Journal of the American Heart Association

ORIGINAL RESEARCH

Effects of Vitamin D Supplementation on Cardiovascular and Glycemic Biomarkers

Jennifer Miao, MD*; Katherine N. Bachmann, MD, MSCI*; Shi Huang, PhD; Yan Ru Su, MD; Jeffery Dusek, PhD; Christopher Newton-Cheh, MD, MPH; Pankaj Arora, MD; Thomas J. Wang, MD

BACKGROUND: Experimental and observational studies have suggested a link between vitamin D and cardiovascular and metabolic disease, but this has not been confirmed in randomized controlled trials. We sought to determine whether vitamin D supplementation reduces biomarkers of insulin resistance, inflammation, neurohormonal activation, and lipids.

METHODS AND RESULTS: This was a prespecified, secondary analysis of the DAYLIGHT (Vitamin D Therapy in Individuals at High Risk of Hypertension) randomized controlled trial. We measured circulating homeostatic model assessment of insulin resistance, hs-CRP (high-sensitivity C-reactive protein), N-terminal pro-B-type natriuretic peptide, renin, aldosterone, and lipids at baseline and at 6 months in 289 individuals with low vitamin D status (25-hydroxyvitamin-D [25-OH-D] ≤25 ng/mL) receiving low-dose (400 IU/d) versus high-dose (4000 IU/d) vitamin D3 for 6 months. A meta-analysis of randomized controlled trials reporting biomarker changes after vitamin D supplementation was then performed. Levels of 25-OH-D increased in the high-dose relative to the low-dose vitamin D group (+15.5 versus +4.6 ng/mL, $P<0.001$). Changes in biomarkers of glycemia, inflammation, and neurohormonal activation did not differ by dose. Lipids did not differ between groups, other than triglycerides, which increased in the high-dose compared with the low-dose group (+11.3 versus −6.2 mg/dL, $P<0.001$). The meta-analysis showed potential modest decreases in homeostatic model assessment of insulin resistance and hs-CRP, but no changes in low-density lipoprotein, after vitamin D supplementation compared with control groups.

CONCLUSIONS: In the DAYLIGHT randomized controlled trial, high-dose vitamin D supplementation did not improve biomarkers of glycemia, inflammation, neurohormonal activation, or lipids.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01240512.

Key Words: high-sensitivity C-reactive protein ■ insulin resistance ■ lipids ■ meta-analysis ■ vitamin D

The potential cardiovascular and metabolic effects of vitamin D have been widely investigated in experimental and epidemiological studies. The active metabolite of vitamin D, 1,25-dihydroxy vitamin D3 ($1,25(OH)_2D_3$), has been shown to regulate an array of extraskeletal signaling pathways, including, but not limited to, those found in vascular smooth muscle cells, pancreatic β cells, myeloid cells, and cardiomyocytes.[1–3] These interactions lead to downstream effects that control vascular function, neurohormonal activation, cytokine production, and lipid and glucose homeostasis. Furthermore, the role of vitamin D in numerous outcomes has been investigated, with observational studies suggesting a correlation between high vitamin D levels and lower risk of cardiovascular disease, type 2 diabetes mellitus, metabolic syndrome, colorectal cancer, and other chronic diseases.[4]

Nonetheless, the hypothesized benefits of vitamin D supplementation have not been prospectively confirmed in randomized controlled trials (RCTs).[5–7]

Correspondence to: Katherine N. Bachmann, MD, MSCI, Division of Diabetes, Endocrinology, and Metabolism, Vanderbilt University Medical Center, 2213 Garland Avenue, MRB IV Suite 7465, Nashville, TN 37232. E-mail: katherine.n.bachmann@vanderbilt.edu

*J. Miao and K. N. Bachmann contributed equally and are co-first authors.

Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.017727

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In this secondary analysis of the DAYLIGHT (Vitamin D Therapy in Individuals at High Risk of Hypertension) randomized controlled trial, individuals randomized to high-dose vitamin D supplementation did not experience improvements in biomarkers of insulin resistance, inflammation, neurohormonal activation, or lipids.

What Are the Clinical Implications?

- High-dose vitamin D supplementation does not improve biomarkers of glycemia, inflammation, neurohormonal activation, or lipids in otherwise healthy individuals with low vitamin D status.

Nonstandard Abbreviations and Acronyms

25-OH-D	25-hydroxyvitamin D
CV	coefficient of variation
DAYLIGHT	Vitamin D Therapy in Individuals at High Risk of Hypertension
HOMA-IR	homeostatic model assessment of insulin resistance
VITAL	Vitamin D and Omega-3 Trial

The largest of these trials, the VITAL (Vitamin D and Omega-3 Trial), found that vitamin D supplementation in a large sample of older individuals did not reduce major cardiovascular events.[6] Smaller trials have suggested that vitamin D supplementation may benefit metabolic risk factors, such as homeostatic model assessment of insulin resistance (HOMA-IR), but results have been inconsistent.

The DAYLIGHT (Vitamin D Therapy in Individuals at High Risk of Hypertension) trial examined the effect of vitamin D supplementation on blood pressure (BP) in individuals with low vitamin D status, as well as prehypertension or untreated stage I hypertension. The trial found no effect of vitamin D supplementation on 24-hour mean systolic BP, the primary end point.[8] We performed a prespecified substudy of the DAYLIGHT trial, to examine the influence of vitamin D supplementation on biological pathways linked to vitamin D metabolism, including glucose handling, inflammation, neurohormonal activation, and lipid storage. One objective of this substudy was to explore whether vitamin D supplementation might have cardiometabolic effects not adequately captured by the composite outcomes in large randomized studies such as VITAL. A second objective was to determine whether discordant effects of vitamin D on biological pathways might account for the overall neutral results with regard to clinical outcomes. These objectives were further pursued through a meta-analysis of RCTs measuring changes in low-density lipoprotein (LDL), hs-CRP (high-sensitivity C-reactive protein), or HOMA-IR following vitamin D supplementation in relatively healthy individuals.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Participants

The DAYLIGHT trial (NCT01240512) was a randomized, double-blind, controlled trial examining the effects of high-dose vitamin D supplementation on ambulatory BP. Details of the design of the trial have been previously described.[8] In the original trial, 534 individuals were randomized to low-dose (400 IU/d) or high-dose (4000 IU/d) vitamin D supplementation for 6 months at 1 of 3 study sites. Individuals were aged between 18 and 50 years, with low vitamin D status (defined as serum 25-hydroxyvitamin D [25-OH-D] ≤25 ng/mL), and with prehypertension or untreated stage I hypertension (systolic BP 120–159 mm Hg and diastolic BP <99 mm Hg). Participants did not use vitamin D supplementation or antihypertensive medications during the prior 3 months or have a history of diabetes mellitus, cardiovascular disease, significant gastrointestinal disease, serum creatinine >2.0 mg/dL, or estimated glomerular filtration rate <30 mL/min.[8] Written informed consent was obtained following approval from the institutional review boards at the participating institutions, and the investigators had full access to all of the data in the study and take responsibility for its integrity and data analysis.

For the present study, we included 289 individuals (54% of the parent study population) who had blood specimens available from baseline and 6 months (144 in the low-dose group and 145 in the high-dose group) (Table S1).

Biomarker Assays

Fasting blood specimens were collected, immediately centrifuged, and aliquoted at each of the study sites. Samples were stored at −80°C until analysis. Unless otherwise noted, all assays were performed in a core laboratory at Vanderbilt University Medical Center, in a single batch to minimize interassay variability from temporal drift in reagents or the platform. NT-proBNP (N-terminal pro-B-type natriuretic peptide) and insulin were assayed using a Roche Cobas e411 analyzer

(Roche Diagnostics). The average intra-assay coefficient of variation (CV) in our laboratory is <2% for both assays. Glucose, hs-CRP, and plasma lipid profile (total cholesterol, triglycerides, LDL, and HDL) were measured on the ACE AXcel platform (Alfa Wassermann Diagnostic Technologies, LLC). The average CV was <6% for all lipid measurements and <2% for glucose. hs-CRP was measured using the C-Reactive Protein High Sensitivity CRP Wide Range Reagent Set (Manufactured for Pointe Scientific, Inc.) by Pointe Scientific, with an intra-assay CV of 3.3%. Renin and aldosterone were measured at DiaSorin (Stillwater, MN) using a fully automated LIAISION chemiluminescent immunoassay with intra-assay CV <4.2% and interassay CV <10%. Serum 25-OH-D was also measured using a chemiluminescence immunoassay at DiaSorin with intra-assay and interassay CVs of <5% and 10%, respectively.

Meta-Analysis

We conducted a meta-analysis including RCTs that reported effects of vitamin D supplementation on HOMA-IR, LDL, or hs-CRP in adults aged 18 years and older (Figure S1). We excluded studies that included participants with end-stage renal disease, chronic kidney disease, heart failure, or cirrhosis. Studies published during January 2009 to January 2020 were identified through Scopus and PubMed literature searches. Search terms included vitamin D and its derivatives or analogs, LDL, cholesterol, low density lipoprotein, hyperlipidemia, HOMA-IR, HOMA-IR assessment, homeostasis model of assessment for insulin resistance, homeostatic model assessment estimate insulin resistance, hs-CRP, C-reactive protein, and CRP under "clinical trial," "clinical trial protocol," and clinical trial phase I to IV search filters. The following data were extracted: first author; year of publication; country where RCT was conducted; sample size; daily dose and duration of vitamin D supplementation (if participants received bolus or weekly doses, the total dose was divided by number of days); baseline serum 25-OH-D levels; and mean change and SD of outcome changes for intervention and placebo groups.

Statistical Analysis

Continuous variables were expressed as median (lower, upper interquartile ranges) and categorical variables as percentage proportions. Pearson chi-square tests were used to compare race and sex, and nonparametric Wilcoxon rank sum tests compared age, baseline 25-OH-D levels, and mean systolic and diastolic BP readings from 2 clinic visits between groups receiving high- versus low-dose vitamin D supplementation. Thus, the changes in circulating biomarker levels from baseline to 6 months between the low-dose versus high-dose groups were compared using Wilcoxon rank sum tests. The changes in circulating biomarker level from baseline to 6 months within a treatment arm were compared using paired Wilcoxon signed rank test. In addition, we examined the 6-month biomarker levels between dose groups after adjusting for baseline biomarker levels, age, race, and sex using multivariable regression analysis. The regression coefficient of the dose group indicated the impact of vitamin D dose on biomarkers. In multiple regression analyses, logarithmic transformation was applied to circulating biomarker levels with skewed distributions (all biomarkers except for total cholesterol, HDL, LDL, and 25-OH-D).

For the meta-analysis, the difference of outcome changes between treatment and placebo groups were analyzed by conducting fixed and random effect meta-analyses. Forest plots, sorted by total vitamin D delivered from lowest cumulative dose to highest, are presented to visualize the individual and overall effects of vitamin D supplementation on hsCRP, HOMA-IR, and LDL. I^2 (as a measure of effect heterogeneity) and P values of Q statistics are reported in the forest plots. Statistical analysis was performed using R version 3.3.1 (R Foundation for Statistical Computing).[9] In addition, we conducted subgroup meta-analyses stratified by vitamin D dose (≥3000 IU/d versus <3000 IU/d), baseline vitamin D status (mean baseline 25-OH-D level ≥20 ng/mL versus <20 ng/mL), supplementation duration (≥12 weeks versus <12 weeks), and patient's diabetes mellitus status (presence or absence of diabetes mellitus) (Figures S2 through S4). Subgroup meta-analyses were not conducted for subgroups of ≤2 studies.

RESULTS

Baseline characteristics of the 289 patients in the substudy did not differ from those in the overall study (Table 1). Patient characteristics for the substudy are presented in Table 2. Demographic and clinical characteristics were similar between the low-dose and high-dose vitamin D groups, except that baseline values of plasma triglycerides were slightly lower in the high-dose group (P=0.03). At 6 months, 25-OH-D concentrations increased in the high-dose supplementation group compared with the low-dose group (mean increase of +15.5 ng/mL versus +4.6 ng/mL, respectively, P<0.001) (Figure 1A). The prevalences of vitamin D deficiency at the end of the trial, defined using a threshold of ≤20 ng/mL, were 24% and 52% in the high-dose and low-dose groups, respectively.

Table 1. Baseline Characteristics of the Substudy Compared With the Overall DAYLIGHT Trial

	Substudy (n=289)	DAYLIGHT Trial (n=534)	*P* Value
Age, y	37.0 (27.0, 45.0)	38.0 (28.0, 45.0)	0.92*
Sex			
Male	0.7	0.7	0.33†
Race			
White	0.45	0.45	
Non-White‡	0.55	0.55	0.92†
BMI, kg/m^2	27.5 (24.4, 30.8)	27.5 (24.2, 31.3)	0.86*
25-OH-D, ng/mL	15.1 (11.1, 19.9)	15.3 (11.1, 20.2)	0.76*
Mean clinic systolic BP, mm Hg	130 (125, 136)	129 (123, 136)	0.22*
Mean clinic diastolic BP, mm Hg	83 (77, 88)	81 (76, 88)	0.096*

Continuous variables are presented as median (lower quartile, upper quartile); categorical variables are presented as percentages of total number. 25-OH-D indicates 25-hydroxyvitamin D; BMI, body mass index; BP, blood pressure; and DAYLIGHT, Vitamin D Therapy in Individuals at High Risk of Hypertension.

**P* value represents nonparametric Wilcoxon rank sum test to compare continuous variables.

†*P* value represents Pearson chi-square test to compare categorical variables.

‡Non-White includes Black, Asian, and Native Hawaiian/Other Pacific Islander.

Changes in hs-CRP, glucose, insulin, HOMA-IR, NT-proBNP, renin, aldosterone, total cholesterol, HDL, and LDL did not differ between the treatment arms (Table 3 and Figure 1B through 1F). An increase in plasma triglycerides was noted in the high-dose group at 6 months (mean change of 11.3 mg/dL versus −6.2 mg/dL for the low-dose group, *P*<0.001) (Figure 1G). Similar results were obtained in regression analyses adjusted for baseline biomarker levels, age, sex, and race.

Subgroup analyses stratified by baseline 25-OH-D levels are reported in Table S2. In addition, we performed post hoc analyses to examine whether the end points differed between individuals who achieved vitamin D sufficiency at the end of the study and those who did not (final 25-OH-D level >20 ng/mL versus ≤20 ng/mL, respectively) (Table S3). Among participants randomized to high-dose vitamin D supplementation who achieved vitamin D sufficiency at the end of the trial, there were no significant changes in any biomarkers, other than an increase in plasma triglycerides (similar to the findings in the high-dose arm as a whole). The directionality and magnitude of changes in biomarkers for the remaining subgroups were similar in those with and those without achievement of vitamin D sufficiency.

A total of 23 studies, including the present study, were included in the final meta-analysis (Figure 2A through 2C, Tables S4 through S6, and Figures S2 through S4). Follow-up duration ranged from 8 weeks to 52 weeks with the exception of a 5-year RCT included in the Women's Health Initiative.[10] Daily vitamin D doses varied from 400 IU/d to 7142 IU/d. The change of circulating levels of LDL was not significantly different between vitamin D supplementation groups and control groups (mean difference, −1.76; 95% CI, −5.07 to 1.55) (Figure 2A[11–32]). Compared with control groups, the vitamin D supplementation groups experienced modest decreases in HOMA-IR (mean difference, −0.53; 95% CI, −0.60 to −0.46) (Figure 2B) and hs-CRP (mean difference, −0.58; 95% CI, −1.00 to −0.15) (Figure 2C).

DISCUSSION

We examined the effects of high- versus low-dose vitamin D supplementation on a large panel of cardiometabolic biomarkers over the course of 6 months in adults with low vitamin D status. Our results from this prespecified, secondary analysis of a randomized trial do not support the hypothesis that vitamin D has a beneficial influence on glycemic, inflammatory, neurohormonal, or lipid pathways.

This study examines the cardiometabolic effects of vitamin D supplementation in the context of a randomized trial. Although prior studies have

Table 2. Baseline Characteristics for Individuals Randomized to Low-Dose (400 IU/d) Versus High-Dose (4000 IU/d) Vitamin D Supplementation

	Low-Dose Vitamin D (n=144)	High-Dose Vitamin D (n=145)	*P* Value
Age, y	36.0 (28.0, 45.0)	38.0 (27.0, 45.0)	0.92*
Sex			
Male	0.7	0.7	0.28†
Race			
White	0.5	0.4	
Non-White‡	0.5	0.6	0.34†
BMI, kg/m^2	28.0 (25.0, 30.7)	26.6 (23.7, 31.0)	0.075*
25-OH-D, ng/mL	15.4 (11.7, 19.6)	14.6 (9.2, 19.7)	0.14*
Mean clinic systolic BP, mm Hg	129 (124, 137)	131 (125, 136)	0.32*
Mean clinic diastolic BP, mm Hg	83 (77, 88)	83 (78, 88)	0.69*

25-OH-D indicates 25-hydroxyvitamin D; BMI, body mass index; and BP, blood pressure. Continuous variables are presented as median (lower quartile, upper quartile); categorical variables are presented as percentages of total number.

**P* value represents nonparametric Wilcoxon rank sum test to compare continuous variables.

†*P* value represents Pearson chi-square test to compare categorical variables.

‡Non-White includes Black,Asian, and Native Hawaiian/Other Pacific Islander.

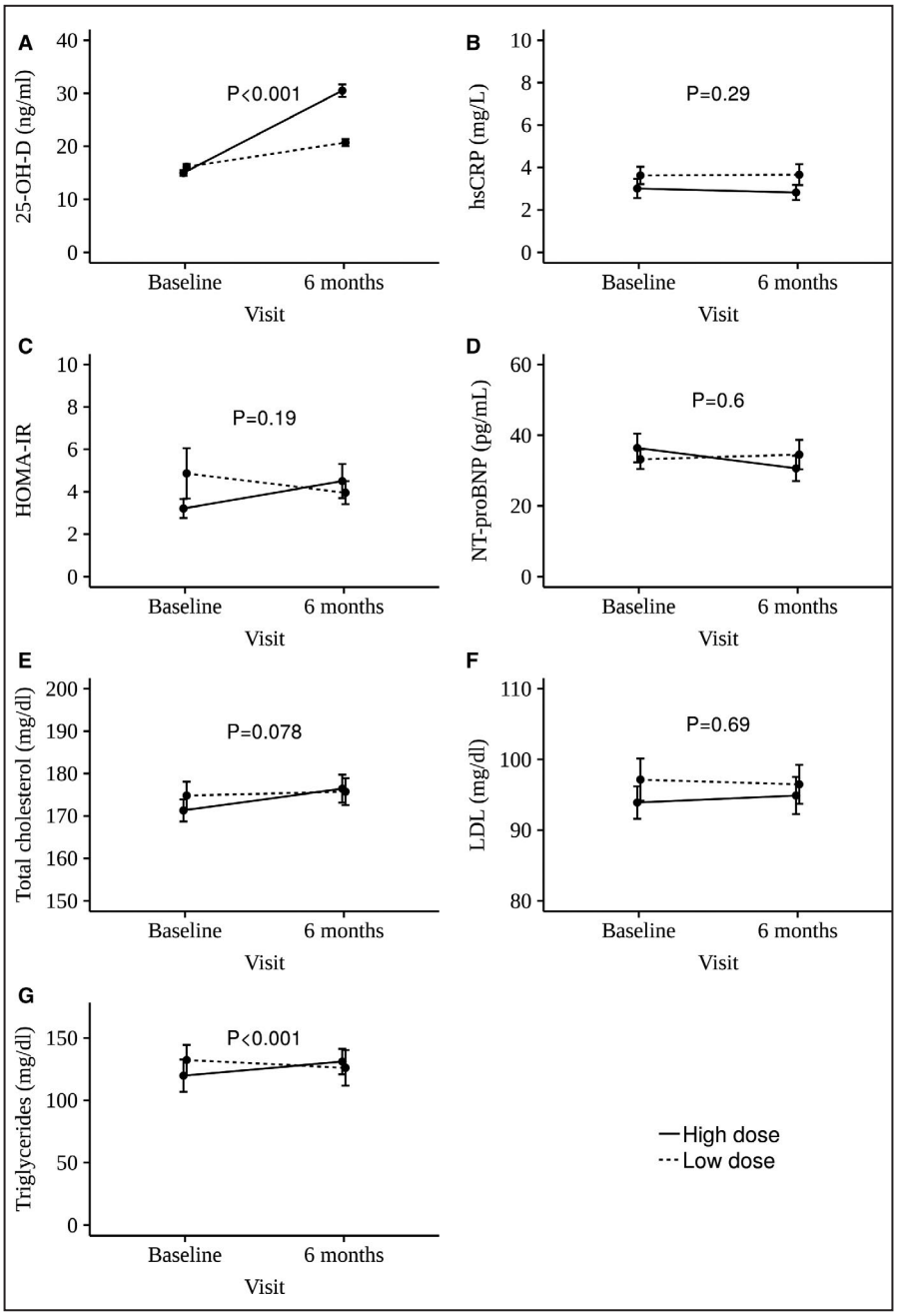

Figure 1. Circulating biomarkers in groups receiving low-dose (400 IU/d) or high-dose (4000 IU/d) vitamin D supplementation over 6 months.
Mean change ±1 standard error in 25-hydroxyvitamin D (25-OH-D; **A**), high-sensitivity C-reactive protein (hs-CRP; **B**), homeostatic model assessment of insulin resistance (HOMA-IR; **C**), NT-proBNP (N-terminal pro-B-type natriuretic peptide; **D**), total cholesterol (**E**), low-density lipoprotein (LDL; **F**), and triglycerides (**G**) in the high-dose and low-dose vitamin D supplementation groups are reported.

examined the effect of vitamin D supplementation on cardiometabolic biomarkers, they have typically had shorter follow-up periods,[33–40] investigated a limited set of biomarkers,[33–37,39,41–44] and had less statistical power.[25,33–40,42–46] Distinctive features of the present study include the large, well-powered sample; assessment of multiple cardiometabolic biomarkers at once; and a large contrast in vitamin D dosing. Additionally, while several RCTs have had negative cardiovascular disease end points, these were studied in select populations[5,47–49] or included individuals without vitamin D deficiency,[6,7] suggesting that adequate

Table 3. Circulating Glycemic, Inflammatory, Neurohormonal, 25-OH-D, and Lipid Biomarkers at Baseline and 6-Month Follow-Up

Biomarker	Low-Dose Vitamin D Group (n=144)				High-Dose Vitamin D Group (n=145)				*P* Value*
	Baseline	Month 6	Change	*P* Value†	Baseline	Month 6	Change	*P* Value†	
hs-CRP, mg/L	1.9 (0.9, 4.4)	1.8 (0.8, 4.0)	−0.1 (−1.2, 0.8)	0.29	1.2 (0.6, 3.3)	1.4 (0.7, 3.3)	0.02 (−0.6, 0.8)	0.67	0.29
Fasting plasma glucose, mg/dL	79 (70, 91)	79 (69, 92)	0.0 (−10.0, 9.5)	0.96	78 (69, 89)	80 (72, 91)	1.0 (−10.0, 13.0)	0.22	0.46
Insulin, μU/mL	10.9 (5.8, 21.6)	9.3 (6.5, 15.9)	0.2 (−5.5, 3.9)	0.38	9.3 (5.5, 15.8)	10.4 (6.0, 18.5)	0.6 (−3.1, 5.2)	0.15	0.10
HOMA-IR	2.1 (1.1, 4.6)	1.8 (1.1, 3.5)	−0.04 (−1.2, 0.9)	0.50	1.7 (1.0, 3.1)	1.9 (1.1, 3.9)	0.09 (−0.7, 1.2)	0.22	0.19
NT-proBNP, pg/mL	22.4 (9.7, 44.3)	14.9 (8.3, 42.0)	−0.7 (−17.4, 8.7)	0.14	20.5 (10.8, 40.0)	19.4 (6.8, 36.5)	−3.5 (−15.0, 5.6)	0.02	0.60
Renin, pg/mL	9.4 (4.1, 16.3)	8.3 (5.1, 14.6)	−0.4 (−6.3, 5.0)	0.58	9.1 (4.2, 14.7)	8.8 (4.4, 15.3)	0.0 (−3.5, 3.5)	0.78	0.83
Aldosterone, ng/dL	11.0 (7.8, 13.7)	10.6 (7.9, 14.7)	−0.4 (−3.0, 2.6)	0.44	9.4 (6.5, 14.1)	10.9 (7.3, 14.5)	0.2 (−2.0, 3.7)	0.25	0.16
Total cholesterol, mg/dL	170 (148, 195)	169 (150, 197)	−1.0 (−14.0, 16.3)	0.84	170 (152, 189)	175 (149, 196)	5.0 (−7.0, 18.8)	0.01	0.078
Triglyceride, mg/dL	100 (71, 148)	98 (68, 132)	−6.5 (−29.0, 16.5)	0.02	88 (68, 118)	96 (68, 141)	7.0 (−16.8, 31.0)	0.02	<0.001
HDL, mg/dL	50 (45, 58)	53 (47, 64)	3.0 (−2.0, 8.0)	<0.001	54 (46, 64)	55 (47, 65)	2.0 (−4.0, 8.0)	0.048	0.25
LDL, mg/dL	91 (72, 121)	94 (72, 113)	1.0 (−13.3, 13.0)	0.94	93 (75, 112)	94 (73, 112)	0.0 (−11.0, 13.0)	0.58	0.69
25-OH-D, ng/mL	15.4 (11.7, 19.6)	20.0 (14.9, 24.8)	4.4 (−0.25, 9.4)	<0.001	14.6 (9.2, 19.7)	29.9 (21.4, 39.2)	14.3 (6.0, 21.8)	<0.001	<0.001

Data are presented as median (lower quartile, upper quartile). 25-OH-D indicates 25-hydroxyvitamin D; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

**P* value represents the Wilcoxon rank sum test of change in circulating biomarker level from baseline to 6 months between the 2 treatment arms.

†*P* value represents the paired Wilcoxon signed rank test of change in circulating biomarker level from baseline to 6 months within a treatment arm.

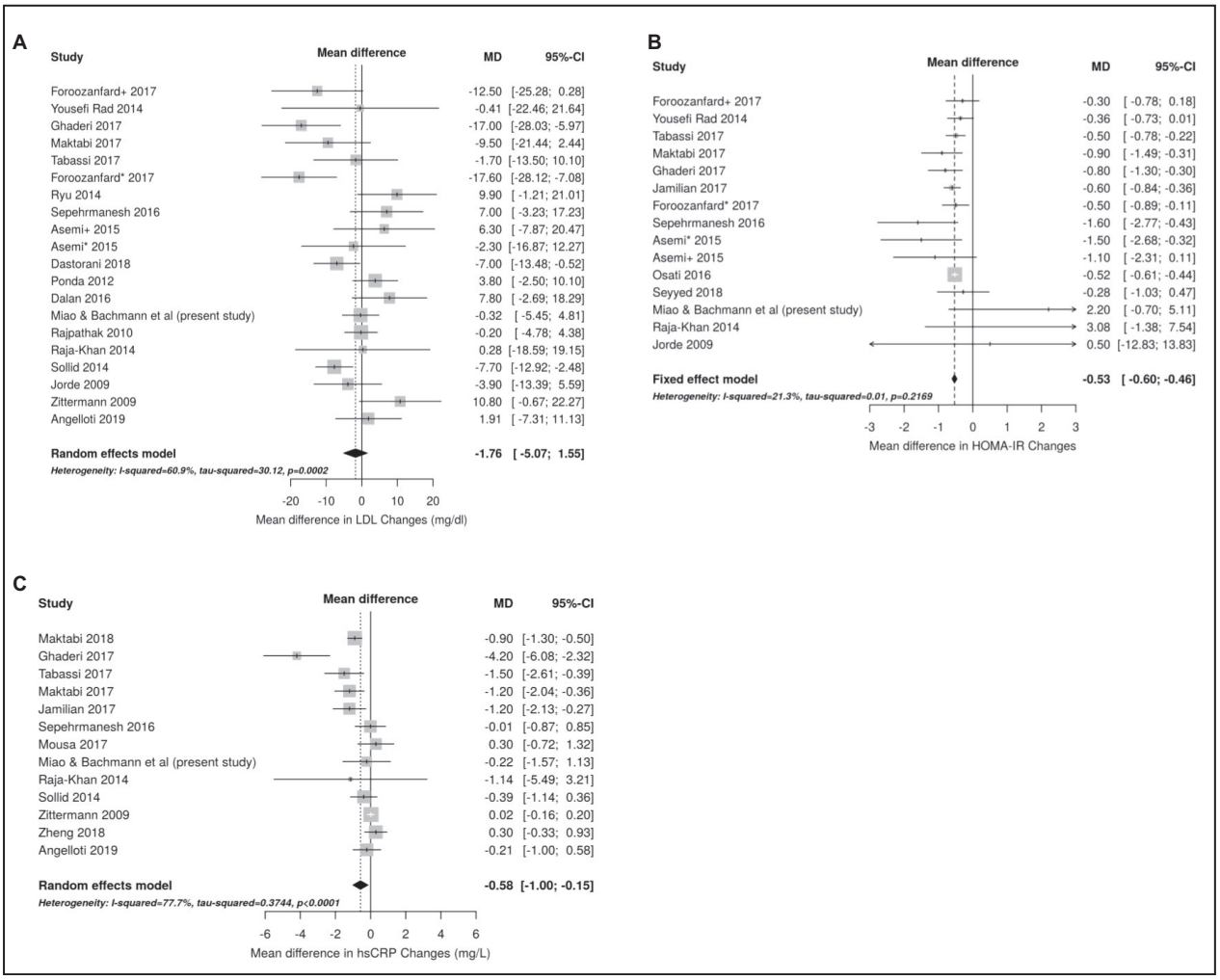

Figure 2. Forest plots comparing effect size for vitamin D supplementation on changes in biomarkers for studies included in the meta-analysis.

Changes in low-density lipoprotein (LDL; **A**), homeostatic model assessment of insulin resistance (HOMA-IR; **B**), and high-sensitivity C-reactive protein (hs-CRP; **C**) are reported. The gray boxes correspond with study precision, and the lines denote 95% CIs. Studies are ordered by the cumulative vitamin D dose delivered during the course of the study (vitamin D dose×duration), from lowest to highest. The asterisks and plus sign denote combinations of vitamin D with or without calcium supplementation for two different treatment arms enrolled in the same RCT. Asemi* indicates vitamin D 50 000 IU weekly+calcium 1000 mg daily whilst Asemi+ group received vitamin D 50 000 IU weekly; Foroozanfard*, vitamin D 4000 IU daily; Foroozanfard+, vitamin D 1000 IU daily; and MD, mean difference. References: Foroozanfard+ 2017,[11] Yousefi Rad 2014,[12] Ghaderi 2017,[13] Maktabi 2017,[14] Tabassi 2017,[15] Foroozanfard* 2017,[11] Ryu 2014,[16] Sepehrmanesh 2016,[17] Asemi+ 2015,[18] Asemi * 2015,[18] Dastorani 2018,[19] Ponda 2012,[20] Dalan 2016,[21] Rajpathak 2010,[10] Raja-Khan 2014,[22] Sollid 2014,[23] Jorde 2009,[24] Zittermann 2009,[25] Angelloti 2019,[26] Jamilian 2017,[27] Osati 2016,[28] Seyyed 2018,[29] Maktabi 2018,[30] Mousa 2017,[31] and Zheng 2018.[32]

baseline levels of circulating vitamin D may alter neurohormonal and metabolic pathways on a molecular basis without directly influencing cardiovascular morbidity and mortality. It remains unclear whether vitamin D may still influence molecular pathways. Thus, we enrolled otherwise healthy individuals with low vitamin D status, rather than all-comers, to examine whether correcting vitamin D deficiency with supplementation may improve intermediate biomarkers in these molecular pathways. We were able to evaluate changes in biomarkers involved in neurohormonal activation in this present analysis, and compare these with previously published findings from the DAYLIGHT trial, which found that vitamin D supplementation did not reduce BP. Further, in contrast to some studies, we were able to achieve a reasonably large difference in circulating 25-OH-D levels between the 2 dose groups, facilitating assessment of the influence of vitamin D on metabolic and cardiovascular pathways.

Prior experimental and observational studies in humans have supported a link between vitamin D and glucose homeostasis. Vitamin D deficiency leads to impaired insulin secretion and insulin sensitivity in animals,[50,51] which is corrected by vitamin D repletion.[51,52]

In humans, observational studies have demonstrated that low vitamin D status is associated with glucose intolerance and type 2 diabetes mellitus.[53,54] Results of prior RCTs have been substantially more mixed. While some randomized studies have suggested that vitamin D supplementation leads to modest improvements in glucose homeostasis,[28,35,38,55,56] others have not.[34,40,41,57–61] Most prior trials have been small to moderately sized, with the exception of a substudy of the Women's Health Initiative, which found no benefit of combined calcium and very low-dose (400 IU) vitamin D supplementation on glucose homeostasis in a large sample of older, postmenopausal women.[60] Our secondary analysis of the DAYLIGHT trial supports the conclusion that vitamin D does not influence glucose homeostasis in a large, demographically diverse sample that includes men and younger individuals. To further examine this question, we performed a meta-analysis of the current study with prior RCTs assessing HOMA-IR as an end point. The meta-analysis results suggest a significant, but modest reduction in HOMA-IR with vitamin D supplementation.

Experimental studies have also suggested a link between vitamin D and other key pathways involved in vascular and cardiac function,[62–66] including the neurohormonal system. In human observational studies, 25-OH-D concentrations are inversely associated with decreased plasma renin activity[67–69] and NT-proBNP levels among patients with heart failure.[70] However, causality cannot be established by observational studies. Prior randomized studies examining the influence of vitamin D supplementation on renin-angiotensin-aldosterone system are limited[71–73] and have largely been negative.[71,72] Similarly, randomized studies of the effects of vitamin D supplementation on B-type natriuretic peptide levels in humans have mostly been limited to patients with significant comorbidities, and have produced inconsistent findings.[72,74,75] Several RCTs have sought to evaluate the effects of vitamin D supplementation on cardiac biomarkers and congestive heart failure end points among patients with chronic heart failure, including 6-minute walk test, left ventricular remodeling, and NT-proBNP levels, with pooled results demonstrating no significant effect of vitamin D supplementation on improvement in NT-proBNP.[76] In our relatively healthy population, we found that vitamin D supplementation does not significantly affect the renin-angiotensin-aldosterone system or natriuretic peptide levels. Our study extends the literature by examining the effects of vitamin D supplementation on cardiac and vascular biomarkers in a large cohort of patients without significant comorbidities that may have influenced the results of prior studies. Moreover, we examined these effects after an extensive duration of adequate vitamin D supplementation that produced a substantial difference in vitamin D levels between dose groups. Our findings that improvements in vitamin D status did not appear to influence neurohormonal activation or cardiac wall stress are concordant with the largely negative findings regarding the effect of vitamin D and/or calcium supplementation on BP, vascular function, and cardiac remodeling.[8,10,77–79]

Similarly, our findings do not support an effect of vitamin D supplementation on lipids. Experimental studies indicate that activated vitamin D decreases oxidized LDL uptake, inhibits foam cell formation, and promotes a lineage shift from M2-predominant macrophages to M1-predominant macrophages.[65,80] While several randomized trials have suggested that vitamin D supplementation may improve lipid profiles in humans, others have not.[25,33,36,37,40] Some of these studies were performed in the setting of weight loss intervention, which could have independently improved lipid profiles.[33,42] We observed an unexpected increase in plasma triglycerides in the high-dose vitamin D group. This is likely a chance finding attributable to the number of statistical tests and differences in the baseline levels of triglycerides between arms, which may have led to regression to the mean. The results of the DAYLIGHT substudy are consistent with our meta-analysis of RCTs examining vitamin D supplementation and LDL concentrations, which found no benefit of vitamin D supplementation.

Systemic inflammation contributes to atherogenesis, and markers of inflammation are robustly related to vascular risk. Activated vitamin D has potent anti-inflammatory effects, inhibiting the production of multiple cytokines, including interleukin (IL) 2 and IL-6.[81] IL-6 induces the production of hs-CRP, a well-established downstream marker of inflammation that is also associated with risk of cardiovascular disease and type 2 diabetes mellitus.[82–84] In the present study, we found no effect of the dose of vitamin D supplementation on levels of hs-CRP. These findings are concordant with those of some prior randomized trials[25,36,40,42,85] but not others.[13–15,27,30] Our meta-analysis demonstrated a borderline significant decrease in hs-CRP following vitamin D supplementation.

The present study has several limitations. Vitamin D deficiency has been traditionally classified based on its relation to bone health. Thus, a limitation with the present study and other vitamin D studies is that the serologic threshold, below which vitamin D levels could influence cardiometabolic parameters, if it does at all, is unknown. Also, DAYLIGHT trial participants were enrolled based on mean systolic BP between 120 mm Hg and 159 mm Hg and diastolic BP <99 mm Hg, off antihypertensive therapies, and without significant comorbidities. Therefore, the overall negative substudy results may not accurately represent the potential downstream effects of vitamin D supplementation on

cardiometabolic biomarkers in other study cohorts with heart failure or chronic kidney disease, for example. Additionally, although the DAYLIGHT trial is one of the larger randomized studies analyzing the effects of vitamin D supplementation on cardiometabolic biomarkers, it is possible that we were still underpowered to detect changes. The point estimates suggest that, even if vitamin D supplementation had a significant influence on any individual biomarker, the effect would likely have been small. Moreover, 6 months may not have been a sufficiently long duration for vitamin D supplementation to lead to improvement in cardiovascular and metabolic parameters. Additionally, the liquid formulation of vitamin D supplementation in the DAYLIGHT trial may differ in bioavailability in comparison to conventional tablets, although this is not well understood. Compliance may also have been a potential limitation, although the liquid formulation permitted accurate assessment of compliance during each visit by weighing bottles on a calibrated gravimetric scale, and noncompliant participants were withdrawn early by study investigators. Nonetheless, the treatment duration was sufficient to correct vitamin D deficiency in a substantial proportion of individuals in the high-dose arm, and experimental studies do not suggest a mechanism for a delayed response with regard to these pathways.

CONCLUSIONS

In our analysis of the DAYLIGHT trial results, we did not find evidence that vitamin D supplementation has beneficial effects on markers of glycemia, neurohormonal activation, inflammation, or lipid status. These findings are concordant with our meta-analysis of studies examining the effects of vitamin D supplementation on LDL. On the other hand, our meta-analysis suggested potential modest improvement in HOMA-IR and hs-CRP following vitamin D supplementation, although these findings are limited by high heterogeneity observed in studies measuring hs-CRP. Additionally, it is difficult to ascertain how much these modest changes can affect clinical outcomes or have a clinically meaningful impact. As further context, we note that several prior studies have been performed using a Mendelian randomization framework, using genetic variation in vitamin D as the exposure and cardiometabolic end points as the outcome.[86,87] These studies have yielded findings that are largely consistent with ours, eg, they have not established a causal relationship between vitamin D genetic variation and cardiometabolic end points.

ARTICLE INFORMATION

Received August 12, 2020; accepted February 15, 2021.

Affiliations

Department of Medicine, Vanderbilt University Medical Center, Nashville, TN (J.M.); Veterans Health Administration, Tennessee Valley Healthcare System, Nashville, TN (K.N.B.); Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN (K.N.B.); Vanderbilt Translational and Clinical Cardiovascular Research Center, Vanderbilt University School of Medicine, Nashville, TN (K.N.B., S.H.); Department of Biostatistics (S.H.); and Division of Cardiovascular Medicine, Department of Medicine (Y.R.S.), Vanderbilt University Medical Center, Nashville, TN; Department of Family Medicine and Community Health, Case Western University Medical Center, Cleveland, OH (J.D.); Department of Cardiology, Massachusetts General Hospital/Harvard Medical School, Boston, MA (C.N.); Division of Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL (P.A.); Section of Cardiology, Birmingham Veterans Affairs Medical Center, Birmingham, AL (P.A.); and Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX (T.J.W.).

Acknowledgments

We would like to thank Kelsey Tomasek for her assistance running the biomarker assays for this work.

Sources of Funding

The parent study was funded by an investigator-initiated grant from DiaSorin Inc. Support for some of the assays in the substudy was provided by Diasorin. Diasorin was not involved in the design or conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation of the article for publication. This work was also supported by Career Development Award #IK2 CX001678 (Bachmann) from the US Department of Veterans Affairs Clinical Sciences Research and Development Program, Vanderbilt University Medical Center Faculty Research Scholars Award (Bachmann), and American Heart Association Career Development Award 18CDA34110135 (Arora).

Disclosures

Dr Wang reports consultant fees from DiaSorin before 2014. Dr Bachmann owns stock in Medtronic, unrelated to the current project. The remaining authors have no disclosures to report.

Supplementary Material

Tables S1–S6
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SUPPLEMENTAL MATERIAL

Table S1. Number of participants (n) at baseline and 6 months for individual biomarkers.

Biomarker	Baseline	Month 6
hsCRP	281	281
Glucose	282	282
Insulin	281	281
HOMA-IR	281	279
NT-proBNP	281	281
Renin	251	274
Aldosterone	264	252
Lipid profile*	282	282
25-OH-D	288	277

*Lipid profile includes total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein; hsCRP, high-sensitivity C-Reactive Protein; Glucose, Fasting Plasma Glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NT-proBNP, N-terminal pro-B-type natriuretic peptide; 25-OH-D, 25-hydroxyvitamin D.

Table S2. Circulating glycemic, inflammatory, neurohormonal, and lipid biomarkers at baseline and 6-month follow-up, stratified by baseline vitamin D level.

Biomarker	Low-dose Vitamin D Group			High-dose Vitamin D Group			*P-value
	Baseline	Month 6	Change	Baseline	Month 6	Change	
Baseline 25-OH-D ≤ 10 ng/ml							
hsCRP (mg/L)	2.6 (1.0, 12.5)	2.9 (1.1, 5.6)	-0.2 (-1.7, 0.5)	2.0 (0.7, 4.1)	1.7 (0.7, 3.5)	0.03 (-1.0, 1.0)	0.4
Glucose (mg/dL)	77.0 (71.0, 88.0)	77.0 (68.0, 94.0)	0.0 (-10.0, 4.0)	79.0 (70.0, 92.0)	81.0 (73.0, 92.0)	4.0 (-8.5, 13.0)	0.3
Insulin (μU/mL)	13.0 (9.2, 23.6)	9.1 (6.0, 23.0)	-0.2 (-7.6, 4.0)	8.0 (4.4, 16.3)	11.6 (5.9, 18.2)	0.5 (-4.4, 5.7)	0.5
HOMA-IR	2.7 (1.5, 6.5)	1.8 (0.9, 5.3)	-0.2 (-1.3, 0.3)	1.5 (0.8, 3.5)	2.4 (1.1, 4.0)	0.06 (-1.1, 1.0)	0.4
NT-proBNP (pg/mL)	27.8 (7.7, 42.4)	21.7 (9.8, 67.1)	1.5 (-6.3, 18.3)	23.6 (11.8, 42.2)	21.4 (7.1, 39.4)	-5.6 (-17.6, 9.0)	0.09
Renin (pg/mL)	6.3 (3.9, 16.1)	7.6 (3.3, 14.5)	-1.2 (-3.5, 3.1)	7.3 (3.5, 10.6)	7.0 (5.1, 16.3)	0.2 (-1.2, 4.2)	0.3
Aldosterone (ng/dL)	10.9 (8.3, 13.1)	9.6 (7.2, 11.9)	-0.5 (-2.9, 1.6)	9.4 (6.3, 12.6)	10.9 (6.3, 13.9)	-0.03 (-1.7, 3.8)	0.3
Total Cholesterol (mg/dL)	172.0 (150.0, 198.0)	160.0 (143.0, 200.0)	-8.0 (-15.0, 8.0)	162.0 (152.0, 193.0)	169.0 (146.0, 205.0)	8.0 (-13.0, 21.0)	0.08
Triglyceride (mg/dL)	102.0 (72.0, 150.0)	94.0 (68.0, 134.0)	-18.0 (-28.0, .0)	85.0 (70.0, 110.0)	97.0 (73.0, 146.0)	7.0 (-19.0, 37.0)	0.04
HDL (mg/dL)	49.0 (44.0, 60.0)	54.0 (44.0, 64.0)	1.5 (-2.0, 7.8)	60.0 (53.0, 74.0)	61.0 (52.0, 72.0)	1.0 (-7.5, 8.0)	0.3
LDL (mg/dL)	94.0 (67.0, 115.0)	94.0 (68.0, 105.0)	-5.0 (-15.3, 5.3)	86.0 (68.0, 100.0)	86.0 (70.0, 110.0)	4.0 (-6.5, 16.5)	0.1
Baseline 25-OH-D > 10 and < 20 ng/ml							
hsCRP (mg/L)	1.9 (1.1, 4.7)	1.9 (0.9, 3.9)	-0.3 (-1.4, 0.5)	1.2 (0.5, 2.5)	1.3 (0.7, 2.5)	-0.2 (-0.7, 0.8)	0.5
Glucose (mg/dL)	79.0 (70.0, 90.0)	80.0 (72.0, 91.0)	-1.0 (-10.0, 13.0)	79.0 (68.0, 89.0)	80.0 (70.0, 88.0)	-0.5 (-10.3, 13.0)	0.9
Insulin (μU/mL)	11.2 (6.1, 22.2)	9.5 (6.2, 14.9)	-0.2 (-7.1, 3.6)	9.3 (6.1, 14.9)	11.0 (6.2, 18.3)	1.1 (-3.1, 5.0)	0.09
HOMA-IR	2.2 (1.1, 4.4)	1.9 (1.2, 3.3)	-0.04 (-1.6, 1.2)	1.6 (1.1, 3.1)	2.1 (1.2, 3.3)	0.1 (-0.6, 1.2)	0.2
NT-proBNP (pg/mL)	20.8 (9.1, 48.8)	14.0 (8.1, 39.6)	-0.07 (20.0, 8.7)	19.0 (10.5, 38.4)	15.4 (5.0, 33.2)	-3.8 (-9.8, 0.6)	0.5
Renin (pg/mL)	9.8 (3.7, 18.5)	8.4 (4.5, 14.5)	-0.4 (-7.0, 4.1)	9.6 (3.4, 15.1)	8.8 (3.7, 14.5)	0.0 (-3.4, 4.0)	0.7
Aldosterone (ng/dL)	10.9 (7.8, 13.6)	9.9 (7.5, 14.4)	-0.7 (-2.9, 2.6)	8.8 (6.5, 13.9)	11.2 (8.1, 15.0)	0.7 (-1.0, 4.5)	0.06
Total Cholesterol (mg/dL)	178.0 (153.0, 196.0)	175.0 (152.0, 199.0)	2.0 (-15.0, 16.0)	173.0 (152.0, 187.0)	179.0 (156.0, 195.0)	7.5 (-5.0, 19.0)	0.07
Triglyceride (mg/dL)	98.0 (74.0, 148.0)	98.0 (70.0, 135.0)	-5.0 (-29.0, 20.0)	89.0 (68.0, 125.0)	100.0 (68.0, 131.0)	7.0 (-6.0, 28.0)	0.045
HDL (mg/dL)	50.0 (45.0, 59.0)	52.0 (45.0, 64.0)	2.0 (-2.0, 7.0)	50.0 (44.0, 59.0)	52.0 (46.0, 64.0)	3.0 (-1.0, 9.0)	0.2
LDL (mg/dL)	97.0 (76.0, 122.0)	96.0 (73.0, 115.0)	2.0 (-16.0, 13.0)	96.0 (74.0, 114.0)	100.0 (76.0, 113.0)	2.0 (-8.8, 11.0)	0.7
Baseline 25-OH-D ≥ 20 ng/ml							
hsCRP (mg/L)	1.4 (0.5, 2.3)	1.1 (0.4, 3.3)	0.1 (-0.6, 1.3)	1.1 (0.5, 2.0)	1.2 (0.6, 3.6)	0.1 (-0.4, 0.8)	0.9
Glucose (mg/dL)	78.0 (72.0, 100.0)	78.0 (70.0, 96.0)	2.0 (-9.5, 8.5)	75.0 (71.0, 84.0)	79.0 (69.0, 94.0)	1.0 (-8.5, 12.8)	0.6
Insulin (μU/mL)	9.1 (5.5, 17.1)	9.3 (6.6, 20.4)	-0.3 (-2.7, 4.4)	8.3 (5.4, 15.2)	9.5 (5.4, 19.6)	1.0 (-2.4, 6.6)	0.7
HOMA-IR	1.5 (1.0, 4.5)	1.8 (1.3, 4.1)	0.07 (-0.5, 1.0)	1.7 (0.9, 3.0)	1.9 (1.0, 4.1)	0.009 (-0.5, 1.3)	0.9
NT-proBNP (pg/mL)	21.0 (14.0, 41.0)	14.8 (9.1, 47.0)	-4.0 (16.4, 2.9)	22.0 (14.0, 38.0)	22.8 (12.2, 43.2)	-1.2 (-11.4, 9.9)	0.2
Renin (pg/mL)	8.6 (5.0, 14.9)	9.2 (6.0, 14.7)	-0.3 (-5.3, 7.5)	10.6 (6.8, 20.1)	9.7 (4.3, 14.2)	-1.4 (-8.6, 2.3)	0.2
Aldosterone (ng/dL)	11.2 (7.3, 16.6)	12.3 (8.9, 17.9)	0.3 (-3.2, 4.4)	11.7 (7.8, 18.2)	10.2 (7.5, 15.4)	-0.7 (-4.5, 2.8)	0.6
Total Cholesterol (mg/dL)	162.0 (140.0, 192.0)	164.0 (151.0, 186.0)	-2.0 (-9.5, 19.5)	172.0 (154.0, 190.0)	175.0 (152.0, 190.0)	1.5 (-13.0, 7.3)	0.5
Triglyceride (mg/dL)	99.0 (65.0, 143.0)	99.0 (62.0, 126.0)	-5.0 (-30.0, 6.5)	88.0 (62.0, 120.0)	94.0 (74.0, 135.0)	3.5 (-10.8, 24.8)	0.03
HDL (mg/dL)	49.0 (45.0, 56.0)	53.0 (49.0, 62.0)	5.0 (0.5, 9.5)	53.0 (47.0, 63.0)	54.0 (47.0, 63.0)	-1.0 (-5.8, 6.0)	0.003
LDL (mg/dL)	82.0 (72.0, 100.0)	86.0 (72.0, 113.0)	2.0 (-9.0, 11.5)	96.0 (82.0, 111.0)	96.0 (74.0, 108.0)	-4.5 (-12.8, 1.5)	0.1

Data presented as median (lower quartile, upper quartile); 25-OH-D, 25-hydroxyvitamin D; hsCRP, high-sensitivity C-Reactive Protein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HDL, High-density Lipoprotein; LDL, Low-density Lipoprotein. **P*-value refers to the difference between the change for low-dose group vs. change for high-dose group.

Table S3. Circulating glycemic, inflammatory, neurohormonal, and lipid biomarkers at baseline and 6-month follow-up, stratified by vitamin D status at the end of the study.

Biomarker	Low-dose Vitamin D Group			High-dose Vitamin D Group		
	Baseline	Month 6	Change	Baseline	Month 6	Change
Final 25-OH-D > 20 ng/ml						
hsCRP (mg/L)	1.9 (0.9, 3.3)	1.5 (0.7, 2.4)	-0.3 (-1.2, 0.5)	1.2 (0.6, 3.2)	1.3 (0.7, 3.2)	0.02 (-0.6, 0.8)
Glucose (mg/dL)	81.0 (74.0, 96.0)	79.0 (69.0, 92.0)	-1.0 (-12.0, 8.0)	78.0 (69.0, 87.0)	79.0 (70.0, 88.0)	0.5 (-9.0, 12.8)
Insulin (µU/mL)	9.8 (5.3, 18.3)	7.5 (5.7, 14.5)	-0.4 (-5.2, 4.2)	8.3 (5.1, 14.0)	7.9 (5.2, 14.5)	-0.1 (-3.4, 4.3)
HOMA-IR	1.7 (1.1, 3.9)	1.5 (1.0, 3.4)	-0.06 (-1.2, 1.1)	1.4 (0.9, 2.9)	1.5 (1.0, 2.9)	-0.05 (-0.7, 0.9)
NT-proBNP (pg/mL)	20.8 (8.6, 40.2)	14.8 (9.5, 41.0)	-0.07 (-16.1, 9.8)	20.5 (10.6, 39.3)	18.3 (6.9, 37.1)	-2.7 (-11.4, 5.5)
Renin (pg/mL)	10.2, (4.5, 17.5)	10.3 (6.7, 15.5)	0.0 (-6.3, 5.1)	8.9 (4.5, 14.5)	9.0 (5.2, 15.6)	0.05 (-3.4, 4.7)
Aldosterone (ng/dL)	12.3 (8.5, 15.7)	11.7 (9.2, 16.3)	-0.05 (-4.5, 3.0)	9.4 (6.5, 14.1)	11.7 (8.1, 14.9)	0.3 (-1.8, 3.9)
Total Cholesterol (mg/dL)	168.0 (144.0, 191.0)	166.0 (147.0, 188.0)	-2.0 (-11.0, 9.0)	170.0 (152.0, 188.0)	175.0 (147.0, 196.0)	5.0 (-12.5, 17.0)
Triglyceride (mg/dL)	99.0 (70.0, 147.0)	92.0 (65.0, 124.0)	-6.0 (-33.0, 11.0)	88.0 (68.0, 122.0)	98.0 (70.0, 140.0)	7.0 (-15.0, 31.0)
HDL (mg/dL)	53.0 (45.0, 59.0)	53.0 (48.0, 64.0)	2.0 (-2.0, 7.0)	54.0 (46.0, 63.0)	54.0 (46.0, 63.0)	1.0 (-4.0, 6.0)
LDL (mg/dL)	83.0 (74.0, 110.0)	88.0 (72.0, 113.0)	0.0 (-10.0, 9.0)	92.0 (75.0, 112.0)	95.0 (73.0, 112.0)	-1.5 (-11.8, 13.8)
Final 25-OH-D ≤ 20 ng/ml						
hsCRP (mg/L)	1.8 (0.7, 5.6)	2.4 (0.9, 5.4)	-0.05 (-1.0, 1.2)	1.2 (0.6, 2.2)	2.0 (0.7, 3.2)	-0.2 (-0.5, 1.1)
Glucose (mg/dL)	77.0 (70.0, 89.0)	77.0 (69.0, 91.0)	0.0 (-8.0, 9.0)	82.0 (73.0, 92.0)	81.0 (75.0, 94.0)	1.0 (-11.0, 13.0)
Insulin (µU/mL)	12.3 (5.9, 28.0)	9.8 (7.1, 16.6)	0.03 (-7.0, 3.7)	11.8 (5.6, 17.2)	14.2 (11.3, 23.2)	3.7 (-2.6, 9.8)
HOMA-IR	2.3 (1.1, 5.3)	1.9 (1.2, 3.5)	-0.04 (-1.5, 0.6)	2.3 (1.3, 4.0)	2.8 (2.1, 5.5)	0.6 (-0.9, 1.8)
NT-proBNP (pg/mL)	25.0 (10.0, 49.0)	14.9 (7.2, 41.7)	-3.5 (-19.5, 6.4)	24.0 (13.0, 41.0)	19.9 (6.7, 33.4)	-6.8 (-17.8, 0.6)
Renin (pg/mL)	9.4 (3.9, 16.1)	7.1 (3.7, 13.0)	-0.6 (-5.8, 3.5)	9.2 (3.7, 17.3)	6.1 (3.0, 14.0)	-0.3 (-4.9, 2.5)
Aldosterone (ng/dL)	10.6 (7.3, 12.4)	9.0 (7.1, 12.9)	-0.6 (-2.6, 2.4)	9.4 (6.9, 14.3)	10.0 (6.8, 12.8)	0.2 (-1.7, 2.7)
Total Cholesterol (mg/dL)	170.0 (152.0, 196.0)	175.0 (156.0, 198.0)	7.0 (-14.5, 20.5)	167.0 (159.0, 189.0)	176.0 (162.0, 205.0)	11.0 (-3.0, 22.0)
Triglyceride (mg/dL)	103.0 (74.0, 148.0)	101.0 (71.0, 144.0)	-10.0 (-25.0, 19.0)	88.0 (68.0, 113.0)	94.0 (66.0, 121.0)	7.0 (-16.0, 29.0)
HDL (mg/dL)	49.0 (45.0, 58.0)	53.0 (45.0, 65.0)	4.0 (-1.0, 9.5)	53.0 (47.0, 64.0)	59.0 (50.0, 70.0)	6.0 (-3.0, 10.0)
LDL (mg/dL)	95.0 (71.0, 122.0)	95.0 (73.0, 113.0)	2.0 (-13.5, 14.0)	94.0 (80.0, 112.0)	95.0 (72.0, 107.0)	3.0 (-4.0, 11.0)

Data presented as median (lower quartile, upper quartile); 25-OH-D, 25-hydroxyvitamin D; hsCRP, high-sensitivity C-Reactive Protein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HDL, High-density Lipoprotein; LDL, Low-density Lipoprotein. **P*-value refers to the difference between the change for low-dose group vs. change for high-dose group.

Table S4. General characteristics of studies investigating effects of vitamin D supplementation on LDL.

Author	Year	Country	Subject Characteristics	Mean Age, Control	Mean Age, Intervention	Sex (n) male	Intervention (n)	Control (n)	Baseline 25 OH-D (ng/mL		Vit D Dose (IU/day)	Duration (Weeks)	Delta, Intervention	Delta, Control	p-value
									Intervention	Control					
Angelloti [26]	2019	USA	T2D	60.3	60.1	89	66	61	25.8 (10.3)	27.5 (12.0)	4000	48	-0.07 (3.3)	-2.0 (3.4)	0.7
Dastorani [19]	2018	Iran	PCOS	30.1	29.9	0	20	20	10.5 (2.5)	11.0 (2.4)	7142	8	-4.5 (10.3)	2.5 (10.6)	0.04
Foroozanfard [11]	2017	Iran	PCOS	NR	NR	0	60	30	13.5 (3.1) (high dose) 14.0 (4.6) (low dose)	14.0 (3.5)	4000	12	-10.8 (8.3) (high dose) -5.7 (21.9) (low dose)	6.8 (28.2)	0.005
Ghaderi [13]	2017	Iran	MMT	42.5	40.1	NR	34	34	13.9 (4.5)	13.5 (4.5)	3571	12	-11.1 (17.9)	5.9 (27.5)	0.004
Dalan [21]	2016	Singapore	T2D	54.8	52.2	33	33	31	18.0 (7.0)	17.0 (11.0)	4000	16	0.1 (0.5)	-0.1 (0.6)	0.2
Maktabi [14]	2017	Iran	PCOS	23.1	22.0	0	35	35	12.8 (4.5)	14.5 (5.1)	3571	12	-6.3 (24.1)	3.2 (26.8)	0.1
Tabassi [15]	2017	Iran	Endometrial hyperplasia	43.2	41.5	0	30	30	NR	NR	3571	12	0.9 (4.0)	3.3 (4.0)	0.7
Sepehrmanesh [17]	2015	Iran	MDD	36.1	36.5	6	20	20	13.6 (7.9) µg/L	9.2 (6.0) µg/L	7142	8	4.0 (17.0)	-3.0 (16.0)	0.3
Asemi [18]	2015	Iran	PCOS, obesity	24.3	25.0	0	26	26	13.9 (2.0) (Calcium) 11.6 (4.7) (Vit D) 15.1 (3.6) (Calcium+ Vit D)	14.0 (4.1)	7142	8	-4.5 (20.8)	-2.2 (31.7)	0.4
Yousefi Rad [12]	2014	Iran	T2D	49.9	50.0	12	28	30	15.6 (1.9)	14.6 (2.2)	4000	8	0.9 (7.2)	1.3 (8.7)	1.0
Ryu [16]	2014	South Korea	T2D	56.7	54.5	NR	32	30	12.3 (3.0)	10.7 (2.6)	2000	24	10.2 (17.5)	0.3 (26.0)	0.09
Sollid [23]	2014	Norway	Prediabetes	61.9	62.3	314	256	255	23.9 (8.8)	24.4 (8.5)	2857	52	-0.1 (0.7)	0.09 (0.66)	<0.05
Raja-Khan [22]	2014	USA	PCOS	28.7	28.2	0	13	15	19.9 (9.5)	22.2 (6.9)	12000	12	-0.1 (20.6)	-0.4 (20.9)	0.9
Ponda [20]	2012	USA	Vit D deficient	47.4	48.4	75	76	75	13.4 (5.3)	14.1 (5.7)	7142	8	-0.3 (18.6)	-4.1 (20.8)	0.1
Rajpathak [10]	2010	USA	Postmenopausal	62.1	61.6	0	592	599	NR	NR	400	260	-6.5 (1.6)	-6.3 (1.7)	NS
Jorde [24]	2009	Norway	T2D	54.8	57.7	18	16	16	24.0 (5.6)	23.4 (8.4)	5714	26	-0.1 (0.4)	0.0 (0.3)	0.6
Zittermann [25]	2009	Germany	Overweight	48.8	47.4	53	82	83	30.0 (17.5)	30.3 (20.1)	3332	52	0.2 (1.0)	-0.09 (0.9)	0.2

Data are presented as mean (SD). LDL, Low-density Lipoprotein; 25-OH-D, 25-hydroxyvitamin D; IU, International Units; PCOS, Polycystic Ovarian Syndrome; MMT, Methadone Maintenance Treatment; MDD, Major Depressive Disorder; T2D, Type 2 Diabetes; NR, Not Reported; NS, Not Significant.

Table S5. General characteristics of included studies investigating effects of vitamin D supplementation on HOMA-IR.

Author	Year	Country	Subject Characteristics	Mean Age, Control	Mean Age, Intervention	Sex (n) male	Intervention (n)	Control (n)	Baseline 25 OH-D (ng/mL)		Vit D Dose (IU/day)	Duration (Weeks)	Delta, Intervention	Delta, Control	p-value
									Intervention	Control					
Seyyed [29]	2018	Iran	PCOS	22.8	26.2	0	22	22	8.65 (4.3)	9.8 (5.1)	7142	8	0.04 (1.2)	0.3 (1.4)	0.3
Maktabi [14]	2017	Iran	PCOS	23.1	22.0	0	35	35	12.8 (4.5)	14.5 (5.1)	3571	12	-0.3 (0.8)	0.6 (1.6)	0.003
Ghaderi [13]	2017	Iran	MMT	42.5	40.1	NR	34	34	13.9 (4.5)	13.5 (4.5)	3571	12	-1.0 (1.3)	-0.2 (0.7)	0.003
Jamilian [27]	2017	Iran	PCOS	25.0	26.0 (low dose) 28.0 (high dose)	0	60	30	12.6 (3.4) (low dose) 12.6 (2.7) (high dose)	12.9 (2.4)	4000 (high dose) 1000 (low dose)	12	-0.5 (0.4) (high dose) -0.3 (0.7) (low dose)	0.1 (2.6)	0.004
Tabassi [15]	2017	Iran	Endometrial hyperplasia	43.2	41.5	0	30	30	NR	NR	3571	12	-0.2 (0.1)	0.3 (0.1)	0.006
Foroozanfard [11]	2017	Iran	PCOS	NR	NR	0	60	30	13.5 (3.1) (high dose) 14.0 (4.6) (low dose)	14.0 (3.5)	4000	12	-0.4 (1.0) (low dose) -0.6 (0.6) (high dose)	-0.1 (0.9)	0.02
Sepehrmanesh [17]	2016	Iran	MDD	36.1	36.5	6	20	20	13.6 (7.9) µg/L	9.2 (6.0) µg/L	7142	8	-1.0 (1.5)	0.6 (2.2)	0.01
Osati [28]	2016	Iran	Vit D deficient	38.0	38.0	49	105	105	13.8 (3.6) mg/dL	13.9 (3.7) mg/dL	7142	8	-0.8 (0.4)	-0.3 (0.3)	<0.001
Asemi [18]	2015	Iran	PCOS, obesity	24.3	25.0	0	26	26	13.9 (2.0) (Calcium) 11.6 (4.7) (Vit D) 15.1 (3.6) (Calcium+ Vit D)	14.0 (4.1)	7142	8	-0.7 (2.4) (Calcium +Vit D)	0.8 (1.9)	0.04
Yousefi Rad [12]	2014	Iran	T2D	49.9	50.0	12	28	30	15.6 (1.9)	14.6 (2.2)	4000	8	-0.1 (0.1)	0.2 (0.1)	0.06
Raja-Khan [22]	2014	USA	PCOS	28.7	28.2	0	13	15	19.9 (9.5)	22.2 (6.9)	12000	12	2.57 (4.9)	-0.5 (4.9)	0.2
Jorde [24]	2009	Norway	T2D	54.8	57.7	18	16	16	24.0 (5.6)	23.4 (8.4)	5714	26	0.3 (23.5)	-0.2 (13.7)	0.6

Data are presented as mean (SD). HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; 25-OH-D, 25-hydroxyvitamin D; IU, International Units; PCOS, Polycystic Ovarian Syndrome; MMT, Methadone Maintenance Treatment; MDD, Major Depressive Disorder; T2D, Type 2 Diabetes; NR, Not Reported.

Table S6. General characteristics of included studies investigating effects of vitamin D supplementation on hsCRP.

Author	Year	Country	Subject Characteristics	Mean Age, Control	Mean Age, Intervention	Sex (n) male	Intervention (n)	Control (n)	Baseline 25 OH-D (ng/mL)		Vit D Dose (IU/day)	Duration (Weeks)	Delta, Intervention	Delta, Control	p-value
									Intervention	Control					
Angelloti [26]	2019	USA	T2D	60.3	60.1	89	66	61	25.8 (10.3)	27.5 (12.0)	4000	48	-0.8 (0.3)	-0.6 (0.3)	0.6
Maktabi [30]	2018	Iran	PCOS	24.8	23.8	0	30	30	10.1 (4.9)	10.8 (4.6)	400	12	-0.7 (0.8)	0.2 (0.8)	<0.001
Zheng [32]	2018	Australia	Knee OA, Vit D deficient	62.8	63.3	93	106	94	17.0 (4.7)	17.4 (5.0)	1667	104	0.3 (2.1)	0.0 (2.4)	0.4
Ghaderi [13]	2017	Iran	MMT	42.5	40.1	NR	34	34	13.9 (4.5)	13.5 (4.5)	3571	12	-2.2 (4.2)	2.0 (3.7)	<0.001
Mousa [31]	2017	Australia	Obese/ Overweight	29.5	30.5	35	28	26	12.6 (5.0)	13.7 (4.0)	4893	16	-0.1 (1.4)	-0.4 (2.3)	0.9
Jamilian [27]	2017	Iran	PCOS	25	26 (low dose) 28 (high dose)	0	30 (low dose) 30 (high dose)	30	12.6 (2.7) (high dose) 12.6 (3.4) (low dose)	12.9 (2.4)	4000 (high dose) 1000 (low dose)	12	-0.7 (1.4) (high dose) -0.5 (0.9) (low dose)	0.5 (2.4)	0.01
Maktabi [14]	2017	Iran	PCOS	23.1	22	0	35	35	12.8 (4.5)	14.5 (5.1)	3571	12	-0.7 (1.4)	0.5 (2.1)	0.009
Tabassi [15]	2017	Iran	Endometrial hyperplasia	43.2	41.5	0	30	30	NR	NR	3571	12	-1.7 (0.4)	-0.2 (0.4)	0.007
Sepehrmanesh [17]	2016	Iran	MDD	36.1	36.5	6	20	20	13.6 (7.9)	9.2 (6.0)	7142	8	0.01 (1.0)	0.02 (1.7)	1.0
Sollid [23]	2014	Norway	Prediabetes	61.9	62.3	314	256	255	24.0 (8.8)	24.4 (8.5)	2857	52	2.7 (3.2)	3.1 (4.1)	NS
Raja-Khan [22]	2014	USA	PCOS	28.7	28.2	0	13	15	19.9 (9.5)	22.2 (6.9)	12000	12	0.9 (4.8)	2.0 (4.8)	0.6
Zittermann [25]	2009	Germany	Overweight	48.8	47.4	53	82	83	30.0 (17.5)	30.3 (20.1)	3332	52	-0.03 (0.5)	-0.05 (0.7)	0.5

Data are presented as mean (SD). hsCRP, high-sensitivity C-reactive protein; 25-OH-D, 25-hydroxyvitamin D; IU, International Units; PCOS, Polycystic Ovarian Syndrome; OA, Osteoarthritis; MMT, Methadone Maintenance Treatment; MDD, Major Depressive Disorder; T2D, Type 2 Diabetes; NR, Not Reported; NS, Not Significant.

Figure S1. Flow diagram detailing study selection for randomized-controlled trials included in the meta-analysis.

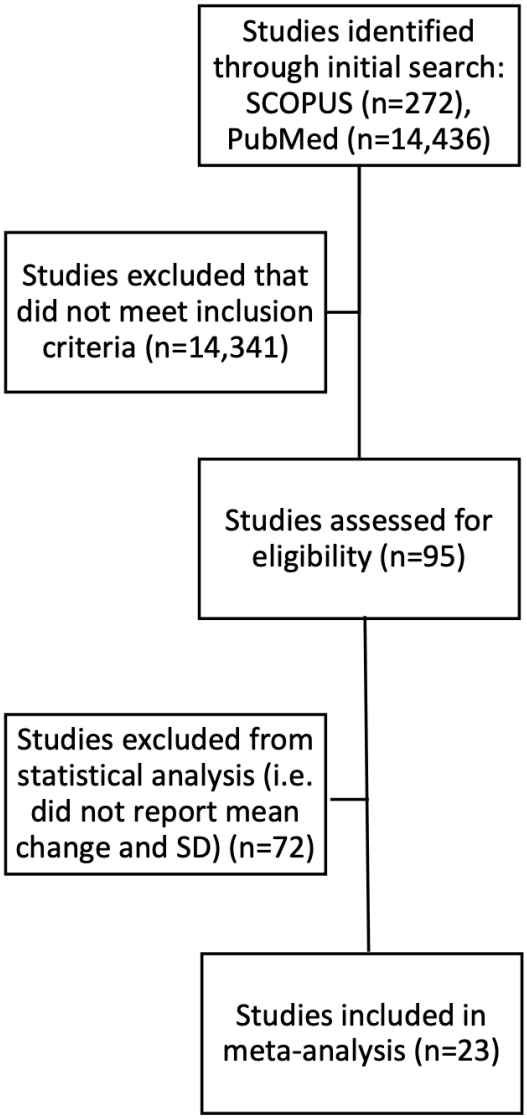
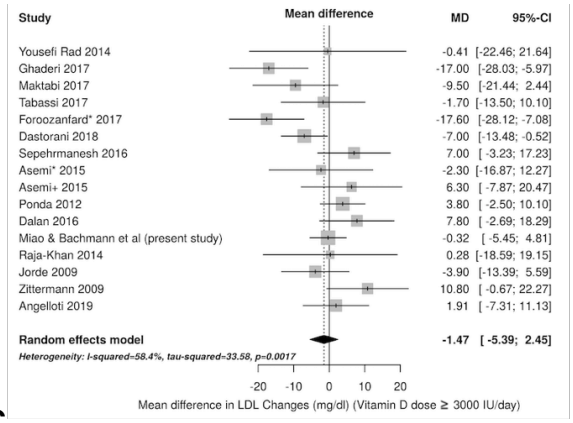


Figure S2. Forest plots comparing effects of vitamin D supplementation on changes in LDL stratified by dose of vitamin D supplementation, mean baseline 25-hydroxyvitamin D concentration, duration of vitamin D supplementation, and diabetes status.

A



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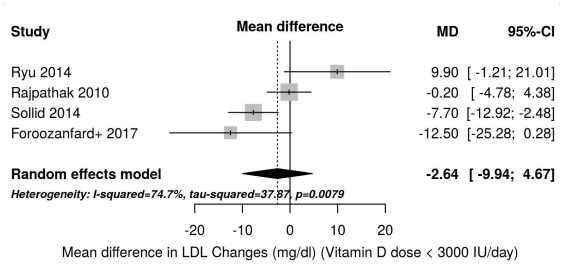

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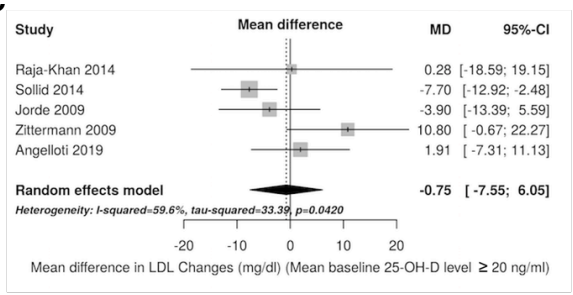

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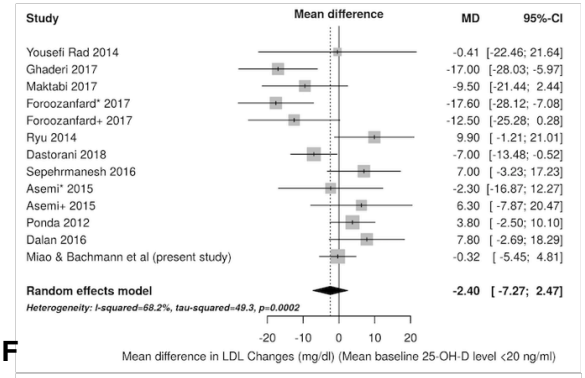

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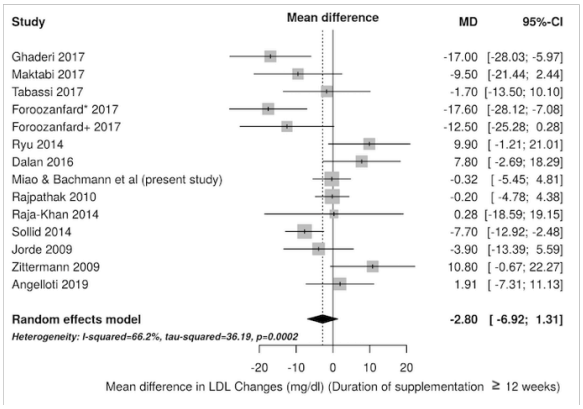

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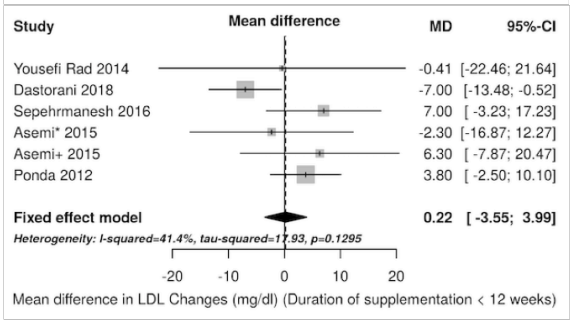

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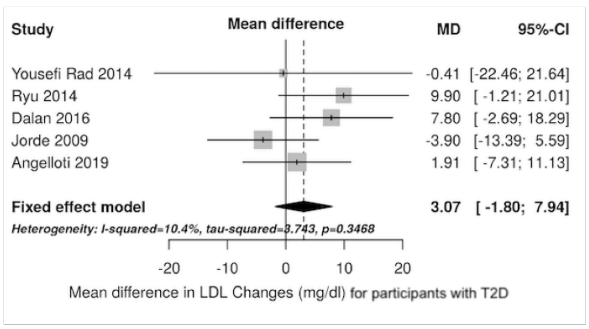

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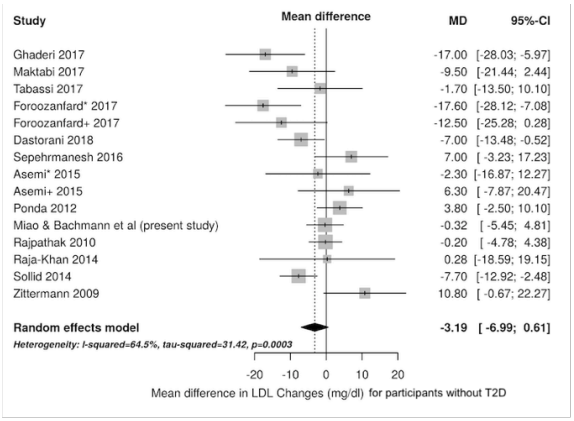
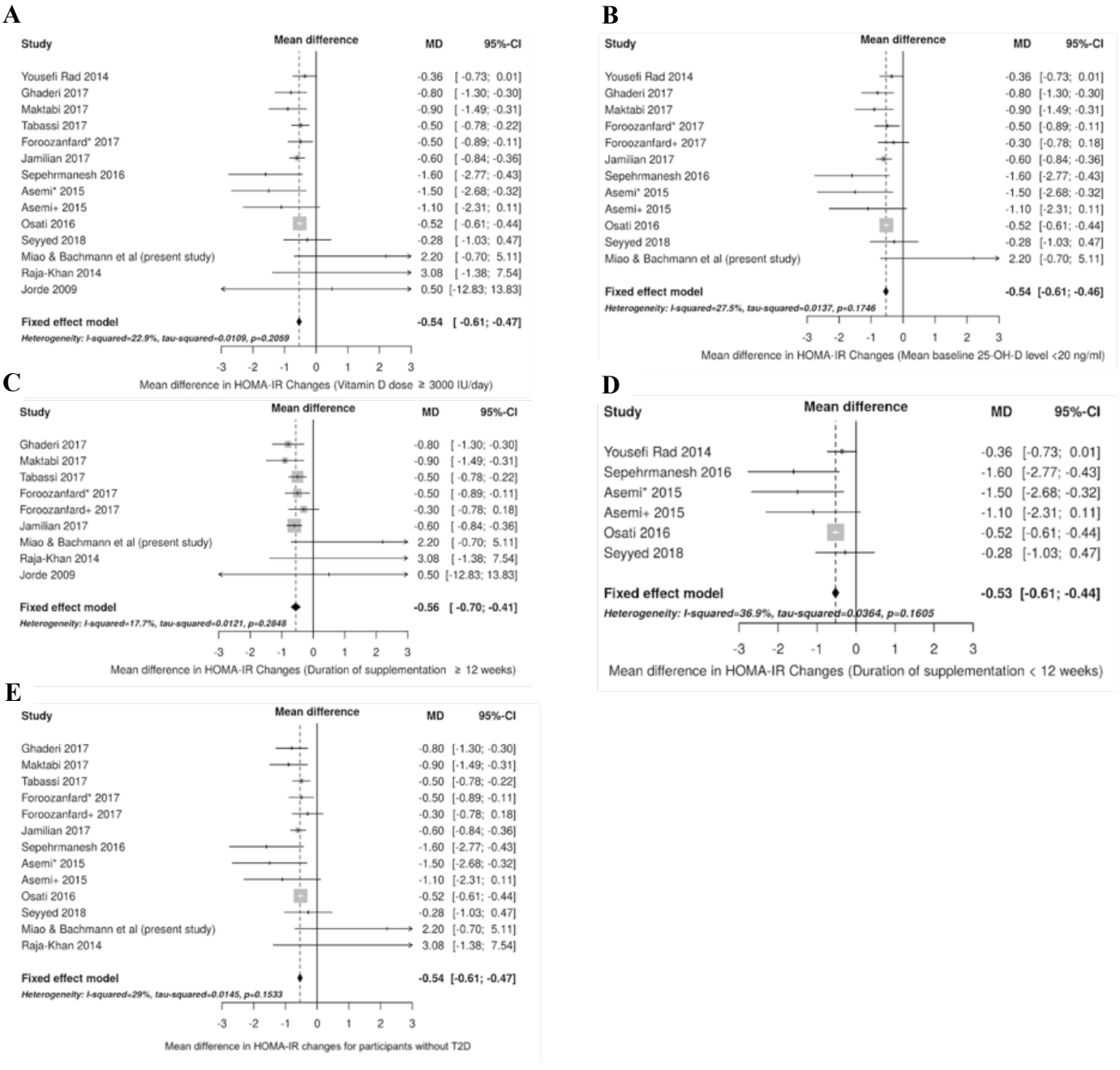


Subgroup analyses are presented for daily vitamin D supplementation dose ≥ or < 3000 IU/day (panels A and B, respectively), mean baseline 25-hydroxyvitamin-D level ≥ or <20 ng/ml (panels C and D, respectively), supplementation duration ≥ or < 12 weeks (panels E and F, respectively), and participants with or without T2D (panels G and H, respectively). Meta-analysis was not performed for subgroups consisting of 2 or fewer studies. The gray boxes correspond with study precision, lines denote 95% CI. MD, mean difference; IU, International Units; 25-OH-D, 25-hydroxyvitamin D; T2D, Type 2 Diabetes; LDL, Low-density Lipoprotein.

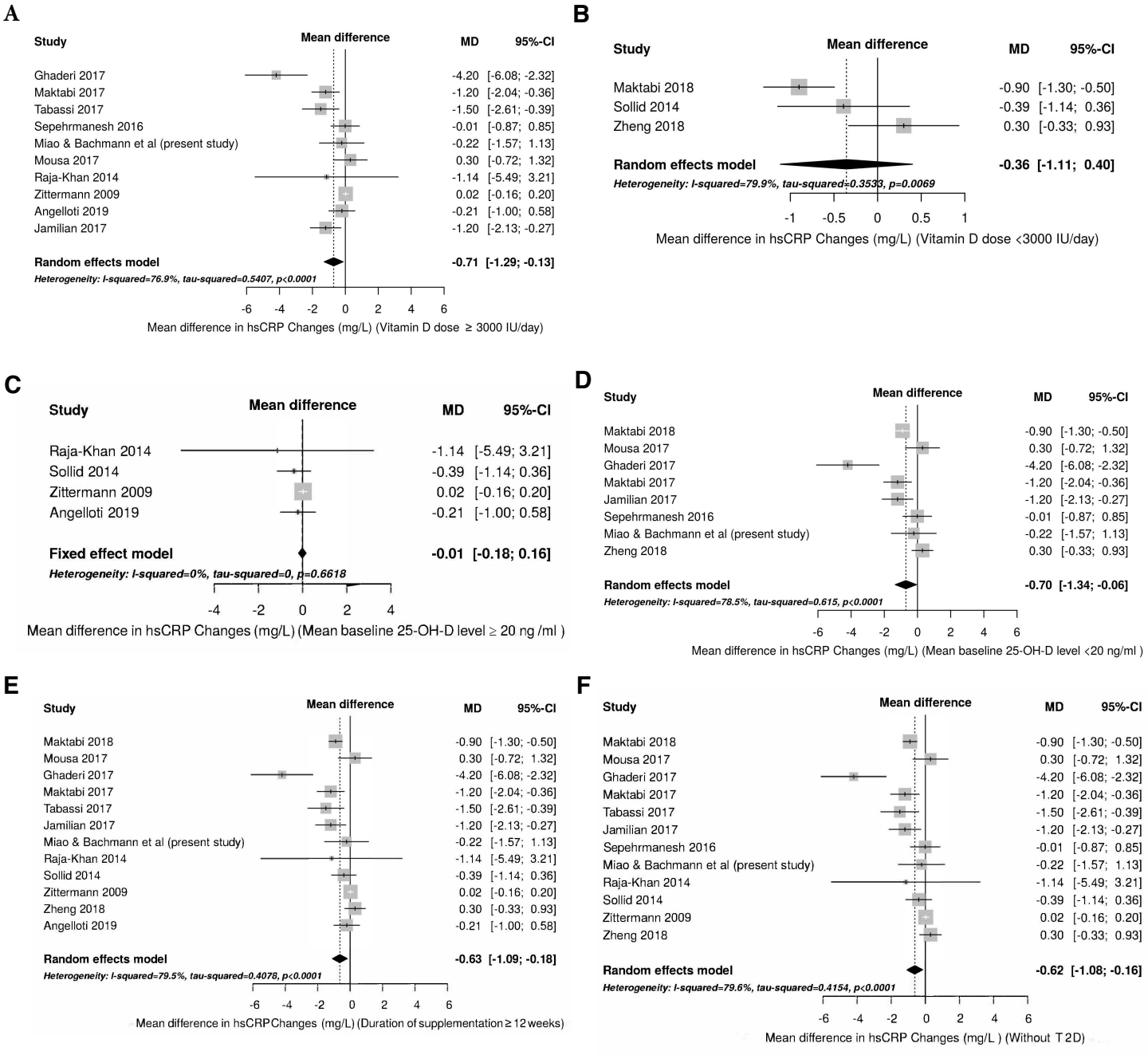
Figure S3. Forest plots comparing effects of vitamin D supplementation on changes in HOMA-IR stratified by dose of vitamin D supplementation, mean baseline 25-hydroxyvitamin D concentration, duration of vitamin D supplementation, and diabetes status.



Subgroup analyses are presented for daily vitamin D supplementation dose ≥3000 IU/day (panel A), mean baseline 25-hydroxyvitamin-D level <20 ng/ml (panel B), supplementation duration ≥ or <12 weeks (panels C and D, respectively), and participants without T2D (panel E). Meta-analysis was not performed for subgroups consisting of 2 or fewer studies. The gray boxes correspond with study precision, lines denote 95% CI. MD,

mean difference; IU, International Units; 25-OH-D, 25-hydroxyvitamin D; T2D, Type 2 Diabetes; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance. Foroozanfard*, Vitamin D 4000 IU daily; Foroozanfard+, Vitamin D 1000 IU daily; Asemi*, Vitamin D 50,000 IU weekly + calcium 1000 mg daily; Asemi+, Vitamin D 50,000 IU weekly.

Figure S4. Forest plots comparing effects of vitamin D supplementation on changes in hsCRP between studies included in the meta-analysis stratified by dose of vitamin D supplementation, mean baseline 25-hydroxyvitamin D concentration, duration of vitamin D supplementation, and diabetes status.



Subgroup analyses are presented for daily vitamin D supplementation dose ≥ or < 3000 IU/day (panels A and B, respectively), mean baseline 25-OH-D level ≥ or <20 ng/ml (panels C and D, respectively), supplementation duration ≥12 weeks (panel E), and participants without T2D (panel F). Meta-analysis was not performed for subgroups consisting of 2 or fewer studies. The gray boxes correspond with study precision, lines denote 95%

CI. MD, mean difference; IU, International Units; 25-OH-D, 25-hydroxyvitamin D; T2D, Type 2 Diabetes; hsCRP, high-sensitivity C-reactive protein.