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Vitamin D for your patients with chronic hepatitis C?

Adeeb H. Rahman, Andrea D. Branch*

Division of Liver Diseases, Mount Sinai School of Medicine, New York, NY, USA

Summary

Vitamin D is increasingly becoming recognized as an important physiological regulator with pleiotropic effects outside of its classical role in skeletal homeostasis. A growing body of clinical evidence highlights the prevalence and risks of vitamin D deficiency in patients suffering from chronic hepatitis C infection, and vitamin D supplementation has been proposed as an adjunct to current standards of care. This review considers the experimental evidence for the anti-inflammatory, antifibrotic and antiviral effects of vitamin D, and discusses the therapeutic potential of vitamin D supplementation to protect against liver disease progression and improve responses to treatment.

Vitamin D metabolism

Unlike most vitamins, vitamin D is neither an enzyme co-factor nor an essential nutrient that must be obtained from food. Rather, it is a precursor of a seco-steroid hormone. Vitamin D can be manufactured endogenously from 7-dehydrocholesterol when skin is exposed to ultraviolet B radiation (Fig. 1). Historically, sun exposure was the main source of vitamin D, but food and supplements are now important sources, especially among urban populations and people who work indoors. During its conversion from a precursor to an active hormone, vitamin D is first modified in the liver by microsomal vitamin D 25-hydroxylases, which form 25-hydroxyvitamin D [25(OH)D], a stable metabolite that is the best single indicator of vitamin D status [1]. A second hydroxylase step, mediated by the mitochondrial cytochrome P450 oxidase, CYP27B1, produces the most biologically active metabolite, 1,25-dihydroxyvitamin D [1,25(OH)2D]. In individuals with adequate renal function, most of the circulating 1,25(OH)2D is produced by the kidney; however, CYP27B1 activity occurs in many extra-renal tissues, including innate immune cells, such as macrophages and dendritic cells. The local metabolism of 25(OH)D by these cells is likely to be an important factor in generating the high local concentrations of 1,25(OH)2D needed for its paracrine and autocrine activities.

1,25(OH)2D mediates most of its biological effects by binding to the vitamin D receptor (VDR), which is expressed at some level in almost all human tissues. In the absence of its ligand, the VDR largely exists as an inactive homodimer. Upon binding 1,25(OH)2D, the VDR is phosphorylated and forms a heterodimer with its preferred binding partner, the retinoid X receptor (RXR), forming a nuclear transcription factor. This VDR/RXR heterodimer binds vitamin D response elements (VDREs) in DNA and recruits co-regulatory protein complexes to modulate the expression of hundreds of genes. In addition to acting as a ligand-activated transcription factor, the VDR is also thought to activate cell signaling pathways independent of its genomic effects [2].

The multiple steps in vitamin D bioactivation are controlled by intricate regulatory pathways [1]. CYP27B1 expression in the renal proximal tubule is stimulated by the parathyroid hormone (PTH), which is regulated by free serum calcium levels. 1,25(OH)2D itself can directly and indirectly inhibit CYP27B1 expression, thereby providing a tight negative feedback loop. CYP27B1 expression in keratinocytes is stimulated by both PTH and inflammatory cytokines such as TNFα and IFNγ. 1,25(OH)2D negatively regulates its own activity in these cells by inducing the expression of the 1,25(OH)2D catabolic enzyme, CYP24A1. The functional expression of CYP27B1 and intracellular
The synthesis of 1,25(OH)2D in macrophages is induced by both inflammatory cytokines, such as IFNγ, and toll-like receptor (TLR) ligands, such as lipopolysaccharide. Because vitamin D metabolism is controlled by multiple factors, the amount of vitamin D consumed in the diet is only one of many variables that determine the local activity of the vitamin D system. The levels of vitamin D binding protein and VDR are additional variables that strongly influence the magnitude of the biological effects of vitamin D.

VDR activation by 1,25(OH)2D has long been known to increase intestinal calcium and phosphate absorption, fostering healthy bones. A growing number of recent studies reveal pleiotropic roles of 1,25(OH)2D beyond bone and calcium metabolism, including the induction of antimicrobial genes and the reduction of inflammation and fibrogenesis [1]. Given the prevalence of bone disease, inflammation, and fibrosis in HCV-positive patients, both classical and newly-discovered effects of vitamin D may be relevant to disease management.

Vitamin D deficiency in patients with liver disease
Knowledge of the widespread physiological importance of vitamin D raises concern about the risks of vitamin D deficiency.
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Focusing on bone, several groups have attempted to identify markers that can be used to determine whether a patient’s vitamin D status is optimal. A panel assembled by the Institute of Medicine (IOM) determined that a serum 25(OH)D concentration of 20 ng/ml is sufficient for the majority of healthy adults, but also determined that a concentration of 16 ng/ml is insufficient for about 50% of healthy adults, suggesting that there is very little margin of error at a 25(OH)D level of 20 ng/ml [3]. A panel from the Endocrine Society concluded that 32 ng/ml should be used as the threshold of 25(OH)D sufficiency in patients with various disease conditions [4]. It is not clear whether the optimal level of 25(OH)D is the same for adults of all racial groups, which may be an important issue when considering these guidelines.

While serum levels of 25(OH)D are the best single surrogate marker of vitamin D status, PTH levels provide a second useful marker, and skeletal homeostasis is influenced by the combined effect of these two hormones. There is high inter-individual variability in the relationship between 25(OH)D and PTH levels. With serial testing, a patient’s vitamin D status can be optimized by increasing vitamin D supplements until a 25(OH)D level is reached that maximally suppresses PTH. This approach is time consuming and costly, and the financial burden of widespread vitamin D testing is becoming recognized as an increasingly significant issue [5]. Some have advocated that screening efforts should be focused on specific high risk individuals; however, a recent study has shown that 25% of a group of young physicians had 25(OH)D levels below 20 ng/ml, despite their lack of established risk factors [6]. Whatever the cost-effectiveness of vitamin D testing in the general public, the benefits to HCV-positive patients are likely to be greater because of their susceptibility to bone disease and increased risk of bone fracture [7–11].

It is important to keep in mind that the estimates of the 25(OH)D levels needed for optimal health are based exclusively on skeletal outcomes. The levels required for the non-classical functions of vitamin D have yet to be established and are under dispute [3,4,12]. The first solid information about the level for a non-skeletal outcome is likely to come from the infectious disease field where intervention trials can be completed in a relatively short period of time. Intriguingly, active tuberculosis increases seasonally as 25(OH)D levels fall [13], suggesting that optimal 25(OH)D levels could be determined for this disease by conducting randomized trials of vitamin D supplements. Meanwhile, a growing body of evidence indicates that higher levels of vitamin D and calcium may protect against colon cancer, and possibly other cancers, including hepatocellular carcinoma; however, definitive trials have not been carried out [3,7].

A large number of studies have examined the relationship between the vitamin D status of patients with chronic hepatitis C and disease outcome [7,14,15]. The majority found that HCV-positive patients have depressed 25(OH)D levels. A large study conducted in Sicily found that mean serum 25(OH)D levels were significantly lower than in healthy, age, and sex-matched controls (25.1 ± 9.9 ng/ml vs. 43.1 ± 10.2 ng/ml; p <0.0001), and that 73% of the HCV-positive patients had 25(OH)D levels below 30 ng/ml, in contrast to only 6% of the control subjects [16]. It is possible that HCV depresses 25(OH)D levels by altering lipid metabolism; a recent study has shown that HCV reduces production of 7-dehydrocholesterol, the precursor of endogenously-produced vitamin D [17]. Most studies indicate that the severity of vitamin D deficiency correlates with the severity of liver disease. In addition to low 25(OH)D levels, a recent study of VDR genetic polymorphisms has found that the b3 (CCA) haplotype is significantly associated with fibrosis progression rate as compared to the b2 (CAA) and bT (TAG) haplotypes [18]. While observational studies cannot establish a causal link between low 25(OH)D levels and liver disease progression, there are many ways in which vitamin D deficiency might plausibly play a role in pathogenesis.

Vitamin D as a down-modulator of inflammation

Chronic HCV infection is associated with intrahepatic inflammation and enhanced circulating levels of multiple inflammatory cytokines including members of the TNF superfamily, a complex group of ligands and receptors that are involved in cell survival, death and differentiation. Circulating TNFα and soluble TNF receptor (TNFR) levels are elevated in patients with chronic HCV relative to controls, and serum TNFR levels correlate with the grade of liver inflammation [19,20]. TNFα is strongly linked to insulin resistance, and the elevated levels of this cytokine in HCV-positive patients are thought to promote the development of type 2 diabetes, which is associated with adverse outcomes [21].

Hepatic TNFα expression is highly correlated with hepatic toll-like receptor (TLR)2 and TLR4 expression in HCV-infected patients [22], and a similar correlation exists between serum levels of TNFα and TLR2 expression on circulating monocytes [23]. Some data suggest that a proinflammatory cytokine milieu promotes HCV replication; sRNA-based silencing of multiple members of the proinflammatory NFκB signaling pathway inhibits HCV RNA replication [24]. Further evidence of the importance of TNFα in HCV infection is provided by a double-blinded randomized, placebo controlled trial of 50 patients with chronic HCV, in which treatment with etanercept, a TNFα antagonist, improved the virologic response to IFN and ribavirin therapy [25].

Another proinflammatory factor that is strongly associated with HCV infection is the chemokine CXCL10 (a.k.a., interferon gamma-inducible protein 10), which exhibits strong chemotactic effects on effector T cells. CXCL10 levels are elevated in HCV-positive patients compared with healthy controls and correlate with viral load, ALT elevations, and the extent of hepatic inflammation [26]. There is a highly statistically significant association between serum CXCL10 levels and the likelihood of treatment failure; however, it is not known if the relationship is causal. A truncated antagonistic form of CXCL10 was recently discovered [27], and more information about this new form is needed to understand the relationship between CXCL10 and treatment outcome.

If proinflammatory cytokines and chemokines promote HCV persistence and disease progression, vitamin D’s anti-inflammatory effects may be beneficial. These effects are illustrated by studies showing that treatment of human monocytes, macrophages and myeloid dendritic cells with 1,25(OH)2D downregulates TLR expression, reduces the production of multiple inflammatory factors, including TNFα and CXCL10, and promotes a more tolerogenic phenotype [28–31]. Consistent with these results, circulating 25(OH)D levels inversely correlate with monocyte TLR expression and with seasonal differences in TLR-induced TNFα production by peripheral blood mononuclear cells [32,33]. Due in part to these tolerogenic effects in antigen presenting cells, vitamin D also inhibits inflammatory T cell responses, reducing the production of IFNγ and IL-17 in favor of IL-4 and IL-10 [34]. These findings suggest that vitamin D deficiency is likely to exacerbate chronic inflammation in HCV-positive patients.
Vitamin D as a downregulator of fibrogenesis

Progressive liver fibrosis is a dreaded consequence of HCV infection. Advanced fibrosis/cirrhosis is associated with reduced virologic response rates [35]. Several association studies demonstrate that vitamin D levels inversely correlate with the liver fibrosis stage [7,14]. This could conceivably be related to the anti-inflammatory effects of vitamin D, given the contribution of inflammatory cell recruitment to hepatic fibrosis progression. In vitro studies establish that 1,25(OH)₂D treatment also directly inhibits the proliferation and pro-fibrotic phenotype of hepatic stellate cells and reduces thiacetamide-induced liver fibrosis in rats [36]. Vitamin D analogues have been found to similarly reduce renal fibrosis through multiple mechanisms in animal models of obstructive nephropathy [37]. These findings suggest that vitamin D deficiency may contribute to liver fibrosis and that vitamin D supplementation may have anti-fibrotic effects in HCV-positive patients.

Vitamin D and the response to antiviral treatment

The anti-inflammatory and anti-fibrotic roles of vitamin D indicate that vitamin D has the potential to reduce HCV-mediated liver disease and, perhaps, to positively contribute to treatment outcome. It is well established that vitamin D plays an important antibacterial role by regulating cathelicidin expression in human monocytes [38]. A number of studies now suggest that vitamin D may also have analogous effects, as evidenced by the fact that vitamin D and its metabolites can synergize with IFN treatment to directly inhibit HCV RNA replication in vitro [39,40]. Consistent with these findings, a growing body of clinical data supports a positive role for vitamin D in the virological response to therapy. Several association studies have examined 25(OH)D levels in patients with chronic HCV and most, though not all, report a positive correlation between 25(OH)D levels and the likelihood of achieving an SVR [7,14]. The VDR hAt (CCA) haplotype has also been found to be negatively associated with a failure to respond to therapy [41], as have polymorphisms in the vitamin D binding protein gene [42]. In addition, a small number of studies have reported the impact of vitamin D supplementation on treatment outcome. A retrospective review of patients in Italy who were treated with interferon and ribavirin for recurrent HCV post-transplantation found that concurrent treatment with vitamin D (for bone disease) resulted in significantly higher SVR rates [43]. In addition, two recent randomized clinical trials examined the effect of vitamin D3 (2000 IU/day) in conjunction with conventional interferon and ribavirin therapy and showed that vitamin D supplementation significantly enhanced the SVR rates of treatment-naive patients infected with HCV genotype 1 (86% for the supplemented group vs. 42% for the controls, p <0.001) and genotypes 2–3 (95% for the supplemented group vs. 77% of the controls, p <0.001) [44,45].

Conclusions

Vitamin D is increasingly becoming recognized as an important physiological regulator with pleiotropic effects. A growing body of experimental and clinical evidence suggests that vitamin D deficiency is a risk factor in HCV-infected patients and that vitamin D supplementation might protect against liver disease progression and improve responses to treatment. There is still a lack of consensus on optimal 25(OH)D target levels and dosing strategies. The existing evidence highlights the need for additional well-designed clinical trials to evaluate the effects of vitamin D supplementation. The outcomes should include effects on the fibrosis progression rate (in patients with ongoing HCV replication and in patients who achieve a SVR), the incidence of hepaticocellular carcinoma, and the incidence of bone fractures. In light of data showing protective effects of vitamin D supplementation in preventing influenza virus infection [46], studies of vitamin D supplementation in HCV patients should also examine vaccine responses and susceptibility to infectious diseases. Given that the relationships between vitamin D and chronic inflammation and progressive hepatic fibrosis are not unique to HCV infection, and that vitamin D deficiency may also be a factor in other liver diseases [7], clinical trials to study the effects of vitamin D supplementation in HCV patients are likely to be broadly relevant to the field of hepatology. Although dose-response data are limited, many liver disease patients will likely require relatively high doses of nutritional vitamin D to achieve 25(OH)D levels above 20 ng/ml. Until clinical data are available, 4000 IU/day is a

Key Points

- Vitamin D production and turnover are highly regulated processes involving a complex array of feedback loops, hormonal inputs, and signal transduction pathways. The pleiotropic effects of vitamin D allow it to integrate mineral homeostasis with inflammatory responses, fibrogenesis, and microbial defenses.

- The main circulating form of vitamin D is 25-hydroxyvitamin D. This metabolite can be converted to the active form, 1,25(OH)₂D, by the enzyme CYP27B1. Local production of 1,25(OH)₂D allows targeted paracrine/autocrine effects.

- Many cohort studies show that the majority of patients with hepatitis C virus (HCV) infection and other liver diseases have levels of vitamin D that increase the risk of bone disease. The severity of vitamin D deficiency correlates with the severity of liver disease, and advanced liver disease is associated with an increased risk of bone fracture. Vitamin D supplements should be prescribed at doses that maintain 25-hydroxyvitamin D levels at, or above 20 ng/ml. Many patients will require 2000 to 4000 IU of nutritional vitamin D per day to achieve this target.

- Several studies suggest that optimal vitamin D levels may increase the likelihood of successful antiviral treatment in HCV-positive patients treated with interferon and ribavirin, but the results are inconsistent and require further confirmation. Polymorphisms in genes in the vitamin D pathway, such as vitamin D binding protein, vitamin D receptor, and enzymes involved in vitamin D metabolism, should be considered when evaluating the significance of vitamin D in interferon-based treatment response.
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reasonable daily dose for patients with baseline 25(OH)D levels below 10 ng/ml and 2000 IU/day is an appropriate starting dose for patients with levels between 10 and 20 ng/ml.

Conflict of interest

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References

