

## Vitamin D and Hepatitis C Virus-related Liver Disease

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### Abstract

Vitamin D through the vitamin D receptor (VDR) is involved in the control of bone and calcium homeostasis, immunoregulation, cellular differentiation, and anti-inflammatory actions. The liver is central in vitamin D synthesis, however the direct involvement of the vitamin D with chronic liver disease, chronic hepatitis C (CHC) infection, and hepatocellular carcinoma (HCC) remains to be evaluated. The purpose of this review is to describe vitamin D metabolism, the mechanisms of homeostatic control, and to address the associations between vitamin D and HCV-related liver disease.

### Introduction

Vitamin D, the “sunshine vitamin”, is an important secosteroid hormone with pleiotropic effects. While its role in the regulation of calcium and bone homeostasis is well established, recently there is increasing recognition that vitamin D has immunomodulatory, anti-inflammatory and anti-fibrotic properties and plays an important role in the regulation of cell proliferation and differentiation. These extraskeletal effects are relevant in the pathogenesis and treatment of many causes of chronic liver disease, including HCV (1).

#### Sources of vitamin D

Several forms of vitamin D exist, the two major forms are vitamin D<sub>2</sub> (ergocalciferol), and vitamin D<sub>3</sub> (cholecalciferol). Vitamin D<sub>3</sub> is produced in the skin from 7-dehydrocholesterol (7-DHC) through a two-step process in which the B ring is broken by ultraviolet (UV) radiation (spectrum 280–320 UVB) from the sunlight, forming previtamin D<sub>3</sub> that isomerizes to vitamin D<sub>3</sub> in a thermosensitive but noncatalytic process (Figure 1). Both UVB intensity and skin pigmentation level contribute

to the rate of vitamin D<sub>3</sub> formation (2). Vitamin D is not widely present in nature; however, its provitamins are common in both plants and animals. The richest food sources of vitamin D are oily fishes (Salmon, Mackerel, Tuna, Herring and Sardines) and their products, dairy products, irradiated mushrooms, and fortified foods oils (1).

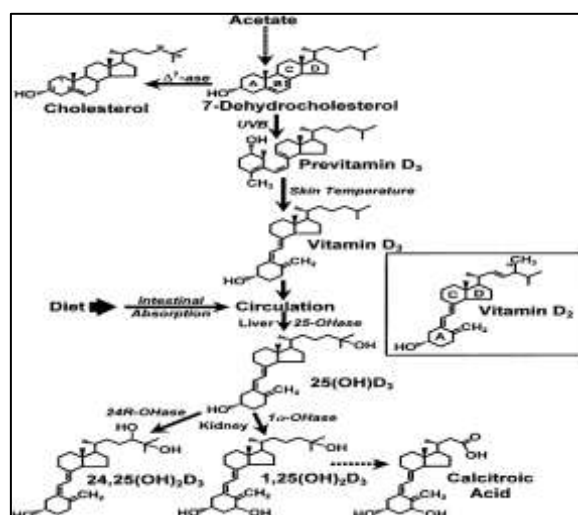
#### Vitamin D metabolism

The two forms of vitamin D (D<sub>3</sub> and D<sub>2</sub>) are biologically inactive; they require activation in the liver and kidney, through 25- and 1 $\alpha$ -hydroxylation to produce the active form 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>, calcitriol). Calcitriol then undergoes catabolism via 24-hydroxylation (Figure 1) (3).

Vitamin D is transported to the liver by binding to carrier proteins, in particular, vitamin D-binding protein (DBP), where it is enzymatically hydroxylated to 25-hydroxyvitamin D<sub>3</sub> (25(OH)D<sub>3</sub>, calcidiol). Hydroxylation is catalyzed by a microsomal cytochrome P450 enzyme CYP2R1 and/or the mitochondrial cytochrome P450 CYP27A1. 25-Hydroxyvitamin D<sub>3</sub>, bound to DBP, is then transported to

the kidneys and is finally hydroxylated by CYP27B1 to hormonally active  $1\alpha,25$ -dihydroxyvitamin  $D_3$  ( $1,25(OH)_2D_3$ , calcitriol). This step is tightly regulated by parathormone (PTH); other regulators are calcium, phosphate, calcitonin, fibroblast growth factor 23 (FGF-23), and calcitriol itself (3).  $25$ -Hydroxyvitamin  $D_3$  can undergo epimerization, through 3-epimerase, at the C3 position to 3-epi- $25(OH)D_3$ . The C-3 epimer of  $25(OH)D_3$  has reduced binding to DBP relative to  $25(OH)D_3$ , and the C-3 epimer of  $1,25(OH)_2D_3$  is catabolized more slowly and has reduced affinity

for the VDR relative to  $1,25(OH)_2D_3$ , thus reducing its transcriptional activity and most biologic effects (4). CYP24A1 is the only established  $24$ -hydroxylase involved with vitamin D metabolism. This enzyme has both  $24$ -hydroxylase and  $23$ -hydroxylase activities. The  $24$ -hydroxylase pathway results in the biologically inactive calcitroic acid, whereas the  $23$ -hydroxylase pathway ends up producing the biologically active  $1,25$ - $26,23$  lactone. All steps are performed by one enzyme (5).



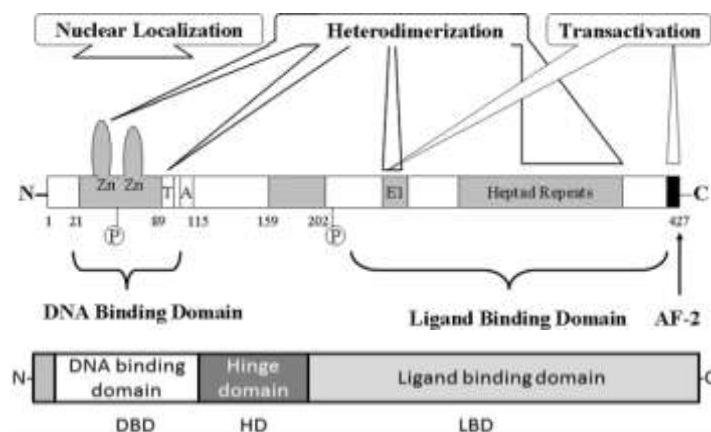
**Figure (1):** Photosynthesis, and metabolic pathways for vitamin  $D_3$ . Specific enzymes abbreviated as follows:  $\Delta^7$ ase, 7-dehydrocholesterol reductase; 25OHase, vitamin D-25-hydroxylase;  $1\alpha$ -OHase,  $25(OH)D_3$ - $1\alpha$ -hydroxylase; 24R-OHase,  $25(OH)D_3$ -24R-hydroxylase (2).

### Vitamin D mechanism of action

#### A) Genomic effects of vitamin D

All genomic actions of  $1,25(OH)_2D_3$  are mediated by the VDR, which is a specific nuclear receptor, that binds  $1,25(OH)_2D_3$  with high affinity, and  $25(OH)D_3$  and  $24,25(OH)_2D_3$  with lower affinity. VDRs have been identified in more than 30 different cell types including

cells involved in calcium homeostasis (bone, kidney, intestine), immune function, endocrine function, hematopoiesis, skin, and tumors. VDR is comprised of three domains; the N-terminal DNA binding domain (DBD), the C-terminal ligand binding domain (LBD), and the hinge region which binds the DBD and LBD together (Figure 2)(1).



**Figure (2):** Functional domains of the VDR. AF-2, activation function-2 (6).

The major steps involved in the control of gene transcription by the VDR include: ligand binding, heterodimerization with retinoid X receptor (RXR), binding of the heterodimer to vitamin D-responsive elements (VDREs) in the promoter of calcitriol-responsive genes, and recruitment of VDR-interacting nuclear proteins (coregulators) into the transcriptional preinitiation complex, which markedly enhance or suppress the rate of gene transcription by the VDR (6).

### **B) Nongenomic effects of vitamin D**

Some responses to vitamin D occur within seconds to minutes of  $1,25(\text{OH})_2\text{D}_3$  exposure and are membrane mediated. This suggests signaling independent of genomic responses, which typically take hours to days (1). The first such response to be recognized is transcaltachia, the rapid transport of calcium across the intestinal mucosa (7). Several other transcription-independent responses have since been demonstrated including phosphoinositide metabolism, cytosolic calcium levels, cGMP levels, phospholipase C, protein kinase C (PKC), mitogen activated protein (MAP) kinases, and the opening of chloride channels(6).

These rapid responses to vitamin D depend on VDR as indicated by their

absence in VDR-null mice. However, evidence indicates the involvement of another receptor, a membrane-associated rapid response steroid-binding protein (MARRS), that appears to be a protein localized in plasma membrane caveolae(1).

### **Classical action of vitamin D: regulation of calcium and phosphate homeostasis**

Calcitriol participates in the regulation of plasma ionized calcium and phosphate levels by acting on their intestinal absorption, renal excretion, and calcium bone mobilization. When serum calcium levels decrease, PTH secretion is stimulated and activates calcitriol synthesis. Both PTH and calcitriol stimulate calcium renal reabsorption and mobilization from bones (bone resorption). Calcitriol without PTH mediation stimulates intestinal calcium absorption(8). In contrast, if serum calcium levels rise, PTH secretion drops, leading to a decrease of calcitriol and calcium mobilization. Indeed, if serum calcium levels become too high, the parafollicular cells of the thyroid secrete calcitonin, which block calcium mobilization from the bone and stimulate calcium and phosphorous excretion, contributing to keep calcium levels within the normal range (8). Calcitriol acts directly on 3 target tissues (intestine, kidney, bone) with

the aim of maintaining optimal serum calcium levels. In addition, through VDR, calcitriol suppresses parathyroid gene expression and parathyroid cell proliferation, reinforcing its direct action on increasing serum calcium levels (8).

#### **Nonclassical action of vitamin D**

The vitamin D endocrine system is involved in a wide variety of biological processes including immunoregulation, anti-inflammatory/anticancer actions, xenobiotic detoxification, oxidative stress reduction, neuroprotective functions, antimicrobial defense, protection against diabetes, and cardiovascular benefits (9).

#### **A) Vitamin D and cell proliferation and differentiation**

Calcitriol and VDR have been shown to control the expression of genes associated with cellular proliferation and differentiation, suggesting a key role in cancer prevention. There is some evidence that vitamin D levels provide a protective status to lower the risk of cancer (specially colon, breast, prostate, and ovarian cancers) (10).

Studies show that calcitriol have antitumor effects through multiple mechanisms including the induction of cell cycle arrest, apoptosis, differentiation, and the suppression of inflammation, angiogenesis, invasion, and metastasis (11). Recently, calcitriol have demonstrated the ability to regulate the hedgehog (Hh) signaling pathway, responsible for tissue differentiation during embryogenesis and maintenance of stem cell populations in certain adult tissues (8).

#### **B) Vitamin D and the immune system**

Calcitriol has important immunomodulatory actions, it acts on both innate and adaptive immunity. Vitamin D exerts an inhibitory action on the adaptive immune system. In particular, calcitriol suppresses

proliferation and immunoglobulin production and retards the differentiation of B cell precursors into plasma cells (12). In addition, calcitriol inhibits T cell proliferation, in particular the T helper (Th)-1 cells capable of producing IFN- $\gamma$  and IL-2 and activating macrophages. These actions prevent further antigen presentation to and recruitment of T lymphocytes, and T lymphocyte proliferation (13). In contrast IL-4, IL-5, and IL10 production can be increased (14), shifting the balance to a Th2 cell phenotype. CD4/CD25 regulatory T cells (Treg) are also increased by calcitriol (15). These actions on T cell proliferation and differentiation stem from actions of calcitriol on dendritic cells to reduce their antigen presenting capability (13). Vitamin D exerts a stimulatory action on the innate immune system. Activation of toll like receptors (TLRs) leads to the induction of antimicrobial peptides and reactive oxygen species (ROS), which kill the organism. Among those antimicrobial peptides is cathelicidin. The expression of this antimicrobial peptide is induced by calcitriol in both myeloid and epithelial cells (16).

#### **Vitamin D and the liver**

The liver is a pivotal organ in the synthesis of vitamin D. It is the site where 25-hydroxylation occurs and where the vast majority of DBP is synthesized (17). In patients with chronic liver disease (CLD) the prevalence of vitamin D insufficiency (<75 nmol/L) is almost universal, with vitamin D deficiency (<50 nmol/L) present in around two-thirds of subjects. Even in the absence of cirrhosis, vitamin D deficiency is present in the majority of subjects. In those with cirrhosis, the prevalence of severe vitamin D deficiency (<25 nmol/L) increases with increasing severity of synthetic liver dysfunction

(18). Notably, in those about to undergo liver transplantation, the frequency of 25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> deficiency is 84% and 77%, respectively, with transplantation resulting in a marked increase in 25(OH)D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, and DBP levels (19).

The high prevalence of vitamin D deficiency in this population occurs regardless of the etiology of liver disease (20). Synthetic liver dysfunction is not entirely responsible, as vitamin D deficiency is still highly prevalent in those with non-cirrhotic liver disease (21). 25(OH)D<sub>3</sub> levels normalize after oral or parenteral administration of vitamin D in patients with cirrhosis, indicating that 25-hydroxylation is preserved in this patient population. Serum DBP levels, are moderately decreased in cirrhosis. However, as only 5% of DBP binding sites are occupied at any one time with vitamin D metabolites, profound liver dysfunction is required for low DBP levels to exert a significant contributing role to vitamin D deficiency in CLD (17).

Vitamin D deficiency in CLD is likely to result from a number of mechanisms. In addition to those described above, those patients with a chronic medical illness such as liver disease are more likely to have lower levels of sunlight exposure and/or inadequate dietary intake of vitamin D. Moreover, luminal absorption of dietary sources of vitamin D may be hindered by intestinal edema complicating portal hypertension and/or impaired bile salt dependent micellar incorporation due to cholestasis. Also, there is increased catabolic removal of 25(OH)D<sub>3</sub> (17).

Vitamin D deficiency in CLD is reported to increase the degree of necroinflammation and exacerbate the progression of liver fibrosis. Moreover, it is partly responsible for the

development of hepatic osteodystrophy, which refers to the specific CLD-associated bone disease and related metabolism abnormalities with both osteopenia and osteoporosis (22).

#### **Vitamin D and chronic hepatitis C**

Hepatitis C virus (HCV) infection is one of the major public health problems worldwide. Estimates indicate that about 71 million people are chronically infected, with three to four million persons are newly infected, and 399,000 deaths occur each year due to all HCV-related causes (23). Chronic hepatitis C (CHC) infection, defined by persistence of HCV RNA in the blood of more than 6 months, is characterized by a high rate of progression to fibrosis, chronic hepatitis, leading to cirrhosis and ultimately to hepatocellular carcinoma (HCC) (24).

Vitamin D deficiency is more prevalent in CHC subjects than healthy controls, even in those with minimal liver fibrosis. The majority of subjects with CHC are vitamin D deficient (<50 nmol/L) with 25% having severe deficiency (<25 nmol/L) (25). Current understanding of the mechanisms underlying the high prevalence of vitamin D deficiency in CHC is incomplete (17). Several studies showing that vitamin D inhibits HCV replication in a dose-dependent manner (26). During the pegylated IFN- $\alpha$ /ribavirin era, an association between baseline vitamin D status and treatment response has been established (17). Pre-treatment vitamin D deficiency is reportedly an independent predictor of failure to achieve sustained virologic response (SVR) in HCV genotype 1,2 and 3 infection (25). Vitamin D status also reportedly correlates with liver histology in CHC. Patients with vitamin D deficiency have a higher grade of hepatic necroinflammation (27), more

advanced fibrosis stage (28) and may possibly have more rapid fibrosis progression (29).

#### Vitamin D and HCC

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the fourth most common cause of cancer death, accounts for 75% to 85% of primary liver cancers.(30). Major risk factors for HCC include viral infections (especially chronic HBV and HCV), cirrhosis, alcohol, and non-alcoholic fatty liver disease (NAFLD). Additional risk factors include aflatoxin, family history and genetic factors, obesity, diabetes and smoking (31).

As mentioned previously, 1,25(OH)<sub>2</sub>D<sub>3</sub> exerts antiproliferative, pro-differentiation, pro-apoptosis effects on many cancer cells which express VDR (32). In terms of HCC, *in vivo* and *in vitro* studies reported inhibitory effect of 1,25(OH)<sub>2</sub>D<sub>3</sub> on HCC cell lines (33). The antiproliferative effect of 1,25(OH)<sub>2</sub>D<sub>3</sub> on HCC is mainly attributable to cell cycle arrest at G0/G1, leading to increased fraction of cells at G0/G1 phase and decreased fraction of cells at S phase (34). Previously, it has been shown that the observed cell cycle arrest at G0/G1, which is characteristic of 1,25(OH)<sub>2</sub>D<sub>3</sub> action, is through the induction of p21 and p27, leading to suppression of cyclins (D1, E and A) and cyclin dependent kinases 2 and 4 in many cancer cell lines (35). The antiproliferative effect of 1,25(OH)<sub>2</sub>D<sub>3</sub> on HCC cells correlate with intracellular VDR level (36). 1,25(OH)<sub>2</sub>D<sub>3</sub> may induce apoptosis either directly or indirectly through effects on the insulin-like growth factor (IGF-I) receptor and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) (37).

#### Conclusion

The vitamin D through VDR is involved in calcium

homeostasis, immunity control and detoxification of xenobiotics or endogenous compounds. The liver, by transforming vitamin D into 25(OH)D<sub>3</sub>, is a key organ in vitamin D synthesis. Vitamin D deficiency is a common problem in chronic liver disease and is closely associated with disease severity. The anti-inflammatory and immune-modulatory properties of vitamin D provide plausible mechanisms by which vitamin D may impact on disease progression and severity, especially in CHC and HCC. Calcitriol has been shown to exert an array of antitumor activities against HCC, including antiproliferation, anti-inflammation, anti-angiogenesis, pro-apoptosis, pro-differentiation, and inhibition of cancer cell invasion. These observations warrant for development of more studies that will clarify the relationship between the vitamin D-VDR axis and the liver.

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