Summary: Vitamin D is not technically a vitamin, since it is not an essential dietary factor. It is rather a prohormone produced photochemically in the skin from 7-dehydrocholesterol. Vitamin D and its metabolites may be categorized as either cholecalciferols or ergocalciferols. Cholecalciferol (vitamin D3) is the parent compound of the naturally occurring family and is produced in the skin from 7-dehydrocholesterol on exposure to the ultraviolet B portion of sunlight. Vitamin D2 (ergocalciferol), the parent compound of the other family, is manufactured by irradiation of ergosterol produced by yeasts and its potency is less than one-third of vitamin D3’s potency. The steps in the vitamin D endocrine system include the following: 1) the photoconversion of 7-dehydrocholesterol to vitamin D3 in the skin or dietary intake of vitamin D3; 2) metabolism of vitamin D3 by the liver to 25-hydroxyvitamin-D3 \([25(OH)D3]\), the major form of vitamin D circulating in the blood compartment; 3) conversion of 25(OH)D3 by the kidney (functioning as an endocrine gland) to the hormone 1,25-dihydroxyvitamin D3 \([1,25(OH)2D3]\); 4) systemic transport of the dihydroxylated metabolite 1,25(OH)2D3 to distal target organs; and 5) binding of 1,25(OH)2D3 to a nuclear receptor (VDR) at target organs, followed by generation of appropriate biological responses. The activation of vitamin D to its hormonal form is mediated by cytochrome P450 enzymes. Six cytochrome P450 (CYP) isoforms have been shown to hydroxylate vitamin D. Four of these, CYP27A1, CYP2R1, CYP3A4 and CYP2J3, are candidates for the enzyme vitamin D 25-hydroxylase that is involved in the first step of activation. Four of these, CYP27A1, CYP2R1, CYP3A4 and CYP2J3, are candidates for the enzyme vitamin D 25-hydroxylase that is involved in the first step of activation. The highly regulated, renal enzyme 25-hydroxyvitamin D-1α-hydroxylase contains the component CYP27B1, which completes the activation pathway to the hormon monal form 1,25(OH)2D3. A five-step inactivation pathway from 1,25(OH)2D3 involves \(\Delta\)-24 hydroxylase activity, 1α-hydroxylase activity and 24,25-hydroxylase activity. CYP24A1, a multifunctional enzyme, hydroxylates 24-hydroxyvitamin D to 24,25-dihydroxyvitamin D, which is further hydroxylated by CYP26B1 to 25-hydroxyvitamin D. The highly regulated, renal enzyme 25-hydroxyvitamin D-1α-hydroxylase contains the component CYP27B1, which completes the activation pathway to the hormon monal form 1,25(OH)2D3. A five-step inactivation pathway from 1,25(OH)2D3 involves \(\Delta\)-24 hydroxylase activity, 1α-hydroxylase activity and 24,25-hydroxylase activity. CYP24A1, a multifunctional enzyme, hydroxylates 24-hydroxyvitamin D to 24,25-dihydroxyvitamin D, which is further hydroxylated by CYP26B1 to 25-hydroxyvitamin D.
Introduction

Vitamin D₃ is not a genuine vitamin, in the sense that it is not an essential element of diet, but it is more of a sterol prohormone produced through photochemical reaction in the skin from 7-dehydrocholesterol. The metabolic action is carried out through its hormonal form, 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), after binding to a nuclear receptor (vitamin D receptor, VDR) which regulates the transcription of a number of target genes in a variety of vitamin D target cells. The presence of activating and inactivating enzymes of vitamin D, as well as VDR in almost all mammalian cells, together with the observation that provitamin D₃ is oxidized almost completely by the mouse and human genome is regulated via the vitamin D pathway, indicates that it is essential for life in higher animals (1). Besides the well established central role in calcium homeostasis, this pluripotent hormone has additional biological actions in the adaptive and innate immune system, insulin secretion by the pancreatic β cells, multifactorial heart functioning and blood pressure regulation, brain and fetal development (2). In accord with the above, the prevailing epidemiological data support the role that vitamin D might play in controlling the risk of many chronic illnesses, including common cancers, myopathy, autoimmune disease, diabetes and the metabolic syndrome, infections, and cardiovascular disease (1, 3).

In this article we summarize the current knowledge of the metabolism and biochemistry of vitamin D, including biological activation and inactivation, mechanisms of action and regulation.

Structure and synthesis of vitamin D

Vitamin D is a secosteroid with broken 9,10 carbon-carbon bond in B ring of the cyclopentano-perhydrophenanthrene structure. Vitamin D and its metabolites may be classified as either cholecalciferol or ergocalciferol. Cholecalciferol (vitamin D₃) is the parent compound of the naturally occurring family and is produced in the skin from 7-dehydrocholesterol on exposure to the ultraviolet B portion of sunlight. Beside the photosynthesis in the skin, vitamin D₃ can also be introduced with certain types of foods, including fatty fish, fish liver oils, and egg yolk. Vitamin D₂ (ergocalciferol) is manufactured by irradiation of ergosterol produced by yeasts. Vitamin D₂ differs from vitamin D₃ by the double bond between carbon 22 and carbon 23 and a methyl group on carbon 24. When vitamin D or its metabolites are written without a subscript, both families are included (4). Vitamin D₂ has only one third of vitamin D₃’s potency regarding its biological actions (5), structure of vitamins D₃ and D₂ is represented in Figure 1.

Metabolism

Vitamin D₃ has no known biological function in its native form. It must be metabolized first to 25-hydroxyvitamin D₃ (25(OH)D₃) in the liver and then to 1,25(OH)₂D₃ by the kidney. In studies of vitamin D metabolism some 37 vitamin D₃ metabolites have been isolated and chemically characterized (6). All of these metabolites can be systematized into three basic pathways of vitamin D₃ metabolism: (a) the main two-step activation pathway in liver and kidney that produces 1,25(OH)₂D₃; (b) an inducible carbon-
oxidation pathway in vitamin D target cells for inactivating 25(OH)D3 to 24,25-dihydroxyvitamin D3 \([24,25(OH)2D3]\) and 1,25(OH)2D3 to calcitriol; (c) and not completely elucidated 26,23-lactone pathway for converting both 25(OH)D3 and 1,25(OH)2D3 to lactone products (7). Vitamin D2 undergoes 25- and 1α-hydroxylation steps by the same enzymes, which produces an analogous form of 1,25(OH)2D2, after the conversion into 25(OH)D2 (8).

The sequencing of the human genome has led to understanding that among a pool of 60 total cytochrome P450s (CYPs) there are three known and possibly other vitamin D-related CYPs linked to the metabolism of vitamin D. The key enzymes in vitamin D metabolism are the hepatic vitamin D-25-hydroxylase (CYP27A1 and CYP2R1), renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1), and 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) (9).

### Vitamin D-25-hydroxylase

Several CYPs are able to catalyze the 25-hydroxylation of vitamin D3 in vitro. No meaningful regulation of CYP27A1 by vitamin D or calcium homeostatic hormones has been reported in the literature, why it is probably not the physiologically relevant 25-hydroxylase. CYP27A1 does not 25-hydroxylate vitamin D2 and the efficient 25-hydroxylation of vitamin D3 has been reported in most mammals in vivo. This enzyme has an essential role in cholesterol and bile acid metabolism and its deficiency does not affect serum levels of vitamin D metabolites. Genetic mutations of CYP27A1 in humans cause cerebrotendinous xanthomatosis, a disorder of bile acid and lipid metabolism that sometimes presents with low 25(OH)D levels and a type of osteoporosis (7).

On the other hand, microsomal CYP2R1 is thought to play a major role due to the absence of sex and species differences and catalytic activity toward both vitamin D2 and D3. CYP2R1 is regiospecific to the C-25 position of a secosteroid in contrast to other polyfunctional CYP enzymes with vitamin D 25-hydroxylase activity (CYP27A1, CYP2C11, and CYP3A4). Moreover, mutation in the human Cyp2r1 gene results in vitamin D-dependent rickets, the only type of rickets that is caused by 25-hydroxylase deficiency. Furthermore, CYP2R1 expression in human
tissues is ubiquitous, which supports the findings that the ability to form 25(OH)D₃ is almost unchanged in patients with liver disorders (10).

**25-hydroxyvitamin D-1α-hydroxylase**

Mitochondrial 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) has a central role in calcium homeostasis. The main control of calcium homeostasis is the calcium-sensing receptor in the parathyroid cell, which regulates the secretion of parathyroid hormone (PTH). In turn, PTH is principal activator of 25-hydroxyvitamin D-1α-hydroxylase gene expression, which represents the mechanism needed to control plasma concentration of 1,25(OH)₂D (11). This regulation is independently amplified by low calcium and phosphorus signals (12). By maintaining tight regulation of the concentration of 1,25(OH)₂D and thereby giving rise to appropriate transcriptional activation of the genes involved in calcium and phosphorus transport and cell differentiation, the 25-hydroxyvitamin D-1α-hydroxylase plays a vital role in vitamin D signaling (7).

It has been shown, however, that CYP27B1 is expressed in various extrarenal sites around the body including the keratinocyte, lung, colon, and macrophages (1). This indicates that extrarenal 25-hydroxyvitamin D-1α-hydroxylase has an autocrine or paracrine role in specific tissue differentiation (13). This was demonstrated by the work of Dusso et al. (14) who have shown that cytokines such as interferon-γ, not PTH, upregulate expression of CYP27B1 in the macrophage. The concept of extrarenal 25-hydroxyvitamin D-1α-hydroxylase has not only physiological implications but also pathological ones. For example, sarcoidosis is a granulomatous condition that often involves hypercalcemia caused by the overproduction of 1,25(OH)₂D in sarcoid macrophages (7).

**25-hydroxyvitamin D-24-hydroxylase**

Mitochondrial 25-hydroxyvitamin D-24-hydroxylase (CYP27A1) is also regulated by 1,25(OH)₂D, but in opposite direction to 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) owing to the transcriptional upregulation in the CYP27A1 promoter. The outcome is induction of CYP27A1 in all vitamin D target cells, which provides exquisite attenuation of the hormonal signal in the individual target cell when the gene transcriptional upregulation of 1,25(OH)₂D need to be turned off (7). Cyp24a1 knockout mice confirm the catabolic role for CYP27B1, because they show poor viability with 50% lethality, showing hypercalcemia, with marked difficulty in excreting a bolus of [1,25(OH)₂D₃ and isolated cultured keratinocytes from these mice fail to synthesize calcitriol, as opposite to heterozygous and wild-type mice (15). In humans, mutations in Cyp24a1 with a complete loss of function, cause idiopathic infantile hypercalcemia (16).

C-24 oxidation represents the pr edominant catabolic pathway for 1,25(OH)₂D₃ in which sequential C-24 hydroxylation, C-24 ketonization, and C-23 hydroxylation, followed by oxidative cleavage to form tetranor-1α, 23-dihydroxyvitamin D₃ and calcitriol, generate inactive, water-soluble product that is excreted in bile (9). The second γ pathway involves C-26 hydroxylation, C-23 hydroxylation, and lactonization to form (23S,25R)-1,25(OH)₂D₃-26,23-lactone. Both pathways have also been shown to participate in the metabolism of 25(OH)D₃ (17). Studies of the recombinant CYP24A1 protein have revealed that CYP24A1 uses multicatalytic activity, facilitating sequential oxidation of C-23, C-24 and C-26 hydroxylation and side-chain cleavage (9).

**Mechanisms of action**

Mechanism of action of vitamin D3, through its hormonal form, 1,25(OH)₂D₃ involves nuclear 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 (18). These regulatory functions are performed by several specific proteins included in vitamin D signal transduction system, which constitutes of VDR and its associated transcriptional coactivators, plasma transport protein (vitamin D binding protein, DBP), the activating CYPs (CYP2R1, CYP27A1, and CYP27B1), and catabolic CYP24A1 (described in previous section) (19).

**Vitamin D binding protein**

Because of its lipophilic nature, vitamin D₃ 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 of calcitriol. After the absorption from the gut, vitamin D enters the circulation on chylomicrons first, and it is only slowly transferred to DBP, for which it has low affinity, between 10⁻⁵ and 10⁻⁷ mol/L. The difference between the transport of dietary and vitamin D synthesized in the skin is that the later is mainly bound to DBP (20). 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. The liver takes up remaining vitamin D from the chylomicron remnant and quickly removes it from the bloodstream. The loss into tissue and liver pools explains the short plasma half-life, ≈4–6 h, and the whole-body half-life ≈2 months of vitamin D (19).
In the liver vitamin D converts into 25(OH)D due to the action of mitochondrial CYP2R1 or mitochondrial CYP27A1, neither of which is subject to tight regulation (21). 25(OH)D quickly enters the plasma pool that constitutes the predominant pool of vitamin D in the body, with a capacity of 4.5 μmol/L. 25(OH)D2 and 25(OH)D3 have a strong affinity for DBP at 5 × 10⁻⁸ mol/L why 25(OH)D₃ has a half-life of 15 days in the cir culation. The normal circulating level of 25(OH)D in the blood is only 25–200 nmo/L, indicating that ligand only occupies 2–5% of DBP in the physiologic state (19).

The dihydroxy-metabolites have different affinities for DBP: 25(OH)D₂-26,23-lactone binding 3–5 times as tightly as 25(OH)D₂ and inactive metabolites, 24,25(OH)₂D₂ and 25,26(OH)₂D₃ bind with equal affinity as 25(OH)D₂. The active for m 1,25(OH)₂D₃ has a half-life of 10–20 h depending on the state of the highly inducible catabolic machinery. The accumulation of these metabolites in the bloodstream is mainly a function of their affinities for DBP, but the rate of synthesis and degradation also plays a partial role (19).

**Vitamin D receptor**

The steroid hormone 1,25(OH)₂D₃, like other steroid hormones, generates biological responses by regulating gene transcription (genomic responses) and by activating a variety of signal transduction pathways at or near plasma membrane (nongenomic rapid responses) (22). The genomic responses are due to stereospecific interaction of 1,25(OH)₂D₃ with its nuclear receptor, VDR. The primary amino acid sequence of the VDR consists, like in other steroid hormone receptors, of 6 functional domains: the variable regions, DNA binding, the hinge region, the ligand binding region, and the transcriptional activation domain (2). Formation of the ligand-receptor complex results in conformational changes in the receptor protein, which allow the ligand-receptor complex to specifically interact with many proteins from the transcriptional machinery. It is estimated that the VDR can regulate the expression of as many as 500 of the 20 488 genes in the human genome (23). The large number of VDR-regulated genes undoubtedly reflects the consequence of the distribution of both the VDR and 25(OH)D₂-1α-hydroxylase to many organs.

»Rapid« or nongenomic responses provoked by 1,25(OH)₂D₃ are mediated through the interaction of the hormone with a receptor located on the cell’s external membrane. This membrane receptor is the classic VDR, found in nucleus and cytosol and associated with caveolae in the plasma membrane of a variety of cells (24). Caveolae are flask-shaped membrane invaginations enriched in sphingolipids and cholesterol that are commonly found in a wide variety of cells (25). Using VDR knockout and wild-type mice, it was found that rapid modulation of osteoblast ion channel responses by 1,25(OH)₂D₃ require the presence of a functional vitamin D nuclear and caveola receptor (26).

Although VDR is expressed in many cells around the body, differences in tissue-, differentiation stage- and gene-specific transcription factors present at the vitamin D-dependent genes allow wide variability in the range of genes that are modulated in each tissue at any given time. Even the direction of the effect of 1,25(OH)₂D₃ on gene transcription, in the sense whether it causes upregulation or downregulation, is gene-specific. For example, various Ca-homeostatic genes (e.g. calbindins, Ca-channel proteins, osteocalcin, osteopontin, RANKL genes) are upregulated, whereas others (e.g. collagen and prolactin genes) are downregulated by 1,25(OH)₂D₃ (7).

VDR is 1,25(OH)₂D₃-dependent transcription factor that controls gene expression by heterodimerizing with retinoid X receptors (RXRs) and associating specifically with VDR responsive elements (VDRE) in target genes. Sequence and promoter analysis of several 1,25(OH)₂D₃-regulated genes have led to the identification of VDREs, short DNA sequences to which the VDR-RXR heterodimer binds and subsequently exerts its influence on transcription. Some VDREs have been identified in genes that are known to be transcriptionally activated by the 1,25(OH)₂D₃ hormone including osteocalcin and osteopontin (expressed in bone osteoblasts), β₂ integrin (found in bone osteoclasts and macrophages), calbindin-D₂₈k (from kidney) and p21 (an inhibitor of cyclin dependent kinase, Cdk, in many tissues) (12).

The activation of the ligand-bound VDR in the intestine, bone, kidney, and parathyroid gland cells results in the maintenance of normal serum calcium and phosphorous levels and their related effects on mineralization and turnover of bone (27). However, 1α-hydroxylase is also present in cells of several extrarenal tissues such as skin, bone, prostate, and many immune cells. The enzyme here is identical to the one expressed in the kidney, but its expression is regulated by immune signals instead of mediators of bone and mineral homeostasis (28). Therefore, the potential actions of 1,25(OH)₂D₃ via its nuclear VDR extend far beyond the bone mineral homeostasis. High local 1,25(OH)₂D₃ concentrations may, independently from serum concentrations, manifest an autocrine and paracrine function since the VDR can act as a receptor for 1,25(OH)₂D₃ in vivo immunomodulatory function since the VDR is widely present in many different cell types and tissues (29). For example, one major target for 1,25(OH)₂D₃ is the immune system, where the mediators of VDR it suppresses IL-1 to IL-6 and interferon-γ in vitro. Moreover, documented in vivo immunomodulatory actions of the hormone are reduced macrophage and lymphocyte function in vitamin D deficient rats. 1,25(OH)₂D₃ functions as a general suppressor of the immune system, especially of Th1 cells, suggesting its potential usefulness in the treatment of autoimmune disorders. In central nervous system, besides

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immunosuppression, 1,25(OH)2D3 has been shown to induce expression of a number of neurotrophic hormones from degenerative processes. Together with the expression of calbindin-D28k which exhibits antiapoptotic effect, 1,25(OH)2D3 may have a possible role in therapeutic intervention for neurodegenerative disorders. Similarly, 1,25(OH)2D3 affects the maturation and function of certain normal and neoplastic cells (e.g. mammary, prostate, and colon), which may be related to the ability of liganded VDR to arrest cells at the G1 stage by influencing cell cycle regulatory proteins, such as p21 and p27, to control cell growth transcription factors, such as c-myc and c-fos, or to elicit apoptosis by down-regulating Bcl-2. 1,25(OH)2D3 also reportedly affects several major endocrine processes, such as TRH/TSH action and pancreatic insulin secretion (12).

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