

# Pharmacokinetics of vitamin D toxicity<sup>1-4</sup>

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## ABSTRACT

Although researchers first identified the fat-soluble vitamin cholecalciferol almost a century ago and studies have now largely elucidated the transcriptional mechanism of action of its hormonal form,  $1\alpha,25$ -dihydroxyvitamin  $D_3$  [ $1\alpha,25(OH)_2D_3$ ], we know surprisingly little about mechanisms of vitamin D toxicity. The lipophilic nature of vitamin D explains its adipose tissue distribution and its slow turnover in the body (half-life  $\approx 2$  mo). Its main transported metabolite, 25-hydroxyvitamin  $D_3$  [ $25(OH)D_3$ ], shows a half-life of  $\approx 15$  d and circulates at a concentration of 25–200 nmol/L, whereas the hormone  $1\alpha,25(OH)_2D_3$  has a half-life of  $\approx 15$  h. Animal experiments involving vitamin  $D_3$  intoxication have established that  $25(OH)D_3$  can reach concentrations up to 2.5  $\mu\text{mol/L}$ , at which it is accompanied by hypercalcemia and other pathological sequelae resulting from a high  $\text{Ca/PO}_4$  product. The rise in  $25(OH)D_3$  is accompanied by elevations of its precursor, vitamin  $D_3$ , as well as by rises in many of its dihydroxy- metabolites [ $24,25(OH)_2D_3$ ;  $25,26(OH)_2D_3$ ; and  $25(OH)D_3$ -26,23-lactone] but not  $1\alpha,25(OH)_2D_3$ . Early assumptions that  $1\alpha,25(OH)_2D_3$  might cause hypercalcemia in vitamin D toxicity have been replaced by the theories that  $25(OH)D_3$  at pharmacologic concentrations can overcome vitamin D receptor affinity disadvantages to directly stimulate transcription or that total vitamin D metabolite concentrations displace  $1\alpha,25(OH)_2D_3$  from vitamin D binding, increasing its free concentration and thus increasing gene transcription. Occasional anecdotal reports from humans intoxicated with vitamin D appear to support the latter mechanism. Although current data support the viewpoint that the biomarker plasma  $25(OH)D$  concentration must rise above 750 nmol/L to produce vitamin D toxicity, the more prudent upper limit of 250 nmol/L might be retained to ensure a wide safety margin. *Am J Clin Nutr* 2008;88(suppl):582S–6S.

## INTRODUCTION

Research has shown that the mechanism of action of vitamin  $D_3$ , through its hormonal form,  $1\alpha,25$ -dihydroxyvitamin  $D_3$  [ $1\alpha,25(OH)_2D_3$ ], involves a nuclear receptor [vitamin D receptor (VDR)] that regulates the transcription of several target genes in a variety of vitamin D target cells that are primarily involved in calcium homeostasis and cell differentiation (1). To allow vitamin D to achieve these important regulatory functions, normal physiology has evolved several specific proteins that constitute the vitamin D signal transduction system (Figure 1).

These specialized proteins, in addition to the VDR and its associated transcriptional coactivators, include the plasma transport protein, vitamin D binding protein (DBP); the activating cytochrome P450s (CYP2R1, CYP27A1, and CYP27B1); and the catabolic cytochrome P450 CYP24A1. CYP24A1 plays a

critical role in the degradation of both 25-hydroxyvitamin  $D_3$  [ $25(OH)D_3$ ] and  $1\alpha,25(OH)_2D_3$  through a side-chain hydroxylation and cleavage pathway known as C-24 oxidation (5). Recent research using the CYP24A1-null mouse has shown that CYP24A1 plays a vital role in vivo in that its absence leads to an inability to degrade vitamin D metabolites to C-24 oxidation products or 26,23-lactone products. This resulted in hypercalcemia, nephrocalcinosis, and death in 50% of animals (6).

Because vitamin D toxicity resulting from excessive vitamin D intakes (hypervitaminosis D) is also accompanied by hypercalcemia, toxicity is remarkably similar to the CYP24A1-null mouse. One can therefore postulate that overactivity of the vitamin D signal transduction system in hypervitaminosis D is also the result of an inability of the catabolic system involving CYP24A1 to keep up with the target cell levels of activated vitamin D metabolites. Indeed, investigators have postulated several variations of this theme, each involving a different metabolite (or metabolites) that, together, overwhelm the vitamin D signal transduction process, to explain the vitamin D toxicity of hypervitaminosis D. I review these explanations of vitamin D toxicity, along with the animal and human data assembled to support them.

## IMPORTANT DETERMINANTS OF VITAMIN D PHARMACOKINETICS RELEVANT TO HYPERVITAMINOSIS D

Vitamin  $D_3$  is a lipophilic molecule similar to its closely related lipid precursor cholesterol, so it requires a protein carrier for solubility in plasma. Its mono-, di-, and tri-hydroxylated metabolites show progressively increasing polarity, culminating in the water-soluble biliary form calcitric acid (7). When absorbed from the gut, vitamin D enters the circulation on chylomicrons first, and it is only slowly transferred to DBP (8). Vitamin D has relatively low affinity for DBP; reviews estimate this at between  $1 \times 10^{-5}$  and  $1 \times 10^{-7}$  mol/L (9, 10).

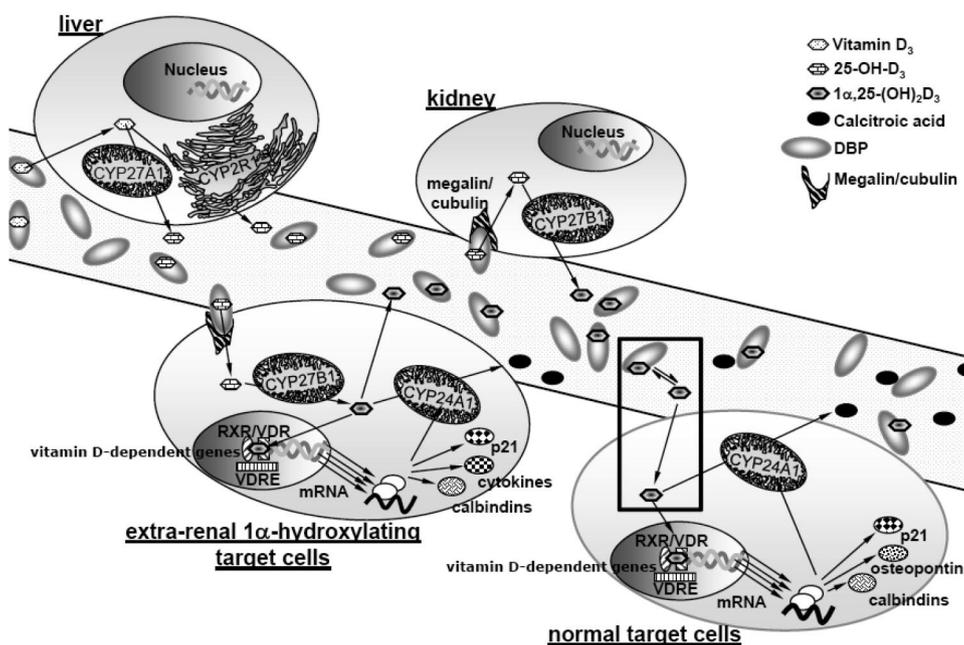
Transport of dietary vitamin D contrasts significantly with that of vitamin  $D_3$  made during skin synthesis, which is mainly bound

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<sup>2</sup> Presented at the National Institutes of Health conference "Vitamin D and Health in the 21st Century: an Update," held in Bethesda, MD, September 5–6, 2007.

<sup>3</sup> Supported by grants from the Canadian Institutes for Health Research and Cytochroma Inc, Markham, Ontario, Canada.

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**FIGURE 1.** Free 1,25-dihydroxyvitamin D theory of target cell entry. This figure depicts most components of vitamin D signal transduction. Vitamin D<sub>3</sub> (upper left side of the diagram) is carried to the liver, where one of a series of 25-hydroxylases—the major ones are the microsomal CYP2R1 and the mitochondrial CYP27A1—activates it. The resulting 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] is then carried on vitamin D binding protein (DBP) to the kidney, where the classic renal CYP27B1 1 $\alpha$ -hydroxylates it and puts it back into circulation as 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> [1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>]. 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> then enters and acts on vitamin D target cells at the level of gene transcription through a VDR-mediated mechanism (cell at bottom right). Note that according to this vitamin D signal transduction, 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> arrives at the target cell bound to DBP, but its entry into the cell is a function of the free pool of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (shown inside the red box) in equilibrium with protein-bound 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>. In hypervitaminosis D, some investigators argue that saturation of the DBP displaces a greater amount of free 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>, thereby increasing gene expression and causing hypercalcemia and other toxicity symptoms. According to the extrarenal 1 $\alpha$ -hydroxylase theory, some target cells express the membrane proteins megalin or cubulin to concentrate the 25(OH)D<sub>3</sub>-DBP complex and express the protein CYP27B1 to 1 $\alpha$ -hydroxylate 25(OH)D. Some believe that in this way, certain target cells (eg, macrophages and monocytes) boost 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> production by augmenting the circulating 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> concentrations produced by the kidney. Although research has not proven the theory, many of its facets are consistent with the observed health advantages of vitamin D and with emerging epidemiologic data (2, 3). Adapted with permission from reference 4. RXR, retinoid X receptor; VDR, vitamin D receptor; VDRE, vitamin D responsive element.

to DBP (8). The consequence of chylomicron transport of dietary vitamin D is the possibility of uptake by peripheral tissues, such as adipose tissue and muscle, due to the action of lipoprotein lipase (11). The liver takes up the vitamin D left in the chylomicron remnant and quickly removes it from the bloodstream.

The loss into tissue and liver pools thus logically explains the short plasma half-life,  $\approx 4$ –6 h, of physiologically relevant doses of vitamin D (11). Similar studies with radiolabeled vitamin D have shown that the whole-body half-life is  $\approx 2$  mo (11).

The liver converts vitamin D into 25(OH)D, a process that some attribute to a microsomal cytochrome P450 enzyme, CYP2R1, or the mitochondrial cytochrome P450 CYP27A1; neither is subject to tight regulation (12). 25(OH)D quickly enters the plasma pool that constitutes the predominant pool of vitamin D in the body, with a capacity of  $\approx 4.5$   $\mu$ mol/L. 25(OH)D<sub>3</sub> and 25(OH)D<sub>2</sub>, its vitamin D<sub>2</sub> counterpart, have a strong affinity for DBP at  $5 \times 10^{-8}$  mol/L, which is at least an order of magnitude higher than that of its vitamin D precursors (9, 10). As a consequence, 25(OH)D<sub>3</sub> has a half-life of 15 d in the circulation. The normal circulating level of 25(OH)D [25(OH)D<sub>3</sub> + 25(OH)D<sub>2</sub>] in the blood is only 25–200 nmol/L (13), indicating that ligand only occupies 2–5% of DBP in the physiologic state.

The dihydroxy- metabolites have widely differing affinities for DBP, with 25(OH)D<sub>3</sub>-26,23-lactone binding 3–5 times as tightly as 25(OH)D<sub>3</sub>. The inactive metabolites 24,25(OH)<sub>2</sub>D<sub>3</sub>

and 25,26(OH)<sub>2</sub>D<sub>3</sub> bind with equal affinity as 25(OH)D<sub>3</sub>, and the active form 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> binds DBP with an affinity of  $2 \times 10^{-7}$  mol/L, an order of magnitude less than its precursor (12, 13). 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> has a half-life of 10–20 h (14, 15), although this depends to some extent on the state of the highly inducible catabolic machinery. The accumulation of these metabolites in the bloodstream is mainly a function of their affinity for DBP, although rate of synthesis and degradation must also play a partial role.

Recent studies (16) have conclusively shown that CYP24A1 is responsible for the formation of several principal accumulating metabolites, 24,25(OH)<sub>2</sub>D<sub>3</sub> and 25(OH)D<sub>3</sub>-26,23-lactone, as well as the catabolite calcitric acid. Research has not identified the origin of the minor metabolite 25,26(OH)<sub>2</sub>D<sub>3</sub>, although studies have shown that several CYPs, such as CYP27A1 and CYP24, hydroxylate at C26 (1, 12) under in vitro conditions. Of course, although research has proven that renal CYP27B1 synthesizes the circulating pool of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>, the amount of vitamin D channeled into this active form is tightly regulated (17) and studies have estimated that it is only a small percentage of the total body pool of vitamin D.

Measurements of the plasma level show that circulating 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> is in the picomolar range, a concentration 1000 times lower than that of 25(OH)D. Molecular probes for CYP27B1 mRNA based on reverse transcriptase–polymerase

chain reaction and for CYP27B1 protein based on immune localization studies have shown that this “activating” CYP is expressed not only in the kidney but also in several extrarenal tissues; in addition, it is subject to different regulation than the renal enzyme (Figure 1; 2, 3). This extrarenal  $1\alpha$ -hydroxylase appears to have a paracrine or autocrine function to augment local production of  $1\alpha,25(\text{OH})_2\text{D}_3$  in selected vitamin D target cells (2, 3), although this remains largely unproven.

Accordingly, vitamin D's lipophilicity, affinity of its metabolites for plasma transport carriers, and rates of synthesis and degradation of its metabolites by the vitamin D-related CYPs largely determine the pharmacokinetics and distribution of vitamin D in the body. The affinity of  $1\alpha,25(\text{OH})_2\text{D}_3$  for the VDR plays a major role in determining its role as the sole active metabolite under physiologic conditions; the other components of the vitamin D signal transduction machinery are equally important in keeping the inactive metabolites in the plasma pool and away from the nuclear transcriptional machinery. However, in vitamin D toxicity associated with hypervitaminosis D, this is almost certainly not the case; vitamin D metabolites other than  $1\alpha,25(\text{OH})_2\text{D}_3$  could interfere with the normal delivery of  $1\alpha,25(\text{OH})_2\text{D}_3$  to the target cell or else have effects themselves.

#### EVIDENCE FROM STUDIES OF VITAMIN D INTOXICATION IN ANIMALS

Researchers have conducted several animal studies involving systematic vitamin D intoxication over the past 3 decades in a variety of different species, including rats, cows, pigs, rabbits, dogs, and horses (18–23). As knowledge of vitamin D metabolism might lead one to expect, vitamin intoxication results in elevation of the plasma concentrations of vitamin  $\text{D}_3$ ;  $25(\text{OH})\text{D}_3$ ;  $24,25(\text{OH})_2\text{D}_3$ ;  $25,26(\text{OH})_2\text{D}_3$ ; and  $25(\text{OH})\text{D}_3$ -26,23-lactone, although it rarely raises plasma  $1\alpha,25(\text{OH})_2\text{D}_3$ . The studies by Littledike and Horst (19, 20) in pigs and dairy cows are quite definitive on this point and suggest that the renal CYP27B1 is effectively turned off.

Focus has therefore shifted to the levels of other vitamin D metabolites correlated with toxicity, especially the plasma threshold of  $25(\text{OH})\text{D}$  that must be exceeded to cause hypercalcemia. One of the most widely cited publications in this area is the work of Shephard and DeLuca (18), who acutely intoxicated rats with graded oral doses of vitamin  $\text{D}_3$  (0.65 to 6500 ng/d for 14 d) or  $25(\text{OH})\text{D}_3$  (0.46 to 4600 ng/d for 14 d). They measured all major vitamin D metabolites by reliable HPLC and competitive binding assays at the end of the dosing period. Vitamin  $\text{D}_3$  and  $25(\text{OH})\text{D}_3$  concentrations rose to micromolar levels in plasma of rats given the highest intakes of vitamin  $\text{D}_3$ , resulting in marked hypercalcemia. All dihydroxylated metabolites, including  $24,25(\text{OH})_2\text{D}_3$ ;  $25,26(\text{OH})_2\text{D}_3$ ; and  $25(\text{OH})\text{D}_3$ -26,23-lactone, also rose to concentrations higher than 100 nmol/L, but the level of plasma  $1\alpha,25(\text{OH})_2\text{D}_3$  remained within the normal range.

The study design did not allow for repeated measurements of plasma vitamin D metabolite levels as the hypercalcemia developed in the animals, leaving open the possibility that plasma  $1\alpha,25(\text{OH})_2\text{D}_3$  initially rose and was subsequently suppressed in response to hypercalcemia. Nevertheless, other animal studies of hypervitaminosis D (19, 20) have also repeatedly failed to demonstrate a significant elevation of the hormonal form.

The 10-fold increments in the dose of vitamin  $\text{D}_3$  used in the study by Shephard and DeLuca (18) did not allow for a very

precise prediction of the  $25(\text{OH})\text{D}_3$  threshold that can be correlated with hypercalcemia (toxicity), because a vitamin  $\text{D}_3$  dose of 65 ng/d resulted in a  $25(\text{OH})\text{D}_3$  concentration of  $74 \pm 15$  ng/mL ( $185 \pm 36$  nmol/L) and no hypercalcemia, whereas a vitamin  $\text{D}_3$  dose of 650 ng/d resulted in a  $25(\text{OH})\text{D}_3$  concentration of  $643 \pm 93$  ng/mL ( $1608 \pm 232$  nmol/L) and profound hypercalcemia (total calcium was 12.4 mg/dL). However, the studies with  $25(\text{OH})\text{D}_3$  revealed a remarkable finding: a dose of 460 ng/d resulted in  $25(\text{OH})\text{D}_3$  concentrations of  $436 \pm 53$  ng/mL ( $1090 \pm 132$  nmol/L) with normocalcemia.

Although this study suggests that rodents can tolerate plasma  $25(\text{OH})\text{D}_3$  concentrations in the range of 250–1000 nmol/L, such tolerance represents a relatively unique situation for  $25(\text{OH})\text{D}_3$  administration, in which high circulating vitamin  $\text{D}_3$  that is present when vitamin  $\text{D}_3$  is the dietary form does not accompany elevated concentrations of  $25(\text{OH})\text{D}_3$ . Based on a variety of studies in several animal species, it appears that the plasma  $25(\text{OH})\text{D}$  concentrations associated with toxicity are always in excess of 375 nmol/L. Furthermore, small differences between various mammalian species used in animal experiments are largely irrelevant to the toxicity issue and render the animal data valuable to our discussion.

#### ANECDOTAL EVIDENCE FROM STUDIES OF VITAMIN D INTOXICATION IN HUMANS

For ethical reasons, no systematic studies have examined vitamin intoxication in humans. But numerous anecdotal reports over the years have described accidental vitamin D intoxication with either vitamin  $\text{D}_3$  or vitamin  $\text{D}_2$  (17, 24–33; many reviewed in 34). Because most of these studies measured  $25(\text{OH})\text{D}$  [and sometimes  $1\alpha,25(\text{OH})_2\text{D}$ ], it is worth reviewing the vitamin D metabolite values reported with overt toxicity symptoms.

Although an occasional report did find evidence of modest elevations of  $1\alpha,25(\text{OH})_2\text{D}$  (17), all reported that  $25(\text{OH})\text{D}$  concentrations were well above the normal range at 710–1587 nmol/L, with several patients exhibiting values consistently around 750 nmol/L. In addition, several reports have documented clusters of patients with vitamin D intoxication due to vitamin D-contaminated food sources (35, 36). In a study of 35 hypervitaminotic patients with hypercalcemia resulting from chronic ingestion of overfortified milk (35), the average  $25(\text{OH})\text{D}$  concentration was 560 nmol/L (range: 140–1490 nmol/L). In an extended family group accidentally intoxicated with a veterinary vitamin D concentrate (peanut oil solution containing  $2 \times 10^6$  IU cholecalciferol), Pettifor et al (36) showed that  $25(\text{OH})\text{D}$  concentrations ranged from 847 to 1652 nmol/L in intoxicated family members, whereas plasma  $1\alpha,25(\text{OH})_2\text{D}$  concentrations were within the normal range in 8 of 11 patients. A perusal of these data and the anecdotal reports leads this reviewer to the same conclusion as that of Vieth (34); namely, that hypercalcemia only results when  $25(\text{OH})\text{D}_3$  concentrations have consistently been above 375–500 nmol/L.

#### THEORIES ABOUT THE MECHANISM OF VITAMIN D TOXICITY

Researchers have proposed 3 major theories about the mechanism of vitamin D toxicity. All involve increased concentrations of a vitamin D metabolite reaching the VDR in the nucleus of target cells and causing exaggerated gene expression. At issue



is the offending vitamin D metabolite and how it becomes elevated. The 3 hypotheses to explain this are as follows:

1) Vitamin D intake raises plasma  $1\alpha,25(\text{OH})_2\text{D}$  concentrations, which increase cellular  $1\alpha,25(\text{OH})_2\text{D}$  concentrations.

2) Vitamin D intake raises plasma  $25(\text{OH})\text{D}$  to  $\mu\text{mol/L}$  concentrations that exceed the DBP binding capacity and “free  $25(\text{OH})\text{D}$ ” enters the cell, where it has direct effects on gene expression.

3) Vitamin D intake raises the concentrations of many vitamin D metabolites, especially vitamin D itself and  $25(\text{OH})\text{D}$ . These concentrations exceed the DBP binding capacity and cause release of “free”  $1\alpha,25(\text{OH})_2\text{D}$ , which enters target cells.

In weighing each of these hypotheses, it is useful to consider other factors, in addition to elevated dietary vitamin D intakes, that result in symptoms of vitamin D toxicity. One disease that can result in vitamin D toxicity is the granulomatous condition sarcoidosis, but only when strong evidence exists that the mechanism involves consistently elevated plasma  $1\alpha,25(\text{OH})_2\text{D}_3$  concentrations due to overexpression of unregulated extrarenal CYP27B1 in the face of normal concentrations of  $25(\text{OH})\text{D}$  (37–39). In addition, the widespread use of calcitriol or its analogues to treat secondary hyperparathyroidism in chronic kidney disease occasionally results in such symptoms as hypercalcemia, hyperphosphatemia, and soft-tissue calcifications; these symptoms are almost certainly the result of overexposure of target cells to active analogue (40).

Thus, toxicity due to  $1\alpha,25(\text{OH})_2\text{D}$  (hypothesis 1 above) is well known but correlated with a high plasma concentration of the hormone. But when we observe toxicity due to high vitamin D intakes, it is rarely accompanied by elevated total  $1\alpha,25(\text{OH})_2\text{D}_3$  concentrations. Thus, although overproduction of the active form due to excessive precursor levels (hypothesis 1) might be the most obvious explanation of hypervitaminosis D, animal and human data do not strongly support it.

Before any discussion of alternate hypotheses 2 or 3, we must return to the model for the normal action of  $1\alpha,25(\text{OH})_2\text{D}_3$ . The low affinity of  $1\alpha,25(\text{OH})_2\text{D}_3$  for the transport protein DBP and its high affinity for VDR dominate normal physiology, making it the only ligand with access to the transcriptional signal transduction machinery. However, in vitamin D intoxication, overloading by various vitamin D metabolites significantly compromises the capacity of the DBP by allowing other metabolites to enter the cell nucleus. Of all the inactive metabolites,  $25(\text{OH})\text{D}_3$  has the strongest affinity for the VDR and thus, at sufficiently high concentrations, could stimulate transcription. Some evidence using highly artificial in vitro transactivation systems indicates that D metabolites other than  $1\alpha,25(\text{OH})_2\text{D}_3$  might directly stimulate gene transcription (41). Whether  $\approx 750$  nmol/L of plasma  $25(\text{OH})\text{D}_3$ , a level associated with hypercalcemia in many human and animal studies (17–33), is sufficiently high enough to overcome plasma DBP binding and enter the cell's nucleus to stimulate gene transcription is debatable.

Considerable discussion in the literature focuses on the exact concentration of  $1\alpha,25(\text{OH})_2\text{D}_3$  required for gene expression. The original theory for classical steroid action postulated that most circulating hormone is protein bound in the plasma and gains entry to cells in a “free” form of the hormone that crosses the cell membrane, binds to the nuclear receptor, and triggers gene expression. Many researchers have assumed that  $1\alpha,25(\text{OH})_2\text{D}_3$ , being a secosteroid, mimics other steroids and thus its “free” form is the most important (Figure 1) (42).

In fact, Bikle et al (43, 44) have pioneered methods for determining free concentrations of  $1\alpha,25(\text{OH})_2\text{D}_3$  and  $25(\text{OH})\text{D}_3$  in clinical samples that are based on measuring DBP and total metabolites. Accordingly, in hypervitaminosis D, in which vitamin D metabolites [such as vitamin  $\text{D}_3$ ;  $25(\text{OH})\text{D}_3$ ;  $24,25(\text{OH})_2\text{D}_3$ ;  $25,26(\text{OH})_2\text{D}_3$ ; and  $25(\text{OH})\text{D}_3$ -26,23-lactone] saturate the DBP in the bloodstream, the free concentrations of some of the most important metabolites, such as  $1\alpha,25(\text{OH})_2\text{D}_3$  and  $25(\text{OH})\text{D}_3$ , could increase significantly.

In the investigation of the family accidentally intoxicated by vitamin  $\text{D}_3$  in peanut oil discussed above (36), the authors used the methods of Bikle (43, 44) to investigate free  $1\alpha,25(\text{OH})_2\text{D}_3$  concentrations. They found that unlike total  $1\alpha,25(\text{OH})_2\text{D}_3$  concentrations, which remained within the normal range in 8 of 11 family members, free  $1\alpha,25(\text{OH})_2\text{D}_3$  concentrations were  $>2$  SDs above the reference range in 6 of 9 patients studied. The authors concluded that the micromolar concentrations of  $25(\text{OH})\text{D}$  and other metabolites displace free  $1\alpha,25(\text{OH})_2\text{D}$  from DBP.

These investigators also simulated in vitro the displacement of  $1\alpha,25(\text{OH})_2\text{D}$  from DBP noted in hypervitaminotic plasma by adding  $25(\text{OH})\text{D}_3$  to normal serum at the 800-nmol/L concentration found in hypervitaminotic plasma. They found a virtual doubling of free  $1\alpha,25(\text{OH})_2\text{D}$  concentrations. Although these results are quite convincing, few other data are available to support hypothesis 3, and one must conclude that the theory remains unproven.

## CONCLUSIONS

Our current understanding of the components of the vitamin D signal transduction machinery (DBP, activating CYPs, VDR, and CYP24) allows us to theorize in broad terms about how vitamin D toxicity might arise from hypervitaminosis D. Of the 3 hypotheses put forward to explain the triggering event for toxicity, increases in total  $25(\text{OH})\text{D}$  and free  $1\alpha,25(\text{OH})_2\text{D}$  concentrations are the most plausible, although they remain unproven. However, even in the absence of definitive evidence to establish the responsible metabolite, the wealth of animal studies and human anecdotal reports of vitamin D intoxication indicate that plasma  $25(\text{OH})\text{D}_3$  is a good biomarker for toxicity, and the threshold for toxic symptoms is  $\approx 750$  nmol/L. This threshold value implies that  $25(\text{OH})\text{D}$  concentrations up to the currently considered upper limit of the normal range, namely 250 nmol/L, are safe and still leave a broad margin for error because values significantly higher than this value have never been associated with toxicity.

David Prosser helped with the artwork used in this review. The author had no conflicts of interest.

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