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Review article
Pregledni članak

BIOCHEMISTRY AND METABOLISM OF VITAMIN D

BIOHEMIJA I METABOLIZAM VITAMINA D

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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 D_3) 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 D_2 (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 D_3's potency. The steps in the vitamin D endocrine system include the following: 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-hydroxyvitamin-D_3 [$25(OH)D_3$], 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 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]; 4) systemic transport of the dihydroxylated metabolite $1,25(OH)_2D_3$ to distal target organs; and 5) binding of $1,25(OH)_2D_3$ 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. The highly regulated, renal enzyme 25-hydroxyvitamin D-1α-hydroxylase contains the component CYP27B1, which completes the activation pathway to the hormonal form $1,25(OH)_2D_3$. A five-step inactivation pathway from

Kratak sadr`aj: Vitamin D nije pravi vitamin, odnosno nije esencijalni dijetetski faktor, već je prehormon koji nastaje fotohemijskom reakcijom u koži iz 7-dehidroholesterola. Vitamin D i njegovi metaboliti mogu da se kategorizuju kao holekalcifer oli ili ergokalciferoli. Holekalciferol (vitamin D_3) je polazno jedinjenje za familiju koja se nalazi u prirodi i produkuje se u koži iz 7-dehidroholesterola pri izlaganju ultraljubičastom B delu spektra sunčeve svetlosti. Vitamin D_2 (ergokalciferol), polazno jedinjenje druge familije, nastaje radijacijom ergosterola koga produkuju kvasci i ima samo jednu trećinu aktivnosti vitamina D_3. Faze u endokrinom sistemu vitamina D su: 1) fotokonverzija 7-dehidroholesterola u vitamin D_3 u koži ili unos vitamina D_3 hranom; 2) metabolizam vitamina D_3 u jetri do 25-hidroksivitamina D_3 [$25(OH)D_3$], glavnog oblika vitamina D u cirkulaciji; 3) konverzija $25(OH)D_3$ u bubregu (koji ovde funkcioniše kao endokrina žlezda) do hormona 1,25-dihidroksivitamin D_3 [$1,25(OH)_2D_3$]; 4) sistemski transport dihidroksi-metabolita do distalnih ciljnih organa; i 5) vezivanje $1,25(OH)_2D_3$ za nuklearni receptor (VDR) u ciljnim organima, što prati odgovarajući biološki odgovor. Aktivacija vitamina D do hormonskog oblika je posredovana citohrom P450 enzimima. Pokazano je da šest izoformi citohroma P450 (CYP) učestvuje u hidroksilaciji vitamina D. Za četiri od njih, CYP27A1, CYP2R1, CYP3A4 i CYP2J3, se pretpostavlja da imaju aktivnost 25-hidroksilaze koja učestvuje u prvom koraku aktivacije. Renalni enzim, 25-hidroksivitamin D-1α-hidroksilaza sa strogo regulisanom aktivnošću, predstavlja CYP27B1, koji završava aktivaciju do hormonskog oblika $1,25(OH)_2D_3$. Proces inaktivacije, koji se sastoji iz pet stupnjeva od $1,25(OH)_2D_3$ do kalcitroične kiseline, obavlja jedan multifunkcionalni CYP, CYP24A1, čija je transkripcija indukovana u ciljnim ćelija-

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$1,25(OH)_2D_3$ to calcitroic acid is attributed to a single multifunctional CYP, CYP24A1, which is transcriptionally induced in vitamin D target cells by the action of $1,25(OH)_2D_3$. An additional key component in the operation of the vitamin D endocrine system is the plasma vitamin D binding protein (DBP), which carries vitamin D_3 and its metabolites to their metabolism and target organs. DBP is a specific, high-affinity transport protein. It is synthesized by the liver and circulates in great excess, with fewer than 5% of the binding sites normally occupied. $1,25(OH)_2D_3$, acts as a ligand for a nuclear transcription factor, vitamin D receptor – VDR, which like all other nuclear receptors, regulates gene transcription and cell function. The widespread presence of VDR, and the key activating (1 α-hydroxylase, CYP27B1) and inactivating (24-hydroxylase, CYP24A1) enzymes in most mammalian cells 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. Thanks to this widespread presence of elements of vitamin D endocrine system, its biological features are being recognized outside bone tissue, i.e. calcium and phosphate metabolism.

Keywords: vitamin D, vitamin D receptor, vitamin D binding protein, cytochrome P450

ma dejstva vitamina D posredstvom $1,25(OH)_2D_3$. Dodatna ključna komponenta u dejstvu vitamin D endokrinog sistema je vitamin D vezujući protein u plazmi (DBP), koji transportuje vitamin D_3 i njegove metabolite do ciljnih i organa gde se odvija njihov metabolizam. DBP je specifičan transportni protein velikog afiniteta. Sintetiše se u jetri i cirkuliše u velikom višku, sa zasićenjem vezujućih mesta manjim od 5%. $1,25(OH)_2D_3$ deluje kao ligand nuklearnog transkripcionog faktora, VDR, koji reguliše transkripciju gena i funkciju ćelija. široka rasprostranjenost VDR i ključnih enzima aktivacije (1 α-hidroksilaza, CYP27B1) i inaktivacije (24-hidroksilaza, CYP24A1) u većini ćelija sisara znači da ćelije u ovim tkivima imaju potencijal za produkovanje bioloških odgovora, zavisno od raspoloživosti dovoljnih količina vitamina D_3. Zahvaljujući rasprostranjenosti elemenata endokrinog sistema vitamina D, njegove biološke osobine se prepoznaju i izvan koštanog sistema, odnosno metabolizma kalcijuma i fosfora.

Ključne reči: vitamin D, receptor za vitamin D, vitamin-D vezujući protein, citohrom P450

Introduction

Vitamin D_3 is not a genuine vitamin, in the sense that it is not an essential element of diet, but it is more of a steroid prohormone produced through photochemical reaction in the skin from 7-dehydrocholesterol. The metabolic action is carried out through its hormonal form, 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], 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 approximately 3% of 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 have 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 accordance, the all growing 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 summmarize 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 cyclopentanoperhydrophenanthrene structure. Vitamin D and its metabolites may be classified as either cholecalciferols or ergocalciferols. Cholecalciferol (vitamin D_3) 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 D_3 can also be introduced with certain types of foods, including fatty fish, fish liver oils, and egg yolk. Vitamin D_2 (ergocalciferol) is manufactured by irradiation of ergosterol produced by yeasts. Vitamin D_2 differs from vitamin D_3 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 D_2 has only one third of vitamin D_3's potency regarding its biological actions (5). Structure of vitamins D_3 and D_2 is represented in *Figure 1*.

Metabolism

Vitamin D_3 has no known biological function in its native form. It must be metabolized first to 25-hydroxyvitamin D_3 [$25(OH)D_3$] in the liver and then to $1,25(OH)_2D_3$ by the kidney. In studies of vitamin D metabolism some 37 vitamin D_3 metabolites have been isolated and chemically characterized (6). All of these metabolites can be systematized into three basic pathways of vitamin D_3 metabolism: (a) the main two-step activation pathway in liver and kidney that produces $1,25(OH)_2D_3$; (b) an inducible carbon-

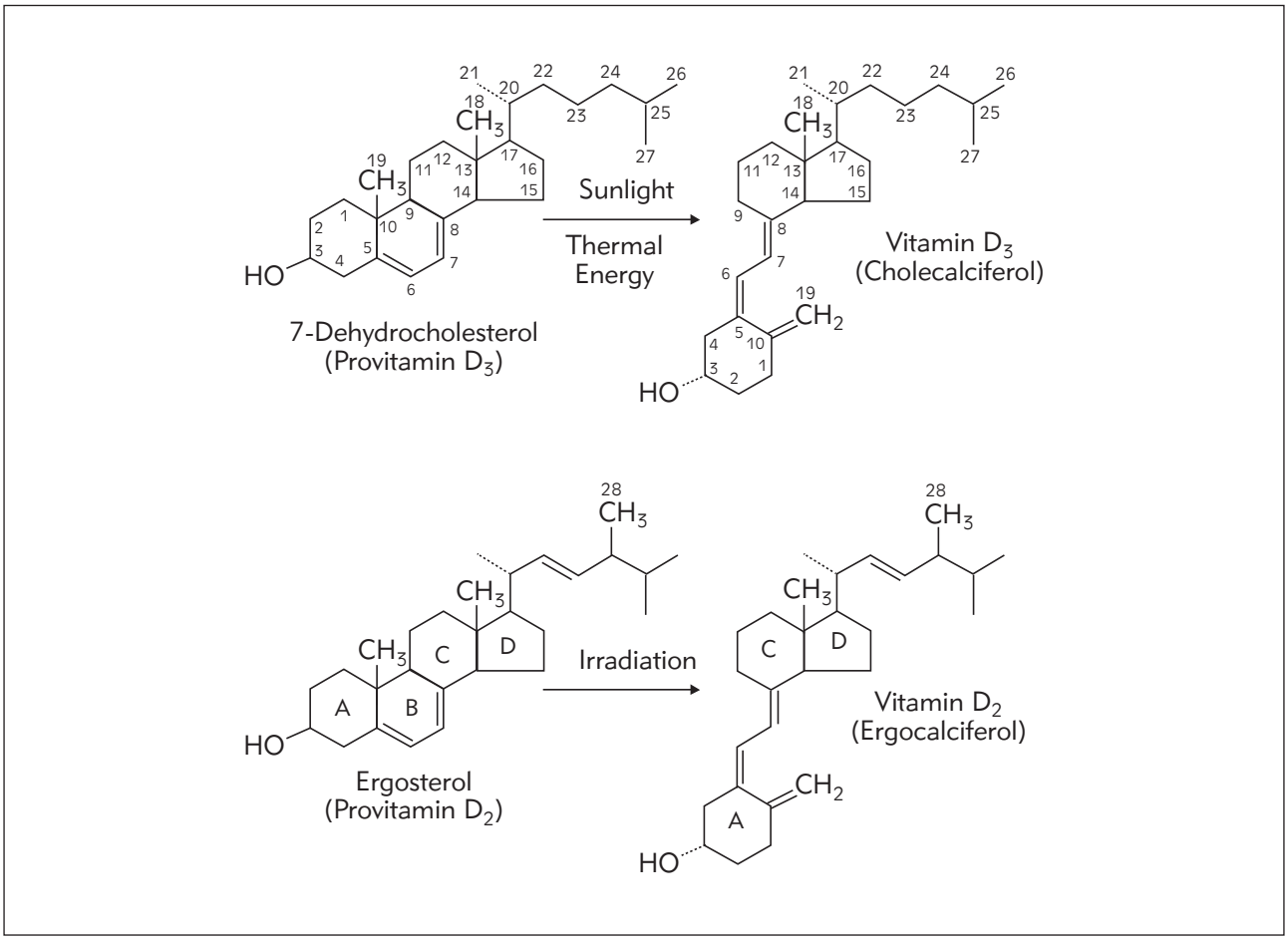

Figure 1 Structure of vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) and their precursors (4).

24 oxidation pathway in vitamin D tar get cells for inactivating 25(OH)D_3 to 24,25-dihydroxyvitamin D_3 [24,25(OH)$_2D_3$] and 1,25(OH)$_2D_3$ to calcitroic acid; (c) and not completely elucidated 26,23-lactone pathway for converting both 25(OH)D_3 and 1,25(OH)$_2D_3$ to lactone pr oducts (7). Vitamin D_2 undergoes 25- and 1α-hydroxylation steps by the same enzymes, which produces an analogous form of 1,25(OH)$_2D_2$, after the conversion into 25(OH)D_2 (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), r enal 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 D_3 *in vitro*. No meaningful regulation of CYP27A1 by vitamin D or calcium homeostatic hormones has been reported in the literature, why it is pr obably not the physiologically r elevant 25-hydroxylase. CYP27A1 does not 25-hydr oxylate vitamin D_2 and the efficient 25-hydr oxylation of vitamin D_2 has been reported in most mammals *in vivo*. This enzyme has an essential r ole in cholester ol and bile acid metabolism and its deficiency does not affect serum levels of vitamin D metabolites. Genetic mutations of CYP27A1 in humans cause cer ebrotendinous xanthomatosis, a disor der of bile acid and lipid metabolism that sometimes pr esents with low 25(OH)D levels and a type of osteopor osis (7).

On the other hand, micr osomal CYP2R1 is thought to play a major r ole due to the absence of sex and species differ ences and catalytic activity towar d both vitamin D_2 and D_3. CYP2R1 is r egiospecific 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-hydr oxylase deficiency. Furthermore, CYP2R1 expr ession in human

tissues is ubiquitous, which supports the findings that the ability to form $25(OH)D_3$ 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 maintainig 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, sarcoidosisis is a granulomatous condition that often involves hypercalciuria and eventually hypercalcemia caused by the overproduction od $1,25(OH)_2D$ in sarcoid macrophages (7).

25-hydroxyvitamin D-24-hydroxylase

Mitochondrial 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) 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 CYP24A1 promoter. The outcome is induction of CYP24A1 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 CYP24A1, because they show poor viability with 50% lethality, showing hypercalcemia, with marked difficulty in excreting a bolus of $[1\beta\text{-}^3H]1\alpha,25\text{-}(OH)_2D_3$ and isolated cultured keratinocytes from these mice fail to synthesize calcitroic acid, 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 predominant catabolic pathway for $1,25(OH)_2D_3$ in which sequential C-24 hydroxylation, C-24 ketonization, and C-23 hydroxylation, followed by oxidative cleavage to form tetranor-1α, 23-dihydroxyvitamin D_3 and calcitroic acid, liberate an inactive, water-soluble product that is excreted in bile (9). The secondary pathway involves C-26 hydroxylation, C-23 hydroxylation, and lactonization to form (23*S*,25*R*)-$1,25(OH)_2D_3$-26,23-lactone. Both patways have also been shown to participate in the metabolism of $25(OH)D_3$ (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 D_3, through its hormonal form, $1,25(OH)_2D_3$ 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 diferentiation (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 D_3 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 calcitroic acid. 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 microsomal 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. $25(OH)D_3$ and $25(OH)D_2$ have a strong affinity for DBP at 5×10^{-8} mol/L why $25(OH)D_3$ has a half-life of 15 days in the circulation. The normal circulating level of 25(OH)D in the blood is only 25–200 nmol/L, indicating that ligand only occupies 2–5% of DBP in the physiologic state (19).

The dihydroxy- metabolites have different affinities for DBP: $25(OH)D_3$-26,23-lactone binding 3–5 times as tightly as $25(OH)D_3$ and inactive metabolites, $24,25(OH)_2D_3$ and $25,26(OH)_2D_3$, bind with equal affinity as $25(OH)D_3$. The active form $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$, 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 $1,25(OH)_2D_3$ 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 $25(OH)D_3$-1α-hydroxylase to many organs.

»Rapid« or nongenomic responses provoked by $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ require the presence of a functional vitamin D nuclear and caveolae 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 $1,25(OH)_2D_3$ 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 pre-pro-PTH genes) are downregulated by $1,25(OH)_2D_3$ (7).

VDR is $1,25(OH)_2D_3$-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 $1,25(OH)_2D_3$-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 $1,25(OH)_2D_3$ hormone including osteocalcin and osteopontin (expressed in bone osteoblasts), β_3 integrin (found in bone osteoclasts and macrophages), calbindin-D_{28k} (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 phosphorus 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 $1,25(OH)_2D_3$ via its nuclear VDR extend far beyond the bone mineral homeostasis. High local $1,25(OH)_2D_3$ concentrations may, independently from serum concentrations, manifest an autocrine and paracrine function since the VDR is widely present in many different cells and tissues (29). For example, one major target for $1,25(OH)_2D_3$ is the immune system, where mediated by 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. $1,25(OH)_2D_3$ 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

immunosuppression, $1,25(OH)_2D_3$ has been shown to induce expression of a number of neurotrophic hormones from degenerative processes. Together with the expression of calbindin-D_{28k} which exhibits antiapoptotic effect, $1,25(OH)_2D_3$ may have a possible role in therapeutic intervention for neurodegenerative disorders. Similarly, $1,25(OH)_2D_3$ 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 downregulating *Bcl-2*. $1,25(OH)_2D_3$ also reportedly affects several major endocrine processes, such as TRH/TSH action and pancreatic insulin secretion (12).

Conclusion

In the era of worldwide vitamin D deficiency, the new roles of vitamin D, beyond well established bone and mineral metabolism, are being recognized. Enzymes involved in the activation of components of this endocrine system, together with elements of catabolic reactions, as well as nuclear receptors, and aspects of autocrine and paracrine actions, have essential roles not only in preserving bone and mineral homeostasis, but in regulation of numerous processes of specific cell differentiation and proliferation. The knowledge of these mechanisms of action of vitamin D endocrine system can be used to help the diagnosis and treatment of diseases involving specific organs and tissues which respond to actions of vitamin D.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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