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Meta-analysis: vitamin D and non-alcoholic fatty liver disease

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SUMMARY

Background
Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition. Emerging evidence suggests that vitamin D may play a role in the pathogenesis of NAFLD.

Aim
To review systematically the association between vitamin D levels, measured as serum 25-hydroxy vitamin D [25(OH)D], and NAFLD.

Methods
We used PubMed and EMBASE databases to identify all studies that assessed the association between vitamin D and NAFLD up until 22 April 2013, without language restrictions. We included studies that compared vitamin D levels between NAFLD cases and controls and also those that compared the odds of vitamin D deficiency by NAFLD status. Pooled standardised differences and odds ratios were calculated using an inverse variance method.

Results
Seventeen cross-sectional and case–control studies have evaluated the association between vitamin D and NAFLD. NAFLD was diagnosed using biopsy (4 studies), ultrasound or CT (10 studies) and liver enzymes (3 studies). Nine studies provided data for a quantitative meta-analysis. Compared to controls, NAFLD patients had 0.36 ng/mL (95% CI: 0.32, 0.40 ng/mL) lower levels of 25(OH)D and were 1.26 times more likely to be vitamin D deficient (OR 1.26, 95% CI: 1.17, 1.35).

Conclusions
NAFLD patients have decreased serum 25(OH)D concentrations, suggesting that vitamin D may play a role in the development of NAFLD. The directionality of this association cannot be determined from cross-sectional studies. Demonstration of a causal role of hypovitaminosis D in NAFLD development in future studies could have important therapeutic implications.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most common form of chronic liver disease in Western countries with a prevalence as high as 30%, already exceeding viral hepatitis and alcoholic fatty liver disease.[1] NAFLD comprises of a wide spectrum of liver damage ranging from simple steatosis to steatohepatitis (NASH), to fibrosis and cirrhosis that can progress to liver failure and hepatocellular carcinoma.[2] Although NAFLD is strongly associated with obesity, insulin resistance, diabetes and the metabolic syndrome, its pathogenesis is incompletely understood. Currently, the pathogenesis of NAFLD and NASH is framed in the 'multiple-hits hypothesis' where a number of diverse parallel processes involving extrahepatic factors (genetic and nutritional) may contribute to the development and progression of liver inflammation.[3]

Vitamin D is a fat-soluble vitamin formed in the skin from 7-dehydrocholesterol during exposure to solar ultraviolet B (UVB) radiation.[4] Although vitamin D can be derived from the diet, few foods naturally contain vitamin D, such as oily fish. Vitamin D from the skin or from diet is metabolised in the liver to 25-hydroxyvitamin D [25(OH)D], which is the major circulating metabolite and the most widely used indicator of vitamin D stores.[5] 25(OH)D is metabolised in the kidneys to the biologically active form $1,25(OH)_2D$, which exerts its functions through binding to its nuclear receptor (vitamin D receptor, VDR). Within the last two decades, VDR has been shown to be present not only in primary target tissues such as bone, kidney and intestine, but also in many other tissues, including the immune and endocrine systems, muscles, brain and liver, therefore expanding the role of vitamin D beyond the skeletal system.[6]

Numerous publications propose that low levels of vitamin D may contribute to the development of insulin resistance, the metabolic syndrome and more recently NAFLD.[7–9] Accordingly, there are reports to suggest increasing prevalence of vitamin D deficiency in parallel to the prevalence of obesity.[10] Although the mechanisms underlying the association of vitamin D with NAFLD are not yet fully understood, recent animal studies have shown that vitamin D has an important role in the regulation of oxidative stress, the production of pro-inflammatory cytokines,[11, 12] hepatocyte apoptosis[13] and even liver fibrosis.[14] As both diseases – NAFLD and vitamin D deficiency – are associated with insulin resistance, type 2 diabetes and cardiovascular disease, many studies have emerged over the past few years examining the relationship of vitamin D status, reflected by 25(OH) vitamin D level, with the development of NAFLD. To this end, we aimed to systematically review and quantify the association between vitamin D levels and NAFLD. We hypothesised that low vitamin D levels were associated with higher prevalence of NAFLD.

METHODS

Search strategy and study selection

We identified all studies that assessed the association between vitamin D and NAFLD in humans. We developed a search engine adapted for PUBMED and EMBASE up until 22 April 2013 (Table S1), with no language restrictions. In addition, we examined the reference lists of relevant original papers and review articles.

We included all studies that reported data on vitamin D levels and NAFLD or examined the association between vitamin D levels and NAFLD in multivariate models. We excluded papers without original data; animal or *in vitro* studies; studies that examined other types of liver disease (e.g. alcoholic liver disease, viral hepatitis, hepatocellular carcinoma, toxin-induced liver injury); and studies whose main focus were conditions primarily affected by vitamin D metabolism (end-stage renal disease, primary hyperparathyroidism) or medications that affect vitamin D metabolism like antiepileptic medications.

Data abstraction

Three investigators (ME, ES, NA) independently reviewed identified abstracts and selected papers for full review. Discrepancies were resolved by a fourth reviewer (RH). For each selected publication, we abstracted key study characteristics, including publication year, country, study design, participant characteristics (age, gender, ethnicity and body mass index), season, method of diagnosis of NAFLD and controls, serum vitamin D assay and the cut-off level for defining vitamin D deficiency. We assessed the quality of the reporting by adapting the *Strengthening the Reporting of Observational studies in Epidemiology* (STROBE) checklist[15] (Table S2), and followed the *Meta-analysis of Observational Studies in Epidemiology* (MOOSE) guidelines for the current meta-analysis.[16] All data were double checked by one investigator (ME).

Statistical methods

For studies that reported mean and standard deviation of vitamin D levels for NAFLD participants and controls,

we combined the standardised mean differences (SMD) using Hedge's adjusted g to correct for small sample bias in a random effects model.[17] For studies that reported dichotomous outcomes (vitamin D deficiency), we pooled the odds ratios (OR) using the inverse variance method with a random effects model. For studies that reported ORs for quartiles/tertiles of vitamin D levels, we obtained a single OR for each study by combining all odds ratios using a fixed effects model and the single OR as the measure for the association between low vitamin D and NAFLD in that study. For two studies that did not report measures of association between low vitamin D and NAFLD,[18, 19] we estimated the unadjusted odds ratio and 95% confidence interval (CI) by creating a 2 × 2 table of NAFLD and controls by vitamin D status (in absolute frequencies).

Recently published reports from Asia[20–23] suggest an ethnic difference between Asian and non-Asian populations in the association between vitamin D and NAFLD. This might be explained by allele frequency of vitamin D receptor (VDR) polymorphism between Chinese and Western populations[24]; therefore, we opted to stratify our analyses in Western (participants from USA, Italy, Canada and Israel), and Eastern (China and Korea) participants.

We assessed statistical heterogeneity with Cochran's *Q*-test and with the I^2 statistic. Publication bias was assessed using Harbord's regression test for funnel-plot asymmetry and Egger's test. All analyses were carried out using the commands metan and metabias, available in STATA 10 (College Station, TX, USA).

RESULTS

Our search identified 805 unique references of which 789 did not meet our inclusion criteria, resulting in 17 papers included for the systematic review. Reasons for exclusions are shown in Figure 1.

Of the 17 studies included,[18–23, 25–35] six originated in North America, four from Asia and the rest in Europe or Israel (Table S3). Six studies were conducted in general population settings, five in out-patient settings, one included both inpatients and out-patients, one only inpatients and two studies did not report the clinical setting.[25, 34] The mean age of the participants was similar amongst the studies with the exception of the study by Katz *et al.*,[29] which was conducted in adolescents. Not unexpectedly, those subjects with NAFLD had higher body mass index (BMI) and homeostasis model assessment-insulin resistance (HOMA-IR) indexes compared with subjects without NAFLD. Half of the studies reported participant ethnicity of which four studies were of Korean or Chinese population.

NAFLD was ascertained by liver biopsy in four studies,[19, 25, 32, 35] by imaging (ultrasound or CT) in ten studies[18, 20–23, 26–28, 33, 34] and by elevated ALT levels in three studies after excluding other causes of abnormal liver enzymes.[29–31] The cut-off level of 25(OH)D for vitamin D deficiency also varied across studies, ranging from 12 to 30 ng/mL; however, most of the studies that reported dichotomous outcomes used the value of 20 ng/mL as the cut-off level. In four studies, the analysis was conducted based on quartiles of vitamin D levels. Three studies did not report the cut-off level of vitamin D deficiency.[19, 25, 30]

Overall, most of the studies showed higher prevalence of vitamin D deficiency in NAFLD subjects compared with controls.[19, 22, 23, 26, 28, 33–35] In two studies,[18, 29] the association of vitamin D and NAFLD was not significant after adjustment for other covariates; and, two other studies[30, 31] showed an inverse association between vitamin D levels and presumed NAFLD (as evident by elevated ALT levels). Using histopathological results, two reports[25, 32] showed that low levels of vitamin D were associated with increased likelihood of fibrosis and inflammation and therefore progression to NASH.

Meta-analysis

Of the 17 studies included in the systematic review, 11 provided data to conduct the pooled analyses: 9 were used for the continuous vitamin D levels[19, 20, 22, 26, 28, 29, 31, 33, 35] (Table 1 and Figure 2) and 9 for the dichotomous exposure (vitamin D deficiency),[18, 20, 22, 23, 26, 28, 29, 31, 35] Figure 3. The reasons for exclusion were the presence of only NAFLD subjects in the study population in two studies[25, 32]; the evaluation of metabolic syndrome as the study outcome[30]; the lack of detailed data on vitamin D levels,[18, 21, 23] the publication of duplicate data from another study from the same investigators[34] and the restriction to hypertensive NAFLD participants that could introduce heterogeneity in the analyses.[27] The meta-analysis for the continuous levels of vitamin D by NAFLD status included 12 794 participants (4855 NAFLD cases and 7939 controls). On average, NAFLD patients had 0.36 ng/mL lower levels of 25(OH)D levels compared to controls (SMD: 0.36 ng/mL, 95% CI: 0.32,0.40 ng/mL) (I^2 99%, $P < 0.01$). Western NAFLD participants had lower levels of vitamin D compared with their Eastern NAFLD counterparts (Figure 2).

For the presence of vitamin D deficiency, nine studies totalling 5202 NAFLD participants and 8520 controls

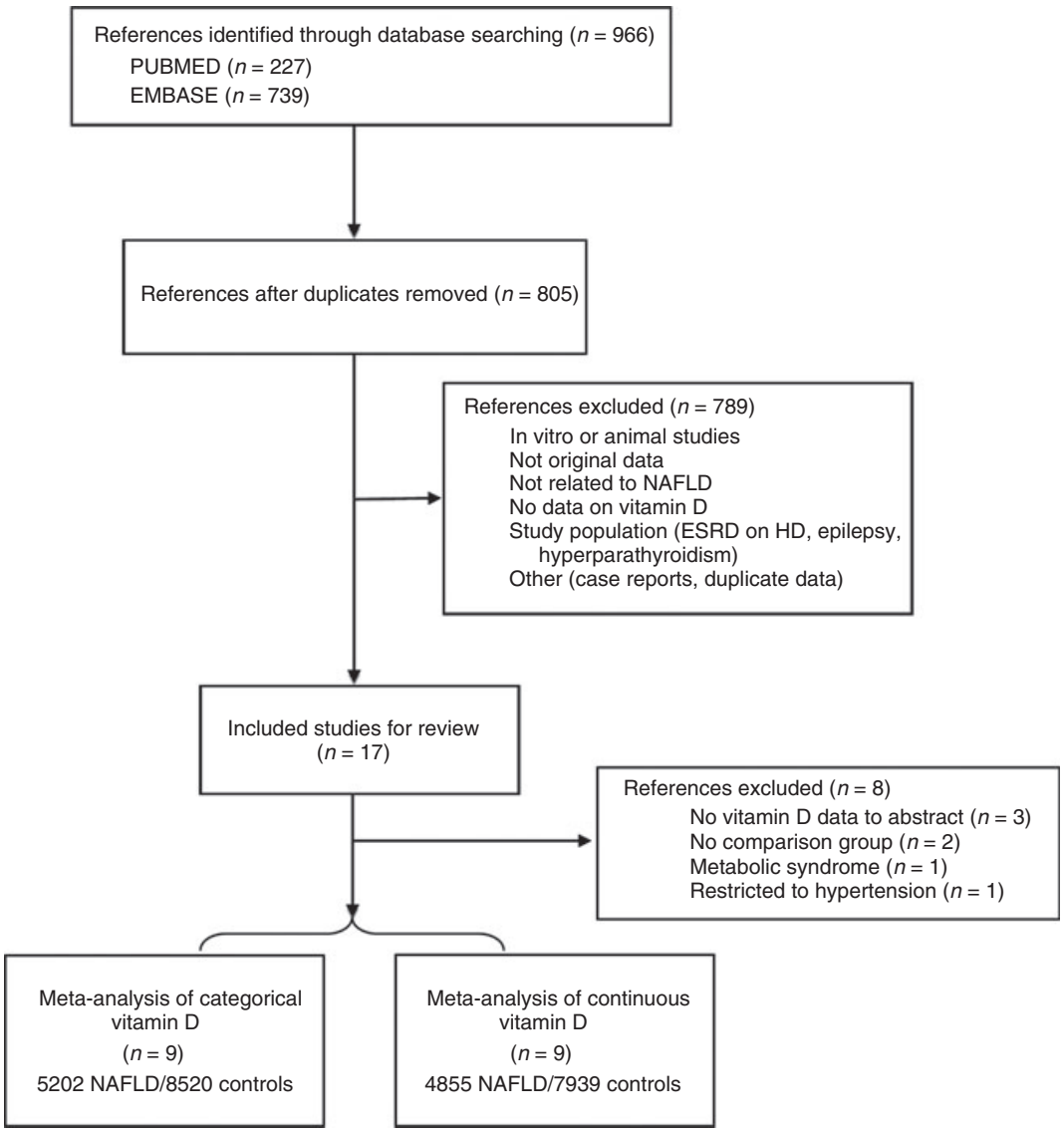


Figure 1 | Flow diagram of the study.

were included. NAFLD participants were 1.26 times more likely to have vitamin D deficiency (OR 1.26, 95% CI: 1.17, 1.35) compared to their controls. After stratifying for ethnic background, Western NAFLD participants were more likely to be vitamin D deficient as compared with their Eastern counterparts (Figure 3).

The exclusion of any individual studies did not markedly affect the overall measure of the association. In addition, different methods of ascertainment on the quantitative levels of vitamin D have not shown changes in the inferences. More specifically, in the dichotomous analysis (presence or absence of vitamin D deficiency), even after using different models of adjustment – from none in Barchetta and Foster[18, 26]; metabolic syndrome, insulin resistance and serum triglycerides in Liangpunsakul[31]; age, gender, race and poverty status in Katz[29] and finally, age, sex, BMI, calcium, creatinine, HOMA-IR and ATP III in Targher[35] – none of the positive associations between NAFLD and low vitamin D deficiency disappeared. We found no evidence of publication bias as evidence by Egger's test *P*-value ($P = 0.32$)

DISCUSSION

In this systematic review and meta-analysis of approximately 5000 NAFLD cases and 8000 controls, we found lower vitamin D levels in NAFLD subjects compared with controls (SMD 0.36 ng/mL, 95% CI: 0.32, 0.40). Moreover, NAFLD subjects were 26% more likely to be

Table 1 | Studies with data on vitamin D levels in NAFLD and controls, chronologically ordered

First Author, year (ref)	NAFLD (*N*)/ Total (*N*)	NAFLD, males,%	NAFLD, years (mean or range)	Assay method	Assay error (CV)	Season of collected samples	25(OH)D in NAFLD, ng/mL (mean ± s.d.)*	25(OH)D in controls, ng/mL (mean ± s.d.)*	*P*-value
Li, 2013[20]	378/1248	69	51	RIA	5.6–6%	May–July	22.1 ± 8.1	22.8 ± 8.4	0.21
Rhee, 2013[22]	2863/6567	100	42	EIA	NR	Model	15.5 ± 3.6	15.9 ± 3.9	<0.01
Jablonski, 2013[28]	607/1,214	26	56	RIA	<10%	NR	26 ± 2.9	29.2 ± 2.8	<0.01
Dasarathy, 2012[19]	87/119	NR	NR	NR	NR	NR	24 ± 10.7	28.3 ± 10.6	NR
Barchetta, 2011[26]	162/262	55	52	EIA	NR	NR	14.8 ± 9.2	20.5 ± 9.7	<0.01
Liangpunsakul, 2011[31]	308/1287	49	39	RIA	10–25%	NR	24.7 ± 10.4	26.8 ± 10.9	<0.01
Nseir, 2011[33]	247/347	45	53	EIA	NR	NR	22.9 ± 9.8	31 ± 6	<0.01
Katz, 2010[29]	143/1630	14	12-19	RIA	6.3–13.2%	NR	23.01 ± 1.39	25.6 ± 0.8	0.03
Targher, 2007[35]	60/120	67	47	EIA	9–12%	Winter	20.43 ± 8.81	29.84 ± 6	<0.01

RIA, radioimmunoassay; EIA, electrochemiluminescence assay; CV, coefficient variation.

* Vitamin D levels reported in ng/mL. Levels that were measured in SI units (nmol/L) were converted to ng/mL by dividing by 2.496.

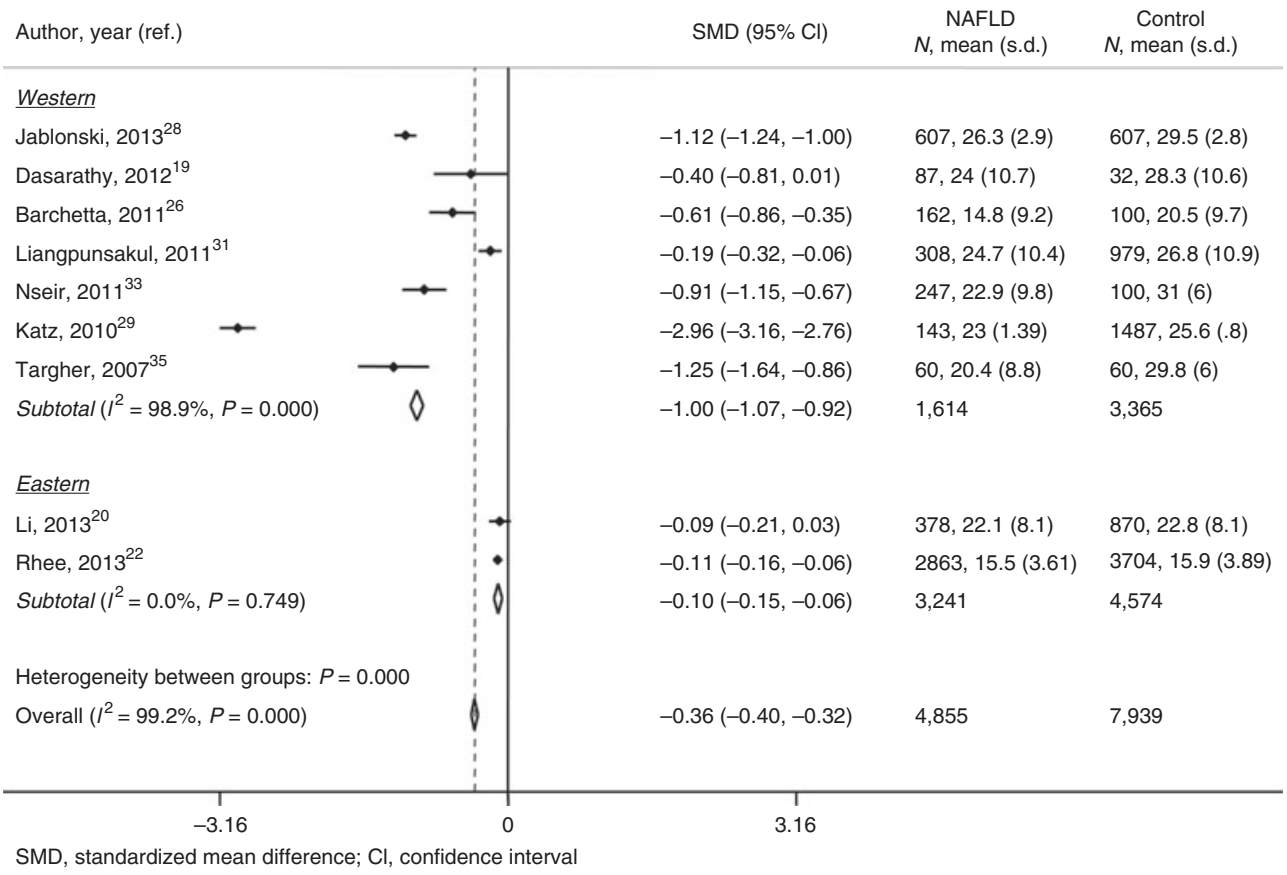
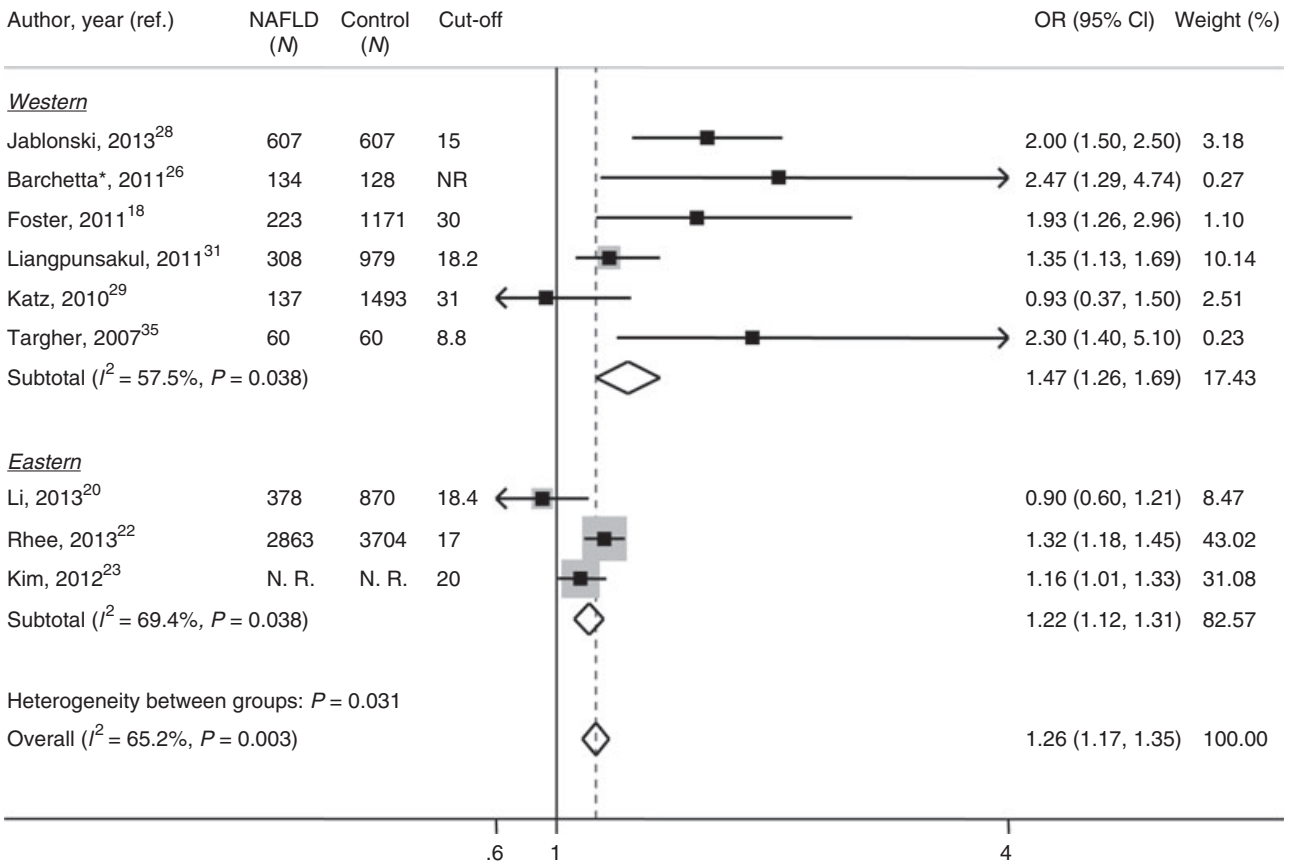


Figure 2 | Meta-analysis of studies reporting 25(OH)D levels in NAFLD vs. controls, standardised mean difference with 95% confidence interval, chronologically ordered.



Adjustments: none (Jablonski, Barchetta, Foster); age, BMI, blood glucose, total cholesterol, HDL-C, triglycerides, uric acid (Li); BMI, waist circumference, triglyceride, HDL-c, LDL-c, diabetes, hypertension (Kim); metabolic syndrome, insulin resistance, and serum triglycerides (Liangpunsakul); age, gender, race, and poverty status (Katz); age, sex, BMI, calcium, creatinine, HOMA-IR score and metabolic syndrome (Targher); N.R.: not reported
* Based on back-calculations obtained from Table 5 in the paper by Barchetta *et al.*[26]

Figure 3 | Meta-analysis of studies reporting dichotomous outcomes of 25(OH)D levels in NAFLD vs. controls and estimated ORs with 95% confidence interval, chronologically ordered.

vitamin D deficient (OR 1.26; 95% CI: 1.17, 1.35). These differences were higher when we stratified the analyses in Western vs. Eastern participants (Figures 2 and 3). Our results suggest that vitamin D levels are indeed low in patients with NAFLD and might be part of the pathogenesis of NAFLD.

Insulin resistance, a key risk factor in the pathogenesis of NAFLD, is linked to the development of oxidative stress and lipotoxicity.[36–38] It is now recognised that pro-inflammatory cytokines such as TNF-α, IL-6 and adipokines such as leptin and adiponectin play a major role in the progression from steatosis to steatohepatitis.[39] Moreover, the nuclear factor κ-B (NF-κB) pathway appears to be an important mediator between insulin resistance and hepatic inflammation.[40]

As already mentioned above, recent years have witnessed a significant scientific interest into the potential role played by vitamin D in the pathophysiology of insulin resistance and diabetes. A recent systematic review found that vitamin D levels >25 ng/mL were associated with a 43% lower risk of type 2 diabetes compared to vitamin D levels <14 ng/mL (95% CI 24, 57%).[41] In the same study, vitamin D treatment improved insulin resistance among patients with baseline glucose intolerance.[41] Similarly, another meta-analysis showed that vitamin D supplementation improved insulin resistance by 0.25 standard deviation (95% confidence interval 0.03–0.48, P = 0.03) compared to placebo.[42] The underlying mechanisms by which vitamin D may preserve glucose tolerance are thought to be related to effects on insulin secretion and sensitivity via the regulation of insulin receptor expression in pancreatic beta cells[43, 44] and in peripheral target tissues (including the liver)[45, 46] as well as via

improvement in systemic inflammation seen in insulin resistance.[47, 48]

Additional evidence from animal studies further supports the notion of an immunomodulatory role of vitamin D in NAFLD. In a recent study, rats fed a vitamin D-deficient Westernised diet had a higher NAFLD activity score on liver histology compared with those on the nonvitamin D-deficient Westernised diet; in addition, they had increased hepatic mRNA levels for resistin, IL-4, IL-6 and TNFα – markers known to be implicated in oxidative stress and hepatic inflammation.[12] Accordingly, in another rat NASH model, phototherapy elevated 25 (OH)D and $1,25(OH)_2D_3$ levels while reducing hepatocyte inflammation, fibrosis and apoptosis compared with controls. Phototherapy also improved insulin resistance and increased serum adiponectin in association with reduced hepatic expression of inflammatory genes TNF-α and TGF-β as well as α-smooth muscle actin (α-SMA) known to be a marker of hepatic stellate cell activation.[49] Furthermore, vitamin D significantly reduced free radical liver peroxidation substances and increased hepatic glucose uptake in a streptozotocin-induced diabetic rat model.[11] These findings suggest that vitamin D deficiency may exacerbate NAFLD at least in part via an inflammatory-mediated pathway.

Vitamin D mediates its intracellular signals via its receptor VDR, which is constitutively expressed in the liver.[50–52] VDR expression on cholangiocytes and hepatocytes from NAFLD subjects was inversely correlated with non-alcoholic fatty liver activity score.[53] In the same study, liver VDR expression was strongly associated with a diagnosis of NASH independently from other metabolic determinants such as BMI, insulin resistance or adiponectin; additionally, CYP27A1 and CYP2R1 expression – enzymes that catalyse the 25-hydroxylation of vitamin D – was preserved in NASH patients.[53] These observations therefore question the hypothesis of a loss of hydroxylation capacity of hepatocytes as a cause of NASH. Conversely, the finding of a direct inverse association of 25 $(OH)D_3$ levels with intra-hepatic ballooning in this study favours a possible hepatoprotective role of vitamin D.

The role of vitamin D in liver fibrosis has also been examined. In rat models of liver fibrosis, $1,25(OH)_2D_3$ – the active form of vitamin D – had protective antifibrotic effects and prevented the development of cirrhosis by thioacetamide (TAA).[54] Similarly, in another *in vitro* study of human hepatic stellate cells, $1,25(OH)_2D_3$ inhibited type I collagen formation, suggesting that correction of vitamin D deficiency could be a pathway to prevent fibrosis in chronic liver disease, including NAFLD.[14]

To our knowledge, this is the first meta-analysis to investigate the association of vitamin D levels with NAFLD. Our search method had no language or date restrictions and, by including EMBASE, we also incorporated grey literature accepted for scientific meetings, thus adding strength to our study. However, our study had several limitations. First, the method of NAFLD diagnosis varied across studies. Only a few studies used liver biopsy, which is considered the gold standard, while some studies used imaging techniques or elevated ALT levels as to identify NAFLD cases. However, based on results from a recent meta-analysis, ultrasound has been proven to be an accurate and reliable imaging technique for the detection of fatty liver disease with an overall sensitivity and specificity of 85% and 94% respectively.[55] Elevated ALT levels are also strongly associated with NAFLD in subjects with the metabolic syndrome,[56] and have been used in clinical and population studies as surrogate markers of NAFLD. The use of imaging and particularly of biochemical methods to assess NAFLD may also be associated with considerable measurement error and may substantially underestimate the association between vitamin D and NAFLD. Second, the cut-off level for defining vitamin D deficiency varied across studies, probably contributing to the heterogeneity in our findings. The normal range of vitamin D remains a controversial area of research and has been the subject of debate over the past several years. While some investigators recommend using 30 ng/mL as the cut-off level,[57] the most recent Institute of Medicine (IOM) committee report endorses the use of 20 ng/mL.[58] Third, many of the original studies did not adjust for potentially important confounders, such as BMI, presence of diabetes or season. Finally, there was evidence of statistical heterogeneity in our analysis, but the current studies suggest no major clinical heterogeneity and, therefore, we thought appropriate to provide the pooled analyses.

In conclusion, we have demonstrated that vitamin D deficiency is prevalent in NAFLD subjects, suggesting that vitamin D may play a role in the development of the disease. The anti-inflammatory and immune-modulatory properties of vitamin D provide plausible mechanisms by which vitamin D may impact on disease progression and severity in NAFLD. Due to the nature of the abstracted cross-sectional studies in our review, directionality of our results cannot be ascertained. Future research should focus on investigating prospectively the association between vitamin D and NAFLD as well as on randomised controlled trials of vitamin D supplementation in NAFLD subjects. Vitamin D is an inexpensive

intervention, well tolerated and widely available with minimal side effects. Hence, vitamin D supplementation may prove to be beneficial in the treatment of NASH in addition to vitamin E.

AUTHORSHIP

Guarantor of the article: Myrto Eliades.

Author contributions: Myrto Eliades, Elias Spyrou and Nidhi Agrawal performed the research. Mariana Lazo and Ruben Hernaez collected and analysed the data. Myrto Eliades and Ruben Hernaez designed the research study and wrote the paper. Frederick Brancati, James Potter, Ayman Koteish, Jean Clark and Eliseo Guallar contributed to the design of the study. All authors approved the final version of the manuscript.

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Declaration of personal and funding interests: None.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Search Engine up to April 22nd, 2013.

Table S2. Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist.

Table S3. Characteristics of observational studies assessing the association between vitamin D and NAFLD, ordered by year of publication.

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