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**TITLE PAGE**

**Title**

Vitamin D and Non-alcoholic Fatty Liver Disease

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**STRUCTURED ABSTRACT**

**Purpose of review**

Vitamin D deficiency may impact disease progression of non-alcoholic fatty liver disease (NAFLD). The aim of this work was to review recent studies examining either vitamin D status or the effects of supplementation in patients with NAFLD, along with investigating the roles of genetic polymorphisms and the gut microbiome.

**Recent findings**

Six heterogeneous observational studies of vitamin D status, and four randomised controlled intervention trials of vitamin D supplementation in NAFLD were conflicting. All studies were hampered by the challenges of diagnosing NAFLD, were underpowered, and lacked data on clinically important outcomes. The results of three cross-sectional studies, including a Mendelian randomisation study, provide limited evidence for a role for genetic modifiers of vitamin D status in NAFLD. Genetic and experimental evidence suggests that vitamin D and the vitamin D receptor (VDR) may influence the gut microbiome in health and disease.

**Summary**

The evidence relating either lower vitamin D status to the prevalence and severity of NAFLD, or examining vitamin D supplementation in patients with NAFLD is inconclusive. Larger, higher quality trials with relevant endpoints are needed. Further mechanistic studies on the roles of vitamin D and VDR in influencing the gut-liver axis in NAFLD are warranted.

**Keywords**

NAFLD, vitamin D status, supplementation, polymorphisms, microbiome

## INTRODUCTION

In parallel with the increasing epidemics of obesity, diabetes and metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) has become the most common cause of liver disease, with an estimated global prevalence of 24% [1]. Defined histologically, NAFLD is a broad spectrum of disease that varies from simple fatty liver to nonalcoholic steatohepatitis (NASH), which can also progress to fibrosis and cirrhosis [2]. Vitamin D is an important secosteroid hormone, well known for its regulation of calcium/phosphate metabolism and bone homeostasis [3]. Experimental research has shown that vitamin D has anti-proliferative, anti-inflammatory and anti-fibrotic properties, which might impact disease progression in chronic liver diseases including NAFLD [4]. However, mechanisms involving vitamin D and NAFLD pathogenesis have only recently begun to be examined and are not completely understood yet. The aim of this review is to give insights into the association between NAFLD and vitamin D status, describe recent vitamin D supplementation trials in patients with NAFLD, and to discuss the roles of genetic polymorphisms and the gut microbiome in influencing both vitamin D status and NAFLD pathogenesis.

### The association of low-serum vitamin D status and NAFLD

Vitamin D from either the diet, or synthesized through sun exposure to skin, is metabolized into its biologically active metabolite, 1$\alpha$,25-dihydroxyvitamin D [1$\alpha$,25(OH)$_2$D], through two critical hydroxylation steps. The first step occurs in the liver resulting in 25-hydroxyvitamin D [25(OH)D], the more stable circulating form of vitamin D and its most widely used status indicator; with the second, 1$\alpha$ -hydroxylation step occurring in the kidneys [4]. Given that vitamin D undergoes this important biotransformation in the liver, abnormal vitamin D metabolism might be expected to be

67 associated with chronic liver diseases, including NAFLD [5]. Previous studies have
68 found vitamin D deficiency associated with components of the metabolic syndrome,
69 such as insulin resistance and dyslipidemia [4]. NAFLD is often considered as the
70 hepatic manifestation of metabolic syndrome [6], and a growing body of research
71 suggests a relationship between vitamin D deficiency and NAFLD, with low levels of
72 25(OH)D associated with hepatic inflammation, and the severity and progression of
73 NAFLD [7, 8]. Whether vitamin D deficiency is a contributing factor to NAFLD, or is
74 symptomatic of associated obesity or impaired liver metabolism capacity in NAFLD
75 remains unclear.

76 Early cross-sectional studies, in two Italian cohorts of 120 [9] and 262 [10]
77 adults with and without NAFLD, suggested lower 25(OH)D was associated with
78 advanced liver steatosis and fibrosis in patients with NAFLD. However, two recent
79 meta-analyses, synthesizing six observational studies in patients with biopsy-proven
80 NAFLD (published prior to September of 2017), concluded that 25(OH)D level was not
81 associated with either NAFLD activity score (NAS, a measure of histological severity)
82 or fibrosis [11, 12]. Here we review more recent studies published since the meta-
83 analyses were done. We searched databases including PubMed, Scopus and
84 Cochrane to identify acceptable observational studies reporting vitamin D status and
85 NAFLD. In total six observational studies were identified examining vitamin D status
86 and NAFLD (Table 1).

87 Studies were heterogeneous in terms of NAFLD diagnosis, the populations
88 examined, and sample size. A key challenge in NAFLD is the diagnosis of patients.
89 While liver biopsy is considered the gold standard for staging disease, biopsies are
90 invasive and not practical for large population studies [2]. Only two of the studies
91 reviewed here (with n=83 and 64) used biopsy for diagnosis [13, 14]. Ultrasound,

although not completely sensitive, reliably detects fatty liver when steatosis is at least 20% and is more amenable for larger studies [15]. Three studies, with sample sizes ranging from 220-789 individuals, used ultrasound for diagnosis [16-18]. Elevated liver enzyme levels in blood are readily measured and are therefore often used to define 'suspected NAFLD' in large population studies; although it is recognized these are neither sensitive nor specific for NAFLD and significantly underestimate prevalence [2]. Here, the largest population study (n= 3878) used elevated serum alanine transaminase (ALT) levels (ALT > 30 U/L) to diagnose NAFLD in adolescents [19].

All studies showed that vitamin D inadequacy was prevalent in both adolescents and adults with NAFLD. However, conclusions relating the lower vitamin D status to the prevalence and severity of NAFLD were conflicting. Of the three studies that compared status between people with and without NAFLD, two larger population studies: a general Korean adolescent population (n=3878; diagnosis based on ALT levels) [19] and a Chinese type 2 diabetes population (n=331; diagnosis by ultrasound) [18]; identified significant differences with lower 25(OH)D status in NAFLD. A third study in Italian adults with type 1 diabetes (n=220; diagnosis by ultrasound) found no differences [16]. In the three studies that examined 25(OH)D status in relation to NAFLD severity, no relationship was identified [13, 14, 17].

It is a challenge to draw a conclusion from such heterogenous studies. In addition, given the observational nature of these studies a causal relationship between vitamin D deficiency and NALFD can not be concluded.

**Supplementation of vitamin D in NAFLD**

At present, there are no pharmaceutical therapeutic agents specific for NAFLD and clinical care is reliant on dietary and/or physical activity changes aimed at inducing

weight loss; however, lifestyle modifications are difficult to implement and sustain [2]. A number of compounds are currently being examined in clinical trials including several nutraceuticals, such as vitamin D [20, 21]. From a pathophysiological point of view, vitamin D supplementation has been shown to improve insulin-sensitivity and glycemic control in people with prediabetics and type 2 diabetes [22, 23], and patients with NAFLD [24]. Additionally, in vitro studies show extensive vitamin D receptor (VDR) expression in non-parenchymal liver cells like macrophages, Kuppfer cells, and hepatic stellate cells (HSCs) suggesting that vitamin D could exert anti-proliferative, anti-inflammatory and anti-fibrotic properties [25]. However, the results of vitamin D supplementation trials in patients with NAFLD are inconclusive. In 2017, both a systematic review with meta-analysis that focused exclusively on randomized controlled trials in NAFLD [26], and a larger Cochrane review that more broadly focused on chronic liver disease [5], concluded that although vitamin D supplementation appeared to have no effects on liver aminotransferases in patients with NAFLD, the evidence base for this (four trials) was extremely weak.

Here we have focused on randomized controlled trials (RCTs) published since the end of the census in the aforementioned meta-analyses. Four RCTs investigating the biochemical and histological benefits of oral vitamin D supplementation in NAFLD patients were identified (Table 2). Interventions ranged from 12-48 weeks, with outcomes of interest including vitamin D status, liver enzymes, hepatic steatosis and/or fibrosis. In each RCT, serum 25(OH)D was measured and was significantly increased in all studies after the intervention. In terms of liver-related outcomes, all four studies measured serum concentrations of liver aminotransferases (ALT and aspartate transaminase, AST). Two studies by Geier [27] and Shidfar [28] and colleagues reported significant decreases in serum levels of ALT and AST at the post-

142 interventional point, consistent with an earlier study of Lorvand Amiri [29]. However,
143 these results contrast not only with the work of Dabbaghmanesh [30] and Taghvaei
144 [31] and colleagues reviewed here (Table 2); but also contrasts to previous studies
145 done by Barchetta [32], Sharifi [33], and coworkers that concluded that
146 supplementation with vitamin D did not significantly change ALT and AST levels. Both
147 studies showing a significant effect of vitamin D supplementation on
148 aminotransferases were small. While the trial of Shidfar and collegues [28] had n=36-
149 37/arm, in the pilot study of Geier and coworkers [27], the sample size was particularly
150 small (placebo n=8, vitamin D n=10). Only two studies [27, 31] evaluated the effects
151 of vitamin D supplementation on hepatic steatosis in NAFLD patients; however,
152 neither showed significant differences between placebo and the supplemented group.
153 This included the study of Geier and coworkers [27], who had liver biopsy specimens
154 pre- and post-treatment from only seven patients (four from placebo group and three
155 from the intervention group). Surprisingly, the authors reported that while the NAS
156 improved in all three patients receiving vitamin D (4,000 IU/d for 48 weeks), it also
157 improved in three of the four placebo-treated patients who had both pre- and post-
158 intervention biopsies. In the work by Taghvaei and workers [31], steatosis and fibrosis
159 were examined by FibroScan® (ultrasound-based transient elastography) and no
160 differences between placebo and vitamin D treated (50,000 IU/wk for 12 weeks;
161 n=20/arm) were observed.

162 In general, while vitamin D treatment in animal and cell models has improved
163 NAFLD-like symptoms, clinical trials of vitamin D supplementation in patients with
164 NAFLD have mostly been under-powered [25]. Similar to earlier studies previously
165 reviewed [34, 35], the trials reviewed here were heterogeneous in terms of populations
166 examined (adolescents, adults, multiple ethnicities, participants with either obesity,

type 1 or type 2 diabetes), the sample size, the type of vitamin D supplementation, duration and dosage used, and the modality used for diagnosis of NAFLD. Cholecalciferol (vitamin $D_3$) raises 25(OH)D levels more than ergocalciferol (calciferol; vitamin $D_2$) and has been primarily the choice of supplement used (Table 2 and [35]). However, the dosing regime of supplementation for both NAFLD and other chronic diseases, continues to be debated, with some evidence in favour of loading dose regimes where megadoses, e.g. 100,000 IU, are given to rapidly elevate 25(OH)D [34]. Among the three 12-week RCTs with ultrasound or FibroScan® diagnosis, the largest sample size was n=36-37/arm, found in the study by Shidfar and collegues [28], which reported significant decreases in ALT levels. Although the study by Geier et al. [27] was hampered by sample size, it was notably the longest intervention, giving 4,000 IU/d for 48 weeks, and also reported improved ALT status. Based on the results of the RCTs to date, accurate diagnosis of NAFLD, duration of intervention and sample size are important considerations for the design of future intervention trials.

**Polymorphisms influencing vitamin D status and NAFLD severity**

Both vitamin D status and NAFLD are complex phenotypes that arise from dynamic interactions between dietary, lifestyle and genetic factors [2, 4]. Multiple environmental factors have been implicated in vitamin D status, including reduced dietary intake, minimal sun exposure related to climate change and modern lifestyles, and age related impairment of hepatic and renal hydroxylation [3, 4]. Equally, hypercaloric diets and sedentary lifestyle are key contributors to the development and progression of NAFLD [2]. In addition to these environment factors, both NAFLD and vitamin D status are influenced by genetic polymorphisms.

Several genetic variants involved in vitamin D metabolism have been found in linkage, candidate gene, and genome-wide association studies (GWAS) to affect circulating vitamin D concentrations [36]. These include variants in the gene for the dehydrocholesterol reductase-7 (DHCR7) enzyme that reduces 7-dehydrocholesterol to cholesterol. DHCR7 is in linkage disequilibrium with the gene for nicotinamide adenine dinucleotide synthetase-1 (NADSYN1) that catalyses the final step of NAD biosynthesis [37]. Polymorphisms in the NADSYN1 gene have also been associated with vitamin D status; along with variants of the group-specific component (GC) gene, which encodes the vitamin D binding protein (DPB) responsible for transporting vitamin D in serum [36]. Furthermore, polymorphisms in multipe genes encoding for cytochrome P450 (CYP) enzymes involved in the formation of 25(OH)D and $1\alpha$,25(OH)$_2$D along with the inactivation of $1\alpha$,25(OH)$_2$D (CYP2R1, CYP27B1 and CYP24A1 respectively) have also been associated with vitamin D status [36, 37]. Additionally, genetic variants encoding the vitamin D receptor (VDR), which mediates the transcriptional effects of vitamin D have also been associated with serum 25(OH)D levels through multiple GWAS [37].

208 However, only a few studies have investigated whether vitamin D-related single
209 nucleotide polymorphisms (SNPs) or hepatic expression of vitamin D-related genes
210 affect the progression and severity of NAFLD and the results are conflicting. For
211 example, Beilfuss et al. [38] found in 106 obese patients with NAFLD and inadequate
212 vitamin D status that VDR SNPs were associated with with altered profibrogenic
213 mRNA expression and liver fibrosis. While a seperate cross-sectional study (control
214 patients n=39, patients with biopsy-proven NAFLD n=244) by Patel and colleagues
215 [39] found no differences in hepatic expression of VDR or other vitamin D-related
216 genes (including CYP24A1 and GC) associated with histological severity of NAFLD.
217 More recently, three recent cross-sectional studies including one mendelian
218 randomization (MR) study have examined the relationship between genetic modifiers
219 of vitamin D status and NAFLD (Table 3). Two of these studies were done in patients
220 with biopsy-proven NAFLD [37, 40]. In a Japanese adult population (n=220), a
221 polymorphism in the VDR gene (rs1544410) was significantly associated with
222 advanced liver fibrosis [40]. Separately in an UK paediatric population (n=103),
223 variants of the NADSYN1 (rs12785878, rs 3829251) and VDR (rs2228570) genes
224 were independently associated with increased steatosis, while a GC gene variant (rs
225 4588) was associated with increased inflammation [37]. On the other hand in a large
226 Chinese population (n=9128) diagnosed by ultrasound, Wang and colleagues notably
227 used bi-directional MR to explore the causal relationship between 25(OH)D and
228 NAFLD [41]. MR uses SNPs that explain trait variance in the general population to
229 make causal inferences regarding the effect of lifetime exposure to that trait with
230 disease incidence or outcome. MR avoids many of the limitations of conventional
231 epidemiological studies (such as residual confounding and reverse causation) as the
232 populations under investigation are randomized from birth based on their genotype

[42]. In this study, the authors examined four variants related to vitamin D status and eight variants related to NAFLD (Table 3). The results showed three SNPs (GCKR rs780094, PNPLA3 rs738409 and PARVB rs5764455) were significantly associatied with NAFLD, and two SNPs (GC rs2282679 and DHCR7 rs12785878) were significantly associated with serum 25(OH)D status. However, in applying MR utilising polygenetic risk scores (for both vitamin D status and NAFLD) the authors concluded that there was no causal association between vitamin D and NAFLD [41].

Based on the current studies, there is limited evidence for a role for the vitamin D-related polymorphisms in NAFLD. The key limitation of the two biopsy-proven observational studies is sample size. In addition, 25(OH)D levels were measured in serum and bioavailability of the active hormone in liver can not be accounted for. Similarly the hepatic expression of genes responsible for vitamin D metabolism may be altered or switched off in the context of significant liver injury, confounding conclusions. While MR is a valuable tool to assess causality of vitamin D status and NAFLD, done in a single population limits conclusions related to ethnicity and the potential contribution of rare variants related to vitamin D and NAFLD heritability. Therefore, further MR studies examing rare variants and large multi-ethnic populations are likely warranted.

**The crosstalk between the gastrointestinal microbiome and the VDR in NAFLD**

The gastrointestinal microbiome is an additional factor that most likely influences the progression of NAFLD, in the first instance through influencing nutrient uptake from the diet and enterohepatic circulation of nutrients and bile acids [43]. In the context of obesity, the metabolic syndrome and NAFLD, dysbiosis or altered gut microflora can result in intestinal permeability and chronic inflammation in patients [6, 44].

Approximately 75% of liver blood comes from the intestine via the portal vein, thus exposing the liver to the gastrointestinal microbiome and its endotoxins, such as peptidoglycan and lipopolysaccharides [44, 45]. Endotoxin exposure can trigger the activation of inflammatory cytokines that contribute to NAFLD pathogenesis. While murine studies have found associations between NAFLD and certain bacteria, studies in human reporting differences in the intestinal bacteria between healthy controls and NAFLD have been largel cross-sectional to date [45]. Therefore, the causal relationships between NAFLD and gastrointestinal microbiome pathology remains uncertain.

However, most interestingly a recent GWAS of the gut microbiota in a German population identified significant associations for overall microbial diversity and individual taxa at multiple genetic loci, including the VDR gene [46]. This is consistent with experimental evidence that vitamin D and its receptor VDR play a vital role in regulating microbiome in health and disease [47]. Genetic deletion of VDR in mice has been shown to influence the intestinal microbiome at both the taxonomic and functional levels, resulting in higher risk of infections, inflammation, cancer and other conditions [48]. Additionally, in pre-clinical models of NAFLD, a vitamin D deficient, high fat diet (HFD) led to gut permeability, dysbiosis, endotoxemia, systemic inflammation, insulin resistance and hepatic steatosis; conversely, dietary vitamin D supplementation attenuated steatosis [49]. These results suggest that further studies of vitamin D and VDR signaling at the genetic and functional levels and its regulation of microbiome in gut-liver axis will provide novel mechanistic insights and potential therapeutic opportunities for NAFLD.

## CONCLUSION

Recent studies either examining vitamin D status in patients with NAFLD, or examining the efficacy of vitamin D supplementation for treating NAFLD, are largely inconclusive. However, only a few heterogeneous trials with an insufficient number of participants have been done to date. These were hampered by the challenges of diagnosing NAFLD and lack of data on clinically important outcomes. The overall quality of evidence is very low. There is limited evidence for a role for genetic modifiers of vitamin D status in NAFLD and a recent MR study suggests there is no causal association between vitamin D and NAFLD. However, there is genetic and experimental evidence that vitamin D and the VDR play important roles in regulating the microbiome in health and disease and further mechanistic studies of this pathway influencing the gut-liver axis in NAFLD are warranted.

**KEY POINTS**

- Experimental research has shown that vitamin D has anti-proliferative, anti-inflammatory and anti-fibrotic properties, which might impact disease progression in chronic liver diseases including NAFLD.
- Vitamin D supplementation has been shown to improve insulin-sensitivity and glycemic control in people with prediabetes and type 2 diabetes and therefore is of plausible benefit to patients with NAFLD.
- Vitamin D supplementation trials in patients with NAFLD have been underpowered and are inconclusive.
- Based on current studies, there is limited evidence for a role for the vitamin D-related polymorphisms in NAFLD.
- Mechanistic studies on the roles of vitamin D and VDR in influencing the gut-liver axis in NAFLD are warranted

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## Conflicts of Interest

There are no conflicts of interest.

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A comprehensive narrative review of NAFLD and its pathogenesis, thoroughly reviewing the roles of genetics and nutrition in NAFLD development and progression. In addition, the paper introduces the concept of systems biology and reviews recent work utilising genome-scale metabolic networks and developing multi-scale models of liver metabolism relevant to NAFLD.

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This comprehensive narrative review included all major cross-sectional studies prior to February 2017 examining the association between vitamin D status and the progression of NAFLD, as well as intervention trials examining effects of vitamin D supplementation. Forty-five studies were reviewed. It usefully discusses key host, environment, and heritability factors that may impact vitamin D status, as well as the conflicting definitions of deficient or optimal vitamin D status and challenges related to the methods of measuring 25(OH)D and the diagnosis of NAFLD.

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This Cochrane review is significant for synthesizing the major randomized controlled trials executed prior to 2017 that assessed the beneficial and harmful effects of vitamin D supplementation in people with chronic liver disease. Four trials that administered vitamin D3 or placebo to patients with NALFD were included. It concluded that there was a paucity of evidence on which to determine the effect of vitamin D supplementation on liver‐related morbidity or mortality.

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This observational study examined the association between vitamin D deficiency and the severity of NAFLD in 64 adults with biopsy-proven NAFLD. All patients had low levels of 25(OH)D, but a significant relationship between vitamin D deficiency and the severity of NALFD was not established. When patients examined as two groups based on vitamin D insufficiency or deficiency, the results showed that, fibrosis and steatohepatitis were independent predictors of low vitamin D concentration.
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This observational study examined the relationship between vitamin D and serum liver enzymes and ultrasound findings in 83 adults with biopsy-proven NAFLD. When divided into two groups, 37 patients with vitamin D deficiency and 46 patients with normal vitamin D levels, serum aspartate transaminase levels were significantly higher in patients with vitamin D deficiency. However, no relationship was identified between 25(OH)D status and NAFLD severity.
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agents and their mechanism of actions that are currently in phase 2 and 3 clinical trials for NAFLD. In addition it notably summarises, the efficacy of those that have been more thoroughly studied (e.g.: vitamin E, pioglitazone, obetocholic acid, elafibranor) and the significant placebo response observed in these trials.

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This narrative review discusses the major barriers to drug development for NASH treatment, evidencing the point that natural supplements can improve cardiometabolic parameters and may be of benefit at an earlier stage of disease. Regulatory, intellectual property, manufacturing, and clinical development steps were reviewed.

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This meta-analysis is significant for including all randomized controlled trials prior to September 2017 that assessed the effects of oral vitamin D supplementation on glycemic control in type 2 diabetes. Twenty trials administered vitamin D or placebo to 2703 participants were synthesized. Conclusions were that vitamin D supplementation resulted in significant improvements to fasting insulin and homeostasis model assessment of insulin resistance as well as serum 25(OH)D levels.

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This meta-analysis is significant for including all randomized controlled clinical trials prior to April 2017 that assessed glycemic outcomes in adults with high risk of type 2 diabetes. In synthesizing 28 trials that administered vitamin D with or without calcium or placebo to 3848 participants it concluded that vitamin D supplementation resulted in significant reductions of glycosylated hemoglobin, fasting plasma glucose level and the homeostasis model assessment of insulin resistance.

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This meta-analysis is significant for including all major randomized trials prior to October 2016 where patients with NAFLD were randomized to treatment with vitamin D or placebo. Notably of the 7 studies with 452 individuals that were included, only 4 examined serum aspartate transaminase and alanine aminotransferase levels and the meta-analysis concluded no significant reduction in these from vitamin D treatment.

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This narrative review focused on four randomised controlled trials and two uncontrolled clinical trials examining the effects of vitamin D supplementation on patients with NAFLD and highlights conflicting results.
*[37] Gibson PS, Quaglia A, Dhawan A et al. Vitamin D status and associated genetic polymorphisms in a cohort of UK children with non-alcoholic fatty liver disease. Pediatr Obes 2018; 13:433-441.
This cross-sectional study is significant for being the first to examine the relationship between vitamin D status, genetic variants known to affect vitamin D status and NAFLD histological severity in a paediatric population. 103 biopsy-proven paediatric patients with NAFLD had low vitamin D status throughout the year and significantly lower 25(OH)D levels in winter.
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This cross-sectional study of 220 patients with biopsy-proven NAFLD examined select polymorphisms in vitamin D related genes in relationship to histopathological severity, suggesting that the vitamin D receptor rs1544410 polymorphism was associated with advanced liver fibrosis.
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This study is significant for being the first study to apply bi-directional mendelian randomization (MR) to explore the causal relationship between 25(OH)D and NAFLD. Examining a Chinese population with 9128 participants, although individual single nucleotide polymorphisms associated with NAFLD; when polygenetic risk scores were applied (for both vitamin D status and NAFLD) the authors concluded no causal association between vitamin D and NAFLD.
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**Table 1 Recent observational studies of vitamin D status in NAFLD**

| Reference; Country | Design; Sample size (NAFLD/NN) | Study population; Age | Diagnosis of NAFLD | BMI (kg/m$^2$) | Vitamin D assay; Definition of inadequacy (nmol/L) | Reporting of date or season of blood draw, or sun exposure | 25(OH)D status (nmol/L) |
|---|---|---|---|---|---|---|---|
| Cho et al., 2019 [19]; Korea | Cross-sectional; 3878 (215[§]/3663) | Adolescent in general population; 12-18 | ALT>30U/L | **NAFLD**: 26.16±0.34[†] **NN**: 20.87±0.07[†] | RIA; **Deficiency:** <50 | **Season report:** 2008 to 2014 **Sun exposure:** nr | **NAFLD***: 35.50±0.83[†] **NN**: 40.70±0.38[†] |
| Clipponeri et al., 2019 [16]; Italy | Cross-sectional; 220 (57/163) | Caucasian adults with type 1 diabetes; 18-60 | Ultrasound | **NAFLD**: 26.6 (24.5-28.5)[¤] **NN**: 23.5 (21.5-26.4)[¤] | RIA; **Sufficiency:** ≥75 **Insufficiency:** 50-75 **Deficiency:** <50 | **Season report:** July 2013 to April 2014 **Sun exposure:** 2h/day | **NAFLD**: 53 (38-70)[¤] **NN:** 50 (34-69)[¤] |
| He et al., 2018 [18]; China | Cross-sectional; 331 (209/122) | Adults with type 2 diabetes; 20-78 | Ultrasound | **NAFLD**: 26.83±3.00[†] **NN**: 23.27±2.47[†] | ECLIA; nr | **Season report:** March 2015 to December 2016 **Sun exposure:** nr | **NAFLD*:** 41.08 (31.75-53.43)[¤] **NN:** 48.98 (34.45-54.65)[¤] |
| Izadi et al., 2019 [14]; Iran | Cross-sectional; 83 (83/0) | Adults with NAFLD; 20-60 | Biopsy | 24.19±4.18[†] | RIA; **Sufficiency:** >75 **Insufficiency:** 50-75 **Deficiency:** <50 | **Season report:** nr **Sun exposure:** nr | 22.01±8.38[†] r=-0.17 for NAS |
| Leitao et al., 2018 [17]; Portugal | Cross-sectional; 789 (280[#]/509) | Adults in general population; 18-79 | Ultrasound | **Steatosis**: 29.7±4.8[†] **NS**: 25.5±3.9[†] | ECLIA; **Insufficiency:** 50-75 **Deficiency:** <50 | **Season report:** 2012 to 2015, measurements reported by seasons **Sun exposure:** 2500 h/year | **Steatosis**: 62.75±21.75[†] **NS**: 66.00±25.75[†] |
| Livadariu et al., 2018 [13]; Romania | Cross-sectional; 64 (64/0) | Adults with obesity; 18-60× | Biopsy | 45.06 [35-58][‡] **Steatosis:** 44.35±6.82[†] **NASH:** 45.82±6.50[†] **NASH+ vanished steatosis:** 43.91±7.56[†] | CLIA; **Sufficiency:** 75-250 **Insufficiency:** 50-75 **Deficiency:** <50 | **Season report:** November 2014 to November 2016 **Sun exposure:** nr | **Steatosis:** 40.48 ±15.63[†] **NASH:** 34.45 ±14.80[†] **NASH+ vanished steatosis:** 32.00 ±19.08[†] |

ALT, alanine aminotransferase; CLIA, chemiluminescence assay; ECLIA, electro-chemiluminescence binding assay; HPLC-UV, high performance liquid chromatography with ultraviolet detector; NAS, NAFLD activity score; NN, non-NAFLD; nr, not reported; NS, no steatosis; RIA, radioimmunoassay

¤Median (Interquartile range); † Mean ± Standard Deviation; ‡ Mean [Range]

[§] Adolescent with suspected NAFLD; [#] Steatosis group includes alcohol (60) and NAFLD (220)

[×] Author communication

* Statistic significant relative to 25(OH)D level

**Table 2 Recent randomised controlled intervention trials of vitamin D supplementation in NAFLD**

| Reference; Country | Design; Arms (n=) | NAFLD diagnosis; Vitamin D cut off; Age | Vitamin D assay and status (nmol/L) | Liver and vitamin D related outcomes | Post-intervention changes |
|---|---|---|---|---|---|
| Dabbaghmanesh et al. 2018[30]; Iran | 12-week 3-arm RCT;<br>**G1:** placebo (n=31)<br>**G2:** 0.25 mg/d calcitriol (n=28)<br>**G3:** 50,000 IU/wk cholecalciferol (n=32) | Ultrasound;<br>25(OH)D <70 nmol/L;<br>20-75 | nr;<br>**Before:**<br>**G1:** 52.8±13.0†, **G2:** 46.5±13.8†, **G3:** 47.3±15.5†<br>**After:**<br>**G1:** 47.0±17.5†, **G2:** 57.3±49.5†, **G3:** 80.5±35.3† | **Liver:**<br>ALT, AST, GTT, ALP<br>**Vitamin D:**<br>25(OH)D | **Liver:**<br>ns<br>**Vitamin D:**<br>25(OH)D↑* |
| Geier et al., 2018[27]; Switzerland | 48-week 2-arm RCT (pilot study);<br>**G1:** placebo (n=10)<br>**G2:** 4,000 IU/d vitamin D (n=8) | ALT and/or biopsy;<br>25(OH)D <70 nmol/L;<br>23-63 | ECLIA;<br>**Before:**<br>**G1:** 50±25†, **G2:** 52.5±30†<br>**After:**<br>**G1:** 40±23†, **G2:** 98±33† | **Liver:**<br>ALT, AST, GTT, ALP, NAS and CK-18 M30<br>**Vitamin D:**<br>25(OH)D | **Liver:**<br>ALT↓*, CK-18 M30↓*<br>**Vitamin D:**<br>25(OH)D↑* |
| Shidfar et al., 2019[28]; Iran | 12-week 3-arm RCT;<br>**G1:** placebo (n=36)<br>**G2:** 1,000 IU/d vitamin D (n=37)<br>**G3:** 500 mg calcium carbonate+1000 IU/d vitamin D (n=37);<br>All three groups given some advice on physical activity, hypocaloric diet and sun-light exposure. | Ultrasound;<br>25(OH)D < 37.5 nmol/L;<br>18-65 | ELISA;<br>**Before**:<br>**G1:** 25.00 ± 1.58†, **G2:** 24.75 ± 1.60†, **G3:** 24.75 ± 2.33†<br>**After:**<br>**G1:** 27.50 ± 1.95†, **G2:** 53.50 ± 1.83†, **G3:** 67.75 ± 2.75† | **Liver:**<br>ALT, AST, ALP<br>**Vitamin D:**<br>25(OH)D | **Liver:**<br>ALT↓*, AST↓*<br>**Vitamin D:**<br>25(OH)D↑* |
| Taghvaei et al., 2018[31]; Iran | 12-week 2-arm RCT;<br>**G1:** placebo (n=20)<br>**G2:** 50,000 IU/wk vitamin D3 (n=20);<br>Both groups were given lifestyle modification advice. | FibroScan®;<br>25(OH)D <70nmol/L;<br>30-70 | ELISA;<br>**Before:**<br>**G1:** 49.45 ± 10.88†, **G2**: 47.90 ± 13.73†<br>**After:**<br>**G1:** 52.13 ± 6.23†, **G2:** 86.00 ± 10.70† | **Liver:**<br>ALT, AST, ALP, CAP score and kPA<br>**Vitamin D**:<br>25(OH)D | **Liver:**<br>ns<br>**Vitamin D:**<br>25(OH)D↑* |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CK-18 M30, cytokeratin 18 M30; ECLIA, electro-chemiluminescence binding assay; ELISA, enzyme-linked immunosorbent assay; GGT, γ-glutamyl transferase; HS, hepatic steatosis; NAS, NAFLD activity score; nr, not reported; ns. not significant; RCT, randomized controlled trial

† Mean±Standard deviation

* Statistic significant

**Table 3 Studies examining genetic polymorphisms related to vitamin D status and NAFLD**

| Reference; Country | Design; Sample Size (NAFLD/NN) | Study Population; Age | Diagnosis of NAFLD | Vitamin D Assay; Definition of Inadequacy; Status (nmol/L) | Vitamin D or NAFLD Related Polymorphisms | Summary of Associations |
|---|---|---|---|---|---|---|
| Arai et al., 2019[40]; Japan | Cross-sectional; 220 (220/0) | Adults with NAFLD; 18-84 | Biopsy | RIA;<br>**Sufficiency:** ≥75<br>**Insufficiency:** 50-75<br>**Deficiency:** <50;<br>45.0 [17.5-97.5]‡ | **Vitamin D related:**<br>**CYP2R1**: rs1993116 rs10741657<br>**DHCR7:** rs7944926, rs12784878<br>**GC:** rs2282670<br>**CYP27B1:** rs10877012<br>**VDR:** rs2228570, rs1544410, rs7975232, rs731236 | **CYP2R1** rs1993116 genotype non-AA and **VDR** rs228570 genotype GG associated with VDD;<br>**VDR** rs1544410 genotype CC associated with advanced liver fibrosis |
| Gibson et al., 2018[37]; United Kingdom | Cross-sectional; 103 (103/0) | Children with NAFLD; 11-16 | Biopsy | CLIA reported by season;<br>**Deficiency:** <25<br>**Insufficiency:** 25-50;<br>**Spring:** 36.6 [30.5-42.1]*<br>**Summer:** 41.8 [36.3-47.2]*<br>**Autumn:** 40.8 [34.2-47.5]*<br>**Winter:** 26.9 [22.7-31.2]* | **Vitamin D related:**<br>**NADSYN1:** rs12785878, rs3829251<br>**GC:** rs2282670, rs7041, rs4588<br>**CYP2R1:** rs10741<br>**VDR:** rs2228570 | **NADSYN1/DHCR7** rs3829251, **CYP2R1** rs10741657 and **VDR** rs2228570 associated with increased steatosis; **GC rs4588** associated with increased inflammation in liver biopsies |
| Wang et al., 2018[41]; China | Cross-sectional, Mendelian Randomization; 9128 | General population; 18-93 | Ultrasound | CLIA;<br>nr;<br>**VD GRS:**<br>**Quartile 1:** 41.8 ± 12.9†<br>**Quartile 2:** 40.4 ± 12.3†<br>**Quartile 3:** 39.6 ± 12.5†<br>**Quartile 4:** 38.7 ± 11.9†<br>**NAFLD GRS:**<br>**Quartile 1:** 40.2 ± 12.4†<br>**Quartile 2:** 40.3 ± 12.7†<br>**Quartile 3:** 40.0 ± 12.2†<br>**Quartile 4:** 40.0 ± 12.4† | **Vitamin D related:**<br>**NADSYN1:** rs12785878§<br>**CYP2R1:** rs10741657<br>**GC:** rs2282679<br>**CYP24A1:** rs6013897<br>**NAFLD related:**<br>**LYPLAL1:** rs12137855<br>**PPP1R3B:** rs4240624<br>**TM6SF2:** rs58542926<br>**PNPLA3:** rs738409<br>**GCKR:** rs780094<br>**SAMM50:** rs738491<br>**PARVB:** rs5764455<br>**COL13A1:** rs1227756 | **GC** rs2282679 and **DHCR7** rs12785878 were associated with 25(OH)D;<br>**GCKR** rs780094, **PNPLA3** rs738409 and **PARVB** rs5764455 associated with NAFLD |

ALT, alanine aminotransferase; CLIA, chemiluminescent immunoassays; COL13A1, collagen type XIII alpha 1 chain; CYP24A1, CYP27B1, cytochrome P450 27B1; cytochrome P450 24A1; CYP2R1, cytochrome P450 2R1; DHCR7, 7-dehydrocholesterol reductase; GC, vitamin D binding protein; GCKR, glucokinase regulatory protein; HS, hepatic steatosis; LYPLAL1, lysophospholipase-like 1;NADSYN1, adenine dinucleotide synthetase-1; NN, Non-NAFLD; nr, not reported; PARVB, parvin beta; PNPLA3, patatin-like phospholipase domain-containing protein 3; PPP1R3B, protein phosphatase 1 regulatory subunit 3b; RIA, radioimmunoassay; SAMM50, sorting and assembly machinery component; TM6SF2, transmembrane 6 superfamily member 2; VDD, vitamin D deficiency; VDR, vitamin D receptor

§ rs12785878 is an intronic variant in the NADSYN1 gene, which is located immediately proximal to DHCR7; previously rs12785878 has been reported as SNP in DHCR7 [41].

‡ Median [Range]; * Mean [95%CI]; † Mean ± Standard Deviation