

Original Article

Association between vitamin D and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: results from a meta-analysis

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Abstract: The prevalence and impact of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) have continued to increase in recent years. Previous reports have shown that hypovitaminosis D is associated with the prevalence and severity of non-alcoholic fatty liver disease (NAFLD). The aim of this study was to systematically evaluate the association of vitamin D levels, as measured by serum 25-hydroxy vitamin D [25(OH)D], with NAFLD and NASH. We searched all of the publications that assessed the association between vitamin D and NAFLD/NASH in the PubMed and EMBASE databases up to November 2014. In total, twenty-nine articles met the eligibility criteria, including twenty-seven studies about NAFLD and four studies about NASH, which were identified and included in the meta-analysis. Twenty-nine cross-sectional and case-control studies evaluated the association between vitamin D and NAFLD/NASH. Twenty-three studies provided data for a quantitative meta-analysis. Compared with the controls, the NAFLD patients had significantly lower levels of 25(OH)D (SMD-0.76; 95% CI-0.97 to -0.54) and were 1.26 times more likely to be vitamin D deficient (OR 1.26, 95% CI: 1.15 to 1.38). Compared with the controls, the NASH patients had significantly lower levels of 25(OH)D (SMD-1.30; 95% CI-2.37 to -0.23). Although the cross-sectional studies did not allow us to determine a causal nexus, our meta-analysis found lower serum 25(OH)D levels in NAFLD/NASH patients than in subjects without NAFLD/NASH, which suggests that hypovitaminosis D could play a role in the pathogenesis of NAFLD/NASH. Further studies are required to establish the causality between vitamin D status and NAFLD.

Keywords: Non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, vitamin D, meta-analysis

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathologic condition that covers simple fatty liver, steatohepatitis, fibrosis and cirrhosis and is observed in individuals with no evidence of excessive alcohol consumption [1]. Non-alcoholic fatty liver disease (NAFLD) has currently become the most common chronic liver disease worldwide, affecting approximately 20-35% of the adults in the general population [2]. Non-alcoholic steatohepatitis (NASH), which is the potentially evolutive variant of NAFLD, occurs in 10-20% of patients with NAFLD and could predispose them to cirrhosis and hepatocellular carcinoma (HCC) [3-5]. NAFLD is a hepatic manifestation of metabolic syndrome, which comprises type 2 diabetes, hyperten-

sion, insulin resistance (IR), obesity and dyslipidaemia [6].

Vitamin D plays a vital role in calcium and phosphorus homeostasis and is implicated in the modulation of immunologic function, hormone secretion, and cellular proliferation and differentiation [7]. Vitamin D is a fat-soluble vitamin that is synthesized in the skin by UV sunlight from 7-dehydrocholesterol. In the liver, vitamin D is metabolized by 25-hydroxylase (CYP2R1) being converted into 25-hydroxyvitamin D [25(OH)D], which is typically used to assess an individual's vitamin D status [8]. 25(OH)D is transported to the kidney, where it is metabolized by 1 α -hydroxylase converting to the biologically active form 1,25(OH)₂D. Vitamin D deficiency is widely considered to be serum 25(OH)

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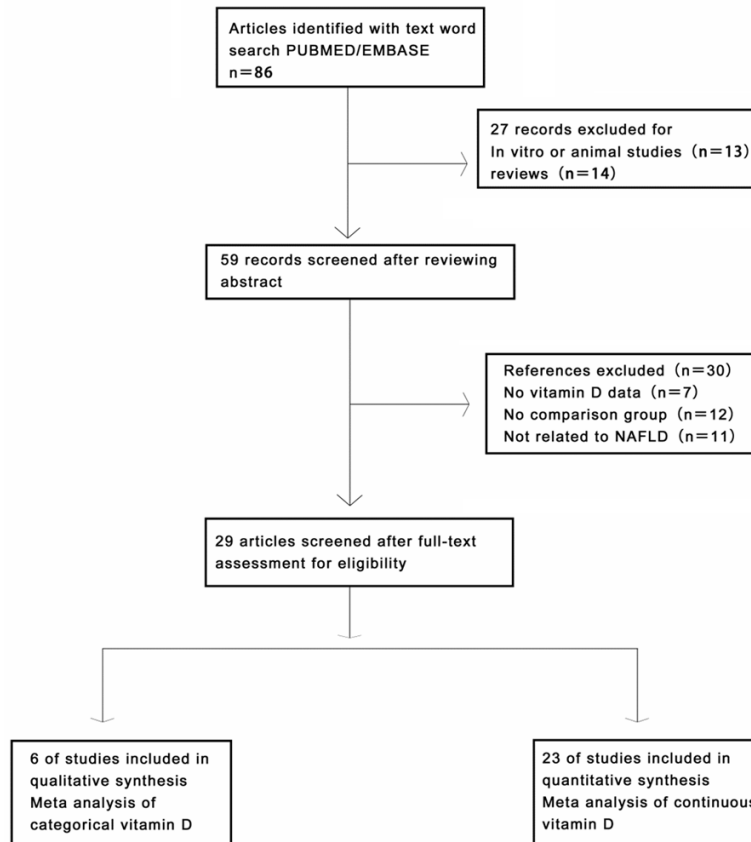


Figure 1. Flowchart that shows the process for the selection of studies in the literature.

D concentrations below 20 ng/ml (50 nmol/l), and insufficiency is considered to be serum 25(OH)D concentrations between 20 and 30 ng/ml (50-75 nmol/l). Vitamin D sufficiency is considered to be serum 25(OH)D concentrations of over 30 ng/ml (75 nmol/l) [9].

Several studies have demonstrated that Hypovitaminosis D could play an important role in the development of insulin resistance, obesity, hypertension, diabetes mellitus, NAFLD, metabolic syndrome, and cardiovascular disease. The relationship between vitamin D levels and NAFLD has been increasingly recognized [10-13]. A previous study confirmed that subjects with biopsy-proven NAFLD have lower serum 25(OH)D concentrations relative to control subjects, and importantly, it assessed the association between liver histology and vitamin D levels [14]. Another study indicated that there is an inverse association between low serum 25(OH)D levels and NAFLD, irrespective of age, sex, race, season of measurement, BMI, history of diabetes, renal disease, peripheral vascular

disease, liver diseases and hypertension [15]. These observations were further confirmed in children with obesity who had hepatosteatosis [16]. However, two Chinese population studies showed that Serum 25(OH)D concentrations were not obviously correlated with the prevalence of NAFLD [17, 18]. Thus, the role of vitamin D in the development of NAFLD/NASH remains controversial.

Based on the findings summarized above, we hypothesized that hypovitaminosis D was associated with a higher morbidity of NAFLD/NASH.

The aim of this study was thus to test our hypothesis with a meta-analysis of the association of vitamin D levels with NAFLD/NASH.

Methods

Search strategy

We identified all of the published articles that evaluated the association between vitamin D and NAFLD or NASH and that were restricted to humans. A literature search was conducted using PubMed and Embase up to November 2014 in the English language. The database searches were performed using the following keywords: (Vitamin D, vitamin d, 25-hydroxyvitamin D, 25 hydroxyvitamin d) and (fatty liver, NAFLD, non-alcoholic fatty liver disease, hepatic steatosis) or (NASH, nonalcoholic steatohepatitis). Two independent investigators performed the literature search, with any discrepancy resolved by mutual negotiation. Reference lists from relevant reviews were examined manually for additional relevant studies.

Inclusion and exclusion criteria

Studies were included if they met the following criteria: (i) Full text published in English; (ii) NAFLD or NASH diagnosed by histology or suggestive imaging features (ultrasound, computed tomography, magnetic resonance imaging),

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Table 1. Characteristics of studies on continuous outcomes of vitamin D levels in NAFLD and controls, chronologically ordered

| First author, year | NAFLD (N)/ Total (N) | NAFLD, males, % | NAFLD, years (mean or range) | 25(OH)D in NAFLD, (mean ± SD) | 25(OH)D in controls, (mean ± SD) | P-value |
|------------------------|-------------------------|--------------------|---------------------------------|----------------------------------|-------------------------------------|---------|
| Dasarathy, 2014 | 148/187 | 28 | 50 | 21.2 ± 10.4 ng/ml | 35.7 ± 6.0 ng/ml | <0.001 |
| Black, 2014 | 156/994 | 38 | 17 | 26.84 ± 8.81 ng/ml | 30.85 ± 9.62 ng/ml | <0.001 |
| Ku "c, u" kazman, 2014 | 154/211 | 43 | 46 | 12.3 ± 8.9 ng/dl | 20.3 ± 13.6 ng/dl | <0.0001 |
| Hao, 2014 | 76/514 | 100 | 54 | 13.46 ± 4.65 ng/ml | 15.65 ± 5.89 ng/ml | 0.002 |
| Yildiz, 2014 | 58/101 | 62 | 12 | 12.6 ± 6.52 ng/ml | 16.4 ± 9.19 ng/ml | 0.005 |
| Beilfuss, 2014 | 56/66 | 26 | 50 | 13.89 ± 0.91 ng/ml | 19.35 ± 1.8 ng/ml | NR |
| Li, 2013 | 378/1248 | 69 | 51 | 22.1 ± 8.1 ng/ml | 22.8 ± 8.4 ng/ml | 0.21 |
| Rhee, 2013 | 2863/6567 | 100 | 42 | 15.5 ± 3.6 ng/ml | 15.9 ± 3.9 ng/ml | <0.01 |
| Cui, 2013 | 119/224 | 39 | 60 | 45.71 ± 20.93 IU/L | 48.03 ± 15.73 IU/L | 0.35 |
| Pirgon, 2013 | 45/87 | 47 | 13 | 29.5 ± 18.4 ng/ml | 41.0 ± 17.9 ng/ml | <0.05 |
| Kasapoglu, 2013 | 338/613 | 23 | 53 | 14.6 ± 9.2 ng/ml | 26.4 ± 9.8 ng/ml | NR |
| Bhatt, 2013 | 162/335 | 80 | 38 | 19.4 ± 8.5 ng/ml | 27.8 ± 9.4 ng/ml | 0.0001 |
| Catena, 2013 | 4/24 | 42 | 53 | 18.11 ± 6.29 ng/ml | 19.27 ± 11.58 ng/ml | 0.33 |
| Jablonski, 2013 | 607/1214 | 26 | 56 | 26.04 ± 12.76 ng/ml | 29.25 ± 12.46 ng/ml | 0.0003 |
| Purnak, 2012 | 102/156 | 51 | 41 | 27.47 ± 7.64 ng/ml | 29 ± 7.83 ng/ml | NR |
| Dasarathy, 2012 | 36/68 | NR | NR | 26.9 ± 12.2 ng/ml | 28.3 ± 10.6 ng/ml | NR |
| Barchetta, 2011 | 162/262 | 55 | 52 | 14.8 ± 9.2 ng/ml | 20.5 ± 9.7 ng/ml | <0.001 |
| Nseir, 2011 | 247/347 | 45 | 53 | 22.9 ± 9.8 ng/ml | 31 ± 6 ng/ml | 0.001 |
| Assy, 2010 | 60/90 | NR | 50 | 13 ± 8 ng/ml | 31 ± 4 ng/ml | <0.001 |
| Barchetta, 2009 | 65/100 | NR | NR | 12.86 ± 7.73 ng/ml | 19.04 ± 8.81 ng/ml | 0.002 |
| Targher, 2007 | 60/120 | 67 | 47 | 20.43 ± 8.81 ng/ml | 29.84 ± 6 ng/ml | <0.001 |

Table 2. Characteristics of studies on continuous outcomes of vitamin D levels in NASH and controls, chronologically ordered

| First author, year | NASH (N)/ Total (N) | NASH, males, % | NASH, years (mean or range) | 25(OH)D in NASH, (mean ± SD) | 25(OH)D in controls, (mean ± SD) | P-value |
|--------------------|------------------------|-------------------|--------------------------------|---------------------------------|-------------------------------------|---------|
| Beilfuss, 2014 | 51/61 | 29 | 49 | 15.93 ± 1.03 ng/ml | 19.35 ± 1.8 ng/ml | NR |
| Bril, 2014 | 127/185 | 85 | 54 | 24.5 ± 2.1 ng/ml | 21.8 ± 1.0 ng/ml | 0.18 |
| Dasarathy, 2012 | 51/83 | NR | NR | 21.9 ± 9.0 ng/ml | 28.3 ± 10.6 ng/ml | NR |
| Barchetta, 2012 | 25/45 | 52 | 49 | 21.92 ± 12.3 ng/ml | 21.19 ± 4.42 ng/ml | NR |

NR, not reported; NASH: non-alcoholic steatohepatitis; SD: standard deviation.

and/or suspected NAFLD diagnosed by elevated ALT levels; (iii) Evaluated the association between Vitamin D and NAFLD/NASH; and (iv) Not limited by design. Studies were excluded if (i) Papers in the final form were not published in English; (ii) Participants were included with alcoholic, infectious (hepatitis B virus and hepatitis C virus), drug-induced, total parenteral nutrition-induced, or hereditary causes of liver injury; (iii) Non-human studies; (iv) Only included NAFLD/NASH individuals without controls; or (v) No adequate data for extraction.

Data extraction and quality assessment

The two researchers independently extracted the required information and reached agree-

ment on all of the items. Any discrepancies were eliminated by discussion with other researchers. The following variables were extracted from the selected studies: the first author's name, publication year, country of origin, participant characteristics (age, gender, ethnicity and body mass index), study design, diagnostic criteria of NAFLD/NASH, number of cases and controls. In cases where the published studies contained insufficient information, attempts were made to contact the corresponding authors to obtain missing data. The Newcastle-Ottawa scale (NOS) was adopted to assess the study quality. Studies that met at least five of the NOS criteria were considered high-quality studies.

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Table 3. Characteristics of studies on the association between vitamin D and NAFLD, ordered by year of publication

| First author, year (ref) | Country | Race/ethnicity | Study type | Setting | Method of NAFLD ascertainment | BMI in NAFLD (mean ± SD) | BMI in controls (mean ± SD) |
|--------------------------|-----------|--------------------------------------|-----------------|--------------------------|---|--------------------------|-----------------------------|
| Dasarathy, 2014 | USA | NR | Case control | Outpatient | Liver biopsy | 35.7 ± 7.0 | 25.5 ± 3.1 |
| Black, 2014 | Australia | Caucasian Non-Caucasian | Cross-sectional | General population | Ultrasound | 27.0 ± 7.4 | 22.0 ± 3.0 |
| Ku "c, u" kazman, 2014 | Turkey | NR | Case control | Outpatient | Ultrasound | 31.7 ± 7.6 | 31.9 ± 4.3 |
| HAO, 2014 | China | Chinese | Case control | General population | Ultrasound | 23.7 ± 1.1 | 22.6 ± 2.1 |
| Yildiz, 2014 | Turkey | Turkish | Case control | pediatrics clinic | Ultrasound | 30.9 ± 3.9 | 29.3 ± 4.4 |
| Beilfuss, 2014 | Germany | NR | Case control | Inpatient | Liver biopsy | 53.2 ± 1.2 | 22.5 ± 1.3 |
| Li, 2013 | China | Chinese | Cross-sectional | General population | Ultrasound | 26.3 ± 2.9 | 22.3 ± 2.9 |
| Rhee, 2013 | Korea | Korean | Cross-sectional | General population | Ultrasound | 26.2 ± 2.6 | 23.6 ± 2.4 |
| Cui, 2013 | China | Chinese | Cross-sectional | Inpatient | Ultrasound | 26.7 ± 4.8 | 23.9 ± 3.3 |
| Pirgon, 2013 | Turkey | Turkish | Case control | Inpatient | Ultrasound | 28.7 ± 4.7 | 28.4 ± 3.6 |
| Kasapoglu, 2013 | Turkey | NR | Cross-sectional | Outpatient | Ultrasound | 27.2 ± 2.8 | 26.3 ± 4.1 |
| Bhatt, 2013 | India | NR | Case control | Inpatient | Ultrasound | 28.1 ± 3.2 | 26.8 ± 3.2 |
| Catena, 2013 | Italy | NR | Cross-sectional | Outpatient | Ultrasound | 24.6 ± 3.5 | 24.1 ± 2.9 |
| Jablonski, 2013 | USA | White 90% Hispanic 8% Other 2% | Case control | Inpatient and outpatient | NAFLD defined by ICD 9 code AND abnormal findings on abdominal ultrasound | 33.4 ± 8.3 | 29.6 ± 7.3 |
| Purnak, 2012 | Turkey | NR | Case control | Inpatient and outpatient | Ultrasound | 28.6 ± 4.4 | 27.7 ± 4.1 |
| Dasarathy, 2012 | USA | NR | Case control | NR | Liver biopsy | NR | NR |
| Barchetta, 2011 | Italy | NR | Cross-sectional | Outpatient adult clinic | Ultrasound | 31.36 ± 5.49 | 25.87 ± 5.1 |
| Nseir, 2011 | Israel | NR | Case control | Hospital | Ultrasound | 33.0 ± 7.0 | 26 ± 3.0 |
| Assy, 2010 | Israel | NR | Case control | NR | Abdominal CT | NR | NR |
| Barchetta, 2009 | Italy | NR | Case control | NR | Ultrasound | NR | NR |
| Targher, 2007 | Italy | NR | Cross-sectional | Outpatient adult clinic | Liver biopsy | 26.3 ± 2.0 | 26 ± 2.0 |

NR, not reported; BMI was measured in kg/m²; ALT: alanine aminotransferase; CT: computerized tomography; NAFLD: non-alcoholic fatty liver disease; SD: standard deviation.

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Table 4. Characteristics of studies on the association between vitamin D and NASH, ordered by year of publication

| First author, year (ref) | Country | Race/ethnicity | Study type | Setting | Method of NASH ascertainment | BMI in NASH (mean ± SD) | BMI in controls (mean ± SD) |
|--------------------------|---------|---------------------------------|-----------------|--------------------|------------------------------|-------------------------|-----------------------------|
| Beilfuss, 2014 | Germany | NR | Case control | Inpatient | Liver biopsy | 53.1 ± 1.3 | 22.5 ± 1.3 |
| Bril, 2014 | USA | Caucasian Hispanic Others | Case control | General population | Liver biopsy | 34.6 ± 0.4 | 33.3 ± 0.6 |
| Dasarathy, 2012 | USA | NR | Case control | NR | Liver biopsy | NR | NR |
| Barchetta, 2012 | Italy | NR | Cross-sectional | Inpatient | Liver biopsy | 30.5 ± 5.5 | 35.8 ± 8.4 |

NR, not reported; BMI was measured in kg/m²; NASH: non-alcoholic steatohepatitis; SD: standard deviation.

Table 5. Meta-regression analyses of continuous outcomes of vitamin D levels

| Meta-regression | | Number of obs = 21 | | | | |
|---|-----------|------------------------|-------|-------|----------------------|-----------|
| REML estimate of between-study variance | | tau2 = .4606 | | | | |
| % residual variation due to heterogeneity | | I-squared_res = 94.54% | | | | |
| Proportion of between-study variance explained | | Adj R-squared = 45.06% | | | | |
| Joint test for all covariates | | Model F (2, 18) = 5.52 | | | | |
| With Knapp-Hartung modification | | Prob > F = 0.0135 | | | | |
| _ES | Coef. | Std. Err. | t | P > t | [95% Conf. Interval] | |
| CT | .8906265 | .9380717 | 0.95 | 0.355 | -1.080189 | 2.861442 |
| Ultrasound | 2.065151 | .8612496 | 2.40 | 0.028 | .2557326 | 3.874569 |
| _Cons | -2.571006 | .8381361 | -3.07 | 0.007 | -4.331864 | -.8101472 |
| Metareg _ES CT Liver biopsy Ultrasound, wsse (seES) bbest (reml) graph knapphartung | | | | | | |

Note: Liver biopsy dropped because of collinearity.

Statistical analysis

Meta-analysis was conducted using the Cochrane Collaboration RevMan 5.3 and STATA package version 13.1 (Stata Corporation, College Station, TX, USA). For studies that reported continuous variables (presented as the mean and standard deviation of vitamin D concentrations) for NAFLD/NASH patients and controls, we combined the standardized mean differences (SMD) using Hedge's adjusted *g* to adjust for a small sample bias. For studies that reported dichotomous variables (presented as OR with 95% CI of vitamin D deficiency), we pooled the odds ratios (OR) using the inverse variance method. A χ^2 -test-based *Q* statistic test at $P < 0.05$ and $I^2 > 50\%$ was performed to evaluate the between-study heterogeneity [19]. A fixed-effects model was used in the presence of $P \geq 0.05$ or $I^2 \leq 50\%$. Otherwise, a random effect model was used ($P < 0.05$ or $I^2 > 50\%$). Analysis of sensitivity was used to assess the stability of the outcomes by sequentially omit-

ting one study each time with the metaninf algorithm in STATA. Finally, any potential publication bias was investigated using a visual inspection of funnel plots, Begg's rank correlation test and Egger's regression test with the meta bias algorithm in STATA. A symmetric inverted funnel shape indicates the absence of publication bias; an asymmetric or incomplete funnel indicates the possible presence of publication bias. $P < 0.05$ was considered the cut-off for statistical significance.

Meta-regression analyses were conducted if there were over ten studies with significant heterogeneity (i.e., $I^2 > 50\%$), using the metareg algorithm in STATA.

Results

Our search identified 86 potentially eligible references, of which 27 were excluded after screening the titles and abstracts. The full text of 59 articles was retrieved for final review, and the references in the involved articles were screened. Altogether, 29 studies that met our inclusion criteria were included in the meta-analysis (Figure 1). The 29 included studies comprised 14 case-control studies and 15 cross-sectional studies (Tables 7 and 8). Tables 1-4, 6 demonstrate that 29 case-control and cross-sectional studies were involved in the analysis, of which 27 studies were used in the analysis of the association between vitamin D and NAFLD; 4 studies were used in the analysis of the association between vitamin D and

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Table 6. Characteristics of studies on the association between vitamin D and NAFLD, ordered by year of publication

| First author, year (ref) | Country | Race/ethnicity | Study type | Setting | Method of NASH ascertainment | BMI in NAFLD (mean ± SD) | BMI in controls (mean ± SD) |
|--------------------------|-----------|--------------------|-----------------|--------------------|------------------------------|--------------------------|-----------------------------|
| Malespin, 2014 | USA | Chinese | Cross-sectional | Outpatient | Elevated ALT | NR | NR |
| L, 2014 | Australia | Australian | Cross-sectional | General population | Ultrasound | NR | NR |
| Kim, 2013 | Korea | Korean | Cross-sectional | General population | Ultrasound | NR | NR |
| Seo, 2013 | Korea | Korean | Cross-sectional | General population | Abdominal CT | NR | NR |
| Foster, 2011 | USA | Caucasian | Cross-sectional | General population | Abdominal CT | NR | NR |
| | | African American | | | | | |
| | | Hispanics | | | | | |
| | | Asian | | | | | |
| Katz, 2010 | USA | Non-HispanicWhite | Cross-sectional | General population | Elevated ALT | NR | NR |
| | | Non-Hispanic Black | | | | | |
| | | Mexican American | | | | | |
| | | Other Hispanics | | | | | |
| | | Other Races | | | | | |

NR, not reported; BMI was measured in kg/m²; ALT: alanine aminotransferase, CT: computerized tomography; NAFLD: non-alcoholic fatty liver disease; SD: standard deviation.

Table 7. Newcastle-Ottawa Scale (NOS) assessment of the quality of the case-control studies

| Study | Selection | | | Comparability | | Exposure | | | Total scores |
|-----------------------|--------------------------|---------------------------------|-----------------------|------------------------|---|---------------------------|---|-------------------|--------------|
| | Case definition adequate | Representativeness of the cases | Selection of controls | Definition of controls | Comparability based on design or analysis | Ascertainment of exposure | Same method of ascertainment for cases and controls | Non-response rate | |
| Dasarathy, 2014 | ★ | ★ | ★ | ★ | | ★ | ★ | | 6 |
| Ku "c_u" kazman, 2014 | ★ | ★ | | ★ | ★★ | | ★ | | 6 |
| HAO, 2014 | ★ | ★ | ★ | ★ | | | ★ | | 5 |
| Yildiz, 2014 | ★ | ★ | ★ | ★ | ★★ | ★ | ★ | | 8 |
| Beilfuss, 2014 | ★ | ★ | | ★ | | | ★ | | 4 |
| Bril, 2014 | ★ | ★ | ★ | ★ | ★★ | | ★ | | 7 |
| Bhatt, 2013 | ★ | ★ | | ★ | ★ | | ★ | | 5 |
| Pirgon, 2013 | ★ | ★ | | ★ | ★★ | | ★ | | 6 |
| Jablonski, 2013 | ★ | ★ | ★ | ★ | ★★ | | ★ | | 7 |
| Purnak, 2012 | ★ | ★ | ★ | ★ | ★★ | | ★ | | 7 |
| Dasarathy, 2012 | ★ | ★ | ★ | ★ | | | ★ | | 5 |
| Nseir, 2011 | ★ | ★ | | ★ | ★★ | | ★ | | 6 |
| Assy, 2010 | ★ | ★ | ★ | ★ | ★ | | ★ | | 6 |
| Barchetta, 2009 | ★ | ★ | ★ | | | | ★ | | 4 |

NASH; 23 studies provided continuous data on the vitamin D levels (Table 1 and Figure 2) [14-18, 20-37]; and 6 studies provided dichotomous data (vitamin D deficiency) (Table 6 and Figure 5) [38-43].

The studies that were used to analyse the continuous vitamin D data for the NAFLD condition included 13524 participants (5896 NAFLD patients and 7628 controls). The NAFLD patients had significantly lower levels of 25(OH)D than did the controls (SMD -0.76; 95% CI -0.97 to -0.54) (I^2 95.6%, $P=0.000$). There was obvi-

ous heterogeneity in the middle of these studies (I^2 95.6%, $P=0.000$) (Figure 2). To evaluate the relationship between vitamin D and NAFLD, a subgroup analysis was conducted based on stratification on the geographic location and body mass index (Figures 2, 3). The NAFLD cases that originated from the western countries had significantly lower levels of vitamin D than did the NAFLD cases that originated from the eastern countries (Figure 2). The NAFLD cases with a BMI of ≥ 30 kg/m² had significantly lower levels of vitamin D than did the NAFLD cases with a BMI of <30 kg/m² (Figure 3).

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Table 8. Newcastle-Ottawa Scale (NOS) assessment of the quality of the cross-sectional studies

| Study | Selection | | | | Comparability | | Exposure | | Total scores |
|-----------------|--------------------------|---------------------------------|-----------------------|------------------------|---|---------------------------|---|-------------------|--------------|
| | Case definition adequate | Representativeness of the cases | Selection of controls | Definition of controls | Comparability based on design or analysis | Ascertainment of exposure | Same method of ascertainment for cases and controls | Non-response rate | |
| Black, 2014 | ★ | ★ | ★ | ★ | ★ | ★ | ★ | | 7 |
| Malespin, 2014 | ★ | ★ | | ★ | ★ | | ★ | | 5 |
| L, 2014 | ★ | ★ | ★ | ★ | ★ | ★ | ★ | | 7 |
| Li, 2013 | ★ | ★ | ★ | ★ | | ★ | ★ | | 6 |
| Rhee, 2013 | ★ | ★ | ★ | ★ | ★ | ★ | ★ | | 7 |
| Cui, 2013 | ★ | ★ | | ★ | ★ | | ★ | | 5 |
| Kasapoglu, 2013 | ★ | ★ | ★ | ★ | ★ | | ★ | | 6 |
| Kim, 2013 | ★ | ★ | ★ | ★ | | ★ | ★ | | 6 |
| Seo, 2013 | ★ | ★ | ★ | ★ | | ★ | ★ | | 6 |
| Catena, 2013 | ★ | ★ | ★ | ★ | | | ★ | | 5 |
| Barchetta, 2012 | ★ | ★ | | ★ | | | ★ | | 4 |
| Foster, 2011 | ★ | ★ | ★ | ★ | | | ★ | | 5 |
| Barchetta, 2011 | ★ | ★ | ★ | ★ | ★★ | | ★ | | 7 |
| Katz, 2010 | ★ | ★ | ★ | ★ | ★ | | ★ | | 6 |
| Targher, 2007 | ★ | ★ | ★ | ★ | ★★ | ★ | ★ | | 8 |

During the sensitivity analysis, the exception (omission) of any research did not change the magnitude and direction of the estimates (**Table 3**), which shows a relatively low sensitivity (**Figure 7**).

The studies on the continuous data of vitamin D with respect to the NASH condition included 374 participants (254 NASH patients and 120 controls). The NASH patients had significantly lower levels of 25(OH)D than did the controls (SMD -1.30; 95% CI -2.37 to -0.23) (I^2 94%, $P=0.02$) (**Figure 4**).

For the dichotomous data (vitamin D deficiency), the NAFLD cases were 1.26 times more likely to have vitamin D deficiency (OR 1.26, 95% CI: 1.15 to 1.38) (I^2 39.7%, $P=0.141$) compared with the controls. Subgroup analysis was conducted based on stratification on the geographic location. There was no significant difference between the NAFLD cases that originated from the western countries and the NAFLD cases that originated from the eastern countries (**Figure 5**). During the sensitivity analysis, the exception of any research did not change the magnitude and direction of the estimates (**Figure 8**), which shows a relatively low sensitivity.

In our study, univariate meta-regression analysis, with the covariates of study design, publica-

tion year, geographic locations, and diagnosis of NAFLD, revealed that the diagnosis of the NAFLD covariate (especially ultrasound-diagnosed NAFLD) had an obvious influence on the between-study heterogeneity (**Table 5**).

The asymmetry of the funnel plot demonstrated the existence of publication bias within studies to some extent (**Figure 6**). The P values for Begg's test and Egger's test were 0.239 and 0.001, respectively.

Discussion

In this systematic review and meta-analysis, 14 case-control studies and 15 cross-sectional studies were included. We found that NAFLD patients had significantly lower levels of 25(OH)D than did the controls (SMD -0.76; 95% CI -0.97 to -0.54) and that the NASH patients had significantly lower levels of 25(OH)D than did the controls (SMD -1.30; 95% CI -2.37 to -0.23). Moreover, the NAFLD cases were 1.26 times more likely to have vitamin D deficiency (OR 1.26, 95% CI: 1.15 to 1.38). These differences were significant when we stratified the analyses based on the geographic location and body mass index (**Figures 2 and 3**). Our findings demonstrate that there are lower levels of 25(OH)D in patients with NAFLD/NASH that might contribute to the development and progression of NAFLD/NASH.

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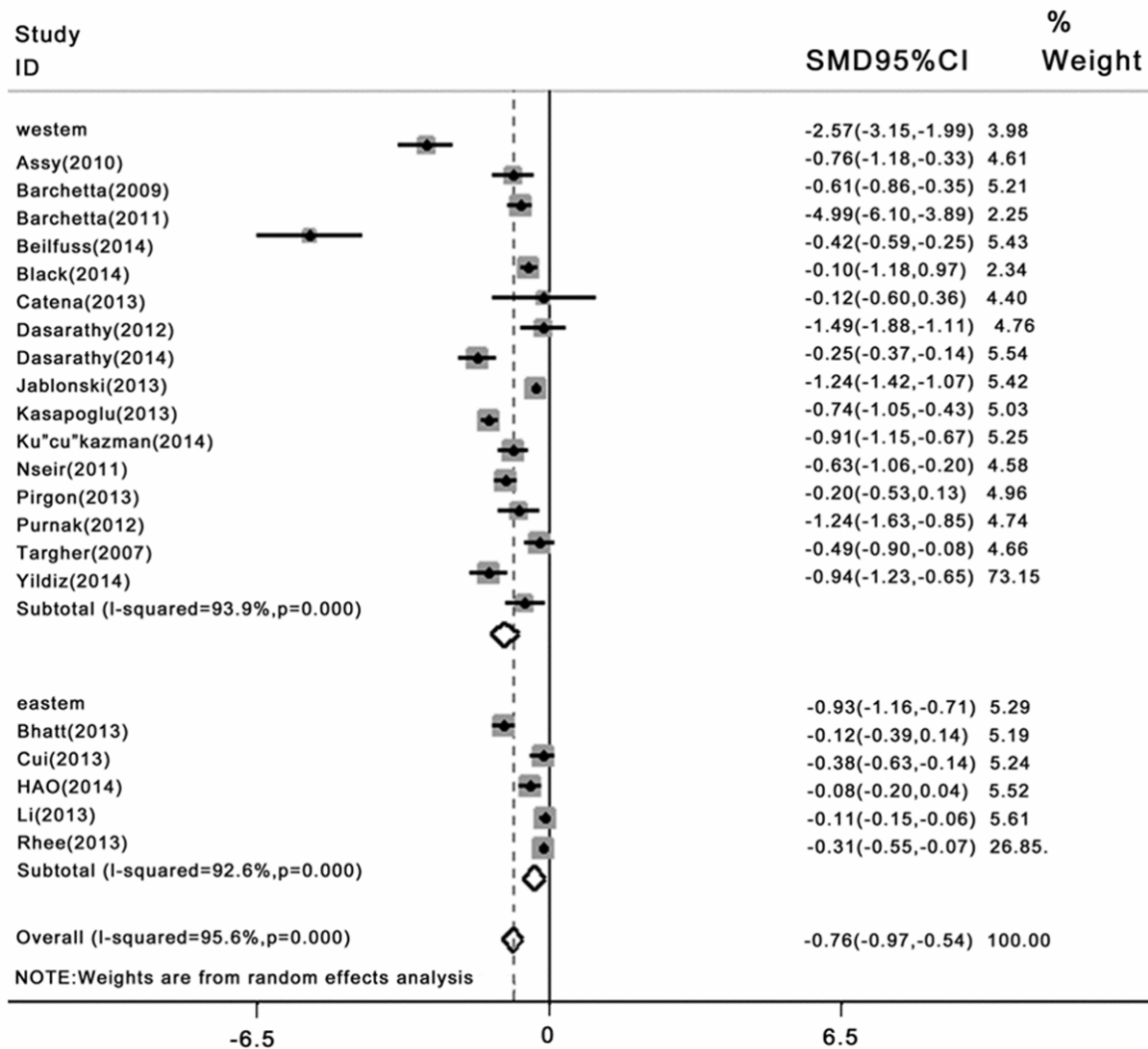


Figure 2. Meta-analysis of the association between vitamin D and NAFLD with a sub-analysis of NAFLD cases that originated from western countries vs. NAFLD cases that originated from eastern countries using a random-effects model and a standardized mean difference with a 95% confidence interval.

Non-alcoholic fatty liver disease (NAFLD), which is the most common cause of chronic liver disease in western nations, with a prevalence of 20-30%, is considered to be the hepatic manifestation of metabolic syndrome [44]. The association of serum vitamin D levels and NAFLD has been increasingly acknowledged. The inherent mechanisms that illustrate the relationship between low serum 25(OH)D levels and NAFLD are still not fully understood. Evidence from animal studies shows that vitamin D deficiency could be involved in the development of NAFLD through increased inflammation. A previous study demonstrated that obese rats that were fed a vitamin D-deficient Western diet deteriorated the development of NAFLD in

part due to increasing inflammation [45]. Additionally, another study showed that when serum 25(OH)D levels are elevated using phototherapy, the progression of NAFLD in the rat model is inhibited, as indicated by reduced hepatocyte inflammation, fibrosis and apoptosis [46]. These findings demonstrate that vitamin D deficiency might play an important role in the development of NAFLD, partly via inhibition of its anti-inflammatory properties. In addition, vitamin D directly modulates the metabolism of FFAs via its action on peroxisome proliferator-activated receptor (PPAR- γ), thereby relieving FFA-induced insulin resistance in vitro. Hence, the increased FFAs flowing in the bloodstream could promote fat deposition into the hepato-

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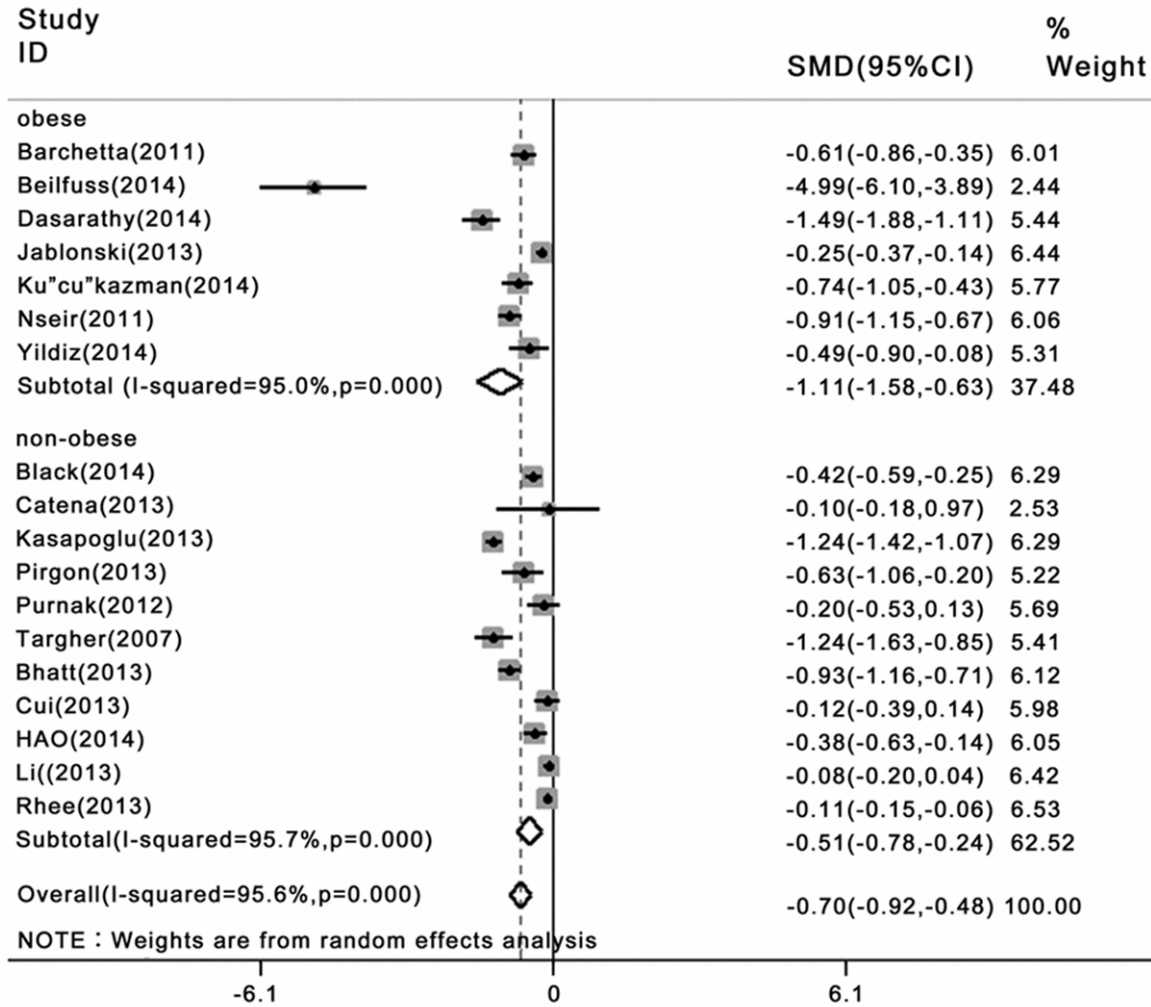


Figure 3. Meta-analysis of the association between vitamin D and NAFLD with the sub-analysis of NAFLD cases with a BMI ≥ 30 kg/m² vs. NAFLD cases with a BMI <30 kg/m² using a random-effects model and standardized mean differences with a 95% confidence interval.

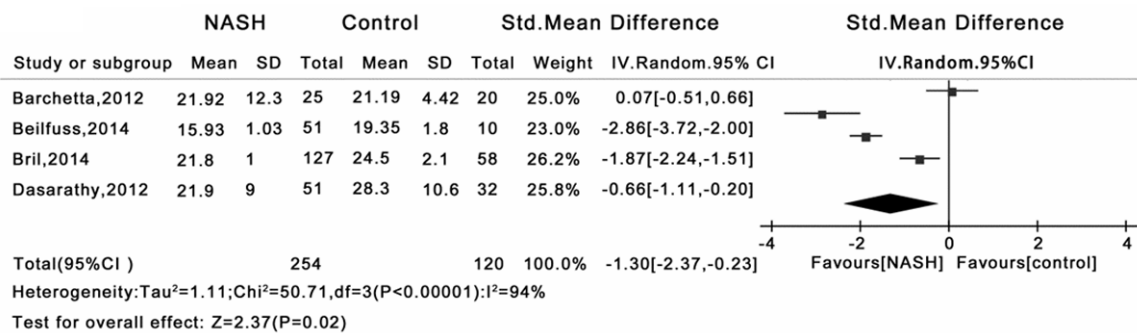


Figure 4. Meta-analysis of the association between vitamin D and NASH using a random-effects model and a standardized mean difference with a 95% confidence interval.

cyte and the progression of NAFLD under the condition of vitamin D deficiency [34].

The function of vitamin D in liver fibrosis has also been reviewed. Collagen deposition and

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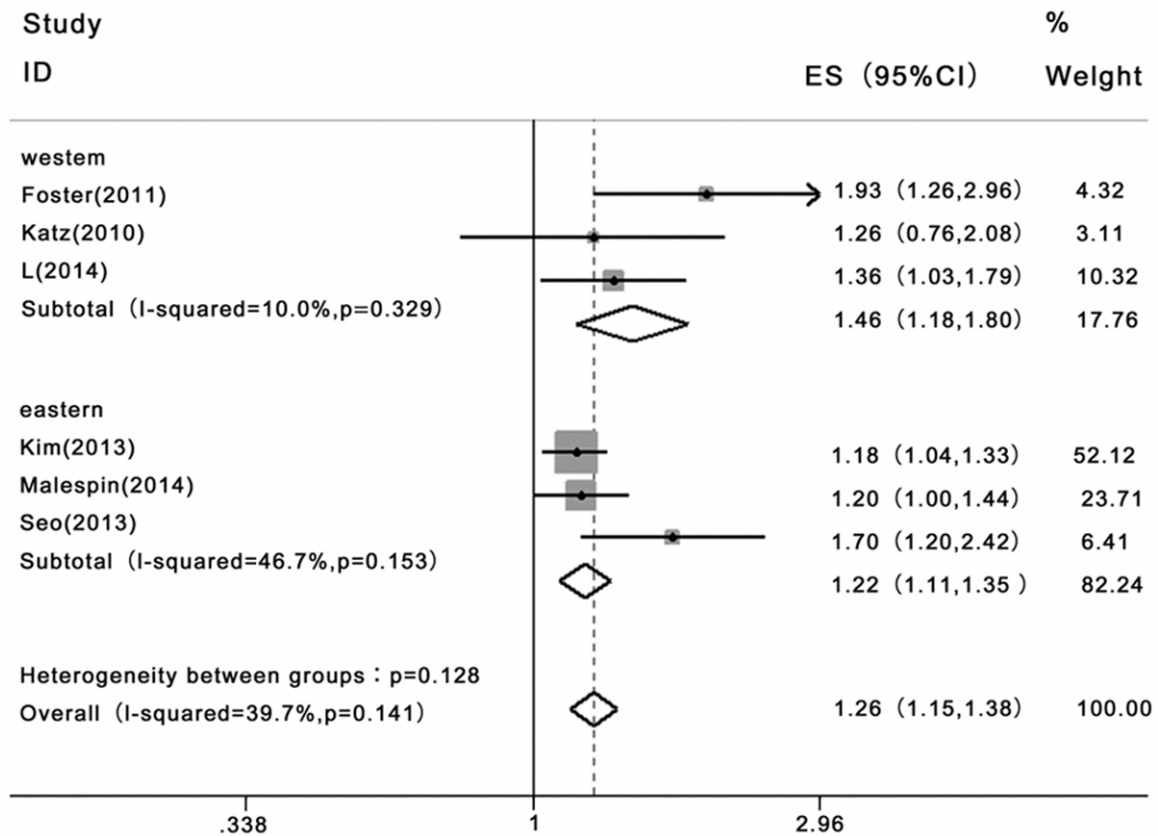


Figure 5. Meta-analysis of the association between lower levels of vitamin D and susceptibility to NAFLD using a fixed-effects model and estimated ORs with a 95% confidence interval.

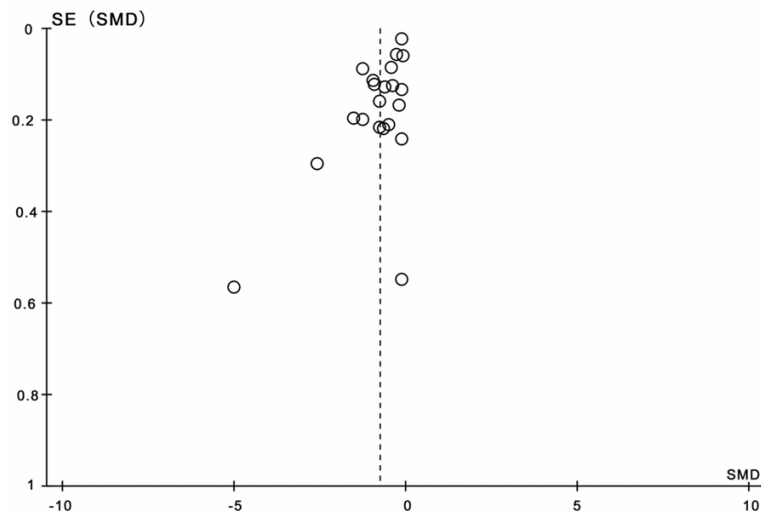


Figure 6. Funnel plot to detect publication bias.

fibrosis can be attributed to hepatic stellate cell (HSC) activation, which induces increased cellular proliferation and transformation into a myofibroblast-like cell, which in turn leads to

increased synthesis and deposition of extracellular matrix proteins, especially type I collagen [47]. Previous research has shown that the suppression of HSC proliferation by vitamin D was associated with antifibrotic effects in the murine model [48]. Another in vitro study demonstrated that even in the presence of FFAs, vitamin D supplementation can inhibit the activity of HSCs [49]. However, the therapeutic impact of vitamin D as an anti-fibrotic agent must be estimated.

Vitamin D through the vitamin D nuclear receptor (VDR) plays an important role in mineralion homeostasis [50]. Vitamin D receptor (VDR) expression on the parenchymal and inflammatory cells from the liver biopsies of patients with NASH

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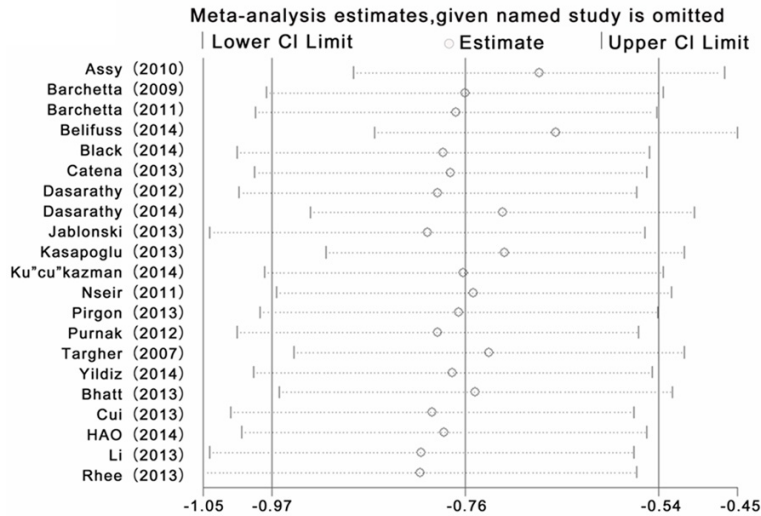


Figure 7. Forest plots of sensitivity of each included publication for continuous outcomes of vitamin D levels.

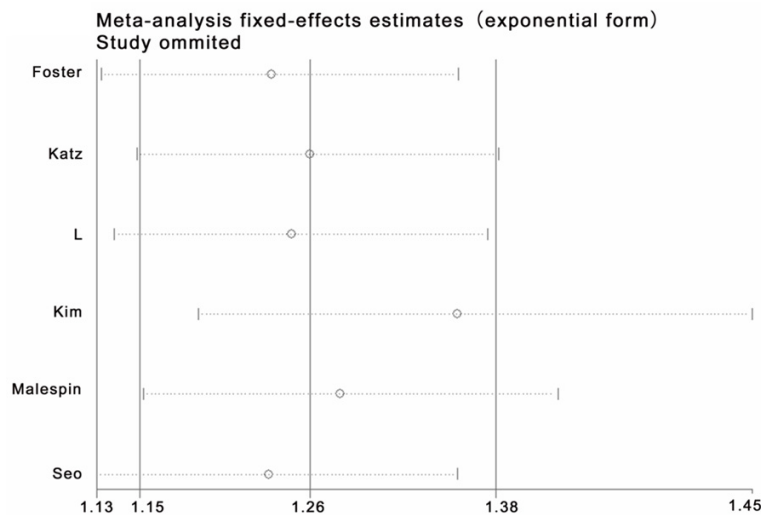


Figure 8. Forest plots of the sensitivity of each included publication for dichotomous outcomes of vitamin D levels.

has been determined [33]. Vitamin D receptor (VDR) gene polymorphisms that are involved in vitamin D synthesis and activation have been determined to be associated with vitamin D status and the severity of liver disease [51, 52]. There is a significant difference in the VDR polymorphisms (FokI, BsmI, ApaI, and TaqI) between Chinese and Western populations [53].

However, our study has some limitations. First, NAFLD was diagnosed and staged mostly by ultrasonography and not by biopsy, though biopsy is considered the gold standard for diagnosis. Ultrasonography is, in fact, a commonly

used method for assessing hepatic steatosis, but it is not the gold standard method for the quantitative assessment of liver fat content. The subjectivity of the ultrasonography is another problem because the value of the reported results is dependent on the operator. In fact, univariate meta-regression analysis demonstrated that the diagnosis of the NAFLD covariate (especially ultrasound-diagnosed NAFLD) made a great contribution to the between-study heterogeneity. The proper training of operators and the use of semi-quantitative ultrasonographic indices could contribute to overcoming this limitation. Second, the 25(OH)D levels can be influenced by UV light exposure, seasons, dairy products and lifestyles, which would most likely contribute to the heterogeneity in our results. Third, this study relates to cross-sectional and case-control design, and thus, the causative nature of the associations between low serum 25(OH)D levels and the presence of NAFLD cannot be determined.

In summary, our meta-analysis has shown that lower serum 25(OH)D levels are prevalent in NAFLD/NASH

patients, which suggests that hypovitaminosis D could play a role in the management of NAFLD/NASH. These findings provide the need for evaluating the role of vitamin D supplementation in the progression of NAFLD/NASH. Further prospective clinical studies and randomized controlled trials are needed to determine the causality between the vitamin D status and NAFLD.

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Disclosure of conflict of interest

None.

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