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The effect of vitamin D supplementation on cardiovascular risk in patients with prediabetes: A secondary analysis of the D2d study

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ABSTRACT

Aims: Low blood 25(OH)D level is associated with increased cardiovascular disease (CVD) risk. Additionally, individuals with prediabetes are at higher risk for CVD than individuals with normoglycemia. We investigated the effects of vitamin D supplementation on CVD outcomes in the vitamin D and type 2 diabetes (D2d) study, a large trial among adults with prediabetes.

Methods: 2423 participants were randomized to 4000 IU/day of vitamin D_3 or placebo and followed for median 3.0 years for new-onset diabetes. In pre-specified secondary analyses, we examined the effect of vitamin D supplementation on composite Major Adverse Cardiovascular Events (MACE); expanded MACE (MACE + revascularization); atherosclerotic CVD (ASCVD) risk score; and individual CVD risk factors (blood pressure, lipids, high-sensitivity C-reactive protein). Cox models compared hazard ratios (HR) between the two groups on MACE and expanded MACE.

Results: Mean age was 60 years, 45 % were women, 13 % had history of CVD. Twenty-one participants assigned to vitamin D and 12 participants assigned to placebo met the MACE outcome (HR 1.81, 95%CI 0.89 to 3.69). There were 27 expanded MACE outcomes in each group (HR 1.02, 95%CI, 0.59 to 1.76). There were no significant differences between vitamin D and placebo in individual CVD risk factors, but change in ASCVD risk score favored the vitamin D group (−0.45 %, 95%CI -0.75 to −0.15).

Conclusions: In people with prediabetes not selected for vitamin D insufficiency and with intermediate CVD risk, vitamin D supplementation did not decrease MACE but had a small favorable effect on ASCVD risk score.

Trial registration: D2d ClinicalTrials.gov number, NCT01942694, prospectively registered September 16, 2013.

1. Introduction

There is an abundance of data from observational studies showing that lower blood 25-hydroxyvitamin D (25(OH)D) levels are associated with increased cardiovascular disease (CVD) risk, as summarized recently.[1] Mechanistic studies support such an association as low vitamin D status (generally, blood 25[OH]D level below 15 ng/mL) is associated with endothelial dysfunction, inflammation, oxidative stress,

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and may also negatively influence lipid profiles, leading to worsening CVD risk.[2] However, results from trials that have tested for an effect of vitamin D supplementation on risk of CVD events have largely shown no beneficial effect among people at average risk for CVD.[3]

Prediabetes, defined as impaired glucose tolerance, impaired fasting glucose, or abnormal hemoglobin A1c (HbA1c), is associated with an increased risk of CVD. In a recent meta-analysis of 129 studies involving >10 million participants, compared with normoglycemia, prediabetes was associated with an increased risk of all-cause mortality and new-onset CVD.[4] However, it is not known whether supplementation with vitamin D decreases CVD risk in people with prediabetes.

The vitamin D and type 2 diabetes (D2d) study is the largest modern diabetes prevention trial enrolling participants with prediabetes, deffned by the latest (2010) American Diabetes Association criteria, and was conducted to test the effect of daily vitamin D supplementation on incident diabetes.[5,6] The D2d-CVD ancillary study is a pre-speciffed secondary analysis in the D2d study to assess whether vitamin D supplementation reduces CVD risk, assessed by clinical outcomes and by ASCVD score and individual risk factors, in people with prediabetes who are at higher risk for CVD than the general population.

2. Materials and methods

2.1. Overview of the D2d study

The D2d study (clinicaltrials.gov NCT 01942694) is a U.S.-based multicenter, randomized, placebo-controlled, diabetes prevention clinical trial with two groups (vitamin D vs. placebo) in participants at-risk for diabetes who were followed for incident diabetes. The design and primary outcome of D2d have been published.[5,6] The study was approved by the institutional review board of each collaborating clinical site and monitored by an independent Data and Safety Monitoring Board. All study participants provided written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice.

2.2. Study population

Participants were recruited from and followed at 22 medical centers across the United States. Recruitment took place from October 2013 to December 2016. Eligible participants met 2 or 3 glycemic criteria for prediabetes as deffned by the 2010 American Diabetes Association guidelines: fasting plasma glucose 100–125 mg/dL (5.6–6.9 mmol/L); plasma glucose 2 h after a 75-g oral glucose load 140–199 mg/dL (7.8–11.0 mmol/L); HbA1c 5.7–6.4 % (39–47 mmol/mol).[7] Other inclusion criteria were age greater than or equal to 30 years (25 years for American-Indian, Alaska Native, Native Hawaiian or Other Pacific Islander) and body mass index 24–42 kg/m^2 (22.5–42 kg/m^2 for Asian). A "low" blood 25(OH)D level was not an inclusion criterion. Key exclusion criteria relevant to this analysis included any glycemic criterion in the diabetes range, use of diabetes or weight-loss medications, use of supplemental vitamin D over 1000 IU per day and unwilling to decrease to >1000 IU per day for the duration of the study, uncontrolled hypertension (systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg), severe symptomatic cardiovascular disease, or recent history of myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, or cerebrovascular disease. The complete list of eligibility criteria and the recruitment and screening process has been described previously.[6,8]

2.3. Intervention and procedures

Participants were randomized to a once-daily soft-gel that contained either 4000 IU of vitamin D_3 (cholecalciferol) or a matching placebo with stratiffcation by site, BMI (<30 or ≥ 30 kg/m^2), and race (White or non-White). Study staff used a web-based, interactive, D2d-speciffc study pill inventory and randomization system to enter the stratiffcation variables, and the system randomly assigned the participant to vitamin or placebo. At randomization and every 6 months, the system generated a specific pill bottle number that study staff dispensed to the participant in a blinded fashion. No unblinding took place during the study, and study staff and participants were notified of the assignment only after the primary results were published.

To maximize the study's ability to observe a treatment effect, participants were asked to limit the use of outside-of-study vitamin D to 1000 IU per day from all supplements, including multivitamins, and to refrain from taking glucose-lowering medications, prior to receiving a diabetes diagnosis, or weight-loss medications during the study. For safety, participants were asked to limit calcium supplements to 600 mg per day. At each follow-up encounter (four times a year), participants were queried about any changes to medications and supplements and amounts of supplemental vitamin D were specifically reviewed and totaled. Participants taking over 1000 IU of supplemental vitamin D daily were asked to reduce intake to <1000 IU. During the study, participants were provided with information on diabetes prevention through information sheets and twice-yearly group meetings.

Participants had in-person follow-up at scheduled visits at month 3, month 6, and twice annually thereafter, with interim phone or email contact between in-person visits after month 6. Follow-up took place until the prespeciffed number of diabetes outcome events was met, with the last follow-up visit in November 2018. Blood pressure was measured at all visits using a standardized procedure in which two measurements were taken ffve minutes apart after the participant sat quietly for ffve minutes and averaged by the electronic data capture system. Serum was collected annually after an 8-h fast and shipped to the central laboratory for long-term storage at −80 °C until analysis. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and high-sensitivity CRP (hsCRP) concentrations were measured in the clinical laboratory at Tufts Medical Center (Boston, MA) using an Abbott Architect c8000 automated analyzer (Abbott Laboratories).

Cardiovascular events were collected as part of serious adverse event reporting during the study. At each contact with study staff, participants were asked if they had any changes to their health or medications, and details were elicited with targeted follow-up questions. For all serious adverse events (death, a new hospitalization, prolongation of an existing hospitalizations, a persistent or significant disability or incapacity, or any other significant hazard based upon the medical judgment by the investigator), medical records were collected for review by the study's safety and outcomes subcommittee. When a site was notified of a participant death, the death certificate, autopsy report, and relevant hospital records were collected, as available. For participants who missed visits and were unable to be contacted, study staff followed a vital status ascertainment checklist which included a search of the electronic medical record (as available) and the National Death Index. For participants without evidence of death but who did not return for a formal end-of-study visit, the last vital status was defined at the participant's last study encounter.

Records for all cardiovascular events and all deaths, regardless of cause, were reviewed by the study's external cardiovascular clinical events adjudicator, who was blinded to participant treatment group. The adjudicator followed operational standardized definitions for event classification.

2.4. Definition of cardiovascular outcomes

Major Adverse Cardiovascular Events (MACE) were defined as cardiovascular death (fatal acute myocardial infarction, sudden cardiac death, fatal heart failure, or fatal stroke) or cardiovascular-related hospitalization (non-fatal acute myocardial infarction, unstable angina, stroke, or transient ischemic attack). An expanded MACE definition additionally included any other cardiovascular death such as due to a cardiovascular procedure or cardiovascular hemorrhage, and hospitalization for cardiovascular procedure, heart failure, cardiovascular hemorrhage, or other cardiovascular reason. Cardiovascular hemorrhage was defined as hemorrhage such as non-stroke intracranial

hemorrhage (e.g., subdural hematoma), non-procedural or non-traumatic vascular rupture (e.g., aortic aneurysm) or hemorrhage causing cardiac tamponade.

The main outcome for this analysis was first occurrence of MACE during the study (i.e., since randomization) as defined above. Secondary outcomes included: first occurrence of the expanded MACE definition; change over time in specific CVD risk factors (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, systolic and diastolic blood pressure, hsCRP); and 10-year atherosclerotic cardiovascular disease (ASCVD) score, as defined in the 2013 ACC/AHA Cardiovascular Risk Assessment Guideline.[9] We did not include data on urine albumin-creatinine ratio as a CVD risk factor because we have already published about this outcome.[10]

Data on potential confounders or effect modifiers were collected. At baseline, age, race, and ethnicity were self-reported and personal health history was captured by targeted questionnaires. At all visits, anthropometric measures, changes to medications (e.g., for hypercholesterolemia, hypertension) or health, and dietary supplement intake were collected. Descriptions of measurements of these and other confounders and variables have been previously published.[6,11]

2.5. Statistical methods

Sample size was determined by the parent D2d study, which was conducted as an event-driven trial for the study's primary outcome of diabetes. A single, planned interim analysis was conducted for the primary outcome of diabetes. End-of-study procedures were initiated when the minimum number of required diabetes events was reached. All randomized participants were included in this analysis.

Descriptive data included means and standard deviations, medians and interquartile ranges, or percentages. Intention-to-treat analyses compared groups defined by the randomization procedure and included all participants and events observed during the study regardless of adherence to assigned treatment. Follow-up time in the study was calculated as time from randomization until the outcome of interest (MACE or expanded MACE) was met. In those individuals free of the outcome, follow-up was censored at end-of-study visit, withdrawal, death, or last completed study encounter for those who did not return for the end-of-study visit.

Cox proportional hazard models were used to calculate the hazard ratio (HR) and 95 % confidence interval (CI) of incident MACE and of expanded MACE comparing the two groups.[12] The model included an indicator for the randomized intervention as its main predictor variable. Study site, race (White or non-White), and BMI <30 or ≥ 30 kg/m^2 at randomization were used as stratifying factors in the baseline hazard function.

Between group differences for the change in continuous variables (individual CVD risk factors and ASCVD score) were determined using a linear mixed-effects model approach to account for within participant correlation across the time-points. An interaction term between treatment assignment and time was used to assess if the change in trajectory by each continuous variable differed significantly between randomization groups. Sensitivity analyses explored potential non-linear changes over the entire follow-up. Missing values for the continuous variables were not imputed. Repeated measures analysis was done on these variables instead.

We also conducted pre-specified analyses that censored follow-up when a participant stopped the trial pills or took out-of-trial vitamin D from supplements above the trial limit of 1000 IU per day ("per-protocol" analysis) to fully capture the effects of vitamin D on cardiovascular pathophysiology compared to placebo without the confounding effects of trial product discontinuation or off-protocol use of vitamin D (out-of-study high-dose vitamin D supplementation).

Before outcomes data were analyzed by treatment group, the statistical analysis plan was reviewed and approved by all co-authors and the study's publications and presentations committee. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). No adjustments were made for multiple comparisons.

3. Results

3.1. Baseline characteristics

All 2423 randomized participants were included in the present intention-to-treat analyses, with 1211 randomized to vitamin D and 1212 randomized to placebo (Fig. 1). At baseline, the mean (±SD) age of the cohort was 60.0 (±9.9) years, mean BMI was 32.1 (±4.5) kg/m^2, and about 13 % of participants had a history of CVD (excluding hypertension), with a mean (±SD) ASCVD risk score of 10.5 % (±8.5). Over half of participants (51 %) had impaired glucose tolerance (plasma glucose 140–199 mg/dL after a 75-g glucose load). There were no significant differences in the baseline characteristics between the vitamin D and placebo groups (Table 1). By month 24, the mean serum 25(OH)D level increased from 27.7 to 54.3 ng/mL ($p < 0.001$) in the vitamin D group and remained unchanged in the placebo group (28.2 ng/mL at baseline and 28.8 ng/mL at 24 months).

3.2. New-onset MACE and expanded MACE (pre-specified)

Over a median follow up of 3.0 years (IQR 2.0 to 3.6) in the vitamin D group and 2.9 years (IQR 2.0 to 3.5) in the placebo group, 33 participants experienced MACE, 21 in the vitamin D group and 12 in the placebo group (HR 1.81, 95 % CI 0.89 to 3.69). A total of 54 participants experienced expanded MACE, 27 in each group (HR 1.02, 95 % CI 0.59 to 1.76) (Fig. 2). Subgroup analyses for MACE were not feasible due to the very low numbers of events in each subgroup. For example, among participants with baseline vitamin D levels <20 ng/mL, only four experienced a MACE.

3.3. Change in individual CVD risk variables (exploratory)

Key cardiovascular risk factors (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, hsCRP, systolic and diastolic blood pressure) and ASCVD score were assessed at baseline and every 12 months through 48 months, and changes over time between the vitamin D and placebo group are shown in Table 2. Both groups showed reductions over time in total cholesterol, LDL cholesterol, triglycerides, and increases in HDL cholesterol. In the vitamin D treated group, LDL cholesterol decreased by 5.5 mg/dL (95% CI 6.5 to 4.5), HDL cholesterol increased by 1.2 mg/dL (95% CI 0.9 to 1.4), and triglycerides decreased by 3.3 mg/dL (95% CI 5.7 to 0.8). The difference between the change over time in the vitamin D group versus the placebo group was not statistically different for LDL cholesterol, triglycerides, and total cholesterol. The HDL cholesterol in the vitamin D group had a marginally larger increase than the placebo group at the end of the study (0.34 mg/dL, 95% CI 0.0 to 0.7).

The ASCVD score increased in both the vitamin D group and the placebo group. However, change in ASCVD score in the vitamin D group was less than the placebo group (−0.45, 95% CI -0.75 to −0.15).

Both groups showed reductions over time in systolic and diastolic blood pressure. The reduction in systolic blood pressure favored the vitamin D group more than in the placebo group (−1.1 mmHg; 95% CI 1.5 to −0.6 for vitamin D vs. -0.4 mmHg; 95% CI -0.9 to 0.1 for placebo) but the result did not reach statistical significance. At baseline, there was a trend towards higher use of ACE inhibitors or ARB in the placebo group (34 %) vs. the vitamin D group (31 %) ($p = 0.085$ for difference in proportions) (Table 3). This difference increased during the follow-up period, such that during the study, significantly more participants in the placebo group were treated with ACE inhibitors or ARB than the vitamin D group (40.3 vs. 36.3 % respectively; $p = 0.042$).

There were no significant differences in adverse events, including hypercalcemia and nephrolithiasis during the trial.[5]

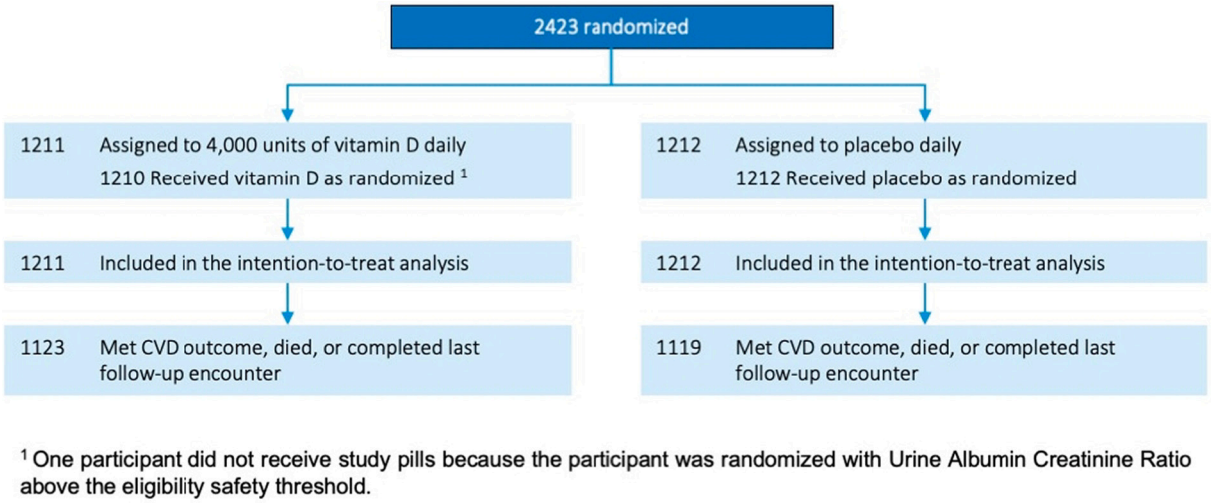

Fig. 1. Flow of participants through the D2d CVD outcomes study.

4. Discussion

4.1. Summary of main result

In this randomized, double-blind, placebo-controlled clinical trial, daily oral vitamin D_3 supplementation in participants at-risk for diabetes followed for a median of 3.0 years (IQR 2.0 to 3.5) did not have an effect on new-onset MACE or expanded MACE. There was a small benefit towards an improved ASCVD risk score and a trend towards improved systolic blood pressure and HDL levels among participants assigned to vitamin D. There was less use of ACE/ARB in the vitamin D group over time.

4.2. Comparison with other vitamin D studies of MACE

Observational studies have reported consistent associations between lower vitamin D levels and increased CVD risk, especially in people with very low vitamin D levels (defined based on blood 25[OH]D absolute thresholds or relative levels within each study).[1] However, trials with vitamin D supplementation that have reported on cardiovascular events have largely shown no beneficial effect. In a trial of adults in Australia, there was no effect of vitamin D supplementation on cardiovascular events.[13] However, that study had limitations, including targeting the general population (not at high risk for CVD), outcomes that were based on ICD codes extracted from medical records and not adjudicated, and administration of very high monthly doses of vitamin D, which are not considered physiologic. The large VITAL study in the U.S. also did not show any reduction in major cardiovascular events with vitamin D supplementation in an older but generally healthy population. The VITAL study also included participants with varying CVD risk and glycemic status, including participants with diabetes, prediabetes, and no diabetes, which may have prevented seeing an effect of vitamin D supplementation on CVD risk.[14] In a recent meta-analysis of 52 randomized controlled trials, vitamin D treatment did not have an effect on cardiovascular mortality or all-cause mortality,[15] while in another meta-analysis of 21 randomized controlled trials, vitamin D treatment did not decrease new-onset MACE[3]; however, such meta-analyses are not conclusive because they combine populations at varying risks and heterogenous study designs (intervention, outcome etc.). In the D2d study among people with prediabetes, there was no significant difference in new-onset MACE or expanded MACE between vitamin D and placebo groups. The wide confidence interval in the hazard ratio for new-onset-MACE which spans from 0.89 (protection) to 3.69 (harm) is due to the small number of events (33 MACE events) and precludes any definitive conclusions. Our results in a population with prediabetes is therefore consistent with the results from other populations at average CVD risk. Although none of these studies were dedicated cardiovascular outcomes trials, except the VITAL study where MACE was a co-primary outcome, the lack of a signal or trend towards cardiovascular protection is consistent in various subsets of populations. However, the D2d study was not designed for cardiovascular outcomes and the present secondary analysis was not powered to detect statistically significant effects.

4.3. Effect of vitamin D supplementation on cardiovascular risk factors and comparison with other studies

Most of the key cardiovascular risk factors we examined (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and systolic and diastolic blood pressure) improved over the course of the study in both groups. This is likely due to frequent lifestyle counseling (as part of the protocol) and/or due to more frequent monitoring and healthcare contact as part of participating in a research study, followed by appropriate medication changes by the participants' providers.[16] There were no differences between vitamin D and placebo in these individual risk factors except for HDL cholesterol, which increased more in the vitamin D group. Post-randomization confounding due to use of cardiovascular medications may also explain the lack of differences in these variables. For example, during the course of the study, more participants in the placebo group were taking an ARB or ACE inhibitor, which may have masked detection of a difference in blood pressure between vitamin D and placebo. It is possible that blood pressure differences would have been detected in favor of the vitamin D group if anti-hypertensive medications use was held constant in both groups of the study. While a recent meta-analysis reported no significant effects of vitamin D supplementation on blood pressure among healthy individuals, there was large heterogeneity among trials and most trials were not designed with blood pressure as the primary outcome.[17]

4.4. ASCVD risk

The ASCVD score is a quantitative risk assessment of developing ASCVD over 10 years that uses individual CVD risk factors in the calculation including age, blood cholesterol, blood pressure, use of blood pressure lowering medications, smoking status, and diabetes status. During follow-up, the ASCVD risk scores increased in both groups, as expected, likely driven primarily by increasing age and development of diabetes. However, the increase in ASCVD score was significantly less in the vitamin D treated group than the placebo group,

Table 1
Baseline characteristics[a].

Characteristic	Overall ($N = 2423$)	Vitamin D ($N = 1211$)	Placebo ($N = 1212$)
Demographics			
Age, years	60.0 ± 9.9	59.6 ± 9.9	60.4 ± 10.0
Women, no. (%)	1086 (44.8)	541 (44.7)	545 (45.0)
Primary race[b], no. (%)			
Asian	130 (5.4)	66 (5.5)	64 (5.3)
Black or African American	616 (25.4)	301 (24.9)	315 (26.0)
White	1616 (66.7)	810 (66.9)	806 (66.5)
Other	61 (2.5)	34 (2.8)	27 (2.2)
Hispanic or Latino ethnicity[b], no. (%)	225 (9.3)	120 (9.9)	105 (8.7)
Clinical characteristics			
Body mass index, kg/m^2	32.1 ± 4.5	32.0 ± 4.5	32.1 ± 4.4
Smoking history, no. (%)			
Never	1410 (58.2)	710 (58.6)	700 (57.8)
Former	838 (34.6)	416 (34.4)	422 (34.8)
Current	155 (6.4)	75 (6.2)	80 (6.6)
Unknown or not reported	20 (0.8)	10 (0.8)	10 (0.8)
Physical activity, total MET hour/week	109.8 ± 158.7	110.7 ± 158.8	109.0 ± 158.6
median (Q1-Q3)	55.8 (25.9–125.9)	58.8 (25–130.8)	53.4 (26.3–121.2)
Medical History[c], no. (%)			
Hypercholesterolemia	1346 (55.6)	661 (54.6)	685 (56.5)
Cardiovascular disease	1379 (56.9)	674 (55.7)	705 (58.2)
Arrhythmias	153 (6.3)	82 (6.8)	71 (5.9)
Chest pain	10 (0.4)	7 (0.6)	3 (0.2)
Congestive heart failure	9 (0.4)	5 (0.4)	4 (0.3)
Aortic or coronary artery disease	74 (3.1)	41 (3.4)	33 (2.7)
CABG / PCI	69 (2.8)	38 (3.1)	31 (2.6)
Myocardial infarction	40 (1.7)	27 (2.2)	13 (1.1)
Palpitations	21 (0.9)	10 (0.8)	11 (0.9)
Peripheral vascular disease	16 (0.7)	7 (0.6)	9 (0.7)
Hypertension	1297 (53.5)	622 (51.4)	675 (55.7)
Cardiovascular disease excluding hypertension	305 (12.6)	161 (13.3)	144 (11.9)
Systolic blood pressure, mmHg	128.4 ± 13.4	128.1 ± 13.3	128.6 ± 13.5
Diastolic blood pressure, mmHg	77.0 ± 9.3	77.2 ± 9.1	76.9 ± 9.4
Medication use[c], no (%)			
Anti-platelet / anti-coagulation	754 (31.1)	364 (30.1)	390 (32.2)
Hypercholesterolemia	1065 (44.0)	532 (43.9)	533 (44.0)
Statin	1027 (42.4)	518 (42.8)	509 (42.0)
Hypertension	1264 (52.2)	621 (51.3)	643 (53.1)
ACE inhibitors	493 (20.3)	231 (19.1)	262 (21.6)
Angiotensin blockers	297 (12.3)	144 (11.9)	153 (12.6)
ACE or ARB	788 (32.5)	374 (30.9)	414 (34.2)
Beta blockers	401 (16.5)	226 (18.7)	174 (14.4)
Calcium channel blockers	338 (13.9)	167 (13.8)	171 (14.1)
ASCVD score, %	10.5 ± 8.5	10.0 ± 8.2	11.0 ± 8.8
Laboratory			
Fasting plasma glucose, mg/dL	107.9 ± 7.4	108.0 ± 7.4	107.8 ± 7.4
2-h post-load plasma glucose, mg/dL	137.2 ± 34.3	136.9 ± 34.3	137.6 ± 34.3
Hemoglobin A1c, %	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2
Pre-diabetes categories[d], no. (%)			
Met all 3 criteria (iA1c + IFG + IGT)	856 (35.3)	427 (35.3)	429 (35.4)
Met two criteria only			
iA1c + IFG only	1184 (48.9)	607 (50.1)	577 (47.6)
IFG + IGT only	152 (6.3)	74 (6.1)	78 (6.4)
IGT + iA1c only	231 (9.5)	103 (8.5)	128 (10.6)
Laboratory – Safety			
Creatinine, mg/dL	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
Estimated Glomerular Filtration Rate[e], mL/min/1.73m^2	87.1 ± 15.7	87.5 ± 15.6	86.7 ± 15.9
≥ 60 mL/min/1.73m^2, no. (%)	2314 (95.5)	1163 (96.0)	1151 (95.0)
45 to 59.9 mL/min/1.73m^2, no. (%)	109 (4.5)	48 (4.0)	61 (5.0)
Serum 25-hydroxyvitamin D, ng/mL	28.0 ± 10.2	27.7 ± 10.2	28.2 ± 10.1
Lipid profile			
Total cholesterol, mg/dL	192.7 ± 40.5	193.1 ± 41.4	192.3 ± 39.5
HDL cholesterol, md/dL	47.7 ± 11.5	47.6 ± 11.6	47.7 ± 11.5
LDL cholesterol, mg/dL	118.5 ± 33.9	118.9 ± 34.3	118.2 ± 33.4
Triglycerides, mg/dL	133.7 ± 81.5	133.8 ± 73.7	133.7 ± 88.6
High-sensitivity C-reactive protein, mg/L	4.5 ± 6.3	4.5 ± 6.2	4.5 ± 6.5

[a] Plus-minus values are means±SD. Percentages may not add up to 100 because of rounding.
[b] Race and ethnicity were reported by the participant. The category "other" includes Asian, American Indian or Alaska Native; Native Hawaiian or other Pacific Islander; or other race. Ethnicity includes any race.
[c] Self-reported.
[d] IFG, impaired fasting glucose defined as fasting plasma glucose 100–125 mg/dL (5.6–6.9 mmol/L); IGT, impaired glucose tolerance defined as 2-h post-load plasma glucose after a 75-g glucose load 140–199 mg/dL (7.8–11.0 mmol/L) or); iA1c, impaired A1c defined as HbA1c 5.7–6.4 % (39–47 mmol/mol).
[e] GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

albeit by a small amount. In part, this may be due to fewer cases of diabetes observed in the vitamin D group.[5]

4.5. Strengths and limitations

Our study had several strengths, including many related to the design and conduct of the parent trial, such as the use of the latest American Diabetes Association criteria in defining prediabetes, high adherence to the study pills (83.2 %), and frequent follow-up. The vitamin D dose of 4000 IU per day, selected to balance safety and efficacy, resulted in a large difference in serum 25(OH)D level between the vitamin D and placebo groups during follow-up.[5] To establish the MACE outcome, medical records were collected and then reviewed and adjudicated by an independent blinded adjudicator using standardized definitions. The main limitation was that the parent study was designed for prevention of diabetes, not for CVD outcomes, and the follow-up time was too short for CVD outcomes; therefore, the analysis for MACE and expanded MACE – although pre-specified – was not powered to detect potentially small effects of vitamin D. For the same reason (i.e., relatively small sample size), we were unable to do subgroup analyses. While the participants recruited for D2d were at risk for diabetes, baseline cardiovascular risk by ASCVD score was considered intermediate, with an average ASCVD score of 10.5 %. Additionally, almost half of participants were taking statins at baseline and 60.2 % were taking either a statin, ACE inhibitor, ARB, or beta blocker. It is also important to note that the serum 25(OH)D level at baseline would be considered sufficient by most guidelines for overall health, which may have prevented detection of an effect of vitamin D supplementation on CVD risk. Among a small subgroup of participants with baseline 25(OH)D level <12 ng/mL, we previously reported a reduction in progression to diabetes and improvement in pancreatic beta cell function,[5,18] however, such subgroup analysis was not possible in the present analysis as the total number of MACE was too low. Our results could be combined with other similar, high-risk pre-diabetes trials in aggregate or individual participant data meta-analyses to detect smaller effects of vitamin D on CVD risk.

5. Conclusions

In conclusion, in a pre-diabetes population not selected for vitamin D insufficiency and with intermediate CVD risk, there was no effect of

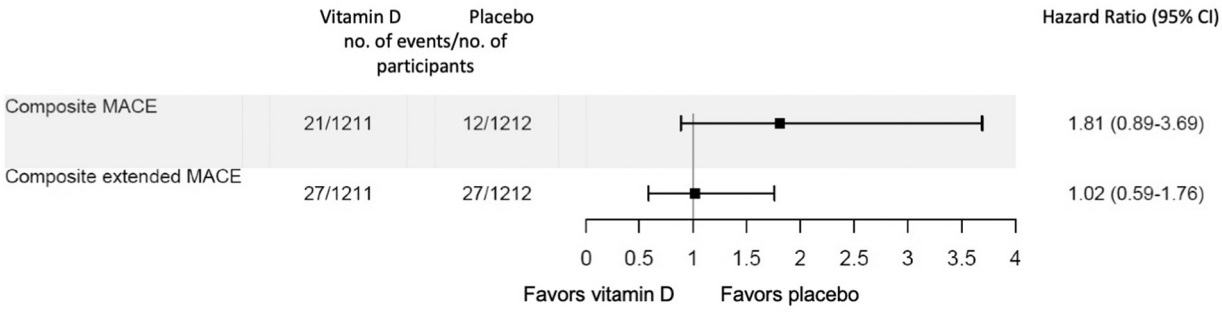

Fig. 2. Effect of vitamin D on composite MACE and expanded composite MACE outcomes.

Table 2
Changes in cardiovascular disease risk factors over time.

	Baseline	Month 12	Month 24	Month 36	Month 48	Mean difference compared to baseline (95 % CI)	Between group difference (95 % CI)
Total cholesterol							
Vitamin D group	193.1 ± 41.4	190 ± 40.7	188.2 ± 40.5	186.7 ± 39.9	187 ± 40.7	−4.93 (−6.08 to −3.78)	−0.14 (−1.77 to 1.49)
No. analyzed	1211	1121	986	608	239		
Placebo group	192.3 ± 39.5	190 ± 40.3	188.9 ± 41.8	186 ± 41.6	182.8 ± 39.2	−4.8 (−5.95 to −3.64)	
No. analyzed	1211	1122	986	593	238		
LDL cholesterol							
Vitamin D group	118.9 ± 34.3	115.7 ± 33.5	114.1 ± 33.9	109.9 ± 32.9	110.1 ± 34.6	−5.49 (−6.47 to −4.5)	−1.01 (−2.4 to 0.38)
No. analyzed	1211	1121	985	608	239		
Placebo group	118.2 ± 33.4	116.8 ± 34.3	115.9 ± 35.7	110 ± 34.6	107.6 ± 32.6	−4.48 (−5.46 to −3.49)	
No. analyzed	1211	1122	986	593	238		
HDL cholesterol							
Vitamin D group	47.6 ± 11.6	48.1 ± 12.2	48 ± 12.3	51.1 ± 12.4	51.8 ± 12.1	1.16 (0.91 to 1.4)	0.34 (−0.01 to 0.69)
No. analyzed	1211	1121	986	608	239		
Placebo group	47.7 ± 11.5	47.9 ± 11.8	48 ± 12.1	51.3 ± 13.1	50.3 ± 12.5	0.82 (0.57 to 1.07)	
No. analyzed	1211	1122	986	593	238		
Triglycerides							
Vitamin D group	133.8 ± 73.7	131.9 ± 71.9	132.1 ± 77.7	128.7 ± 67.8	125.3 ± 59.6	−3.25 (−5.74 to −0.76)	2.55 (−0.98 to 6.08)
No. analyzed	1211	1120	986	608	239		
Placebo group	133.7 ± 88.6	128.4 ± 71.3	126.8 ± 72.7	125.8 ± 70.1	126.1 ± 72	−5.8 (−8.3 to −3.3)	
No. analyzed	1211	1122	986	593	238		
hsCRP							
Vitamin D group	4.5 ± 6.2	4.4 ± 5.6	4.4 ± 6.5	4.7 ± 6.7	5.4 ± 8.6	0.01 (−0.23 to 0.24)	0.01 (−0.32 to 0.34)
No. analyzed	1211	1121	986	608	239		
Placebo group	4.5 ± 6.5	4.3 ± 5.8	4.5 ± 6	4.3 ± 7.1	5.3 ± 9.4	0 (−0.23 to 0.24)	
No. analyzed	1211	1122	986	593	238		
Systolic blood pressure							
Vitamin D group	128.1 ± 13.3	127.1 ± 14.5	127 ± 13.8	126.7 ± 14.5	126.5 ± 14.7	−1.05 (−1.54 to −0.57)	−0.64 (−1.33 to 0.04)
No. analyzed	1211	1126	996	614	244		
Placebo group	128.6 ± 13.5	128.5 ± 13.8	128.5 ± 14	127.2 ± 14.3	126.7 ± 13.5	−0.41 (−0.89 to 0.07)	
No. analyzed	1212	1132	1002	601	238		
Diastolic blood pressure							
Vitamin D group	77.2 ± 9.1	76.1 ± 9.9	75.5 ± 9.6	75.2 ± 9.9	75.5 ± 9.6	−1.37 (−1.67 to −1.07)	−0.34 (−0.76 to 0.08)
No. analyzed	1211	1126	996	614	244		
Placebo group	76.9 ± 9.4	76 ± 9.6	75.7 ± 10.1	74.5 ± 9.4	74.3 ± 9.5	−1.04 (−1.33 to −0.74)	
No. analyzed	1212	1132	1002	601	238		
ASCVD score							
Vitamin D group	10 ± 8.2	11.6 ± 9.8	13.1 ± 10.8	13.4 ± 11.5	14.3 ± 12.2	2.70 (2.48 to 2.91)	−0.45 (−0.75 to −0.15)
No. analyzed	1208	1118	982	603	237		
Placebo group	11 ± 8.8	12.9 ± 10.3	15 ± 11.7	15.7 ± 13.1	16.3 ± 13.2	3.14 (2.93 to 3.36)	
No. analyzed	1206	1119	979	589	235		

Table 3
Changes over time in medication use (potentially a "competing risk").

Medication category, n (%)	Baseline	Month 3	Month 12	Month 24	Month 36	Month 48	Mean difference
Any anti-hypertensive medication							
Vitamin D group	621 (51.3)	618 (52.2)	608 (52.8)	557 (53.7)	338 (52.2)	128 (47.9)	697 (57.6)
Placebo group	643 (53.1)	643 (54.4)	641 (55.7)	595 (57.2)	360 (56.2)	145 (56.6)	733 (60.5)
P value for mean difference proportion							0.14
Angiotensin II receptor blockers or ACE inhibitors							
Vitamin D group	374 (30.9)	374 (31.6)	369 (32)	336 (32.4)	196 (30.3)	66 (24.7)	440 (36.3)
Placebo group	414 (34.2)	415 (35.1)	408 (35.4)	379 (36.4)	238 (37.1)	96 (37.5)	489 (40.3)
P value for mean difference proportion							0.04
Any cholesterol medication							
Vitamin D group	532 (43.9)	529 (44.7)	530 (46)	486 (46.8)	284 (43.9)	116 (43.4)	621 (51.3)
Placebo group	533 (44)	526 (44.5)	530 (46)	491 (47.2)	272 (42.4)	112 (43.8)	626 (51.7)
P value for mean difference proportion							0.86
Any diabetes medication							
Vitamin D group	3 (0.3)	2 (0.2)	26 (2.3)	49 (4.7)	48 (7.4)	26 (9.7)	75 (6.2)
Placebo group	0 (0)	1 (0.1)	15 (1.3)	47 (4.5)	51 (8)	16 (6.3)	78 (6.4)
P value for mean difference proportion							0.80

vitamin D supplementation for 3 years on MACE but there was a small beneficial effect on global CVD risk, assessed by the ASCVD risk estimator.

CRediT authorship contribution statement

Cyrus Desouza: Conceptualization, Investigation, Methodology, Writing – original draft and review and editing. Ranee Chatterjee: Investigation, Writing – review and editing. Ellen Vickery: Conceptualization, Date Curation, Project administration, Visualization, Writing – original draft and review and editing. Jason Nelson: Data curation, Formal analysis, Visualization, Writing – review and editing. Karen Johnson: Investigation, Writing – review and editing. Sangeeta Kashyap: Investigation, Writing – review and editing. Michael Lewis: Investigation, Writing – review and editing. Karen Margolis: Investigation, Writing – review and editing. Richard Pratley: Investigation, Writing – review and editing. Neda Rasouli Investigation, Writing – review and editing. Patricia Sheehan: Conceptualization, Data Curation, Methodology, Project administration, Supervision, Writing – review and editing. Anastassios Pittas: Conceptualization, Data Curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft and review and editing.

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Declaration of competing interest

The authors have nothing to disclose.

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Data sharing statement

The data underlying this article and the associated data dictionary are not publicly available. Requests for datasets analyzed in the current study can be made after acceptance for publication by bona fide researchers by submitting a research proposal to the D2d Publications Committee for review. Individual participant data will be shared in a de-identified/anonymized format using a specialized SAS data platform. Protocol synopsis, contact details, publications, and the process for collaboration and data requests can be found on the website (d2dstudy.org).

Appendix A

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