From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health[1–4]

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ABSTRACT

New knowledge of the biological and clinical importance of the steroid hormone 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] and its receptor, the vitamin D receptor (VDR), has resulted in significant contributions to good bone health. However, worldwide reports have highlighted a variety of vitamin D insufficiency and deficiency diseases. Despite many publications and scientific meetings reporting advances in vitamin D science, a disturbing realization is growing that the newer scientific and clinical knowledge is not being translated into better human health. Over the past several decades, the biological sphere of influence of vitamin D_3, as defined by the tissue distribution of the VDR, has broadened at least 9-fold from the target organs required for calcium homeostasis (intestine, bone, kidney, and parathyroid). Now, research has shown that the pluripotent steroid hormone 1α,25$(OH)_2D_3$ initiates the physiologic responses of $\geq$36 cell types that possess the VDR. In addition to the kidney's endocrine production of circulating 1α,25$(OH)_2D_3$, researchers have found a paracrine production of this steroid hormone in $\geq$10 extrarenal organs. This article identifies the fundamentals of the vitamin D endocrine system, including its potential for contributions to good health in 5 physiologic arenas in which investigators have clearly documented new biological actions of 1α,25$(OH)_2D_3$ through the VDR. As a consequence, the nutritional guidelines for vitamin D_3 intake (defined by serum hydroxyvitamin D_3 concentrations) should be reevaluated, taking into account the contributions to good health that all 36 VDR target organs can provide. *Am J Clin Nutr* 2008;88(suppl):491S–9S.

INTRODUCTION

Vitamin D_3 is essential for life in higher animals. Research has shown, for example, that vitamin D_3 is one of the primary biological regulators of calcium homeostasis. Vitamin D_3's important biological effects occur only as a consequence of its metabolism into a family of daughter metabolites, including the key kidney-produced metabolite 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. Researchers consider 1α,25$(OH)_2D_3$ to be a steroid hormone and believe that it functions the same way as other steroid hormones— by interacting with its cognate vitamin D receptor (VDR) (1).

The role of vitamin D_3 as a vitamin or essential dietary component, in concert with the biological and clinical importance of the steroid hormone 1α,25$(OH)_2D_3$ and the VDR, has achieved increasing prominence over the past 3 to 4 decades in the public health arena because of its contributions to good health in the general public. However, despite the plethora of publications and scientific meetings focusing on advances in vitamin D science, there is the disturbing realization that all is not "well" with the translation of the newer scientific and clinical knowledge into the achievement of better health. Scientists and nutrition experts at the 13th Vitamin D Workshop held in 2006 agreed in a consensus statement that "about half of the elderly in North America and two-thirds of the rest of the world are not getting enough vitamin D to maintain healthy bone density, lower their risks for fractures and improve tooth attachment. Such vitamin D insufficiency also decreases muscle strength and increases the risk for falls and is even associated with increased risk for colorectal and other major cancer" (2).

The purpose of this article is to identify the fundamentals of the vitamin D endocrine system and the actions of 1α,25$(OH)_2D_3$ that are essential for good health, keeping in mind that the totality of their contributions depends on the adequate availability of 25-hydroxyvitamin D_3 [25$(OH)D_3$], which in turn depends on appropriate vitamin D nutritional status as determined by ultraviolet (UV) radiation exposure and dietary intake of vitamin D_3.

SOURCES OF VITAMIN D

Vitamin D is not technically a vitamin, ie, it is not an essential dietary factor; rather, it is a prohormone produced photochemically in the skin from 7-dehydrocholesterol. The molecular structure of vitamin D is closely allied to that of classic steroid hormones (eg, estradiol, cortisol, and aldosterone) in that they have the same root cyclopentanoperhydrophenanthrene ring structure. Technically, vitamin D is a secosteroid because one of the rings of its cyclopentanoperhydrophenanthrene structure has a broken carbon-carbon bond; in vitamin D, this occurs in the 9,10 carbon-carbon bond of ring B (**Figure 1**). Given that fact as a starting point, the reader must have access to some of the details of the sunlight-mediated photochemical conversion of 7-dehydrocholesterol into vitamin D_3; this information is provided in Figure 1.

The skin produces vitamin D_3 photochemically from the provitamin D, 7-dehydrocholesterol, which is present in the epidermis or skin of higher animals, by the action of sunlight in most geographical locations or of artificial UV light. The conjugated double-bond system in ring B (*see* Figure 1) allows the absorption of light quanta at

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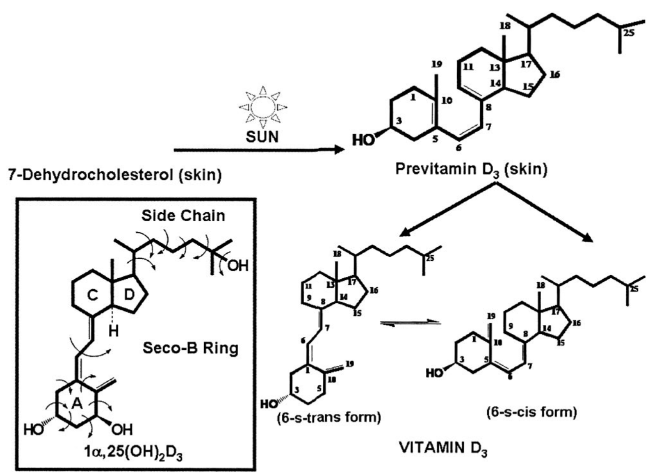

FIGURE 1. Chemistry and irradiation pathway for the production of vitamin D_3. The provitamin, which is characterized by the presence in the B ring of a $\Delta5$, $\Delta7$ conjugated double bond system, is converted to a seco B previtamin steroid after exposure to ultraviolet light and the 9,10 carbon-carbon bond is broken. Next, in a process independent of ultraviolet light, the previtamin D thermally isomerizes to the vitamin form, which is characterized by a $\Delta6,7$, $\Delta8,9$, $\Delta10,19$ conjugated triple bond system. The main portion of the figure also illustrates the 2 principal conformations of the molecule that result from rotation about the 6,7 carbon single bond of the seco B ring: the 6-s-*cis* conformer (the steroid-like shape) and the 6-s-*trans* conformer (the extended shape). The interconversion of the 2 conformers occurs millions of times per second. The extreme conformational flexibility potential of all vitamin D metabolites is illustrated in the inset for the principal metabolite 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$]. Each arrow indicates carbon-carbon single bonds (in the side chain, the seco B ring, and the A ring) that have 360 ° rotational freedom. For all vitamin D molecules, this results in a multitude of different shapes in solution and in biological systems (8).

certain wavelengths in the UV range, initiating a complex series of transformations of the provitamin (partially summarized in Figure 1) that ultimately result in the generation of vitamin D_3. Thus, vitamin D_3 can be endogenously produced. As long as the animal (or human) has access to adequate sunlight on a regular basis, the animal (or human) might not need to obtain this vitamin from the diet. However, dermatologists are concerned that individuals with extensive UV radiation exposure could have an increased risk of skin cancer or melanoma (3). A discussion of the parameters affecting the balance between sun exposure and dietary vitamin D to meet bodily requirements is available elsewhere (4). Because vitamin D_3 is also a vitamin, animals and humans can meet some or all of their vitamin D_3 needs through the diet.

For the past 6 decades, the literature has claimed that vitamin D_3 and vitamin D_2 (*see* **Figure 2** for their structures) have equivalent biological effects in humans. However, with the realization that the serum 25(OH)D clinical assay provides the best assessment of vitamin D nutritional status (5), researchers needed to determine whether vitamin D_2 is as effective in elevating serum 25(OH)D concentrations in humans as is vitamin D_3. Heaney and others recently confirmed earlier data suggesting that vitamin D_3 is substantially more effective than vitamin D_2 (6, 7). In a study of 20 healthy human volunteers, these investigators found that vitamin D_2's potency was less than one-third of vitamin D_3'spotency on the basis of ability to elevate serum 25(OH)D concentrations.

VITAMIN D ENDOCRINE SYSTEM

Vitamin D_3 does not have any known intrinsic biological activity. It must first be metabolized to $25(OH)D_3$ in the liver and then to $1\alpha,25(OH)_2D_3$, $24R$,25-dihydroxyvitamin D_3 [$24R,25(OH)_2D_3$], or both by the kidney. Researchers have isolated and chemically characterized some 37 vitamin D_3 metabolites (8). Investigators have also established that humans and some other animals can metabolize vitamin D_2 to $25(OH)D_2$ and $1\alpha,25(OH)_2D_2$ and many other similar cognate metabolites (9).

The steps in the vitamin D endocrine system (8) include the following (**Figure 3**): *1*) the photoconversion of 7-dehydrocholesterol to vitamin D_3 in the skin or dietary intake of vitamin D_3; *2*) metabolism of vitamin D_3 by the liver to $25(OH)D_3$, the major form of vitamin D circulating in the blood compartment; *3*) conversion of $25(OH)D_3$ by the kidney (functioning as an endocrine gland) to the hormone $1\alpha,25(OH)_2D_3$ and the candidate hormone $24R,25(OH)_2D_3$; *4*) systemic transport of the dihydroxylated metabolites $24R,25(OH)_2D_3$ and $1\alpha,25(OH)_2D_3$ to distal target organs; and *5*) binding of $1,25(OH)_2D_3$ to a nuclear receptor, plasma membrane receptor, or both at the target organs, followed by generation of appropriate biological responses. An additional key component in the operation of the vitamin D endocrine system is the plasma vitamin D binding protein, which carries vitamin D_3 and its metabolites to their metabolism and target organs (10).

The most important regulation point in the classic vitamin D endocrine system occurs through the stringent control of the circulating concentration of the steroid hormone $1\alpha,25(OH)_2D_3$; typically, its production is modulated according to the organism's calcium and other endocrine needs. The chief regulatory factors are $1\alpha,25(OH)_2D_3$ itself, which down-regulates its own production; parathyroid hormone, which stimulates the renal production of $1,25(OH)_2D_3$; fetal growth factor 23; and serum concentrations of calcium and phosphate (11). Probably the most important determinant of the 25(OH)D-1α-hydroxylase activity is the animal's vitamin D nutritional status (11). When the circulating concentration of $1\alpha,25(OH)_2D_3$ is low, production of $1\alpha,25(OH)_2D_3$ by the kidney is high; when the circulating concentration of $1\alpha,25(OH)_2D_3$ is high, the output of $1\alpha,25(OH)_2D_3$ by the kidney decreases sharply (11).

The pervasive contributions of the VDR in collaboration with its ligand, $1\alpha,25(OH)_2D_3$, to the functioning of the vitamin D endocrine system are illustrated in Figure 3 and listed in **Table 1**. Thirty-six tissues definitively possess the VDR, which means that the cells in these tissues have the potential to produce biological responses, depending on the availability of appropriate amounts of vitamin D_3.

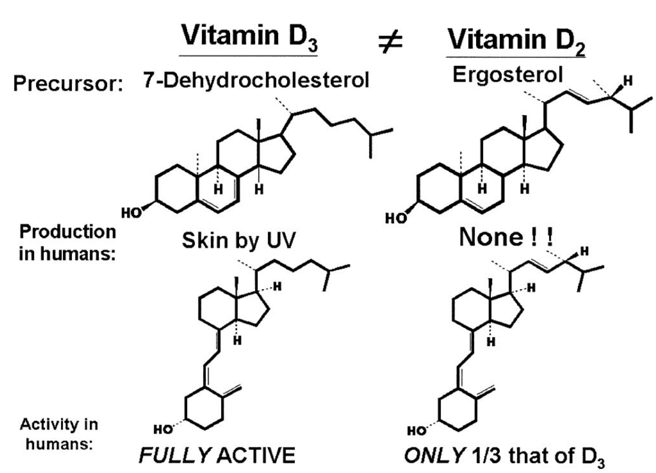

FIGURE 2. Structural and biological similarities and differences between vitamin D_3 and vitamin D_2.

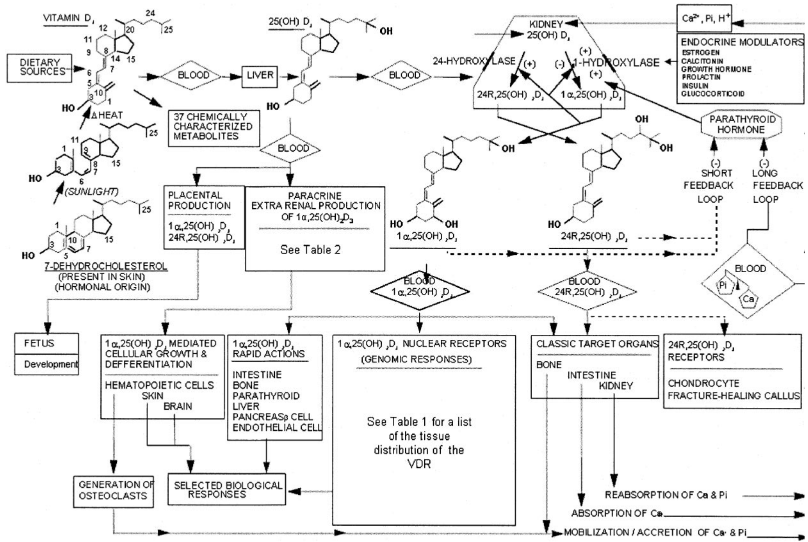

FIGURE 3. The vitamin D endocrine system. In this system, the biologically inactive vitamin D$_3$ is activated, first in the liver to produce 25-hydroxyvitamin D$_3$ [25(OH)D$_3$], and the endocrine gland (the kidney) converts it to the hormones 1α,25-dihydroxyvitamin D$_3$ [1α,25(OH)$_2$D$_3$] and 24*R*,25-dihydroxyvitamin D$_3$ [24*R*,25(OH)$_2$D$_3$]. Researchers have identified ≥36 target organs, defined by the presence of the vitamin D receptor (VDR), which is the receptor for the steroid hormone 1α,25(OH)$_2$D$_3$; *see* Table 1 for a list of these target organs. Table 2 lists the 10 extrarenal tissues that investigators have shown possess the 1α-hydroxylase, the enzyme that converts 25(OH)D$_3$ to the steroid hormone 1α,25(OH)$_2$D$_3$. Pi, inorganic phosphate.

Barbour et al (12) discovered the first extrarenal 25(OH)D$_3$-1α-hydroxylase in 1981 in a hypercalcemic anephric patient with sarcoidosis. Researchers now recognize that in individuals with any of a number of granulomatous diseases, the locally produced 1α,25(OH)$_2$D$_3$ frequently spills over into the general circulation, which results in hypercalcemia. Researchers have shown that the enzyme that converts 25(OH)D$_3$ into 1α,25(OH)$_2$D$_3$, namely the 25(OH)D$_3$-1α-hydroxylase, is present in a paracrine fashion in at least 10 tissues in addition to the proximal tubule of the kidney (**Table 2**). Thus, cells that express a functional 25(OH)D$_3$-1α-hydroxylase acquire the ability to produce local concentrations of the steroid hormone 1α,25(OH)$_2$D$_3$. This "local" or modest production of 1α,25(OH)$_2$D$_3$ then generates biological responses in the local cellular neighborhood. It is believed that this paracrine-generated 1α,25(OH)$_2$D$_3$ does not normally spill over into the circulatory system; thus, the plasma concentration of 1α,25(OH)$_2$D$_3$ does not increase in a measurable way. The ability of locally produced 1α,25(OH)$_2$D$_3$ to promote cell differentiation in prostate cancer (24) and colon cancer cells (25) are examples demonstrating its potential biological importance.

TABLE 1

Tissues that express the vitamin D receptor for the steroid hormone 1α, 25-dihydroxyvitamin D$_3$[1]

Tissue distribution	
Adipose	Muscle, embryonic
Adrenal	Muscle, smooth
Bone	Osteoblast
Bone marrow	Ovary
Brain	Pancreas β cell
Breast	Parathyroid
Cancer cells	Parotid
Cartilage	Pituitary
Colon	Placenta
Eggshell gland	Prostate
Epididymis	Retina
Hair follicle	Skin
Intestine	Stomach
Kidney	Testis
Liver (fetal)	Thymus
Lung	Thyroid
Lymphocytes (B & T)	Uterus
Muscle, cardiac	Yolk sac (bird)

[1] For reference citations on the distribution of the vitamin D receptor, *see* reference 8.

EXPANSION OF VITAMIN D KNOWLEDGE

The consequences that the new knowledge of the vitamin D endocrine system and the various vitamin D metabolites has had on the rate of publication of peer-reviewed articles on vitamin D

TABLE 2

Sites of extrarenal 1α,25-dihydroxyvitamin D$_3$ production in humans[1]

Tissue	mRNA[2]	Protein[2]	Enzymatic activity[3]	Reference
Colon	Y	Y	Y	(13)
Dendritic cells	Y	—	—	(14)
Endothelial cells	Y	Y	Y	(15)
Human brain	—	Y	—	(16)
Mammary, breast	Y	Y	Y	(17)
Pancreatic islets	Y	Y	Y	(18)
Parathyroid glands	Y	Y	Y	(19)
Placenta, decidua	Y	Y	Y	(20)
Prostate	Y	Y	Y	(21)
Skin, keratinocytes	Y	Y	Y	(22, 23)

[1] Based on the presence of the 25-hydroxyvitamin D$_3$-1α-hydroxylase enzyme. Hewison and colleagues have studied the tissue localization of the extrarenal 25-hydroxyvitamin D$_3$-1α-hydroxylase enzyme extensively in humans (18, 20).

[2] Detection of mRNA for the 1α-hydroxylase and the 1α-hydroxylase protein.

[3] Presence of measurable enzymatic activity of the 25-hydroxyvitamin D$_3$-1α-hydroxylase.

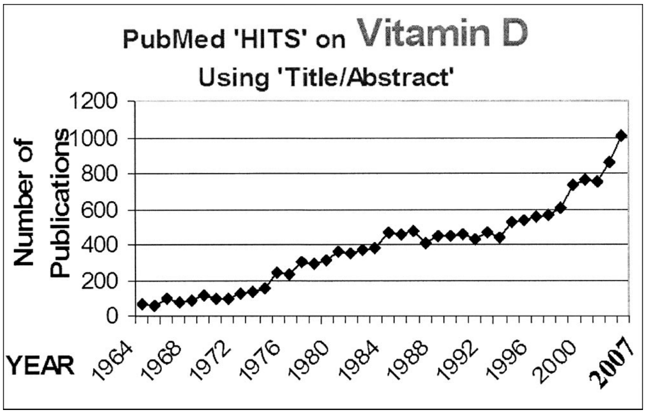

FIGURE 4. Growth in the number of articles published each year with the term *vitamin D* in the title or abstract, as reported in PUBMED (National Library of Medicine, Bethesda, MD).

is illustrated in **Figure 4**. In 1975, journals published only ≈100 articles per year that included the term *vitamin D* in the title or abstract; by 2007, the rate of publication had increased to >1400 articles per year. Another driving factor for this increased publication rate was the chemical synthesis by academic chemists and by 4 pharmaceutical companies of >2000 analogues of 1α,25(OH)$_2$D; most of these analogues were targeted toward selective responses in diseases such as osteoporosis, renal osteodystrophy, and psoriasis, and the authors reported their biological properties in a multitude of publications (8, 26).

Thus, an enormous body of scientific literature currently exists for vitamin D. PUBMED (National Library of Medicine, Bethesda, MD) lists >20 700 publications that use the term *vitamin D* in either the title or abstract from 1950 to the present. This total includes articles that combine the use of *vitamin D* with one of the following terms: *bone* (>6300 articles), *deficiency* (>2900), *cancer* (>1500), *renal failure* (>700), *intestine* (>700), *cardiovascular/heart* (>600), *diabetes* (>470), *insulin* (>450), or *brain* (>270). Finally, PUBMED lists >4500 publications with the term *vitamin* D_3 in the title or abstract, >660 articles with *vitamin* D_2, and >2600 articles with *calcitriol* [a synonym for 1α,25(OH)$_2$D$_3$].

Unfortunately, the proliferation of published articles on vitamin D occurred at the same time as the use by many authors of less precise terminology to describe 3 key vitamin D molecules, namely, vitamin D$_3$, vitamin D$_2$, and 1α,25(OH)$_2$D$_3$. Figure 2 emphasizes the structural differences between vitamins D$_3$ and D$_2$ and provides a clear description and comparison of the biological properties of these 2 key molecules. This figure shows that *1*) vitamin D$_3$ is the only naturally occurring form of vitamin D in humans and other animals and *2*) vitamin D$_2$ has only one-third the biological activity of vitamin D$_3$ in humans (7). Given the significant differences in the biological activity of vitamin D$_3$ and vitamin D$_2$, describing a clinical trial by writing that "the patients received 10 μg (400 IU) of vitamin D" is not a best practice when the patients actually received vitamin D$_2$, whose biological activity is equivalent to only 3.25 μg (130 IU) vitamin D$_3$.

Another frequent error occurs when authors use *vitamin D* as a synonym for 1α,25(OH)$_2$D$_3$, sometimes even in the methods sections of articles. The very significant structural and biological differences between 1α,25(OH)$_2$D$_3$ and vitamin D$_3$ are emphasized in **Figure 5**, which clearly shows why authors must not use *vitamin D* to refer to 1α,25(OH)$_2$D$_3$. If, for example, a reader sees the following statement in the results or discussion section of an article, "the animals or subjects received a standard vitamin D dose that would not cause hypercalcemia," he or she would make a serious error of interpretation if he or she had not carefully read the methods section to learn that "all subjects received a dose of 1.5 micrograms of 1α,25(OH)$_2$vitamin D$_3$."

MODE OF ACTION OF 1α,25(OH)$_2$D$_3$: GENOMIC ACTIONS

The steroid hormone 1α,25(OH)$_2$D$_3$ and many other steroid hormones (eg, estradiol, progesterone, testosterone, cortisol, and aldosterone) generate biological responses both by regulating gene transcription (the classic genomic responses) and by rapidly activating a variety of signal transduction pathways at or near the plasma membrane (rapid or nongenotropic responses) (27). The genomic responses to 1α,25(OH)$_2$D$_3$ result from its stereospecific interaction with its nuclear receptor, VDR$_{nuc}$ (**Figure 6**). The VDR$_{nuc}$ is a protein of 50 kDa, which binds 1α,25(OH)$_2$D$_3$ with high affinity (K_d ≈ 0.5 nmol/L). The VDR$_{nuc}$ does not bind the parent vitamin D$_3$ or vitamin D$_2$; 25(OH)D$_3$ and 1α(OH)D$_3$ only bind 0.1–0.3% as well as 1α,25(OH)$_2$D$_3$. As is true for all nuclear receptors for the steroid hormones involved, the primary amino acid sequence of the VDR$_{nuc}$ consists of 6 functional domains: the variable regions (A and B domains), DNA binding (the C domain), the hinge region (D domain), the ligand-binding region (E domain), and transcriptional activation (domain F) (28). A detailed discussion of the VDR$_{nuc}$ and its participation in the regulation of gene transcription is available elsewhere (28).

Nuclear-receptor–mediated regulation of gene transcription depends on the exquisite structural relation between the unoccupied receptor, which is transcriptionally inactive, and its cognate ligand 1α,25(OH)$_2$D$_3$. Formation of the ligand-receptor complex, which results in conformational changes in the receptor protein, then allows the ligand-receptor complex to specifically interact with the many proteins that collectively constitute the transcriptional machinery. The complementarity of the ligand shape with that of the interior surface of the nuclear VDR ligand binding domain is key not only to the structural basis of receptor action and its formation of heterodimers and interactions with coactivators (**Figure 7**), but also to designing new drug forms of

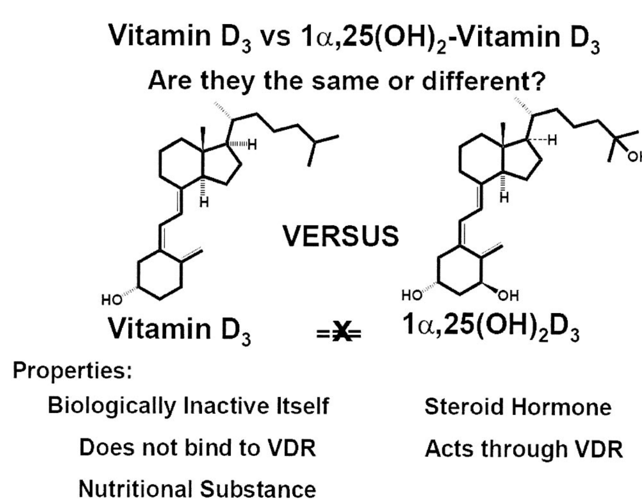

FIGURE 5. Structural and biological similarities and differences between vitamin D$_3$ and 1α,25(OH)$_2$D$_3$. VDR, vitamin D receptor.

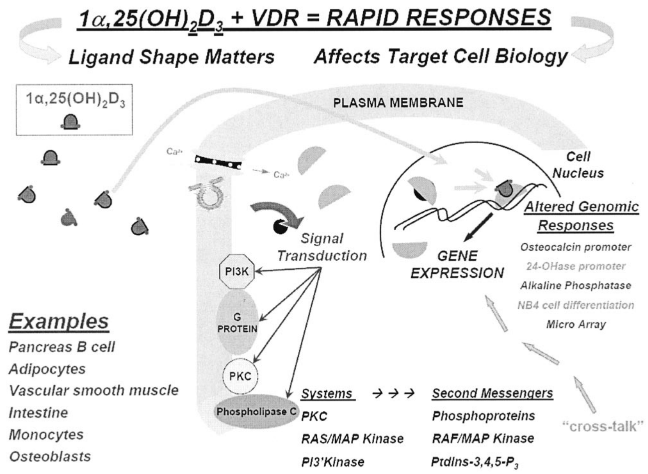

FIGURE 6. Schematic model showing how the conformationally flexible 1α,25-dihydroxyvtamin D$_3$ [1α,25(OH)$_2$D$_3$] can interact with the vitamin D receptor (VDR) localized in the cell nucleus to generate genomic responses and in caveolae with the plasma membrane VDR to generate rapid responses. Binding of 1α,25(OH)$_2$D$_3$ to the caveolae-associated VDR may result in the activation of one or more second messenger systems, including phospholipase C (PKC), protein kinase C, G protein-coupled receptors, or phosphatidylinositol-3-kinase (PI3K). The many possible outcomes include opening the voltage-gated calcium or chloride channels or generating the indicated second messengers. Some of these second messengers, particularly RAF/MAPK, can engage in crosstalk with the nucleus to modulate gene expression. PtdIns-3,4,5-P3, phosphatidylinositol-3,4,5-trisphosphate.

the various hormones, including 1α,25(OH)$_2$D$_3$. Researchers estimate that the VDR can regulate the expression of as many as 500 of the ≈20 488 genes in the human genome (29). The large number of VDR-regulated genes undoubtedly reflects the consequence of the distribution of both the VDR and 25(OH)D$_3$-1α-hydroxylase to many organs.

MODE OF ACTION OF 1α,25(OH)$_2$D$_3$: RAPID RESPONSES

Investigators originally postulated that the "rapid" or nongenomic responses mediated by 1α,25(OH)$_2$D$_3$ were mediated through the interaction of the secosteroid with a novel protein receptor located on the cell's external membrane. Researchers have shown more recently that this membrane receptor is the classic VDR (previously found primarily in the nucleus and cytosol) associated with caveolae present in the plasma membrane of a variety of cells (30). Caveolae are flask-shaped membrane invaginations enriched in sphingolipids and cholesterol that are commonly found in a wide variety of cells (31). Using VDR knockout and wild-type mice, researchers found that rapid modulation of osteoblast ion channel responses by 1α,25(OH)$_2$D$_3$ require the presence of a functional vitamin D nuclear and caveolae VDR receptor (32, 33).

Careful research using a variety of structural analogues of 1,25(OH)$_2$D$_3$ has shown that the genomic and nongenomic responses to this conformationally flexible steroid hormone have different requirements for ligand structure (34). For example, a key consideration is the position of rotation about the 6,7 single carbon-carbon bond that can be either the 6-s-*cis* or 6-s-*trans* orientation (*see* Figure 1). The preferred shape of the ligand for VDR$_{nuc}$, determined from the X-ray crystal structure of the receptor occupied with the ligand 1α,25(OH)$_2$D$_3$, is a 6-s-*trans* shaped bowl with the A-ring 30 ° above the plane of the C/D rings. In contrast, structure-function studies of rapid nongenomic actions of 1,25(OH)$_2$D$_3$ and its analogues show that the VDR$_{mem}$ preferentially binds a ligand with a 6-s-*cis* shape (27). This new ligand structure-function knowledge will allow chemists to synthesize analogues of 1α,25(OH)$_2$D$_3$ that are selective for either genomic or rapid responses, depending on the ligand's overall shape.

DETERMINATION OF VITAMIN D STATUS

As stated in the Introduction, the actions of 1α,25(OH)$_2$D$_3$ essential for good health depend on the vitamin D endocrine system. The operation of the vitamin D endocrine system to produce the steroid hormone 1α,25(OH)$_2$D$_3$ depends on the circulating concentration of 25(OH)D$_3$; this key metabolite is the substrate for the 25(OH)D$_3$-1α-hydroxylase enzyme that produces 1α,25(OH)$_2$D$_3$. As is the case for any enzyme, the activity of the 1α-hydroxylase depends on the absolute concentration of its substrate. The K_m or substrate concentration of 25(OH)D$_3$ required for 50% maximal activity for the 1α-hydroxylase is ≈100 nmol/L (11). As emphasized in Figure 2, the availability of 25(OH)D$_3$ depends on adequate access to vitamin D$_3$. Thus, determining vitamin D nutritional status becomes a critical issue in optimizing the prospects for those aspects of "good health" that 1α,25(OH)$_2$D$_3$ can mediate or to which it can contribute.

Surprisingly, despite extensive efforts, no routine clinical assay is available for determining the serum concentration of either vitamin D$_3$ or vitamin D$_2$. Furthermore, researchers are unlikely to develop a routine serum vitamin D clinical assay in the future. However, the US Institute of Medicine has endorsed the view that the circulating concentration of 25(OH)D$_3$ is an acceptable functional measure of vitamin D nutritional status (5, 35).

Information on obtaining insight into vitamin D nutritional status by determining the serum concentrations of several vitamin D metabolites is presented in **Tables 3** and **4**. Table 34 presents a tabulation of the circulating serum concentrations of the 3 major vitamin D metabolites: 25(OH)D$_3$, 24,25(OH)D$_3$, and 1α,25(OH)$_2$D$_3$. The molar ratio of the total (not free steroid) serum concentrations of these metabolites is 830:77:1 for 25(OH)D to 24,25(OH)D$_3$ to 1α,25(OH)$_2$D$_3$. Investigators primarily measure circulating concentrations of 24,25(OH)D$_3$ for

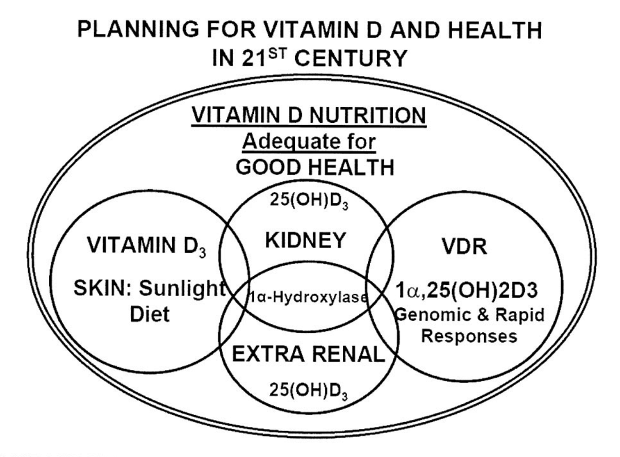

FIGURE 7. Fundamentals of the vitamin D endocrine system that contribute to the daily maintenance of a vitamin D nutritional status that is essential for achieving good health. 25(OH)D$_3$, 25-hydroxyvitamin D$_3$; 1α,25(OH)2D3, 1α,25-dihydroxyvtamin D$_3$.

experimental animal studies or selected clinical studies; such measurements are not available through commercial laboratories. Many, but not all, clinical chemistry laboratories can measure $1\alpha,25(OH)_2D_3$ concentrations. Because serum $1\alpha,25(OH)_2D_3$ values do not correlate with clinical disease status, information on serum $1\alpha,25(OH)_2D_3$ concentration does not usually help with clinical diagnosis and treatment (48).

Most researchers agree that the range of the serum concentration of $25(OH)D_3$ in a population of healthy subjects is the best indicator for assessing the vitamin D status in patients with a vitamin D–related disease. The factors supporting this include: *1*) no vitamin D clinical assay is available; *2*) the metabolism of vitamin D_3 into $25(OH)D_3$ by the liver vitamin D–25-hydroxylase is not regulated, so the serum concentration of $25(OH)D_3$ is an accurate "reporter" of both cutaneous UV-stimulated synthesis and dietary intake of vitamin D_3; *3*) a variety of clinical assays are available to measure 25(OH)D; and *4*) the plasma concentrations of $25(OH)D_3$ correlate with many clinical diseases (6, 46). Hollis et al (41) argued that the relation between vitamin D_3 and $25(OH)D_3$ is *not* linear, but rather saturable and controlled. They conclude that "optimal vitamin D status was achieved when $25(OH)D_3$ was > 40 ng/mL or > 100 nmol/L," which is approximately equivalent to the K_m of the $25(OH)D_3$-1α-hydroxylase.

Presented in Table 4 is a classification of circulating levels of 25(OH)D in relation to vitamin D nutritional status that was largely obtained from clinical studies relating to calcium homeostasis (intestinal calcium absorption, bone mineral density, parathyroid hormone concentrations, etc). In a large population of vitamin D–replete subjects, the normal range of 25(OH)D was found to be 25–137 nmol/L. But it was also noted that the lower limit of the normal range can vary among populations, ranging from as low as 20 up to 50 nmol/L (5). Undoubtedly, it will ultimately be essential to determine the normal 25(OH)D range in all ethnic groups and geographical populations of the world (at all latitudes to reflect differing UV exposures). I believe that a major goal for the vitamin D field is to agree on the "normal 25(OH)D serum levels" that support all 36 VDR-containing target organs in all the world's population groups.

FUNDAMENTALS OF VITAMIN D AND ITS ENDOCRINE SYSTEM FOR GOOD HEALTH

The purpose of the Adequate Intake recommendations for vitamin D put forth by the Food and Nutrition Board of the Institute of Medicine (5) in 1999 was to provide guidelines of vitamin D_3 intake to achieve normal serum levels of 25(OH)D. This was a very difficult goal to achieve, however, given that a quantitative relation of vitamin D's (ie, operation of the vitamin D endocrine system) contribution to good health was not clearly appreciated by 1997.

Tables 5 and **6** focus on the new biological actions of the steroid hormone $1\alpha,25(OH)_2D_3$ that must be carefully studied to appreciate their dependency on an adequate availability of vitamin D_3 to generate biological responses that are mediated by $1\alpha,25(OH)_2D_3$ to be *fully* compatible with proper health for each individual in the population. The 2 historical roles of vitamin D, namely stimulation of intestinal calcium absorption and increasing the mineral content and the remodeling of bone, are summarized in Table 5. For each process (intestine or bone), historical reference citations are provided to create the foundation that vitamin D is crucial to bone mineral content and intestinal calcium absorption. Thus, between 1922 and 1924, the pioneers Mellanby (54), McCollum (55), and Goldblatt (56) made the separate bone-related discoveries, respectively, that *1*) the treatment or prevention of rickets could be mediated by cod liver oil; *2*) by feeding a new vitamin, termed vitamin D; or *3*) by exposure of skin to UV irradiation. Then in 1937, Nicolayson (49) showed the potent actions of vitamin D_3 on stimulating intestinal calcium absorption in rats. Also included in Table 5 are comparable modern observations implicating the participation of the VDR and $1\alpha,25(OH)_2D_3$ in both intestinal calcium absorption and bone remodeling. It is these actions of $1\alpha,25(OH)_2D_3$ and its VDR that were largely addressed in 1999 by the Food and Nutrition Boards' guidelines for Adequate Intake of vitamin D_3 .

Summarized in Table 6 are the 5 physiologic arenas in which new biological actions of $1\alpha,25(OH)_2D_3$ and the VDR have been

TABLE 3
Serum circulating concentrations of key vitamin D metabolites[1]

Vitamin D metabolite	Vitamin D concentration	Reference
Vitamin D	Not routinely measured[2]	—
25(OH)D	50–100 nmol/L (20–40 ng/ml)	(36, 41)
$24R,25(OH)_2D$	5–12 nmol/L (2–5 ng/ml)	(37, 38)
$1\alpha,25(OH)_2D$	50–125 pmol/L (20–50 pg/ml)	(39)

[1] 25(OH)D, 25-hydroxyvitamin D; $24R,25(OH)_2D$, $24R$,25-dihydroxyvitamin D; $1\alpha,25(OH)_2D$, 1α,25-dihydroxyvitamin D_3. Vitamin D (both D_2 and D_3) is quite difficult to measure because of its hydrophobicity, so vitamin D measurements require extensive HPLC (40).

A US Institute of Medicine report has endorsed the view that the circulating concentration of 25(OH)D is a functional measure of vitamin D nutritional status (5); *see* also reference 36.

TABLE 4
Circulating concentrations of 25-hydroxyvitamin D [25(OH)D] by vitamin D nutritional status[1]

Serum 25(OH)D range[2]	Vitamin D nutritional status	Reference
>75 nmol/L (>30 ng/mL)	Sufficiency	(42)
>50 nmol/L (>20 ng/mL)	Sufficiency	(2, 43)
30–50 nmol/L (12–20 ng/mL)	Insufficiency	(44)
12–30 nmol/L (5–12 ng/mL)	Deficiency	(43)
<12 nmol/L (<5 ng/mL)[2]	Severe deficiency	(43)

[1] The classification of 25(OH)D concentrations into sufficiency, insufficiency, deficiency, and severe deficiency represents the author's interpretation of definitions in the publications listed. Researchers have suggested 2 distinct minimum serum concentrations of 25(OH)D for vitamin D sufficiency: >50 nmol/L and >75 nmol/L. The serum concentrations listed in the table refer to the sums of the concentrations of $25(OH)D_3$ and $25(OH)D_2$. Certain methods for measuring 25(OH)D concentration yield information on both $25(OH)D_3$ and $25(OH)D_2$ without distinguishing between the two. However, mass spectrometry provides discrete values for each form of 25(OH)D. The use of liquid chromatography–tandem mass spectrometry has made it possible to simultaneously and routinely determine the amount of $25(OH)D_3$ and $25(OH)D_2$ in a small blood sample (45–47); the major drawback is that this costs more than $100 per determination. In addition, many physicians cannot interpret differences between $25(OH)D_3$ and $25(OH)D_2$ concentrations.

[2] Persons with a $25(OH)D_3$ concentration <20 nmol/L probably have rickets or osteomalacia (5).

TABLE 5
Roles of vitamin D identified in the early 20th century[1]

VDR-dependent system	VDR present or involved	Observation	Key reference
Intestinal calcium absorption	Not yet discovered	Vitamin D stimulates intestinal calcium and phosphorus absorption.	(49)
Intestinal calcium absorption	Yes	$1\alpha,25(OH)_2D_3$ stimulates intestinal calcium absorption in chicks and humans.	(50–52)
Bone formation and resorption (remodeling)	Not yet discovered	Giving the patient a new dietary supplement, called vitamin D, or exposing the skin to ultraviolet irradiation can prevent or treat rickets.	(54–56)
Bone formation and resorption (remodeling)	VDR	The actions of $1\alpha,25(OH)_2D_3$ on the osteoblast (bone formation) and crosstalk with the osteoclast result in bone resorption and overall bone remodeling.	(53)

[1] VDR, vitamin D receptor; $1\alpha,25(OH)_2D$, 1α,25-dihydroxyvitamin D_3.

clearly documented and which have the capability of contributing to better health through improved vitamin D nutritional status. These include understanding at the molecular level the involvement of the VDR and $1\alpha,25(OH)_2D_3$ (in both genomic and rapid responses) in *1*) the functioning of B and T lymphocytes, which constitutes the adaptive immune system; *2*) the innate immune system; *3*) secretion of insulin by the pancreatic β cell; *4*) the multifactorial functioning of the heart and blood pressure regulation; and *5*) the multitude of activities of the brain. There are also multiple opportunities to address how the actions of the VDR and $1\alpha,25(OH)_2D_3$ can affect several diseases. These include prevention and treatment of several cancers (breast, colon, and prostate) through prodifferentiation, antiproliferation, or induction of apoptosis effects, hypertension (infant heart failure), immunomodulation (psoriasis, type 1 diabetes, inflammatory bowel disease, periodontal disease, multiple sclerosis, and possibly rheumatoid arthritis), and neuromuscular effects (muscle strength and better balance). Another critical goal is to understand the role of proper vitamin D nutrition and the molecular actions of the VDR with $1\alpha,25(OH)_2D_3$ for all the steps of fetal development.

The important points made in this presentation are summarized in Figure 7. Researchers must understand the fundamentals of the vitamin D endocrine system, which include *1*) vitamin D nutritional status (dietary intake and sunlight exposure), which define the circulating concentration of $25(OH)D_3$ that is available to *2*) the kidney functioning as an endocrine gland, which is complimented by *3*) the paracrine 1α-hydroxylase activities in 10 tissues to produce the steroid hormone $1\alpha,25(OH)_2D_3$, which will *4*) interact with the VDR in $\geq$36 target tissues to generate optimal responsess in the calcium homeostasis system. Researchers must also understand the 5 new physiologic systems that collectively comprise the vitamin D endocrine system.

TABLE 6
Newly identified biological actions of 1α, $25(OH)_2D_3$ with relevance to vitamin D nutritional status and resulting good health[1]

VDR-dependent system	VDR present or involved	Observation	Key reference
B and T lymphocytes	Yes	VDR is present in activated human mononuclear leukocytes and lymphocytes.	(57, 58)
Adaptive immune system	Yes	1,25-dihydroxyvitamin D_3 plays an immunoregulatory role.	(59)
Innate immune system	Yes	$1\alpha,25(OH)_2D_3$ induces cathelicidin, an antimicrobial peptide, through VDR-mediated gene expression.	(60)
Innate immune system	Yes	Toll-like receptor activation of human macrophages upregulates expression of the VDR and the 25(OH)D3-1α,-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular *Mycobacterium tuberculosis.*	(61)
Innate immune system Innate immune system	Yes	Skin injury enhances antimicrobial peptide synthesis through the VDR and the $25(OH)D_3$-1α-hydroxylase in keratinocytes.	(62)
Pancreas β cells	Yes	Vitamin D deficiency inhibits pancreatic secretion of insulin and $1\alpha,25(OH)_2D_3$ restores it.	(63, 64)
Pancreas β cells	Yes	Vitamin D deficiency in early life accelerates development of type 1 diabetes in nonobese diabetic mice.	(65, 66)
Pancreas β cells	Yes	In humans, 25(OH)D concentration has a positive correlation with insulin sensitivity and hypovitaminosis D has a negative effect on β cell function.	(67)
Brain	Yes	The VDR and 1α-hydroxylase are distributed in human brain.	(16)
Brain	Yes	Vitamin D deficiency in utero alters adult behavior in mice. Researchers have suggested that in humans, fetal deprivation of vitamin D_3 could be associated with adverse neuropsychiatric outcomes.	(68)
Brain	Yes	Combined prenatal and chronic postnatal vitamin D deficiency in rats impairs prepulse inhibition of acoustic startle.	(69)
Heart function and blood pressure regulation	Yes	Research characterized heart size and blood pressure in the VDR knockout mouse.	(70)
	Yes	Hypocalcaemia and vitamin D deficiency is an important but preventable cause of life-threatening infant heart failure.	(71)
	Yes	$1\alpha,25(OH)_2D_3$ is a negative endocrine regulator of the renin-angiotensin system and blood pressure.	(72)

[1] VDR, vitamin D receptor; $1\alpha,25(OH)_2D$, 1α,25-dihydroxyvitamin D_3.

One can derive at least 3 important conclusions, one prediction, and one expectation from the discoveries made over the past 30 y pertaining to the wide tissue distribution of both the VDR and the extrarenal $25(OH)D_3$-1α-hydroxylase

Conclusions

1) Recent research has shown that vitamin D_3's biological sphere of influence is much broader than researchers originally thought, as shown by the tissue distribution of the VDR, from mediating only calcium homeostasis (intestine, bone, kidney, and parathyroid) to functioning as a pluripotent hormone in 5 physiologic arenas in which researchers have clearly identified additional biological actions of $1\alpha,25(OH)_2D_3$ through the VDR. These physiologic arenas are the adaptive immune system, the innate immune system, insulin secretion by the pancreatic β cell, multifactorial heart functioning and blood pressure regulation, and brain and fetal development.

2) Researchers have also expanded the parent vitamin D_3's nutritional sphere of influence from a focus on bone health to include 5 additional physiologic systems.

3) The nutritional guidelines for vitamin D_3 intake must be carefully reevaluated to determine the adequate intake (balancing sunlight exposure with dietary intake) to achieve good health by involving all 36 target organs rather than just the first 4 target organs (intestine, kidney, bone, and parathyroid gland) that are considered for calcium homeostasis.

Corollary

Given that vitamin D_2 is significantly less biologically active in humans than is vitamin D_3 (7), its biological use as a dietary supplement in the United States should be discontinued and its use in a high-dose form [eg, 500 000 IU/mL of ergocalciferol (vitamin D_2)] in clinical studies (73, 74) and as described in the *Physicians Desk Reference* (75) should be replaced by a new formulation of high-dose vitamin D_3.

Prediction

Given the large expansion of the vitamin D endocrine system, the number of identified diseases or consequences of vitamin D deficiency or insufficiency will greatly increase to reflect the fact that the number of target organs for $1\alpha,25(OH)_2D_3$ has increased $\approx$9-fold since the discovery of the VDR in intestine, bone, kidney, and parathyroid tissues in the early 1970s.

Expectation or hope

More than ever, we need to increase the amount of research on vitamin D (ie, increase funding from government agencies and pharmaceutical companies) to meet the challenge of maximizing the knowledge of how to use vitamin D in the context of the vitamin D endocrine system to preserve or improve the health of everyone on the planet.

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