Overview of general physiologic features and functions of vitamin D[1–4]

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ABSTRACT

Vitamin D_3 is a prohormone produced in skin through ultraviolet irradiation of 7-dehydrocholesterol. It is biologically inert and must be metabolized to 25-hydroxyvitamin D_3 in the liver and then to 1α,25-dihydroxyvitamin D_3 in the kidney before function. The hormonal form of vitamin D_3, ie, 1α,25-dihydroxyvitamin D_3, acts through a nuclear receptor to carry out its many functions, including calcium absorption, phosphate absorption in the intestine, calcium mobilization in bone, and calcium reabsorption in the kidney. It also has several noncalcemic functions in the body. This overview provides a brief description of the physiologic, endocrinologic, and molecular biologic characteristics of vitamin D. It also provides information on new selective analogs of 1α,25-dihydroyvitamin D_3 for therapy. *Am J Clin Nutr* 2004;80(suppl):1689S–96S.

KEY WORDS Vitamin D metabolism, bone, calcium homeostasis, tetany, vitamin D endocrine system, autoimmune diseases

INTRODUCTION

The discovery of vitamin D and the elimination of rickets as a major medical problem must rank as one of medicine's great achievements (1). From the early studies of McCollum and Davis (2) in 1913, when the first vitamin was discovered, until 1940, the work leading to the identification of vitamin D and its role in bone formation and prevention of hypocalcemic tetany included many outstanding contributions. Most noteworthy was the work by Sir Edward Mellanby, who demonstrated that he could produce rickets in dogs by feeding them the diet characteristic of Scotland, ie, oatmeal; unknown to Sir Edward Mellanby was the fact that he deprived those dogs of sunlight. Because of the work of McCollum and Davis in discovering fat-soluble vitamin A, Mellanby attributed the ability of cod liver oil to cure the rachitic condition in dogs as being another property of vitamin A (3). McCollum very cleverly destroyed the vitamin A activity of cod liver oil by bubbling oxygen through the solution and heating it, but the ability to cure rickets remained in the preparation. McCollum correctly concluded that this represented a new vitamin, called vitamin D (4). Huldshinsky (5) and Chick et al (6) independently demonstrated that rachitic children could be cured with exposure to sunlight or artificially produced ultraviolet light. The puzzle was ultimately solved when Steenbock and Black (7) discovered that irradiation not only of the skin of animals but also of the food they consumed imparted antirachitic activity to either the animals or their food. Furthermore, Goldblatt and Soames (8) showed that livers taken from irradiated rats could heal rickets in rats. Therefore, 2 important discoveries occurred. First, Steenbock and Black (7) conceived that foods could be irradiated to impart vitamin D and rickets as a major medical problem would disappear. Second, the irradiation of fat-soluble substances extracted from tissues could be used to generate large amounts of vitamin D for later characterization. The structure of vitamin D_2 was deduced in 1931 by Askew et al (9), and the structure of vitamin D_3 was determined through synthetic means by Windaus et al (10). Vitamin D was discovered with many other vitamins and is classed as a vitamin even now. However, findings from the second half of the 20th century showed that vitamin D is truly a prohormone and not a vitamin. Vitamin D is virtually absent from the food supply. It is not found in plant materials (eg, vegetables, fruits, or grains) and is present in low abundance in meats and other animal food sources, except in rare cases such as fish liver oils and plants such as waxy-leaf nightshade (*Solanum glaucophyllum*).

PRODUCTION AND METABOLISM OF VITAMIN D

Vitamin D is normally produced in skin through a robust photolytic process acting on a derivative of cholesterol (ie, 7-dehydrocholesterol) to produce previtamin D, which is then slowly isomerized to vitamin D_3 (11). Vitamin D_3 is the natural form of vitamin D produced in skin, and vitamin D_2 is derived from irradiation of ergosterol, which occurs to some degree in plankton under natural conditions and is used to produce vitamin D_2 from the mold ergot (which contains as much as 2% ergosterol). We must move away from the concept that vitamin D is a vitamin.

Another important fact is that vitamin D is required throughout life. It not only is needed for the formation of bone but also likely plays an important role in several other physiologic systems. Its use may well prevent several degenerative diseases, and it may also play a role as an anticancer agent.

The structure of vitamin D_3 and its numbering system are indicated in **Figure 1**. We now know that vitamin D_3 itself is biologically inert, as clearly indicated by genetic defects that result in the disease rickets despite normal intakes of vitamin D (12). By 1967, the concept that vitamin D is converted to an active form had appeared (13, 14). By 1969, the circulating form

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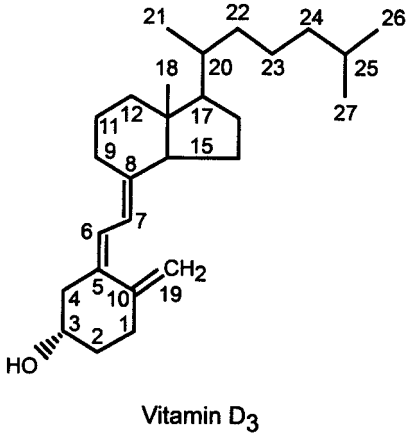


FIGURE 1. Structure of vitamin D_3, or cholecalciferol, and its numbering system.

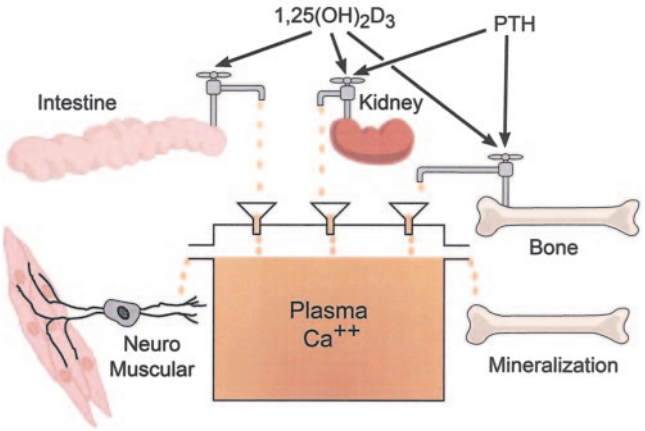


FIGURE 3. Diagrammatic representation of the role of the vitamin D hormone and the parathyroid hormone (PTH) in increasing plasma calcium concentrations to prevent hypocalcemic tetany (neuromuscular) and to provide for mineralization of the skeleton.

of vitamin D had been isolated, chemically identified, and synthesized (15, 16). This compound, 25-hydroxyvitamin D_3 [$25(OH)D_3$], is now currently monitored in serum to indicate the vitamin D status of patients, as discussed below. However, $25(OH)D_3$ itself is metabolically inactive and must be modified before function. The final active hormone derived from vitamin D was isolated and identified in 1971, and its structure was deduced as 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] (17) and confirmed by synthesis (18). The pathway that vitamin D must follow is illustrated in **Figure 2** and forms the basis of the vitamin D endocrine system. For ~2 decades, there was consistent revisitation of the concept that more than one hormone was derived from vitamin D, and ~33 metabolites of vitamin D were identified (19). However, it soon became clear that all metabolites were either less active or rapidly cleared and were thus intermediates in the degradation of this important molecule. The most important of these metabolites are 24,25-dihydroxyvitamin D_3 and 1α,24(*R*),25-trihydroxyvitamin D_3 produced by the enzyme CYP24, which is induced by the vitamin D hormone itself (20).

Much is known about the enzymes that produce $1,25(OH)_2D_3$ and their regulation, but a great deal remains to be learned (20). Two enzymes are thought to function in the 25-hydroxylation step. They are not exclusively hepatic but are largely functionally active in the liver. The mitochondrial enzyme, which is not specific for vitamin D, has been cloned and a knockout mouse strain has been prepared, without any apparent effect on vitamin D metabolism, which suggests that there is an alternate 25-hydroxylase (21). A microsomal hydroxylase was recently cloned and could represent the missing enzyme (22). The $25(OH)D_3$ 1α-hydroxylase was cloned by 3 different laboratories (reviewed in ref 20), and the sites of vitamin D-dependent rickets type I were identified in several studies (20). Very important was the generation of 1α-hydroxylase knockout mice, which exhibit a phenotype virtually identical to the human vitamin D-dependent rickets type I phenotype. Therefore, the enzymes that activate vitamin D have been identified.

Of major metabolic importance is the mode of disposal of vitamin D and its hormonal forms. The cytochrome P-450 enzyme now called CYP24 was isolated in pure form by Ohyama and Okuda (23) and the complementary DNA and gene were cloned, which yielded a 24-hydroxylase-null mutant (reviewed in 20). No significant phenotype resulted except for a large accumulation of $1,25(OH)_2D_3$ in the circulation, which produced secondary effects on cartilaginous growth (20, 24). CYP24 is an extremely active enzyme, but the gene remains silent in vitamin D deficiency; it is induced by the hormonal form of vitamin D itself. Therefore, pulses of the vitamin D hormone program its own death through induction of the 24-hydroxylase. The 24-hydroxylase is able to metabolize vitamin D to its excretion product calcitroic acid (20). $25(OH)D_3$ can also be degraded through this pathway. 24-Hydroxylase and its regulation are important factors in the determination of the circulating concentrations of the hormonal form of vitamin D.

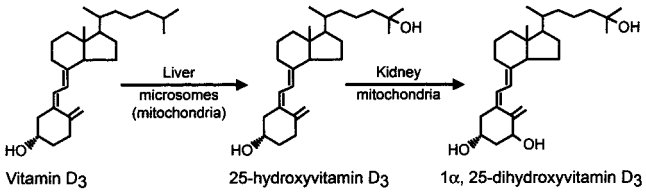


FIGURE 2. Metabolic activation of vitamin D_3 to its hormonal form, $1,25(OH)_2D_3$.

PHYSIOLOGIC FUNCTIONS OF VITAMIN D

A diagrammatic explanation of the role of the vitamin D hormone in mineralizing the skeleton and preventing hypocalcemic tetany is presented in **Figure 3** (20). Plasma calcium concentrations are maintained at a very constant level, and this level is supersaturating with respect to bone mineral. If the plasma becomes less than saturated with respect to calcium and phosphate, then mineralization fails, which results in rickets among children and osteomalacia among adults (24). The vitamin D hormone functions to increase serum calcium concentrations through 3 separate activities. First, it is the only hormone known to induce the proteins involved in active intestinal calcium absorption. Furthermore, it stimulates active intestinal absorption of phosphate. Second, blood calcium concentrations remain in the normal range even when an animal is placed on a no-calcium diet. Therefore, an animal must possess the ability to mobilize calcium in the absence of calcium coming from the environment, ie,

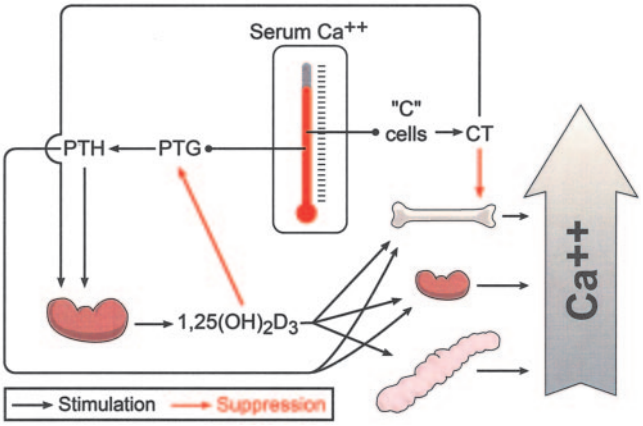


FIGURE 4. Diagrammatic representation of the vitamin D endocrine system. Red arrows indicate suppression; black arrows indicate stimulation. Serum calcium concentrations are represented by a thermometer. Low serum calcium concentrations are detected by a calcium sensor in the parathyroid gland, which initiates synthesis and secretion of parathyroid hormone. The calcium sensor for high concentrations is in the C cells of the thyroid gland and initiates secretion of calcitonin (CT).

through enterocytes. Two mechanisms play a role in increasing blood calcium concentrations, especially in the absence of intestinal calcium absorption. Vitamin D hormone stimulates osteoblasts to produce receptor activator nuclear factor-κB ligand (RANKL) (25). RANKL then stimulates osteoclastogenesis and activates resting osteoclasts for bone resorption (25). Therefore, the vitamin D hormone plays an important role in allowing individuals to mobilize calcium from bone when it is absent from the diet. It is very important to note, however, that in vivo both vitamin D and parathyroid hormone are required for this mobilization event (26, 27). Therefore, 2 keys are required, similar to a safety deposit box. Third, the distal renal tubule is responsible for reabsorption of the last 1% of the filtered load of calcium, and the 2 hormones interact to stimulate the reabsorption of this last 1% of the filtered load (28). Because 7 g of calcium are filtered every day among humans, this represents a major contribution to the calcium pool. Again, both parathyroid hormone and the vitamin D hormone are required. Calcium physiologic processes are such that a single low concentration of the vitamin D hormone stimulates enterocytes to absorb calcium and phosphate. If the plasma calcium concentration fails to respond, then the parathyroid glands continue to secrete parathyroid hormone, which increases production of the vitamin D hormone to mobilize bone calcium (acting with parathyroid hormone). Under normal circumstances, environmental calcium is used first; if environmental calcium is absent, then internal stores are used.

VITAMIN D ENDOCRINE SYSTEM

A diagrammatic representation of the endocrine regulation of calcium concentrations in the plasma and the vitamin D endocrine system is presented in **Figure 4**. Calcium-sensing proteins that sense plasma calcium concentrations are found in the parathyroid gland (29, 30). When calcium concentrations decrease below normal, even slightly, then these transmembrane proteins, coupled to a G protein system, stimulate the secretion of parathyroid hormone. Parathyroid hormone then proceeds to the osteoblasts and to the proximal convoluted tubule cells within seconds. Most importantly, in the convoluted tubule cells that

The vitamin D response elements found in target genes

Gene	Sequence	Position
CaBP 9K	GGGTGT CGG AAGCCC	-488 to -474
Rat osteocalcin	GGGTGA ATG AGGACA	-456 to -442
Human osteocalcin	GGGTGA ACG GGGGCA	-511 to -486
Mouse osteopontin	GGTTCA CGA GGTTCA	-757 to -743
Rat 24-OHase distal	GGTTCA GCG GGTGCG	-262 to -238
Human 24-OHase distal	ACTTCA CCG GGTGTG	-293 to -273
Rat 24-OHase prox.	GAGTCA GCG AGGTGA GTG AGGGCG	-151 to -125
Human 24-OHase prox.	GAGTCA GCG AGGTGA GCG AGGGCG	-171 to -143
Mouse CaBP 28K	GGGGAT GTG AGGAGA	-198 to -182
Human PTH	TCAACT ATA GGTTCA AAG CAGACA	-121 to -99
Rat PTHrp	GGTGGA GAG GGGTGA	-1121 to -1075

For referral information, see reference 20.

FIGURE 5. Partial list of VDREs found in the promoter regions of target genes. Most prominent is the VDRE found in the 24-hydroxylase (CYP24) promoter.

serve as the endocrine gland for the vitamin D hormone, 1α-hydroxylase concentrations are markedly elevated (30, 31). This signals the vitamin D hormone, which by itself stimulates intestinal absorption of calcium or together with parathyroid hormone, at higher concentrations, stimulates mobilization of bone calcium and renal reabsorption of calcium. The increase in serum calcium concentrations exceeds the set point of the calcium-sensing system, shutting down the parathyroid gland-induced cascade of events. If the plasma calcium concentrations overshoot, then the C-cells of the thyroid gland secrete the 32-amino acid peptide calcitonin, which blocks bone calcium mobilization (32). Calcitonin also stimulates the renal 1α-hydroxylase to provide the vitamin D hormone for noncalcemic needs under normocalcemic conditions (33). The molecular mechanisms have not been entirely determined, except for the vitamin D hormone induction of 24-hydroxylase (CYP24).

An important aspect of the vitamin D endocrine system is that dietary calcium is favored to support serum calcium concentrations under normal conditions but, when this fails, the system mediates calcium mobilization from bone and reabsorption in the kidney to satisfy the needs of the organism. This results in loss of calcium from the skeleton and can ultimately lead to osteoporosis. Another important aspect is that, except for stimulating mineralization of the skeleton, the vitamin D hormone has not been found to be anabolic on bone by itself.

MOLECULAR MECHANISMS OF VITAMIN D ACTIONS

The vitamin D hormone functions through a single vitamin D receptor (VDR), which has been cloned for several species including humans, rats, and chickens. It is a member of the class II steroid hormones, being closely related to the retinoic acid receptor and the thyroid hormone receptor (reviewed in ref 20). It, like other receptors, has a DNA-binding domain called the C-domain, a ligand-binding domain called the E-domain, and an F-domain, which is one of the activating domains. Despite many statements to the contrary in the literature, a single receptor appears to mediate all of the functions of vitamin D, which complicates the preparation of analogs for one specific function rather than another. The human receptor is a 427-amino acid peptide, whereas the rat receptor contains 423 amino acids and the chicken receptor contains 451 amino acids. This receptor acts through vitamin D-responsive elements (VDREs), which are usually found within 1 kilobase of the start site of the target gene. The VDREs, which are shown in **Figure 5**, are repeat sequences

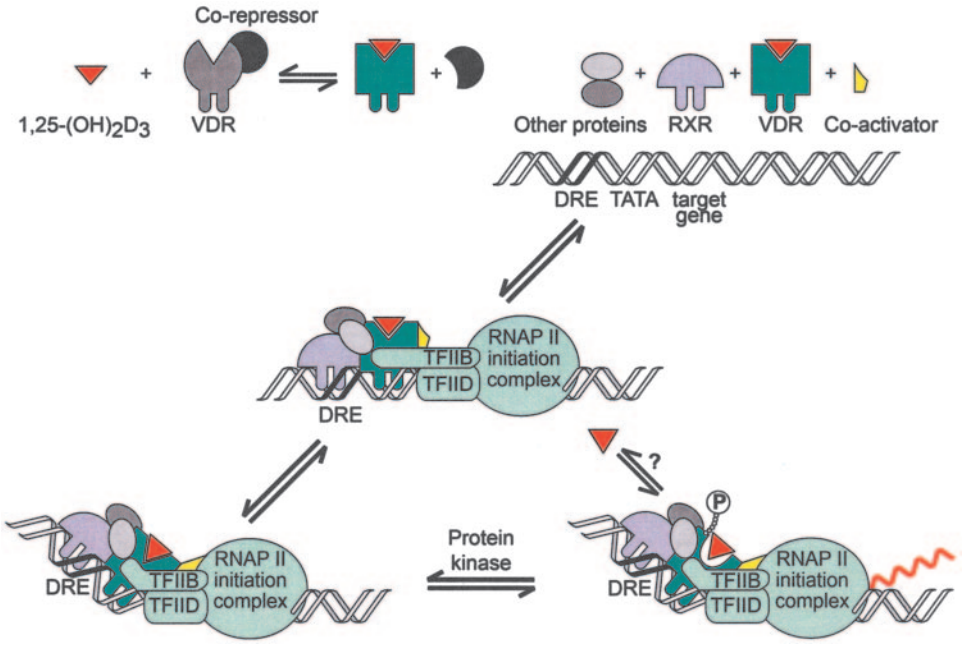


FIGURE 6. Diagrammatic representation of the known molecular events in the regulation of gene expression by the vitamin D hormone, $1,25(OH)_2D_3$, acting through its receptor, VDR. The result of regulation may be either suppression or activation. RXR, retinoid X receptor; DRE, VDRE (see Figure 5); TFIIB, transcription factor IIB; TFIID, transcription factor IID; RNAP, RNA polymerase.

of 6 nucleotides separated by 3 nonspecified bases. It is now clear that the 5' arm of this sequence binds the retinoic acid X receptor and the 3' arm binds the VDR. Of all of the genes identified to date, the most powerfully regulated is the CYP24 or 24-hydroxylase enzyme, which is responsible for the degradation of vitamin D (20). The programming of its own destruction is thus an important aspect of this endocrine system, which uses one of the most potent ligands known.

A diagram that describes how the VDR with its ligand affects the transcription of target genes is presented in **Figure 6**. Although there is little evidence for a co-repressor, we think that co-repressors will eventually be found for the VDR. When the VDR interacts with the ligand, the repressor is no longer able to bind to the receptor, and the receptor changes conformation. Together with the required RXR, the VDR forms a heterodimer at the VDREs (20). At the same time, it binds several other proteins required in the transcription complex and, most importantly, acquires an activator (20). To date, at least 3 coactivators have been identified, ie, SARC1, -2, and -3 (34) and DRIP205 (35). There may be additional coactivators, and there may be selectivity among the coactivators with respect to which gene is being expressed. Much attention is being focused on this aspect for selective regulation of target genes. Once the complex is formed, the DNA bends (36), phosphorylation on serine-205 occurs (37), and transcription is either initiated or suppressed, depending on the gene. To date, it is unclear whether the phosphorylation plays a functional role in transcription.

FUNCTIONS OF VITAMIN D UNRELATED TO CALCIUM

One of the most important findings after discovery of the receptor was that the receptor appeared not only in the target cells of enterocytes, osteoblasts, and distal renal tubule cells but also in parathyroid gland cells, skin keratinocytes, promyelocytes, lymphocytes, colon cells, pituitary gland cells, and ovarian cells (20). The expression of VDRs in these cells and not in skeletal muscle, heart muscle, and liver suggests that they must serve a function there (20). Although VDRs have been reported in liver, heart, and skeletal muscle (38–42), we and other groups failed to confirm those reports, with the use of specific monoclonal antibodies and other methods (43, 44). This led to the investigation and discovery of functions of vitamin D not previously appreciated, which takes the vitamin D system beyond bone.

An important discovery was made by Suda et al (25), who demonstrated that the vitamin D hormone plays an important role in the terminal differentiation of promyelocytes to monocytes, which are precursors of the giant osteoclasts. Those authors also found that, when the cells differentiated into a functional cell line, growth ceased. This function did not involve calcium and phosphorus and was later shown to be fundamental to vitamin D-induced production of osteoclasts through the RANKL system (for review, see reference 25).

Of great importance is the finding of the VDR in the parathyroid glands. We now know, through the treatment of renal osteodystrophy with the vitamin D hormone and its analogs, that an essential site for this therapy is the VDR in the parathyroid glands (20). An important function of the vitamin D hormone is to keep the production of the preproparathyroid gene under control and reasonably suppressed (20, 45). Furthermore, the vitamin D hormone, through its receptor, in some way functions to prevent proliferation of parathyroid gland cells. Therefore, an important function of the vitamin D hormone among normal subjects is to maintain normal parathyroid status. Among patients with renal failure, the site of production of the vitamin D hormone is destroyed and the parathyroid gland becomes vitamin D deficient; in the presence of adequate amounts of calcium in the circulation,

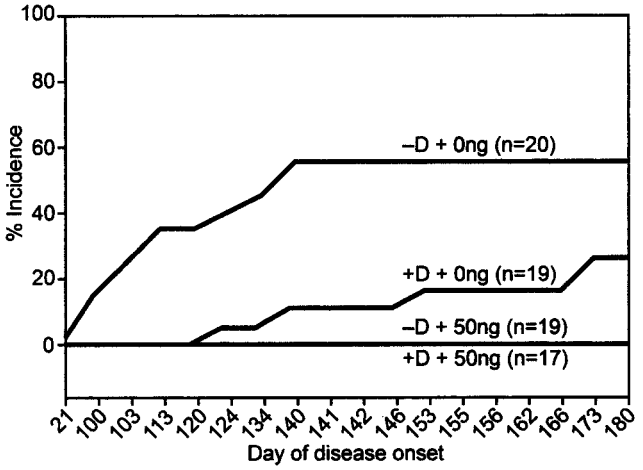
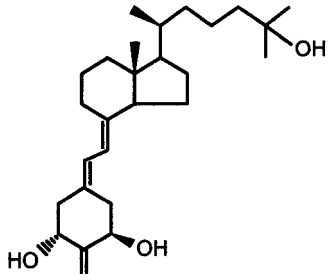


FIGURE 7. Time course of the development of diabetes among NOD/LTJ female mice. Weanling mice were fed either a vitamin D-deficient diet containing normal amounts of calcium and phosphorus or a vitamin D-sufficient diet containing, in addition to the components of the vitamin D-deficient diet, vitamin D_3. In addition, the diet was supplemented with 50 ng of $1,25(OH)_2D_3$ in a daily ration for the mice. The incidence of diabetes is shown on the left, whereas the daily onset is shown on the *x*-axis. There is no doubt that $1,25(OH)_2D_3$ can prevent the onset of diabetes among NOD/Ltj female mice.



2-Methylene-19-nor-20S
1α,25-Dihydroxyvitamin D_3

FIGURE 8. Structure and name of the carbon-2–modified analog of $1,25(OH)_2D_3$ that is selective in its activity on the osteoblasts of bone (2MD).

the parathyroid gland hyperproliferates and hypersecretes parathyroid hormone, resulting in secondary hyperparathyroidism (46). An important available treatment is the use of the vitamin D hormone and its analogs to manage the parathyroid glands of patients undergoing dialysis.

Another important area of investigation has been the immune system. Clearly, vitamin D deficiency affects the immune system, especially T cell-mediated immunity, whereas vitamin D in excess actually suppresses certain aspects of the immune system (47, 48). This has led to investigation of the use of vitamin D compounds to suppress certain autoimmune disorders. The first autoimmune disorder to come under scrutiny is multiple sclerosis, and experimental autoimmune encephalomyelitis has been used as an animal model. This disease can be suppressed or eliminated at any stage of development with adequate amounts of the vitamin D hormone administered orally each day (49). However, hypercalcemia occurs with this therapy. We now know that the increase in serum calcium concentrations plays some role in this therapeutic response. A clearer example of an autoimmune disease that is regulated by the vitamin D hormone is type 1 diabetes mellitus (50). Among nonobese diabetic rats, vitamin D deficiency caused a marked increase in incidence and a marked decrease in the lag time required for the initiation of diabetes (**Figure 7**). Very important is the fact that large doses of the vitamin D hormone could suppress type 1 diabetes mellitus completely (50), preventing the destruction of islet cells. Similar results were obtained with models of systemic lupus (51), inflammatory bowel disease (52), and rheumatoid arthritis (53). It is likely that the suppression of these autoimmune diseases involves the vitamin D hormone interacting with T helper lymphocytes, which in turn suppress the inflammatory responses of T helper type 1 lymphocytes. Alternative ideas have also been put forth, such as suppression of the dendritic cells that present antigens to the T cells (54). Although the mechanisms of this regulation of autoimmune diseases are not understood, the results are sufficient to warrant investigation of vitamin D analogs for the treatment of such diseases. As an extension of this function of vitamin D, the utility of $1,25(OH)_2D_3$ in helping prevent transplant rejection has been demonstrated with both vascular and nonvascular transplants (55).

ANALOGS OF $1,25(OH)_2D_3$

We now come to the area of the design of $1,25(OH)_2D_3$ analogs with selective activities for use against specific diseases. The major problem is that the primary role of $1,25(OH)_2D_3$ is to adjust serum calcium and phosphorus concentrations. This is its dominant role, and the design of any analog to treat a disease other than osteoporosis or osteomalacia must include the elimination or marked suppression of the plasma calcium-increasing activity. Most analog development in this field has been performed with that in mind. Years of experience with modifications of $1,25(OH)_2D_3$ and assessments of the consequent physiologic effects have yielded some information that is very useful for designing analogs for particular uses. A recent development has been the understanding that the carbon-2 position of vitamin D not only is tolerated but actually produces a much more stable transcription complex, compared with vitamin D analogs without carbon-2 modifications (56, 57). Our group has developed analogs that are selective for actions on osteoblasts, particularly the anabolic or bone-forming actions of that cell type. The most promising of the compounds studied is 2-methylene-19-nor-20S-1α,25-dihydroxyvitamin D_3 (2MD) (**Figure 8**). This compound is very selective for its action on bone, being ~80–100 times more effective than the native hormone in stimulating bone calcium mobilization while being equally effective in the intestine. Demonstrating that osteoblastic activity is favored by this analog, incubation of 2MD with human osteoblasts caused formation of bone nodules within 2 wk (**Figure 9**) (58). However, incubation of the same cells with even high concentrations of $1,25(OH)_2D_3$ produced little or no change. These results suggest strong bone-forming activity of 2MD. To test whether this analog could cause bone synthesis in vivo, 2MD was given to aged female rats that had been ovariectomized to ensure a loss of bone mass (osteoporosis). 2MD caused a marked increase in the synthesis of new bone, yielding a high bone mass value; samples tested for breaking strength proved to be extremely strong. In the

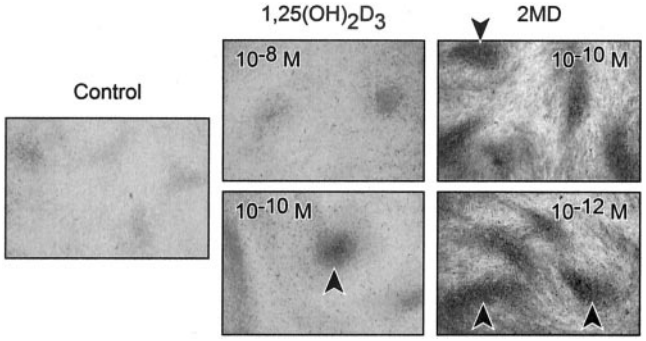
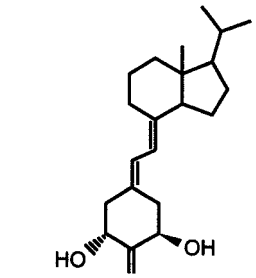


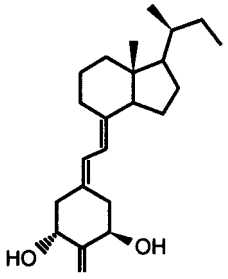
FIGURE 9. Incubation of human osteoblasts with 2MD, resulting in the appearance of marked mineralized bone nodules. Osteoblasts and osteoblast precursors harvested from pediatric bone samples discarded during surgery were incubated at 10^5 cells per plate with 10^{-8} mol/L $1,25(OH)_2D_3$, 10^{-10} or 10^{-12} mol/L 2MD, or no vitamin D for a period of 7 d. Fresh medium was presented to the cells every 3 d. After 1 wk of incubation, the medium was replaced with ascorbic acid/β-glycerol phosphate-containing medium (without vitamin D compounds). After a 2-wk period, the cells were stained with silver nitrate to reveal bone nodules (arrows).

same model, $1,25(OH)_2D_3$ administered at much higher doses was unable to induce the same levels of bone synthesis and bone mass. 2MD is now in phase 2 of development for osteoporosis and appears extremely promising as an anabolic agent for bone growth.

Two other analogs modified at carbon-2 are shown in **Figure 10** (59). These compounds bind very well to the receptor and are active in transcription but, even when given orally to animals at doses as high as 70 μg/kg, are unable to increase serum calcium



1α-hydroxy-2-methylene-19-nor-homopregnacalciferol
2MP



(20S)1α-hydroxy-2-methylene-19-nor-bishomopregnacalciferol
2MbisP

FIGURE 10. Structures of noncalcemic, carbon-2–modified analogs of $1,25(OH)_2D_3$.

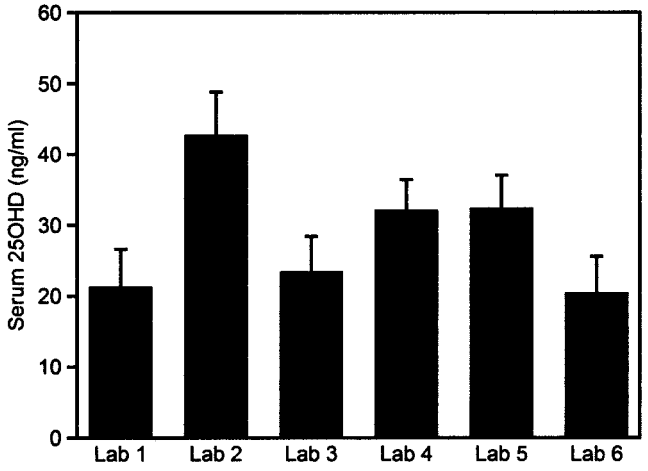


FIGURE 11. $25(OH)D_3$ concentrations in 20 serum samples, as measured with assay kits from 5 different commercial laboratories and with HPLC in our own laboratory (Lab 6). The results indicate very clearly that the laboratory values differ according to the laboratory performing the tests, some of which are antibody-based tests. Only 2 methods (Lab 1 and Lab 3) yielded values approaching the values obtained with the accurate chemical or HPLC method. These results demonstrate a need for assay certification before public health parameters can be set on the basis of $25(OH)D_3$ concentrations in plasma.

concentrations. However, much lower doses of the same compounds are able to suppress plasma parathyroid hormone concentrations, which shows that they are systemically active. Therefore, there is great promise that new analogs with very selective activities can be prepared. One group of analogs is very selective in stimulating bone synthesis. Another pair of analogs are devoid of calcium activity while retaining systemic activity for the parathyroid glands. The latter group might be used to treat autoimmune diseases and cancer and to suppress secondary hyperparathyroidism among patients undergoing dialysis.

ASSESMENT OF VITAMIN D STATUS

One of the most important facets of vitamin D is its many physiologic activities. The importance of vitamin D being continually available is obvious. Vitamin D deficiencies compromise not only bone mineralization but also many other biological activities. It is well known that vitamin D deficiency rickets is appearing even in highly developed countries. In Europe, vitamin D fortification of foods is largely absent, and little vitamin D is made in the skin of individuals in the northern and southern regions of the planet during the winter months. To protect against bone diseases and other kinds of degenerative diseases and autoimmune diseases, adequate concentrations of vitamin D are extremely important. In the view of many scientists in the vitamin D field, the recommended dietary allowance is too low. Supplementation with vitamin D_3 at 2000 IU/d should be considered and should be perfectly safe. To determine safety, an assessment of vitamin D status is required. Generally, $25(OH)D_3$ concentrations are considered the best measure of vitamin D status. Unfortunately, commercially available assays for $25(OH)D_3$ yielded widely differing results (**Figure 11**) (60). To determine which of these values truly represents the $25(OH)D_3$ concentration, we measured $25(OH)D_3$ concentrations in the same serum samples with a chemical method and we found that only 2 values agreed with the value determined chemically. Therefore, there is

a great need to develop a standard and highly reliable $25(OH)D_3$ assay before supplementation levels can be set on the basis of serum $25(OH)D_3$ concentrations. It is well known that, when large amounts of vitamin D are given to a patient, much of the vitamin D is stored in adipose tissues. Once these sites have been saturated, the vitamin D remains in serum and is converted to $25(OH)D_3$, which is toxic as an analog of $1,25(OH)_2D_3$ (61). When the dietary levels of vitamin D_3 needed to achieve normal concentrations of $25(OH)D_3$ in the plasma are being determined, vitamin D_3 itself should be measured, to confirm that vitamin D_3 is not being accumulated to an extent that would result in vitamin D intoxication. These measurements with well-established, precise methods, together with a careful, clinical, dose-escalation study, should allow setting of a supplementation level that is safe and can help prevent degenerative diseases, as well as preventing or reducing the risk of autoimmune diseases.

CONCLUSIONS

Vitamin D has yielded a class of compounds that can be used for the treatment of a variety of diseases. Vitamin D_3 itself is converted, in a 2-step process, to $1,25(OH)_2D_3$. This hormone reacts with a single nuclear type 2 receptor to facilitate the activation or suppression of target genes. The proteins produced in response to the hormone then carry out classic and nonclassic functions of vitamin D. In addition to causing mineralization of the skeleton and increasing serum calcium and phosphorus concentrations, vitamin D is known to regulate parathyroid growth and parathyroid hormone production; it plays a role in the islet cells of the pancreas, has a significant effect on the immune system, and can help in suppression of certain autoimmune diseases and certain cancers. To obtain maximal benefits of dietary vitamin D and to reduce the risks of these diseases, intakes of vitamin D higher than currently recommended are in order. Furthermore, a standardized $25(OH)D_3$ assay that provides true values must be developed; findings could provide a basis for understanding what levels of supplementation must be used to yield adequate amounts of $25(OH)D_3$.

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REFERENCES

1. Steenbock H. The induction of growth promoting and calcifying properties in a ration by exposure to light. Science 1924;60:224–5.
2. McCollum EV, Davis M. The necessity of certain lipins in the diet during growth. J Biol Chem 1913;25:167–231.
3. Mellanby E. An experimental investigation on rickets. Lancet 1919;1: 407–12.
4. McCollum EV, Simmonds N, Becker JE, Shipley PG. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J Biol Chem 1922;53:293–8.
5. Huldshinsky K. Heilung von Rachitis durch kunstalich Hohen-sonne. (The healing of rickets with artificial high altitude sun.) Dtsch Med Wochenschr 1919;45:712–3 (in German).
6. Chick H, Dolyell EJ, Hume EM. Studies of rickets in Vienna 1919–1922. Med Res Counc (GB) Spec Rep Ser 1923;77.
7. Steenbock H, Black A. Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultraviolet light. J Biol Chem 1924;61:405–22.
8. Goldblatt H, Soames KM. Studies on the fat-soluble growth-promoting factor. Biochem J 1923;17:446–53.
9. Askew FA, Bourdillon RB, Bruce HM, Jenkins RGC, Webster TA. The distillation of vitamin D. Proc R Soc Lond 1931;8107:76–90.
10. Windaus A, Schenck F, von Werder F. Uber das antirachitisch wirksame Bestrahlungs-produkt aus 7-Dehydrocholesterin. (Concerning the antirachitic activity of the irradiation product of 7-dehydrocholesterol.) Hoppe-Seyler's Z Physiol Chem 1936;241:100–3 (in German).
11. Velluz L, Amiard G. Chimie organique-equilibre de réaction entre précalciférol et calciférol. (The organic chemical equilibrium of the reaction between precalciferol and calciferol.) C R Assoc Anat 1949;228:853–5 (in French).
12. Prader A, Illig R, Heierli E. Eine besondere Form der primären Vitamin D-resistenten Rachitis mit Hypocalcämie und autosomaldominantem Erbgang: Die hereditäre Pseudo-mangelrachitis. (A special form of primary vitamin D-resistant rickets with hypocalcemia and autosomal receiver inheritance: The hereditary pseudodeficiency rickets.) Helv Paediatr Acta 1961;16:452–68 (in German).
13. Lund J, DeLuca HF. Biologically active metabolite of vitamin D_3 from bone, liver, and blood serum. J Lipid Res 1966;7:739–44.
14. Morri H, Lund J, Neville PF, DeLuca HF. Biological activity of a vitamin D metabolite. Arch Biochem Biophys 1967;120:508–12.
15. Blunt JW, DeLuca HF, Schnoes HK. 25-Hydroxycholecalciferol: a biologically active metabolite of vitamin D_3. Biochemistry 1968;7:3317–22.
16. Blunt JW, DeLuca HF. The synthesis of 25-hydroxycholecalciferol: a biologically active metabolite of vitamin D_3. Biochemistry 1969;8: 671–5.
17. Holick MF, Schnoes HK, DeLuca HF, Suda T, Cousins RJ. Isolation and identification of 1,25-dihydroxycholecalciferol: a metabolite of vitamin D active in intestine. Biochemistry 1971;10:2799–804.
18. Semmler EJ, Holick MF, Schnoes HK, DeLuca HF. The synthesis of 1α,25-dihydroxycholecalciferol: a metabolically active form of vitamin D_3. Tetrahedron Lett 1972;40:4147–50.
19. DeLuca HF, Schnoes HK. Vitamin D: recent advances. Annu Rev Biochem 1983;52:411–39.
20. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev 1998;78:1193–231.
21. Rosen H, Reshef A, Maeda N, et al. Markedly reduced bile acid synthesis but maintained levels of cholesterol and vitamin D metabolites in mice with disrupted sterol 25-hydroxylase gene. J Biol Chem 1998;273:14805–12.
22. Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxylase. J Biol Chem 2003;278:38084–93.
23. Ohyama Y, Okuda K. Isolation and characterization of a cytochrome P-450 from rat kidney mitochondria that catalyzes the 24-hydroxylation of 25-hydroxyvitamin D_3. J Biol Chem 1991;266:8690–5.
24. Underwood JL, DeLuca HF. Vitamin D is not directly necessary for bone growth and mineralization. Am J Physiol 1984;246:E493–8.
25. Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. J Cell Biochem 2002;88:259–66.
26. Garabedian M, Holick MF, DeLuca HF, Boyle IT. Control of 25-hydroxycholecalciferol metabolism by the parathyroid glands. Proc Natl Acad Sci USA 1972;69:1673–6.
27. Garabedian M, Tanaka Y, Holick MF, DeLuca HF. Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D_3 in thyroparathyroidectomized rats. Endocrinology 1974;94:1022–7.
28. Yamamoto M, Kawanobe Y, Takahashi H, Shimazawa E, Kimura S, Ogata E. Vitamin D deficiency and renal calcium transport in the rat. J Clin Invest 1984;74:507–13.
29. Brown EM, Gamba G, Riccardi R, et al. Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. Nature 1993;366:575–80.
30. Tanaka Y, DeLuca HF. Rat renal 25-hydroxyvitamin D_3-1- and 24-hydroxylases: their in vivo regulation. Am J Physiol 1984;246:E168–73.
31. Brenza HL, DeLuca HF. Regulation of 25-hydroxyvitamin D_3 1α-hydroxylase gene expression by parathyroid hormone and 1,25-dihydroxyvitamin D_3. Arch Biochem Biophys 2000;381:143–52.
32. Chambers TJ, Magnus CJ. Calcitonin alters behaviour of isolated osteoclasts. J Pathol 1982;136:27–39.
33. Shinki T, Ueno Y, DeLuca HF, Suda T. Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D_3-1α-hydroxylase gene in normocalcemic rats. Proc Natl Acad Sci USA 1999;96:8253–8.
34. Sutton AL, MacDonald PN. Vitamin D: more than a "bone-a-fide" hormone. Mol Endocrinol 2003;17:777–91.
35. Rachez C, Suldan Z, Ward J, et al. A novel protein complex that interacts with the vitamin D_3 receptor in a ligand-dependent manner and enhances

VDR transactivation in a cell-free system. Genes Dev 1998;12:1787–800.
36. Kimmel-Jehan C, Darwish HM, Strugnell SA, Jehan F, Wiefling B, DeLuca HF. DNA binding is induced by binding of vitamin D receptor-retinoid X receptor heterodimers to vitamin D response elements. J Cell Biochem 1999;74:220–8.
37. Brown TA, DeLuca HF. Phosphorylation of the 1,25-dihydroxyvitamin D_3 receptor: a primary event in 1,25-dihydroxyvitamin D_3 action. J Biol Chem 1990;265:10025–9.
38. Segura C, Alonso M, Fraga C, Garcia-Caballero T, Dieguez C, Perez-Fernandez R. Vitamin D receptor ontogenesis in rat liver. Histochem Cell Biol 1999;112:163–7.
39. Fraga C, Blanco M, Vigo E, Segura C, Garcia-Caballero T, Perez-Fernandez R. Ontogensis of the vitamin D receptor in rat heart. Histochem Cell Biol 2002;117:547–50.
40. Bischoff HA, Borchers M, Gudat F, et al. In situ detection of 1,25-dihydroxyvitamin D_3 receptor in human skeletal muscle tissue. Histochem J 2001;33:19–24.
41. Prufer K, Veenstra TD, Jirikowski GF, Kumar R. Distribution of 1,25-dihydroxyvitamin D_3 receptor immunoreactivity in the rat brain and spinal cord. J Chem Neuroanat 1999;16:135–45.
42. Gascon-Barre M, Demers C, Mirshahi A, Neron S, Zalzal S, Nanci A. The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells. Hepatology 2003;37:1034–42.
43. Pike JW, Goozé LL, Haussler MR. Biochemical evidence for 1,25-dihydroxyvitamin D receptor macromolecules in parathyroid, pancreatic pituitary and placental tissues. Life Sci 1979;26:407–14.
44. Sandgren ME, Bronnegard M, DeLuca HF. Tissue distribution of the 1,25-dihydroxyvitamin D_3 receptor in the male rat. Biochem Biophys Res Commun 1991;181:611–6.
45. Darwish HM, DeLuca HF. Identification of a transcription factor that binds to the promoter region of the human parathyroid hormone gene. Arch Biochem Biophys 1999;365:123–30.
46. Slatopolsky E, Gonzalez E, Martin K. Pathogenesis and treatment of renal osteodystrophy. Blood Purif 2003;21:318–26.
47. Yang S, Smith C, Prahl JM, DeLuca HF. Vitamin D deficiency suppresses cell-mediated immunity in vivo. Arch Biochem Biophys 1993; 303:98–106.
48. Yang S, Smith C, DeLuca HF. 1α,25-Dihydroxyvitamin D_3 and 19-nor-1α,25-dihydroxyvitamin D_2 suppress immunoglobulin production and thymic lymphocyte proliferation in vivo. Biochim Biophys Acta 1993; 1158:269–86.
49. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D_3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci USA 1996;93:7861–4.
50. Zella JB, DeLuca HF. Vitamin D and autoimmune diabetes. J Cell Biochem 2003;88:216–22.
51. Lemire JM, Ince A, Takashima M. 1,25-Dihydroxyvitamin D_3 attenuates the expression of experimental murine lupus of MRL/1 mice. Autoimmunity 1992;12:143–8.
52. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr 2000;130:2648–52.
53. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D_3 prevents and ameliorates symptoms in two experimental model of human arthritis. J Nutr 1998;128:68–72.
54. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1α,25-dihydroxyvitamin D_3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. Proc Natl Acad Sci USA 2001; 98:6800–5.
55. Hullett DA, Cantorna MT, Redaelli C, et al. Prolongation of allograft survival by 1,25-dihydroxyvitamin D_3. Transplantation 1998;66:824–8.
56. Sicinski RR, Prahl JM, Smith CM, DeLuca HF. New 1α,25-dihydroxy-19-norvitamin D_3 compounds of high biological activity: synthesis and biological evaluation of 2-hydroxymethyl, 2-methyl and 2-methylene analogs. J Med Chem 1998;41:4662–74.
57. Yamamoto H, Shevde NK, Warrier A, Plum LA, DeLuca HF, Pike JW. 2-Methylene-19-nor-(20*S*)-1,25-dihydroxyvitamin D_3 potently stimulates gene-specific DNA binding of the vitamin D receptor in osteoblasts. J Biol Chem 2003;278:31756–65.
58. Shevde NK, Plum LA, Clagett-Dame M, Yamamoto H, Pike JW, DeLuca HF. A novel potent analog of 1α,25-dihydroxyvitamin D_3 selectively induces bone formation. Proc Natl Acad Sci USA 2002;99:13487–91.
59. Plum LA, Prahl JM, Ma X, et al. Biologically active noncalcemic analogs of 1α,25-dihydroxyvitamin D with an abbreviated side chain containing no hydroxyl. Proc Natl Acad Sci USA 2004;101:6900–4.
60. Binkley N, Krueger D, Cowgill CS, et al. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. J Clin Endocrinol Metab 2004;88:3152–7.
61. Shepard RM, DeLuca HF. Plasma concentrations of vitamin D_3 and its metabolites in the rat as influenced by vitamin D_3 or 25-hydroxyvitamin D_3 intakes. Arch Biochem Biophys 1980;202:43–53.