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Review

Vitamin D in Preclinical Models of Fatty Liver Disease

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Abstract. *Simple steatosis in non-alcoholic fatty liver disease (NAFLD) progresses to non-alcoholic steatohepatitis (NASH) when excessive fat accumulation is accompanied by ballooning, inflammation, and progressive hepatocellular injury. Due to the increasing global incidence of NAFLD/NASH and the lack of effective drugs, current treatment options are currently dominated by lifestyle interventions, including dietary and physical activity modifications. In this regard, vitamin D has received widespread attention in recent years. In line with its pleiotropic physiological effects, preclinical animal models and patient cohorts have demonstrated anti-inflammatory, anti-fibrotic and anti-proliferative effects of vitamin D on NAFLD and NASH. Several animal models have confirmed the association of vitamin D deficiency and NALFD/NASH severity in humans and revealed potential benefits of dietary vitamin D supplementation. These preclinical models also provide critical guidance to define the roles and therapeutic potential of vitamin D as well as its downstream functional mechanisms in the pathogenesis of fatty liver disease. This review summarizes vitamin D research in currently available animal models of fatty liver disease.*

Non-alcoholic steatohepatitis (NASH) was first introduced to medical terminology by Ludwig *et al.* in 1980 to describe an unknown liver disease that histologically mimics alcoholic fatty liver disease in patients with no history of alcohol abuse (1). Since then, increasing evidence has demonstrated that NASH is a severe form of non-alcoholic fatty liver disease (NAFLD), which is distinct from simple fatty liver (hepatic steatosis), since it is characterized by necro-inflammation, hepatocellular ballooning and progressive tissue damage in addition to hepatic fat deposition, which may lead to liver cirrhosis and hepatocellular carcinoma. Due to the global increasing incidence of NAFLD/NASH (2) and limited therapeutic options, lifestyle modifications such as increased physical activity and dietary-related interventions remain important treatment strategies (3). Currently, vitamin D, with its direct anti-inflammatory, anti-proliferative and anti-fibrotic effects shown in experimental studies (4, 5), is a promising nutrient for the treatment of NAFLD/NASH, since it is commonly deficient in chronic liver diseases (6). Moreover, vitamin D has been reported to be associated with NAFLD/NASH in several epidemiological studies (7, 8). Longitudinal cohort studies and randomized controlled trials offer ideal methodological designs to confirm these protective effects of vitamin D on liver diseases (9). However, such clinical trials are to a great extent hampered by the slow nature of disease progression (10) and the wide spectrum of disease stages of NAFLD/NASH, which are difficult to differentiate non-invasively (11). Hence, various animal models of fatty liver disease that mimic human pathobiology have been developed for vitamin D research. As summarized in Table I, these preclinical models largely depend on diet-induced liver injury, however a limited number of chemically induced and alcohol-induced fatty liver disease models are also listed as well as those that depend on the genetic background of the animals. The aim of this review is to present an overview of vitamin D research in current experimental fatty liver disease models to better understand the role and therapeutic potential of vitamin D and its effects on the pathogenesis and the course of the disease.

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Vitamin D in Fatty Liver Disease

Vitamin D is a fat-soluble secosteroid that is metabolized in the liver and kidney and is converted to the $1,25(OH)_2D_3$ active form (calcitriol) in the body (Figure 1). As calcitriol, vitamin D acts as a sterol hormone that regulates diverse

Table I. *Vitamin D research in preclinical models of fatty liver disease*.

Disease	Animal model	Cause for liver injury	VitD suppl	VitD ther	Notes	Ref.
Diet-induced liver injury						
NAFLD NASH	BALB/c mice *Vdr*$^{-/-}$ mice	• Control chow with VD_3 • Vitamin D depleted control chow • High fat diet with VD_3 • HFD without VD_3	+	–		(32)
NASH	Sprague-Dawley rats	• HFD	–	+		(33)
NAFLD	Sprague-Dawley rats	• HFD	–	+		(53)
NAFLD	C57BL/6 mice	• Low fat vitamin D replete (LFD+) • Low fat vitamin D deficient (LFD–) • High fat vitamin D replete (HFD+) • High fat vitamin D deficient (HFD–)	+	–		(54)
Hypercholesterolemia	*Fxr*$^{-/-}$ mice *Shp*$^{-/-}$ mice C57BL/6 mice	• Western diet	–	+		(37)
NASH	Balb/C mice	• HFD • VD_3 depleted HFD	–	+		(34)
Metabolic syndrome	Wistar rats	• HFD	–	+	Vitamin D is administered alone and in combination with metformin	(55)
NASH	Lewis rats	• Choline-deficient, L-amino acid-defined and iron-supplemented diet (CDAA)	–	+	Vitamin D is applied either orally or by phototherapy	(41)
Obesity-related NASH	C57BL/6 mice	• LFD • High fat/high sugar Surwit diet (HFHS) • HFHS + VD_3 diet	+	–		(56)
NAFLD NASH	C57BL/6 mice *ApoE*$^{-/-}$ mice *Vdr*$^{-/-}$ mice *ApoE*$^{-/-}$*Vdr*$^{-/-}$ mice	• Methionine and choline-deficient diet (MCD) • HFD	–	+		(44)
Hyperinsulinemia and steatosis	ICR mice	• Vitamin D depleted control diet • HFD • Vitamin D depleted HFD	–	–		(57)
Diabetes induced liver injury	Sprague-Dawley rats	• HFHS	–	+	Streptozotocin is applied to induce diabetes	(58)
Obesity-related NAFLD	Sprague-Dawley rats	• HFD • Western diet (high fructose corn syrup (HFCS) + HFD) • Vitamin D depleted Western diet or HFD	–	–		(42)
NASH	Wistar rats	• Choline-deficient diet	+	+		(35)
NAFLD	Wistar rats	• High fat/high fructose diet (HFHF) • HFHF + vitamin D	+	–	Vitamin D administration in diet is supplemented by IP injections of metformin	(59)
Steatosis	Wistar rats	• HFHF	+	–	Vitamin D is applied together with calcium in increasing doses	(60)
Steatosis	C57BL/6 mice	• HFD	–	+		(38)
Steatosis	Sprague-Dawley rats	• 35% fructose solution • 35% fructose solution + vitamin D	–	+	Rats are fed standard chow diet and solutions are applied by oral gavage	(52)
Metabolic syndrome	*Ldlr*$^{-/-}$ and *Ldlr*$^{+/+}$ C57BL/6 mice	• Diabetogenic diet (DD) • DD + vitamin D	+	–		(61)
Steatosis	Sprague-Dawley rats	• HFD	–	+		(39)

Table I. *Continued*

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Disease	Animal model	Cause for liver injury	VitD suppl	VitD ther	Notes	Ref.
Chemically-induced liver injury						
Liver fibrosis	Wistar rats	Thioacetamide	–	+	Bile duct ligation is also applied for induction of liver fibrosis	(62)
Hepatocarcinogenesis	Wistar rats	Diethylnitrosamine followed by CCl_4 injections	–	+		(63)
Diabetes induced liver injury	Sprague-Dawley rats	Streptozotocin	–	+		(43)
Monogenic liver injury						
Obesity-related NASH	Zucker *fa/fa* rats	–	–	+	Vitamin D is applied *via* phototherapy	(41)
Cholestatic liver disease	*Abcb4*$^{-/-}$ FVB/NJ mice	–	–	+		(30)
Alcoholic liver injury						
ND	Long Evans rats	Liquid diet containing Sustacal and ethanol for 10 months	–	+		(64)
Alcoholic liver disease	C57BL/6 mice	Ethanol diet for 6 weeks	–	+		(65)

Abcb4: ATP binding cassette transporter B4; *Apoe*: apolipoprotein E; CDAA: choline-deficient, L-amino acid-defined diet; DD: diabetogenic diet; *Fxr*: farnesoid X receptor (bile acid receptor); HFD: high fat diet; HFHF: high fat, high fructose diet; HFHS: high fat, high sucrose diet; IP: intraperitoneal; *Ldlr*: low-density lipoprotein receptor; LFD: low fat diet; MCD: methionine and choline-deficient diet; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; *Shp*: short heterodimer partner; VD_3: vitamin D_3 (calcitriol); *Vdr*: vitamin D receptor; VitD suppl: vitamin D supplementation prior to/during establishment of liver injury; VitD ther: vitamin D rescue therapy after the establishment of liver injury; ND: not determined; Ref: reference.

biological events, ranging from host immune responses to cellular differentiation in addition to its classical role in calcium and bone metabolism (12). Therefore, it is not surprising that a growing body of evidence links vitamin D deficiency (VDD) with multiple inflammatory (13) and chronic disorders (14), including chronic liver diseases (15). In this regard, various studies have demonstrated that VDD is more common and associated with the severity and disease progression in patients with NAFLD/NASH (16-22). Although two meta-analyses (7, 8) and a recent study in a large cohort of Korean patients with NAFLD (23) confirmed these findings, it is noteworthy to state that several studies have presented contradictory results with no significant association between serum vitamin D concentrations and fatty liver disease phenotypes (24-27). These controversies may be, at least in part, explained by the heterogeneity of the criteria used for NAFLD diagnosis and VDD, as well as differences in vitamin D assays. Nevertheless, future prospective randomized clinical trials in patients with NAFLD/NASH can be envisioned.

Vitamin D Research in Preclinical Models of Fatty Liver Disease

Given the conflicting results in human studies, animal models of fatty liver disease gain more importance when it comes to the potential to provide critical guidance in delineating causal roles and the therapeutic potential of vitamin D on disease pathogenesis and progression. Several *in vivo* and *in vitro* studies have revealed that vitamin D and its receptor (VDR) are involved in repressing hepatic stellate cell (HSC) activation, thereby inhibiting the primary driver of hepatic fibrogenesis (4, 28, 29). Consistent with these findings, we have demonstrated that low-vitamin D diet-fed *Abcb4* knock-out mice develop more advanced biliary fibrosis and elevated collagen deposition (30). Feeding these mice a high-vitamin D diet led to an increase in serum vitamin D concentrations, which simultaneously lowered liver enzyme activities, altered the expression levels of profibrogenic genes, and ameliorated, in part, liver injury. A more recent *in vitro* study has further confirmed the inhibition of HSC activation by vitamin D

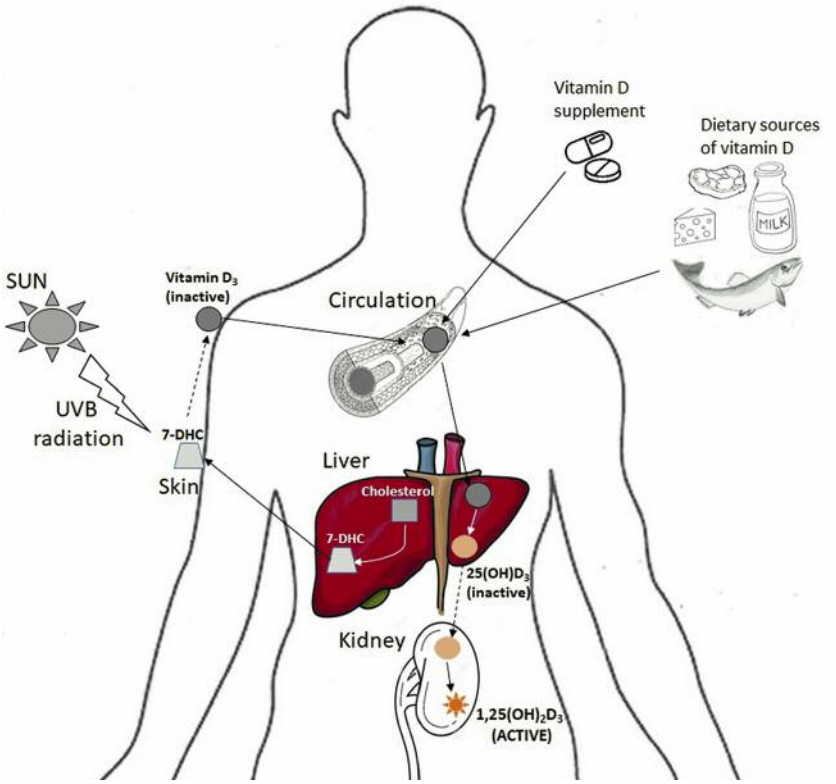

Figure 1. *Vitamin D synthesis and activation. Cholesterol is metabolized to 7-dehydrocholesterol (7-DHC) in liver, which is further converted to vitamin D_3 (cholecalciferol, an inactive form of vitamin D) in the skin when exposed to UVB radiation via sunlight. In addition to photosynthesis, vitamin D is also acquired by dietary intake and supplementation, entering the blood stream after intestinal absorption. Vitamin D is then converted to $1,25(OH)_2D_3$ (active form) through sequential hydroxylation steps in liver and kidney. 7-DHC: 7-dehydrocholesterol; $25(OH)D_3$: 25-hydroxyvitamin D_3 (calcidiol); $1,25(OH)_2D_3$: 1,25 dihydroxyvitamin D_3 (calcitriol, active form of vitamin D); UVB: ultraviolet radiation in wavelength region B (320-290 nm).*

through transforming growth factor (TGF)-β signaling in primary human HSC, supporting the anti-fibrotic effect of vitamin D (31).

Su *et al*. (32) have demonstrated that feeding BALB/c male mice a vitamin D-deficient, high fat diet resulted in overt insulin resistance and NASH phenotypes with aggravated systemic and local inflammation, whereas sufficient vitamin D supplementation ameliorated the histopathological changes with less insulin resistance and moderate hepatic steatosis. This study also revealed a critical role of the vitamin D/VDR axis in suppressing NASH progression by maintaining intestinal integrity through the induction of defensins and tight junction genes. Similarly, a recent study has reported the reduction of tumor necrosis factor (TNF)-α-mediated immunological abnormalities and hepatic steatosis in a high fat diet rodent model of NASH with chronic calcitriol supplementation (33). Another experimental study has demonstrated that VDD plus high fat diet impairs the enterohepatic circulation of bile acids, resulting in NASH in BALB/c inbred mice *via* repression of the ileal apical sodium-dependent bile acid cotransporter [*Asbt*=Solute carrier 10A2 (*Slc10a2*)] (34). Of note, the NASH phenotype was corrected by administration of calcitriol, which restored ileal *Slc10a2* expression and reduced hepatic inflammation and steatosis. In line with these findings, Han *et al*. (35) have demonstrated that $1,25(OH)_2D_3$ supplementation in rats with NASH induced by choline-deficient diet resulted in the reduction of cytokeratin 18 apoptotic fragment M30, which is a *bona fide* marker for liver injury (36). Additionally, a shift of bile acid metabolism to lower cholesterol levels by down-regulation of small heterodimer protein (*Shp*=Nuclear receptor 0B2) and up-regulation of cholesterol 7α-hydroxylase (*Cyp7a1*) through intraperitoneal (IP) injections of $1,25(OH)_2D_3$ was observed in mice on Western diet (37). Moreover, $1,25(OH)_2D_3$ has also been shown to ameliorate hepatic steatosis by autophagy through the induction of autophagy-related-16-like 1 (*Atg16l1*) protein (38), the inhibition of lipogenesis and the promotion of fatty acid oxidation *in vivo* (39). These preventive and

therapeutic effects of vitamin D have also been reported in a number of experiments using phototherapy, which increased the concentrations of active circulating vitamin D in serum (40, 41). Nakano *et al*. (41) have reported the suppression of apoptosis, inflammation and fibrosis in NASH models Lewis rats on choline-deficient, L-amino acid-defined and iron-supplemented diet (CDAA) as well as in obesity-related NASH (Zucker *fa/fa* rats). These changes were reflected by lower serum aminotransferase activities and hepatic triglyceride levels, the suppression of inflammatory and fibrotic genes such as TNF-α, TGF-β and α-Smooth muscle actin, and an increase in serum adiponectin levels. Interestingly, both skin exposure to ultraviolet radiation and the supplementation of CDAA-fed rats with $1\alpha(OH)D_3$ (0.4 μg/kg orally 3 times/week) resulted in a similar phenotype. Roth *et al*. (42) have provided additional evidence on the immune-regulatory role of vitamin D in NAFLD in obese Sprague-Dawley rats. Liver histopathology in these rats revealed that vitamin D-depleted Western diet resulted in increased lobular inflammation and higher NAFLD activity scores, accompanied by elevated expression of hepatic resistin, interleukins (IL) 1β, 4 and 6 and Toll-like receptors (TLR) 2, 4 and 9 as well as the oxidative stress marker heme oxygenase 1 (HO-1), suggesting that VDD may lead to elevated endotoxin exposure, exacerbated inflammatory pathways, and increased oxidative stress, which subsequently aggravate NAFLD. In line with these results, suppression of TLR4-mediated inflammation and amelioration of liver injury have been observed in diabetic rats treated with $1,25(OH)_2D_3$ (43).

Factors Involved in Vitamin D Metabolism in Experimental Models of Fatty Liver Disease

Further studies in experimental models suggest that not only vitamin D but also related factors involved in vitamin D metabolism are associated with NAFLD/NASH. Bosic *et al*. (44) have investigated hepatic *Vdr* expression in two mouse models of fatty liver disease, *i.e*. apolipoprotein E knock-out ($Apoe^{-/-}$) mice on a high fat diet and wild-type mice on methionine and choline-deficient (MCD) diet as well as in patients with NAFLD and NASH. Similar to the mouse models, hepatic *VDR* expression in livers from NAFLD patients was markedly increased in the setting of hepatosteatosis, but decreased in NASH, suggesting an early role of *VDR* induction in the pathogenesis of fatty liver diseases. Early induction of *VDR* in NAFLD was also found to modulate key hepatic lipid regulatory genes with decreased expression of *CD36*, *DGAT2*, *C/EBPα* and *FGF21*, and increased expression of *PNPLA2*, *LIPIN1* and *PGC1α*. Moreover, using a double knock-out $ApoE^{-/-}Vdr^{-/-}$ mice, they have demonstrated that the deletion of VDR prevents diet-induced fatty liver. Convincing evidence on the key role of VDR in NAFLD pathogenesis has been provided by Ding *et al*. (45) in a preclinical model of $Vdr^{-/-}$ mice, showing that VDR ligands inhibit HSC activation by abrogating TGF-β-induced fibrotic gene signatures through a VDR/SMAD "genomic circuit", whereas *Vdr* knock-out mice spontaneously develop hepatic fibrosis.

Furthermore, normalization of VDR and SMAD3 binding in the presence of both calcipotriol and TGFβ1 to their basal levels revealed that the occupancy of VDR and SMAD3 were inversely correlated, suggesting TGFβ-induced chromatin accessibility produces a genomic architecture that facilitates VDR to reverse SMAD activation.

Another factor in vitamin D metabolism, vitamin D binding protein (DBP), binds to vitamin D metabolites and acts as a reservoir, in particular for 25(OH)D (46, 47). DBP thereby helps to ameliorate VDD. Proteomics analysis in a NASH model of C57BL/6J male mice fed an MCD diet revealed DBP to be among the most differentially expressed proteins (48). Interestingly, a genome-wide association study of 928 adolescents assessed for NAFLD revealed a highly significant association of NAFLD with the single nucleotide polymorphism rs222054 in the group-specific globulin gene (*GC*), which encodes DBP (49). In relation to these findings, we have demonstrated that liver steatosis is associated with low serum 25(OH)D concentrations in a cohort of 241 patients with chronic liver diseases, who were assessed non-invasively by controlled attenuation parameter (CAP) to quantify hepatic steatosis (50). In addition, the rare allele of 7-dehydrocholesterol reductase (*DHCR7*) variant (rs12785878) has also been shown to be associated with increased liver stiffness (51). However, no associations with CAP were found for other common vitamin D pathway gene variants, namely *GC* rs7041, cytochrome P450 2R1 (*CYP2R1*) rs10741657, DHCR7 rs12785878 or *VDR* rs7974353 (52).

Conclusion

A growing body of evidence links abnormalities in vitamin D metabolism to fatty liver disease. Although the preclinical models in this review do not replicate the whole spectrum of human disease, they are of relevance in elucidating the roles and pathways of vitamin D and related factors involved in the pathogenesis of NAFLD and NASH, as well as for providing a better mechanistic understanding of the potential of vitamin D-based therapies. However, the utility of these models in verifying hypotheses on disease pathogenesis or intervention studies does not eliminate the need for well-designed clinical trials in (vitamin D-deficient) patients, particularly due to the broad spectrum of alterations in NAFLD.

Conflicts of Interest

The Authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

All the Authors contributed in the drafting, intellectual and structural development and critical review of this manuscript.

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