supporting multiple myeloma cell growth and adhesion to stromal cells/osteoblasts in the bone marrow (226). In order to grow in bone, myeloma cells must secrete bone-resorbing factors. A number of such factors have been implicated including MIP-1 α , IL-1, IL-6, TNF- β (lymphotoxin) and hepatocyte growth factor (HGF). Increased RANKL expression by stromal cells with decreased OPG expression also occurs in multiple myeloma and this correlates with the extent of the bone resorption (227). Although bone resorption is stimulated there is little active new bone formation. Consequently, the serum alkaline phosphatase, a marker of osteoblast function is usually normal and bone scans may be negative. Production by myeloma cells of Dickkopf-1 (DKK-1) protein, a soluble inhibitor of signaling via the Wnt pathway, an important growth factor pathway for osteoblasts, has been implicated in the suppression of osteoblast differentiation (228). Other Wnt signaling antagonists, such as soluble-frizzled-related protein (229) and sclerostin (230) have also been implicated in inhibition of osteoblast differentiation in myeloma. All patients with myeloma therefore have extensive bone destruction which may be discrete and focal or diffuse throughout the axial skeleton. Consequently, bone pain is a frequent complaint (80% of cases) and pathological fractures are a disabling consequence.

Although all patients develop osteolysis, hypercalcemia occurs in only about 30% of patients. It is likely that as long as renal function is intact and no circulating factor is produced which enhances renal calcium re-absorption (PTHrP is rarely produced by myeloma cells), increased renal excretion of calcium can accommodate the increased filtered load consequent to bone resorption. Impairment of renal function can occur however due to Bence Jones nephropathy or "myeloma kidney" (in which free light chain fragments of immunoglobulins are filtered and damage glomerular and tubular function), or due to amyloidosis, uric acid nephropathy, or recurrent infections. At this time hypercalcemia may become evident and be associated, because of the renal damage, with hyperphosphatemia rather than the hypophosphatemia occurring in other disorders causing MAH. Therapy aimed at inhibiting bone resorption (e.g., bisphosphonates) may therefore have a special effect in Myeloma, not only in reducing hypercalcemia but also in limiting tumor growth.

Therapeutic Considerations for MAH

Therapy of MAH should be directed primarily at treating the hypercalcemia, which may be of acute onset and considerable magnitude, and at treating the underlying tumor burden. Several approaches have been directed at reducing PTHrP production by those tumors in which PTHrP hypersecretion occurs. These include immunoneutralization (193), antisense inhibition, inhibition of growth factor stimulation through farnesyl transferase inhibition (77), inhibition of gene transcription with low calcemic vitamin D analogs (82), and convertase inhibition (84). To date these remain experimental but if PTHrP contributes to the local growth of the tumor, which some studies have reported, reduction of PTHrP levels may contribute not only to the long-term amelioration of skeletal and calcium homeostasis but also to a reduction in tumor burden.

INFLAMMATORY DISORDERS CAUSING HYPERCALCEMIA

Granulomatous Disorders

Both infectious and non-infectious granuloma-forming disorders have been associated with $1,25(\text{OH})_2\text{D}$ -mediated hypercalcemia (231). Noninfectious disorders include sarcoidosis, silicone-induced granulomatosis, paraffin-induced granulomatosis, berylliosis, Wegener's granulomatosis, eosinophilic granuloma, NOD2 pediatric granulomatous arthritis, Crohn's disease, Langerhan cell histiocytosis, and infantile fat necrosis. Infectious disorders include tuberculosis, candidiasis, cryptococcosis, leprosy, histoplasmosis, coccidiomycosis, and Bartonella Hensalae infection (cat-scratch disease). The disorder in which the hypercalcemia was first noted, is perhaps best documented, and has best been studied is sarcoidosis. Consequently, this will be discussed as a prototype of granulomatous diseases.

Up to 50% of patients with sarcoidosis will become hypercalciuric during the course of their disease and mild to severe hypercalcemia will be detected in 10% (232). Hypercalciuria and hypercalcemia generally occur in patients who have widespread disease and high serum angiotensin-converting enzyme activity. Normocalcemic patients with sarcoidosis are prone to the development of hypercalciuria and hypercalcemia after receiving small amounts of dietary vitamin D or after exposure to UV light (233). This is due to the fact the serum $1,25(\text{OH})_2\text{D}$ levels in active sarcoidosis are exquisitely sensitive to increases in the $25(\text{OH})\text{D}$ substrate levels. This leads to inappropriately elevated serum $1,25(\text{OH})_2\text{D}$ concentrations and absorption of high fractions of dietary calcium (Fig. 7). PTH is suppressed and its calcium reabsorptive effect in the kidney is lost leading to hypercalciuria. However urinary calcium often exceeds dietary calcium intake suggesting a role for $1,25(\text{OH})_2\text{D}$ -mediated bone

resorption as an alternate or additional source of filtered calcium and indeed accelerated trabecular bone loss and decreased bone mass has been documented in patients with active sarcoidosis (234,235). The source of the inappropriate $1,25(\text{OH})_2\text{D}$ is believed to be an extra-renal $1\alpha(\text{OH})\text{ase}$ (as in malignant lymphoproliferative disease) produced by macrophages which are prominent components of the sarcoid granuloma

(236). This enzyme exhibits similar kinetics and substrate specificity as the renal $1\alpha(\text{OH})\text{ase}$ but is clearly not regulated as is the renal $1\alpha(\text{OH})\text{ase}$ by PTH, $1,25(\text{OH})_2\text{D}$, calcium, or phosphorus. This extra-renal $1\alpha(\text{OH})\text{ase}$ does however appear to be suppressible by glucocorticoids (237), chloroquine (238) analogs, and cytochrome P-450 inhibitors such as ketoconazole (239).

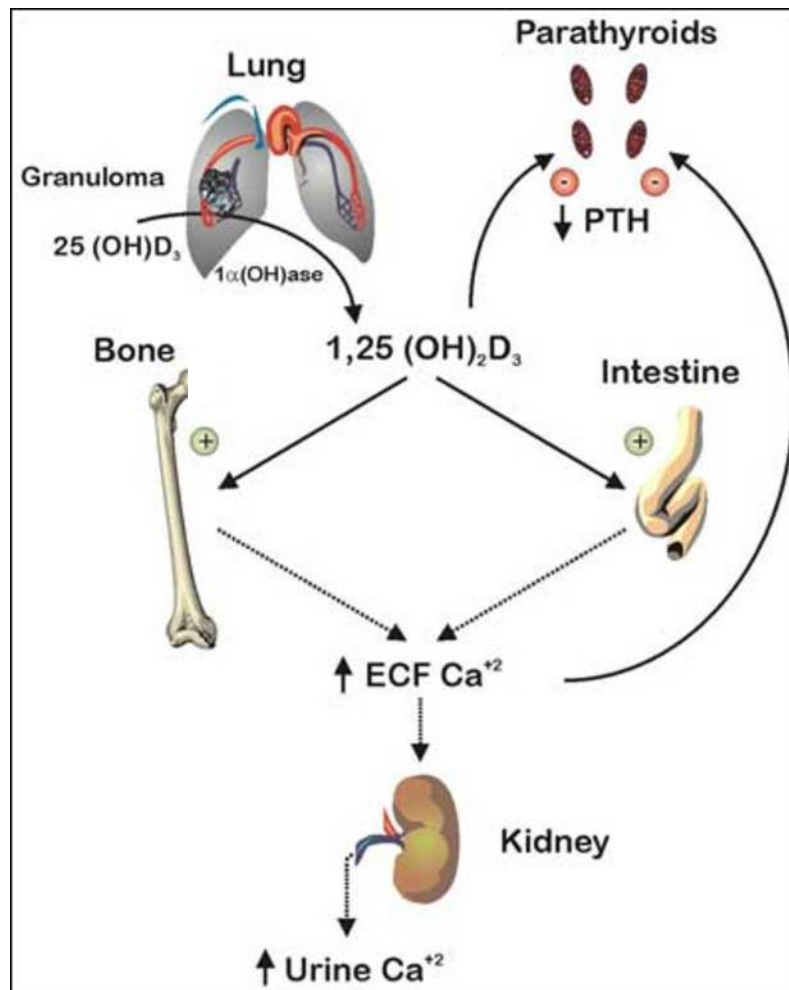


Figure 7. Disordered calcium homeostasis in granulomatous disease. Production of an extra-renal $1\alpha(\text{OH})\text{ase}$ by macrophages in a granuloma can increase conversion of circulating $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$. This secosteroid will increase Ca^{+2} absorption from the gut and Ca^{+2} resorption from bone resulting in an increased ECF Ca^{+2} . The increased ECF Ca^{+2} and $1,25(\text{OH})_2\text{D}$ will inhibit PTH production by the parathyroid glands. The increased filtered load of Ca^{+2} through the kidney and suppressed PTH will contribute to hypercalciuria.

Therapy of hypercalcemia associated with granulomatous disease is therefore aimed at reducing dietary intake of calcium and vitamin D, limiting sunlight exposure, and treating the underlying disease. Glucocorticoid therapy, if not already indicated for treating the underlying disease, or chloroquine analogs or ketoconazole should be considered to specifically decrease $1,25(\text{OH})_2\text{D}$ concentrations.

Viral Syndromes: Autoimmune Deficiency Syndrome: HIV and CMV Infections

A number of mechanisms may contribute to causing hypercalcemia in people living with AIDS however direct skeletal resorption has been described due to human immunodeficiency virus (HIV), HTLV-III, or cytomegalovirus (CMV) infections of the skeleton (240). Use of foscarnet as an antiviral agent has also been associated with hypercalcemia (241).

PEDIATRIC SYNDROMES

Williams Syndrome

Williams Syndrome (William-Beuren Syndrome) is a sporadic disorder characterized by dysmorphic features including “elfin facies”, cardiac abnormalities, the most typical of which is supravalvular aortic stenosis, neurologic deficits, musculoskeletal abnormalities, and hypercalcemia (242). Hypercalcemia occurs in about 15% of cases and may be associated with increased sensitivity to vitamin D (243). Williams Syndrome has been associated with loss of genetic material at 7q11.23 which likely represents a continuous gene deletion that includes the elastin gene (*ELN*) and *LIM-KINASE* gene (244). The hypercalcemia typically occurs during infancy and resolves between 2 and 4 years of age. The pathophysiology is not well understood. but both

abnormal $1,25(\text{OH})_2\text{D}$ metabolism and decreased calcitonin production have been suggested (245).

Idiopathic Infantile Hypercalcemia

Idiopathic Infantile Hypercalcemia is a disorder in which patients lack phenotypic features of Williams Syndrome and do not have a 7q11.13 deletion. However, they also manifest hypercalcemia in infancy which is associated with apparent vitamin D sensitivity and which resolves in the first few years of life (246). Loss-of-function mutations in *CYP 24A1*, the gene encoding the 24-hydroxylase may occur. Consequently, inactivation of the active form of vitamin D, $1,25(\text{OH})_2\text{D}$ is impaired. This results in increased $1,25(\text{OH})_2\text{D}$ levels and enhanced calcium absorption and hypercalcemia (247). Loss-of-function mutations of *SLC34A1*, encoding the renal proximal tubular sodium-phosphate cotransporter, Na/Pi-IIa result in phosphaturia, hypophosphatemia, increased FGF23, stimulation of *CYP27B1* and inhibition of *CYP24A1*, causing increased $1,25(\text{OH})_2\text{D}$ and hypercalcemia hypercalciuria, and nephrocalcinosis which may also manifest as IIH

Hypophosphatasia

Hypophosphatasia (HPP) is characterized by loss of function mutations in the gene *ALPL* (chromosome 1) encoding the tissue nonspecific alkaline phosphatase (TNSALP) (248). Hypercalcemia is generally present mainly in the infantile form of the disease, where the hypercalcemia appears to result from reduced deposition of calcium in bone matrix. Hypercalcemia may resolve spontaneously within the first year of life or following targeted asfotase alfa enzyme replacement. Hypercalcemia may also occur in adults with HPP who have been immobilized

Congenital Lactase Deficiency

Congenital lactase deficiency CLD, is an autosomal recessive disorder (73–76) caused by a mutation of the lactase-phlorizin hydrolase gene. Infants with CLD may develop increased calcium absorption in the ileum in the presence of nonhydrolyzed lactose, and hypercalcemia and medullary nephrocalcinosis may ensue. The hypercalcemia generally resolves quickly after a lactose-free diet is initiated but nephrocalcinosis may persist (249).

Congenital Sucrase-Isomaltase Deficiency

Hypercalcemia may possibly occur due to increased bone mobilization of calcium secondary to chronic metabolic acidosis. There may also be roles for dehydration, along with potential increase calcium absorption (250).

MEDICATION-INDUCED HYPERCALCEMIA

Thiazide Diuretics

Thiazides reduce renal calcium clearance, however in the presence of a normal calcium homeostatic system (e.g., in the absence of primary hyperparathyroidism) this should not produce sustained hypercalcemia (251). Thiazides have however been reported to produce hypercalcemia in anephric individuals. Overall, therefore the mechanism is unknown although "unmasking" of mild underlying primary hyperparathyroidism has been suggested as a possible mechanism. Hypercalcemia is however a rare event in thiazide use and is rapidly reversed by discontinuing the medication.

Lithium

Lithium carbonate, at 900 to 1500 mg/day, has occasionally (5% of cases) been reported to cause hypercalcemia. Lithium may reduce renal calcium

clearance and may also alter the set-point for PTH secretion such that higher ECF calcium levels than normal are required to suppress PTH (252). The hypercalcemia is generally reversible with discontinuation of therapy.

Vitamin D and Analogues

Excessive intake of vitamin D per se, of dihydrotachysterol, of 25(OH)D₃, or of 1,25(OH)₂D₃ can all cause hypercalciuria and hypercalcemia by increasing gut absorption of calcium and bone resorption (253). Only vitamin D, (vitamin D₂ or vitamin D₃) is available without prescription. Vitamin D per se, is more lipid soluble and has a much longer retention time in the body (weeks to months) than the hydroxylated analogues (hours to days). Therapy consists of hydration, calciuresis, and if needed glucocorticoids and an anti-resorptive agent (bisphosphonate or denosumab).

Vitamin A and Analogues

Vitamin A, in greater than 50,000 IU/day, and its analogues cis-retinoic acid and all-trans-retinoic acid (used for the treatment of dermatologic and neoplastic disorders) have been associated with hypercalcemia (254-256). This appears to be due to enhanced bone resorption. Discontinuation of the medication, hydration and administration of an anti-resorptive agent would appear to be the treatments of choice.

Antiestrogens (Tamoxifen)

Hypercalcemia may occur when antiestrogens (257) such as tamoxifen are used to treat breast cancer metastatic to the skeleton. Increased bone resorption associated with osteolytic lesions appears to be the major mechanism possibly induced by cytokines and growth factors released when the tumor undergoes lysis. The "flare" hypercalcemia may require acute treatment but is usually self-limiting (258,259).

Theophylline/Aminophylline

Hypercalcemia has been reported with theophylline usage for chronic obstructive pulmonary disease or asthma and appears reversible with cessation of therapy or amenable to treatment with beta – adrenergic antagonists (260).

Aluminum Intoxication

Aluminum intoxication was observed when large amounts of aluminum-containing phosphate-binding agents were prescribed to patients with chronic renal failure to control hyperphosphatemia. Alternatively, clustered outbreaks of aluminum intoxication occurred when inadequately purified water was employed for dialysis or for total parenteral nutrition (261). Aluminum intoxication can cause adynamic bone disease in patients with renal failure, and hypercalcemia possibly due to inadequate deposition of calcium in bone. In chronic kidney disease, removal of aluminum by treating with the chelating agent desferioxamine is effective in reducing serum calcium levels and improving mineralization. Less frequent use of aluminum-containing medications has considerably diminished the frequency of this disorder.

Milk-Alkali Syndrome

The classic milk-alkali syndrome causing hypercalcemia occurred in the past when large quantities of milk and bicarbonate were ingested together to treat peptic ulcers. The modern-day equivalent appears to be consumption of large quantities of milk or other dairy products with calcium carbonate (262). Quantities of calcium that must be ingested to cause the syndrome are at least 3 g per day or more. Classically hypercalcemia is accompanied by alkalosis, nephrocalcinosis, and ultimately by renal failure. The alkali may enhance precipitation of calcium in renal tissue. Discontinuation

of the calcium and antacid, rehydration and rarely, hemodialysis, can be useful for treatment.

SGLT2 Inhibitors

Case reports have documented reversible hypercalcemia with sodium-glucose cotransporter protein 2 inhibitors, likely due to osmotic diuresis and volume contraction. Underlying risk factors (dehydration, high calcium intake, thiazides, acidosis, undiagnosed PHPT) were typically present.

Immune Checkpoint Inhibitors

Hypercalcemia has been reported infrequently with immune checkpoint inhibitors (ICIs). Mechanisms may include ICI-induced endocrine disorders (hyperthyroidism, adrenal insufficiency), sarcoid-like granuloma formation, ICI-related PTHrP production, or transient ICI-related “hyperprogression” of disease.

Denosumab

Hypercalcemia may occur after denosumab discontinuation due to “rebound” osteoclastic bone resorption. Most cases involve children younger than 18 years and those using denosumab for bone tumors and fibrous dysplasia. A few cases have been reported in adults treated for osteoporosis.

Teriparatide, Abaloparatide

Transient hypercalcemia can occur during teriparatide [PTH (1-34)] treatment for osteoporosis, and usually resolves within about 16 h after administration. Abaloparatide, a synthetic analog of human PTHrP (1-34), also used as osteoporosis therapy, exhibits lesser hypercalcemic effects than PTH (1-34) because of faster dissociation of the PTHrP-PTHr1 agonist-receptor complex, than of the PTH-PTHr1 complex.

Foscarnet

Foscarnet is an antiviral medication, commonly used in the treatment and CMV infections. There have been rare reports of hypercalcemia in recipients.

Ketogenic Diet

A small number of children with epilepsy who were following a ketogenic diet have been reported to develop hypercalcemia. The mechanism, while not fully delineated, may be due to impaired osteoblast activity and decreased bone formation.

ALTERATIONS IN MUSCLE AND BONE

Immobilization

Immobilized patients, in association with reduced mechanical load on the skeleton, continue to resorb bone whereas bone formation is inhibited. Thus, high bone resorption with negative calcium balance leading to osteopenia, osteoporosis, and hypercalcemia may occur from prolonged immobilization after burns, spinal injury, major stroke, hip fracture, and bariatric surgery (263). More severe hypercalcemia and hypercalciuria may occur in immobilized individuals with already high bone turnover such as growing children, patients with Paget's Disease, or patients with primary hyperparathyroidism or MAH (264).

Intense Exercise

Hypercalcemia has been described in some individuals after hours of intense exercise; bone resorption markers increased and correlated with elevations in serum calcium and vasopressin levels.

Rhabdomyolysis

In the oliguric early phase of rhabdomyolysis (the rapid breakdown of damaged skeletal muscle), calcium and

phosphate complex deposition in muscle may occur; in the later polyuric phase, the calcium and phosphate complexes in muscle may be mobilized, and redistributed, causing hypercalcemia.

CLINICAL ASSESSMENT OF THE HYPERCALCEMIC PATIENT

This discussion of the clinical assessment of the hypercalcemic patient will focus primarily on adult patients. Although many of the approaches are relevant to childhood and even neonates, detailed discussion of the issues relevant exclusively to the pediatric age group is beyond the scope of this chapter.

History and Physical Examination

The approach to the history and physical examination of the hypercalcemic patient should focus on the signs and symptoms which are relevant to hypercalcemia, and the signs and symptoms which are relevant to the causal disorder. Both duration and severity of symptoms should be ascertained.

Hypercalcemic manifestations will vary depending on whether the hypercalcemia is of acute onset and severe (greater than 12 mg/dL or 3 mM) or whether it is chronic and relatively mild (Table 2). Patients may also tolerate higher serum calcium levels more readily if the onset is relatively gradual, but at concentrations above 14 mg/dL (3.5 mM) most patients are symptomatic. In both acute and chronic cases, the major manifestations affect gastrointestinal, renal, and neuromuscular function. Patients with acute hypercalcemia commonly present with anorexia, nausea, vomiting, polyuria, polydipsia, dehydration, weakness, and depression and confusion which may proceed to stupor and coma. As well the QT interval on EKG may be shortened by hypercalcemia due to the increased rate of cardiac repolarization. Arrhythmias such as bradycardia and first-degree

atrioventricular block, as well as digitalis sensitivity may occur. Acute hypercalcemia, therefore, can represent a life-threatening medical emergency. Patients with chronic hypercalcemia may have a

history of constipation, dyspepsia (generally not due to a true ulcer), pancreatitis, and nephrolithiasis but few other signs or symptoms.

Table 2. Manifestations of Hypercalcemia

	Acute	Chronic
Gastrointestinal	Anorexia, nausea, vomiting	Dyspepsia, constipation, pancreatitis
Renal	Polyuria, polydipsia, dehydration, renal insufficiency	Nephrolithiasis, nephrocalcinosis, renal insufficiency
Neuro-muscular	Depression, confusion, hyporeflexia, stupor, coma	Weakness, lethargy
Cardiac	Prolonged PR interval, short QT interval, widened QRS complex, bradycardia, digitalis sensitivity	Hypertension

The most frequent underlying causes (over 90%) of hypercalcemia are primary sporadic hyperparathyroidism and malignancy-associated hypercalcemia (MAH). In the West, the most frequent presentation of primary sporadic hyperparathyroidism is that of relatively "asymptomatic" disease with only intermittently or mildly (<12 mg/dL or 3 mM) elevated serum calcium concentrations (140). Occasionally a history is obtained of having passed a kidney stone either recently or in the remote past. Neck masses are unusual in primary hyperparathyroidism unless the patient has a particularly large adenoma or a parathyroid carcinoma. In contrast, the most frequent presentation of MAH is of acute, severe hypercalcemia with some or all of the manifestations of this mineral ion abnormality that are noted above. In view of the fact that hypercalcemia is generally a manifestation of advanced disease, tumors causing hypercalcemia are rarely occult. Consequently, evidence for an underlying malignancy may be obtained or suspected on history or physical examination. Endocrine disorders such as hyperthyroidism or hypoadrenalism should be suspected from a careful history and physical examination, and a history of ingestion of medication

and supplements (e.g., calcium, vitamin D, thiazide diuretics, and vitamin A) which have been reported to cause hypercalcemia should be obtained. The presence of chronic granulomatous disease could be suspected on the basis of an accurate history and physical examination targeted to the known granulomatous diseases that cause hypercalcemia. Finally, a careful family history should provide clues as to whether the patient manifests any of the variants of familial hyperparathyroidism.

Laboratory Examination

Laboratory testing should be guided by the results of a careful history and a detailed physical examination and should be geared toward assessing the extent of the alteration in calcium homeostasis and toward establishing the underlying diagnosis and determining its severity. Useful laboratory screening may include a complete blood count (CBC), serum total and ionized calcium, PTH, 25(OH)D, 1,25(OH)₂D, phosphorus, serum creatinine and calculation of estimated glomerular filtration rate (GFR), urinalysis and 24-hour urine collection for calcium and creatinine.

To establish the diagnosis of PHPT the most common cause of hypercalcemia in the ambulatory setting, documentation of at least two elevated corrected (or ionized) serum calcium levels with concomitant elevated, or at least normal, serum PTH levels, at least 2 weeks apart, is required (levels of PTH within the normal range are “inappropriate” and consistent with PHPT, because serum PTH should be suppressed in the setting of hypercalcemia,) (Figure1). A serum PTH level is the most useful initial test to distinguish between PTH-dependent and PTH-independent hypercalcemia. Two site assays for PTH are currently the method of choice (265), and the sensitivity of second-generation intact and third-generation “whole” PTH 2-site immunometric assays is similar and is approximately 90%. PTH levels are elevated in approximately 80% of patients, although temporal trends suggest lower levels compared with past decades. Normocalcemic PHPT may be diagnosed if normal corrected total calcium and normal ionized calcium concentrations occur in association with an elevated intact serum PTH on at least two occasions over 3–6 months, and all causes of secondary hyperparathyroidism have been excluded.

FHH, should also be considered in the presence of hypercalcemia and normal or elevated PTH however FHH patients generally exhibit a urinary calcium /creatinine clearance ratio <0.01 if testing serum and urine calcium in three relatives discloses hypercalcemia and relative hypocalciuria, then this diagnosis is likely and parathyroid surgery is to be avoided. Lithium and thiazide diuretics may also be associated with hypercalcemia and elevated PTH, as may ectopic PTH secretion by tumors,

To evaluate renal involvement, estimated glomerular filtration rate (eGFR) or preferably, creatinine clearance, 24-hour urinary calcium and imaging for nephrolithiasis/nephrocalcinosis should be obtained. To evaluate skeletal involvement, bone mineral density (BMD) should be determined by dual-energy

X-ray absorptiometry (DXA) scans of the lumbar spine, hip, and distal 1/3 radius, and imaging should be performed for vertebral fractures (vertebral fracture assessment [VFA] or vertebral X-rays); trabecular bone score (TBS) could be included if available. These studies should provide a baseline of disease extent before parathyroidectomy. Pre-operative localization of a parathyroid adenoma, generally by nuclear imaging (MIBI scans) or ultrasound has been helpful (266). Ultimately an experienced surgeon is the best guarantee for a successful neck exploration.

The presence of a family history of hypercalcemia or of kidney stones should raise suspicion of MEN1 or MEN2a or MEN4. If, in addition to HPT in the proband, one or more first-degree relatives are found have at least one of the three tumors characterizing MEN1 (parathyroid, pituitary, pancreas) or MEN2a (parathyroid, medullary thyroid carcinoma, pheochromocytoma) then it is highly likely that the disease is familial. Documentation of familial HPT should be transmitted to the surgeon so that multigland disease can be dealt with. The presence of ossifying fibromas of the mandible and maxilla, and renal lesions such as cysts and hamartomas in addition to HPT would suggest HPT-jaw tumor syndrome.

If hypercalcemia is associated with very low or suppressed serum PTH levels, then malignancy would be an important consideration, either in association with elevated serum PTHrP or in its absence, in which case it is generally as a result of the production of other cytokines. Hypercalcemia is however frequently a late manifestation of malignancy and the presentation of hypercalcemia is often acute and severe. When malignancy-associated hypercalcemia is suspected then an appropriate malignancy screen should be done including skeletal imaging to identify skeletal metastases. As well appropriate biochemical assessment such as a complete blood count, serum creatinine, and serum and urine protein

electrophoresis to exclude multiple myeloma would be appropriate. Detection of elevated serum 1,25(OH)₂D levels may point toward the need for a search for lymphoma or for infectious or non-infectious granulomatous disease. Other testing (e.g., a TSH level) could be done for specific clinical disorders based on the findings on the history and physical examination. Although increased PTHrP may be associated with pheochromocytoma, serum PTH

levels are suppressed in hypercalcemia in association with thyrotoxicosis, pheochromocytoma, VIPoma, and hypoadrenalism. Although these disorders may be suspected from clinical examination, detailed biochemical evaluation is required for confirmation.

An approach to laboratory assessment of the hypercalcemic patient is shown in Figure 8.

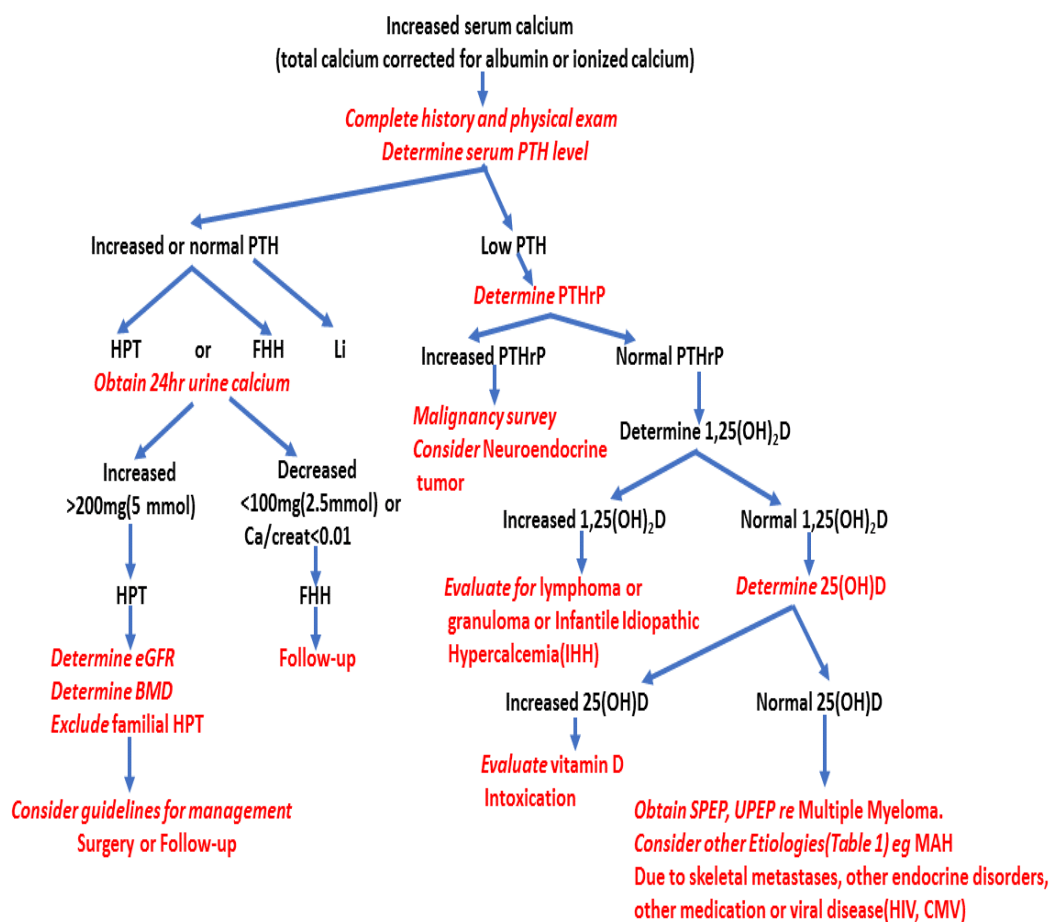


Figure 8. Laboratory approach to the diagnosis of hypercalcemia. Abbreviations used are: BMD= bone mineral density, eGFR=estimated glomerular filtration rate, Li=lithium therapy MAH=malignancy-associated hypercalcemia, PHPT=primary hyperparathyroidism, SPEP=serum protein electrophoresis, UPEP=urine protein electrophoresis.

MANAGEMENT OF HYPERCALCEMIA

If the patient is asymptomatic and the patient's serum calcium concentration is less than 12 mg/dL (3 mM) then treatment of the hypercalcemia should be aimed solely at treatment of the underlying disorder. Nevertheless, calcium intake of greater than 1000

mg/d, and immobilization should be avoided. If feasible, thiazide diuretics should be discontinued.

If the patient has symptoms and signs of acute hypercalcemia as described above and serum calcium is greater than 12 mg/dL (3mM) then a series of urgent measures should be instituted (Table 3). These measures are almost always required with a serum calcium above 14 mg/dL (3.5 mM)

Table 3. Management of Acute Hypercalcemia

1. Hydration
2. Inhibition of Bone Resorption
3. Calciuresis
4. Reduction of GI calcium absorption
5. Calcimimetics
6. Dialysis
7. Mobilization

Hydration to Restore Euvolemia

Hydration with normal saline is necessary in every patient with acute, severe hypercalcemia to correct the ECF deficit due to nausea, vomiting, and polyuria (267). This may require a bolus infusion of 0.9% sodium chloride followed by an infusion of 3 to 4 L over 24 to 48 hours (e.g., an initial rate of 200-300 mL/h subsequently adjusted to maintain a urine output at 100-150 mL/h). Hydration can enhance urinary calcium excretion by increasing the glomerular filtration of calcium and decreasing tubular reabsorption of sodium and calcium. This form of therapy, although always required, should however be used cautiously in patients with compromised cardiovascular or renal function. Hydration alone might be sufficient when the cause is known and readily reversible (e.g., milk-alkali syndrome), but is typically insufficient with other etiologies such as MAH.

Inhibition of Bone Resorption

Accelerated bone resorption is an important factor in the pathogenesis of hypercalcemia in the majority of patients with acute, severe hypercalcemia and treatment with denosumab (Dmab) or an intravenous (IV) bisphosphonate (BP) is the treatment of choice for inhibition of bone resorption (268). Consequently, preferably after the patient is rehydrated, denosumab 120 mg can be given subcutaneously and, if needed, repeated 1, 2 and 4 weeks later and monthly thereafter, to maintain the desired calcium level. In contrast to bisphosphonates, denosumab is not cleared by the kidney, and therefore can be used in patients with severe or chronic kidney disease. Denosumab may, rarely, cause rash or infection. Transient hypocalcemia, may occur particularly in patients with vitamin D deficiency; consequently, low serum 25(OH)D, if present, should be corrected before administering denosumab. Alternatively, nitrogen-containing bisphosphonates may be administered intravenously (IV). Thus, zoledronic acid, 4 mg, can be given Ivin 5 ml of 0.9% saline or 5% dextrose in water over 15 min (269) and can be repeated in 7 days, if

necessary and every 3 to 4 weeks thereafter; alternatively pamidronate, 60-90 mg, may be administered IV in 500 ml of 0.9% saline or 5% dextrose in water over 2-4 hours (270). Bisphosphonates may cause transient fevers, flu-like symptoms, or myalgias for a day or two and transient hypocalcemia and/or hypophosphatemia may result. After a single dose both agents may only reduce serum calcium to normal levels after 4 days but the duration of the effect may last from days to 8 weeks. Salmon calcitonin is a peptide hormone which is a safe therapeutic agent when acutely administered. Calcitonin can inhibit osteoclastic resorption and can also increase calcium excretion (271). It has a more rapid onset of action than either denosumab or the intravenous bisphosphonates, causing serum calcium to fall generally by 1-2 mg/dL (0.25 to 0.50 mM) within 2 to 6 hours of administration. Consequently, it may be used in concert with a bisphosphonate or denosumab to more rapidly reduce the hypercalcemia (within 2-6 hours) (272). It is usually given intramuscularly or subcutaneously at a dose of 4 to 8 IU/kg, and can be repeated every 6 to 12 hours for 48 to 72 hours. Unfortunately, this agent is not as potent as the most potent bisphosphonates or denosumab and tachyphylaxis may occur after 48-72 hours.

Calciuresis

If PTHrP or PTH is suspected to be a pathogenetic mediator of the presenting hypercalcemia then renal calcium retention may contribute to the maintenance of the hypercalcemia and inhibition of bone resorption alone may be insufficient to normalize serum calcium (273). In this case, a loop diuretic i.e., furosemide may be added, but, importantly, only after rehydration. Loop diuretics inhibit both sodium and calcium reabsorption at the CTAL of the kidney. Furosemide, 20 to 40 mg may be administered IV both to control clinical manifestations of volume excess and to promote calciuria, but the possibility of low potassium, or worsening kidney function should be monitored.

Glucocorticoids

Glucocorticoids decrease gastrointestinal absorption of calcium and can also increase urine calcium excretion. Importantly, glucocorticoids can also inhibit $1,25(\text{OH})_2\text{D}$ synthesis by mononuclear cells in patients with granulomatous diseases (237) and by proliferative cells in hematologic malignancies such as lymphoma or myeloma (274). Glucocorticoids (e.g., hydrocortisone 200 to 400 mg intravenously over 24 hours for 3 to 5 days) may therefore be an initial adjunctive therapy for treatment of severe hypercalcemia in those with known or highly suspected vitamin D-mediated hypercalcemia, but may also be used as an additional therapy in refractory life-threatening hypercalcemia of any cause. Once the acute hypercalcemia is controlled and the cause identified, treatment can be tailored to the pathophysiological mechanism, e.g., by the use of oral prednisone 20 mg/d to 40 mg/d or higher, if necessary. Chronic glucocorticoid use may however result in hyperglycemia, altered mental status, hypertension, myopathy, infection, bone loss and/or avascular necrosis of bone.

Ketoconazole

Ketoconazole, is an imidazole antifungal agent, that inhibits $1,25(\text{OH})_2\text{D}$ production in sarcoidosis, tuberculosis, silicone-related granulomatous disease, and in individuals with inactivating CYP24A1 variants (79-82). Ketoconazole may be used instead of glucocorticoids or used in combination with lower doses of glucocorticoids in hypercalcemia associated with these disorders.

Patients with disorders causing increased calcium absorption should also decrease dietary calcium and vitamin D intake, maintain hydration and avoid exposure to sun, in view of the fact that seasonal increases in serum calcium have been described in patients with sarcoid and CYP24A1 variants.

CaSR Agonism (Calcimimetics)

The calcimimetic, cinacalcet, is a CaSR agonist which increases CaSR sensitivity to ECF calcium and reduces PTH secretion. It may be used in doses starting at 30 mg twice daily orally to as high as 90 mg 4 times daily for the treatment of severe, chronic hypercalcemia due to primary HPT, especially if caused by a parathyroid carcinoma, and if the patient is not a surgical candidate (275). Bisphosphonates or denosumab can be used in combination with cinacalcet, if necessary to lower the serum calcium and to increase BMD in these conditions.

Dialysis

Dialysis is usually reserved for severely hypercalcemic patient's refractory to other therapies or who have renal insufficiency. Both peritoneal dialysis (276) and hemodialysis (277) can be effective in removing ionized calcium from the extracellular fluid, over the course of hours.

Mobilization

Finally, the patient should be mobilized as rapidly as possible (278). If mobilization is not possible then continued treatment with antiresorptive agents may be necessary (279).

Once the acute episode of hypercalcemia has been managed, careful attention must be paid to addressing the underlying hypercalcemic disorder per se.

REFERENCES

1. Walser M. Ion association: VI. Interactions between calcium, magnesium, inorganic phosphate, citrate, and protein in normal human plasma. *J. Clin. Invest.* 1961;40:723-730.
2. Parfitt AM, Kleerekoper M. Clinical disorders of calcium, phosphorus and magnesium metabolism. in Maxwell MH, Kleeman CR. (eds): *Clinical disorders of fluid and electrolyte metabolism*, 3rd ed. New York, McGraw-Hill, 1980, pp 947-1153.
3. Stewart AF, Broadus AE: Mineral metabolism. in Felig P, Baxter ID, Broadus AE, Frohman LA. (eds): *Endocrinology and metabolism*, 2nd ed. New York, McGraw-Hill, 1987, pp 1317-1453.
4. Bringhurst FR, Demay MB, Kronenberg HM. Hormones and disorders of mineral metabolism., in Wilson JD, Foster DW, Kronenberg HM, Larsen PR. (eds): *Williams textbook of endocrinology*, 9th ed. Philadelphia, Saunders, 1998, pp 1155-1200.
5. Brown EM: Physiology of calcium homeostasis., in Bilezikian JP, Marcus R, Levine MA. (eds): *The parathyroids: basic and clinical concepts*, 2nd ed. San Diego, Academic Press, 2001, pp 167-181.
6. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* 1993;366:575-580.
7. Fraser DR, Kodicek E. Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. *Nat. New. Biol.* 1973;241:163-166.
8. Potts JT Jr, Jueppner H. Parathyroid hormone and parathyroid hormone-related peptide in calcium homeostasis, bone metabolism, and bone development: the proteins, their genes, and receptors., in Avioli LV, Krane SM. (eds): *Metabolic bone disease*, 3rd ed. New York, Academic Press, 1997, pp 51-84.
9. Grant FD, Conlin PR, Brown EM. Rate and concentration dependence of parathyroid hormone dynamics during stepwise changes in serum ionized calcium in normal humans. *J. Clin. Endocrinol. Metab.* 1990;71:370-378.
10. Mayer GP, Keaton JA, Hurst JG, Habener JF. Effects of plasma calcium concentration on the relative proportion of hormone and carboxyl fragments in parathyroid venous blood. *Endocrinology* 1979;104:1778-1784.

11. Hanley DA, Ayer LM. Calcium-dependent release of carboxyl-terminal fragments of parathyroid hormone by hyperplastic human parathyroid tissue in vitro. *J. Clin. Endocrinol. Metab.* 1986;63:1075-1079.
12. D'Amour P, Palardy J, Bahsali G, Mallette LE, DeLéan A, Lepage R. The modulation of circulating parathyroid hormone immunoheterogeneity in man by ionized calcium concentration. *J. Clin. Endocrinol. Metab.* 1992;74:525-532.
13. Segre GV, D'Amour P, Hultman A, Potts JT Jr. Effects of hepatectomy, nephrectomy, and nephrectomy/uremia on the metabolism of parathyroid hormone in the rat. *J. Clin. Invest.* 1981;67:439-448.
14. Yamamoto M, Igarishi T, Muramatsu M, Fukagawa M, Motokura T, Ogata E. Hypocalcemia increases and hypercalcemia decreases the steady state level of parathyroid hormone messenger RNA in the rat. *J. Clin. Invest.* 1989;83:1053-1056.
15. Naveh-Many T, Silver J. Regulation of parathyroid hormone gene expression by hypocalcemia, hypercalcemia, and vitamin D in the rat. *J. Clin. Invest.* 1990;86:1313-1319.
16. Kremer R, Bolivar I, Goltzman D, Hendy GN. Influence of calcium and 1,25-dihydroxycholecalciferol on proliferation and proto-oncogene expression in primary cultures of bovine parathyroid cells. *Endocrinology* 1989;125:935-941.
17. Xu M, Choudhary S, Goltzman D, Ledgard F, Adams D, Gronowicz G, Koczon-Jaremko B, Raisz L, Pilbeam C. Do cyclooxygenase-2 knockout mice have primary hyperparathyroidism? *Endocrinology* 2005;146:1843-1853.
18. Dusso A, Cozzolino M, Lu Y, Sato T, Slatopolsky E. 1,25-Dihydroxyvitamin D downregulation of TGF α /EGFR expression and growth signaling: a mechanism for the antiproliferative actions of the sterol in parathyroid hyperplasia of renal failure. *J. Steroid Biochem Mol. Biol.* 2004;89-90:507-511.
19. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuroo M, Mohammadi M, Sirkis R, Naveh-Many T, Silver J. The parathyroid is a target organ for FGF23 in rats. *J Clin Invest.* 2007;117:4003-4008.
20. Chan YL, McKay C, Dye E, Slatopolsky E. The effect of 1,25 dihydroxycholecalciferol on parathyroid hormone secretion by monolayer cultures of bovine parathyroid cells. *Calcif. Tiss. Int.* 1986;38:27-32.
21. Goltzman D, Miao D, Panda DK, Hendy GN. Effects of calcium and of the vitamin D system on skeletal and calcium homeostasis: lessons from genetic models. *J. Steroid Biochem. Mol. Biol.* 2004;89-90:485-489.
22. Friedman PA, Gesek FA. Cellular calcium transport in renal epithelia: Measurement, mechanisms, and regulation. *Physiol. Rev.* 1995;75:429-471.
23. Nordin BE, Peacock M. Role of kidney in regulation of plasma-calcium. *Lancet* 1969;2:1280-1283.
24. Rouse D, Suki WN. Renal control of extracellular calcium. *Kidney Int.* 1995;38:700-708.
25. Rouleau MF, Warshawsky H, Goltzman D. Parathyroid hormone binding in vivo to renal, hepatic, and skeletal tissues of the rat using a radioautographic approach. *Endocrinology* 1986;118:919-931.
26. Juppner H, Abou-Samra AB, Freeman MW, Kong SF, Schipani E, Richards J, Kolakowski LF Jr., Hock J, Potts JT Jr., Kronenberg HM, Segre GV. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. *Science* 1991;254:1024-1026.
27. Abou-Samra AB, Juppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Potts JT Jr. Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol triphosphates and increases intracellular free calcium. *Proc. Natl. Acad. Sci. USA* 1992;89:2732-2736.
28. Amizuka N, Lee HS, Kwan MY, Arazani A, Warshawsky H, Hendy GN, Ozawa H, White JH, Goltzman D. Cell-specific expression of the parathyroid hormone (PTH)/PTH-related peptide receptor gene in kidney from kidney-specific and ubiquitous promoters. *Endocrinology* 1997;138:469-481.
29. Keusch I, Traebert M, Lotscher M, Kaissling B, Murer H, Biber J. Parathyroid hormone and dietary phosphate provoke a lysosomal routing of the proximal tubular Na/Pi-cotransporter type II. *Kidney Int.* 1998;54:1224-1232.
30. Brenza HL, Kimmel-Jehan C, Jehan F, Shinki T, Wakino S, Anazawa H, Suda T, DeLuca HF. Parathyroid hormone activation of the 25-hydroxyvitamin D3-1 α -hydroxylase gene promoter. *Proc. Natl. Acad. Sci. USA* 1998;95:1387-1391.
31. Azarani A, Goltzman D, Orlowski J. Parathyroid hormone and parathyroid hormone-related peptide inhibit the apical Na⁺/H⁺ exchanger NHE-3 isoform in renal cells (OK) via a dual signaling cascade involving protein kinase A and C. *J. Biol. Chem.* 1995;270:20004-20010.
32. Derrickson BH, Mandel LJ. Parathyroid hormone inhibits Na⁺(+)-K⁺(+)-ATPase through Gq/G11 and the calcium-independent phospholipase A2. *Am. J. Physiol.* 1997;272:F781-F788.
33. Morel F, Chabardes D, Imbert-Teboul M, Le Bouffant F, Hus-Citharel A, Montégut M. Multiple hormonal control of

- adenylate cyclase in distal segments of the rat kidney. *Kidney Int.* 1982;11:555-562.
34. De Rouffignac C, Quamme GA. Renal magnesium handling and its hormonal control. *Physiol. Rev.* 1994;74:305-322.
35. Hebert SC. Extracellular calcium-sensing receptor: Implications for calcium and magnesium handling in the kidney. *Kidney Int.* 1996;50:2129-2139.
36. Hoenderop JGJ, Nilius B, Bindels RJM. Calcium absorption across epithelia. *Physiol Rev.* 2005;85:373-422..
37. Amizuka N, Karaplis AC, Henderson HE, Warshawsky H, Lipman ML, Matsuki Y, Ejiri S, Tanaka M, Izumi N, Ozawa H, Goltzman D. Haploinsufficiency of parathyroid hormone-related peptide (PTHrP) results in abnormal postnatal bone development. *Dev. Biol.* 1996;175:166-176.
38. Rouleau MF, Mitchell J, Goltzman D. In vivo distribution of parathyroid hormone receptors in bone: Evidence that a predominant osseous target cell is not the mature osteoblast. *Endocrinology* 1988;123:187-191.
39. Bellido T, Saini V, Pajevic PD. Effects of PTH on osteocyte function. *Bone.* 2013;54(2):250-7.
40. Lee SK, Lorenzo JA. Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: Correlation with osteoclast-like cell formation. *Endocrinology* 1999;140:3552-3561.
41. Takahashi N, Udagawa N, Takami M, Suda T. Cells of bone: osteoclast generation., in Bilezikian JP, Raisz LG, Rodan GA. (eds): *Principles of bone biology*, 2nd ed. San Diego, Academic Press, 2002, pp109-126.
42. Miao D, He B, Karaplis AC, Goltzman D. Parathyroid hormone is essential for normal fetal bone formation. *J. Clin. Invest.* 2002;109:1173-1182.
43. Goltzman D. Studies on the mechanisms of the skeletal anabolic action of endogenous and exogenous parathyroid hormone. *Arch. Biochem. Biophys.* 2008;473:218-224.
44. Monzem S, Valkani D, Evans LAE, Chang YM, Pitsillides AA. Regional modular responses in different bone compartments to the anabolic effect of PTH (1-34) and axial loading in mice. *Bone.* 2023;170:116720
45. McCarthy TL, Centrella M, Canalis E. Parathyroid hormone enhances the transcript and polypeptide levels of insulin-like growth factor I in osteoblast-enriched cultures from fetal rat bone. *Endocrinology* 1989;124:1247-1253.
46. Jilka R, Weinstein R, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *J. Clin. Invest.* 1999;104:439-446.
47. Tam C, Heersche J, Murray T, Parsons JA. Parathyroid hormone stimulates the bone apposition rate independently of its resorptive action: differential effects of intermittent and continuous administration. *Endocrinology* 1982;110:506-512.
48. Holick MF. Vitamin D: Photobiology, metabolism and clinical applications., in DeGroot L, et al (eds): *Endocrinology*. Philadelphia, Saunders, 1995, pp 990
49. Bouillon R, Okamura WH, Norman AW. Structure-function relationships in the vitamin D endocrine system. *Endo. Revs.* 1995;16:200-257.
50. Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, Goltzman D. Targeted ablation of the 25-hydroxyvitamin D 1 α -hydroxylase enzyme: Evidence for skeletal, reproductive, and immune dysfunction. *Proc. Natl. Acad. Sci. USA* 2001;98:7498-7503.
51. Nguyen-Yamamoto L, Karaplis AC, St-Arnaud R, Goltzman D. Fibroblast growth factor 23 regulation by systemic and local osteoblast-synthesized 1,25-dihydroxyvitamin D. *J Am Soc Nephrol.* 2017;8(2): 586-597.
52. Hewison M, Zehnder D, Bland R, Stewart PM. 1 α -hydroxylase and the action of vitamin D. *J. Mol. Endocrinol.* 2000;25(2):141-148.
53. St-Arnaud R, Arabian A, Travers R, Barletta F, Raval-Pandya M, Chapin K, Depovere J, Mathieu C, Christakos S, Demay MB, Glorieux FH. Deficient mineralization of intramembranous bone in vitamin D-24-hydroxylase-ablated mice is due to elevated 1,25-dihydroxyvitamin D and not to the absence of 24, 25-dihydroxyvitamin D. *Endocrinology* 2000;141:2658- 2666.
54. Jurutka PW, Whitfield GK, Hsieh JC, Thompson PD, Haussler CA, Haussler MR. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Rev. Endocr. Metab. Disord.* 2001;2(2):203-216.
55. Favus MF. Intestinal absorption of calcium, magnesium and phosphorus., in Coe FL, Favus MJ. (eds): *Disorders of bone and mineral metabolism*. New York, Raven, 1992, pp 57
56. Van de Graaf SFJ, Boullart I, Hoenderop JGJ, Bindels RJM. Regulation of the epithelial Ca²⁺ channels TRPV5 and TRPV6 by 1 α ,25-dihydroxy Vitamin D3 and dietary Ca²⁺. *J. Steroid Biochem. Molec. Biol.* 2004;89-90: 303-308.
57. Panda DK, Miao D, Bolivar I, Li J, Huo R, Hendy GN, Goltzman D. Inactivation of the 25-dihydroxyvitamin D-1 α -hydroxylase and vitamin D receptor demonstrates independent effects of calcium and vitamin D on skeletal and mineral homeostasis. *J. Biol Chem.* 2004;279:16754-16766.

58. Christakos S. Recent advances in our understanding of 1,25-dihydroxyvitamin D(3) regulation of intestinal calcium absorption. *Arch. Biochem. Biophys.* 2012;523(1):73-76.
59. Christakos S, Li S, De La Cruz J, Shroyer NF, Criss ZK, Verzi MP, Fleet JC. Vitamin D and the intestine: Review and update. *J Steroid Biochem Mol Biol.* 2020;196:105501
60. Li YC, Pirro, AE, Amling M, Delling G, Baron R, Bronson R, Demay MB. Targeted ablation of the vitamin D receptor: An animal model of vitamin D-dependent rickets type II with alopecia. *Proc. Natl. Acad. Sci. USA.* 1997;94:9831-9835.
61. Carmeliet G, Dermauw V, Bouillon R. Vitamin D signaling in calcium and bone homeostasis: a delicate balance. *Best Pract. Res. Clin. Endocrinol. Metab.* 2015;29(4):621-631
62. Xue Y, Karaplis AC, Hendy GN, Goltzman D, Miao D. Genetic models show that parathyroid hormone and 1,25-dihydroxyvitamin D3 play distinct and synergistic roles in postnatal mineral ion homeostasis and skeletal development. *Hum. Mol. Genet.* 2005;14:1515-1528.
63. Stewart AF, Horst R, Deftos LJ, Cadman EC, Lang R, Broadus AE. Biochemical evaluation of patients with cancer-associated hypercalcemia: Evidence for humoral and non-humoral groups. *N. Engl. J. Med.* 1980;303:1377-1383.
64. Yasuda T, Banville D, Hendy GN, Goltzman D.: Characterization of the human parathyroid hormone-like peptide gene. *J. Biol. Chem.* 1989;264:7720-7725.
65. Mangin M, Ikeda K, Dreyer BE, Broadus AE. Isolation and characterization of the human parathyroid hormone-like peptide gene. *Proc. Natl. Acad. Sci. USA.* 1989;86:2408-2412.
66. Rabbani SA, Mitchell J, Roy DR, Hendy GN, Goltzman D. Influence of the amino-terminus on in vitro and in vivo biological activity of synthetic parathyroid hormone and parathyroid hormone-like peptides of malignancy. *Endocrinology* 1988;123:2709-2716.
67. Usdin TB, Hoare SR, Wang T, Mezey E, Kowalak JA. TIP39: a new neuropeptide and PTH2-receptor agonist from hypothalamus. *Nat. Neurosci.* 1999;2(11):941-943.
68. Usdin TB, Gruber C, Bonner TI. Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. *J. Biol. Chem.* 1995;270:15455-15458.
69. Keller D, Tsuda MC, Usdin TB, Dobolyi A. Behavioural actions of tuberoinfundibular peptide 39 (parathyroid hormone 2). *J Neuroendocrinol.* 2022;34(9):e13130
70. Goltzman D, Hendy GN, Banville D. Parathyroid hormone-like peptide: Molecular characterization and biological properties. *Trends Endocrinol. Metab.* 1989;1:39-44.
71. Rabbani SA, Haq M, Goltzman D. Biosynthesis and processing of endogenous parathyroid hormone-related peptide (PTHrP) by the rat Leydig cell tumor H-500. *Biochemistry* 1993;32:4931-4937.
72. Plawner LL, Philbrick WM, Burtis WJ, Broadus AE, Stewart AF. Cell type-specific secretion of parathyroid hormone-related protein via the regulated versus the constitutive secretory pathway. *J. Biol. Chem.* 1995;270:14078-14084.
73. Eto M, Akishita M, Ishikawa M, Kozaki K, Yoshizumi M, Hashimoto M, Ako J, Sugimoto N, Nagano K, Sudoh N, Toba K, Ouchi Y. Cytokine-induced expression of parathyroid hormone-related peptide in cultured human vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 1998;249:339-343.
74. Kremer R, Karaplis AC, Henderson JE, Gulliver W, Banville D, Hendy GN, Goltzman D. Regulation of parathyroid hormone-like peptide in cultured normal human keratinocytes. *J. Clin. Invest.* 1991;87:884-893.
75. Sebag M, Henderson JE, Goltzman D, Kremer R. Regulation of parathyroid hormone-related peptide production in normal human mammary epithelial cells in vitro. *Am. J. Physiol.* 1994;267:723-730.
76. Casey ML, Mike M, Erk A, MacDonald PC. Transforming growth factor-B1 stimulation of parathyroid hormone-related protein expression in human uterine cells in culture: mRNA levels and protein secretion. *J. Clin. Endocrinol. Metab.* 1992;74:950952.
77. Aklilu F, Park M, Goltzman D, Rabbani SA: Induction of parathyroid hormone related peptide by the Ras oncogene: Role of Ras farnesylation inhibitors as potential therapeutic agents for hypercalcemia of malignancy. *Cancer Res.* 1997;57:4517-4522.
78. Kremer R, Sebag M, Champigny C, Meerovitch K, Hendy GN, White J, Goltzman D. Identification and characterization of 1,25-dihydroxyvitamin D3-responsive repressor sequences in the rat parathyroid hormone-related peptide gene. *J. Biol. Chem.* 1996;271:16310-16316.
79. Lu C, Ikeda K, Deftos LJ, Gazdar AF, Mangin M, Broadus AE. Glucocorticoid regulation of parathyroid hormone-related peptide gene transcription in a human neuroendocrine cell line. *Mol. Endocrinol.* 1989;3:2034-2040.
80. Liu B, Goltzman D, Rabbani SA: Regulation of parathyroid hormone-related peptide production in vitro by the rat hypercalcemic Leydig cell tumor H-500. *Endocrinology* 1993;132:1658-1664.
81. Haq M, Kremer R, Goltzman D, Rabbani SA. A vitamin D analogue (EB1089) inhibits parathyroid hormone-related peptide production and prevents the development of

- malignancy-associated hypercalcemia in vivo. *J. Clin. Invest.* 1993;91:2416-2422.
82. El Abdaimi K, Papavasiliou V, Rabbani SA, Rhim JS, Goltzman D, Kremer R. Reversal of hypercalcemia with the vitamin D analog EB1089 in a human model of squamous cancer. *Cancer Res.* 1999;59:3325-3328.
83. Liu B, Goltzman D, Rabbani SA. Processing of pro-PTHrP by the prohormone convertase, furin: Effect on biological activity. *Am. J. Physiol.* 1995;268:E832-E838.
84. Liu B, Amizuka N, Goltzman D, Rabbani SA. Inhibition of processing of parathyroid hormone-related peptide by antisense furin: Effect in vitro and in vivo on rat Leydig (H-500) tumor cells. *Int. J. Cancer* 1995;63:276-281.
85. Care AD, Abbas SL, Pickard DW, Barri M, Drinkhill M, Findlay JB, White IR, Caple IW. Stimulation of ovine placental transport of calcium and magnesium by mid-molecule fragments of human parathyroid hormone-related protein. *Exp. Physiol.* 1990;5:605-608.
86. Fenton AJ, Kemp BE, Hammonds RG, Mitchelhill K, Moseley JM, Martin TJ, Nicholson GC. A potent inhibitor of osteoclastic bone resorption within a highly conserved pentapeptide region of PTHrP (107-111). *Endocrinology* 1991;129:3424-3426.
87. Philbrick WM, Dreyer BE, Nakchbandi IA, Karaplis AC. Parathyroid hormone-related protein is required for tooth eruption. *Proc. Natl. Acad. Sci. USA.* 1998;95:11846-11851.
88. Henderson JE, Amizuka H, Warshawsky H, Biasotto D, Lanske BM, Goltzman D, Karaplis AC. Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. *Mol. Cell. Biol.* 1995;15:4064-4075.
89. Lam MHC, House CM, Tiganis T, Mitchelhill KI, Sarcevic B, Cures A, Ramsay R, Kemp BE, Martin TJ, Gillespie MT. Phosphorylation of the cyclin-dependent kinases site (Thr85) of parathyroid hormone-related protein negatively regulates its nuclear localization. *J. Biol. Chem.* 1999;274:18559-18566.
90. Aarts MM, Rix A, Guo J, Bringhurst R, Henderson JE. The nucleolar targeting signal (NTS) of parathyroid hormone-related protein mediates endocytosis and nuclear translocation. *J. Bone Miner. Res.* 1999;14:1493-1503.
91. Meerovitch K, Wing W, Goltzman D. Parathyroid hormone related protein is associated with the chaperone protein BiP and undergoes proteasome mediated degradation. *J. Biol. Chem.* 1998;273:21024-21030.
92. Nguyen M, He B, Karaplis A. Nuclear forms of parathyroid hormone-related peptide are translated from non-AUG start sites downstream from the initiator methionine. *Endocrinology* 2001;142:694-703.
93. Miao D, Su H, He B, Gao J, Xia Q, Zhu M, Gu Z, Goltzman D, Karaplis AC. Severe growth retardation and early lethality in mice lacking the nuclear localization sequence and C-terminus of PTH-related protein. *Proc. Natl. Acad. Sci. U S A.* 2008;105(51):20309-20314.
94. Toribio RE, Brown HA, Novince CM, Marlow B, Hernon K, Lanigan LG, Hildreth BE 3rd, Werbeck JL, Shu ST, Lorch G, Carlton M, Foley J, Boyaka P, McCauley LK, Rosol TJ. The midregion, nuclear localization sequence, and C terminus of PTHrP regulate skeletal development, hematopoiesis, and survival in mice. *FASEB J.* 2010;24(6):1947-1957.
95. Kovacs CS, Lanske B, Hunzelman JL, Guo J, Karaplis AC, Kronenberg HM. Parathyroid hormone-related peptide (PTHrP) regulates fetal-placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. *Proc. Natl. Acad. Sci. USA* 1996;93:15233-15238.
96. Takahashi K, Inoue D, Ando K, Matsumoto T, Ikeda K, Fujita T. Parathyroid hormone-related peptide as a locally produced vasorelaxant regulation of its mRNA by hypertension in rats. *Biochem. Biophys. Res. Commun.* 1995;208:447-455.
97. Morimoto T, Devora GA, Mibe M, Casey ML, MacDonald PC. Parathyroid hormone-related protein and human myometrial cells: Action and regulation. *Mol. Cell. Endocrinol.* 1997;129:91-99.
98. Yamamoto M, Harm SC, Grasser WA, Thiede MA. Parathyroid hormone-related protein in the rat urinary bladder: A smooth muscle relaxant produced locally in response to mechanical stretch. *Proc. Natl. Acad. Sci. USA* 1992;89:5326-5330.
99. Wysolmerski JJ, McCaugherncarucci JF, Daifotis AG, Broadus AE, Philbrick WM. Overexpression of parathyroid hormone-related protein or parathyroid hormone in transgenic mice impairs branching morphogenesis during mammary gland development. *Development* 1995;121:3539-3547.
100. Holick MF, Ray S, Chen TC, Tian X, Persons KS. A parathyroid hormone antagonist stimulates epidermal proliferation and hair growth in mice. *Proc. Natl. Acad. Sci. USA* 1994;91:8014-8016.
101. Fukayama S, Tashjian AH Jr, Davis JN, Chisholm JC. Signaling by N- and C-terminal sequences of parathyroid hormone-related protein in hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 1995;92:10182-10186.
102. Vasavada R, Cavaliere C, D'Ercole AJ, Dann P, Burtis WJ, Madlener AL, Zawulich K, Zawulich W, Philbrick W, Stewart AF. Overexpression of PTHrP in the pancreatic islets of

- transgenic mice causes hypoglycemia, hyperinsulinemia and islet hyperplasia. *J. Biol. Chem* 1996;271:1200-1208.
103. Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM, Mulligan RC. Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. *Genes Dev.* 1994;8:277-289.
 104. Amizuka N, Karaplis AC, Henderson JE, Warshawsky H, Lipman ML, Matsuki Y, Ejiri S, Tanaka M, Izumi N, Ozawa H, Goltzman D. Haploinsufficiency of parathyroid hormone-related peptide (PTHrP) results in abnormal postnatal bone development. *Dev. Biol.* 1996;175:166-176.
 105. Lanske B, Amling M, Neff L, Guiducci J, Baron R, Kronenberg HM. Ablation of the PTHrP gene or the PTH/PTHrP receptor gene leads to distinct abnormalities in bone development. *J. Clin. Invest.* 1999;104:399-407.
 106. Zhang P, Jobert AS, Couvineau A, Silve C. A homozygous inactivating mutation in the parathyroid hormone/parathyroid hormone-related peptide receptor causing Blomstrand chondrodysplasia. *J. Clin. Endocrinol. Metab.* 1998;83:3365-3368.
 107. Karaplis AC, He B, Nguyen MT, Young ID, Semeraro D, Ozawa H, Amizuka N. Inactivating mutation in the human parathyroid hormone receptor type I gene in Blomstrand chondrodysplasia. *Endocrinology* 1998;139:5255-5258.
 108. Miao D, He B, Jiang Y, Kobayashi T, Sorocéanu MA, Zhao J, Su H, Tong X, Amizuka N, Gupta A, Genant HK, Kronenberg HM, Goltzman D, Karaplis AC. Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1-34. *J. Clin. Invest.* 2005;115:2402-2411.
 109. Miao D, Li J, Xue Y, Su H, Karaplis AC, Goltzman D. Parathyroid hormone-related peptide is required for increased trabecular bone mass in parathyroid hormone-null mice. *Endocrinology* 2004;145:3554-3562.
 110. Miao D, Su H, He B, Gao J, Xia Q, Zhu M, Gu Z, Goltzman D, Karaplis AC. Severe growth retardation and early lethality in mice lacking the nuclear localization sequence and C-terminus of PTH-related protein. *Proc. Natl. Acad. Sci. U S A.* 2008;105(51):20309-20314.
 111. Mundy GR. Bone remodeling. in Favus MJ. (ed): *Primer on the metabolic bone diseases and disorders of mineral metabolism*, fourth edition. Philadelphia, Lippincott, Williams and Wilkins, 1999, pp 30-38.
 112. Xie H, Cui Z, Wang L, Xia Z, Hu Y, Xian L, Li C, Xie L, Crane J, Wan M, Zhen G, Bian Q, Yu B, Chang W, Qiu T, Pickarski M, Duong LT, Windle JJ, Luo X, Liao E, Cao X. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat. Med.* 2014;20:1270-7822.
 113. Sims NA, Martin TJ. Osteoclasts Provide Coupling Signals to Osteoblast Lineage Cells Through Multiple Mechanisms. *Annu Rev Physiol.* 2020;82:507-529.
 114. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N. Engl. J. Med.* 2003;349:2483-2494.
 115. Milne M, Kang MI, Cardona G, Quail JM, Braverman LE, Chin WW, Baran DT. Expression of multiple thyroid hormone receptor isoforms in rat femoral and vertebral bone and in bone marrow osteogenic cultures. *J. Cell Biochem.* 1999;74:684-693.
 116. Fell HB, Mellanby E. The effect of hypervitaminosis A on embryonic limb bones cultured in vitro. *J. Physiol.* 1952;116:320-349.
 117. Horowitz MC, Lorenzo JA: Local regulators of bone. in Bilezikian JP, Raisz LG, Rodan GA.(eds): *Principles of Bone Biology*, second edition. San Diego, Academic Press, 2002, pp 961-978.
 118. Pilbeam CC, Harrison JR, Raisz LG. *Principles of Bone Biology*, second edition. San Diego, Academic Press, 2002, pp 979-994.
 119. Goltzman D. Nonparathyroid Hypercalcemia. *Front Horm Res.* 2019;51:77-90.
 120. Marx SJ, Goltzman D. Evolution of Our Understanding of the Hyperparathyroid Syndromes: A Historical Perspective. *J Bone Miner Res.* 2019;34(1):22-37
 121. 114Krebs LJ, Arnold A. Molecular basis of hyperparathyroidism and potential targets for drug development. *Curr. Drug Targets Immune Endocr Metabol Disord.* 2002;2:167-179.
 122. Brewer K, Costa-Guda J, Arnold A. Molecular genetic insights into sporadic primary hyperparathyroidism. *Endocr Relat Cancer.* 2019;26(2):R53-R72 3
 123. Carpten JD, Robbins CM, Villablanca A, Forsberg L, Presciuttini S, Bailey-Wilson J, Simonds WF, Gillanders EM, Kennedy AM, Chen JD, Agarwal SK, Sood R, Jones MP, Moses TY, Haven C, Petillo D, Leotlela PD, Harding B, Cameron D, Pannett AA, Höög A, Heath H 3rd, James-Newton LA, Robinson B, Zarbo RJ, Cavaco BM, Wassif W, Perrier ND, Rosen IB, Kristoffersson U, Turnpenny PD, Farnebo LO, Besser GM, Jackson CE, Morreau H, Trent JM, Thakker RV, Marx SJ, Teh BT, Larsson C, Hobbs MR. HRPT2 encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. *Nature Genet.* 2002;32:676-680..
 124. Shattuck TM, Välimäki S, Obara T, Gaz RD, Clark OH, Shoback D, Wierman ME, Tojo K, Robbins CM, Carpten

- JD, Farnebo L-O, Larsson C, Arnold A. Somatic and germline mutations of the HRPT2 gene in sporadic parathyroid carcinoma. *N. Engl. J. Med.* 2003;349:1722-1729.
125. Costa-Guda J, Soong CP, Parekh VI, Agarwal SK, Arnold A. Germline and somatic mutations in cyclin-dependent kinase inhibitor genes *CDKN1A*, *CDKN2B*, and *CDKN2C* in sporadic parathyroid adenomas. *Horm Cancer.* 2013;4(5):301–307.
 126. D'Agruma L, Coco M, Guarnieri V, Battista C, Canaff L, Salcuni AS, Corbetta S, Cetani F, Minisola S, Chiodini I, Eller-Vainicher C, Spada A, Marcocci C, Guglielmi G, Zini M, Clemente R, Wong BY, de Martino D, Scillitani A, Hendy GN, Cole DE. Increased prevalence of the *GCM2* polymorphism, Y282D, in primary hyperparathyroidism analysis of three Italian cohorts. *J Clin Endocrinol Metab.* 2014;99(12):E2794–E2798.
 127. Guan B, Welch JM, Sapp JC, Ling H, Li Y, Johnston JJ, Kebebew E, Biesecker LG, Simonds WF, Marx SJ, Agarwal SK. *GCM2*-activating mutations in familial isolated hyperparathyroidism. *Am J Hum Genet.* 2016;99(5):1034–1044.
 128. Simonds WF, Marx SJ, Agarwal SK. Ethnicity of patients with germline *GCM2*-activating variants and primary hyperparathyroidism. *J Endocr Soc.* 2017;1(5):488–499.
 129. Canaff L, Guarnieri V, Kim Y, Wong BYL, Nolin-Lapalme A, Cole DEC, Minisola S, Eller-Vainicher C, Cetani F, Repaci A, Turchetti D, Corbetta S, Scillitani A, Goltzman D. Novel Glial Cells Missing-2 (*GCM2*) variants in parathyroid disorders. *Eur J Endocrinol.* 2022;186(3):351-366.)
 130. Kifor O, Moore, FD, Delaney M, Garber J, Hendy GN, Butters R, Gao P, Cantor TL, Kifor I, Brown EM, Wysolmerski J. A syndrome of hypocalciuric hypercalcemia caused by antibodies directed against the calcium-sensing receptor. *J. Clin. Endocrinol. Metab.* 2003;88:60-72.
 131. Walker MD, Shane E. Hypercalcemia: A Review. *JAMA.* 2022;328(16):1624-1636
 132. Eastell R, Arnold A, Brandi ML, Brown EM, D'Amour P, Hanley DA, Rao DS, Rubin MR, Goltzman D, Silverberg SJ, Marx SJ, Peacock M, Mosekilde L, Bouillon R, Lewiecki EM. Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J. Clin. Endocrinol. Metab.* 2009;94(2):340-350.
 133. Agarwal A, Mishra SK, Gujral RB: Advanced skeletal manifestations in primary hyperparathyroidism. *Can. J. Surg.* 1998;41:342-343.
 134. Bandeira F, Griz L, Caldas G, Macedo G, Bandeira C. Characteristics of primary hyperparathyroidism in one institution in northeast Brazil. *Bone.* 1998;5:S380.
 135. Biyabani SR, Talati J. Bone and renal stone disease in patients operated for primary hyperparathyroidism in Pakistan: Is the pattern of disease different from the west? *J. Pakistan Med. Assoc.* 1999;49:194-198.
 136. Chan TB, Lee KO, Rauff A, Tan L, Gwee HM. Primary hyperparathyroidism at the Singapore general hospital. *Singapore Med. J.* 27:154-157.
 137. Harinarayan CV, Gupta N, Kochupillai N. Vitamin D status in primary hyperparathyroidism in India. *Clin. Endocrinol.* 1995;43:351-358.
 138. Silverberg SJ, Shane E, De LaCruz L, Dempster DW, Feldman F, Seldin D, Jacobs TP, Siris ES, Cafferty M, Parisien MV, et al. Skeletal disease in primary hyperparathyroidism. *J. Bone Miner. Res.* 1989;4:283-291.
 139. Silverberg, SJ, Shane E, Jacobs TP, Siris ES, Gartenberg F, Seldin D, Clemens TL, Bilezikian JP. Nephrolithiasis and bone involvement in primary hyperparathyroidism. *Am. J. Med.* 1990;89:327-334.
 140. Silverberg SJ, Shane E, Jacobs TP, Siris E, Bilezikian JP. A 10-year prospective study of primary hyperparathyroidism with or without parathyroid surgery. *N. Engl. J. Med.* 1999;341:1249-1255.
 141. Zhao L, Liu JM, He XY, Zhao H-Y, Sun L-H, Tao B, Zhang M-J, Chen X, Wang W-Q, Ning G. The changing clinical patterns of primary hyperparathyroidism in Chinese patients: data from 2000 to 2010 in a single clinical center. *J Clin Endocrinol Metab.* 2013;98(2):721-728; .
 142. Bilezikian JP, Khan AA, Silverberg SJ, Fuleihan GE, Marcocci C, Minisola S, Perrier N, Sitges-Serra A, Thakker RV, Guyatt G, Mannstadt M, Potts JT, Clarke BL, Brandi ML; International Workshop on Primary Hyperparathyroidism. Evaluation and Management of Primary Hyperparathyroidism: Summary Statement and Guidelines from the Fifth International Workshop. *J Bone Miner Res.* 2022;37(11):2293-2314
 143. Fitzpatrick LA, Bilezikian JP. Acute primary hyperparathyroidism. *Am. J. Med.* 1987;82:275-282.
 144. Bilezikian JP, Silverberg SJ, Bandeira F, Cetani F, Chandran M, Cusano NE, Ebeling PR, Formenti AM, Frost M, Gosnell J, Lewiecki EM, Singer FR, Gittoes N, Khan AA, Marcocci C, Rejnmark L, Ye Z, Guyatt G, Potts JT. Management of primary hyperparathyroidism. *J Bone Miner Res.* 2022;37(11):2391-2403.
 145. Bilezikian JP, Khan AA, Potts JT Jr. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. *J Clin Endocrinol Metab.* 2009;94(2):335-339.

146. Silverberg SJ, Bone HG III, Marriott TB, Locker FG, Thys-Jacobs S, Dziem G, Kaatz S, Sanguinetti EL, Bilezikian JP. Short-term inhibition of parathyroid hormone secretion by a calcium-receptor agonist in patients with primary hyperparathyroidism. *N. Eng. J. Med.* 1997;337:1506-1510.
147. Adami S, Mian M, Bertoldo F, Rossini M, Jayawerra P, O'Riordan JL, Lo Cascio V. Regulation of calcium-parathyroid hormone feedback in primary hyperparathyroidism: Effects of bisphosphonate treatment. *Clin. Endocrinol.* 1990;33:391-397.
148. Gallagher JC, Nordin BEC. Treatment with oestrogens of primary hyperparathyroidism in postmenopausal women. *Lancet* 1972;1:503-507.
149. Rosenthal NR, Insogna KL, Godsall JW, Smaldone L, Waldron JA, Stewart AF. Elevations in circulating 1,25(OH)₂D in three patients with lymphoma-associated hypercalcemia. *J. Clin. Endocrinol. Metab.* 1985;60:29-33.
150. Seymour JF, Gagel RF, Hagemester FB, Dimopoulos MA, Cabanillas F. Calcitriol production in hypercalcemia and normocalcemia patients with non-Hodgkin lymphoma. *Ann. Intern. Med.* 1994;121:633-640.
151. Nagai M, Kyakumoto S, Sato N. Cancer cells responsible for humoral hypercalcemia express mRNA encoding a secreted form of ODF/TRANCE that induces osteoclast formation. *Biochem. Biophys. Res. Commun.* 2000;269:532-536..
152. Mariathasan S, Andrews KA, Thompson E, Challis BG, Wilcox S, Pierce H, Hale J, Spiden S, Fuller G, Simpson HL, Fish B, Jani P, Seetho I, Armstrong R, Izatt L, Joshi M, Velusamy A, Park SM, Casey RT. Genetic testing for hereditary hyperparathyroidism and familial hypocalciuric hypercalcemia in a large UK cohort. *Clin Endocrinol (Oxf).* 2020;93(4):409-418.
153. Li Y, Simonds WF. Endocrine neoplasms in familial syndromes of hyperparathyroidism. *Endocr. Relat. Cancer* 2016;23(6):R229-47.
154. Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ. Positional cloning of the gene for multiple endocrine neoplasia type 1. *Science* 1997;276:404-407.
155. Marx SJ: Multiple endocrine neoplasia type I., in Bilezikian JP, Marcus R, Levine MA. (eds): *The parathyroids: basic and clinical concepts*, second edition. San Diego, Academic Press, 2001, pp 535-584.
156. Sipple JH: The association of pheochromocytoma with carcinoma of the thyroid gland. *Am. J. Med.* 1961;31:163-166.
157. Gagel RF: Multiple endocrine neoplasia., in Wilson JD, Foster DW, Larsen PR, et al. (eds): *Williams textbook of endocrinology*, 9th edition. Philadelphia, Saunders, 1997, pp 1627-1649.
158. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunnacliffe A, Ponder BAJ. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993;363:458-460.
159. Carney JA, Go VL, Sizemore GW, Hayles AB. Alimentary-tract ganglioneuromatosis. A major component of the syndrome of multiple endocrine neoplasia, type 2b. *N. Engl. J. Med.* 1976;295:1287-1291.
160. Mulligan LM, Eng C, Attie T, Lyonnet S, Marsh DJ, Hyland VJ, Robinson BG, Frilling A, Verellen-Dumoulin C, Safar A, Venter DJ, Munnich A, Ponder BAJ. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. *Hum. Mol. Genet.* 1994;3:2163-2168.
161. Goltzman D, Potts JT Jr, Ridgway RC, Maloof F. Calcitonin as a tumor marker. Use of the radioimmunoassay for calcitonin in the postoperative evaluation of patients with medullary thyroid carcinoma. *N. Engl. J. Med.* 1974;290:1035-1039.
162. Mallette LE, Malini S, Rappaport MP, Kirkland JL. Familial cystic parathyroid adenomatosis. *Ann. Intern. Med.* 1987;107:54-60.
163. Jackson CE, Norman RA, Boyd SB, Talpos GB, Wilson SD, Taggart RT, Mallette LE. Hereditary hyperparathyroidism and multiple ossifying jaw fibromas: A clinically and genetically distinct syndrome. *Surgery* 1990;108:1006-1012.
164. Simonds WF, Robbins CM, Agarwal SK, Hendy GN, Carpten JD, Marx SJ. Familial isolated hyperparathyroidism is rarely caused by germline mutation in HRPT2, the gene for the hyperparathyroidism-jaw tumor syndrome. *J Clin Endocrinol Metab.* 2004;89:96-102.
165. Canaff L, Guarnieri V, Kim Y, Wong BYL, Nolin-Lapalme A, Cole DEC, Minisola S, Eller-Vainicher C, Cetani F, Repaci A, Turchetti D, Corbetta S, Scillitani A, Goltzman D. Novel Glial Cells Missing-2 (GCM2) variants in parathyroid disorders. *Eur J Endocrinol.* 2022;186(3):351-366
166. Marx SJ, Attie MF, Levine MA, Spiegel AM, Downs RW Jr, Lasker RD. The hypocalciuric or benign variant of familial hypercalcemia: Clinical and biochemical features in fifteen kindreds. *Medicine* 1981;60:397-412.

167. Heath H III: Familial benign (Hypocalciuric) hypercalcemia. A troublesome mimic of mild primary hyperparathyroidism. *Endocrinol. Metab. Clin. North Am.* 1989;18:723-740.
168. Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, Levi T, Seidman CE, Seidman JG. Mutations in the human Ca^{2+} -sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Cell* 1993;75:1297-1303.
169. Marx SJ, Attie MF, Spiegel AM, Levine MA, Lasker RD, Fox M. An association between neonatal severe primary hyperparathyroidism and familial hypocalciuric hypercalcemia in three kindreds. *N. Eng. J. Med.* 1982;306:257-64.
170. Kifor O, Moore FD Jr, Delaney M, Garber J, Hendy GN, Butters R, Gao P, Cantor TL, Kifor I, Brown EM, Wysolmerski J. A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab.* 2003;88(1):60-72
171. Parfitt AM. Parathyroid growth: normal and abnormal. in Bilezikian JP, Marcus R, Levine MA. (eds): *The parathyroids: basic and clinical concepts*, second edition. San Diego, Academic press, 2001, pp 293-329.
172. Burman KD, Monchick JM, Earl JM, Wartofsky L. Ionized and total serum calcium and parathyroid hormone in hyperthyroidism. *Ann. Intern. Med.* 1976;84:668-671.
173. Britto JM, Fenton AJ, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. *Endocrinology* 123:169-176.
174. Rosen HN, Moses AC, Gundberg C, Kung VT, Seyedin SM, Chen T, Holick M, Greenspan SL. Therapy with parenteral pamidronate prevents thyroid hormone-induced bone turnover in humans. *J. Clin. Endocrinol. Metab.* 1993;77:664-669.
175. Rude RK, Oldham SB, Singer FR, Nicoloff JT. Treatment of thyrotoxic hypercalcemia with propranolol. *N. Eng. J. Med.* 1976;294:431-433.
176. Ross DS, Nussbaum SR: Reciprocal changes in parathyroid hormone and thyroid function after radioiodine treatment of hyperthyroidism. *J. Clin. Endocrinol. Metab.* 1989;68:1216-1219.
177. Kimura S, Nishimura Y, Yamaguchi K, Nagasaki K, Shimada K, Uchida H. A case of pheochromocytoma producing parathyroid hormone-related protein and presenting with hypercalcemia. *J. Clin. Endocrinol. Metab.* 1990;70: 1559–1563.
178. Mune T, Katakami H, Kato Y, Yasuda K, Matsukura S, Miura K. Production and secretion of parathyroid hormone-related protein in pheochromocytoma: participation of an alpha-adrenergic mechanism. *J Clin Endocrinol Metab.* 1993;76(3):757-762.
179. Ghaferi AA, Chojnacki KA, Long WD, Cameron JL, Yeo CJ. Pancreatic VIPomas: subject review and one institutional experience. *J. Gastrointest. Surg.* 2008;12(2):382-393.
180. Vasikaran SD, Tallis GA, Braund WJ: Secondary hypoadrenalism presenting with hypercalcemia. *Clin. Endocrinol.* 1994;41:261-264.
181. Diamond T, Thornley S: Addisonian crisis and hypercalcemia. *Aust. N.Z. J. Med.* 1994;24:316.
182. Schipiani E, Kruse K, Jhpnner H. A constitutively active mutant PTH-PTHrp receptor in Jansen-type metaphyseal chondrodysplasia. *Science* 1995;268:98-100.
183. Zondek H, Petrow H, Siebert W. Die Bedeutung der Calciumbestimmung im Blute fhr die Diagnose der Niereninsuffizienz. *Z. Klin. Med.* 1924;9:129-138.
184. Gutman Ab, Tyson TL, Gutman EB. Serum calcium, inorganic phosphorus, and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic disease of bones. *Arch. Int. Med.* 1936;7:379-413.
185. Albright R. Case records of the Massachusetts General Hospital (Case 27401). *N. Engl. J. Med.* 1941;225:789-791
186. Lafferty FW. Pseudohyperparathyroidism. *Medicine* 1966;45:247-260.
187. Powell D, Singer FR, Murray TM, Minkin C, Potts JR Jr. Non-parathyroid humoral hypercalcemia in patients with neoplastic disease. *N. Engl. J. Med.* 1973;89:176-181.
188. Simpson EL, Mundy GR, D'Souza SM, Ibbotson KJ, Bockman R, Jacobs JW. Absence of parathyroid hormone messenger RNA in non-parathyroid tumors associated with hypercalcemia. *N. Engl. J. Med.* 1983;309:325-330.
189. Goltzman D, Stewart AF, Broadus AE. Malignancy-associated hypercalcemia evaluation with a cytochemical bioassay for parathyroid hormone. *J. Clin. Endocrinol. Metab.* 1981;53:899-904.
190. Stewart AF, Insogna KL, Goltzman D, Broadus AE. Identification of adenylate cyclase-stimulating activity and cytochemical glucose-6-phosphatedehydrogenase-stimulating activity in extracts of tumors from patients with hypercalcemia of malignancy. *Proc. Natl. Acad. Sci. USA.* 1983;80:1454-1458.
191. Suva LJ, Winslow GA, Wettenhall REH, Hammonds RG, Moseley JM, Diefenbach-Jagger H, Rodda CP, Kemp BE, Rodriguez H, Chen EY, et al. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. *Science* 1987;237:893-896.
192. Kukreja SC, Schavin DH, Winbuscus S, Ebeling PR, Danks JA, Rodda CP, Wood WI, Martin TJ. Antibodies to

- parathyroid hormone-related protein lower serum calcium in athymic mouse models of malignancy associated hypercalcemia due to human tumors. *J. Clin. Invest.* 1988;82:1798-1802.
193. Henderson JE, Bernier S, D'Amour P, Goltzan D. Effects of passive immunization against parathyroid hormone (PTH)-like peptide and PTH in hypercalcemic tumor-bearing rats and normocalcemic controls. *Endocrinology* 1990;127:1310-1318.
 194. Fraher LJ, Hodsman AB, Jonas K, Saunders D, Rose CI, Henderson JE, Hendy GN, Goltzman D. A comparison of the in vivo biochemical responses to exogenous parathyroid hormone (1-34) [PTH 1-34] and PTH-related peptide (1-34) in man. *J. Clin. Endocrinol. Metab.* 1992;75:417-423.
 195. Yamato H, Nagai Y, Inoue D, Ohnishi Y, Ueyama Y, Ohno H, Matsumoto T, Ogata E, Ikeda K. In vivo evidence for progressive activation of parathyroid hormone-related peptide gene transcription with tumor growth and stimulation of osteoblastic bone formation at an early stage of humoral hypercalcemia of malignancy. *J. Bone Miner. Res.* 1995;10:36-44.
 196. Budayr AA, Nissenson RA, Klein RF, Pun KK, Clark OH, Diep D, Arnaud CD, Strewler GJ. Increased serum levels of parathyroid hormone-like protein in malignancy-associated hypercalcemia. *Ann. Intern. Med.* 1989;111:807-812.
 197. Burtis WJ, Brady TG, Orloff JJ, Ersbak JB, Warrell RP Jr., Olson BR, Wu TL, Mitnick ME, Broadus AE, Stewart AF. Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. *N. Eng. J. Med.* 1990;322:1106-1112.
 198. Henderson JE, Shustik C, Kremer R, Rabbani SA, Hendy GN, Goltzman D. Circulating concentrations of parathyroid hormone-like peptide in malignancy and hyperparathyroidism. *J. Bone Miner. Res.* 1990;5:105-113.
 199. Ratcliffe WA, Norbury C, Stott RA, Heath DA, Ratcliffe JG. Immunoreactivity of plasma parathyrin-related peptide: Three region specific radioimmunoassays and a two-site immunoradiometric assay compared. *Clin. Chem.* 1991;37:1781-1787.
 200. Grill V, Ho P, Body JJ, Lee SC, Kukreja SC, Moseley JM, Martin TJ. Parathyroid hormone-related protein: elevated levels in both humoral hypercalcemia of malignancy and hypercalcemia complicating metastatic breast cancer. *J. Clin. Endocrinol. Metab.* 1991;73:1309-1315.
 201. Holt EH, Vasavada R, Bander NH, Broadus AE, Philbrick WM. Region-specific methylation of the PTH-related peptide gene determines its expression in human renal carcinoma lines. *J. Biol. Chem.* 1993;268:20639-20645.
 202. Sidler B, Alpert L, Henderson JE, Deckelbaum R, Amizuka N, Silva JE, Goltzman D, Karaplis AC. Overexpression of parathyroid hormone-related peptide (PTHrP) by gene amplification in colonic carcinoma. *J. Clin. Endocrinol. Metab.* 1996;81:2841-2847.
 203. Grunbaum A, Kremer R. Parathyroid hormone-related protein (PTHrP) and malignancy. *Vitam Horm.* 2022;120:133-177
 204. Truong NU, deB Edwardes MD, Papavasiliou V, Goltzman D, Kremer R. Parathyroid hormone-related peptide and survival of patients with cancer and hypercalcemia. *Am. J. Med.* 2003;115:115-121.
 205. Soki FN, Park SI, McCauley LK. The multifaceted actions of PTHrP in skeletal metastasis. *Future Oncol.* 2012;8(7):803-817.
 206. Li J, Karaplis AC, Huang DC, Siegel PM, Camirand A, Yang XF, Muller WJ, Kremer R. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. *J Clin Invest* 12011;21(12): 4655–4669.
 207. Hirbe AC, Morgan EA, Weilbaecher KN. The CXCR4/SDF-1 chemokine axis: A potential therapeutic target for bone metastases? *Current Pharmaceutical Design.* 2010;16(11):1284–1290.
 208. Goltzman D. Non-parathyroid hypercalcemia. In: *Frontiers of Hormone Research: Parathyroid Disorders:Focusing on Unmet Needs.* ML Brandi(ed) Karger Basel, Switzerland 2019;vol 51 pp77-90.
 209. Goltzman D. Pathophysiology of hypercalcemia. *Endocrinol Metab Clin North Am.* 2021;50(4):591-607.
 210. Nakayama K, Fukumoto S, Takeda S, Takeuchi Y, Ishikawa T, Miura M, Hata K, Hane M, Tamura Y, Tanaka Y, Kitaoka M, Obara T, Ogata E, Matsumoto T. Differences in bone and vitamin D metabolism between primary hyperparathyroidism and malignancy-associated hypercalcemia. *J. Clin. Endocrinol. Metab.* 1996;81:607-611.
 211. Stewart AF, Vignery A, Silvergate A, Ravin ND, LiVolsi V, Broadus AE, Baron R. Quantitative bone histomorphometry in humoral hypercalcemia of malignancy. *J. Clin. Endocrinol. Metab.* 1982;55:219-227.
 212. Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, Yoneda T, Mundy GR. Evidence for a causal role of parathyroid hormone related protein in the pathogenesis of human breast cancer-mediated osteolysis. *J. Clin. Invest.* 1996;98:1544-1549.
 213. Rabbani SA, Gladu J, Harakidas P, Jamison B, Goltzman D. Overproduction of parathyroid hormone related peptide

- results in increased osteolytic skeletal metastasis by prostate cancer cells in vivo. *Int. J. Cancer* 1999;80:257-264.
214. Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massagué J, Mundy GR, Guise TA. TGF- β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *J. Clin. Invest.* 1999;103:197-206.
 215. Li X, Loberg R, Liao J, Ying C, Snyder LA, Pienta KJ, McCauley LK. A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. *Cancer Res.* 2009;69(4):1685–1692.
 216. Kremer R, Shustik C, Tabak T, Papavasiliou V, Goltzman D. Parathyroid hormone related peptide in hematologic malignancies. *Am. J. Med.* 1996;100:406-411.
 217. Firkin F, Seymour JF, Watson AM, Grill V, Martin TJ. Parathyroid hormone related protein in hypercalcemia associated with haematological malignancy. *Br. J. Haematol.* 1996;94:486-492.
 218. Watanabe T, Yamaguchi K, Takatsuki K, Osame M, Yoshida M. Constitutive expression of parathyroid hormone-related protein gene in human T cell leukemic virus type I (HTLV1) carriers and adult T cell leukemic patients that can be transactivated by HTLV-1 tax gene. *J. Exp. Med.* 1990;172:759-765.
 219. Grill V, Murray RML, Ho PWM, Santamaria JD, Pitt P, Potts C, Jerums G, Martin TJ. Circulating PTH and PTHrP levels before and after treatment of tumor induced hypercalcemia with pamidronate disodium (APD). *J. Clin. Endocrinol. Metab.* 1992;74:1468-1470.
 220. Truong NU, de B Edwardes MD, Papavasiliou V, Goltzman D, Kremer R. Parathyroid hormone-related peptide and survival of patients with cancer and hypercalcemia. *Am. J. Med.* 2003;115:115-121.
 221. Breslau NA, McGuire JL, Zerwekh JE, Frenkel EP, Pak CY. Hypercalcemia associated with increased serum calcitriol levels in three patients with lymphoma. *Ann. Intern. Med.* 1984;100:1-6.
 222. Nussbaum SR, Gaz RD, Arnold A: Hypercalcemia and ectopic secretion of parathyroid hormone by an ovarian carcinoma with rearrangement of the gene for PTH. *N. Engl. J. Med.* 1990;323:1324-1328.
 223. Iguchi H, Miyagi C, Tomita K, Kawauchi S, Nozuka Y, Tsuneyoshi M, Wakasugi H. Hypercalcemia caused by ectopic production of parathyroid hormone in a patient with papillary adenocarcinoma of the thyroid gland. *J. Clin. Endocrinol. Metab.* 1998;83:2653-2657.
 224. Nakajima K, Tamai M, Okaniwa S, Nakamura Y, Kobayashi M, Niwa T, Horigome N, Ito N, Suzuki S, Nishio S, Komatsu M. Humoral hypercalcemia associated with gastric carcinoma secreting parathyroid hormone: a case report and review of the literature. *Endocr J.* 2013;60(5):557-562.
 225. Mundy GR, Yoneda T, Guise TA, et al: Local factors in skeletal malignancy., in Bilezikian JP, Raisz LJ, Rodan GA. (eds): *Principles of Bone Biology*, second edition. San Diego, Academic Press, 2002, pp 1093-1104.
 226. Roodman GD. Genes associate with abnormal bone cell activity in bone metastasis. *Cancer Metastasis Rev.* 2012;31(3-4):569-578.
 227. Pearce RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF, Colman N, Michaeli J, Epstein J, Choi Y. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. *Proc. Natl. Acad. Sci USA* 2001;98:11581-11586.
 228. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N. Engl. J. Med.* 2003;349: 2483-2494.
 229. Oshima T, Abe M, Asano J, Hara T, Kitazoe K, Sekimoto E, Tanaka Y, Shibata H, Hashimoto T, Ozaki S, Kido S, Inoue D, Matsumoto T. Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. *Blood*, 2005;106(9):3160–3165.
 230. Terpos E, Christoulas D, Katodritou E, Bratengeier C, Gkotsamanidou M, Michalis E, Delimpasi S, Pouli A, Meletis J, Kastritis E, Zervas K, Dimopoulos MA. Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: reduction post-bortezomib monotherapy. *Int J Cancer.* 2012;131(6):1466-71.
 231. Adams JS: Hypercalcemia due to granuloma-forming disorders., in Favus MJ. (ed): *Primer on the metabolic bone diseases and disorders of mineral metabolism*, fourth edition. Philadelphia, Lippincott, Williams and Wilkins, 1999, pp 212-214.
 232. Studdy PR, Bird R, Neville E, James DG. Biochemical findings in sarcoidosis. *J. Clin. Pathol.* 1980;33:528-533.
 233. Bell NH, Gill JR Jr, Bartter FC: On the abnormal calcium absorption in sarcoidosis: evidence for increased sensitivity to vitamin D. *Am. J. Med.* 1964;36:500-513.
 234. Fallon MD, Perry HM III, Teitelbaum SL: Skeletal sarcoidosis with osteopenia. *Metab. Bone Dis. Res.* 1981; 3:171-174.
 235. Rizzato G, Montemurro L, Fraioli P: Bone mineral content in sarcoidosis. *Semin. Resp. Med.* 1992;13:411-423.
 236. Adams JS, Singer FR, Gacad MA, Sharma OP, Hayes MJ, Vouros P, Holick MF. Isolation and structural identification of 1,25-dihydroxyvitamin D3 produced by cultured alveolar

- macrophages in sarcoidosis. *J. Clin. Endocrinol. Metab.* 1985;60:960-966.
237. Sandler LM, Wineals CG, Fraher LJ, Clemens TL, Smith R, O'Riordan JL. Studies of the hypercalcemia of sarcoidosis: effects of steroids and exogenous vitamin D3 on the circulating concentration of 1,25-dihydroxyvitamin D3. *Q. J. Med.* 1984;53:165-180.
 238. Adams JS, Diz MM, Sharma OP: Effective reduction in the serum 1,25-dihydroxyvitamin D and calcium concentration in sarcoidosis-associated hypercalcemia with short-course chloroquine therapy. *Ann. Intern. Med.* 1989;111:437-438.
 239. Adams JS, Sharma OP, Diz MM, Endres DB. Ketoconazole decreases the serum 1,25-dihydroxyvitamin D and calcium concentration in sarcoidosis-associated hypercalcemia. *J. Clin. Endocrinol. Metab.* 1990;70:1090-1095.
 240. Zaloga GP, Chernow B, Eil C. Hypercalcemia and disseminated cyto-megalovirus infection in the acquired immunodeficiency syndrome. *Ann. Int. Med.* 1985;102:331-333.
 241. Gayet S, Ville E, Durand JM, Mars ME, Morange S, Kaplanski G, Gallais H, Soubeyrand J. Foscarnet-induced hypercalcemia in AIDS. *AIDS* 1997;11:1068-1070.
 242. Preus M. The Williams syndrome: objective definition and diagnosis. *Clin. Genet.* 1984;25:422-428.
 243. Taylor AB, Stern PH, Bell NH. Abnormal regulation of circulating 25OHD in the Williams syndrome. *N. Engl. J. Med.* 1982;306:972-975.
 244. Curran ME, Atkinson DL, Ewart AK, Morris CA, Leppert MF, Keating MT. The elastin gene is disrupted by a translocation associated with supraaortic stenosis. *Cell* 1993;73:159-168.
 245. Stokes VJ, Nielsen MF, Hannan FM, Thakker RV. Hypercalcemic Disorders in Children. *J Bone Miner Res.* 2017;32(11):2157-2170
 246. Martin NDT, Snodgrass GJAI, Cohen RD. Idiopathic infantile hypercalcemia: a continuing enigma. *Arch. Dis. Child.* 1984;59:605-613.
 247. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, Misselwitz J, Klaus G, Kuwertz-Bröking E, Fehrenbach H, Wingen AM, Güran T, Hoenderop JG, Bindels RJ, Prosser DE, Jones G, Konrad M. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N. Engl. J. Med.* 2011;365(5):410-421.
 248. Saponaro F. Rare Causes of Hypercalcemia. *Endocrinol Metab Clin North Am.* 2021;50(4):769-779
 249. Saarela T, Similä S, Koivisto M. Hypercalcemia and nephrocalcinosis in patients with congenital lactase deficiency. *J. Pediatr.* 1995;127:920-923.
 250. Belmont JW, Reid B, Taylor W, Baker SS, Moore WH, Morriss MC, Podrebarac SM, Glass N, Schwartz ID. Congenital sucrase-isomaltase deficiency presenting with failure to thrive, hypercalcemia, and nephrocalcinosis. *BMC Pediatr* 2002;2:4.
 251. Porter RH, Cox BG, Heaney D, Hostetter TH, Stinebaugh BJ, Wadi N, Suki WN. Treatment of hypoparathyroid patients with chlorthalidone. *N. Engl. J. Med.* 1978;298:577-581.
 252. Haden ST, Stoll AL, McCormick S, Scott J, Fuleihan G el-H. Alterations in parathyroid dynamics in lithium-treated subjects. *J. Clin. Endocrinol. Metab.* 1979;82:2844-2848.
 253. Pettifor JM, Bikle DD, Cavallero M, Zachen D, Kamdar MC, Ross FP. Serum levels of free 1,25-dihydroxyvitamin D in vitamin D toxicity. *Ann. Intern. Med.* 1995;122:511-513.
 254. Valente JD, Elias AN, Weinstein GD. Hypercalcemia associated with oral isotretinoin in the treatment of severe acne. *JAMA* 1983;290:1899-1900.
 255. Suzumiya J, Asahara F, Katakami H, Kimuran N, Hisano S, Okumura M, Ohno R. Hypercalcemia caused by all trans-retinoic acid treatment of acute promyelocytic leukaemia: case report. *Eur. J. Haematol.* 1994;53:126-127.
 256. Villablanca J, Khan AA, Avramis VI, Seeger RC, Matthay KK, Ramsay NK, Reynolds CP. Phase I trial of 13-cis-retinoic acid in children with neuroblastoma following bone marrow transplantation. *J. Clin. Oncol.* 1995;13:894-901.
 257. Nikolic-Temasevic Z, Jelic S, Popov I, Radosavljevic D, Mitrovic L. Tumor "flare" hypercalcemia – an additional indication for bisphosphonates? *Oncology* 2001;60:123–126.
 258. Arumugam GP, Sundravel S, Shanthi P, Sachdanandam P. Tamoxifen flare hypercalcemia: an additional support for gallium nitrate usage. *J Bone Miner Metab.* 2006;24(3):243-7
 259. Legha SS, Powell K, Buzdar AU, Blumenschein GR. Tamoxifen-induced hypercalcemia in breast cancer. *Cancer* 1981;47:2803-2806.
 260. McPherson ML, Prince SR, Atamer ER, Maxwell DB, Ross-Clunis H, Estep HL. Theophylline-induced hypercalcemia. *Ann Intern Med.* 1986;105(1):52-54.
 261. Ott SM, Maloney NA, Klein GL, Alfrey AC, Ament ME, Coburn JW, Sherrard DJ. Aluminum is associated with low bone formation in patients receiving chronic parenteral nutrition. *Ann. Intern. Med.* 1983;96:910-914.
 262. Beall DP, Scofield RH: Milk-alkali syndrome associated with calcium carbonate consumption. *Medicine* 1995;74: 89-96.

-
263. Tsai WC, Wang WJ, Chen WL, Tsao YT, Tsao YT. Surviving a crisis of immobilization hypercalcemia. *J. Am. Geriatr. Soc.* 2012;60(9):1778-1780.
264. Stewart AF, Adler M, Byers CM, Segre GV, Broadus AE. Calcium homeostasis in immobilization: An example of resorptive hypercalciuria. *N. Engl. J. Med.* 1982;306:1136-1140.
265. Nussbaum Sr, Zahradnik RK, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY, Keutmann HT, Wang CA, Potts JT Jr, Segre GV. Highly sensitive two-site immunoradiometric: assay of parathyrin and its clinical utility in evaluating patients with hypercalcemia. *Clin. Chem.* 1987;33:1364-1367.
266. Wei JP, Burke GJ: Cost utility of routine imaging with Tc-99m-sestamibi in primary hyperthyroidism before initial surgery. *Amer. Surg.* 1997;63(12):1097-1100.
267. Hosking DJ, Cowley A, Bucknall CA. Rehydration in the treatment of severe hypercalcemia. *Q.J. Med.* 1981;200:473-481.
268. El-Hajj Fuleihan G, Clines GA, Hu MI, Marcocci C, Murad MH, Piggott T, Van Poznak C, Wu JY, Drake MT. Treatment of Hypercalcemia of Malignancy in Adults: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2023;108(3):507-528.
269. Body JJ, Lortholary A, Romieu G, Vigneron AM, Ford J. A dose-finding study of zoledronate in hypercalcemic cancer patients. *J. Bone Miner. Res.* 1999;14:1557-1561.
270. Nussbaum SR, Younger J, VandePol CJ, Gagel RF, Zubler MA, Chapman R, Henderson IC, Mallette LE. Single dose intravenous therapy with pamidronate for the treatment of hypercalcemia of malignancy: Comparison of 30-60-, and 90 mg dosages. *Am. J. Med.* 1993;95:297-304.
271. Silva O, Becker KL. Salmon calcitonin in the treatment of hypercalcemia. *Arch. Intern. Med.* 1973;132:337-339.
272. Ralston SH, Alzaid AA, Gardner MD, Boyle IT. Treatment of cancer associated hypercalcemia with combined aminohydroxypropylidene diphosphonate and calcitonin. *Br. Med. J.* 1986;292:1549-1550.
273. Gurney H, Grill V, Martin TJ. Parathyroid hormone-related protein and response to pamidronate in tumour-induced hypercalcemia. *Lancet* 341:1611-1613.
274. Percival RC, Yates AJP, Gray RES, Neal FE, Forrest AR, Kanis JA. The role of glucocorticoids in the management of malignant hypercalcemia. *Br. Med. J.* 1984;289:287.
275. Bilezikian JP, Khan AA, Clarke BL, Mannstadt M, Potts JT, Brandi ML. The Fifth International Workshop on the Evaluation and Management of Primary Hyperparathyroidism. *J Bone Miner Res.* 2022;37(11):2290-2292
276. Heyburn PJ, Selby PL, Peacock M, Sandler LR, Parsons FM. Peritoneal dialysis in the management of severe hypercalcemia. *Br. Med. J.* 1980;280:525-526.
277. Cardella CJ, Birkin BL, Rapoport A. Role of dialysis in the treatment of severe hypercalcemia: Report of two cases successfully treated with hemodialysis and review of the literature. *Clin. Nephrol.* 1979;12:285-290.
278. Bergstrom WH. Hypercalciuria and hypercalcemia complicating immobilization. *Am. J. Dis. Child.* 1978;132:553-554.
279. McIntyre HD, Cameron DP, Urquhart SM, Davies WE. Immobilization hypercalcemia responding to intravenous pamidronate sodium therapy. *Postgrad. Med. J.* 1989;65:244-246.