



UNIVERSITY OF LEEDS

This is a repository copy of *Vitamin D and nonalcoholic fatty liver disease*.

White Rose Research Online URL for this paper:

<http://eprints.whiterose.ac.uk/151893/>

Version: Accepted Version

Article:

Zhang, Z, Thorne, JL orcid.org/0000-0002-3037-8528 and Moore, JB (2019) Vitamin D and nonalcoholic fatty liver disease. *Current Opinion in Clinical Nutrition and Metabolic Care*, 22 (6). pp. 449-458. ISSN 1363-1950

<https://doi.org/10.1097/MCO.0000000000000605>

© 2019 Wolters Kluwer Health, Inc. All rights reserved. This is an author produced version of an article published in *Current Opinion in Clinical Nutrition & Metabolic Care*. Uploaded in accordance with the publisher's self-archiving policy.

Reuse

See Attached

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

1 **TITLE PAGE**

2 **Title**

3 Vitamin D and Non-alcoholic Fatty Liver Disease

4 **Authors**

5 Zixuan Zhang, James L Thorne and J. Bernadette Moore

6 **Affiliation**

7 School of Food Science & Nutrition, University of Leeds, Leeds, LS2 9JT, United
8 Kingdom

9 **Corresponding author**

10 Dr. J Bernadette Moore

11 School of Food Science and Nutrition,

12 University of Leeds,

13 Leeds,

14 West Yorkshire

15 LS2 9JT

16 T: +44(0)11334 39900

17 E: J.B.Moore@leeds.ac.uk

18 **STRUCTURED ABSTRACT**

19 **Purpose of review**

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

24 **Recent findings**

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

33 **Summary**

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

39

40 **Keywords**

41 NAFLD, vitamin D status, supplementation, polymorphisms, microbiome

42 INTRODUCTION

43 In parallel with the increasing epidemics of obesity, diabetes and metabolic syndrome,
44 non-alcoholic fatty liver disease (NAFLD) has become the most common cause of liver
45 disease, with an estimated global prevalence of 24% [1]. Defined histologically,
46 NAFLD is a broad spectrum of disease that varies from simple fatty liver to
47 nonalcoholic steatohepatitis (NASH), which can also progress to fibrosis and cirrhosis
48 [2]. Vitamin D is an important secosteroid hormone, well known for its regulation of
49 calcium/phosphate metabolism and bone homeostasis [3]. Experimental research has
50 shown that vitamin D has anti-proliferative, anti-inflammatory and anti-fibrotic
51 properties, which might impact disease progression in chronic liver diseases including
52 NAFLD [4]. However, mechanisms involving vitamin D and NAFLD pathogenesis have
53 only recently begun to be examined and are not completely understood yet. The aim
54 of this review is to give insights into the association between NAFLD and vitamin D
55 status, describe recent vitamin D supplementation trials in patients with NAFLD, and
56 to discuss the roles of genetic polymorphisms and the gut microbiome in influencing
57 both vitamin D status and NAFLD pathogenesis.

58

59 **The association of low-serum vitamin D status and NAFLD**

60 Vitamin D from either the diet, or synthesized through sun exposure to skin, is
61 metabolized into its biologically active metabolite, $1\alpha,25$ -dihydroxyvitamin D
62 [$1\alpha,25(\text{OH})_2\text{D}$], through two critical hydroxylation steps. The first step occurs in the
63 liver resulting in 25-hydroxyvitamin D [$25(\text{OH})\text{D}$], the more stable circulating form of
64 vitamin D and its most widely used status indicator; with the second, 1α -hydroxylation
65 step occurring in the kidneys [4]. Given that vitamin D undergoes this important
66 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,

92 although not completely sensitive, reliably detects fatty liver when steatosis is at least
93 20% and is more amenable for larger studies [15]. Three studies, with sample sizes
94 ranging from 220-789 individuals, used ultrasound for diagnosis [16-18]. Elevated liver
95 enzyme levels in blood are readily measured and are therefore often used to define
96 'suspected NAFLD' in large population studies; although it is recognized these are
97 neither sensitive nor specific for NAFLD and significantly underestimate prevalence
98 [2]. Here, the largest population study (n= 3878) used elevated serum alanine
99 transaminase (ALT) levels (ALT > 30 U/L) to diagnose NAFLD in adolescents [19].

100 All studies showed that vitamin D inadequacy was prevalent in both
101 adolescents and adults with NAFLD. However, conclusions relating the lower vitamin
102 D status to the prevalence and severity of NAFLD were conflicting. Of the three studies
103 that compared status between people with and without NAFLD, two larger population
104 studies: a general Korean adolescent population (n=3878; diagnosis based on ALT
105 levels) [19] and a Chinese type 2 diabetes population (n=331; diagnosis by ultrasound)
106 [18]; identified significant differences with lower 25(OH)D status in NAFLD. A third
107 study in Italian adults with type 1 diabetes (n=220; diagnosis by ultrasound) found no
108 differences [16]. In the three studies that examined 25(OH)D status in relation to
109 NAFLD severity, no relationship was identified [13, 14, 17].

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

113

114 **Supplementation of vitamin D in NAFLD**

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

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

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

167 type 1 or type 2 diabetes), the sample size, the type of vitamin D supplementation,
168 duration and dosage used, and the modality used for diagnosis of NAFLD.
169 Cholecalciferol (vitamin D₃) raises 25(OH)D levels more than ergocalciferol (calciferol;
170 vitamin D₂) and has been primarily the choice of supplement used (Table 2 and [35]).
171 However, the dosing regime of supplementation for both NAFLD and other chronic
172 diseases, continues to be debated, with some evidence in favour of loading dose
173 regimes where megadoses, e.g. 100,000 IU, are given to rapidly elevate 25(OH)D [34].
174 Among the three 12-week RCTs with ultrasound or FibroScan® diagnosis, the largest
175 sample size was n=36-37/arm, found in the study by Shidfar and colleagues [28], which
176 reported significant decreases in ALT levels. Although the study by Geier et al. [27]
177 was hampered by sample size, it was notably the longest intervention, giving 4,000
178 IU/d for 48 weeks, and also reported improved ALT status. Based on the results of the
179 RCTs to date, accurate diagnosis of NAFLD, duration of intervention and sample size
180 are important considerations for the design of future intervention trials.

181

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

183 Both vitamin D status and NAFLD are complex phenotypes that arise from dynamic
184 interactions between dietary, lifestyle and genetic factors [2, 4]. Multiple environmental
185 factors have been implicated in vitamin D status, including reduced dietary intake,
186 minimal sun exposure related to climate change and modern lifestyles, and age related
187 impairment of hepatic and renal hydroxylation [3, 4]. Equally, hypercaloric diets and
188 sedentary lifestyle are key contributors to the development and progression of NAFLD
189 [2]. In addition to these environment factors, both NAFLD and vitamin D status are
190 influenced by genetic polymorphisms.

191 Several genetic variants involved in vitamin D metabolism have been found in
192 linkage, candidate gene, and genome-wide association studies (GWAS) to affect
193 circulating vitamin D concentrations [36]. These include variants in the gene for the
194 dehydrocholesterol reductase-7 (DHCR7) enzyme that reduces 7-dehydrocholesterol
195 to cholesterol. DHCR7 is in linkage disequilibrium with the gene for nicotinamide
196 adenine dinucleotide synthetase-1 (NADSYN1) that catalyses the final step of NAD
197 biosynthesis [37]. Polymorphisms in the NADSYN1 gene have also been associated
198 with vitamin D status; along with variants of the group-specific component (GC) gene,
199 which encodes the vitamin D binding protein (DPB) responsible for transporting
200 vitamin D in serum [36]. Furthermore, polymorphisms in multiple genes encoding for
201 cytochrome P450 (CYP) enzymes involved in the formation of 25(OH)D and
202 $1\alpha,25(\text{OH})_2\text{D}$ along with the inactivation of $1\alpha,25(\text{OH})_2\text{D}$ (CYP2R1, CYP27B1 and
203 CYP24A1 respectively) have also been associated with vitamin D status [36, 37].
204 Additionally, genetic variants encoding the vitamin D receptor (VDR), which mediates
205 the transcriptional effects of vitamin D have also been associated with serum 25(OH)D
206 levels through multiple GWAS [37].
207

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 separate 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

233 [42]. In this study, the authors examined four variants related to vitamin D status and
234 eight variants related to NAFLD (Table 3). The results showed three SNPs (GCKR
235 rs780094, PNPLA3 rs738409 and PARVB rs5764455) were significantly associated
236 with NAFLD, and two SNPs (GC rs2282679 and DHCR7 rs12785878) were
237 significantly associated with serum 25(OH)D status. However, in applying MR utilising
238 polygenetic risk scores (for both vitamin D status and NAFLD) the authors concluded
239 that there was no causal association between vitamin D and NAFLD [41].

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

251

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

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

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

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

281

282 **CONCLUSION**

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

294

295 **KEY POINTS**

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

308

309 **Acknowledgements**

310 None.

311 **Financial Support and Sponsorship**

312 None.

313 **Conflicts of Interest**

314 There are no conflicts of interest.

315

316 **References:**

- 317 [1] Younossi Z, Anstee QM, Marietti M et al. Global burden of NAFLD and NASH:
318 trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*
319 2018; 15:11-20.
- 320 **[2] Moore JB. From sugar to liver fat and public health: systems biology driven
321 studies in understanding non-alcoholic fatty liver disease pathogenesis. *Proc*
322 *Nutr Soc* 2019; 78:290–304.
- 323 A comprehensive narrative review of NAFLD and its pathogenesis, thoroughly
324 reviewing the roles of genetics and nutrition in NAFLD development and progression.
325 In addition, the paper introduces the concept of systems biology and reviews recent
326 work utilising genome-scale metabolic networks and developing multi-scale models of
327 liver metabolism relevant to NAFLD.
- 328 [3] Mendes MM, Darling AL, Hart KH et al. Impact of high latitude, urban living and
329 ethnicity on 25-hydroxyvitamin D status: a need for multidisciplinary action? *J*
330 *Steroid Biochem Mol Biol* 2019; 188:95-102.
- 331 **[4] Pacifico L, Osborn JF, Bonci E et al. Association between vitamin D levels and
332 nonalcoholic fatty liver disease: potential confounding variables. *Mini Rev Med*
333 *Chem* 2019; 19:310-332.
- 334 This comprehensive narrative review included all major cross-sectional studies prior
335 to February 2017 examining the association between vitamin D status and the
336 progression of NAFLD, as well as intervention trials examining effects of vitamin D
337 supplementation. Forty-five studies were reviewed. It usefully discusses key host,
338 environment, and heritability factors that may impact vitamin D status, as well as the
339 conflicting definitions of deficient or optimal vitamin D status and challenges related to
340 the methods of measuring 25(OH)D and the diagnosis of NAFLD.
- 341 *[5] Bjelakovic G, Nikolova D, Bjelakovic M et al. Vitamin D supplementation for
342 chronic liver diseases in adults. *Cochrane Database Syst Rev* 2017;
343 11:Cd011564.
- 344 This Cochrane review is significant for synthesizing the major randomized controlled
345 trials executed prior to 2017 that assessed the beneficial and harmful effects of vitamin
346 D supplementation in people with chronic liver disease. Four trials that administered
347 vitamin D3 or placebo to patients with NALFD were included. It concluded that there
348 was a paucity of evidence on which to determine the effect of vitamin D
349 supplementation on liver - related morbidity or mortality.
- 350 [6] Jayakumar S, Loomba R. Review article: emerging role of the gut microbiome in
351 the progression of nonalcoholic fatty liver disease and potential therapeutic
352 implications. *Aliment Pharmacol Ther* 2019. <https://doi.org/10.1111/apt.15314>
- 353 [7] Wang X, Li W, Zhang Y et al. Association between vitamin D and non-alcoholic
354 fatty liver disease/non-alcoholic steatohepatitis: results from a meta-analysis.
355 *Int J Clin Exp Med* 2015; 8:17221-17234.
- 356 [8] Cicero AFG, Colletti A, Bellentani S. Nutraceutical approach to non-alcoholic fatty
357 liver disease (NAFLD): the available clinical evidence. *Nutrients* 2018; 10:1153.
- 358 [9] Targher G, Bertolini L, Scala L et al. Associations between serum 25-
359 hydroxyvitamin D3 concentrations and liver histology in patients with non-
360 alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; 17:517-524.
- 361 [10] Barchetta I, Angelico F, Del Ben M et al. Strong association between non alcoholic
362 fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult
363 population with normal serum liver enzymes. *BMC Med* 2011; 9:85.

364 [11] Jaruvongvanich V, Ahuja W, Sanguankeo A et al. Vitamin D and histologic
365 severity of nonalcoholic fatty liver disease: a systematic review and meta-
366 analysis. *Dig Liver Dis* 2017; 49:618-622.

367 [12] Saberi B, Dadabhai AS, Nanavati J et al. Vitamin D levels do not predict the stage
368 of hepatic fibrosis in patients with non-alcoholic fatty liver disease: A PRISMA
369 compliant systematic review and meta-analysis of pooled data. *World J Hepatol*
370 2018; 10:142-154.

371 *[13] Livadariu R, Timofte D, Trifan A et al. Vitamin D deficiency, a noninvasive marker
372 of steatohepatitis in patients with obesity and biopsy proven nonalcoholic fatty
373 liver disease. *Acta Endocrinol* 2018; 14:76-84.

374 This observational study examined the association between vitamin D deficiency and
375 the severity of NAFLD in 64 adults with biopsy-proven NAFLD. All patients had low
376 levels of 25(OH)D, but a significant relationship between vitamin D deficiency and the
377 severity of NALFD was not established. When patients examined as two groups based
378 on vitamin D insufficiency or deficiency, the results showed that, fibrosis and
379 steatohepatitis were independent predictors of low vitamin D concentration.

380 *[14] Izadi A, Aliasghari F, Gargari BP et al. Strong association between serum vitamin
381 D and vaspin levels, AIP, VAI and liver enzymes in NAFLD patients. *Int J Vitam*
382 *Nutr Res* 2019:1-8.

383 This observational study examined the relationship between vitamin D and serum liver
384 enzymes and ultrasound findings in 83 adults with biopsy-proven NAFLD. When
385 divided into two groups, 37 patients with vitamin D deficiency and 46 patients with
386 normal vitamin D levels, serum aspartate transaminase levels were significantly higher
387 in patients with vitamin D deficiency. However, no relationship was identified between
388 25(OH)D status and NAFLD severity.

389 [15] Zhou JH, Cai JJ, She ZG et al. Noninvasive evaluation of nonalcoholic fatty liver
390 disease: Current evidence and practice. *World J Gastroenterol* 2019; 25:1307-
391 1326.

392 [16] Cipponeri E, Vitturi N, Mariano V et al. Vitamin D status and non-alcoholic fatty
393 liver disease in patients with type 1 diabetes. *J Endocrinol Invest* 2019.
394 <https://doi.org/10.1007/s40618-019-01031-8>

395 [17] Leitao J, Carvalhana S, Silva AP et al. No evidence for lower levels of serum
396 vitamin D in the presence of hepatic steatosis. A study on the portuguese
397 general population. *Int J Med Sci* 2018; 15:1778-1786.

398 [18] He X, Shen Y, Ma X et al. The association of serum FGF23 and non-alcoholic
399 fatty liver disease is independent of vitamin D in type 2 diabetes patients. *Clin*
400 *Exp Pharmacol Physiol* 2018; 45:668-674.

401 *[19] Cho YH, Kim JW, Shim JO et al. Association between vitamin D deficiency and
402 suspected nonalcoholic fatty liver disease in an adolescent population. *Pediatr*
403 *Gastroenterol Hepatol Nutr* 2019; 22:233-241.

404 This observational study examined the relationship between vitamin D deficiency and
405 NAFLD in 3,878 adolescents in the Korean National Health and Nutrition Survey. It
406 significantly found that adolescents with 'suspected NAFLD' based on elevated serum
407 alanine aminotransferase levels had lower 25(OH)D levels in comparison to
408 adolescents with normal serum levels of liver enzymes.

409 *[20] Konerman MA, Jones JC, Harrison SA. Pharmacotherapy for NASH: current and
410 emerging. *J Hepatol* 2018; 68:362-375.

411 This narrative review highlights the challenges and considerations in clinical trial
412 design for testing potential therapeutic agents for the treatment of non-alcoholic
413 steatohepatitis. and NAFLD. It is an excellent review of existing pharmacological

414 agents and their mechanism of actions that are currently in phase 2 and 3 clinical
415 trials for NAFLD. In addition it notably summarises, the efficacy of those that have
416 been more thoroughly studied (e.g.: vitamin E, pioglitazone, obetocholic acid,
417 elafibranor) and the significant placebo response observed in these trials.

418 *[21] Ilan Y. Future of Treatment for Nonalcoholic Steatohepatitis: Can the Use of Safe,
419 Evidence-Based, Clinically Proven Supplements Provide the Answer to the
420 Unmet Need? *Dig Dis Sci* 2018; 63:1726-1736.

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

425 *[22] Li X, Liu Y, Zheng Y et al. The effect of vitamin D supplementation on glycemic
426 control in type 2 diabetes patients: a systematic review and meta-analysis.
427 *Nutrients* 2018; 10:375.

428 This meta-analysis is significant for including all randomized controlled trials prior to
429 September 2017 that assessed the effects of oral vitamin D supplementation on
430 glycemic control in type 2 diabetes. Twenty trials administered vitamin D or placebo to
431 2703 participants were synthesized. Conclusions were that vitamin D supplementation
432 resulted in significant improvements to fasting insulin and homeostasis model
433 assessment of insulin resistance as well as serum 25(OH)D levels.

434 *[23] Mirhosseini N, Vatanparast H, Mazidi M et al. Vitamin D supplementation,
435 glycemic control, and insulin resistance in prediabetics: a meta-analysis. *J*
436 *Endocr Soc* 2018; 2:687-709.

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

443 [24] Foroughi M, Maghsoudi Z, Askari G. The effect of vitamin D supplementation on
444 blood sugar and different indices of insulin resistance in patients with non-
445 alcoholic fatty liver disease (NAFLD). *Iran J Nurs Midwifery Res* 2016; 21:100-
446 104.

447 [25] Keane JT, Elangovan H, Stokes RA et al. Vitamin D and the liver-correlation or
448 cause? *Nutrients* 2018; 10:496.

449 *[26] Tabrizi R, Moosazadeh M, Lankarani KB et al. The effects of vitamin D
450 supplementation on metabolic profiles and liver function in patients with non-
451 alcoholic fatty liver disease: A systematic review and meta-analysis of
452 randomized controlled trials. *Diabetes Metab Syndr* 2017; 11 Suppl 2:S975-
453 S982.

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

460 [27] Geier A, Eichinger M, Stirnimann G et al. Treatment of non-alcoholic
461 steatohepatitis patients with vitamin D: a double-blinded, randomized, placebo-
462 controlled pilot study. *Scand J Gastroenterol* 2018; 53:1114-1120.

- 463 [28] Shidfar F, Mousavi SN, Agah S et al. Reduction of some atherogenic indices in
464 patients with non-alcoholic fatty liver by vitamin D and calcium co-
465 supplementation: a double blind randomized controlled clinical trial. *Iran J*
466 *Pharm Res* 2019; 18:496-505.
- 467 [29] Lorvand Amiri H, Agah S, Tolouei Azar J et al. Effect of daily calcitriol
468 supplementation with and without calcium on disease regression in non-
469 alcoholic fatty liver patients following an energy-restricted diet: Randomized,
470 controlled, double-blind trial. *Clin Nutr* 2017; 36:1490-1497.
- 471 [30] Dabbaghmanesh MH, Danafar F, Eshraghian A et al. Vitamin D supplementation
472 for the treatment of non-alcoholic fatty liver disease: a randomized double blind
473 placebo controlled trial. *Diabetes Metab Syndr* 2018; 12:513-517.
- 474 [31] Taghvaei T, Akha, O., Mouodi, M., Fakheri, H.T., Kashi, Z., Maleki, I. &
475 Mohammadpour, R. Effects of vitamin d supplementation on patients with non-
476 alcoholic fatty liver disease (NAFLD). *Acta Medica Mediterranea* 2018; 34:415-
477 422.
- 478 [32] Barchetta I, Del Ben M, Angelico F et al. No effects of oral vitamin D
479 supplementation on non-alcoholic fatty liver disease in patients with type 2
480 diabetes: a randomized, double-blind, placebo-controlled trial. *BMC Med* 2016;
481 14:92.
- 482 [33] Sharifi N, Amani R, Hajjani E et al. Does vitamin D improve liver enzymes,
483 oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty
484 liver disease? A randomized clinical trial. *Endocrine* 2014; 47:70-80.
- 485 [34] Barchetta I, Cimini FA, Cavallo MG. Vitamin D supplementation and non-alcoholic
486 fatty liver disease: present and future. *Nutrients* 2017; 9:1015.
- 487 *This is a useful narrative review that expounds on the evidence in relation to vitamin
488 D dosing regimes in NAFLD.
- 489 [35] Sharifi N, Amani R. Vitamin D supplementation and non-alcoholic fatty liver
490 disease: a critical and systematic review of clinical trials. *Crit Rev Food Sci Nutr*
491 2019; 59:693-703.
- 492 *[36] Bahrami A, Sadeghnia HR, Tabatabaeizadeh SA et al. Genetic and epigenetic
493 factors influencing vitamin D status. *J Cell Physiol* 2018; 233:4033-4043.
- 494 This narrative review focused on four randomised controlled trials and two
495 uncontrolled clinical trials examining the effects of vitamin D supplementation on
496 patients with NAFLD and highlights conflicting results.
- 497 *[37] Gibson PS, Quaglia A, Dhawan A et al. Vitamin D status and associated genetic
498 polymorphisms in a cohort of UK children with non-alcoholic fatty liver disease.
499 *Pediatr Obes* 2018; 13:433-441.
- 500 This cross-sectional study is significant for being the first to examine the relationship
501 between vitamin D status, genetic variants known to affect vitamin D status and
502 NAFLD histological severity in a paediatric population. 103 biopsy-proven paediatric
503 patients with NAFLD had low vitamin D status throughout the year and significantly
504 lower 25(OH)D levels in winter.
- 505 [38] Beilfuss A, Sowa JP, Sydor S et al. Vitamin D counteracts fibrogenic TGF-beta
506 signalling in human hepatic stellate cells both receptor-dependently and
507 independently. *Gut* 2015; 64:791-799.
- 508 [39] Patel YA, Henao R, Moylan CA et al. Vitamin D is not associated with severity in
509 NAFLD: results of a paired clinical and gene expression profile analysis. *Am J*
510 *Gastroenterol* 2016; 111:1591-1598.

511 *[40] Arai T, Atsukawa M, Tsubota A et al. Association of vitamin D levels and vitamin
512 D-related gene polymorphisms with liver fibrosis in patients with biopsy-proven
513 nonalcoholic fatty liver disease. *Dig Liver Dis* 2019; 51:1036-1042.
514 This cross-sectional study of 220 patients with biopsy-proven NAFLD examined
515 select polymorphisms in vitamin D related genes in relationship to histopathological
516 severity, suggesting that the vitamin D receptor rs1544410 polymorphism was
517 associated with advanced liver fibrosis.

518 **[41] Wang N, Chen C, Zhao L et al. Vitamin D and nonalcoholic fatty liver disease:
519 bi-directional mendelian randomization analysis. *EBioMedicine* 2018; 28:187-
520 193.
521 This study is significant for being the first study to apply bi-directional mendelian
522 randomization (MR) to explore the causal relationship between 25(OH)D and
523 NAFLD. Examining a Chinese population with 9128 participants, although individual
524 single nucleotide polymorphisms associated with NAFLD; when polygenetic risk
525 scores were applied (for both vitamin D status and NAFLD) the authors concluded
526 no causal association between vitamin D and NAFLD.

527 [42] Dimou NL, Tsilidis KK. A Primer in Mendelian Randomization Methodology with
528 a Focus on Utilizing Published Summary Association Data. *Methods Mol Biol*
529 2018; 1793:211-230.

530 [43] Tripathi A, Debelius J, Brenner DA et al. The gut-liver axis and the intersection
531 with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; 15:397-411.

532 [44] Safari Z, Gerard P. The links between the gut microbiome and non-alcoholic fatty
533 liver disease (NAFLD). *Cell Mol Life Sci* 2019; 76:1541-1558.

534 [45] Schwenger KJP, Bolzon CM, Li C et al. Non-alcoholic fatty liver disease and
535 obesity: the role of the gut bacteria. *Eur J Nutr* 2018.

536 [46] Wang J, Thingholm LB, Skieceviciene J et al. Genome-wide association analysis
537 identifies variation in vitamin D receptor and other host factors influencing the
538 gut microbiota. *Nat Genet* 2016; 48:1396-1406.

539 [47] Sun J. Dietary vitamin D, vitamin D receptor, and microbiome. *Curr Opin Clin Nutr*
540 *Metab Care* 2018; 21:471-474.

541 [48] Jin D, Wu S, Zhang YG et al. Lack of vitamin D receptor causes dysbiosis and
542 changes the functions of the murine intestinal microbiome. *Clin Ther* 2015;
543 37:996-1009.e1007.

544 [49] Su D, Nie Y, Zhu A et al. Vitamin D signaling through Induction of paneth cell
545 defensins maintains gut microbiota and Improves metabolic disorders and
546 hepatic steatosis in animal models. *Front Physiol* 2016; 7:498.
547

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 ^x	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)

^x 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; G1: placebo (n=31) G2: 0.25 mg/d calcitriol (n=28) G3: 50,000 IU/wk cholecalciferol (n=32)	Ultrasound; 25(OH)D <70 nmol/L; 20-75	nr; Before: G1: 52.8±13.0 [†] , G2: 46.5±13.8 [†] , G3: 47.3±15.5 [†] After: G1: 47.0±17.5 [†] , G2: 57.3±49.5 [†] , G3: 80.5±35.3 [†]	Liver: ALT, AST, GTT, ALP Vitamin D: 25(OH)D	Liver: ns Vitamin D: 25(OH)D↑*
Geier et al., 2018[27]; Switzerland	48-week 2-arm RCT (pilot study); G1: placebo (n=10) G2: 4,000 IU/d vitamin D (n=8)	ALT and/or biopsy; 25(OH)D <70 nmol/L; 23-63	ECLIA; Before: G1: 50±25 [†] , G2: 52.5±30 [†] After: G1: 40±23 [†] , G2: 98±33 [†]	Liver: ALT, AST, GTT, ALP, NAS and CK-18 M30 Vitamin D: 25(OH)D	Liver: ALT↓*, CK-18 M30↓* Vitamin D: 25(OH)D↑*
Shidfar et al., 2019[28]; Iran	12-week 3-arm RCT; G1: placebo (n=36) G2: 1,000 IU/d vitamin D (n=37) G3: 500 mg calcium carbonate+1000 IU/d vitamin D (n=37); All three groups given some advice on physical activity, hypocaloric diet and sun-light exposure.	Ultrasound; 25(OH)D < 37.5 nmol/L; 18-65	ELISA; Before: G1: 25.00 ± 1.58 [†] , G2: 24.75 ± 1.60 [†] , G3: 24.75 ± 2.33 [†] After: G1: 27.50 ± 1.95 [†] , G2: 53.50 ± 1.83 [†] , G3: 67.75 ± 2.75 [†]	Liver: ALT, AST, ALP Vitamin D: 25(OH)D	Liver: ALT↓*, AST↓* Vitamin D: 25(OH)D↑*
Taghvaei et al., 2018[31]; Iran	12-week 2-arm RCT; G1: placebo (n=20) G2: 50,000 IU/wk vitamin D3 (n=20); Both groups were given lifestyle modification advice.	FibroScan®; 25(OH)D <70nmol/L; 30-70	ELISA; Before: G1: 49.45 ± 10.88 [†] , G2: 47.90 ± 13.73 [†] After: G1: 52.13 ± 6.23 [†] , G2: 86.00 ± 10.70 [†]	Liver: ALT, AST, ALP, CAP score and kPA Vitamin D: 25(OH)D	Liver: ns Vitamin D: 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; Sufficiency: ≥75 Insufficiency: 50-75 Deficiency: <50; 45.0 [17.5-97.5]‡	Vitamin D related: CYP2R1: rs1993116 rs10741657 DHCR7: rs7944926, rs12784878 GC: rs2282670 CYP27B1: rs10877012 VDR: rs2228570, rs1544410, rs7975232, rs731236	CYP2R1 rs1993116 genotype non-AA and VDR rs228570 genotype GG associated with VDD; 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; Deficiency: <25 Insufficiency: 25-50; Spring: 36.6 [30.5-42.1]* Summer: 41.8 [36.3-47.2]* Autumn: 40.8 [34.2-47.5]* Winter: 26.9 [22.7-31.2]*	Vitamin D related: NADSYN1: rs12785878, rs3829251 GC: rs2282670, rs7041, rs4588 CYP2R1: rs10741 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; nr; VD GRS: Quartile 1: 41.8 ± 12.9 [†] Quartile 2: 40.4 ± 12.3 [†] Quartile 3: 39.6 ± 12.5 [†] Quartile 4: 38.7 ± 11.9 [†] NAFLD GRS: Quartile 1: 40.2 ± 12.4 [†] Quartile 2: 40.3 ± 12.7 [†] Quartile 3: 40.0 ± 12.2 [†] Quartile 4: 40.0 ± 12.4 [†]	Vitamin D related: NADSYN1: rs12785878 [§] CYP2R1: rs10741657 GC: rs2282679 CYP24A1: rs6013897 NAFLD related: LYPLAL1: rs12137855 PPP1R3B: rs4240624 TM6SF2: rs58542926 PNPLA3: rs738409 GCKR: rs780094 SAMM50: rs738491 PARVB: rs5764455 COL13A1: rs1227756	GC rs2282679 and DHCR7 rs12785878 were associated with 25(OH)D; 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