

# The immunological implication of the new vitamin D metabolism

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## Abstract

*Vitamin D is a neuro-hormone regulating calcium-phosphate homeostasis, cell proliferation, and immunomodulation. Exogenous and endogenous vitamin D is inactive, and two hydroxylations are required to produce the active hormone. The first hydroxylation is unique to the liver, while the second step occurs in kidney, brain, lung, prostate, placenta, and immune cells. Kidney-derived calcitriol regulates calcium homeostasis. Active hormone produced by brain and immune cells mediates immune system response; lung calcitriol is involved in fighting respiratory tract infections; finally, prostate and placenta vitamin D regulates cells growth and proliferation within such tissues. Immune modulation by vitamin D includes enhancing innate immune response, attenuating and stimulating Th1 and Th2 cell proliferation, respectively, and promoting self-tolerance. Hypovitaminosis D is a common finding in several autoimmune diseases. It is unclear whether hypovitaminosis D could be a consequence or a cause of autoimmune diseases and whether vitamin D supplementation has an impact on these patients. Moreover, there is no consensus on oral cholecalciferol dosage for supplementation. More interventional studies are required to better define how vitamin D could represent both a causation agent in autoimmunity and a target for therapeutic strategies in autoimmune patients.*

**Key words:** vitamin D, CYPs, VDR, immunomodulation, autoimmunity.

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## Introduction

Vitamin D refers to two compounds, vitamin D<sub>2</sub> and vitamin D<sub>3</sub>. Vitamin D<sub>2</sub> derives from irradiation of the plant sterol, ergosterol, and is provided by the diet. Vitamin D<sub>3</sub> originates from a cutaneous compound by endogenous synthesis. The only significant sources of vitamin D are fatty fish, animal liver, fish oils, and egg yolks [1].

Vitamin D was initially described at the time of the industrial revolution, when England faced rickets; since then, the relation between vitamin D and calcium-phosphorus metabolism has never been discussed. However, over the last four decades, other activities of vitamin D have been highlighted. Actually, vitamin D is considered as a neuro-hormone that regulates cell growth and proliferation and immunomodulation.

## Vitamin D metabolism

Exogenous and endogenous vitamin D is inactive. Two hydroxylation steps, 25-hydroxylation and 1 $\alpha$ -hydroxylation, are required to produce the active hormone;

24-hydroxylation is involved in vitamin D inactivation and catabolism. Cytochrome P450 (CYP) enzymes carry out all hydroxylation steps. Although CYPs are mostly known to carry out oxidation/reduction reactions and liver drug catabolism, they are considered as responsible for vitamin D metabolism [2-4]. This starts from a cutaneous compound, named 7-dehydro-cholesterol, which is converted in cholecalciferol by the exposure to ultraviolet B (UVB) radiation. Exposure length and characteristics of the skin can influence the amount of vitamin D production. Classic and novel actions of vitamin D can be summarised according to the tissue where the second hydroxylation takes places.

## Liver hydroxylation

Once cholecalciferol is produced in the skin, it enters the circulation and reaches the liver, where 25-hydroxylation generates 25-OH-cholecalciferol or calcidiol. 25-hydroxylase is physiologically encoded by CYP2R1, but CYP27A1 and CYP3A4 are also involved in cholecalciferol 25-hydroxylation. Once synthesised, calcidiol binds the vitamin D binding protein (DBP) and is secreted into blood, where it

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represents the main circulating form of vitamin D. Calcidiol is considered as a reliable marker of vitamin D status.

### Kidney hydroxylation

1 $\alpha$ -hydroxylation takes place both in the kidney (renal hydroxylation) and within other tissues and cell types (extra-renal hydroxylation). Kidney 1 $\alpha$ -hydroxylase originates as a hormone with endocrine effects, responsible for calcium homeostasis (calcaemic actions of vitamin D). Active vitamin D reaches the gut, where it induces intestinal calcium absorption. Renal 1 $\alpha$ -hydroxylase is encoded by CYP27B1 and strictly regulated by parathyroid hormone (PTH) [5].

### Extra-renal hydroxylation

Extra-renal tissues having 1 $\alpha$ -hydroxylases (lung, prostate, brain, immune cells, and placenta) produce a hormone with paracrine and autocrine effects [6]. Extra-renal calcitriol is responsible for non-calcaemic actions of vitamin D, including regulation of cell growth and proliferation and immune system modulation. Macrophages, monocytes, and T and B lymphocytes produce active vitamin D, which induces or inhibits immune cell proliferation and cytokine production. Vitamin D expressed by epithelial lung cells has been related to respiratory tract infections, in which its pathogenic role has been strongly suggested, while its role as a biomarker gained less attention, due to the availability of well-established biomarkers [7, 8]. Finally, prostate and placenta-vitamin D regulates cells growth and proliferation within such tissues. Extra-renal 1 $\alpha$ -hydroxylase is stimulated by cytokines and growth factor but not by PTH [9].

### Vitamin D receptors

Most of the roles of vitamin D3 depend on the nuclear vitamin D receptor (VDR). Neo-synthesis of mRNA and protein and cell proliferation take place after vitamin D3 binds VDR. The interaction between VDR and the retinoic acid receptor (RXR) determines the activation of response elements (VDREs) present in the promoter region of several genes. The vitamin D/VDR-RXR-VDREs complex is deemed to regulate about 900 genes [10]. Some non-genomic rapid actions of vitamin D3, such as regulation of adenylate cyclase activity, phospholipase C, and C kinase, are mediated by the surface receptor 1,25-(OH)2D-MARRS (membrane-associated rapid response steroid binding), also called ERp57/Grp58 [11]. Moreover, a membrane VDR (MVDR), which is similar to the nuclear VDR (NVDR), can mediate other non-genomic rapid actions, such as activation of the phosphatidylinositol 3-kinase and the endothelial nitric oxide synthase [12]. Apart from immune cell types, the prime location of VDR is the brain, hence Vitamin D is defined as a neuro-hormone [13].

### Immunomodulation by vitamin D

It has been suggested that vitamin D action within immune cells resembles that of cytokines, being both a transcription- and a growth factor; as a cytokine, the neuro-hormone displays pleiotropy, synergy, redundancy, and interaction with surface receptors [14]. Vitamin D plays a pivotal role in promoting innate immune response, by enhancing the production of antimicrobial agents by monocyte and neutrophils, and it is able to modulate the specific immune response. Briefly, the hormone acts by skewing T cell to Th2 polarisation, attenuating and stimulating Th1 and Th2 cell proliferation, respectively. Vitamin D can inhibit the synthesis, secretion, and release of Th1 cells anti-inflammatory cytokines (IL-4 and IL-10) whilst inducing those of Th2 cell pro-inflammatory cytokines (IL-1, TNF- $\alpha$ , IFN- $\gamma$ ). Furthermore, vitamin D can suppress IL-17 production by Th17 cells and has been proven to promote self-tolerance. Macrophages, monocytes, and B and T lymphocytes show, even during quiescence, a low expression of VDR, which increases considerably as a result of inflammatory and immunological stimuli [15-19].

### Vitamin D and autoimmunity

Either vitamin D plasma levels or some VDR allelic variants have been largely investigated in immunological disorders, and hypovitaminosis D is a common finding in several autoimmune diseases [20-24].

VDR polymorphisms (mainly TaqI, FokI, BsmI, and ApaI) and CYP27B1 allelic variants have been investigated in multiple sclerosis (MS), with controversial results. In a case-control study [25] performed in 2016, Abdollahzadeh *et al.* found a significant difference in ApaI, BsmI, and TaqI but not in FokI allele frequency between the cases and controls. However, Agnello *et al.* [26] reported no significant difference in allele frequencies and genotype distribution between case and controls when analysing the same VDR polymorphisms. Zhang *et al.* [27] recently performed a meta-analysis on 24 case control studies, including 4013 patients and 4218 healthy controls. The authors found no association between any of the VDR polymorphisms and risk of MS between Asian and Caucasian populations.

A high prevalence of vitamin D deficiency (< 20 ng/ml) has been reported in systemic lupus erythematosus (SLE) [28]. Borba *et al.* also found an association between hypovitaminosis D and disease activity [29]. However, Amital *et al.* [30] reported this association to be weak, one year later. When comparing these results, it should be noted that the Amital population included 378 SLE patients while Borba analysed 36 patients. In his recent review [22] on vitamin D in SLE patients, Shoenfeld *et al.* concluded that vitamin D deficiency could be a consequence of the disease rather than a cause. Moreover, the authors stated that

vitamin D supplementation in these patients has very little impact on disease progression.

Controversial findings have been reported by studies evaluating the association between systemic sclerosis and vitamin D deficiency. A recent review showed that the association between low vitamin D serum levels and skin fibrosis, pulmonary fibrosis, gastrointestinal involvement, and autoantibodies pattern is deeply controversial [21]. However, a recent study [31] performed on 154 patients showed an association between vitamin D deficiency and such clinical features. Because systemic sclerosis is rare, 154 enrolled patients represent a remarkable sample size. Of note, supplementation with oral cholecalciferol was found to be ineffective in increasing 25 (OH) D serum concentrations.

### Randomised controlled trials on vitamin D supplementation

To establish whether there is clinical benefit associated from any treatment, randomised controlled trials (RCTs) are required.

Evidence from RCTs on vitamin D supplementation in MS patients is controversial, as well as in SLE patients.

A few studies performed on MS patients demonstrated that vitamin D supplementation had an effect on the reduction of gadolinium-enhancing lesions on brain magnetic resonance imaging (MRI) [32, 33]. However, previous work found contrasting results, reporting no effect of high-dose supplementation on MRI lesions and clinical outcome [34]. The largest RCT on MS patients was performed by Smolders *et al.* in 2016 and included 229 subjects [35]. The primary endpoint was defined as the proportion of subjects without evidence of disease activity after 48 weeks. No difference in relapse rate was found between supplementation and placebo groups, but a statistically significant difference in combined unique lesions on MRI (as secondary endpoint) was reported.

Recent evidence from RCTs addressing supplementation in SLE patients demonstrated no impact of vitamin D supplementation on disease activity [36, 37]. In their double-blind, placebo RCT, Aranow *et al.* analysed 57 SLE patients for the effect of supplementation on interferon (IFN) signature, which is a biomarker of SLE response. The authors concluded that vitamin D supplementation fails to diminish the IFN signature in these patients. Contrasting results were obtained by Lima *et al.* in the same year; the authors randomised 40 adolescent and young SLE patients to receive oral vitamin D or placebo. The primary endpoint was defined as disease activity measured by the SLE Disease Activity Index (SLEDAI). The authors found significant improvement in SLEDAI in the vitamin D group compared to placebo, concluding that supplementation is effective in decreasing disease activity.

### Conclusions

There is wide uncertainty on the role of vitamin D in the pathogenesis of autoimmune diseases because it is unclear whether hypovitaminosis D could be a consequence or a cause of autoimmunity. Conflicting evidence on the effect of vitamin D supplementation on these patients emerges from RCT findings. Furthermore, there is a lack of consensus on oral cholecalciferol dosage. More interventional studies are required to better define how vitamin D could represent both a causation agent and a target for therapeutic strategies in autoimmune patients.

*The authors declare no conflict of interest.*

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