Chapter 4. APPROACH TO HYPERCALCEMIA

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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 D3, to the active moiety 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] which then will enhance intestinal calcium absorption. These mechanisms restore the serum calcium to normal and inhibit further production of PTH and 1,25(OH)2D3. Normal serum concentrations of total calcium generally range between 8.5 and 10.6 mg/dL (2.12 to 2.65 mM) and ionized calcium between 4.65-5.30 mg/dL (1.16-1.31 mM). Decreased PTH and decreased 1,25(OH)2D3 should accompany hypercalcemia unless PTH or 1,25(OH)2D is causal. Hypercalcemia may be caused by: Endocrine Disorders with Excess PTH including primary sporadic and familial hyperparathyroidism, 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(OH)2D3 in lymphomas); Inflammatory Disorders including Granulomatous Diseases, where excess 1,25(OH)2D3 production may be causal, and HIV/AIDS; Pediatric Syndromes including Williams Syndrome and Idiopathic Infantile Hypercalcemia, where inappropriate levels of 1,25(OH)2D3 may occur due to a mutation in the 24-hydroxylase 25-hydroxyvitamin D gene; Medication, including thiazide diuretics, lithium, vitamin D, vitamin A, estrogens and antiestrogens, and theophylline; and prolonged immobilization, particularly in states of high bone turnover. Treatment should be aimed the underlying disorder, however, if serum calcium exceeds 12 to 14mg/dL (3 to 3.5mM), acute hydration and agents that inhibit bone resorption are required. Under selected conditions, calcimimetics, calciuresis, glucocorticoids, or dialysis may be needed.

DEFINITION

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 10.6 mg/dL (2.12 to 2.65 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 4.65-5.30 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. 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. 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 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. Ordinarily, as a result of normal bone turnover, approximately 500 mg of calcium is released from bone per day and the equivalent amount is accreted per day (Fig. 1).

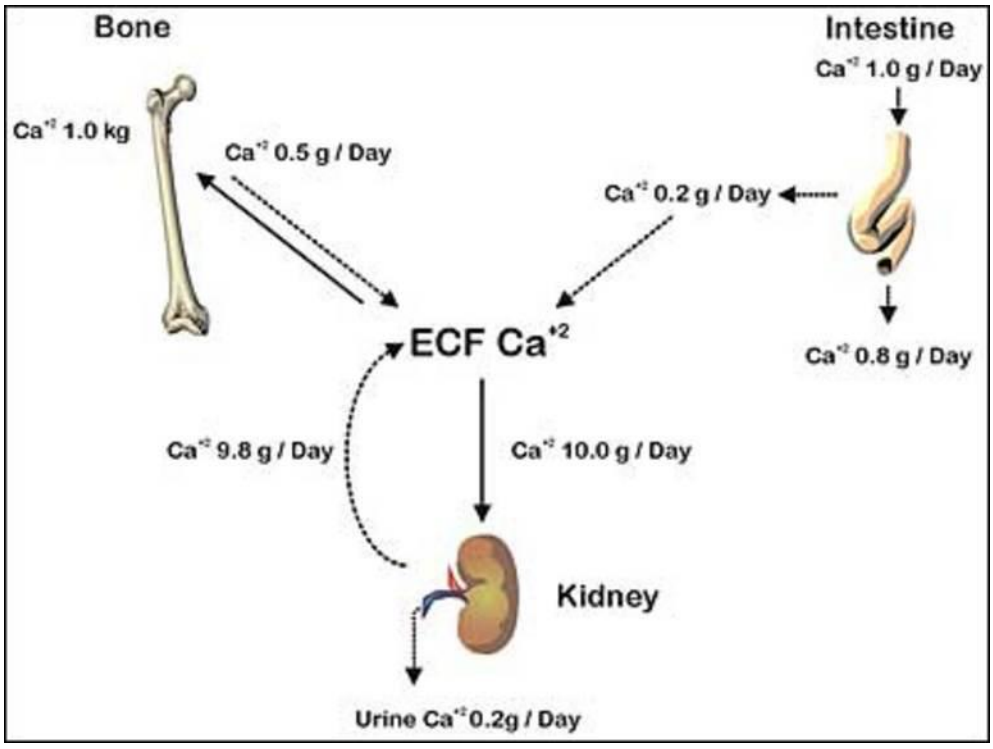

Figure 1. Calcium balance. On average, if, in a typical adult approximately 1g of elemental calcium (Ca+2) is ingested per day, about 200mg/day will be absorbed and 800mg/day excreted. Approximately 1kg of Ca+2 is stored in bone and about 500mg/day is released by resorption or deposited during bone formation. 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. Thus a reduction in ECF calcium can stimulate release of parathyroid hormone (PTH) from the parathyroid glands in the neck. This hormone can then act to enhance calcium reabsorption in the kidney. while at the same time inhibit phosphate reabsorption producing phosphaturia .PTH and hypocalcemia per se can both stimulate the conversion of the inert metabolite of vitamin D, 25-hydroxyvitamin D3 [25(OH)D3], to the active moiety 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] (6) which in turn will enhance intestinal calcium, and to a lesser extent phosphate reabsorption. 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)2D3. The opposite sequence of events i.e. diminished PTH and 1,25(OH)2D3 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

Parathyroid Hormone

Regulation of 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) can mimic many of its actions. The major regulator of PTH secretion from the parathyroid glands is the ECF calcium. 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 (7). 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 (8).

The parathyroid glands detect ECF calcium via a calcium-sensing receptor (CaSR) (9). This receptor 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, 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 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 (TGFa) (18) have been implicated in the regulation of PTH secretion (5). Recently it has been reported that the phosphaturic factor, FGF23, suppresses PTH gene expression and secretion (19). One of the most physiologically relevant regulators is 1,25(OH)2D3 which appears capable of tonically reducing PTH release (20), decreasing PTH gene expression (15) and inhibiting 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 (PTHR) 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-transporter leading to decreased phosphate reabsorption and phosphaturia (29).

In this nephron region, PTH can, after binding to the PTHR, also stimulate CYP27B1, the 25(OH)D3-1a hydroxylase [1a(OH)ase], leading to increased synthesis of 1,25(OH)2D3 (30). A reduction in ECF calcium can itself stimulate 1,25(OH)2D3 production but the precise mechanism of this action is presently unknown. Finally PTH can also inhibit Na+ and HC03- reabsorption in the proximal tubule by inhibiting the apical type 3 Na+/H+ exchanger (31), and the basolateral Na+/K+-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 the PTHR (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 Na/K/2Cl 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 A2, reducing the activity of the Na/K/2Cl 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. ECF calcium may therefore act in a manner analogous to "loop" diuretics such as furosemide.

In the DCT, PTH can also influence transcellular calcium transport (36). This is a multistep process involving transfer of luminal Ca2+ into the renal tubule cell via the transient receptor potential channel (TRPV5), translocation of Ca2+ across the cell from apical to basolateral surface a process involving proteins such as calbindin-D28K, and finally active extrusion of Ca2+ from the cell into the blood via a Na+/Ca2+ exchanger, designated NCX1. PTH markedly stimulates Ca2+ reabsorption in the DCT primarily by augmenting NCX1 activity via a cyclic AMP-mediated mechanism.

Skeletal Actions

In bone, the PTHR is localized on cells of the osteoblast phenotype 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 PTHR 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 on trabecular bone formation in both the fetus and neonate (42,43) PTH has been reported to increase growth factor production, notably insulin-like growth factor-1 (IGF-1) production, which may contribute to this (44). 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. PTH has been suggested to elicit increases in production and activity of cells of the osteoblast pathway and to decrease osteoblast apoptosis (45). 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 (46).

Vitamin D

Regulation of Production

Vitamin D3 is a biologically inert secosteroid that is made in the skin (47). 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) 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 D3 can be converted to 25(OH)D3 by a cytochrome P450-vitamin D 25-hydroxylase which is poorly regulated and converts vitamin D3 to 25(OH)D3 almost constitutively (48).

Consequently serum 25(OH)D3 is the most abundant circulating metabolite of vitamin D3, 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)D3 is also biologically inert and is transported, bound to DBP, to the kidney where it is converted by the cytochrome P450- monooxygenase,

25(OH)D3-1a hydroxylase (CYP27B1) to the active moiety, 1,25(OH)2D3 (49). Although the kidney is the major source of circulating hormonal 1,25(OH)2D3, a variety of extra-renal cells have been reported to synthesize 1,25(OH)2D3, notably skin cells, monocytes/macrophages, and the placenta during pregnancy (50). The 1,25(OH)2D3 produced by many of these non-renal tissues may act in a paracrine/autocrine fashion to regulate cell growth and differentiation. The renal production of 1,25(OH)2D3 is stimulated by hypocalcemia, hypophosphatemia and elevated PTH levels. The renal 1a(OH)ase is potently inhibited by the phosphaturic hormone, fibroblast growth factor (FGF) 23 and also by 1,25(OH)2D3 per se in a negative feedback loop. As well, FGF23 and 1,25(OH)2D3 can both stimulate a 24-hydroxylase enzyme (CYP24A1). This cytochrome P450 monooxygenase produces 24,25(OH)2D3 and 1,24,25(OH)3D3 from 25(OH)D and 1,25(OH)2D3 respectively (51). These metabolites are 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 Ac**tions**

The unbound active form of vitamin D, 1,25(OH)2D3 can enter target cells and interact with the ligand-binding domain of a specific nuclear receptor (VDR) (52). The 1,25(OH)2D3-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)2D3 have been reported their physiologic significance is currently unclear.

A major biologic function of circulating 1,25(OH)2D3 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 accounts for absorption of 10-15% of a dietary load (53). 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 Ca2+ channel TRPV6, or via other calcium channels, intracellular diffusion, mediated by the Ca2+-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 (54). 1,25(OH)2D3, by interacting with the VDR (55) mainly 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 (3654). 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 $1,25(OH)_2D_3$ of active transport of calcium by distal as well as proximal segments of the intestine and, as well, of paracellular calcium transport (56). Reductions in dietary intake of calcium can lead to increased PTH secretion and increased 1,25(OH)2D3 production which can enhance

fractional calcium absorption and compensate for the dietary reduction. Although 1,25(OH)2D3 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)2D3.

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)2D3 on bone appears to be to enhance mobilization of calcium stores when dietary calcium is insufficient to maintain a normal ECF calcium (57). As with PTH, 1,25(OH)2D3 enhances osteoclastic bone resorption by binding to receptors in cells of the osteoblast lineage and stimulating the RANK/RANK 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 (58). Endogenous 1,25(OH)2D3 has also been reported to have an anabolic role in vivo (54,59).

Although effects of 1,25(OH)2D3 on both calcium and phosphorus handling in the kidney have been reported, it remains controversial whether 1,25(OH)2D3 plays a major role in altering renal tubular reabsorption or excretion of these ions in humans.

Parathyroid Hormone Relation Peptide (PTHrP)

PTHrP was discovered as the mediator of the syndrome of "humoral hypercalcemia of malignancy" (HHM) (60). 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 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 (61,62). 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. Several common structural features of these genes however 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 the peptides (63), such that synthetic PTH(1-34) and synthetic PTHrP (1-34) interact with a common receptor (PTHR) (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 (64) and can interact at a second PTH receptor termed the type II receptor (65) to which PTHrP does not bind

(Fig. 2). The physiologic role of TIP 39 and of the type II receptor remain to be elucidated.

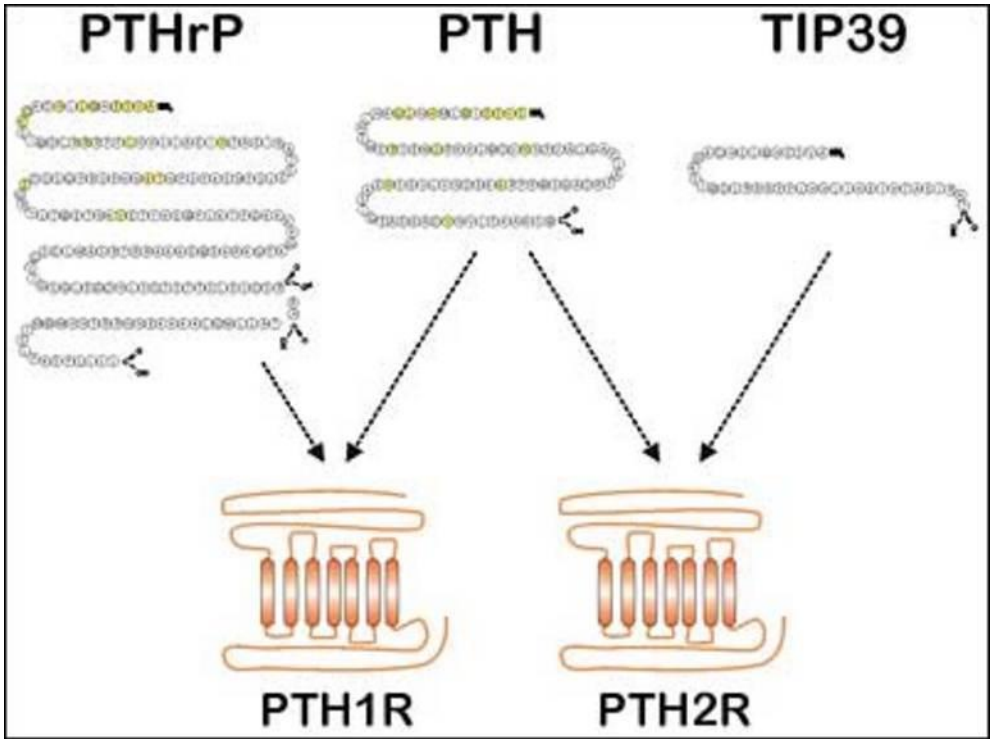

Figure 2. PTH and PTHR gene families: PTHrP, PTH and TIP39 appear to be members of a single gene family. Although only nine amino acids in the NH2-terminal domains of these three peptides are conserved these are functionally important residues. The receptors for these peptides, PTH1R and PTH2R, are both 7 transmembrane-spanning G protein-coupled receptors which seem to be members of a single gene family. PTHrP binds and activates PTH1R; it binds weakly to PTH2R and does not activate it. PTH can bind and activate both PTH1R and PTH2R. TIP39 can bind to and activate PTH2R but not PTH1R.

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 (66). 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 (67,68).

Key stimulators of gene transcription are a variety of growth factors and cytokines (69) including epidermal growth factor (EGF) (70), IGF-1 (71), transforming growth factor b (TGFb) (72). 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 (73). Hypercalcemia associated with these tumors was also diminished.

Several steroidal hormones including 1,25(OH)2D3 (74), glucocorticoids (75) and androgens (76) have been reported to be potent inhibitors of PTHrP gene expression. This prompted the use of 1,25(OH)2D3 (77) and of low calcemic analogues of vitamin

D (78) 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 (79). This processing locus was attacked using a furin antisense approach to block prohormone convertase activity in an animal tumor model which overproduces PTHrP (80). Bioactive PTHrP production was diminished with this intervention, and, in vivo, hypercalcemia associated with the control tumor was not observed.

PTHrP is considerably longer than PTH with three isoforms of 139, 141 and 173 amino acids whose sequences are identical through residue 139. Serine proteases may also act internally in various cell types to cleave an NH2 terminal fragment, a midregion fragment (81) and carboxyl terminal fragments (82) 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 PTHR linked to adenylate cyclase or phospholyase C. In some developing tissues, e.g. teeth, PTHrP is expressed in epithelial cells whereas the PTHR is in adjacent mesenchymal cells facilitating epithelial-mesenchymal interactions (83).

A mid-region domain of PTHrP (37-86) has been implicated in placental calcium transport (81) and a COOH terminal region (107-139) has been reported to inhibit osteoclasts (82). 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 (84). 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 (85). 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 (86). Reverse transport of PTHrP from the endoplasmic reticulum to the cytoplasm has been reported in other studies (87). 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 (88). In vitro studies have suggested that nuclear localization of PTHrP may be involved in its proliferative activity and/or in inhibition of apoptosis (84), 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 the type 1 PTH/PTHrP receptor. 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 (89,90).

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 ie endocrine mechanisms.

With respect to ion homeostasis PTHrP can modulate placental calcium transport and appears necessary for normal fetal calcium homeostasis (91). 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 (92) (leading to dilatation), uterus (93) and bladder (94). The physiologic significance of these effects however remain 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 (95), skin (96), nervous tissue (97) and pancreatic islets (98) 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 (99,100), 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 PTHR itself (101) 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 PTHR produces a similar lethal chondro-osseous dysplasia termed Blomstrand's Syndrome (102,103). 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 (104). Furthermore hypoparathyroid mice that have PTHrP haploinsufficiency do not develop the increased trabecular bone mass that is a characteristic of hypoparathyroidism (105). 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 (106). In humans, variants of the gene PTHLP that encodes PTHrP 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 the PTH1R 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 (107). This is characterized by sequences of activation of osteoclasts followed by osteoclastic bone resorption followed by osteoblastic bone formation. These sequential cellular activities occur 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. TGFb, IGF-1 and fibroblast growth factor (FGF) which can induce osteoclast apoptosis and also induce osteoblast chemotaxis proliferation and differentiation at the site of repair. In addition, direct activation of cells of the osteoblast phenotype by osteoclast family members appears to occur, although the molecular signals regulating this direct interaction remain elusive. A number of 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 Dickoff (Dkk) which inhibit osteoblast production (108), 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)2D3 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 (109). Vitamin A has a direct stimulatory effect on osteoclasts and can induce bone resorption as well (110).

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. TNFa is released by monocytic cells, TNFb (lymphotoxin) by activated T lymphocytes, and interleukin-6 (IL-6) by osteoclastic cells (111). 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 (112). 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.

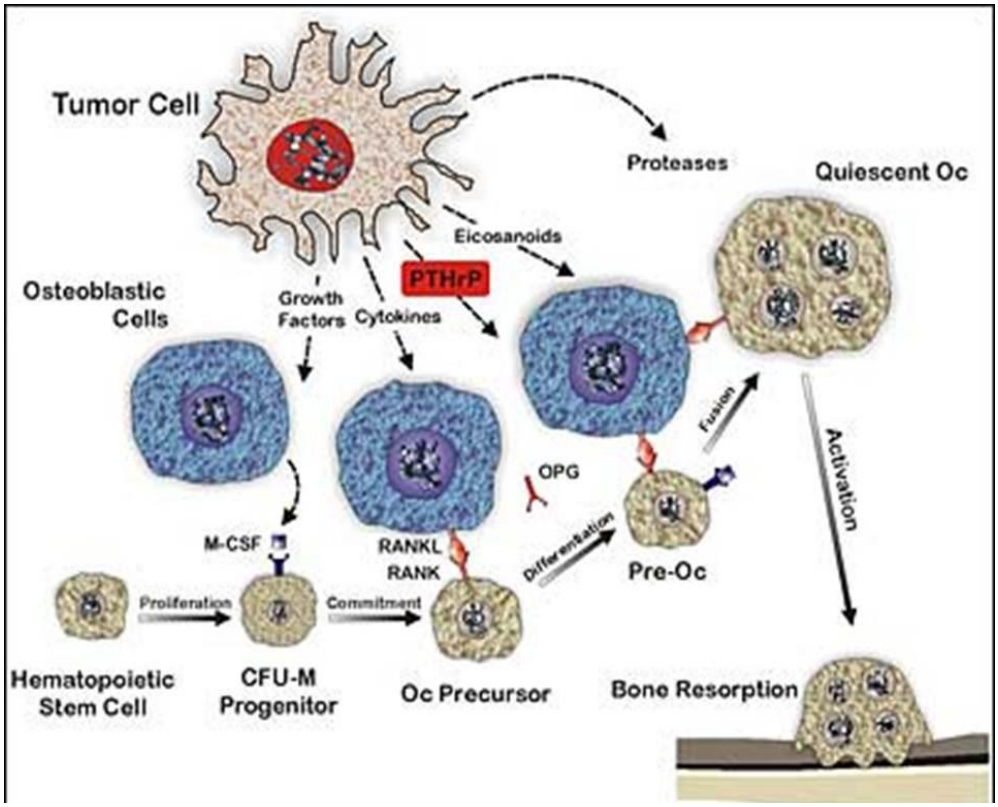

Figure 3. Production of bone resorbing substances by neoplasms. Tumor cells may release proteases which can facilitate tumor cell progression through unmineralized matrix. Tumors cells can also release PTHrP, cytokines, eicosanoids and growth factors (eg EGF) which can act on cells of the osteoblastic 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).

Table 1. Hypercalcemic Disorders

A. Endocrine Disorders Associated with Hypercalcemia **1. Endocrine Disorders with Excess PTH Production** **2. Endocrine Disorders without Excess PTH Production**
B. Malignancy-Associated Hypercalcemia (MAH)

1. MAH with Elevated PTHrP **2. MAH with Elevation of Other Systemic Factors**
C. Inflammatory Disorders Causing Hypercalcemia **1. Granulomatous Disorders** **2. AIDS**
D. Pediatric Syndromes **1.Williams Syndrome** **2. Idiopathic Infantile Hypercalcemia**
E. Medication-Induced **1. Thiazides** **2. Lithium** **3. Vitamin D** **4. Vitamin A** **5. Estrogens and Antiestrogens** **6.Theophylline** **7. Aluminium Intoxication** **8. Milk-Alkali Syndrome**
F. Immobilization

Endocrine Disorders Associated with Hypercalcemia

Endocrine Disorders with Excess PTH Production

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

Sporadic Primary Hyperparathyroidism

Sporadic primary hyperparathyroidism (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. 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 only genes definitively implicated in sporadic benign PHPT are an oncogene that encodes a key regulator of the cell cycle and *MEN1*, a tumor suppressor gene, also implicated in familial multiple endocrine neoplasia type I (113). *HRPT2*, a tumor suppressor gene associated with the Hyperparathyroidism-Jaw Tumor syndrome (114), has also been implicated in most sporadic parathyroid carcinomas (115). 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)2D3, Ca^{+2} and phosphate. Rarely, sporadic hyperparathyroidism with hypocalciuria may

occur, caused by antibodies to the calcium-sensing receptor. This syndrome has been termed Autoimmune Hypocalciuric Hypercalcemia (116).

The clinical manifestations of these disorders are caused by the overproduction of PTH and its effect on bone resorption and formation, on its capacity to stimulate renal 1,25(OH)2D3 production and on the resultant effect on ECF calcium which can modify the filtered renal load of calcium (Fig. 4). About 80% of cases of the most common form of PHPT i.e. benign sporadic PHPT present as mild or “asymptomatic” hyperparathyroidism in which hypercalemia is generally less than 1mg/dL (0.25 mM) above the upper limit of normal and may be normal intermittently (117) 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, these manifestations are rarely seen in Western nations (2% of cases) but were common manifestations in the past and appear to be common in the East (118-121). 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 (122), remains to be determined. The more common skeletal manifestation of excess circulating PTH in the West now appears to be resorption of cortical bone, reflecting the "catabolic bone activity" of PTH, with relative preservation of trabecular bone, reflecting its "anabolic activity" (123). Consequently the severity of bone disease in the West 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 occurs in 15-20% (124). 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 the East, 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 in the West of benign sporadic PHPT in women and in an older age group (125) also appears to distinguish the presentation of this disorder in the West relative to the East.

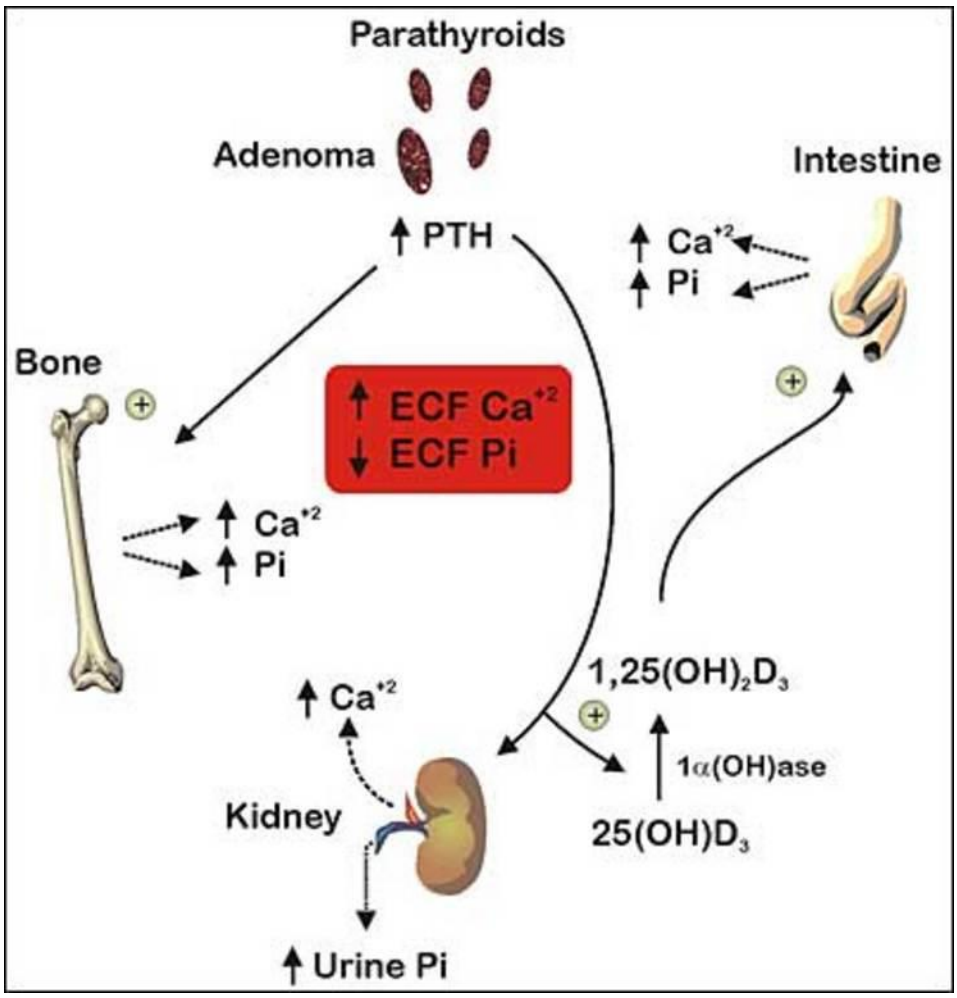

Figure 4. Disordered mineral homeostasis in hyperparathyroidism. In primary sporadic hyperparathyroidism PTH is generally overproduced by a single parathyroid adenoma. Increased PTH secretion leads to a net increase in skeletal resorption with release of Ca+2 and Pi (inorganic phosphate) from bone. PTH also increases renal 1□(OH)ase activity leading to increased production of 1,25(OH)2D3 from 25(OH)D3 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 peptic ulcer 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 popula-

tion at large and difficult to ascribe to hyperparathyroidism. Rarely, primary sporadic PHPT may present with severe acute hypercalcemia (parathyroid crisis) (126).

Although estrogen therapy has been advocated for the treatment of PHPT in postmenopausal women (127) potential adverse effects of estrogen therapy, including breast cancer and cardiovascular complications, make this option unattractive. Although selective estrogen receptor modulators may be an alternative, few long term studies have been done to assess this. Bisphosphonates (128) may increase bone mineral density (BMD) at the lumbar spine and hip regions but generally do not substantially reduce the hypercalcemia. Calcimimetic agents (those that mimic or potentiate the action of calcium at the CaSR) (129) can effectively reduce hypercalcemia and have been approved for use in parathyroid carcinomas but generally do not significantly improve the skeletal abnormalities. Calcimimetic agents may also be useful for the treatment of severe hypercalcemia in patients with PHPT who are unable to undergo parathyroidectomy.

Surgical removal of the parathyroid adenoma currently remains the treatment of choice if the ECF calcium is 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], if creatinine clearance (calculated) is reduced to <60 ml/min or if the patient is less than age 50. Surgery is also indicated in patients for whom medical surveillance is either not desired nor not possible (130). In addition, although hypercalciuria is only one of several risk factors affecting the development of kidney stones, some physicians still regard 24-hour urinary calcium excretion of greater than 400 mg as an indication for surgery.

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. sestamibi scanning) and ultrasound. Computed tomography, 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 or total parathyroidectomy with or without a parathyroid autograft is indicated. Severe, chronic hypercalcemia is more commonly associated with parathyroid carcinoma. Complete resection of the primary lesion is urgent in this case.

Familial Primary Hyperparathyroidism

Multiple Endocrine Neoplasia, Type I (MENI)

MENI is a familial disorder with an autosomal dominant pattern of inheritance which is characterized by tumors in pituitary, parathyroid and enteropancreatic endocrine cells (although tumors in several other endocrine and non-endocrine tissues may also be associated with the syndrome) (131) (Fig. 5). Patients exhibit loss-of-function germline mutations in the tumor suppressor gene, MENI, which encodes the nuclear protein, menin (132).

Tumors in at least 2 of these sites in a proband 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) (133). In contrast to sporadic PHPT however MENI 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 MENI. Enteropancreatic tumors are usually multiple and gastrinomas are the most common. These 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, glucogonomas, 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.

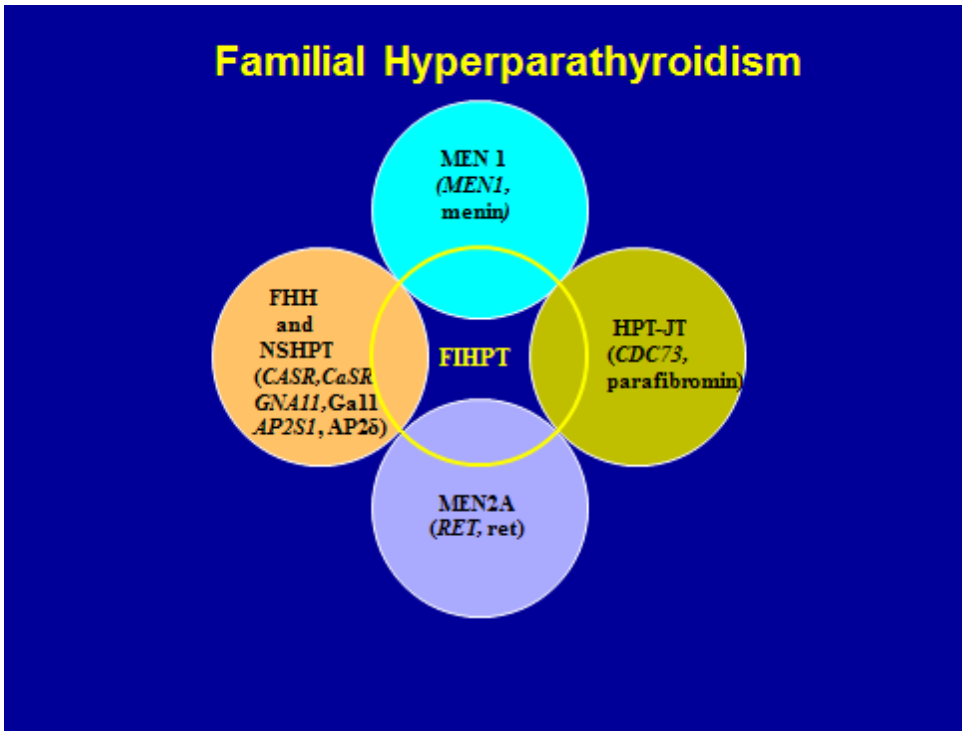

Figure 5. Familial hyperparathyroidism. Familial hyperparathyroidism (FHPT) can occur in the MENI Syndrome, in which MEN1 is mutated; in the MEN2A Syndrome, in which RET is mutated; in FHH and NSHPT in which CaSR, GNA11 or AP2S1 is mutated; and in the Hyperparathyroidism-Jaw Tumor (HPT-JT) Syndrome in 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.

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

parathyroidectomy is recommended. The latter may be accompanied by autotransplantation of resected parathyroid gland fragments.

Multiple Endocrine Neoplasia Type IIA (MENIIA)

MENIIA is an autosomal dominant familial syndrome characterized by medullary thyroid carcinoma (MTC) bilateral pheochromocytomas and hyperparathyroidism (134,135). This syndrome results from activating germline mutations in the rearranged during transfection (RET) protooncogene which is a receptor tyrosine kinase (136). Two variants of this disorder are MENIIB which includes mucosal and intestinal neuromas and a Marfanoid habitus but no hyperparathyroidism (137), and familial medullary thyroid carcinoma. Two other variants of MENII include MENII with cutaneous lichen amyloidosis, and MENII with Hirschsprung's disease. Analyses of RET mutations in these syndromes have provided good genotype-phenotype correlations (138).

The dominant feature of the MENIIA syndrome is MTC, a calcitonin-secreting neoplasm of thyroid C cells. 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 have low malignant potential.

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

Hyperparathyroidism - Jaw Tumor Syndrome

Hyperparathyroidism - Jaw Tumor Syndrome (HPT-JT) is an autosomal-dominant syndrome with incomplete penetrance and variable expression, caused by inactivating germline mutation of 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" (139,140). 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 (114), in sporadic parathyroid cancer (115), and in a minority of families with isolated hyperparathyroidism (141). Genetic testing in relatives can result in identification of individuals at risk for parathyroid carcinoma, enabling preventative or curative parathyroidectomy.

Familial Hypocalciuric hypercalcemia (FHH) and Neonatal Severe Primary hyperparathyroidism (NSHPT)

Familial hypocalciuric hypercalcemia (FHH) (142), also called Familial Benign Hypercalcemia (FBH) (143) is an autosomal dominant trait characterized by moderate hypercalcemia and relative hypocalciuria i.e. urine calcium that is low in relationship to the prevailing hypercalcemia. The molecular basis, in most cases, is a loss-of-function mutation in the calcium-sensing receptor (CaSR) gene (144). The protein product, CaSR, is a G-protein coupled receptor that predominantly signals via the G-protein subunit alpha-11 (Ga11) to regulate calcium homeostasis. As a consequence, in

heterozygotes, diminished ability of the CaSR in the CTAL of the kidney to detect ECF calcium occurs leading to enhanced renal tubular reabsorption of calcium and magnesium, to hypercalcemia and often hypermagnesemia. Loss-of-function mutations in GNA11, the gene encoding Ga11, have also been reported to cause the syndrome, as have loss-of-function mutations of the gene encoding adaptor protein-2 δ-subunit (AP2 δ), which plays a pivotal role in clathrin-mediated endocytosis of CaSR. The calcium to creatinine clearance ratio is usually low i.e. below 0.01 in patients with FHH but above this level in sporadic PHPT. This increased ECF calcium is also inadequately sensed by altered CaSR function in the parathyroid gland so that mild hyperplasia may occur and "normal" levels or elevated levels of PTH are secreted despite the hypercalcemia. Patients are generally asymptomatic. Testing serum and urine calcium in three relatives may be of value in establishing the diagnosis in a proband, however genetic testing may be of 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 renal lesion 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 NSHPT (145). 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 including demineralization, widening of the metaphyses, osteitis fibrosa and fractures. Inasmuch as the course of NSHPT can be self-limited, aggressive medical management is first indicated in all case of NSHPT, but prompt surgical intervention including total parathyroidectomy with immediate or delayed parathyroid autotransplantation should be performed in patients who deteriorate.

Finally a number of cases of FIH have been reported in which familial PHPT occurs in the absence of any other manifestation of the familial disorders described. Undoubtedly additional research will uncover new genetic mutations which contribute to the pathogenesis of these cases.

Tertiary Hyperparathyroidism

Tertiary hyperparathyroidism appears to represent the autonomous function of parathyroid tissue that develops in the face-of-long-standing secondary hyperparathyroidism (146). This 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(OH)2D3 deficiency, hyperphosphatemia and hypocalcemia produce chronic stimulation of the parathyroid glands. However, hypercalcemic hyperparathyroidism 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. In symptomatic patients,

surgical treatment, i.e. either sub-total removal of the parathyroid mass or total parathyroidectomy with autografting of parathyroid tissue is indicated.

Persistent Hyperparathryoidism after Renal Transplantation

After renal transplantation for end-stage kidney disease, pre-existing parathyroid gland hyperplasia associated with pre-transplant chronic kidney disease may persist over a period of months to years. This persistent hyperparathyroidism, in the presence of restored renal calcitriol production and normalized phosphate balance may lead to transient or prolonged hypercalcemia.

Endocrine Disorders Without Excess PTH Production

Hyperthyroidism

Hypercalcemia has been reported in as many as 50% of patients with thyrotoxicosis (147). Due to direct effects of increased triiodothyronine on bone, bone turnover and resorption are increased (148,149). The liberated calcium appears to suppress PTH release so that 1,25(OH)2D3 levels are reduced and renal calcium reabsorption is diminished. Therapy of the hyperthyroidism reverses the hypercalcemia (150,151).

Pheochromocytoma

Hypercalcemia has been reported with pheochromocytomas and may be due to excess PTHrP production (152).

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 (153).

Hypoadrenalism

Although both primary and secondary hypoadrenalism have been associated with hypercalcemia (154,155), the underlying etiology is unclear. Ionized calcium appears to be elevated and PTH and 1,25(OH)2D3 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 chondro-dysplasia, hypercalcemia and hypophosphatemia which is lifelong (156). 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 PTHR 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 Consideration

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 (157). An association between neoplasia and hypercalcemia was later reported in the English medical literature (158). 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 (159). 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 lead to the terms ectopic hyperparathyroidism (160) or pseudohyperparathyroidism. Nevertheless immunoreactive PTH could not be detected in the circulation of these patients (161) and PTH mRNA could not be detected in their tumors (162). To circumvent these issues, bioassays sensitive to PTH were employed to identify PTH-like bioactivity in blood and tumor extracts (163,164) and eventually to identify a novel protein (165), PTHrP. Despite the limited homology of PTH and PTHrP within the NH2-terminal 13 amino acids, PTH(1-34) and PTHrP(1-34) exhibited 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 subsequently shown to be cross-reactivity at the PTHR. In animals models of MAH associated with high PTHrP secretion, passive immunization with PTHrP antiserum reduced hypercalcemia (166,167). 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 (167). 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)2D3 levels (168) and effects on bone characterized, for both PTHrP and PTH, by enhanced bone turnover and increased bone formation as well as resorption (169).

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 (170-174). 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.

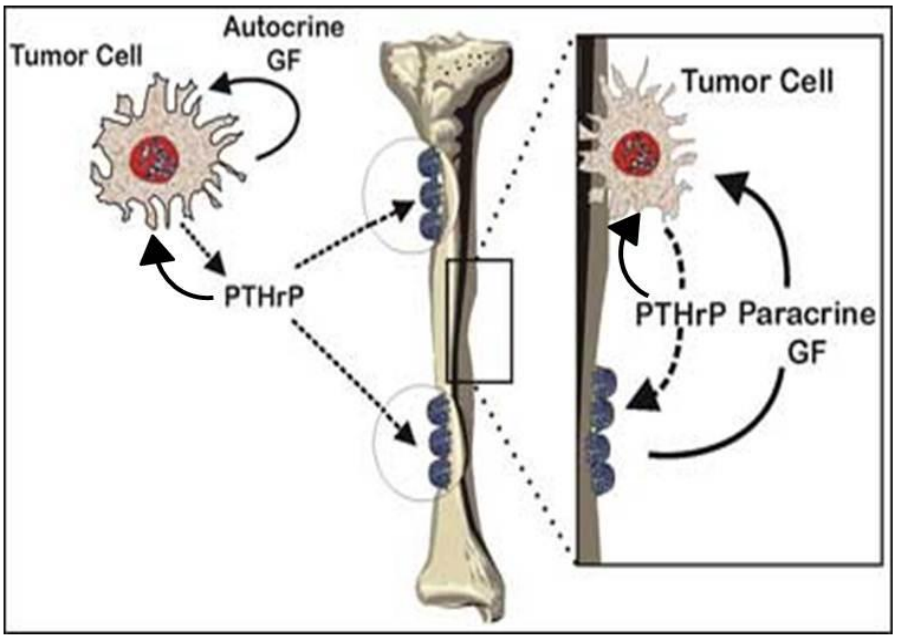

Figure 6. Growth factor-regulated PTHrP production in tumor states. Tumor cells at a distance from bone may be stimulated by autocrine growth factors (GF) to increase production of PTHrP which can then travel to bone via the circulation and enhance bone resorption. Tumor cells metastatic to bone (inset) 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 (175) and gene amplification (176) have been involved 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.

Patients who manifest hypercalcemia usually present with advanced disease. These tumors are generally obvious clinically, and carry a poor prognosis. Elevated PTHrP per se may be an independent prognostic factor signaling a poor prognosis (177).This appears to be because in addition to its role in hypercalcemia, (178) PTHrP produced by tumor cells acts in an intracrine manner, increasing cell survival, apoptosis resistance and anoikis evasion, and in autocrine manner via the PTH1R 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. 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.

Hypercalcemia in association with malignancy is generally more acute and severe than in association with primary hyperparathyroidism. 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)2D3 concentrations which are generally high or high normal in hyperparathyroidism are frequently low or low normal in HHM (179). This may reflect the higher levels of ambient calcium observed in HHM which may directly inhibit the renal 1a(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 (180). The reasons for this uncoupling is 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 (170,172,174). 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 (172,173) 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 (181,182). 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 (178). 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 TGFb (183), 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 (184). 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 (185,186) 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 lymphotrophic virus type I (HTLV-I). This tumor can be associated with hypercalcemia and increased PTHrP production (187). 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 (186). 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 (172,188). 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 (189). Nevertheless further work is necessary to understand the identity of PTHrP fragments which circulate 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)2D3

Although concentrations of 1,25(OH)2D3 are generally normal or low in most patients with MAH, elevated serum concentrations have been reported in some cases of non-Hodgkin's and Hodgkin's lymphoma in association with hypercalcemia (190-192). In view of the fact that extra-renal production of 1,25(OH)2D3 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 1a(OH)ase enzyme in granulomatous tissue. Although it is likely that elevated 1,25(OH)2D3 contributed to the hypercalcemia, co-production of PTHrP has also been reported in some cases (185). Consequently production of

1,25(OH)2D3, 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 (193). 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 (194,195). These tumors include ovarian, lung, thyroid, thymus and gastric malignancies (196). 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 (197). 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-1a 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 (198). In order to grow in bone, myeloma cells must secrete bone-resorbing factors. A number of such factors have been implicated including MIP-1a, IL-1, IL-6, TNF-b (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 (199). 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 (200). Other Wnt signaling antagonists, such as soluble-frizzled-related protein (201) and sclerostin (202) 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 (167), antisense inhibition, inhibition of growth factor stimulation through farnesyl transferase inhibition (73), inhibition of gene transcription with low calcemic vitamin D analogs (78), and convertase inhibition (80). 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(OH)2D3-mediated hypercalcemia (203).

Noninfectious disorders include sarcoidosis, silicone-induced granulomatosis, paraffin-induced granulomatosis, berylliosis, Wegener's granulomatosis, eosinophilic granuloma and infantile fat necrosis. Infectious disorders include tuberculosis, candidiasis, 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% (204). Hypercalciuria and hypercalcemia generally occurs 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 (205). This is due to the fact the serum 1,25(OH)2D3 levels in active sarcoidosis are exquisitely sensitive to increases in the 25(OH)D3 substrate levels. This leads to inappropriately elevated serum 1,25(OH)2D3 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(OH)2D3-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 (206,207). The source of the inappropriate 1,25(OH)2D3 is believed to be an extra-renal 1a(OH)ase (as in malignant lymphoproliferative disease) produced by macrophages which are prominent components of the sarcoid granuloma (208). This enzyme exhibits similar kinetics and substrate specificity as the renal 1a(OH)ase but is clearly not regulated as is the renal 1a(OH)ase by PTH, 1,25(OH)2D3, calcium, or phosphorus. This extra-renal 1a(OH)ase does however appear to be suppressible by glucocorticoids (209), chloroquine (210) analogs and cytochrome P-450 inhibitors such as ketoconazole (211).

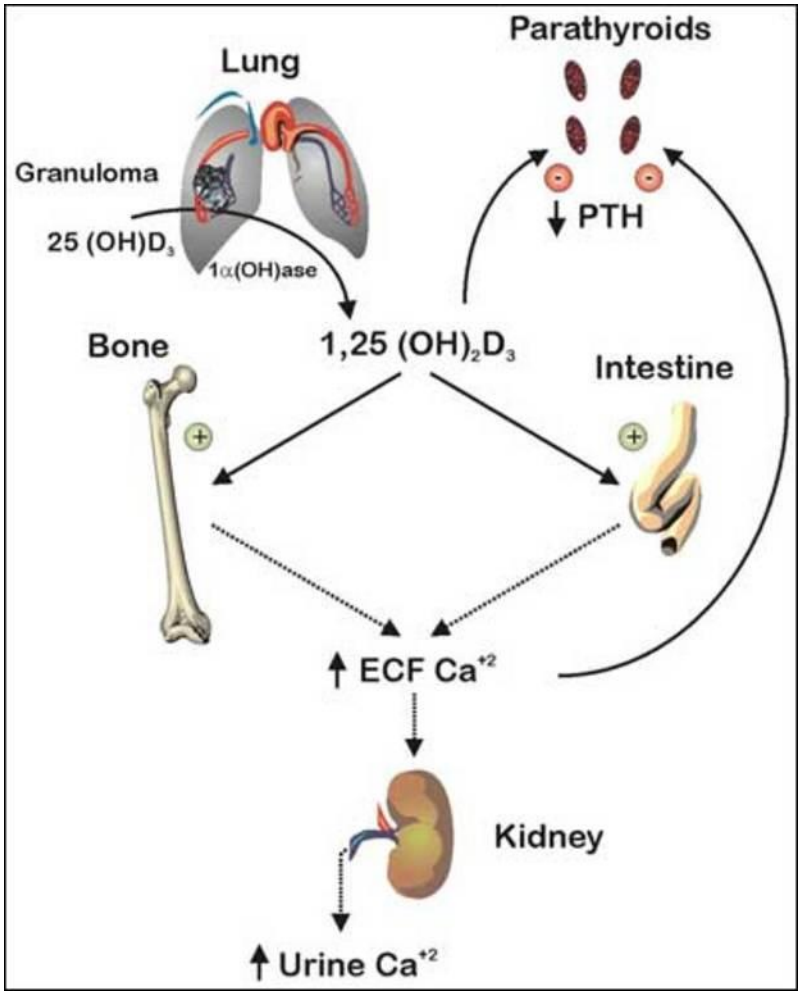

Figure 7. Disordered calcium homeostasis in granulomatous disease. Production of an extra-renal 1α(OH)ase by macrophages in a granuloma can increase conversion of circulating 25(OH)D3 to 1,25(OH)2D3. This secosteroid will increase Ca+2 absorption from the gut and Ca+2 resorption from bone resulting in an increased ECF Ca+2. The increased ECF Ca+2 and 1,25(OH)2D3 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(OH)2D3 concentrations.

Autoimmune Deficiency Syndrome (AIDS)

A number of mechanisms may contribute to causing hypercalcemia in AIDS however direct skeletal resorption has been described due to human immunodeficiency virus (HIV), HTLV-III or cytomegalovirus (CMV) infections of the skeleton (212). Use of foscarnet as an antiviral agent has also been associated with hypercalcemia (213).

Pediatric Syndromes

Williams Syndrome.

Williams Syndrome (William-Beuren Syndrome) is a sporadic disorder characterized by dysmorphic features including an "elfin facies", cardiac abnormalities, the most typical of which is supravalvar aortic stenosis, neurologic deficits, musculoskeletal abnormalities, and hypercalcemia (214). Hypercalcemia occurs in about 15% of cases and may be associated with increased sensitivity to vitamin D (215). Williams Syndrome has been associated with loss of genetic material at 7qll.23 which likely represents a continuous gene deletion that includes the elastin gene (ELN) (216). The hypercalcemia typically occurs during infancy and resolves between 2 and 4 years of age. The pathophysiology is not well understood.

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 (217). Loss-of-function mutations in CYP 24A1, the gene encoding the 24-hydroxylase appear to occur. Consequently inactivation of the active form of vitamin D, 1,25(OH)2D is impaired. This results in increased 1,25(OH)2D levels and enhanced calcium absorption and hypercalcemia (218).

Congenital lactase deficiency. Infants with congenital lactase deficiency may develop increase 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 (219).

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 (220). 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 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 case) 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 (221). 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 (222). 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 (223-225). 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.

Estrogens and Antiestrogens

In up to 30% of patients, hypercalcemia may occur when estrogens or antiestrogens (226) are used to treat breast cancer metastatic to the skeleton. Increased bone resorption appears to be the major mechanism possibly induced by cytokines and growth factors released when the tumor undergoes lysis. The hypercalcemia may require acute treatment but is usually self-limiting.

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Theophylline

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 –blockers and (227).

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 (228). 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 (229). 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.

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 (230). 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 (231).

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.

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	Nephrolithiasis, nephrocalcinosis
Neuro-muscular	Depression, confusion, stupor, coma	Weakness
Cardiac	Bradycardia, first degree atrio-ventricular	Hypertensionblock, digitalis sensitivity

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 (125). 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 as should a history of ingestion of medication which have been reported to cause hypercalcemia. 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 clinic, documentation of at least two elevated corrected (or ionized) serum calcium levels with concomitant elevated (or at least normal) serum PTH levels is required (Figure1). Two site assays for PTH are currently the method of choice (232). If mild hyperparathyroidism is documented, then in addition to the level of urine calcium, bone densitometry, calculation of estimated GFR and a renal ultrasound or renal CT scan for evidence of nephrolithiasis may help determine the extent of the disease. For severe hyperparathyroidism, appropriate skeletal X-rays would be indicated to 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 (233). 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. 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. In all patients with documented HPT, a 24 hour urine calcium and creatinine level should be obtained to exclude FHH. If the urine calcium to creatinine ratio is less than 0.01 and if testing serum and urine calcium in three relatives discloses hypercalcemia and relative hypocalciuria in other family members, then this diagnosis is likely and parathyroid surgery is to be avoided. If the urine calcium to creatinine ratio is greater than 0.01 then a BMD test should be performed and guidelines for treatment of primary HPTH should be considered (see below).

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, pheochromocytome, 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 patient is provided in Figure 8.

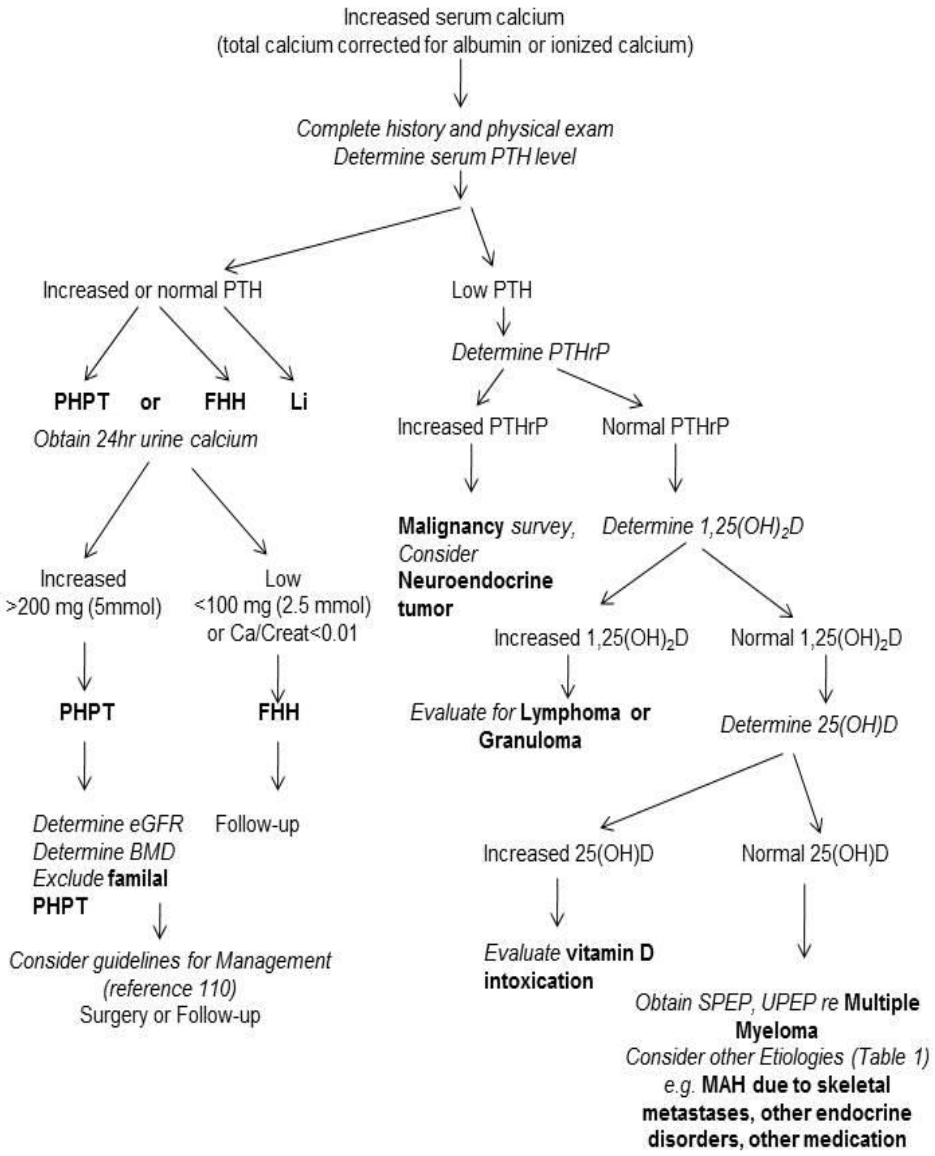
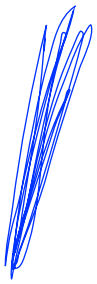


Figure 8. Laboratory approach to the diagnosis of hypercalcemia. Abbreviations used are: BMD= bone mineral density, eGFR=estimated glomerular filtration rate,Li=lithium therapy MAH=mailgnancy-associated hypercalcemia, PHPT=primary hyperparathyroidism, SPEP=serum protein electrophoresis, UPEP=urine protein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



MANAGEMENT OF HYPERCALCEMIA

If 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. 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.Glucocorticoids (when indicated)
5. Dialysis (in renal failure)
6. Calcimimetics
7. Mobilization

Hydration with normal saline is necessary in every patient with acute, severe hypercalcemia to correct the ECF deficit due to nausea, vomiting and polyuria (234). This may require infusion of 3 to 4 L of 0.9% sodium chloride 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.

Accelerated bone resorption is an important factor in the pathogenesis of hypercalcemia in the majority of patients with acute hypercalcemia and a bisphosphonate is the treatment of choice for inhibition of bone resorption. Consequently, after the patient is rehydrated, zoledronate 4 mg intravenously in 5 ml over 15 min (235) or pamidronate, 90 mg, intravenously in 500 ml of 0.9% saline or 5% dextrose in water over 4 hours (236) or zoledronate 4 mg intravenously in 5 ml over 15 minutes may be administered. These agents 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. A second treatment is not recommended for at least 8 days. Denosumab (initial dose 60 mg subcutaneously, with repeat dosing based upon response) is an alternative option and in contrast to bisphosphonates is not cleared by the kidney, and therefore can be used in patients with chronic kidney disease. Calcitonin is a peptide hormone which is a safe therapeutic agent when acutely administered. Calcitonin can inhibit osteoclastic resorption and also increase calcium excretion (237). It has a rapid onset of action, causing serum calcium to fall generally by 2 mg/dL within 2 to 6 hours of administration. Consequently it may be used in concert with a bisphosphonate to more rapidly reduce the hypercalcemia (within 2-6 hours) (238). It is usually given intramuscularly or subcutaneously at a dose of 4 to 8

IU/kg. Unfortunately this agent is not as potent as the most potent bisphosphonates and tachyphylaxis may occur after 24-48 hours.

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 (239). In this case, a loop diuretic i.e. furosemide may be added, but also only after rehydration. Loop diuretics inhibit both sodium and calcium reabsorption at the CTAL of the kidney. Small doses of furosemide may be administered (10 to 20 mg) intravenously both to control clinical manifestations of volume excess and to promote calciuria.

Glucocorticoids (e.g. hydrocortisone 200 to 300 mg intravenously over 24 hours for 3 to 5 days) may be administered, particularly if the underlying disorder is known to be responsive to this agent. Thus patients with hypercalcemia due to hematologic malignancies such as lymphoma or myeloma may benefit (240) as may patients with vitamin D intoxication or granulomatous disease (209) where 1,25(OH)2D3 production and action may be inhibited.

Dialysis is usually reserved for severely hypercalcemic patients refractory to other therapies or who have renal insufficiency. Both peritoneal dialysis (241) and hemodialysis (242) can be effective.

The calcimimetic, cinacalcet, may be used in doses starting from 30 mg twice daily orally to as high as 90 mg 4 times daily for the treatment of hypercalcemia due to parathyroid carcinoma.

Finally, the patient should be mobilized as rapidly as possible. (243). If mobilization is not possible then continued treatment with antiresorptive agents may be necessary (244).

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

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