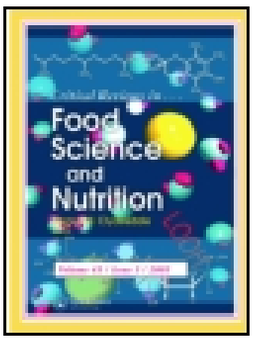

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Title: Vitamin D Supplementation and Non-alcoholic fatty liver disease: A Critical and Systematic Review of Clinical Trials

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Abstract

Previous observational studies have found a relationship between vitamin D deficiency and non-alcoholic fatty liver disease (NAFLD). However, this type of study could not show the causal relationship between these two conditions. Therefore, we systematically and critically reviewed the available clinical trials to elucidate such relationship. We searched databases such as Medline, Scopus and Cochrane to identify the clinical trials that assessed the effects of vitamin D supplementation in adults with NAFLD. The outcome variables of interest were indicators of hepatic steatosis, liver enzymes, insulin resistance, inflammation and oxidative stress. A total of 6 studies were included in the qualitative analysis. Only in two studies the grade of hepatic steatosis decreased significantly after vitamin D supplementation. The changes in insulin resistance parameters were reported significant only in one. Of the 3 included studies that measured biomarkers of inflammation and oxidative stress, one revealed a significant decrease in these biomarkers after vitamin D supplementation. Findings from current review study provided new insight into the factors that could affect the therapeutic role of vitamin D in NAFLD. Factors such as gender differences, baseline serum status of vitamin D, co-supplementation with calcium and gene polymorphism should be considered when designing future clinical trials.

Keywords: vitamin D, supplementation, Non-alcoholic fatty liver disease, clinical trials, systematic review

1. INTRODUCTION

Recent evidence indicates that vitamin D may exert immunomodulatory, anti inflammatory and antioxidant effect in both animal models and human (Zhu et al., 2017; Dankers et al., 2016; Eliades and Spyrou, 2015). What distinguishes vitamin D from other vitamins is its function as a hormone in the body. The biological roles of vitamin D are mediated via a nuclear vitamin D receptor (VDR) (Picciano, 2010). Following recent advances in cellular and molecular methods, it has been shown that VDR is expressed in various tissues of the human body (Altieri et al., 2017). Vitamin D only in its active form is able to bind to VDR (Picciano, 2010). The active form of vitamin D is produced in two steps in the body. First, vitamin D, originated from dietary sources or endogenously synthesized in the body following ultra violet radiation, undergoes 25-hydroxylation in the liver. Then, the enzyme1-α hydroxylase in the kidneys converts 25-hydroxyvitamin D (25 (OH) D) to the active form 1,25-dihydroxyvitamin D [1,25(OH)2D] (Holick, 2007; Prosser and Jones, 2004). Interestingly, it has been found that in addition to the kidneys, the cells of other tissues such as pancreas β cells, macrophages and immune cells also produce 1-α hydroxylase, resulting in the local production of $1,25(OH)_2D$ in corresponding tissues (Dankers et al., 2016; Takiishi et al., 2012). This evidence would support the proposed roles of vitamin D in various diseases, such as different types of infectious and autoimmune diseases as well as cancers, diabetes and metabolic syndrome (Adams and Hewison, 2010).

The results of some previous studies showed a relationship between vitamin D deficiency (VDD) and metabolic syndrome's components such as insulin resistance and dyslipidemia (Challoumas, 2014; Hyppönen et al., 2008; Pittas and Dawson-Hughes, 2010). This relationship proposed a new hypothesis that vitamin D deficiency might be associated with non-alcoholic fatty liver disease (NAFLD) as a hepatic feature of metabolic syndrome. NAFLD occurs when triglyceride is accumulated in hepatic cells due to metabolic causes other than excessive alcohol use(Clark et al., 2002). The spectrum of disease includes simple steatosis, non-alcoholic steatohepatitis (NASH) and liver cirrhosis (Farrell and Larter, 2006). Evidence from previous cellular and experimental studies has suggested various mechanisms by which vitamin D deficiency could be connected to NAFLD pathogenesis. Vitamin D might improve insulin secretion and function (Maestro et al., 2003; Maestro et al., 2002). Additionally, extensive VDR expression in non-parenchymal liver cells like macrophages, Kuppfer cells, and hepatic stellate cells (HSC) represents this fact that vitamin D could exert anti-inflammatory, anti fibrotic and anti-proliferative properties (Zúñiga et al., 2011; Geier, 2011). As a result, vitamin D might prevent NAFLD progression from simple steatosis to more advanced form of steatohepatitis and fibrosis, independently from other risk factors such as BMI (Eliades and Spyrou, 2015). Furthermore, vitamin D would have beneficial effects on NAFLD through modulation of adipokines such as adiponectin (Abenavoli et al., 2016; Nakano et al., 2011).

The first cross-sectional study that evaluated the association between vitamin D deficiency and NAFLD was the study by Targher et al in 2007 (Targher et al., 2007).They found that low serum levels of 25 (OH) D were independently associated with severity of hepatic injuries in NAFLD patients(Targher et al., 2007). From that time up to the present, the results of many case-control

and cross-sectional studies regarding the association between vitamin D deficiency and NAFLD have been published (Barchetta et al., 2011; Nelson et al., 2016; Wang et al., 2016a). Furthermore, the results of a recent meta-analysis that systematically evaluated case-control and cross-sectional studies, revealed that the risk of vitamin D deficiency in NAFLD patients was 1.26 times more than healthy controls (OR 1.26, 95% CI: 1.17, 1.35)(Eliades et al., 2013). However, the observational studies, especially the cross-sectional ones cannot derive the causal relationship between vitamin D deficiency and NAFLD. Meanwhile, whether vitamin D deficiency is one of the causes of NAFLD or the result of physiological and metabolic disturbances involved in the disease is not yet clear. The best study design that can clarify such relationship is clinical trial. If the findings of well-designed clinical trials indicate no effect of vitamin D supplementation on NAFLD, it can be concluded that the results of previousobservational studies that found the association between vitamin D deficiency and NAFLD could probably be due to confounding factors. As a result, in present study, we critically and systematically reviewed the clinical trials that evaluated the effects of vitamin D supplementation on NAFLD, aiming to develop hypotheses that can integrate findings.

2. Materials and Methods

2.1 Search strategy

A systematic literature search was performed for randomized controlled trials (RCTs), non-randomized interventions and uncontrolled clinical trials (UCTs), reporting the effects of vitamin D supplementation on hepatic steatosis, liver enzymes, and/or insulin sensitivity in adults with NAFLD, including non-alcoholic steatohepatitis but no cirrhosis or hepatocellular carcinoma.

It was considered sufficient for studies to provide their own diagnostic criteria based on one or more of the following: (1) histological examination of biopsies; (2) proton magnetic resonance spectroscopy (MRS); (3) computed tomography (CT); (4) ultrasound; and/or (5) blood concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

The primary outcomes of interest included changes in hepatic steatosis assessed by liver biopsy, MRS, CT, or ultrasound, and histologicalindicators of inflammation and fibrosis. Blood ALT and/or ASTconcentrations were also considered. The secondary outcomes were glucose tolerance and/or insulin sensitivity assesseddirectly by insulin clamp techniques or oral glucose tolerancetests, or inferred by validated formulasuch as the homeostasis model assessment of insulin resistance (HOMA-IR). The other secondary outcome included inflammatory and oxidative stress biomarkers.

A systematic literature search was performed independently by two authors (RA and NS), from inception to April 31- 2017, to identifyand appraise studies of vitamin D supplementation in NAFLD.

The following databases were searched: Medline (Pubmed), Scopus, Cochrane and Google Scholar. The selected search terms included: (NAFLD or ‘‘nonalcoholic fatty liver’’ or ‘‘non-alcoholic fatty liver’’ or ‘‘nonalcoholic steatohepatitis’’ or ‘‘non-alcoholic steatohepatitis’’or ‘‘nonalcoholic steatosis’’ or‘‘non-alcoholic liver steatosis’’ or ‘‘non-alcoholic steatosis’’ or ‘‘non-alcoholic hepatic steatosis’’ or ‘‘nonalcoholic hepatic steatosis’’ or ‘‘nonalcoholic liver steatosis’’) AND (“vitamin D” or “25-hydroxyvitamin D” or “25(OH)D” or “cholecalciferol” or “ergocalciferol” or “calcitriol”).

2.2 Inclusion and exclusion criteria

Two researchers also independently determined studies eligible for review. Included studies employed trials involving vitamin D supplementation versus placebo, vitamin D supplementation and calcium supplementation versus calcium alone and/or placebo.Additional inclusion criteria were: (i) the study population consisted of patients with NAFLD and/or NASH; (ii) supplementation of vitamin D2 (ergocalciferol) or vitamin D3 (cholecalciferol) for intervention; (iii) Hepatic steatosis (assessed by liver biopsy, MRS, CT, or ultrasound), liver enzymes and histologicalindicators of inflammation and fibrosis had to be a primary or secondary outcome; (iv) the study performed in subjects≥18 years; (vi) published in English. Studies were excluded if they used 1,25-dihydroxyvitamin D and studies performed in patients with alcoholic, drug-induced, total parenteral nutrition-induced, viral or genetic causes of liver injury. The study types such as case-reports, review, commentary or editorial were not included. Bibliographic references of searched articles were also examined to look for additional studies.

2.3 Data items

The items that were considered from each report included: study design; country; sex; age; diagnostic criteria for NAFLD/NASH; randomization and blinding; comparability of groups at baseline; type and dose of vitamin D supplementation; baseline and changes in serum 25(OH)D; definitionof subject adherence; intervention protocol; reported adherence; assessment the dietary intakes andphysical activity; assessment of hepatic steatosis; serum ALT and/or AST concentrations; measures of glycemia and insulin resistance parameters; inflammatory and oxidative stress biomarkers.

2.4 Data extraction

Data extraction from individual studies was performed separately by the authors and any differences in data extraction were discussed between them and resolved.Itemized tables were used to record relevant data from the reports. To facilitate comparison between studies, results were converted to same units and values of changes from baseline converted to percentages.

2.5 Study quality assessment

The methodological quality of the articleswas assessed by both authors. A modified Downs and Black's checklist provided a standardized scale to assess the quality of both randomized and non-randomized controlled trials (Downs and Black, 1998).This checklist with its items has been presented in Supplementary Table 1. This checklist has several categories: reporting, external validity, internal validity (bias), internal validity (confounding), and power. Each category generated a score; the scores were then summed for a total quality score. In the modified checklist, scores greater than or equal to 20 are considered high quality studies, scores between 15 and 19 and scores of 14 and below are considered fair and poor, respectively.In thepresent study, the population for checklist items was defined as participants aged ≥18 diagnosed with NAFLD or NASH. Potential confounders (item 5) were age, gender, BMI, season, baseline dietary and biochemistry values, change in dietary intake/exercise and medications. For item 27, the study power was adjusted to score using a 3-option scale. The scores of 1 or 2 were given if a study mentioned conducting power analysis to determine the sample size neededto detect a significant difference in effect size for one or more outcome measures, respectively. The score of

0 was given if the study did not report power analysis. As a result, the total score of the Downs and Black checklist changed from 32 to 29points (Supplementary Table 1: Modified Downs and Black Checklist).

To evaluate the quality of uncontrolled clinical trial (UCT),we used the *Quality Assessment Tool for Before-After (Pre-Post) Studies with no Control Group*that consists of 11 'yes/no' items. Each item receives one point for each affirmative answer (Supplementary Table 2).

3. RESULTS

A total of 122 articles were identified using the search of databases. Of these articles, 110 were excluded after screening the abstract because these papers did not clearly meet the criteria or the full texts were not available. The full texts ofthe remaining 12 articles were reviewed, with 6 publications excluded because they did not meet the inclusion criteria (Figure 1). Six articleswere included in the final review(four RCTs and two UCTs studies) with a total of 330 patients(Barchetta et al., 2016; Foroughi et al., 2014; Kitson et al., 2016; Lorvand Amiri et al., 2016; Papapostoli et al., 2016; Sharifi et al., 2014).

The included articles were individually scored for their methodological quality using the two aforementionedchecklists. The average score across 4RCTswas 22/29 on the modified Downs and Black's checklist(Downs and Black, 1998)(Table 1). The reduced quality across studies could be due to inadequate reporting of randomization, blinding, loss to follow-upor controlling for confounding variables by statistical analyses. The results of quality assessment for two UCTs were presented in Table 2(Kitson et al., 2016; Papapostoli et al., 2016).

3.1 Exposure measurements

The form of serum vitamin D that was measured in all of the included studies was 25(OH)D. The method for serum assessment of vitamin D was ELISA, chemiluminescence and colorimetric assay. The cut off for deficiency was defined as <20 ng/mL for 25(OH)D, however, only in one study the values lower than 15 ng/mL of serum vitamin D was set as inclusion criteria(Lorvand Amiri et al., 2016).The prevalence of vitamin D deficiency was 65% (70 out of 108 participants)among patients with NAFLD that had participated in 2 RCTs in which vitamin D deficiency was not among the inclusion criteria of them(Barchetta et al., 2016; Sharifi et al., 2014).

Averaging the results of the studies revealed that serum levels of 25(OH) D increased by 125% after vitamin D supplementation. The form of the vitamin D that was used in all these studies was cholecalciferol (vitamin D_3).

Vitamin D supplementation interval was different between the studies. Participants in two studies (Barchetta et al., 2016; Lorvand Amiri et al., 2016) received vitamin D supplements in daily basis whereas, in four studies(Foroughi et al., 2014; Kitson et al., 2016; Papapostoli et al., 2016; Sharifi et al., 2014), supplementation was done at weekly intervals.

The duration of vitamin D supplementation ranged from 10 to 24 weeks among the reviewed studies. Only Lorvand Amiri et al., evaluated the effects of vitamin D supplementation, with and without calcium, on biochemical and metabolic parameters in patients with NAFLD (Lorvand Amiri et al., 2016).

3.2 Outcomes measurements

3.2.1 Effects of vitamin D on hepatic steatosis and liver enzymes

All 6 studies had evaluated the effects of vitamin D supplementation on hepatic steatosis in patients with NAFLD. The method for assessing the changes in liver fat was ultrasound imaging in 3 studies (Foroughi et al., 2014; Lorvand Amiri et al., 2016; Sharifi et al., 2014). In a pilot study by Kitson et al., liver biopsy was used to detect histopathological changes in patients with NASH who received vitamin D supplementation(Kitson et al., 2016). In one study, transient elastography was applied to assess the changes in hepatic steatosis and fibrosis(Papapostoli et al., 2016). In addition to ultrasound, cytokeratin 18 and transforming growth factor β1 (TGF-β1) were evaluated as non-invasive biomarkers of NAFLD by Barchetta et al. and Sharifi et al, respectively (Barchetta et al., 2016; Sharifi et al., 2014).Of the six included studies, only in Lorvand-Amiri et al. and Papapostoli et al., the grade of hepatic steatosis decreased significantly after vitamin D supplementation(Lorvand Amiri et al., 2016; Papapostoli et al., 2016). However, the study by Papapostoli et al. was an uncontrolled clinical trial (Papapostoli et al., 2016).

All six researches measured serum concentrations of liver aminotransferases (ALT and AST).Serum levels of ALT and AST significantly decreased after vitamin D supplementation in two studies (Lorvand Amiri et al., 2016; Papapostoli et al., 2016) . In Lorvand Amiri et al. study, vitamin D supplementation, both alone and in combination with calcium, significantly lowered the serum levels of aminotransferases in NAFLD patients with vitamin D deficiency (Lorvand Amiri et al., 2016).

3.2.2 Effects of vitamin D on insulin resistance, oxidative stress and inflammatory biomarkers

Glucose metabolism parameters were measured in 5 studies, however, only in one study, their changes were significant (Lorvand Amiri et al., 2016). Lorvand Amiri et al. found that daily supplementation with 1000 IU vitamin D both alone and in combination with calcium could significantly decrease HOMA-IR and serum fasting blood glucose (FBG) in patients with NAFLD (Lorvand Amiri et al., 2016). Particularly, the decrease was more pronounced in the study group that received calcium with vitamin D supplements (Table 3).

The inflammatory biomarkers that were assessed as secondary outcomes in most studies were high-sensitive C-reactive protein (hs-CRP) and tumor necrosis factor α (TNF-α). Among the 3 studies that measured serum hs-CRP (Barchetta et al., 2016; Foroughi et al., 2014; Sharifi et al., 2014) , only the results of study by Sharifi et al. showed a near significant decrease in this biomarker post- supplementation(Sharifi et al., 2014).

The oxidative stress biomarkers were measured in one of the six reviewed studies(Sharifi et al., 2014). The results of Sharifi et al. study revealed that supplementation with vitamin D could significantly decrease serum levels of malondialdehyde (MDA), a lipid peroxidation biomarker, in patients with NAFLD(Sharifi et al., 2014). However, the change in serum total antioxidant capacity was not significant when compared to the control group(Sharifi et al., 2014).

3.2.3Effects of vitamin D on adiponectin and pro-fibrotic biomarkers

The obtained results from three studies, that measured serum levels of adiponectin, did not demonstrate any significant changes in this adipokine after vitamin D supplementation (Barchetta et al., 2016; Kitson et al., 2015; Sharifi et al., 2014). Transforming growth factor β1 (TGF-β1) and procollagen III propeptide were evaluated as biomarkers of liver fibrosis by Sharifi et al. and Barchetta et al., respectively (Sharifi et al., 2014; Barchetta et al., 2016). However, they did not find significant changes in these biomarkers post-supplementation.

3.3 Confounding Variables

Age, sex, body mass index, percent of body fat, dietary intakes, physical activity and seasonal variation were determined from the literature as potential confounders of the association between vitamin D and NAFLD. Out of the six studies, three adjusted for age and sex (Barchetta et al., 2016; Lorvand Amiri et al., 2016; Sharifi et al., 2014), five for BMI(Barchetta et al., 2016; Foroughi et al., 2014; Kitson et al., 2016; Lorvand Amiri et al., 2016; Sharifi et al., 2014), three for percent of body fat(Kitson et al., 2016; Lorvand Amiri et al., 2016; Sharifi et al., 2014), three for dietary intakes and two for physical activity(Foroughi et al., 2014; Lorvand Amiri et al., 2016; Sharifi et al., 2014). Only in two studies the effect of seasonal variation on variable changes was examined by comparing data collected in different seasons(Papapostoli et al., 2016; Sharifi et al., 2014). Also, Barchetta et al. compared the used medications between cases and controls as a confounding variable(Barchetta et al., 2016). Foroughi et al. reported overall data

regarding sex and age but did not elaborate on the statistical significance of these data when compared the groups at baseline(Foroughi et al., 2014).

4. DISCUSSION

For the first time, present study reviewed critically and systematically the available clinical trials that have evaluated the effects of vitamin D supplementation on hepatic steatosis, liver enzymes, insulin resistance and biomarkers of inflammation and oxidative stress in patients with NAFLD. In four studies, vitamin D supplementation did not significantly affect liver steatosis.Only in two studies the grade of hepatic steatosis and liver enzymes decreased significantly post-supplementation(Lorvand Amiri et al., 2016; Papapostoli et al., 2016). Lorvand-Amiri et al. conducted a 12-week RCT in 120 NAFLD patients that randomly assigned to receive 1000 IU vitamin D_3 (n = 37), 500 mg calcium carbonate plus 1000 IU vitamin D3 (n = 37), or placebo (n = 36) every day following a weight-loss program. At the end of the study, vitamin D supplement, both alone and in combination with calcium, significantly improved the grade offatty liver (measured by ultrasound imaging) and serum concentrations of aminotransferases(Lorvand Amiri et al., 2016). The study design of Lorvand-Amiri et al. differs from that of the other reviewed trials in some ways (Lorvand Amiri et al., 2016). They included patients with NAFLD who had vitamin D deficiency (serum levels < 15 ng/mL) and moderate dietary calcium intake (700-800 mg/d)(Lorvand Amiri et al., 2016). Moreover, the participants followed a 500-kcal energy deficit diet throughout the intervention. These factors, along with vitamin D supplementation, might have an impact on the resultant improvement in the grade of fatty liver. Some evidence suggests that the most beneficial effects of a nutrient supplementation occur

during the process of restoring its reserves in the body(Heaney, 2008). In other words, giving additional amounts of vitamin D to participants who already have sufficient serum levels might not produce expected outcomes(Heaney, 2012). The study by Papapostoli et al., that found a decrease in the grade of hepatic steatosis after vitamin D supplementation, also included NAFLD patients with vitamin D deficiency (Papapostoli et al., 2016). However, low sample size and the lack of control group were the limitations of their study. On the other hand, Barchetta et al. performed a post-hoc analysis to examine the hypothesis that vitamin D supplementation could exert beneficial effects on hepatic steatosis only in patients affected by hypovitaminosis D. However, the obtained results did not confirm this hypothesis (Barchetta et al., 2016).

It should be noted that some of the indirect biological effects of vitamin D are mediated through its role in regulating the homeostasis of the calcium ion in the body (Major et al., 2009; Pittas et al., 2007). Therefore, the amount of calcium intake might influence the effects of vitamin D on metabolic profiles and hepatic steatosis in patients with NAFLD. The dietary intake of calcium was reported only in the studies by Lorvand-Amiri et al. and Sharifi et al (Lorvand Amiri et al., 2016; Sharifi et al., 2014). Because these two clinical trials differed in study design, it is not possible to interpret and compare their results based on theparticipants' baseline calcium intake. To examine the hypothesis that baseline dietary intake of calcium could influence the effects of vitamin D on primary or secondary outcomes in patients with NAFLD, it is needed to design a well-powered randomized controlled trial with groups of participants with different levels of calcium intake.

Applying different methods to assess hepatic steatosis and fibrosis could influence the results. Four clinical trials in our review used ultrasound to diagnose and measured the degree of

steatosis. It should be noted that ultrasound method subjectively measures hepatic steatosis and could not detect any liver histological changes as precisely as detected by liver biopsy(Saleh and Abu-Rashed, 2007). Liver biopsy remains the gold standard. However, its use is restricted in population-based studies and large clinical trials as a result of cost and ethical issues(Saleh and Abu-Rashed, 2007). Only Kitson et al. applied liver biopsy to evaluate the impact of high-dose oral vitamin D_3 supplement (25 000 IU/week) over 24 weeks on liver histology and metabolic profile in 12 non-cirrhotic patients with NASH(Kitson et al., 2016). Their findings showed no impact of vitamin D supplementation on NAFLD activity score and liver biochemistry, insulin resistance or adipocytokine profile (Kitson et al., 2016). Low sample size and lacking control group were among the limitations of their study.

Among the newer non-invasive imaging methods, magnetic resonance spectroscopy (MRS) is an accurate and quantitative method for NAFLD diagnosis(Cobbold et al., 2012). None of the included studies used MRS as a method of monitoring the changes in liver steatosis. Transient elastography (Fibroscan) has also recently applied for detecting and quantifying steatosis, by using an index named *Controlled Attenuation Parameter* (CAP)(de Ledinghen et al., 2010). This technique is able to detect and quantify the hepatic steatosis even when the level of accumulated fat is as low as 10% (Dowman et al., 2011). In this review, Papapostoli et al. used transient elastography to measure hepatic steatosis (Papapostoli et al., 2016). They included 40 patients with significant liver fat accumulation (CAP value $\geq$ 280 dB/m). The patients received 20,000 IU vitamin D/week for six months. At the end of study, restoration of serum vitamin D levels was accompanied by significant reduction in CAP values relative to baseline(Papapostoli et al.,

2016). However, as the author indicated, the lack of control group was the main limitation of their study.

Measuring the non-invasive serum biomarkers of NAFLD along with imaging techniques can help increase the quality and accuracy of the study results (Dowman et al., 2011; Fitzpatrick and Dhawan, 2014). Barchetta et al. conducted a double-blind, placebo-controlled trial to assess the beneficial effects of oral vitamin D_3 (2000 IU/day) or placebo for six months in type 2 diabetic patients with NAFLD(Barchetta et al., 2016). To measure the changes in severity of NAFLD, they considered serum levels of cytokeratin 18 M-30 (marker of hepatocytes apoptosis)(Fitzpatrick and Dhawan, 2014), procollagen III propeptide (marker of liver fibrosis), transaminases and fatty liver index (FLI) as primary outcomes in addition to the monitoring the hepatic steatosis by magnetic resonance imaging (MRI). They concluded that oral vitamin D supplementation over 24 weeks did not improve hepatic steatosis and metabolic parameters in type 2 diabetic patients with NAFLD. Of note, this clinical trial has received one of the highest scores regarding methodological quality in present systematic review.

Insulin resistance (IR) is one of the important key factors in development and progress of NAFLD(Dowman et al., 2010). Most of the included studies did not demonstrate any significant changes in IR or other glucose metabolism parameters after vitamin D supplementation in NAFLD patients. However, only in one research, daily supplementation with 1000 IU vitamin D for 12 weeks, both alone and in combination with calcium, could significantly decrease HOMA-IR and fasting serum glucose in patients with NAFLD(Lorvand Amiri et al., 2016).Additionally, the decrease was more pronounced in the study group that received calcium in addition to vitamin D supplements(Lorvand Amiri et al., 2016). This finding might confirm the proposed

mechanism that vitamin D could improve insulin secretion and action indirectly via regulating extracellular calcium and calcium flux(Pittas et al., 2007). In other words, co-supplementation of vitamin D and calcium may have synergistic effects on insulin secretion and action. On the other hand, to better elucidate the effect of vitamin D on insulin resistance. Barchetta et al. included NAFLD patients with type 2 diabetes (Barchetta et al., 2016). Although they used a higher dose of vitamin D supplement and longer duration of intervention than those of the clinical trial by Lorvand-Amiri et al., they did not find any significant changes in insulin resistance or sensitivity indices(Barchetta et al., 2016; Lorvand Amiri et al., 2016). Previous studies also reported conflicting results regarding the effects of vitamin D on insulin resistance in other chronic diseases(Pittas and Dawson-Hughes, 2010). Duration of vitamin D supplementation, measuring methods of IR and vitamin D receptor (VDR) gene polymorphisms are among the potential reasons for finding conflicting results regarding the effect of vitamin D on IR(Pittas and Dawson-Hughes, 2010). None of the included trials in our review had used more valid and accurate methods such as euglycemic clamp technique or intravenous glucose tolerance test to assess IR and glucose metabolism. However, application of these methods for epidemiologic studies and clinical trials are not feasible because they are time-consuming, labor-intensive, expensive, and requires an experienced operator to manage technical difficulties(Hannon et al., 2017).Several polymorphisms of the *VDR* gene have been identified, namely *Bsm*I, *Apa*I, *Taq*I, and *Fok*I (Alexandra et al., 2013). In some population, *Taq*I and *Fok*I polymorphisms were related to type2 diabetes and insulin resistance(Nosratabadi et al., 2015). Interestingly, some genotype of VDR polymorphism were slow to respond to vitamin D intake in terms of circulating 25(OH)D, IR and inflammatory markers(Shab-Bidar et al., 2011). Therefore the

longer intervention duration is needed to find an expected effect of vitamin D supplementation on some metabolic profile such as IR in populations who have specific VDR gene polymorphisms.

Inflammation and oxidative stress play important roles in the progression from simple steatosis to more advanced form of NAFLD(Jou et al., 2008). Of the trials, only in one, that was published by our research group, supplementation with vitamin D (50000 IU/bi-weekly) for 16 weeks led to near the significantdecline in serum levels of hs-CRP (a biomarker of inflammation) and decreased serum MDA (a biomarker of lipid peroxidation) significantly in adults patients with NAFLD (Sharifi et al., 2014). The other two studies that used serum biomarkers of inflammation as secondary outcomes, did not find any beneficial effects of vitamin D supplementation on these biomarkers(Barchetta et al., 2016; Foroughi et al., 2014). Such inconsistency also exists between the results of previous studies that assessed the effects of vitamin D on systemic inflammation among patients with other chronic diseases that might be due to differences in diseases, subjects, type and doses of vitamin D supplementation and follow-up duration (Beilfuss et al., 2012; Zittermann et al., 2009).

Fibrosis is the advanced form of NAFLD that predisposes patients to liver cirrhosis (Cheah et al., 2017). The activation of hepatic stellate cells (HSC), during chronic liver injury, is responsible for liver fibrosis(Consolo et al., 2009). In addition to collagen production, HSC also secrete the inhibitors of metalloproteinase enzyme, thereby preventing the degradation of extracellular matrix proteins (Consolo et al., 2009). These cells also secrete transforming growth factor β1 (TGF-β1), which contributes to the activation of other adjacent HSC from the paracrine pathways (Breitkopf et al., 2006). It has been proposed that vitamin D can decrease TGF-β1

production via both VDR and non-VDR pathways, and may reduce the progression of liver fibrogenesis, especially in the early stages of NAFLD (Beilfuss et al., 2014). In present systematic review, Barchetta et al. And Sharifi et al., 2014 evaluated the effects of vitamin D supplementation on serum fibrosis biomarkers such as TGF-β1 and procollagen III propeptide. However, they did not find significant changes in these serum biomarkers post supplementation (Barchetta et al., 2016; Sharifi et al., 2014).Further studies will reveal more facts in this area

Recently, the interactions between the liver and the gut have been investigated as one of the underlying causes of NAFLD onset and progression (Abenavoli et al., 2016; Eliades and Spyrou, 2015). Increased intestinal permeabilityleads to the translocation of bacterial products from lumen to the portal circulation(Poeta et al., 2017). Bacterial products such as lipopolysaccharides (LPS) trigger liver inflammation and inducing the innate immune response (Cani et al., 2008). Toll Like receptor-4 (TLR-4) is activated in response to bacterial LPS exposure (Miura and Ohnishi, 2014). Evidence revealed a significant association between TLR-4 activation and NAFLD (Miura and Ohnishi, 2014). Since VDR is widely expressed in non-parenchymal liver cells like macrophages and Kuppfer cells, it seems that vitamin D can modulate innate immunity via VDR signalling in these types of hepatic cells (Zúñiga et al., 2011). However, there are no randomized controlled trials studying whether vitamin D replacement is beneficial in modulating the immunity responses that are arisen from the gut-liver axis. This can be a potential research topic for future studies.

Some previous cross-sectional studies reported a significant association between serum levels of vitamin D and NAFLD only in men (Park et al., 2017; Zhai et al., 2016). Gender differences

have been proposed as a factor that might affect the relationships between serum vitamin D and NAFLD (Wang et al., 2016b). The mechanisms for such interaction have not been completely understood. Some reports referred the mechanisms to the existence of differences in sex hormones related factors between the genders (Wang et al., 2016b). We previously conducted a post hoc subgroup analysis within our clinical trial in NAFLD patients to determinethe effects of vitamin D on hepatic steatosis and other metabolic profiles separately in men and women (Sharifi et al., 2016). Our results indicated that improved vitamin D status might decrease serum total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) levels as well as hs-CRP in women with NAFLD. However, it might increase serum TC in men(Sharifi et al., 2016). The other clinical trials reviewed here did not perform subgroup analysis to evaluate the gender differences.

Various doses and durations of vitamin D supplementation have been used by reviewed clinical trials in patients with NAFLD. Moreover, controversies exist in the obtained results from limited number of studies. As a result, it seems premature to recommend vitamin D supplementation for the specific treatment of NAFLD based on current limited data. Further randomized, placebo-controlled trials are needed to better determine the appropriate doses and the efficacy of vitamin D supplementation in patients with NAFLD. Until then, it is recommended to follow *The Endocrine Society's Clinical Guidelines in The Treatment and Prevention of Vitamin D Deficiency*, in order to correct serum vitamin D levels in patients with NAFLD suffering from vitamin D deficiency (Holick et al., 2011).

Most recently, a systematic review and meta-analysis study has been published that, aimed to investigate clinical trials related to the effects of vitamin D on NAFLD (Tabrizi et al., 2017). However, our study has some strengths that distinguish it from the meta-analysis study by Tabrizi et al (Tabrizi et al., 2017).We applied extensive literature search and strict adherence to PRISMA guidelines to identify all the relevant articles(Liberati et al., 2009). When evaluating studies in order to enter them into our systematic review, we found several papers from the same research group that reported data from the same group of patients. Therefore, in order to prevent possible errors, we considered the similar published papers derived from the same research group as a single study and did not include them as separate ones in our systematic review. Moreover, we included clinical trials from different countries while all trials in meta-analysis by Tabrizi et al were from a same country that could restrict generalizability of their results. Additionally, to explore the methodological quality of the included studies, we applied Downs and Black checklist not only for the quality of reporting, internal validity (bias and confounding) and power, but also for external validity (Downs and Black, 1998). Due to the limited number of clinical trials published on vitamin D and NAFLD, it was anticipated that the heterogeneity would be high among them. Therefore, we did not perform meta-analysis, because the meta-analysis findings are not conclusive when considerable heterogeneity exists among the included studies (Higgins et al., 2003). Indeed, one of the important goals of the present study was to critically discuss and compare the design and findings of clinical trials in order to provide hypotheses and suggestions that help design future studies to better elucidate the therapatic role of vitamin D in NAFLD.

4.1 Future direction

Based on the results from of clinical trials in the present study, it seems that additional questions remain to be addressed in future studies regarding the effects of vitamin D supplementation on NAFLD. In order to avoid repeating studies with similar design we would suggest the following points:

- If possible, more accurate measurement methods for monitoring the primary (e.g. liver biopsy, MRS or Fibroscan) or secondary outcomes (e.g. euglycemic clamp technique for IR) should be applied.
- It would be better to compare the effects of vitamin D supplementation on NAFLD outcomes between study groups with different subtypes of steatosis, NASH and liver fibrosis.
- At least one of the non-invasive biomarkers of NAFLD should be measured to increase the quality and accuracy of the study results
- Study with the specific objective to compare the effects of vitamin D supplementation on NAFLD between subjects with vitamin D deficiency and those with sufficient vitamin D would be more inclusive.
- Study with the specific objective to compare the effects of vitamin D supplementation on NAFLD between the study groups with different levels of dietary calcium intake is suggested.

- Conducting a single large randomized controlled trial that simultaneously evaluates the effects of vitamin D and calcium supplementation, alone or in combination, on NAFLD parameters would bring advanced results.
- Considering the effects of gender differences on relationship between vitamin D supplementation and outcomes of NAFLD can illustrate more specific therapeutic approach.
- Evaluating the effects of vitamin D supplementation on serum biomarkers of inflammation and oxidative stress along with other outcomes of NAFLD is suggested.
- Investigating the effects of VDR gene polymorphism on association between vitamin D supplementation and NAFLD is a new field to assess.

4.2 Conclusion

The results obtained from this review, provide new insight into the factors that could affect the therapeutic role of vitamin D in NAFLD. Factors such as gender differences, baseline serum status of vitamin D, baseline dietary intake of calcium, gene polymorphism, different subtypes of NAFLD, co-supplementation with calcium, as well as using accurate outcome measurement methods and longer period of intervention should be considered when designing future randomized clinical trials in this field.

Conflict of interest: The authors declare no conflicts of interest.

Author contributions: NS designed the study, extracted data,did the analysis and prepared the first draft. RA designed the study, supervised the work and edited the manuscript.

REFERENCES

Quality Assessment Tool for Before-After (Pre-Post) Studies with no Control Group National Institutes of Health, National Heart, Lung and Blood Istitute.

Abenavoli, L., Milic, N., Di Renzo, L., Preveden, T., Medic-Stojanoska, M., De Lorenzo, A., (2016). Metabolic aspects of adult patients with nonalcoholic fatty liver disease. *World J Gastroenterol*. **22**: 7006-7016.

Adams, J. S., Hewison, M., (2010). Update in vitamin D. *J Clin Endocrinol Metab*.**95**: 471-478.

Alexandra, S.-G., Georgiana, D. C., Nicoleta, C., Daniela, P. M., Traian, S., Veronica, S. (2013). Apa I and Taq I polymorphisms of VDR (vitamin D receptor) gene in association with susceptibility to tuberculosis in the Romanian population. *Rom Biotech Lett*. **18**:7956.

Altieri, B., Grant, W. B., Della Casa, S., Orio, F., Pontecorvi, A., Colao, A., Sarno, G., Muscogiuri, G.(2017). Vitamin D and pancreas: The role of sunshine vitamin in the pathogenesis of diabetes mellitus and pancreatic cancer. *Crit Rev Food Sci Nutr***57**: 3472-3488.

Barchetta, I., Angelico, F., Del Ben, M., Baroni, M. G., Pozzilli, P., Morini, S., Cavallo, M. G.(2011). Strong association between non alcoholic fatty liver disease (NAFLD) and low

25(OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC Med*. **9**:85.

Barchetta, I., Del Ben, M., Angelico, F., Di Martino, M., Fraioli, A., La Torre, G., Saulle, R., Perri, L., Morini, S., Tiberti, C., Bertoccini, L., Cimini, F. A., Panimolle, F., Catalano, C., Baroni, M. G., Cavallo, M. G.(2016). No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *BMC Med*. **14**: 92.

Beilfuss, J., Berg, V., Sneve, M., Jorde, R., Kamycheva, E.(2012). Effects of a 1-year supplementation with cholecalciferol on interleukin-6, tumor necrosis factor-alpha and insulin resistance in overweight and obese subjects. *Cytokine*.**60**(3):870-874.

Beilfuss, A., Sowa, J.-P., Sydor, S., Beste, M., Bechmann, L. P., Schlattjan, M., Syn, W.-K., Wedemeyer, I., Mathé, Z., Jochum, C., Gerken, G., Gieseler, R. K., Canbay, A., (2014). Vitamin D counteracts fibrogenic TGF-β signalling in human hepatic stellate cells both receptor-dependently and independently. *Gut*.**64**(5):791-799.

Breitkopf, K., Godoy, P., Ciuclan, L., Singer, M. V., Dooley, S., (2006). TGF-beta/Smad signaling in the injured liver. *Z Gastroenterol*. **44**: 57-66.

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., Burcelin, R., (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. **57**: 1470-1481.

Challoumas, D.(2014). Vitamin D supplementation and lipid profile: What does the best available evidence show? *Atherosclerosis*. **235**:130-139.

Cheah, M. C., McCullough, A. J., Goh, G. B., (2017). Current Modalities of Fibrosis Assessment in Non-alcoholic Fatty Liver Disease. *J Clin Transl Hepatol*. **5**: 261-271.

Clark, J. M., Brancati, F. L., Diehl, A. M..(2002). Nonalcoholic fatty liver disease. *Gastroenterology*. **122:** 1649-1657.

Cobbold, J. F., Patel, D., Taylor-Robinson, S. D.(2012). Assessment of inflammation and fibrosis in non-alcoholic fatty liver disease by imaging-based techniques. *J Gastroenterol Hepatol*. **27**:1281-1292.

Consolo, M., Amoroso, A., Spandidos, D., Mazzarino, M., (2009). Matrix metalloproteinases and their inhibitors as markers of inflammation and fibrosis in chronic liver disease. *Int J Mol Med*. **24**: 143-152.

Dankers, W., Colin, E. M., van Hamburg, J. P., Lubberts, E.,(2016). Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. *Front Immunol*. **7**: 697.

Das, S., Vasudevan, D., (2008). Genesis of hepatic fibrosis and its biochemical markers. *Scand J Clin Lab Invest*. **68**: 260-269.

de Ledinghen, V., Vergniol, J., Foucher, J., El-Hajbi, F., Merrouche, W., Rigalleau, V.(2010). Feasibility of liver transient elastography with FibroScan using a new probe for obese patients. *Liver Int*. **30**: 1043-1048.

Dowman, J. K., Tomlinson, J. W., Newsome, P. N.(2010). Pathogenesis of non-alcoholic fatty liver disease. *Qjm*. **103**:71-83.

Dowman, J. K., Tomlinson, J. W., Newsome, P. N. (2011). Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. **33**: 525-540.

Downs, S. H., Black, N.(1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *J Epidemiol Community Health*. **52**: 377-384.

Eliades, M., Spyrou, E., Agrawal, N., Lazo, M., Brancati, F. L., Potter, J. J., Koteish, A. A., Clark, J. M., Guallar, E., Hernaez, R. (2013). Meta-analysis: vitamin D and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. **38:** 246-254.

Eliades, M., Spyrou, E., (2015). Vitamin D: a new player in non-alcoholic fatty liver disease? *World J Gastroenterol*. **21**: 1718-1727.

Farrell, G. C., Larter, C. Z. (2006). Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology*. **43**:s99-s112.

Fitzpatrick, E., Dhawan, A. (2014). Noninvasive biomarkers in non-alcoholic fatty liver disease: current status and a glimpse of the future. *World J Gastroenterol*. **20**:10851-10863.

Foroughi, M., Maghsoudi, Z., Ghiasvand, R., Iraj, B., Askari, G. (2014). Effect of Vitamin D Supplementation on C-reactive Protein in Patients with Nonalcoholic Fatty Liver. *Int J Prev Med*. **5**: 969-975.

Gascon- Barré, M., Demers, C., Mirshahi, A., Néron, S., Zalzal, S., Nanci, A., (2003). The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells. *Hepatology*. **37**: 1034-1042.

Geier, A., (2011). Shedding new light on vitamin D and fatty liver disease. *J Hepatol* . **55**: 273-275.

Halder, S. K., Goodwin, J. S., Al-Hendy, A., (2011). 1,25-Dihydroxyvitamin D3 reduces TGF-beta3-induced fibrosis-related gene expression in human uterine leiomyoma cells. *J Clin Endocrinol Metab*. **96**: 2010-2131.

Hannon, T. S., Kahn, S. E., Utzschneider, K. M., Buchanan, T. A., Nadeau, K. J., Zeitler, P. S., Ehrmann, D. A., Arslanian, S. A., Caprio, S., Edelstein, S. L., Savage, P. J., Mather, K. J.(2017). Review of methods for measuring beta-cell function: Design considerations from the Restoring Insulin Secretion (RISE) Consortium. *Diabetes Obes Metab*.doi: 10.1111/dom.13005

Heaney, R. P. (2008). Nutrients, endpoints, and the problem of proof. *J Nutr*. **138**: 1591-1595.

Heaney, R. P. (2012). Vitamin D--baseline status and effective dose. *N Engl J Med*. **367**: 77-78.

Higgins, J. P., Thompson, S. G., Deeks, J. J., Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *Bmj*. **327**: 557-560.

Holick, M. F.(2007). Vitamin D deficiency. *N Engl J Med*. **357**: 266-281.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M., (2011). Evaluation, treatment, and prevention of vitamin d deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. **96**: 1911-1930.

Hyppönen, E., Boucher, B. J., Berry, D. J., Power, C. (2008). 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age. *Diabetes*.**57**: 298-305.

Jou, J., Choi, S. S., Diehl, A. M. (2008). Mechanisms of disease progression in nonalcoholic fatty liver disease. *Semin Liver Dis*. **28**: 370-379.

Kitson, M. T., Pham, A., Gordon, A., Kemp, W., Roberts, S. K. (2016). High-dose vitamin D supplementation and liver histology in NASH. *Gut*. **65**: 717-718.

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gotzsche, P. C., Ioannidis, J. P., Clarke, M., Devereaux, P. J., Kleijnen, J., Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med*. **6**: e1000100.

Lorvand Amiri, H., Agah, S., Tolouei Azar, J., Hosseini, S., Shidfar, F., Mousavi, S. N. (2016). Effect of daily calcitriol supplementation with and without calcium on disease regression in non-alcoholic fatty liver patients following an energy-restricted diet: Randomized, controlled, double-blind trial. *Clin Nutr*. doi: 10.1016/j.clnu.2016.09.020

Maestro, B., Molero, S., Bajo, S., Dávila, N., Calle, C.,(2002). Transcriptional activation of the human insulin receptor gene by 1, 25- dihydroxyvitamin D3. *Cell Biochem Funct*.**20**: 227-232.

Maestro, B., Dávila, N., Carranza, M. C., Calle, C., (2003). Identification of a Vitamin D response element in the human insulin receptor gene promoter. *J Steroid Biochem Mol Biol*. **84**: 223-230.

Major, G. C., Alarie, F. P., Dore, J., Tremblay, A. (2009). Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control. *Br J Nutr*. **101:** 659-663.

Miura, K., Ohnishi, H., (2014). Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. *World J Gastroenterol*. **20**: 7381-7391.

Nakano, T., Cheng, Y. F., Lai, C. Y., Hsu, L. W., Chang, Y. C., Deng, J. Y., Huang, Y. Z., Honda, H., Chen, K. D., Wang, C. C., (2011). Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. *J Hepatol*. **55**:415 - 425.

Nelson, J. E., Roth, C. L., Wilson, L. A., Yates, K. P., Aouizerat, B., Morgan-Stevenson, V., Whalen, E., Hoofnagle, A., Mason, M., Gersuk, V., Yeh, M. M., Kowdley, K. V.(2016). Vitamin D Deficiency Is Associated With Increased Risk of Non-alcoholic Steatohepatitis in Adults With Non-alcoholic Fatty Liver Disease: Possible Role for MAPK and NF-kappaB? *Am J Gastroenterol*. **111**:852-863.

Nosratabadi, R., Arababadi, M. K., Salehabad, V. A. (2015). Vitamin D receptor polymorphisms in type 2 diabetes in southeastern Iranian patients. *Lab Med*. **42**: 32-34.

Papapostoli, I., Lammert, F., Stokes, C. S. (2016). Effect of Short-Term Vitamin D Correction on Hepatic Steatosis as Quantified by Controlled Attenuation Parameter (CAP). *J Gastrointestin Liver Dis*. **25**: 175-181.

Park, D., Kwon, H., Oh, S. W., Joh, H. K., Hwang, S. S., Park, J. H., Yun, J. M., Lee, H., Chung, G. E., Ze, S., Park, J. H., Bae, Y., Lee, A. (2017). Is Vitamin D an Independent Risk Factor of Nonalcoholic Fatty Liver Disease?: a Cross-Sectional Study of the Healthy Population. *J Korean Med Sci*. **32**: 95-101.

Picciano, M. F. (2010). Vitamin D Status and Health. *Crit Rev Food Sci Nutr*. **50**: 24-25.

Pittas, A. G., Lau, J., Hu, F. B., Dawson-Hughes, B. (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*. **92**: 2017-29.

Pittas, A. G., Dawson-Hughes, B. (2010). Vitamin D and diabetes. *J Steroid Biochem Mol Biol*. **121**:425-429.

Poeta, M., Pierri, L., Vajro, P., (2017). Gut-Liver Axis Derangement in Non-Alcoholic Fatty Liver Disease. *Children (Basel)*. **4**(8):doi: 10.3390/children4080066

Prosser, D. E., Jones, G., (2004). Enzymes involved in the activation and inactivation of vitamin D. *Trends Biochem Sci*. **29**: 664-673.

Saleh, H. A., Abu-Rashed, A. H. (2007). Liver biopsy remains the gold standard for evaluation of chronic hepatitis and fibrosis. *J Gastrointestin Liver Dis*. **16**: 425-426.

Shab-Bidar, S., Neyestani, T. R., Djazayery, A.(2011). Efficacy of vitamin D3-fortified-yogurt drink on anthropometric, metabolic, inflammatory and oxidative stress biomarkers according to vitamin D receptor gene polymorphisms in type 2 diabetic patients: a study protocol for a randomized controlled clinical trial. *BMC Endocr Disord*. **11**: 12.

Sharifi, N., Amani, R., Hajiani, E., Cheraghian, B.(2014). Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. *Endocrine*. **47**: 70-80.

Sharifi, N., Amani, R., Hajiani, E., Cheraghian, B. (2016). Women may respond different from men to vitamin D supplementation regarding cardiometabolic biomarkers. *Exp Biol Med* (Maywood). **241**: 830-838.

Tabrizi, R., Moosazadeh, M., Lankarani, K. B., Akbari, M., Heydari, S. T., Kolahdooz, F., Samimi, M., Asemi, Z. (2017). The effects of vitamin D supplementation on metabolic profiles and liver function in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. *Diabetes Metab Syndr*.doi:10.1016/j.dsx.2017.07.025

Takiishi, T., Gysemans, C., Bouillon, R., Mathieu, C., (2012). Vitamin D and diabetes. *Rheum Dis Clin North Am*. **38**: 179-206.

Targher, G., Bertolini, L., Scala, L., Cigolini, M., Zenari, L., Falezza, G., Arcaro, G.(2007). Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis*. **17**: 517-524.

Wang, D., Lin, H., Xia, M., Aleteng, Q., Li, X., Ma, H., Pan, B., Gao, J., Gao, X.(2016a). Vitamin D Levels Are Inversely Associated with Liver Fat Content and Risk of Non-Alcoholic Fatty Liver Disease in a Chinese Middle-Aged and Elderly Population: The Shanghai Changfeng Study. *PLoS One*. **11**:e0157515.

Wang, N., Zhai, H., Zhu, C., Li, Q., Han, B., Chen, Y., Zhu, C., Chen, Y., Xia, F., Lin, D., Lu, Y.(2016b). Combined Association of Vitamin D and Sex Hormone Binding Globulin With Nonalcoholic Fatty Liver Disease in Men and Postmenopausal Women: A Cross-Sectional Study. *Medicine* (Baltimore). **95**:e2621.

Zhai, H. L., Wang, N. J., Han, B., Li, Q., Chen, Y., Zhu, C. F., Chen, Y. C., Xia, F. Z., Cang, Z., Zhu, C. X., Lu, M., Lu, Y. L.(2016). Low vitamin D levels and non-alcoholic fatty liver disease, evidence for their independent association in men in East China: a cross-

sectional study (Survey on Prevalence in East China for Metabolic Diseases and Risk Factors (SPECT-China)). *Br J Nutr*. **115**:1352-1359.

Zhu, C. G., Liu, Y. X., Wang, H., Wang, B. P., Qu, H. Q., Wang, B. L., Zhu, M., (2017). Active form of vitamin D ameliorates non-alcoholic fatty liver disease by alleviating oxidative stress in a high-fat diet rat model. *Endocr J*. **64**: 663-673.

Zittermann, A., Frisch, S., Berthold, H. K., Götting, C., Kuhn, J., Kleesiek, K., Stehle, P., Koertke, H., Koerfer, R.(2009). Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *Am J Clin Nutr*. **89**:1321-1327.

Zúñiga, S., Firrincieli, D., Housset, C., Chignard, N., (2011). Vitamin D and the vitamin D receptor in liver pathophysiology. *Clin Res Hepatol Gastroenterol*. **35**: 295-302.

Table 1: Quality scores of the included RCTs assessed by Downs and Black checklist.

Author, year	Reporting Total score:10	External validity Total score:3	Internal validity-biased Total score:7	Internal validity-confounding Total score:6	Power Total score:3	Total quality score <14 = poor 15–19 = fair >20 = good
Lorvand Amiri et al., 2016	9	2	5	5	1	22/29
Barchetta et al., 2016	10	2	7	5	1	25/29
Foroughi et al., 2014	7	1	6	3	1	18/29
Sharifi et al., 2014	9	1	7	6	1	24/29

RCTs, Randomized controlled trial

Table 2:Quality scores of the included UCTs assessed by *Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group

Author, year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Total Score
Papapostoli et al., 2016	Y	Y	N	Y	CD	Y	Y	NR	Y	Y	Y	8/11
Kitson et al., 2016	Y	N	Y	N	N	Y	Y	NR	NA	Y	N	5/11

* Each question receives one point for each affirmative answer. For more detail about each question please refer to Supplementary Table 2.

CD, can not determine; NR, not reported; N, no; Q, question; UCT, uncontrolled trial; Y,yes

Table 3:Studies that evaluated the effect of vitamin D supplementation on NAFLD outcomes

Author, year,	Country	Design	Total Sample size (M/F)	Study Population	Age years	BMI (kg/m^2)	Intervention and study groups	Duration (wks)	Serum vitamin D levels (ng/mL) Before (BI) and After intervention(AI)	Outcomes	Results
Lorvand Amiri et al., 2016	Iran	RCT	110 (68/42)	NAFLD with VDD	39.8±11	30.3±0.64	**G 1**: Vitamin D3 (1000 IU/d)+ placebo+ weight loss diet **G2** : Vitamin D3+calcium carbonate (1000 IU/d + 500 mg/d)+weight loss diet **G 3**: Placebo+weight loss diet	12	**BI** G1: 9.9±0.64 G2: 9.9±0.64 G3: 10± 0.63 **AI** G1:21.4±0.73 G2: 27.1±1.1 G3: 11± 0.87	HS (Ultrasound), ALT,AST ,FBG,HOMA-IR, BMI	**Relative to baseline:** **Group 1**: HS ↓ in 89% of subject, Vitamin D ↑116% ALT ↓7%, AST↓14%, HOMA-IR ↓19%, FPG↓2% , BMI↓3% **Group 2**: HS ↓ in 73% of subject, Vitamin D ↑174% ALT ↓28%, AST↓17%, HOMA-IR ↓31%, FPG↓9%, BMI↓4% **Group 3:** HS ↓ in 11% of subject, BMI↓4%, ALT, AST, HOMA-IR, FPG : n/s
Barchetta et al., 2016	Italy	RCT	55 (35/20)	NAFLD subjects with Type2 diabetes	58.7±9.9	29.3±4.4	Vitamin D3(2000 IU/d) Placebo	24	**BI**: 17.24 **AI**: 34.32	HS (Ultrasound & MRI), FLI, ALT,AST , CK-M30, γ-GT, FBG,HOMA-IR, hs-CRP, Adiponectin	**Relative to baseline:** Vitamin D ↑100% No significant changes in any of the outcomes' measures
Papapostoli,et	Germany	UCT	40	Patient with	54.9±12	29.5±3.0	Vitamin D3	24	**BI**: 11.8±4.8	HS (Transient	**Relative to baseline:**

al. 2016			(21/19)	hepatic steatosis and VDD	.1		(20,000 IU/week)		**AI**: 34.8±9.8	elastography),ALT, AST, ALP, γ-GT, fat-mass, Ca, PTH	HS ↓ 7% , Vitamin D ↑195% PTH↓ 26% ALT,AST, ALP, γ-GT, fat-mass, Ca: n/s
Kitson et al., 2015	Australia	UCT (pilot study)	12 (n/r)	Non-cirrhotic patients with biopsy proven NASH	n/r	n/r	Vitamin D3 (25.000 IU/week)	24	**BI**: 25.2±12.6 **AI**: 43.9 ±6.24	NAFLD activity score, ALT,AST , γ-GT, HbA1c, HOMA-IR, Adiponectin	**Relative to baseline:** Vitamin D ↑74% No significant changes in values of other outcomes
Foroughi, et al., 2014	Iran	RCT	60 (29/31)	Patients with NAFLD	48.5	31.2±4	Vitamin D3 (50,000 IU/week) Placebo	10	**BI**: 19.6±1 **AI**: 46.8±13	HS (Ultrasound), ALT,AST , hs-CRP, Ca	**Relative to baseline:** Vitamin D ↑138% , Ca ↑37% HS,ALT,AST, hs-CRP: n/s
Sharifi, et al., 2014	Iran	RCT	53 (26/27)	Patients with NAFLD	42.1±9.2	30.8±4.6	Vitamin D3 (50,000 IU/bi weekly)+ Recommendation for lifestyle modification Placebo+ Recommendation for lifestyle modification	16	**BI**: 16.4±9.7 **AI**: 37.4 ±16.3	HS (Ultrasound), ALT,AST , ALP,FBG , HOMA-IR,hs-CRP, TNF-α, TGF β1, MDA, Adiponectin	**Relative to baseline:** Vitamin D ↑128% hs-CRP ↓13% , MDA ↓ 51%, No significant changes in values of other outcomes

Data are mean ± standard errors usually rounded to the nearest full figure; Sample size reflects those in the final analysis.
AI, after intervention; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase ;BI, before intervention; BMI, bod max index (kg/m2); Ca, calcium; CK-M30, cytokeratin 18; d, day(s); F, female; FBG, fasting blood glucose; FLI, fatty liver index; G1, group1; G2, group 2; G3, group 3; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment; HS,hepatic steatosis; hs-CRP, high-sensitive C reactive protein; M, male; MDA, malondialdehyde; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; n/r, data not reported; n.s., result not statistically significant; PTH, parathyroid hormone; RCT, randomised controlled trial; TGF β1, transforming factor β1; TNF-

tumor necrosis factor -α ; UCT, uncontrolled trial; γ-GT, gamma glutamyltransferase

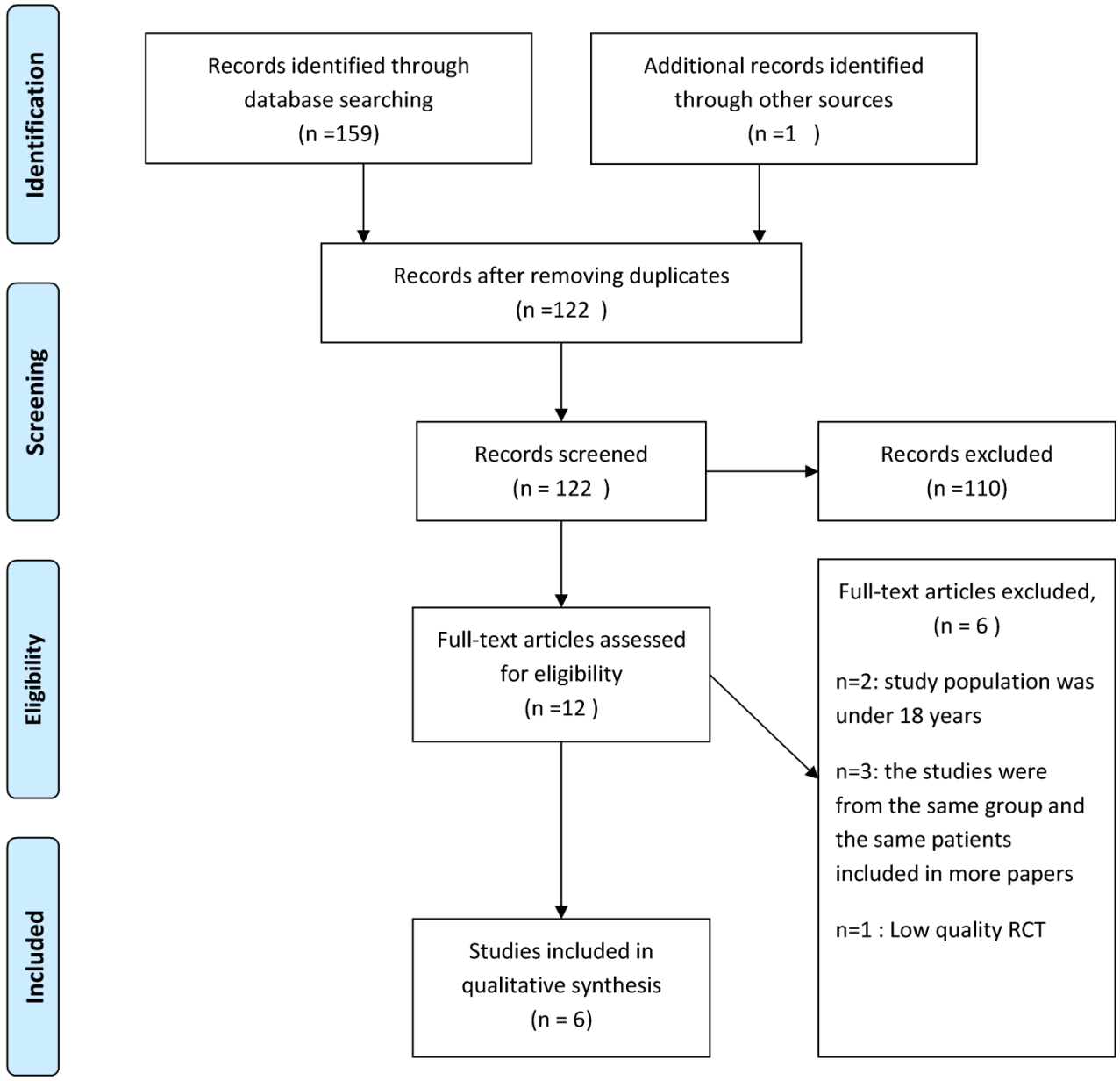

Figure 1.