Vitamin D-Mediated Hypercalcemia: Mechanisms, Diagnosis, and Treatment

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Hypercalcemia occurs in up to 4% of the population in association with malignancy, primary hyperparathyroidism, ingestion of excessive calcium and/or vitamin D, ectopic production of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], and impaired degradation of 1,25$(OH)_2$D. The ingestion of excessive amounts of vitamin D_3 (or vitamin D_2) results in hypercalcemia and hypercalciuria due to the formation of supraphysiological amounts of 25-hydroxyvitamin D [25(OH)D] that bind to the vitamin D receptor, albeit with lower affinity than the active form of the vitamin, 1,25$(OH)_2$D, and the formation of 5,6-*trans* 25(OH)D, which binds to the vitamin D receptor more tightly than 25(OH)D. In patients with granulomatous disease such as sarcoidosis or tuberculosis and tumors such as lymphomas, hypercalcemia occurs as a result of the activity of ectopic 25(OH)D-1-hydroxylase (CYP27B1) expressed in macrophages or tumor cells and the formation of excessive amounts of 1,25$(OH)_2$D. Recent work has identified a novel cause of non-PTH-mediated hypercalcemia that occurs when the degradation of 1,25$(OH)_2$D is impaired as a result of mutations of the 1,25$(OH)_2$D-24-hydroxylase cytochrome P450 (CYP24A1). Patients with biallelic and, in some instances, monoallelic mutations of the *CYP24A1* gene have elevated serum calcium concentrations associated with elevated serum 1,25$(OH)_2$D, suppressed PTH concentrations, hypercalciuria, nephrocalcinosis, nephrolithiasis, and on occasion, reduced bone density. Of interest, first-time calcium renal stone formers have elevated 1,25$(OH)_2$D and evidence of impaired 24-hydroxylase-mediated 1,25$(OH)_2$D degradation. We will describe the biochemical processes associated with the synthesis and degradation of various vitamin D metabolites, the clinical features of the vitamin D-mediated hypercalcemia, their biochemical diagnosis, and treatment. **(*Endocrine Reviews* 37: 521–547, 2016)**

I. Introduction

Hypercalcemia is encountered in 0.2 to 4% of community-dwelling subjects and hospital patients (1–8). The incidence of hypercalcemia is dependent upon whether serum calcium measurements are performed in free-living subjects in a community (1), in a hospital population (2–4), or in patients seen in an emergency department (5, 6, 8). Causes of hypercalcemia are listed in Table 1. Cancer-associated hypercalcemia and primary hyperparathyroidism are the most frequent causes of hypercalcemia. Their relative frequency depends upon whether the diagnosis of hypercalcemia is made in a hospital setting (where cancer-associated hypercalcemia is most frequent) or within the context of an outpatient practice (where the diagnosis of primary hyperparathyroidism predominates) (9).

From a diagnostic and therapeutic perspective, it is useful to think of hypercalcemia as a PTH-dependent or PTH-independent process. Increases in PTH concentrations in association with hypercalcemia indicate the presence of primary (10–14), tertiary (15–28), and post-transplant hyperparathyroidism (3, 21, 25, 26, 28–38) or severe neonatal hyperparathyroidism (associated with homozygous mutations of the calcium-sensing receptor) (39–42),

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Abbreviations: HTLV, human T lymphotropic virus; IIH, idiopathic infantile hypercalcemia; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PAM, pulmonary alveolar macrophage; VDBP, vitamin D binding protein.

Table 1. Causes of Hypercalcemia

PTH-Mediated
Primary hyperparathyroidism
Tertiary hyperparathyroidism
Post-transplant hyperparathyroidism
Familial hypocalciuric hypercalcemia/severe neonatal hyperparathyroidism
Humoral hypercalcemia of malignancy—PTH-mediated
Non-PTH-Mediated
Endocrine
Hypothyroidism
Hypoadrenalism/Addison's syndrome
VIPoma
Pheochromocytoma
Pregnancy/lactation-associated (PTHrP-mediated)
Malignancy
Humoral hypercalcemia of malignancy
PTH-related peptide
1,25-dihydroxyvitamin D
Lytic bone lesions
Drug-related
Thiazide diuretics
Vitamin D or vitamin D analogs
Vitamin K
Calcium
Aluminum
Beryllium
Theophylline
Vitamin A intoxication
Vitamin D-mediated
Excessive cholecalciferol or ergocalciferol indigestion
Ingestion or administration of excessive calcitriol (or other 1α-hydroxylated vitamin D analogs)
Ectopic 1,25-dihydroxyvitamin D production
Granulomatous disease
Sarcoidosis
Tuberculosis
Fungal diseases
Leprosy
Other granulomatous lesions
Lymphoma
Inactivating mutations of the *CYP24A1* gene in children and adults
Miscellaneous conditions
Post-acute renal failure
William's syndrome
Paget's disease
Immobilization
Jansen's metaphyseal chondrodysplasia
Hypophosphatasia
Milk-alkali syndrome

whereas hypercalcemia in association with a low or suppressed PTH concentration indicates the presence of PTH-independent mechanisms causing hypercalcemia. In the latter category, cancer-associated hypercalcemia is predominant. In vitamin D-associated hypercalcemia, PTH concentrations are appropriately reduced.

II. Vitamin D-Associated Hypercalcemia

A review of vitamin D metabolism will assist in the understanding of mechanisms associated with vitamin D-mediated hypercalcemia and the utility of measurements of vitamin D metabolites when a diagnosis of vitamin-associated hypercalcemia is made.

A. Vitamin D metabolism

The major physiological role of vitamin D through the activity of its active metabolite 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D] is the maintenance of normal calcium and phosphorus balance (43–46). 1α,25$(OH)_2$D also mediates several other biological effects such as the modulation of immune function (47, 48), muscle function (49–51), and cell growth and differentiation (52–54). A brief review of the metabolism, regulation, and mechanism of action of vitamin D follows. For more detailed information, readers are referred to prior reviews in *Endocrine Reviews* (49, 50, 54–64) and other journals (48, 65, 67–69).

Figure 1A summarizes the salient biochemical transformations that occur during the formation and metabolism of vitamin D metabolites. The endogenous form of vitamin D, vitamin D_3 (cholecalciferol), is formed in the skin as a result of photolysis of the precursor sterol, 7-dehydrocholesterol (70–78). Under the influence of ultraviolet light (optimal wave lengths for photolysis, 295–300 nm), the B-ring of the sterol is cleaved, giving rise to previtamin D_3, which undergoes thermal equilibration to vitamin D_3 (76–78). Vitamin D_3, bound to vitamin D-binding protein, to which it preferentially binds relative to its precursor, previtamin D_3, exits the skin and enters the circulation (77). Similar biochemical transformations occur with the plant sterol, ergosterol, which upon photolysis gives rise to vitamin D_2, or ergocalciferol (79). Although there are interspecies differences in the biological activity of vitamin D_3 vs vitamin D_2 (for example, vitamin D_2 is much less active in birds than mammals) (80), the major metabolic transformations of vitamin D_3 and vitamin D_2 are similar. For the purposes of this review, we will use the term "vitamin D_3" throughout. Unless specified, the reader may assume that the similar metabolic transformations occur in the case of vitamin D_3 and vitamin D_2. The term "vitamin D" will be used to refer to both vitamin D_2 and vitamin D_3 metabolites.

Vitamin D_3 is metabolized in the liver microsomes and mitochondria to 25-hydroxyvitamin D_3 [25$(OH)D_3$] by the vitamin D_3-25-hydroxylase (81–92). The vitamin D_3-25-hydroxylase is only partially inhibited by its product, and hence, increasing amounts of administered vitamin D_3 are associated with increases in the amount of product, namely, 25$(OH)D_3$, and hence, increasing concentrations of vitamin D_3 in the serum are associated with proportional increases in serum 25$(OH)D_3$. 25$(OH)D_3$ (both free and bound to vitamin D-binding protein) is the major circulating vitamin D_3 metabolite (43, 68, 69, 74, 94–95), and measurements of this vitamin D metabolite are widely used as an index of nutritional vitamin D status (96–99). The CYP2R1 is the cytochrome P450 of the microsomal

Figure 1.

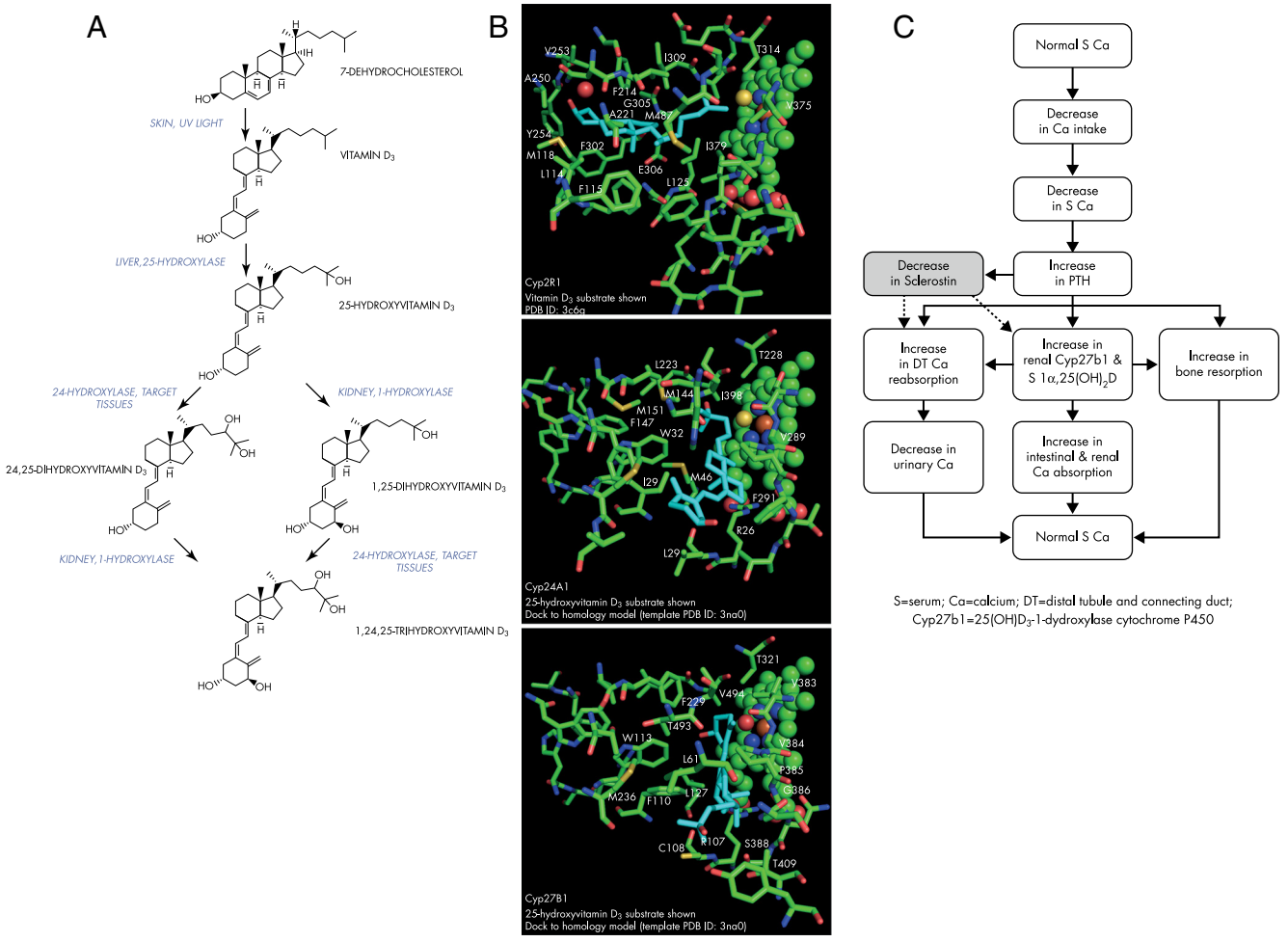

Figure 1. A, The formation and metabolism of vitamin D_3. B, Docking studies may explain ligand specificity of cytochrome P450s for vitamin D_3 and its metabolites. Top panel, Crystal structure of the Cyp2R1 bound to vitamin D_3 (cyan) (PDB ID 3c6g) (100). Middle panel, Homology model of Cyp24A1 (-9.2 kcal/mol) bound to substrate 25(OH)D_3 (cyan). Bottom panel, Homology model of Cyp27B1 (-9.4 kcal/mol) bound to 25(OH)D_3 (cyan). The heme, heme iron, and bound oxygen (yellow) positions in these cytochrome P450 cavities are shown as spheres at right. The amino acids nearby or in the positions of all three ligands are shown for comparison. Residue names and numbers are provided only if one or more enzyme atoms fall within a 4 Å distance of a ligand atom. The homology models shown were based on the closed ligand cavity conformation observed for the crystal structure of Cyp11A1 (PDB ID 3na0). (All protein structural figures and modeling are courtesy of Dr. James R. Thompson.) C, Physiological changes in response to decreases in serum calcium concentrations.

vitamin D_3-25 hydroxylase, a mutant form of which was identified in a human subject with low circulating concentrations of 25-hydroxyvitamin D [25(OH)D] and classic symptoms of vitamin D deficiency (90). In the patient studied, homozygous mutations in exon 2 of the *CYP2R1* gene on chromosome 11p15.2 resulted in the substitution of a proline for an evolutionarily conserved leucine at amino acid 99 in the CYP2R1 protein and reduced vitamin D_3-25 hydroxylase activity. Other vitamin D_3-25 hydroxylases are also likely to play a role in the transformation of vitamin D_3 to 25(OH)D_3 because *Cyp2r1*$^{-/-}$ mice have only a partial (~50%) reduction in serum 25(OH)D_3 concentrations and lack overt rickets and hypocalcemia (92). The structure of the Cyp2A1 cytochrome P450 bound to its ligand, vitamin D_3, has been solved by x-ray crystallography (100). Vitamin D_3 is bound in an elongated conformation with the aliphatic side-chain pointing toward the heme group (Figure 1B, top panel). The active site is lined by conserved, mostly hydrophobic residues.

The further metabolism of 25(OH)D_3 is dependent upon the calcium and phosphorus requirements of the individual. In states of calcium demand, 25(OH)D_3 is metabolized by the 25-hydroxyvitamin D_3-1α-hydroxylase to the biologically active vitamin D metabolite, 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$], in the kidney by PTH-dependent processes (Figure 1C) (43, 65, 74, 101–

112). Changes in PTH alter multiple processes including renal calcium reabsorption (directly and indirectly through changes in sclerostin expression) (111–114), 25(OH)D 1α-hydroxylase activity, and bone resorption mechanisms (43, 65, 74, 101–112). In states of calcium sufficiency, the synthesis of 1α,25$(OH)_2D_3$ is reduced, and the synthesis of 24*R*,25-dihydroxyvitamin D_3 (24*R*,25$(OH)_2D_3$) (115–117), an inert vitamin D metabolite, is increased. The synthesis of 24*R*,25$(OH)_2D_3$ is mediated by the 25$(OH)D_3$-24-hydroxylase that is present in several target tissues of 1α,25$(OH)_2D_3$ including the intestine and the kidney (115, 118–120). This enzyme is induced by 1α,25$(OH)_2D_3$ (118, 121). Serum phosphate concentrations also regulate the synthesis of 1α,25$(OH)_2D_3$ by PTH-independent mechanisms (122). Thus, in states of phosphorous demand, 25$(OH)D_3$ is metabolized to 1α,25$(OH)_2D_3$, and the synthesis of 24*R*,25$(OH)_2D_3$ is reduced (69, 103, 123–126). The converse occurs in hyperphosphatemic states. Numerous factors other than calcium and phosphorus alter the activity of the 25(OH)D-1α-hydroxylase, and the reader is referred to reviews on this matter (104, 127–131). As noted in Figure 1A, 1α,25$(OH)_2D_3$ and 24*R*,25$(OH)_2D_3$ are metabolized to 1α,24*R*,25$(OH)_3D_3$ by the 24 and 1α-hydroxylases.

The 25$(OH)D_3$-24-hydroxylase is a mitochondrial, multicomponent enzyme with a terminal cytochrome P450, the CYP24A1, which uses molecular oxygen to hydroxylate 25$(OH)D_3$ at C-24 on the side chain of the sterol (132). The *Cyp24A1/CYP24A1* gene has been cloned from rats (133–136) and humans (137, 138). As we will discuss in later sections, deletions or mutations in the mouse and human *CYP24A1* gene are responsible for hypercalcemia as a result of elevated 1α,25$(OH)_2D_3$ concentrations (69, 139–147). Shown in Figure 1B, middle panel, is a model of the Cyp24A1 protein bound to 25$(OH)D_3$. Note the proximity of the side chain to oxygen and heme groups. Models of the Cyp24A1 and Cyp27B1 were generated by threading Cyp24A1 and Cyp27B1 amino acid sequences onto the backbone polypeptide positions to form three cytochrome p450 structures: rat 24-hydroxylase (Cyp24A1; PDB ID 3k9v), human cholesterol side-chain cleavage enzyme (Cyp11A1; PDB ID 3na0), and human 11-β-hydroxylase (Cyp11B1; PDB ID 4fdh) (148–154). The 25$(OH)D_3$-1α-hydroxylase is a mitochondrial, multicomponent enzyme with a terminal cytochrome P450 (155–158), CYP27B1, which uses molecular oxygen to hydroxylate 25$(OH)D_3$ at C-1 on the A ring of the sterol (159–162). Mutations of the *CYP27B1* gene are responsible for vitamin D dependency rickets, type 1 (163–166), and deletion of the *Cyp27B1* gene in mice confers a rachitic phenotype (167). Shown in Figure 1B, lower panel, is a model of the Cyp27B1 cytochrome P450 protein bound to 25$(OH)D_3$. Note the proximity of the A ring to oxygen and heme groups. The enzyme is also responsible for the conversion of 24R,25$(OH)_2D_3$ to the metabolite, 1α, 24R, 25-trihydroxyviatamin D_3. Besides metabolism to 1α,24,25$(OH)_3D_3$, 1α,25$(OH)_2D_3$ is also metabolized to polar steroids (glucuronides and sulfates) in the liver and excreted in bile [about 30–40% of an administered dose of 1α,25$(OH)_2D_3$] (55, 104, 168–172); to calcitroic acid that is excreted in the bile as a polar metabolite (about 20–25% of an administered dose of 1α,25$(OH)_2D_3$) (173–176); and to 1α,25R$(OH)_2D_3$-26,23S-lactone (177–179).

The bioactivity of vitamin D_3 is dependent on the formation of 1α,25$(OH)_2D_3$. Pharmacological amounts of precursors such as vitamin D_3 itself or intermediary metabolites such as 25$(OH)D_3$ are required to elicit a biological response in anephric animals and patients (109, 180, 181). In such individuals, 1α,25$(OH)_2D_3$ readily increases intestinal calcium transport (105, 106) and mobilizes calcium from bone (181). The actions of 1α,25$(OH)_2D_3$ require the presence of the vitamin D receptor, a steroid hormone receptor that binds 1α,25$(OH)_2D_3$ with high affinity and binds other vitamin D metabolites with lower affinities (182–185). After binding of the ligand, 1α,25$(OH)_2D_3$, to the ligand-binding domain of the receptor, a conformational change in the receptor is associated with the recruitment of other steroid hormone receptors such as the RXRα and various coactivator (or corepressor) proteins to the transcription start site of genes regulated by 1α,25$(OH)_2D_3$ (186–194). The vitamin D receptor binds DNA binding elements of varied nucleotide structures within vitamin D-regulated genes via its amino-terminal DNA binding domain (195–199). Numerous calcium-regulating genes are induced or repressed in vitamin D-responsive target tissues such as the intestine, kidney, and bone (45, 51, 200–205).

Absorption of dietary calcium by the intestine is essential for the maintenance of normal calcium homeostasis (206) and is a major factor contributing to hypercalcemia in patients with vitamin D intoxication. The efficiency of calcium absorption increases or decreases inversely with the amount of dietary calcium, and adaptations to changes in calcium intake are dependent upon vitamin D and its active metabolite, 1α,25$(OH)_2D_3$ (206, 207). Calcium is absorbed by the intestine (predominantly in the duodenum and proximal small intestine) by two mechanisms, a passive paracellular mechanism, and an active transcellular one (206, 208, 209). Active calcium absorption initially involves the movement of calcium across the apical border of the intestinal cell into the cell down a concentration gradient (the interior of the intestinal cell has a calcium concentration in the high nanomolar range) and

Figure 2.

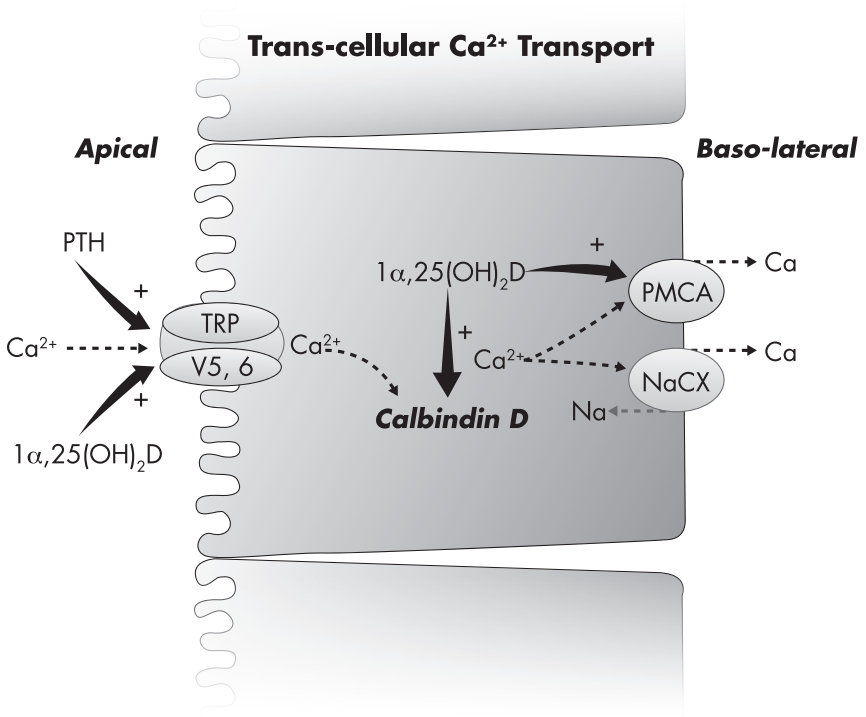

Figure 2. Integrated model of active Ca^{2+} reabsorption in the intestine and distal part of the nephron. Apical entry of Ca^{2+} is facilitated by Transient Receptor Potential Cation Channel Subfamily V Members 5,6 (TRPV 5,6)/Epithelial calcium channel (EcaC); Ca^{2+} then binds to calbindin-D_{28K}, and this complex diffuses through the cytosol to the basolateral membrane, where Ca^{2+} is extruded by a Na^+/Ca^{2+} exchanger and a plasma membrane Ca^{2+}-ATPase. The individually controlled steps in the activation process of the rate-limiting Ca^{2+} entry channel include $1\alpha,25(OH)_2D_3$-mediated transcriptional and translational activation, shuttling to the apical membrane, and subsequent activation of apically located channels by ambient Ca^{2+} concentration, direct phosphorylation, and/or accessory proteins. (Modified from Kumar and Vallon; Ref. 112.)

an electrical gradient (the interior of the cell is electronegative relative to the lumen). It does not require the expenditure of energy (210, 211). The extrusion of calcium out of the intestinal cell at the basolateral membrane is against an electrical and concentration gradient and requires the expenditure of energy (210, 211). Essential to the process of active calcium transport are several vitamin D dependent proteins, each with a specific function. These include the epithelial calcium channel, calbindin D_{9K} andD_{28K}, and the plasma membrane calcium pump (212). In the duodenal enterocyte, apically situated TRPV 5/6 cation channels mediate the increase in calcium uptake from the lumen into the cell (213); intracellular calcium binding proteins such as calbindin D_{9K} and D_{28K} facilitate the movement of calcium across the cell (209, 210); and the basal-lateral plasma membrane calcium pump (214–216) and the sodium-calcium exchanger (217) assist in the extrusion of calcium from within the cell into the extracellular fluid (Figure 2). The sodium gradient for the activity of the sodium-calcium exchanger is maintained by the Na-K ATPase. Intestinal transcellular calcium transport is regulated by vitamin D through its active metabolite, $1\alpha,25(OH)_2D_3$, which increases the expression of TRPV 6 channels (218), the intracellular concentrations of calbindin D_{9K} and D_{28K} (210, 219–221), and the expression of the plasma membrane pump, isoform 1 (222, 223) (Figure 2). The requirement of various intestinal calcium transporter proteins in transcellular calcium transport in vivo has been examined in knockout mice. Deletions of *TrpV6* and *calbindin* D_{9K} genes are not associated with alterations in intestinal calcium transport in vivo in the basal state and after the administration of $1\alpha,25(OH)_2D_3$ (224, 225), although one report suggests that basal calcium transport on an adequate calcium diet is normal in *TrpV6* knockout mice but adaptations to a low-calcium diet are impaired (226). We recently showed that deletion of the *Pmca1* in the intestine is associated with reduced growth and bone mineralization and a failure to up-regulate calcium absorption in response to $1\alpha,25(OH)_2D_3$, thereby establishing the essential role of the pump in transcellular calcium transport (227).

$1\alpha,25(OH)_2D_3$, PTH, and the phosphatonin, fibroblast growth factor-23 (FGF-23), regulate and maintain normal phosphorus concentrations (212, 228, 229). Changes in serum phosphate concentrations are associated with changes in $1\alpha,25(OH)_2D_3$ concentrations. A decrease in serum phosphate concentration is associated with an increase in ionized calcium, a decrease in PTH secretion, and a subsequent decrease in renal phosphate excretion. An increase in renal 25(OH)D 1α-hydroxylase activity, increased $1\alpha,25(OH)_2D_3$ synthesis, and increased phosphorus absorption in the intestine and reabsorption in the kidney occur (122, 126, 230–237). In the intestine and kidney, $1\alpha,25(OH)_2D_3$ regulates the expression of the sodium-phosphate cotransporters IIb, and IIA and IIc, respectively, thereby regulating the efficiency of

inorganic phosphate absorption in enterocytes and proximal tubule cells (212, 238–240).

B. Prevalence and clinical manifestations of vitamin D-mediated hypercalcemia

Although relatively uncommon in comparison to cancer-associated hypercalcemia and primary hyperparathyroidism, the true prevalence of vitamin D-mediated hypercalcemia is unknown. With the increase in vitamin D supplementation in the general population and with new information becoming available on the prevalence of *CYP24A1* mutations (139–147) in the general population (241), it is likely that the prevalence of vitamin D-mediated hypercalcemia will increase. Table 2 summarizes the causes and mechanisms associated with the development of vitamin D-associated hypercalcemia.

C. Hypercalcemia associated with excessive ingestion of vitamin D and active vitamin D metabolites/analogs

1. Vitamin D intake and hypercalcemia

The upper tolerable limit, defined as the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population, for vitamin D_3 is 1000 IU/d in infants ages 0–6 months, 1500 IU/d in infants ages 6–12 months; 2500 IU/d in children ages 1–5 years; 3000 IU/d in children ages 4–8 years, and 4000 IU/d in adolescents and adults (97, 99). The short-term ingestion of up to 10 000 IU/d of vitamin D_3 is associated with the maintenance of 25(OH)D serum concentrations below 50 ng/mL (125 nmol/L) (240), a concentration below which toxicity has not been observed. In a study of 40 patients with metastatic breast tumors, daily doses of 10 000 IU vitamin D_3 for 4 months were not associated with hypercalcemia although small increases in serum calcium and decreases in PTH were observed (243). Ingestion of amounts of vitamin D_3 or vitamin D_2 higher than 10 000 IU/d in an adult (and lower amounts in children) should raise the suspicion of vitamin D intoxication, especially in the context of hypercalciuria and/or hypercalcemia because the serum 25(OH)D concentration rises steeply at intakes >10,000 IU/d. The duration of ingestion of vitamin D, the starting 25(OH)D concentration before the ingestion of vitamin D_3, and the underlying reason for therapy are important in considering the contribution of vitamin D ingestion to changes in 25(OH)D concentrations (see Ref. 242 for a summary of multiple studies). Generally, vitamin D-associated hypercalcemia occurs only when extremely large doses of vitamin D (often several hundred-fold the recommended intake) are ingested (244–257).

Table 2. Vitamin D-Associated Hypercalcemia

Exogenous Vitamin D Toxicity
Administration of excessive amounts of vitamin D_3 or vitamin D_2
Administration of excessive amounts of 25(OH)D_3
Administration of excessive amounts of 1α,25$(OH)_2D_3$, other 1α-hydroxylated vitamin D analogs such as 1α(OH)D_3, paricalcitol, and doxercalciferol in the context of chronic renal failure, end-stage renal disease, and hemodialysis therapy
Excessive Production of Vitamin D Metabolites
Congenital disorders: excessive production of 25(OH)D and 1,25$(OH)_2D_3$, eg, in Williams-Beuren syndrome with mutations of the Williams Syndrome Transcription Factor
Granulomatous disease: excessive production of 1,25$(OH)_2D_3$: sarcoidosis, tuberculosis, leprosy, histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, candidiasis, cat-scratch disease, *Pneumocystis jiroveci* or *P. carinii* pneumonia, *Mycobacterium avium* complex, Wegener's granulomatosis, Crohn's disease, infantile sc fat necrosis, giant cell polymyositis, berylliosis, silicone-induced granuloma, paraffin-induced granulomatosis, talc granuloma.
Lymphomas and malignant lymphoproliferative disease: excessive production of 1,25$(OH)_2D_3$: lymphoma, non-Hodgkin lymphoma, lymphomatoid, granulomatosis, inflammatory myofibroblastic tumor, dysgerminoma
Mutations in Enzymes Associated With Vitamin D Metabolite Degradation
Mutations of the *CYP24A1* gene: reduced degradation of 1,25$(OH)_2D_3$: infantile and adult hypercalcemia

2. Diagnosis of hypervitaminosis D

The clinical symptoms of vitamin D toxicity are the result of hypercalcemia and hypercalciuria and are similar to those of hypercalcemia due to any other cause. Symptoms include neuropsychiatric manifestations such as lethargy, confusion, irritability, depression, hallucinations, and in extreme cases, stupor, and coma; gastrointestinal symptoms such as anorexia, nausea, vomiting, and constipation; cardiovascular manifestations such as ectopy; and renal symptoms such as polyuria and renal colic from the passage of renal stones.

Reports suggest that the administration of vitamin D_3 in large amounts is associated with an increased risk of falls and fractures (258–261). For example, in a 1-year, double-blind, randomized clinical trial conducted in Switzerland among community-dwelling men and women 70 years of age and older, groups of subjects receiving monthly treatment with 60 000 IU of vitamin D_3, and 24 000 IU of vitamin D_3 plus 300 μg of calcifediol [25$(OH)D_3$], had a higher incidence of falls than a the group receiving 24 000 IU of vitamin D_3 (261). In another study, in which older women received a single annual oral dose of 500 000 IU of vitamin D_3, the relative risk of falling in the vitamin D group vs the placebo group was 1.31 in the first 3 months after dosing and 1.13 during the following 9 months (258). Thus, among older community-dwelling women, annual oral administration of high-dose cholecalciferol resulted in an increased risk of falls and fractures.

Laboratory findings other than hypercalcemia include hyperphosphatemia and suppressed serum PTH concentrations. The presence of hyperphosphatemia is a clue to the presence of hypervitaminosis D. It occurs as a result of an increase in intestinal and renal phosphate absorption. In contrast, patients with primary hyperparathyroidism have hypercalcemia and hypophosphatemia on account of PTH-mediated losses of phosphate in the urine. Hypercalciuria is frequently present. Urinary calcium excretion is generally elevated before the development of hypercalcemia in patients with hypervitaminosis D. Urine osmolality may be low on account of a renal concentrating defect that occurs as a result of resistance to the effects of antidiuretic hormone and resultant nephrogenic diabetes insipidus. Three mechanisms have been proposed to mediate the diabetes insipidus associated with hypercalcemia. Activation of the calcium-sensing receptor in the thick ascending limb with attendant inhibition of sodium chloride reabsorption and countercurrent multiplication results in a dilute urine (262). In addition, the sensing of the increased Ca^{2+} concentrations in the urine in the terminal collecting duct by calcium-sensing receptors facing the urinary space is believed to reduce antidiuretic hormone-stimulated water reabsorption from urine to medullary interstitial fluid (262). Decreased aquaporin-2 expression and apical plasma membrane delivery in kidney collecting ducts also contributes to the polyuria seen with hypercalcemia (263). Elevated serum creatinine and blood urea nitrogen, and nephrocalcinosis on radiographic examination of the kidneys are frequently present. Electrocardiogram findings include a shortened QTc, ST segment coving, T wave broadening, and first degree heart block.

Although vitamin D (vitamin D_3 and vitamin D_2) can be measured in serum and plasma and quantitated by various methods such as ultraviolet spectroscopy and competitive protein binding, its measurement is technically difficult, and few reports have appeared on its use in the measurement of vitamin D in patients with hypervitaminosis D (264, 265). Measurements of serum 25(OH)D, which can be performed by a variety of methods—including competitive protein binding assay (266–268), RIA (269–272), HPLC/ultraviolet spectroscopy (273, 274), automated, antibody-, and microparticle-based, chemiluminescent immunoassay (275), and liquid chromatography mass spectrometry (272, 276–279)—are widely used in the assessment of vitamin D status. Various epimers contribute to the total 25(OH)D measurement and appear to be most prominent in infants and very young patients in whom C-3 epimers of 25(OH)D can account for a significant proportion of 25(OH)D measured by liquid chromatography-tandem mass spectrometry unless measures are taken to separate metabolites by chromatography (279). In hypervitaminosis D [$25(OH)D_3$ >64–439 ng/mL], the mean relative contribution of 3-epi-$25(OH)D_3$ was <4%, and concentrations ranged from 2–28.6 ng/mL (280). Serum levels of the C-3 epimer correlate with serum $25(OH)D_3$ concentrations. In subjects with $25(OH)D_3$ concentrations indicative of hypervitaminosis D, the presence and concentrations of the C-3 epimer were unrelated to age, serum markers of renal and liver function, acute-phase reactants, and the presence of hypercalcemia. Subjects with significant PTH suppression (<14 pg/mL) showed higher concentrations of 3-epi-$25(OH)D_3$.

It is challenging to assign an absolute serum vitamin D concentration over which toxicity is always present. Some patients can have 25(OH)D concentrations well over 80 ng/mL without hypercalcemia or hypercalciuria. However, in general, serum total 25(OH)D concentrations >80 ng/mL (200 nmol/L) are necessary to result in vitamin D toxicity, with concentrations typically severalfold higher than 80 ng/mL in those who present with symptomatic hypercalcemia (244–257). In most cases, serum $1\alpha,25(OH)_2D_3$ concentrations are normal. The vitamin D concentration at which an individual develops hypercalcemia or hypercalciuria is likely influenced by the amount of dietary calcium intake. As a result, serum and urine calcium concentrations may be quite variable, despite concentrations of serum 25(OH)D that might be regarded as elevated. A report by Adams and Lee (281) suggested that concentrations of 25(OH)D as low as 50 ng/mL (125 nmol/L) were associated with hypercalciuria. In addition, results of the Women's Health Initiative found that modest vitamin D and calcium supplementation resulted in a higher risk of nephrolithiasis compared to placebo (282). Although 25(OH)D concentrations of approximately 50 ng/mL (125 nmol/L) may increase urinary calcium excretion and the risk of nephrolithiasis, it should be remembered that normal individuals exposed to sunlight for short or long periods of time can have 25(OH)D serum concentrations as high as 65 ng/mL (163 nmol/L) without ill effects or hypercalcemia (248, 266, 283–295). Concentrations of 25(OH)D >80 ng/mL in the presence of hypercalcemia and the clinical setting of excessive vitamin D ingestion should raise the suspicion of vitamin D intoxication.

In serum, 25(OH)D is tightly bound to vitamin D binding protein (VDBP) (296–298), and only a small percentage of total serum 25(OH)D is free or unbound (95, 299–301). The role of VDBP in determining the amount of bioavailable 25(OH)D has recently been investigated by Powe et al (302), who reported that community-dwelling black Americans, as compared with whites, had low levels of total 25(OH)D and vitamin D-binding protein but similar concentrations of estimated bioavailable 25(OH)D. Subsequent studies using mass spectrometry or a poly-

Figure 3.

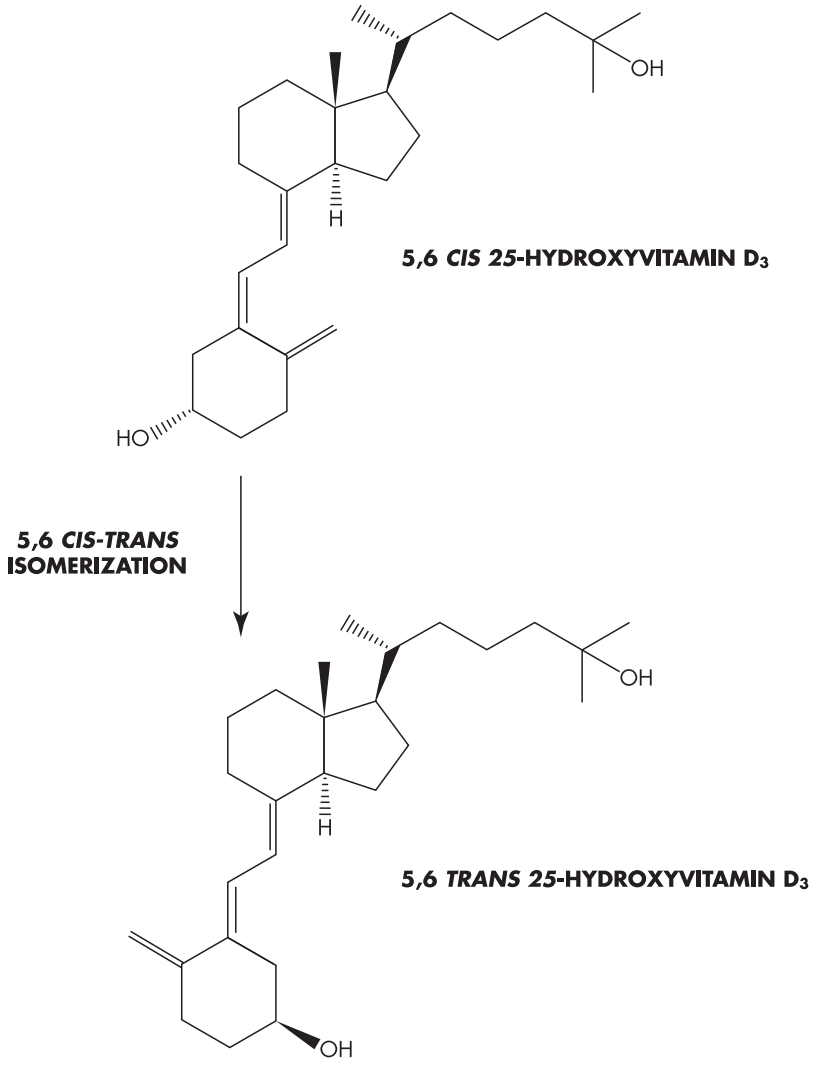

Figure 3. 5,6-*trans*-25(OH)D_3 is produced from 25(OH)D_3 in animal models administered large amounts of vitamin D_3. Because of the presence of a C-1 hydroxyl group, 5,6-*trans*-25(OH)D_3 binds to the vitamin D receptor with higher affinity than 25(OH)D_3.

were unexpectedly *less susceptible* to hypercalcemia and its toxic effects.

3. Mechanism of hypercalcemia in hypervitaminosis D

Hypercalcemia occurs as a result of increased calcium absorption from the intestine and increased bone mobilization. The 25(OH)D_3 or 25(OH)D_2 which are present in increased amounts bind to the vitamin D receptor in sufficient amounts to induce processes that enhance intestinal calcium absorption and enhance bone mobilization (81, 82, 83, 308, 309, 311). In in vitro radioligand binding assays with the vitamin D receptor, the B50 (B50 value is defined as the concentration of material necessary to cause 50% displacement of the radiolabel from the protein) of 1α,25(OH)$_2D_3$ is approximately 1.62×10^{-10} M, whereas, the B50 of 25(OH)D for the vitamin D receptor is approximately 1.38×10^{-7} M. These concentrations of 25(OH)D may be present in vitamin D target tissues in hypervitaminosis D. A second possible mechanism is the endogenous production of 5,6-*trans*-25(OH)D_3, which has a 1α hydroxyl group and which binds to the vitamin D receptor with increased affinity (312) (Figure 3). We have shown that 5,6-*trans*-25(OH)D_3 is present in the serum of rats administered large doses of vitamin D_3 (312). Because of the presence of a 1α hydroxyl group, binding of 5,6-*trans*-25(OH)D_3 to the vitamin D receptor is increased—6.9×10^{-8} M for 5,6-*trans*-25(OH)D_3, 1.95×10^{-7} M for 25(OH)D_3, and 2.2×10^{-10} M for 1α,25(OH)D_3 (312). It should be noted, however, that although we have isolated 5,6-*trans*-25(OH)D_3 from the serum of rats dosed with vitamin D_3, it is not known whether this metabolite is present in the serum of humans with hypervitaminosis D.

clonal antiserum against VDBP (instead of an anti-VDBP monoclonal antibody) to measure VDBP failed to validate the previous report and concluded that total 25(OH)D was an appropriate measure of vitamin D nutritional status (303–306). There is a paucity of information about the role of VDBP in human vitamin D toxicity. The biological role of VDBP was explored in mice in which the VDBP gene had been deleted (307). On vitamin D-replete diets, $DBP^{-/-}$ mice had low levels of total serum vitamin D metabolites but were otherwise normal. When maintained on vitamin D-deficient diets, the $DBP^{-/-}$, but not $DBP^{+/+}$, mice developed secondary hyperparathyroidism and the accompanying bone changes associated with vitamin D deficiency. After an overload of vitamin D, $DBP^{-/-}$ mice

4. Hypercalcemia associated with the administration of 1α-hydroxylated vitamin D metabolites and analogs

Several 1α-hydroxylated vitamin D compounds are available for the treatment of secondary hyperparathyroidism seen in the context of chronic renal failure and

Table 3. Usual Daily Dose Requirement for the Treatment of Secondary Hyperparathyroidism and Duration of Toxicity of Vitamin D Analogs in Chronic Renal Failure

Analog	Potency Relative to Vitamin D_3	Daily Dose, μg	Duration of Toxicity, d
Vitamin D_3	1	750–10 000	17–30
Dihydrotachysterol	10	200–1000	17–30
25(OH)D_3	50	50–200	15–30
1α(OH)D_3	5000	0.5–2.0	5–15
1α,25(OH)$_2D_3$	5000	0.25–2.0	2–7

Data are from Ref. 310.

end-stage renal disease and in various forms of inherited rickets. 1α,25(OH)$_2D_3$ (calcitriol), 1α,(OH)D_3 (alfacalcidol), doxercalciferol (Hectrol), paricalcitol (Zemplar), and 22-oxacalcitriol are examples of such drugs that are available in the United States and Europe. Other drugs, such as dihydrotachysterol (313, 314) and 5,6-*trans*-25(OH)D_3 (312), also have hydroxyl groups in the 1α configuration in the A-ring of the sterol. All are capable of causing hypercalcemia when administered in excess. Some drugs, such as paricalcitol, are believed to be less hypercalcemic than others, such as calcitriol (315–322). Table 3 shows the relative potencies of various vitamin D analogs in chronic renal failure and the duration of toxicity.

5. Treatment of hypercalcemia associated with hypervitaminosis D

Treatment of hypercalcemia associated with hypervitaminosis D includes withholding the vitamin D preparation. In individuals with no previous renal dysfunction, the administration of isotonic fluids with or without a loop diuretic such as furosemide and the administration of glucocorticoids are usually effective in reducing serum calcium concentrations. In patients with chronic renal failure receiving 1α-hydroxylated vitamin D analogs, withholding the drug may be sufficient. If sufficient renal function is still present, administration of isotonic fluids and a loop diuretic will be of value. Glucocorticoids, which act by inhibiting intestinal calcium absorption through the inhibition of enterocyte basolateral membrane calcium extrusion and inhibition of intestinal cell RNA polymerase activity (323–325), will also help in this circumstance. Patients on hemodialysis will need to have the offending drug withheld and, if hypercalcemia persists, may require dialysis against a low calcium hemodialysis bath (2 mEq/L calcium).

D. Hypercalcemia associated with granulomatous disease

As noted in Tables 1 and 2, granulomatous disease is associated with hypercalcemia.

1. Sarcoidosis

Hypercalcemia in sarcoidosis has been described since the 1930s (326, 327). Up to 10% of patients with sarcoidosis have hypercalcemia of varying degrees (328, 329). The association of sunlight exposure with hypercalcemia raised the possibility that abnormal vitamin D metabolism might play a role in the pathogenesis of hypercalcemia (330). Hypercalciuria responsive to cortisone and sodium phytate therapy suggested abnormal intestinal calcium metabolism and hypervitaminosis D (331, 332). Bell and Bartter (333) suggested the presence of increased sensitivity of bone to vitamin D in patients with sarcoidosis. The finding of increased serum 1α,25(OH)$_2D_3$ concentrations in patients with sarcoidosis explained many of the prior findings (334, 335). Investigators demonstrated the presence of elevated serum concentrations of 1α,25(OH)$_2D_3$ in an anephric subject and patients with end-stage renal disease, thus establishing that the kidney was not the source of the elevated serum concentrations of 1α,25(OH)$_2D_3$ (336, 337). The earlier observations of Bell and Bartter (338) that hypercalcemia in sarcoidosis persisted after the occurrence of concomitant nephritis are consistent with the presence of a nonrenal source of 1α,25(OH)$_2D_3$ production (338).

Mason et al (339) described the metabolic conversion of 25(OH)D_3 to 1,25(OH)$_2D_3$ by sarcoid lymph node homogenates but not by normal lymph nodes. Adams et al (340–343) showed that pulmonary alveolar macrophages derived from patients with sarcoidosis metabolized 25(OH)D_3 to 1α,25(OH)$_2D_3$. The 25(OH)D_3-1α-hydroxylase present in sarcoid-associated pulmonary alveolar macrophages (PAMs) has properties distinct from that of the native renal enzyme. PAM 25(OH)D_3-1α-hydroxylase is stimulated by γ-interferon and is not inhibited by 1,25(OH)$_2D_3$ or calcium (340, 3444–347). The enzyme is not stimulated by PTH (343), and 1,25(OH)$_2D_3$ does not induce 25(OH)D_3-24-hydroxylase activity in PAMs (340).

The symptoms and signs of hypercalcemia in the context of sarcoidosis are similar to those found in hypercalcemia due to excessive exogenous vitamin D intake. Laboratory findings are similar except for the presence of increases in serum angiotensin-converting enzyme concentrations that are also found in other granulomatous diseases such as leprosy also found in other granulomatous diseases, and generally correlate with disease activity in sarcoidosis (328, 348–355). Serum 25(OH)D concentrations are normal, whereas 1α,25(OH)$_2$D concentrations are elevated (334, 335, 337, 341, 345, 356). Treatment regimens are similar to those used for the treatment of hypercalcemia. Glucocorticoids are effective in suppressing the activity of the PAM 25(OH)D_3-1α-hydrox-

ylase (340) and reducing hypercalcemia, as well as reducing other manifestations of sarcoid activity (357, 358). Ketoconazole, an inhibitor of 25(OH)D_3-1α-hydroxylase activity, has also been effectively used to treat the hypercalcemia of sarcoidosis (135, 359–365).

2. Tuberculosis

Hypercalcemia occurs in patients with tuberculosis. The prevalence is quite variable in patients with the disease, varying from approximately 2.3% in some studies (366) to 10–48% in other studies (367–371). The precise reason for the variability is uncertain, although vitamin D and calcium intake may play a role. It should be kept in mind that rifampin and isoniazid, drugs used in the treatment of tuberculosis, may alter concentrations of serum 25(OH)D and 1,25$(OH)_2$D and thereby reduce the degree of hypercalcemia (372–376). Rifampin induces several enzymes (Cyp3A4, Cyp24A1, and uridine 5′-diphosphoglucuronyltransferases) that degrade 25(OH)D (373–376) and, by reducing substrate, reduce 1,25$(OH)_2$D concentrations. In contrast, isoniazid inhibits 1,25$(OH)_2$D synthesis (371). As in sarcoidosis, pulmonary alveolar macrophages and lymphocytes, as well as macrophages isolated from pleural fluid, express the 25(OH)D_3-1α-hydroxylase (377–381). The pleural fluid:serum 1,25$(OH)_2D_3$ gradient is approximately 2:1, suggesting 1,25$(OH)_2D_3$ production by cells in the pleural cavity (381). Toll-like receptor activation of human macrophages up-regulates expression of the vitamin D receptor and the 25(OH)D_3-1α-hydroxylase genes (382), the latter increasing 1,25$(OH)_2D_3$ synthesis. Additionally, pleural fluid contains substances such as γ-interferon that potentiate 25(OH)D_3-1α-hydroxylase expression (380). 1,25$(OH)_2D_3$ potentiates macrophage killing of *Mycobacterium tuberculosis* bacteria through the generation of antimicrobial peptides, the cathelicidins (382, 383).

The symptoms and signs of hypercalcemia in the context of tuberculosis are similar to those found in hypercalcemia due to excessive exogenous vitamin D intake. Laboratory findings include suppressed PTH, elevated 1α,25$(OH)_2$D, and usually normal 25(OH)D concentrations. Treatment regimens are similar to those used for the treatment of hypercalcemia. Patients with tuberculosis frequently receive vitamin D supplements, which should be eliminated. Ketoconazole, an inhibitor of 25(OH)D_3-1α-hydroxylase activity, has also been effectively used to treat the hypercalcemia of tuberculosis (365, 384).

3. Leprosy, fungal diseases, and other granulomatous disorders

Hypercalcemia has been reported in association with infections such as leprosy (385–389), *Mycobacterium avium* complex (390–395), Bacille Calmette Guérin administration (396, 397), a variety of fungal infections (see Table 2) (398–411), cat-scratch disease (412), and *Pneumocystis* pneumonia (244, 413–417). A number of noninfectious granulomatous conditions are also associated with hypercalcemia, including Wegener's granulomatosis (418), Crohn's disease (419–421), infantile subcutaneous fat necrosis (422, 423), giant cell polymyositis (424), berylliosis (365, 425), silicone-induced granuloma (426–428), paraffin-associated granulomas (429, 430), and talc granuloma (431). The mechanism for the hypercalcemia in these disorders is the ectopic production of 1α,25$(OH)_2D_3$.

4. Lymphomas

Hodgkin, non-Hodgkin, and adult T-cell leukemia/lymphoma are associated with hypercalcemia (432–435). Hypercalcemia occurs in approximately 13% of non-Hodgkin lymphomas and 5% of Hodgkin lymphomas (432–435). Lymphoma patients with hypercalcemia tend to have more extensive disease and reduced survival (436). Increased serum levels of 1α,25$(OH)_2D_3$ have been implicated in the pathogenesis of hypercalcemia in virtually all cases of Hodgkin lymphoma and in 30–40% non-Hodgkin lymphoma (434). It is likely that the production of 1α,25$(OH)_2D_3$ occurs at extrarenal sites inasmuch as patients with lymphoma have had elevated 1α,25$(OH)_2D_3$ despite the presence of renal failure (437). The production of 1α,25$(OH)_2D_3$ in vitro in lymph node homogenates supports the concept of extrarenal production of the hormone (438).

Adult T-cell leukemia/lymphoma is associated with hypercalcemia in 50–70% of patients with this disease, but the mechanism of hypercalcemia is independent of vitamin D and is often associated with the expression of PTHrP (439, 440) or other cytokines (441–443). The human T lymphotropic virus (HTLV) that is often associated with adult T-cell leukemia/lymphoma elaborates a protein (HTLV-1 transactivator protein, tax) that binds to and activates the PTHrP promoter (444–450). IL-2-mediated stimulation of the PTHrP promoter has been reported in HTLV-1 infected cells (451). Osteoclastogenesis and activity may be influenced by increased expression of Wnt5 and Dkk1 and inhibition of expression of osteoprotegerin by HTLV-1 transfected cells (452–454).

E. Hypercalcemia associated with CYP24A1 mutations

1. Inactivating CYP24A1 mutations and hypercalcemia

Idiopathic infantile hypercalcemia (IIH) is characterized by hypercalcemia, hypercalciuria, nephrocalcinosis, and failure to thrive. The role of vitamin D in IIH was

considered in the United Kingdom in the 1950s when over 200 children were diagnosed with this condition (112, 65). At that time, infants routinely received up to 4000 IU of vitamin D per day between fortified milk powder, infant cereal, and supplementation with cod liver oil (65). As a result, a reduction in vitamin D intake for infants was recommended. In the 1960s, the Committee on Nutrition of the American Academy of Pediatrics also provided guidance on vitamin D fortification for infant formula, suggesting a limit of 400 IU per day in an effort to prevent rickets while avoiding possible toxicity (67, 68).

In 2011, Schlingmann et al (136) described 10 patients with IIH due to loss of function mutations in the *CYP24A1* gene. The majority of the patients were symptomatic at the time of diagnosis with failure to thrive, dehydration, hypotonia, and lethargy. All experienced hypercalciuria and/or nephrocalcinosis. Several of the patients were receiving only modest doses of vitamin D daily (500 IU/d), whereas others had received high doses less frequently (600 000 IU/dose).

In 2012, we described the presence of a similar syndrome in adults (140). Since these original reports, numerous groups have collectively described the clinical and biochemical phenotype of over 100 patients with mono- or biallelic mutations in the *CYP24A1* gene (49, 50, 55, 56, 60–64, 141, 147, 455–459).

2. The syndrome of hypercalcemia, hypercalciuria, nephrocalcinosis, and nephrolithiasis due to CYP24A1 mutations

The clinical manifestations of this disease depend largely on the age at diagnosis. As noted above, infants present with weight loss or failure to thrive, vomiting, dehydration, lethargy, and hypotonia (50, 62, 139, 140, 146, 456, 457). Some infants and children have been asymptomatic at diagnosis and were discovered only after evaluation due to positive family history (63, 139, 140). In some cases, this was attributed to avoidance of vitamin D supplementation in a younger child due to hypercalcemia experienced by the older sibling. Adults with *CYP24A1* mutations most frequently present with renal manifestations such as nephrolithiasis and/or nephrocalcinosis and may experience polyuria. The degree of hypercalcemia (and symptoms) can vary from mild and intermittent to severe but in general is less pronounced compared to those who manifest disease during infancy. As with other causes of vitamin D-mediated hypercalcemia, adults may develop neuropsychiatric symptoms such as lethargy, confusion, and irritability. Gastrointestinal symptoms can include abdominal pain, nausea, vomiting, and constipation. Additional features described in adults with *CYP24A1* mutations include hypertension (56, 63, 142, 144, 458) and pancreatitis (56). Exposure to ultraviolet radiation due to seasonal changes or tanning bed use has been implicated as a factor altering disease severity in some patients (49, 55, 62). It should be noted that pregnancy is a time when this condition may initially manifest or progress as a result of increased $1\alpha,25(OH)_2D$ production. Worsening hypercalcemia during pregnancy or shortly after delivery has been described in several recent reports (56, 141, 146). It has long been recognized that calcitriol concentrations are elevated during normal pregnancy (129, 460), which will lead to exacerbation of hypercalcemia and hypercalciuria in women lacking an adequate calcitriol disposal pathway due to *CYP24A1* mutations. A review of the changes in mineral and bone metabolism during pregnancy has been recently published and details the changes in calcium, PTH, and vitamin D concentrations during gestation (131).

The effect of this condition on bone health and bone density is not clear. The few reports that included bone mineral density assessment have yielded conflicting results ranging from low, to normal, to clearly elevated bone mineral density (49, 140, 142, 145, 455). Infants with IIH are frequently treated with a low-calcium diet, which conceivably could lead to low bone density over time. Alternatively, a lifetime of suppressed PTH due to intestinal calcium hyperabsorption and hypercalcemia may produce an elevated bone density such as that seen in patients with acquired hypoparathyroidism. More data are needed to understand the effect of the underlying disease and its treatment on bone health.

Distinguishing laboratory findings include variable degrees of hypercalcemia, low PTH, and an inappropriate $1,25(OH)_2D$ concentration (upper normal or elevated). Infants who are symptomatic nearly universally have moderate to severe hypercalcemia sometimes exceeding 20 mg/dL. Most adults have serum calcium concentrations in the 10–15 mg/dL range. Because the underlying mechanism is an inadequate disposal pathway for active vitamin D and not excessive substrate, 25(OH)D concentrations can be low, normal, or elevated. A family history of hypercalcemia or a personal history of overzealous vitamin D supplementation would be helpful but is not always readily apparent. The biochemical profile of hypercalcemia, low PTH, and elevated $1,25(OH)_2D$ is indistinguishable from patients with endogenous overproduction of $1,25(OH)_2D$ due to granulomatous disease and lymphoma described above.

Low serum concentrations of $24,25(OH)_2D$ have proved useful in identifying patients with *CYP24A1* mutations (93). We recently developed and validated a liquid chromatography-tandem mass spectrometry assay for the measurement of serum $24,25(OH)_2D$ (93). The limits of detection for $24,25(OH)_2D_3$ and $24,25(OH)_2D_2$ were

Figure 4.

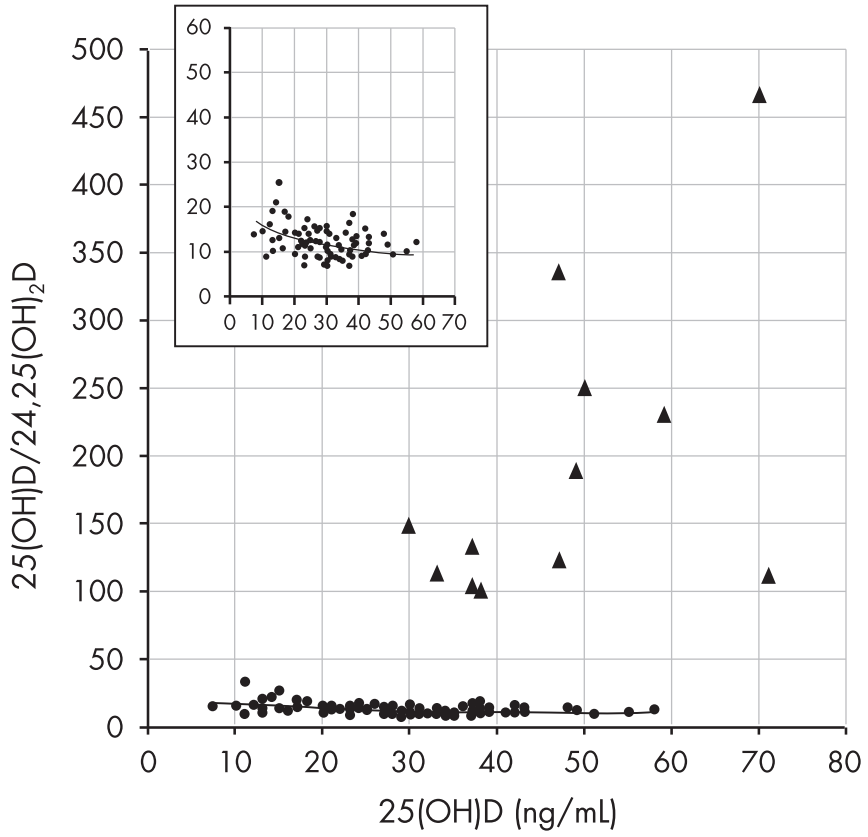

Figure 4. Association between 25(OH)D/24,25$(OH)_2$D in healthy individuals (●) and patients with *CYP24A1* mutations (▲). The inset shows that although the 25(OH)D/24,25$(OH)_2$D ratio at 25(OH)D <20 ng/mL is higher, it still distinguishes between unaffected and affected individuals (93). Modified Figure 4B from: Kumar R, Vallon V. Reduced renal calcium excretion in the absence of sclerostin expression: evidence for a novel calcium–regulating bone kidney axis. J Am Soc Nephrol. 2014 Oct;25(10):2159–68. doi: 10.1681/ASN.2014020166. Epub 2014 May 29. Review. PubMed PMID: 24876121; PubMed Central PMCID: PMC4178449.

0.03 ng/mL (0.2 nmol/L) and 0.1 ng/mL (0.23 nmol/L), respectively; the corresponding limits of quantification were 0.1 ng/mL (0.2 nmol/L) and 0.5 ng/mL (1.2 nmol/L). On the basis of the limits of quantification and the highest calibrators used, the analytical measurement range for undiluted samples was set at 0.1–25 ng/mL (0.2–60 nmol/L) for 24,25$(OH)_2D_3$ and 0.5–25 ng/mL (1.2–58.3 nmol/L) for 24,25$(OH)_2D_2$. Across this range, intra-assay imprecision was 3.1–6.2% for 24,25$(OH)_2D_3$ and 11.7–14.8% for 24,25$(OH)_2D_2$. The corresponding interassay values were 4.5–8.3% and 3.0–10.1%. Recovery of exogenous 24,25$(OH)_2D_3$ and 24,25$(OH)_2D_2$ spiked into samples was 94–100% and 90–94%, respectively. 24,25$(OH)_2D_3$ showed very low cross-reactivity (0.6%) with the spiked 25(OH)D, and 24,25$(OH)_2D_2$ showed 4% cross-reactivity. We observed <5% signal suppression for both 24,25$(OH)_2D_2$ and 24,25$(OH)_2D_3$. 25(OH)D/24,25$(OH)_2$D ratios of 7–35 were observed in healthy subjects. In these individuals, serum 24,25$(OH)_2D_3$ concentrations correlated with 25$(OH)D_3$ concentrations of 7–60 ng/mL (17.5–150 nmol/L): 24,25$(OH)_2D_3 = 0.10 \times 25(OH)D_3 - 0.32$; $r^2 = 0.75$; n = 91 (Figure 4). It should be noted that in the presence of vitamin D excess or deficiency when substrate, namely 25(OH)D, concentrations are high or low, 24,25$(OH)_2$D increases or decreases, but the 25(OH)D/24,25$(OH)_2$D ratio does not change significantly. In patients with Cyp24A1 mutations, 24,25$(OH)_2$D is low as a result of reduced 24-hydroxylase activity, despite the presence of adequate amounts of substrate. As a result, the ratio of 25(OH)D to 24,25$(OH)_2$D measured on a simultaneous sample is elevated. Hence, the assessment of the 25(OH)D/24,25$(OH)_2$D ratio is necessary for the interpretation of 24,25$(OH)_2$D concentrations and the assessment 24-hydroxylase activity. A 25(OH)D/24,25$(OH)_2$D ratio of 7–35 was observed in healthy subjects, whereas in patients with *CYP24A1* mutations, 25(OH)D/24,25$(OH)_2$D was significantly increased (99–467; $P < .001$) (Figure 4). A 25(OH)D/24,25$(OH)_2$D ratio >99 identified patients who were candidates for *CYP24A1* genetic testing (Table 4). Nearly all patients described to date with biallelic disease have a 25(OH)D/24,25$(OH)_2$D ratio >80 (60,140–144, 458). Unaffected patients and most heterozygotes have a ratio <30.

3. Biallelic vs monoallelic disease

Patients with biallelic disease (homozygous or compound heterozygous mutations) consistently demonstrate the clinical and biochemical phenotype described above. It is less clear whether individuals with monoallelic gene changes are asymptomatic carriers or manifest an attenuated condition. We have described two kindreds with some, but not all, monoallelic members having symptomatic disease including IIH (calcium as high as 16 mg/dL), hypercalcemia, hypercalciuria, nephrolithiasis, and/or nephrocalcinosis (60, 140). Other groups have described asymptomatic family members with monoallelic disease

Table 4. Serum 25(OH)D, 1,25$(OH)_2$D, PTH, and 25(OH)D/24,25$(OH)_2$D Ratio in Patients With Genetically Confirmed Cyp24A1 Mutations

Patient No.	25(OH)D, ng/mL	1,25$(OH)_2$D, pg/mL	PTH, pg/mL	25(OH)D/24, 25$(OH)_2$D
1	47	79	24	336
2	70	70	14	467
3	50	123	8.1	250
4	37	101	13	103
5	47	104	22	124
6	37	66	<1	132
7	29.7	82	11	149
8	49	86	9	189
9–11	39–59	83–160	3–10	130–230
12	71	79–121	3	112
13	38			99
14	32.5			113
Reference interval	20–80	22–65	15–65	7–35

Data are from Ref. 93.

who have normal biochemical findings including a normal 25(OH)D/24,25$(OH)_2$D ratio (53, 143). Cools et al (455) recently described heterozygous members of a family and reviewed the available literature regarding the biochemical and clinical phenotype of patients with monoallelic mutations in *CYP24A1*. It should be noted that not all monoallelic patients reported in the literature have been fully phenotyped, leaving much to be learned about this population. In those with available data, however, five of 28 were hypercalcemic (calcium >10.6 mg/dL), seven of 22 had 1,25$(OH)_2$D concentrations >80 pg/mL, nine of 26 had a low PTH (<15 pg/mL), three of 15 had an elevated 25(OH)D:24,25$(OH)_2$D ratio, and eight of 40 had nephrolithiasis and/or nephrocalcinosis. These findings suggest that patients with monoallelic mutations can become symptomatic. It is likely that environment (calcium and vitamin D intake) and other genetic factors affect disease expressivity in this group.

4. Treatment of hypercalcemia and hypercalciuria due to inactivating CYP24A1 mutations

Initial treatment of severe, symptomatic hypercalcemia caused by *CYP24A1* mutations is the same as any other cause of hypercalcemia and should begin with intravenous isotonic saline. A loop diuretic can be added once the patient is adequately hydrated. Intravenous bisphosphonates, calcitonin, and glucocorticoids have been used in the acute setting with variable results (49, 50, 55, 56, 63, 139, 141, 142, 144–146). It is difficult to attribute improvement in hypercalcemia to any single treatment when multiple therapies are provided in the acute setting. However, based on available reports, bisphosphonate therapy appears to be more effective, and glucocorticoids have a limited, if any, role in the acute (or chronic) management of these patients.

Acute management of hypercalcemia during pregnancy is more problematic because some of the available medications are contraindicated. In this population, focusing efforts on calcium restriction and hydration seems prudent. The effects of hypercalcemia are not limited to the mother because intrauterine hypercalcemia can lead to fetal/neonatal PTH suppression with resulting severe and sometimes prolonged hypoparathyroidism and hypocalcemia after birth (61, 66, 88, 131). Infants born to mothers with hypercalcemia should be monitored closely for hypocalcemia, especially if maternal hypercalcemia was moderate to severe.

Long-term management is focused on eliminating or minimizing symptoms of hypercalcemia and reducing hypercalciuria (and thus nephrocalcinosis/nephrolithiasis). Because the underlying mechanism of disease is intestinal calcium hyperabsorption, a low-calcium and vitamin D diet is the cornerstone of therapy. Although this is sufficient for some patients, others will remain hypercalcemic with ongoing active renal stone disease. A variety of long-term strategies have been described including glucocorticoids, loop and thiazide diuretics, phosphate supplementation, proton pump inhibitors, and antifungals such as ketoconazole and fluconazole. Glucocorticoids have not consistently been shown to be effective and would not be a desirable long-term solution due to a multitude of toxicities associated with glucocorticoid exposure (49, 141, 146, 457). Thiazide diuretics may reduce urine calcium without exacerbating hypercalcemia in some patients (49) but have been implicated for producing significant hypercalcemia in others (144). Normalization of serum calcium with reductions in urine calcium have been described in several patients treated with the azole drugs, ketoconazole or fluconazole, acting to inhibit 25(OH)D-1-hydroxylase (55, 140, 141, 143–145). Toxicity and off-target P450 enzyme blockade from azole drugs will limit their long-term use in many patients. It is interesting that patients do not sufficiently down-regulate 25(OH)D-1-hydroxylase activity sufficiently despite the suppression of PTH and elevation in serum calcium concentrations. This would suggest that 25(OH)D-1-hydroxylase is to some degree constitutively active. A selective, titratable inhibitor of 25(OH)D-1-hydroxylase would be optimal but is not currently available.

III. Summary and Conclusions

Vitamin D-mediated hypercalcemia occurs as a result of diverse mechanisms including excessive ingestion of vita-

min D and its metabolites, ectopic enzyme overexpression, and mutations of inactivating enzymes. Diagnosis of vitamin D-mediated hypercalcemia is usually based on the presence of hypercalcemia, elevated concentrations of various vitamin D metabolites in the presence of a suppressed concentration of PTH. A few select biochemical tests will allow the diagnosis to be established. Treatment with various drugs or by withholding vitamin D and/or calcium is usually successful in treating vitamin D-mediated hypercalcemia.

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