**D-livering the message: The importance of vitamin D status in chronic liver disease**

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**Summary**

Vitamin D is synthesized predominantly in the liver and functions as an important secosteroid hormone with pleiotropic effects. While its key regulatory role in calcium and bone homeostasis is well established, recently there is increasing recognition that vitamin D has immunomodulatory, anti-inflammatory and anti-fibrotic properties. These non-skeletal effects are relevant in the pathogenesis and treatment of many causes of chronic liver disease. Vitamin D deficiency is frequently present in chronic liver disease and may predict non-response to antiviral therapy in chronic hepatitis C. Small studies suggest that vitamin D supplementation improves sustained viral response rates, while 1α-hydroxylase polymorphisms and vitamin D-binding protein functions independent of its role as the carrier of vitamin D. These extraskeletal effects are relevant in the pathogenesis and treatment of many causes of chronic liver disease.

**Vitamin D synthesis and metabolism**

Vitamin D undergoes a 3-step activation process before it interacts with the vitamin D receptor. The majority of circulating vitamin D is synthesized in the skin as a result of exposure to sunlight. The initial step involves ultraviolet-B radiation (wavelength 290–315 nm) converting the cholesterol metabolite 7-dehydrocholesterol into previtamin D3 in the lower epidermis, which is rapidly converted to vitamin D3 in a heat-dependent process. However, excessive sunlight exposure does not cause vitamin D intoxication because excess vitamin D3 is destroyed by sunlight [1]. Only a small proportion of vitamin D is obtained from dietary sources such as fatty fish, eggs, UV-irradiated mushrooms, supplements, and artificially fortified foods (Table 2). Dietary-derived vitamins D2 (ergocalciferol) and D3 (cholecalciferol) are absorbed via a bile-acid dependent process whereby vitamin D is incorporated into micelles in the intestinal lumen, then absorbed by enterocytes and packaged into chylomicrons that are then transported to the venous circulation via lymphatic drainage. Vitamin D from both skin synthesis and dietary sources can either be stored in adipocytes or undergo 25-hydroxylation in the liver. This process is mediated by the 25-hydroxylases, which are cytochrome P450 isozymes that include the important microsomal CYP2R1 and the mitochondrial CYP27A1 enzymes. This produces the main circulating, though biologically inactive, form 25-hydroxyvitamin D [25(OH)D], or calcidiol, which has a long half-life of 2–3 weeks and is therefore used to assess vitamin D status. The vast majority (88%) of serum 25(OH)D is bound to vitamin D-binding protein (DBP), which is also known as Gc or the group-specific component of globulin. DBP is a 58 kDa α-macroglobulin almost exclusively synthesized by the liver and a member of the albumin gene family located on chromosome 4, with high sequence homology to albumin and α-fetoprotein [2]. It is highly polymorphic, having three common isoforms, Gc1F, Gc1S, and Gc2, that display marked racial variation [3], with the Gc1F isoform having the highest affinity for vitamin D metabolites. DBP has anti-inflammatory and immunomodulatory functions independent of its role as the carrier of vitamin D [4,5].

Keywords: Vitamin D; Cholecalciferol; Liver disease; Liver fibrosis.
The final step in the synthesis of vitamin D is 1α-hydroxyl-
dation that predominantly occurs in the proximal tubule of the
kidney but also to a lesser extent in lymphocytes and parathyroid
tissue. It is mediated by 1α-hydroxylase (CYP27B1) that produces
the active form 1α,25-dihydroxyvitamin D [1α,25(OH)2D] or
calcitriol, which is also highly bound to DBP (85%) [2] and has a
half-life of only 4 h. 1α,25(OH)2D is the ligand that activates
the vitamin D receptor (VDR). This then forms a heterodimer with
the retinoid X receptor that acts as a transcription factor that
binds to vitamin D response elements in the promoter region of
target genes. 1α-hydroxylation is under the influence of factors
such as serum phosphate and calcium concentration, parathyroid
hormone (PTH), fibroblast growth factor 23 and genetic polymor-
phisms of CYP27B1. 1α,25(OH)2D acts in a negative feedback loop
to decrease its own synthesis and increase the expression of
25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which catabo-
lizes 1α,25(OH)2D into calcitroic acid, a biologically inert agent
excreted in the bile (Fig. 1).

VDR is expressed in most tissues and cells of the human body,
including liver, pancreas, and several immune cells including
monocytes, macrophages, T lymphocytes, B lymphocytes, natural
killer (NK) cells, and dendritic cells (DC), with expression most
abundant on the epithelial cells of the gastrointestinal tract. As
a transcription factor activated by 1α,25(OH)2D, VDR directly or
indirectly regulates the expression of more than 200 genes that
influence cell proliferation, differentiation and apoptosis, as well
as immunomodulation and angiogenesis [6]. Studies in VDR null
mice highlight the broad physiologic function of vitamin D [7].

Vitamin D deficiency

Vitamin D deficiency is broadly defined as a serum 25(OH)D level
<50 nmol/L (<20 ng/ml). Levels between 75 and 125 nmol/L
(30–50 ng/ml) are considered optimal as PTH levels rise when
25(OH)D is <75 nmol/L (30 ng/ml); hence, levels between 50

<table>
<thead>
<tr>
<th>Target</th>
<th>Action</th>
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<tbody>
<tr>
<td>Hepatic</td>
<td>Inhibits in vitro HCV replication in a dose-dependent manner [30-32]</td>
</tr>
<tr>
<td>Extra-hepatic</td>
<td>Supplementation may improve SVR rate in HCV [36-38]</td>
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<tr>
<td>Mortality</td>
<td>Vitamin D-binding protein is one of 3 metaproteins associated with SVR in HCV [40]</td>
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<tr>
<td>Calcium and bone homeostasis</td>
<td>Supplementation/phototherapy improves liver histology in preclinical studies of NAFLD [58]</td>
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<tr>
<td>Pancreas/adipocytes</td>
<td>Supplementation prevents liver fibrosis in preclinical studies [62,63]</td>
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<tr>
<td>Immune system</td>
<td>Supplementation decreases risk of acute rejection post-transplantation [125]</td>
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<tr>
<td>Innate</td>
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<td>Activates macrophage TLR response to TB infection [95]</td>
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<tr>
<td>Adaptative</td>
<td>Hastens sputum culture conversion in pulmonary TB in those with tt TaqI VDR allele [100]</td>
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<td>Downregulates expression of TLR2, TLR4 and TLR9 [107-110]</td>
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<tr>
<td>Necessary for NK cell development and function [114]</td>
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<td>Enhances NK cell cytotoxicity [115]</td>
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<tr>
<td>Promotes tolerant DC phenotype by suppressing DC maturation [116]</td>
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<tr>
<td>Enhances secretion of IL10 and decreases secretion of IL12 from DCs [122]</td>
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<tr>
<td>Carcinogenesis</td>
<td>Supplementation decreases risk of developing MS in women [85] and type 1 diabetes in children [86]</td>
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<tr>
<td>Higher 25(OH)D levels associated with lower incidence of colorectal adenoma [135]</td>
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<td>Sunlight exposure associated with reduced risk of NHL [145]</td>
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Vitamin D and chronic liver disease

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. In those 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 [17,18]. Notably, in those about to undergo liver transplantation, the frequency of 25(OH)D and 1α,25(OH)2D deficiency is 84% and 77%, respectively, with transplantation resulting in a marked increase in 25(OH)D, 1α,25(OH)2D, and DBP levels [19].

The high prevalence of vitamin D deficiency in this population occurs regardless of the etiology of liver disease [20,21]. Synthetic liver dysfunction is not entirely responsible, as vitamin D deficiency is still highly prevalent in those with non-cirrhotic liver disease [17]. 25(OH)D levels normalize after oral or parenteral administration of vitamin D in patients with cirrhosis, indicating that 25-hydroxylation is preserved in this patient population [22,23]. Serum DBP levels, which play a critical role in the transport and bioavailability of vitamin D, are moderately decreased in cirrhosis [24,25]. However, as only 5% of DBP binding sites are occupied at any one time with vitamin D metabolites [2], profound liver dysfunction is required for low DBP levels to exert a significant contributing role to vitamin D deficiency in chronic liver disease.

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.

Vitamin D and chronic hepatitis C

Around 170 million people worldwide have chronic hepatitis C (CHC) infection [26], causing a substantial burden of chronic liver disease globally [27]. 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) [28,29]. Current understanding of the mechanisms underlying the high prevalence of vitamin D deficiency in CHC is incomplete.

Nevertheless, recent evidence suggests that vitamin D may impact upon clinical outcomes and treatment response. Fundamental to this are several in vitro studies showing that vitamin D inhibits hepatitis C virus (HCV) replication in a dose-dependent manner [30–32]. Moreover, an association between baseline vitamin D status and treatment response to pegylated-interferon (PEG-IFN) and ribavirin (RBV) has recently been established (Fig. 2). Pre-treatment vitamin D deficiency is reportedly an independent predictor of failure to achieve a sustained virologic response (SVR) in HCV genotype 1 (HCV-1), [28,33], and 2/3 infection [29]. However, 25(OH)D level is not associated with SVR in HCV–HIV co-infection [34]. In HCV-1 infection, the rs12979860 C/T polymorphism upstream of the interleukin-28B (IL28B) gene on chromosome 19 is the strongest pre-treatment predictor of SVR [35]. Baseline vitamin D status is independent of, but additive to, the IL28B genotype in predicting SVR in HCV-1. The highest SVR rate occurs in subjects who have the favorable CC genotype and 25(OH)D levels >50 nmol/L [33].

To date, there is limited data evaluating vitamin D supplementation in CHC treatment. Two small prospective randomized controlled studies from Israel showed that those subjects who received vitamin D3 supplementation of 2000 IU/day, targeting a 25(OH)D level >80 nmol/L in addition to PEG-IFN/RBV combination therapy, had higher rates of rapid virologic response (RVR; 44% vs. 17%, p <0.001), complete early virologic response (cEVR; 94% vs. 48%, p <0.001) and SVR (86% vs. 42%; OR 2.5, 95% CI 2.0–4.9, p <0.001) in HCV-1 [36] and SVR (95% vs. 77%, p <0.001) in HCV-2/3 infection [37] compared to subjects treated with standard therapy. Moreover, recipients of vitamin D3 supplementation were less likely to be relapsers or non-responders to antiviral therapy, and had improved insulin resistance indices.
Similarly, a small retrospective Italian study showed vitamin D3 supplementation improved SVR rate in the treatment of recurrent hepatitis C post liver transplantation (53.3% vs. 18.5%, \( p = 0.02 \)) [38]. It remains unclear whether these improvements in the clearance of HCV with vitamin D supplementation are the result of an alteration in innate and/or adaptive immune function, or are mediated via improvement in insulin resistance. Large, prospective, placebo-controlled studies are thus required to assess the impact of vitamin D supplementation on viral response in CHC treatment. However, these studies now seem unlikely to occur in the new and rapidly evolving era of direct acting viral therapy.

Vitamin D status also reportedly correlates with liver histology in CHC. Patients with vitamin D deficiency have a higher grade of hepatic necroinflammation [28,33], more advanced fibrosis stage [28,29,34] and may possibly have more rapid fibrosis progression [39]. At a cellular level, vitamin D deficiency is associated with downregulation of the 25-hydroxylase enzyme CYP27A1 in liver tissue. This may have pathogenetic relevance, given the established inverse relationship between CYP27A1 expression and the severity of necroinflammatory activity [28].

The above findings highlight the potential role that proteins and enzymes involved in the synthesis and metabolism of vitamin D may have in liver inflammation and response to anti-viral therapy.

Fig. 1. Vitamin D synthesis.
therapy. Genetic variation in the rs10877012 A/C polymorphism in the promoter region of the 1α-hydroxylase enzyme CYP27B1, but not the rs10735810 FokI VDR polymorphism, is associated with SVR in HCV-1 infection. Subjects with the AA genotype have higher SVR rate and 1α,25(OH)2D level than those with the AC or CC genotype [29], suggesting a key role of vitamin D in CHC infection. Moreover, a recently published proteomic study has shown vitamin DBP to be one of three metaproteins associated with SVR [40]. DBP levels are significantly lower in subjects with significant or advanced fibrosis (METAVIR F2-4) compared with those with absent or minimal fibrosis (F0/1) and healthy controls [41,42].

Thus, vitamin D deficiency appears to be common in CHC and may be associated with adverse outcomes such as lower treatment response, more advanced fibrosis stage and increased severity of necroinflammation. It remains, however, uncertain as to whether vitamin D supplementation improves the SVR rate in patients receiving combination anti-viral therapy with PEG-IFN and RBV. Still, the findings of a significant association between the CYP27B1 rs10877012 A/C polymorphism, higher 1α,25(OH)2D levels, and SVR rate, as well as the association between vitamin D-binding protein and SVR suggest that higher 25(OH)D and 1α,25(OH)2D levels directly improve the virologic response to PEG-IFN and RBV therapy, presumably by impacting on the downstream regulation of vitamin D target gene transcription. DBP determines how much free 25(OH)D substrate is available for 1α-hydroxylase as well as the amount of free 1α,25(OH)2D ligand available to activate the VDR and influences
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downstream gene transcription. Hepatic 1α-hydroxylase activity levels therefore represent a major additional factor regulating 1α,25(OH)2D concentration in the liver.

Vitamin D and non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome. It is the most common liver disease in the developed world, with a prevalence of 20–30% [43]. Thirty percent of subjects with NAFLD have histologic evidence of non-alcoholic steatohepatitis (NASH) [44] and are at risk of disease progression and development of cirrhosis. The pathogenesis of NAFLD is yet to be fully elucidated, but insulin resistance (IR) is implicated as the key mechanism leading to hepatic steatosis. Apart from lifestyle modification that results in significant weight loss [45], there is currently no safe, effective therapy for NASH.

Vitamin D levels decrease by 1.3 nmol/L with each 1 kg/m2 increase in body mass index (BMI) [46]. Normal vitamin D status is associated with a two-thirds lower prevalence of metabolic syndrome compared to those with reduced levels [47]. In non-diabetic Caucasians low vitamin D levels are independently associated with insulin resistance [48] and are a predictor of increased 10-year risk of developing hyperglycemia and insulin resistance [49]. A vitamin D response element is present in the insulin gene promoter region, and 1α,25(OH)2D activates transcription of the insulin gene [50]. Both 1α-hydroxylase and the vitamin D receptor are expressed on pancreatic β cells, with an association between low vitamin D levels and impaired β cell function having been suggested [50,51]. Two randomized placebo-controlled trials have shown that high dose vitamin D supplementation improved insulin sensitivity in non-diabetic South Asians [52,53]. A large prospective cohort study of women demonstrated that those who received vitamin D supplementation had a significantly lower risk of developing type 2 diabetes [54].

Subjects with NAFLD have lower vitamin D levels when compared to controls. Low vitamin D levels are closely associated with histologic severity of steatosis, necroinflammation, and fibrosis in NAFLD. independent of age, gender, BMI, Homeostatic Model Assessment (HOMA)-IR score and presence of metabolic syndrome [55,56]. These findings have been confirmed in children with NAFLD [57].

In a recent study of Lewis rats with diet-induced (choline-deficient and iron-supplemented l-amino acid or CDAA) NASH, phototherapy elevated 25(OH)D, and 1α,25(OH)2D levels while reducing hepatocyte inflammation, fibrosis, and apoptosis when compared to controls. Phototherapy also improved insulin resistance and increased serum adiponectin in association with reduced hepatic expression of the profibrotic transforming growth factor (TGF)-β and α-smooth muscle actin (α-SMA), a marker of hepatic stellate cell activation. In addition, oral vitamin D3 supplementation reportedly improved liver histology in a dose-dependent manner [58]. Furthermore, in a rodent high fat diet model of NAFLD, vitamin D deficiency exacerbated histologic features of NAFLD, increased insulin resistance, and upregulated liver tissue expression of genes involved in hepatic inflammation and oxidative stress [59]. Given the above findings, prospective studies that assess the impact of vitamin D supplementation on the histologic features of NASH are warranted as a priority, given the lack of an effective therapy for this condition.

Vitamin D and liver fibrosis

1α,25(OH)2D has anti-fibrotic effects in lung fibroblasts and mesenchymal multipotent cells in vitro [60,61], as well as anti-proliferative and anti-fibrotic effects in both in vitro and in vivo rat models of liver fibrosis. VDR is expressed by hepatic stellate cells (HSC) and this expression is upregulated by 1α,25(OH)2D. In addition, 1α,25(OH)2D suppresses HSC proliferation, and expression of cyclin D1, tissue inhibitor of metalloproteinase 1 and collagen 1α1 in vitro. In vivo, 1α,25(OH)2D decreases α-SMA expression and collagen levels, and prevents the development of cirrhosis by thioacetamide (TAA) [62,63]. A vitamin D level >50 nmol/L may be associated with a decreased frequency of rapid fibrosis progression in CHC [39]. However, the clinical importance of vitamin D as an anti-fibrotic agent remains to be determined.

Vitamin D receptor polymorphisms and liver disease

The vitamin D receptor (VDR) gene is located on chromosome 12. It encodes a 48 kDa soluble protein that is a member of the nuclear receptor family of ligand-activated transcription factors. Common single nucleotide polymorphisms (SNP) of the VDR gene include FokI (rs10735810), BsmI (rs1544410), Apal (rs7975232), and TaqI (rs731236). There is a marked racial variation in the allele frequency of these VDR polymorphisms [64], but their influence on VDR function and signaling is unknown [65]. The BsmI, Apal, and TaqI SNPs are all in the 3’ region of the VDR gene and are in linkage disequilibrium with each other [66].

In CHC infection, the bAT [CCA]-haplotype of the BsmI, Apal, and TaqI alleles, and the CC genotype of the Apal allele are associated with rapid fibrosis progression, cirrhosis and increased intrahepatic expression of the fibrosis marker gene MMP-9 [39]. In chronic hepatitis B (HBV) infection, the variation in allele frequency of BsmI, Apal, and TaqI is associated with HBeAg positivity and HBV flare [67]. Variation in Apal, and to a lesser extent TaqI, is also associated with a higher HBV viral load and more severe fibrosis and necroinflammation [68]. Variation in the TaqI VDR polymorphism is also associated with both chronic HBV infection [69] and occult HBV infection [70], in which there is a low degree of HBV replication present in HBsAg negative subjects.

In hepatocellular carcinoma (HCC), complicating cirrhosis variation in the allele frequency of the BsmI, Apal, and TaqI, but not FokI VDR polymorphisms is associated with HCC development when compared to cirrhotic patients without HCC. This association is most marked in subjects with alcohol-related cirrhosis, where carriage of the BsmI–Apal–TaqI A–T–C and G–T–T haplotypes is independently associated with an increased risk of HCC. Furthermore, there is a significant difference in allele frequency of these VDR polymorphisms in alcohol-related cirrhosis compared to cirrhosis complicating chronic viral hepatitis [66].

Multiple studies have confirmed an association between VDR polymorphisms and autoimmune liver disease in both European and Asian populations. Variation in the allele frequency of the BsmI polymorphism is associated with primary biliary cirrhosis [71,72], while variation of the FokI polymorphism is associated with autoimmune hepatitis [64,73]. Furthermore, carriage of the VDR BsmI–TaqI G–T/G–T diplotype is an independent predictor of acute cellular rejection post-liver transplantation [74]. Similarly, VDR polymorphisms are associated with a variety of other autoimmune and immune-mediated diseases, including type 1
diabetes [75], leprosy [76], Crohn’s disease [77], tuberculosis [69,78], psoriasis [79], multiple sclerosis [80], and Graves’ disease [81] (Table 4).

Vitamin D, the immune system, and the liver

There is an increased incidence and prevalence of autoimmune diseases such as type I diabetes, multiple sclerosis (MS) and Crohn’s disease in geographic regions at higher latitude [82,83]. This phenomenon is suggested to be related to lower 25(OH)D levels resulting from decreased ultraviolet sunlight exposure. In support of this hypothesis, the incidence of MS decreases with increasing 25(OH)D levels [84] and vitamin D supplementation decreases the risk of developing both MS in women [85] and type 1 diabetes in children by 80% [86]. In this context, vitamin D has an important role in both the innate and adaptive immune system [87]. Macrophages, T cells, and DCs express both 1α-hydroxylase and VDR receptor, and are thus direct targets of 25(OH)D and 1,25(OH)2D [88–90].

Innate immunity

The innate immune response is mediated by pattern-recognition receptors (PRR). Toll-like receptors (TLR) are a family of transmembrane PRRs with broad specificity, expressed on immune cells such as polymorphonuclear cells, monocytes, and macrophages. They interact with pathogen-associated molecular patterns such as viral nucleic acids, and bacterial and fungal products, to trigger an inflammatory (TNF, IL-1β, and IL-6) or antimicrobial response in the host [91]. Several data from studies focusing on the immunology of mycobacterial infection suggest vitamin D and DBP play a significant part in the activation of the innate immune response. The risk of Mycobacterium tuberculosis (TB) infection is increased in subjects with vitamin D deficiency, with the greatest risk observed in subjects with the lowest 25(OH)D levels [78,92–94]. At a cellular level, macrophages infected with M. tuberculosis initiate a TLR2/1 response that enhances 1α-hydroxylase and VDR expression and induction of the anti-microbial peptide cathelicidin. The anti-microbial activity of macrophages occurs via a vitamin D-dependent process. Addition of 1α,25(OH)2D, that varies according to the DBP genotype.

Table 4. Genetic variation in vitamin D and disease.

<table>
<thead>
<tr>
<th>Target</th>
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<tr>
<td>Hepatic</td>
<td>1α-hydroxylase (CYP27B1) gene:</td>
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<tr>
<td></td>
<td>rs10877012 A/C SNP associated with responsiveness to therapy in HCV-1</td>
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<tr>
<td></td>
<td>AA genotype has higher SVR rate and 1α,25(OH)2D level than AC or CC genotype [29]</td>
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<td>VDR gene polymorphism associations:</td>
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<td>bAt [CCA]-haplotype of BsmI, Apal and TaqI alleles, and CC genotype of the Apal allele predicts rapid fibrosis progression, cirrhosis and increased intrahepatic expression of fibrosis marker gene MMP-9 [39]</td>
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<td>HBV: eAg positivity and flare [67], higher viral load, more severe fibrosis and necroinflammation [68], chronic infection [69] and occult infection [70]</td>
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<td>BsmI-Apal-TaqI A-T-C and G-T-T haplotypes associated with HCC in alcohol-related cirrhosis [66]</td>
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<td></td>
<td>BsmI and PBC [71,72]</td>
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<td></td>
<td>FokI and AIH [64,73]</td>
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<td></td>
<td>BsmI-TaqI G-T/G-T diplotype predicts acute rejection post-liver transplantation [74]</td>
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<td>Extra-hepatic</td>
<td>VDR gene polymorphism associations:</td>
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<td>Immune-mediated diseases: type 1 diabetes [75], leprosy [76], Crohn’s disease [77], TB [69,78,100], psoriasis [79], MS [80] and Graves’ disease [81]</td>
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<td>Malignancies: melanoma [148] and cancer of the colon [146], ovary [147], breast, prostate and kidney [148]</td>
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<td>DBP gene associations:</td>
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<td>Gc1F, Gc1S and Gc2 isoforms of DBP have differing affinities for vitamin D [133,134] and result in variable responses to vitamin D supplementation [132,133]</td>
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<td></td>
<td>Vitamin D dependent antimicrobial response of monocytes varies with DBP genotype [96]</td>
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<td>Gc2 isoform associated with lower 25(OH)D level [97,98], reduced macrophage function [4] and increased susceptibility to active TB in the presence of severe vitamin D deficiency [99]</td>
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with TLR4 in the liver is crucial during hepatic fibrogenesis. Hepatocytes, hepatic stellate cells, sinusoidal endothelial cells, biliary epithelial cells, and hepatic DCs also express TLR4 and are responsive to LPS. The interaction of LPS with TLR4 is the main ligand of TLR4. The role of vitamin D in innate immunity has implications on liver disease. Chronic liver disease is characterized by ongoing increased exposure of the liver via the portal circulation to bacterial products such as lipopolysaccharide (LPS). Contributing factors include increased intestinal mucosal permeability, alcohol ingestion, and small bowel bacterial overgrowth [101–103]. Dietary factors, such as a high-fat diet that predisposes to NAFLD, may also contribute to increased intestinal permeability and result in increased hepatic exposure to LPS [104]. Kupffer cells, the resident macrophages of the liver, represent 80–90% of the macrophages in the body [105], and their innate immune vitamin D-dependent antimicrobial response is also likely to be influenced by the vitamin D status and genetic polymorphisms in DBP. They also express TLR2, TLR4, and TLR9, and are responsive to LPS, the main ligand of TLR4. Hepatocytes, hepatic stellate cells, sinusoidal epithelial cells, biliary epithelial cells, and hepatic DCs also express TLR4 and are responsive to LPS. The interaction of LPS with TLR4 in the liver is crucial during hepatic fibrogenesis [101,106]. Serum vitamin D levels are inversely proportional to TLR2 and TLR4 expression in monocytes, with administration of 1α,25(OH)2D downregulating expression of TLR2, TLR4, and TLR9 [107–110]. Intestinal microbiota play an essential role in hepatic fat accumulation. TLR2, TLR4, and TLR9 are implicated in the pathogenesis of NAFLD, with TLR4 and TLR9 signaling associated with worsening steatosis, inflammation and fibrosis [111,112]. In obese rats, vitamin D deficiency increases hepatic mRNA levels of TLR2, TLR4, and TLR9, and the endotoxin receptor CD14, which is implicated in worsening histologic features of NAFLD [59]. In CHC infection, increasing hepatic necroinflammatory activity correlates with increasing hepatic mRNA expression of TLR2 and TLR4, and hepatic TNFα mRNA is also closely correlated with TLR2 and TLR4 mRNA expression [113]. Furthermore, the antiviral effect of vitamin D on hepatitis C inoculated HuH7.5 hepatoma cells is mediated by innate immune system activation of the interferon-mediated signaling pathways [30]. NK cells and DCs are both important innate immune effector cells. Studies in VDR knockout mice have shown that expression of VDR is necessary for NK cell development and function [114]. 1α,25(OH)2D enhances NK cell cytotoxicity [115] and suppresses DC maturation, inducing a more tolerant DC phenotype which, at the interface of the innate and adaptive immune systems, promotes T regulatory (Treg, CD4+CD25+) cell activity [116].

Adaptive immunity

Vitamin D is an important modulator of T cell response to pathogens, which is a key component of adaptive immunity. In particular, activation of naïve T cells is a vitamin D-dependent process. In the inactivated state, naïve T cells do not express VDR and express almost no phospholipase C-γ1 (PLCγ1), which is a key molecule required for subsequent classical T cell receptor signaling and T cell activation. Following stimulus exposure, VDR is expressed on T cells through T cell receptor signaling via the alternative mitogen-activated kinase p38 pathway. The VDR complex, activated by binding of 1α,25(OH)2D3, upregulates transcription of the gene encoding PLCγ1 and results in a 75-fold increase in PLCγ1 expression, enabling activation of naïve T cells. T cells in patients with lower 25(OH)D and 1α,25(OH)2D levels have a lower proliferation index after stimulation than T cells from patients with normal 25(OH)D and 1α,25(OH)2D levels; this pattern is overcome by exogenous administration of 1α,25(OH)2D3 [117].

1α,25(OH)2D also has an anti-proliferative effect on adaptive immunity. It inhibits proliferation of T helper type 1 (Th1) lymphocytes, which produce interferon (IFN)-γ, interleukin (IL)-2, and activate macrophages [118], and shifts the balance to a T helper type 2 (Th2) phenotype with increased production of IL-4, IL-5, and IL-10 [119]. 1α,25(OH)2D3 increases Treg cells [120,121], enhances DC secretion of IL-10, decreases DC secretion of IL-12, a critical cytokine in Th1 development [122], and inhibits Th17 development via inhibition of IL-6 and IL-23 production [121]. In patients with MS, 25(OH)D, but not 1α,25(OH)2D3 levels, correlate with the ability of Treg cells to suppress the proliferation of activated T responder cells and inversely correlated with Th1/Th2 ratio [123]. IL-2, IL-10, and IL-12 genes in T cells have regions which bind to VDR and 1α,25(OH)2D3 may directly play a role in the transcription of these cytokines in T cells [124]. The ability of 1α,25(OH)2D3 to modulate the adaptive immune system may explain the association of vitamin D supplementation and higher 25(OH)D levels with a lower risk of multiple autoimmune diseases.

In orthotopic liver transplant recipients, severe 25(OH)D deficiency (<12.5 nmol/L) and VDR BsmI–Taql G→T/G→T diplotype are independent predictors of moderate-severe acute cellular rejection, whilst vitamin D3 supplementation decreases the risk of acute rejection by 60% [74,125]. These findings highlight the importance of optimizing the vitamin D status in liver transplant recipients, not only to prevent bone loss, but also to reduce the risk of T cell-mediated acute rejection. A lower Th1/Th2 ratio is an independent predictor of SVR in treatment of HCV-1 [126], which possibly explains why vitamin D supplementation may improve therapeutic outcomes with PEG-IFN plus RBV. The

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**Table 5. Key future research requirements.**

<table>
<thead>
<tr>
<th>Identification of optimal 25(OH)D level in CLD</th>
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<tr>
<td>Effect of vitamin D supplementation on:</td>
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<td>NAFLD</td>
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<td>Liver fibrosis and fibrogenesis</td>
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<td>Prospective, randomized, placebo-controlled studies of vitamin D supplementation as an adjunct to HCV anti-viral therapy</td>
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<td>Associations and relevance of genetic polymorphisms in DBP, VDR, 25-hydroxyloside and 1-hydroxyloside with HBV, HCV, NAFLD and HCC</td>
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</table>
immune tolerant phenotype promoted by vitamin D may also be of therapeutic benefit in NASH, where activation of both innate and adaptive immunity is implicated in its pathogenesis.

Genome wide association studies of vitamin D

Only about a quarter of vitamin D variability between individuals is explained by factors such as reported dietary intake, latitude and season of measurement [127,128]. Twin and family studies suggest that genetic factors play a significant role in the wide variation of vitamin D levels observed within and between populations [129]. Polymorphisms of the hydroxylases, DBP, and VDR may have a profound influence on serum vitamin D levels and the efficacy of vitamin D as a hormone. Two large genome wide association studies (GWAS), involving patients of European ancestry [130,131], of SNPs and their association with 25(OH)D levels have revealed important information about genetic variation in the enzymes and carrier proteins which are integral to the synthesis and metabolism of vitamin D.

The NADSYN1/DHCR7 locus is closely related to the de novo synthesis of vitamin D3 in the skin from the precursor 7-dehydrocholesterol. There is an association between 25(OH)D levels and several SNPs including rs12785878, rs12800438, rs3794060, rs4945008, and rs4944957 of this locus [124]. SNPs in the 25-hydroxylase CYP2R1 locus rs10741657, rs2060793, rs12794714, rs10500804, and rs7116978 are also significantly associated with 25(OH)D levels [128,129].

The highly polymorphic vitamin D-binding protein binds the majority of 25(OH)D and 1α,25(OH)2D. DBP is predominantly produced in the liver, but also in kidney, gonads, fat, and neutrophils. SNPs in the DBP locus associated with 25(OH)D levels are rs2282679, rs7041, rs3755967, rs17467825, rs2298850, and rs1155563 [124–126]. Response to vitamin D supplementation may vary with differing genotypes of DBP [132,133]. In addition to the three common isoforms Gc1F, Gc1S, and Gc2, there are >120 rare variants of DBP. Haplotypes of the SNPs rs4588 and rs7041 in exon 11 of the gene result in the Gc1F, Gc1S, and Gc2 isoforms, which have differing affinities for vitamin D [134]. The DBP SNP rs2282679, which has the strongest association with vitamin D levels, lies in intron 12 near the actin subdomain III and may affect DBP binding of 25(OH)D [131].

24-hydroxylase (CYP24A1) is primarily responsible for the inactivation of 25(OH)D and 1α,25(OH)2D. The SNP rs6013897 from this locus is also associated with vitamin D levels [130].

These studies highlight the importance of genetic variation in vitamin D status and may explain in part the varying response seen to vitamin D supplementation. Polymorphisms in four specific loci involved in vitamin D synthesis and metabolism have a significant impact on circulating 25(OH)D levels in patients of European ancestry. Further GWAS that include patients of more diverse racial backgrounds may reveal more genetic associations with the vitamin D status.

Vitamin D and cancer

Vitamin D is also associated with the development of neoplasia. Higher 25(OH)D levels are associated with a lower risk of incident left-sided colorectal adenomas [135]. Multiple meta-analy-

Key Points

- Extra-skeletal effects of vitamin D include immunomodulatory, anti-inflammatory, and anti-fibrotic properties
- Vitamin D deficiency is frequently present in CLD
- Vitamin D deficiency may independently predict non-response to antiviral therapy in CHC
- Vitamin D supplementation may improve SVR to interferon-based antiviral therapy in CHC genotypes 1, 2 and 3
- Vitamin D deficiency is associated with the histologic severity of NAFLD
- Vitamin D is a plausible therapy for NASH because of its insulin-sensitizing, immunomodulatory, anti-inflammatory, and anti-fibrotic properties

Conclusions

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 NASH. However, there are few prospective studies evaluating the effect of vitamin D supplementation in chronic liver disease and these are clearly warranted in the areas of NASH and CHC based on preclinical, and limited retrospective and prospective clinical data. Genetic polymorphisms of the vitamin D receptor and of proteins and enzymes involved in vitamin D synthesis and activation have an association with vitamin D status and severity of liver disease. Further studies are also warranted in this area, to confirm known associations and evaluate other genetic polymorphisms, especially in the vitamin D binding protein, which plays a key role in vitamin D synthesis, activity and bioavailability (Table 5). In the interim period, we recommend vitamin D status to be assessed in all patients with CLD and, if deficiency is present (<50 nmol/L or 20 ng/ml), supplementation with 1000–4000 IU/day of vitamin D3 should be initiated, with the initial dose dependent upon baseline 25(OH)D levels. However, increasing evidence suggests that supplementation should be considered for a 25(OH)D level <75 nmol/L, especially in those considering interferon-based antiviral therapy for CHC. Further prospective studies are required to identify an optimal 25(OH)D level in subjects with CLD.
References


von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. J Hepatol 2011;54:5638A.


Artaza JN, Norris JC. Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. J Endocrinol 2009;200:207–221.


Vogel A, Strassburg CP, Manns MP. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. J Hepatol 2011;54:5638A.


Review


