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Effect of vitamin D on regression to normal glucose regulation and individual glycemic measures: A secondary analysis among participants adherent to the trial protocol in the randomized clinical trial vitamin D and type 2 diabetes (D2d) study

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ABSTRACT

Aims: To examine the effect of vitamin D on regression to normal glucose regulation (NGR) and individual glycemic measures in the D2d study.
Methods: In per-protocol analyses, we examined time to new-onset diabetes; time to new-onset NGR defined as first occurrence of: 2-or-3 glycemic criteria in the normal range (NGR-1) or fasting plasma glucose (FPG) and 2-hour post-load-glucose (2hPG) in the normal range (NGR-2); proportion meeting NGR at the last study visit; and change in FPG, 2hPG, and HbA1c.
Results: Among 2423 participants, hazard ratio [HR] for diabetes was 0.84 [95%CI, 0.71, 0.99]). HR (95%CI) was 1.16 (0.99, 1.36) for new-onset NGR-1 and 1.06 (0.87, 1.30) for NGR-2. At the last visit, NGR-1 occurred in 12.4% vs. 9.5% participants in the vitamin D vs. placebo group (rate ratio for vitamin D 1.31 [1.02, 1.70]); whereas, NGR-2 occurred in 8.7% vs. 6.0% (rate ratio for vitamin D 1.45 [1.05, 2.00]). During follow-up, FPG, HbA1c, and 2hPG increased in both groups. Mean difference in FPG favored vitamin D (-0.80 mg/dL; 95%CI, −1.26, −0.33).
Conclusions: In secondary analyses among participants adherent to the trial protocol, vitamin D lowered risk of developing diabetes and increased likelihood of NGR at the end of the study.

1. Introduction

Prediabetes is defined as having glycemic parameters above normal but below diabetes thresholds (fasting plasma glucose [FPG] 100–125 mg/dL, 2-hour glucose after a 75-gram oral glucose load [2hPG] 140–199 mg/dL, or hemoglobin A1c [HbA1c] 39–47 mmol/mol [5.7–6.4%]) and has been associated with an annualized progression to diabetes of 5–10% [1]. While the different prediabetes criteria define overlapping, but not identical, diabetes risk categories, hyperglycemia is a continuum; prediabetes cannot be considered a benign condition as it has been associated with diabetes-specific complications such as nephropathy, small fiber neuropathy, retinopathy, and risk of macrovascular disease [2]. In clinical trials, intensive lifestyle modifications and pharmacologic interventions have shown significant reductions

(~30–70%) in risk of developing diabetes in people with prediabetes defined by impaired glucose tolerance following a 75-gram oral glucose tolerance test [3–7]; however, real-life effectiveness of lifestyle modifications is disappointingly low, and additional approaches are needed.

Vitamin D supplementation has garnered attention for prevention of type 2 diabetes as longitudinal observational studies have shown a consistent association between higher blood 25-hydroxyvitamin D [25(OH)D] levels and lower risk of developing diabetes [8,9]. Potential mechanisms by which vitamin D may affect glucose metabolism include stimulating insulin secretion through vitamin D receptors on pancreatic beta cells, decreasing systemic inflammation, and improving insulin resistance in muscle and the liver [10]. Moreover, insufficient vitamin D status could contribute to hypophosphatemia, which has been implicated in impaired glucose tolerance and decreased insulin sensitivity [11].

There are three trials that were specifically designed and conducted to test whether vitamin D supplementation reduces the rate of progression to diabetes in people with prediabetes [12–14]. In the vitamin D and type 2 diabetes (D2d), Tromsø, and Diabetes Prevention with active Vitamin D (DPVD) trials, vitamin D consistently lowered risk of developing diabetes compared to placebo in a remarkably similar degree; however, observed differences in intention-to-treat (ITT) analyses in each individual trial did not reach statistical significance. A meta-analysis that combined individual participant data from these three trials reported, in ITT analyses, a statistically significant 15% lower risk of developing diabetes with vitamin D among people with prediabetes [15]. ITT analysis estimates the effect of being assigned to an intervention regardless of adherence or protocol fidelity and addresses the effectiveness of an intervention, approximating clinical practice. However, ITT analyses do not address post-randomization biases due to nonadherence, which is the objective of per-protocol (as treated) analyses [16–19].

Although diabetes prevention trials typically focus on delaying progression to diabetes, regression to normal glucose regulation (NGR) is a critical outcome because NGR is associated with lower risk of vascular disease than prediabetes due to lower glycemic exposure over time [20]. In addition to potentially reducing progression from prediabetes to clinical diabetes, vitamin D may increase the likelihood of regression to NGR, which is important because even transient regression to NGR confers lower risk of developing diabetes [21–23]. For example, participants in the Diabetes Prevention Program (DPP) who regressed to NGR at least once during the intervention period (median 3.2 years) had a 56% lower risk of developing diabetes compared to those who consistently had prediabetes during a median follow-up of 5.7 years [21]. Furthermore, regression to NGR during DPP was associated with a 22–30% decreased prevalence of microvascular disease [20], which further highlights the importance of interventions that promote reaching and maintaining NGR.

The D2d study is the largest vitamin D diabetes prevention trial and the only diabetes prevention trial that used all three modern glycemia criteria (FPG, 2hPG, HbA1c) to define glycemic states for eligibility and outcome assessment [12]. The purpose of this secondary analysis of D2d data is to examine the effect of vitamin D on progression to diabetes, regression to NGR, and change in the individual glycemic variables of FPG, 2hPG and HbA1c, based on per-protocol (as treated) analyses among participants adherent to the trial protocol defined as those on study treatment and prior to the introduction of a rescue medications (diabetes/weight-loss medications or out-of-study high-dose vitamin D).

2. Research design and methods

2.1. Overview of the D2d study

The D2d study is a U.S.-based randomized, double-blind, placebo-controlled clinical trial conducted at 22 collaborating sites (d2dstudy.org/sites) testing whether vitamin D_3 reduces diabetes risk in adults at

high-risk for type 2 diabetes [12]. Participants were recruited from October 2013 through February 2017 and follow-up continued until November 2018, when the pre-specified number of diabetes events was met. The design of D2d, including eligibility criteria, when and where data was collected, a description of the intervention, and how randomization was implemented, and main results have been published (the protocol is available at d2dstudy.org and is summarized below). The study was approved by the Institutional Review Board of each collaborating site and monitored by an independent Data and Safety Monitoring Board. All participants provided written informed consent.

Eligible participants met at least 2-of-3 glycemic criteria for pre-diabetes as defined by the 2010 American Diabetes Association (ADA) guidelines [24]: FPG 100–125 mg/dL (5.6–6.9 mmol/L); 2hPG 140–199 mg/dL (7.8–11.0 mmol/L); HbA1c 39–47 mmol/mol (5.7–6.4%), and not meeting any of the criteria for diabetes. Other inclusion criteria were age ≥ 30 years (25 years for American Indians, Alaska Natives, Native Hawaiians, or other Pacific Islanders) and body-mass index of 24–42 kg/m^2 (22.5–42 kg/m^2 for Asian Americans). We selected these age criteria to avoid recruiting people who have prediabetes for a different reason (e.g., monogenic diabetes, type 1 diabetes) other than being at risk for type 2 diabetes. Blood 25(OH)D level was not an eligibility criterion. Key exclusion criteria included: conditions (other than hyperglycemia and race) affecting HbA1c assessment, use of diabetes or weight-loss medications, hyperparathyroidism, nephrolithiasis, hypercalcemia, pregnancy and bariatric surgery [25]. People with serum liver transaminases (ALT or AST) higher than 3 times the normal range for the clinical site's laboratory and those with chronic kidney disease defined as estimated glomerular filtration rate [GFR] < 50 mL/min were excluded. The complete list of eligibility criteria and the recruitment and screening process and how participant flow through the study have been described previously [12,26].

Participants were randomized to take a once-daily single soft-gel containing 4000 IU of vitamin D_3 (cholecalciferol) or identical placebo. The vitamin D dose was selected to balance safety and efficacy and resulted in a large difference in the serum 25(OH)D level between the trial groups in the first 2 years of follow-up. Randomization was block-stratified by site, body-mass index (<30 or ≥30 kg/m^2), and race (White or non-White). Participants and study staff were blinded to randomization assignment. Participants were asked to bring their pill bottles with them to all scheduled visits for adherence assessment. Pill counts were completed by study staff at each visit. Participants also received information and tips to promote adherence with study pills.

To maximize the study's ability to observe a treatment effect, participants were asked to refrain from using diabetes-specific and weight-loss medications during the study and to limit the use of outside-of-study vitamin D to 1000 IU per day from all supplements, including multivitamins. During the study, participants were provided with information on diabetes prevention through information sheets and optional twice-yearly group meetings.

2.2. Follow up and laboratory testing

Glycemic status was assessed annually with FPG, 2hPG, and HbA1c and semi-annually with FPG and HbA1c over a median follow up of 2.5 years, as previously described [25]. HbA1c was measured with an ion-exchange high-performance liquid chromatography method certified by the National Glycohemoglobin Standardization Program. Plasma glucose was measured with the use of a hexokinase method. At the conclusion of the study, stored serum samples from the baseline and yearly visits were used to measure 25(OH)D by liquid chromatography–tandem mass spectrometry, as previously described [12]. Adverse events were assessed at every visit and no differences were noted between the vitamin D and placebo groups [12,27].

2.3. Outcomes

For the present analysis, the outcomes of interest were time to new-onset diabetes, time to new-onset NGR, proportion of participants with NGR at the last visit, and change from baseline in FPG, 2hPG and HbA1c (as continuous variables).

New-onset diabetes, which was the primary outcome of D2d, was defined as when two or three of the glycemic measures met the ADA thresholds for diabetes (FPG ≥ 126 mg/dL [7.0 mmol/L], 2hPG ≥ 200 mg/dL [11.1 mmol/L] or HbA1c ≥ 48 mmol/mol [6.5%]) at a scheduled visit [25]. When only one glycemic measure met the threshold, a confirmatory visit to repeat the same glycemic test that was positive was completed within 8 weeks. A diagnosis of diabetes would only be made if the same glycemic test was again positive within 8 weeks. A diagnosis of diabetes made outside of the study was validated by in-study laboratory testing or adjudicated by an independent clinical outcomes committee based on review of medical records that included data on FPG, HbA1c or 2hPG obtained in routine clinical practice. During the adjudication process, which was blinded to treatment assignment, the committee members were asked to follow as closely as possible the in-study glycemic algorithms when making a diagnosis of diabetes. Given that the diagnosis of diabetes by study procedures (e.g., screening for diabetes at regular intervals, use of common laboratory criteria assessed in a central laboratory) is robust and unbiased compared to a diagnosis outside of the study that depends on many random, uncontrolled factors, we also evaluated the rate of new-onset diabetes according to trial-specific glycemic criteria only, i.e., diagnoses of diabetes made outside of the study were excluded, and a sensitivity analysis was conducted as a comparison.

Regression to NGR was not a pre-specified D2d outcome, and there is no uniformly accepted definition for NGR. We used two definitions. First, to parallel the definition of the primary outcome of time to new-onset diabetes, we defined regression to NGR as the first occurrence of two or three glycemic criteria in the normal range and none in the diabetes range (NGR-1). In addition, we defined NGR as having both FPG and 2hPG within the normal range regardless of HbA1c (NGR-2). This latter definition is consistent with how other diabetes prevention trials have defined NGR, including the DPP study [22,28].

Because new-onset NGR during the study is dynamic and participants can move between the NGR and pre-diabetes categories during follow-up, and to assess for a longer-term effect of vitamin D, we examined whether participants met the NGR definitions at their last study visit.

Change from baseline in FPG, 2hPG, and HbA1c was calculated as described below. Data for FPG, 2hPG and HbA1c were included until a diabetes medication was started, beyond the D2d-defined diabetes outcome, to better assess the long-term effect of vitamin D on glycemia. This approach allowed follow-up to extend until the study end and modeled a similar analysis done in the DPP study [4].

2.4. Data analyses

The analytical cohort included all 2,423 randomized participants in the D2d study. The sample size for the trial was estimated based on the primary outcome, incident diabetes. Comparisons between the two groups (vitamin D vs. placebo) at baseline and with respect to the rate of withdrawal, discontinuation of trial pills, use of diabetes or weight-loss medications, and supplemental intake above the trial limit used Fisher's exact test, the chi-square test, the Wilcoxon rank-sum test, or the pooled-variance *t*-test. Descriptive statistics included percentage, means ± SD, or medians (interquartile range; Q1-Q3) for non-normally distributed data.

Because the present secondary analyses focus on the effect of vitamin D on glycemia among participants adherent to the trial protocol, all analyses censored follow-up data when a participant stopped trial pills, started a diabetes or weight-loss medication, or took out-of-study supplemental vitamin D above the study limit of 1000 IU per day, similar to prior analyses from the D2d study [12,29]. This per-protocol analysis aims to capture the effects of the intervention using data obtained while on treatment and prior to introducing a rescue medication (diabetes/weight-loss medications or out-of-study high-dose vitamin D) [30].

Follow-up time for all analyses was calculated as time from randomization until the occurrence of death, withdrawal, last follow-up visit, or the first occurrence of the event of interest in the time-to-event analyses (new-onset diabetes, new-onset NGR-1/NGR-2). No imputation was performed for missing data, but we conducted a sensitivity analysis to assess for noninformative censoring of incomplete data. For all analyses, models included group assignment as its main predictor variable and the stratification variables (trial site, body-mass index, and race).

Kaplan-Meier estimates were plotted for each group (vitamin D vs. placebo) for time-to-event analyses (new-onset diabetes, new-onset NGR-1/NGR-2). After finding no evidence that the proportionality of hazards assumption was violated, Cox proportional hazard models were used to compare the hazard rates of new-onset diabetes and new-onset NGR-1/NGR-2 between the vitamin D and placebo groups. For the diabetes outcome, an HR < 1.00 indicates lower risk of diabetes; for the NGR outcomes, an HR > 1.00 indicates higher risk for NGR, i.e., favors regression to NGR. Participants who met the NGR outcomes during follow-up could be diagnosed with diabetes subsequently; hence, we conducted a sensitivity analysis of new-onset NGR-1/NGR-2 only among participants who did not subsequently develop diabetes. To assess the effect of vitamin D on regression to NGR at the end of the study, we calculated the rate ratio (and 95%CI) for NGR-1 and NGR-2 and compared between the vitamin D vs. placebo groups.

Between group differences for the change in continuous variables (FPG, 2hPG and HbA1c) from baseline were determined using a linear mixed-effects model approach to account for within participant correlation across the time-points, and an overall least-squares mean difference for vitamin D vs. placebo from baseline to the end of study was calculated. An interaction term between treatment assignment and time from baseline visit was used to assess if the change trajectories in variable levels differed significantly between randomization groups. Sensitivity analyses explored potential non-linear changes during follow-up. Since participants with very low baseline levels of serum 25(OH)D would be expected to benefit more from vitamin D [12,15,29], we examined differences between vitamin D and placebo for the change in continuous variables (FPG, 2hPG and HbA1c) among participants with baseline serum 25(OH)D levels of <12 ng/mL (30 nmol/L).

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc). No adjustments were made for multiple comparisons; therefore, only point estimates and 95%CIs are presented without P-values.

3. Results

3.1. Baseline characteristics

The baseline characteristics (Table 1) were comparable between the two groups. Overall, 170 participants in the vitamin D group and 172 in the placebo group were censored at some point during follow-up because they met a per-protocol censoring event. These participants contributed data until censoring.

3.2. New-onset diabetes

As previously reported based on the per-protocol analysis [12], the overall diabetes event rate was 9.19 per 100 person-years in the vitamin D group and 10.98 per 100 person-years in the placebo group (Fig. 1) with a hazard ratio (HR) (95%CI) of 0.84 (0.71 to 0.99). In the small subgroup of participants with a baseline 25(OH)D level <12 ng/mL (n = 103), the hazard ratio in the per-protocol analysis (95%CI) was 0.17 (0.04 to 0.79) for diabetes in the vitamin D vs placebo. In a sensitivity analysis that excluded cases of diabetes diagnosed by adjudication (N =

Table 1
Baseline characteristics of D2d participants.

	Overall (n = 2,423)	Vitamin D (N = 1,211)	Placebo (N = 1,212)
Characteristic			
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)
Race, no. (%) [2]			
White	1616 (66.7)	810 (66.9)	806 (66.5)
Black	616 (25.4)	301 (24.9)	315 (26.0)
Asian	130 (5.4)	66 (5.5)	64 (5.3)
Other	61 (2.5)	34 (2.8)	27 (2.3)
Hispanic or Latino Ethnicity, no. (%) [a]	225 (9.3)	120 (9.9)	105 (8.7)
Family history of diabetes (1st degree relative), no. (%)	1514 (62.5)	759 (62.7)	755 (62.3)
Dietary supplement use [b]			
Vitamin D			
Participants taking vitamin D supplements, no. (%)	1037 (42.8)	508 (41.9)	529 (43.6)
Vitamin D intake from supplements among all participants, IU/day [c]	313 ± 398	310 ± 401	316 ± 397
Vitamin D intake among participants using supplements, IU/day	732 ± 254	739 ± 256	725 ± 253
Calcium			
Participants taking calcium supplements, no. (%)	804 (33.2)	385 (31.8)	419 (34.6)
Calcium intake from supplements among all participants, mg/day [c]	103 ± 176	100 ± 175	107 ± 176
Calcium intake among participants using supplements, mg/day	312 ± 167	316 ± 168	308 ± 166
Body-mass index, kg/m²	32.1 ± 4.5	32.0 ± 4.5	32.1 ± 4.4
Laboratory			
Fasting plasma glucose (FPG), mg/dL	107.9 ± 7.4	108.0 ± 7.4	107.8 ± 7.4
Glucose 2 h after a 75-gram oral glucose load (2hPG), mg/dL	137.2 ± 34.3	136.9 ± 34.3	137.6 ± 34.3
Hemoglobin A1c, mmol/mol	41.14 ± 2.30	41.21 ± 2.34	41.08 ± 2.26
Hemoglobin A1c, %	5.91 ± 0.21	5.92 ± 0.21	5.91 ± 0.21
Pre-diabetes categories, no. (%)			
Met all 3 glycemic criteria (IGT + *i*A1c + IFG) [d]	856 (35.3)	427 (35.3)	429 (35.4)
Met two glycemic criteria only			
IGT + IFG	152 (6.3)	74 (6.1)	78 (6.4)
IGT + *i*A1c	231 (9.5)	103 (8.5)	128 (10.6)
IFG + *i*A1c	1184 (48.9)	607 (50.1)	577 (47.6)
Meeting individual glycemic criterion [e]			
IFG	2192 (90.5)	1108 (91.5)	1084 (89.4)
IGT	1239 (51.1)	604 (49.9)	635 (52.4)
*i*A1c	2271 (93.7)	1137 (93.9)	1134 (93.6)
Serum 25-hydroxyvitamin D, ng/mL	28.0 ± 10.2	27.7 ± 10.2	28.2 ± 10.1
Serum 25-hydroxyvitamin D categories, no. (%)			
< 12 ng/mL	103 (4.3)	60 (5.0)	43 (3.6)
12–19 ng/mL	422 (17.4)	216 (17.8)	206 (17.0)
20–29 ng/mL	876 (36.2)	453 (37.4)	423 (34.9)
≥ 30 ng/mL	1021 (42.2)	482 (39.8)	539 (44.5)

Plus-minus values are means ± SD. Percentages may not add up to 100 because of rounding. There were statistically significant differences between the two groups in mean age (p = 0.042) and serum 25-hydroxyvitamin D ≥ 30 ng/mL (p = 0.019).

[a] Race and ethnicity were reported by the participant. The category "other" includes American Indian or Alaska Native (n = 13); Native Hawaiian or other Pacific Islander (n = 5); or other race (n = 43). Ethnicity includes any race.

[b] Data on vitamin D and calcium intake are derived from a question about dietary supplements, including multivitamins.

[c] Value shown is among all participants regardless of whether they reported use of supplements or not.

[d] IFG, impaired fasting glucose defined as fasting plasma glucose 100–125 mg per deciliter (5.6–6.9 mmol/L); IGT, impaired glucose tolerance defined as 2-hour post-load plasma glucose after a 75-gram glucose load 140–199 mg/dL (7.8–11.0 mmol/L) or; *i*A1c, impaired A1c defined as HbA1c 5.7–6.4% (39–47 mmol/mol).

[e] Percentages do not add up to 100 because categories are not mutually exclusive, participants are in more than one category.
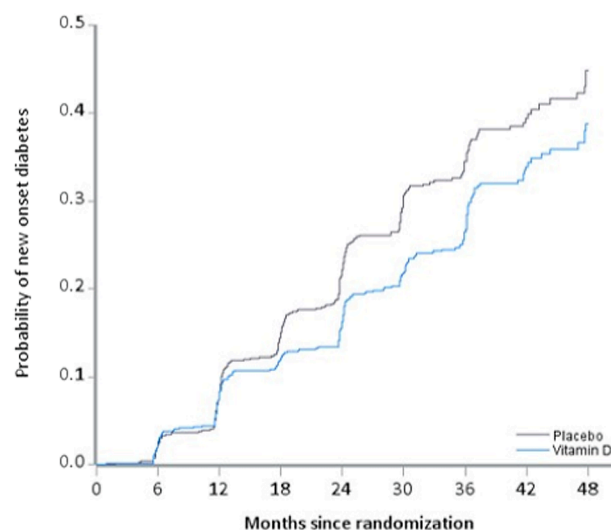
Fig. 1. Time to new-onset diabetes in per-protocol analysis In the per-protocol analysis, follow-up is censored when a participant stopped trial pills, started a diabetes or weight-loss medication or took out-of-study vitamin D from supplements above the study limit of 1000 IU per day. This analysis aims to capture the effects of the intervention using data obtained while on treatment and prior to onset of introducing a rescue medication (diabetes/weight-loss medications or out-of-study high-dose vitamin D).

12 in vitamin D and N = 15 in placebo), the diabetes event rate per 100 person-years was 8.77 in the vitamin D group and 10.44 in the placebo group with a hazard ratio (95%CI) of 0.85 (0.71 to 1.00).

3.3. Regression to normal glucose regulation

Over a median follow-up of 1.9 years (inter-quartile range, 1.0 to 2.5), the NGR-1 outcome occurred in 343 of 1211 participants in the vitamin D group and 295 of 1212 participants in the placebo group (15.3 events and 13.3 events per 100 person-years, respectively). The HR (95%CI) for vitamin D was 1.16 (0.99 to 1.36) (Fig. 2A). Twenty six out of 343 participants (7.6%) in the vitamin D group and 26 out of 295 participants (8.8%) in the placebo group who met the NGR-1 outcome during follow-up were subsequently diagnosed with diabetes. Among participants who never met NGR-1 during follow-up (N = 1785), 29% developed diabetes compared to 8.2% in those who met NGR-1 during follow-up (p < 0.01). Baseline characteristics did not differ between participants who met NGR-1 vs. those that did not meet NGR-1 (results not shown).

Over a median follow-up of 2.0 years (inter-quartile range, 1.0 to 3.0), the NGR-2 outcome occurred in 213 of 1211 participants in the vitamin D group and 189 of 1212 participants in the placebo group (8.5 events and 7.8 events per 100 person-years, respectively). The HR (95% CI) for vitamin D was 1.09 (0.89 to 1.32) (Fig. 2B). 14 out of 213 participants (6.6%) in the vitamin D group and 9 out of 189 participants (4.8%) in the placebo group who met the NGR-2 outcome during follow-up were subsequently diagnosed with diabetes. Among participants who never met NGR-2 during follow-up (N = 2021), 27.0% developed diabetes compared to 5.7% in those who met NGR-2 during follow-up (p <

A. Using NGR-1 definition
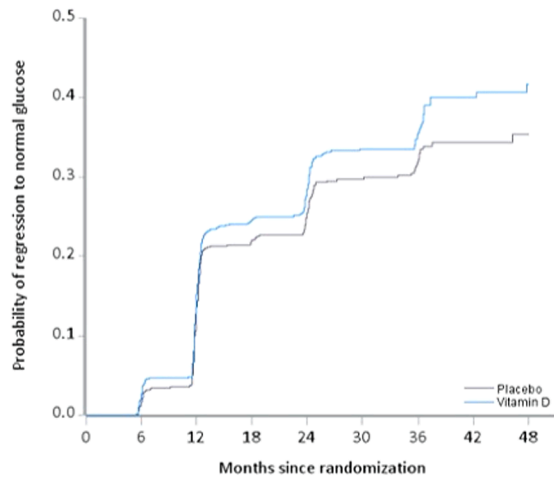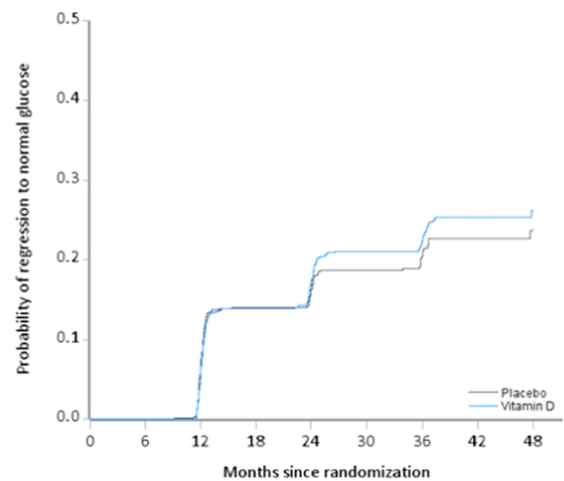
B. Using NGR-2 definition

Fig. 2. Time to first occurrence of normal glucose regulation *Panel A. Using NGR-1 definition* Time to first occurrence of normal glucose regulation (NGR-1) defined as the first occurrence of two or three glycemic criteria (FPG, 2hPG or HbA1c) in the normal range, and none in the diabetes range *Panel B. Using NGR-2 definition* Time to first occurrence of to normal glucose regulation (NGR-2) defined as the first occurrence of FPG and 2hPG normal, regardless of A1c.

0.01). Baseline characteristics did not differ between participants who met NGR-2 vs. those that did not meet NGR-2 (results not shown).

At the last visit, over a median follow-up of 2.5 years (inter-quartile range, 2.0 to 3.2), the NGR-1 outcome was observed in 12.4% of participants in the vitamin D group and 9.5% of participants in the placebo group (Table 2). The rate ratio for NGR-1 for vitamin D vs placebo (95% CI) was 1.31 (1.02 to 1.70). At the last visit, the NGR-2 outcome occurred in 8.7% of participants in the vitamin D group and 6.0% of participants in the placebo group. The rate ratio for NGR-2 for vitamin D vs placebo (95%CI) was 1.45 (1.05, 2.00).

In the small subgroup of participants with a baseline 25(OH)D level <12 ng/mL (n = 103), at the last visit, the rate ratio for NGR-1 for vitamin D vs placebo (95%CI) was 1.06 (0.34 to 3.33) and the rate ratio for NGR-2 for vitamin D vs placebo (95%CI) was 1.83 (0.47 to 7.08).

3.4. Change in continuous glucose variables

At baseline, mean FPG was similar between the vitamin D and

placebo groups (Table 1 and eTable 1). While FPG increased during follow-up in both groups, the overall mean difference in change from baseline favored the vitamin D group compared to the placebo group (−0.80 mg/dL; 95%CI, −1.26 to −0.33). In the small subgroup of participants with a baseline 25(OH)D level <12 ng/mL (n = 103), the overall mean difference in change from baseline in FPG favored the vitamin D group compared to the placebo group (−4.32 mg/dL; 95%CI, −6.39 to −2.25) (eTable 2).

At baseline, mean HbA1c was similar between the vitamin D and placebo groups (eTable 1). During follow-up, HbA1c increased in both groups, and the overall mean difference in change from baseline was not different between groups (-0.045 mmol/mol; 95%CI, −0.163 to 0.073). At baseline, mean 2hPG was similar between the vitamin D and placebo groups (eTable 1). During follow-up, 2hPG increased in both groups, and the overall mean difference in change from baseline was not different between groups (0.13 mg/dL; 95%CI, −2.00 to 2.26). In the small subgroup of participants with a baseline 25(OH)D level < 12 ng/mL (n = 103), the overall mean difference in change from baseline was not significantly different for HbA1c or 2hPG (eTable 2).

During follow-up, the mean change in body weight did not differ between vitamin D and placebo.

4. Discussion

In this secondary, per-protocol analysis among D2d participants adherent to the trial protocol, which provides a different estimand of treatment effect than ITT analyses, vitamin D lowered risk of new-onset diabetes, increased the likelihood of regression to NGR at the last visit, and had a small benefit in FPG especially among those with baseline serum 25(OH)D level <12 ng/mL.

4.1. Incident diabetes, ITT vs. per-protocol

In long-term trials, biases emerge during follow-up due to non-adherence to the trial intervention, use of rescue medications, or differential loss to follow-up leading to post-randomization confounding, which may influence the estimate of treatment effect and study power. Previously, when using an ITT analysis that included all D2d participants regardless of adherence to the protocol, the risk of diabetes was 12% lower in the vitamin D group than the placebo group, but the

Table 2
Normal glucose regulation at the last study encounter.

	Vitamin D	Placebo	
	No. events/ Total	No. events/ Total	Rate Ratio for Vitamin D vs. Placebo (95%CI)
Normal glucose regulation – definition 1: *2 or 3 glycemic criteria (FPG, 2hPG, HbA1c) in the normal range and none in the diabetes range*	133/1069 (12.4%)	102/1078 (9.5%)	1.31 (1.02, 1.70)
Normal glucose regulation – definition 2: *FPG and 2hPG normal, regardless of HbA1c*	90/1037 (8.7%)	63/1050 (6.0%)	1.45 (1.05, 2.00)

Normal Glucose Regulation 1 (NGR-1) and Normal Glucose Regulation 2 (NGR-2) were evaluated at the last annual-type visit (when all three glycemic criteria were ascertained) or last annual-type visit prior to last semi-annual encounter if the last encounter was a semi-annual type (when only FPG and HbA1c were ascertained).
FPG, fasting plasma glucose; 2hPG, plasma glucose 2 h after a 75-gram glucose load; HbA1c, hemoglobin A1c.

difference was not statistically significant (HR 0.88; 95%CI 0.75 to 1.04) [12].

In contrast to an ITT analysis, a per-protocol analysis addresses post-randomization biases and captures the effects of the intervention while on treatment and prior to introducing a rescue medication (diabetes/weight-loss medication) or personal use of supplemental vitamin D over the study allowable limit. For example, if the hypothesis that vitamin D lowers diabetes risk vs. placebo is true, a participant in the placebo group would progress towards the diabetes threshold and a diabetes diagnosis would have been captured during follow-up by in-study testing; however, if the participant's physician started metformin, then the participant's glycemia would be improved and the diabetes outcome would never be captured during follow-up, falsely shifting the relative risk reduction towards null in the ITT analysis. In the per-protocol analysis, this participant still contributes time free of diabetes until the censoring event. Therefore, per-protocol analyses provide a different estimand of the treatment effect, which is useful to clinicians and patients to fully understand the range of potential treatment effects of an intervention, such as vitamin D, when taken as prescribed [18,19].

The results of the present per-protocol analysis on diabetes risk, which we previously reported [12], show that vitamin D in the D2d study reduced risk of diabetes by 16% among participants adherent to the trial protocol. Among participants with blood 25(OH)D lower than 12 ng/mL, per-protocol analysis showed a 93% risk reduction in new-onset diabetes, which is consistent with the 62% reduction by ITT analyses, which we previously reported [12]. Moreover, in a previous analysis we reported that participants who reached and maintained intra-trial serum 25(OH)D $\geq$ 50 ng/mL had a 59% lower risk for conversion to diabetes [31]. It is important to note that despite its strengths, a per-protocol analysis is less conservative than the ITT analysis that aims to captures the benefit of randomization to reduce potential bias of unknown direction.

The per-protocol and ITT results from the D2d study are consistent with results from two other trials which were also specifically designed and conducted to test the effect of vitamin D for diabetes prevention, the Tromsø study (Norway, cholecalciferol 20,000 IU weekly) [13] and the DPVD study (Japan, eldecalcitol 0.75 μg daily). In a meta-analysis that combined individual participant data from the D2d, Tromsø and DPVD trials, vitamin D significantly reduced risk of diabetes by 15% compared to placebo in an ITT analysis (HR0.85; 95%CI 0.75 to 0.96) and 17% in a per-protocol analysis (HR 0.83; 95%CI 0.73 to 0.94) [15]. These results are consistent with our per-protocol analyses and indicate a modest reduced risk of diabetes with vitamin D in people with prediabetes not selected for vitamin D deficiency and a larger effect among those with very low blood 25(OH)D levels.

4.2. NGR-1 and NGR-2

Two definitions of NGR were used in this secondary analysis. To mirror the primary D2d outcome, we defined NGR-1 as reaching two or three glycemic criteria in the normal range with none in the diabetes range. Participants randomized to vitamin D were 16% more likely to reach NGR-1 during follow-up, but the result missed statistical significance. When we examined the effect of vitamin D on NGR-2, the result was in the same direction, but it was also not statistically significant between groups. Participants who met either the NGR-1 or NGR-2 criterion had a lower risk of developing diabetes compared to those who never met the NGR-1 or NGR-2 outcome, respectively. However, regression to NGR is not a required intermediate state in lowering the risk of developing diabetes, as participants may stay in the prediabetes category and never progress to diabetes.

Because NGR may be a transient phenomenon that reverses over time, and to assess for a longer-term effect of vitamin D, we examined the effect of vitamin D on the proportion of participants regressing to NGR at the last study visit. Participants in the vitamin D group were significantly more likely to have reached NGR-1 (31% more likely vs.

placebo) or NGR-2 (45% more likely vs. placebo) at the end of the study. Staying in the NGR state confers clinical benefit as this glycemic category is associated with fewer vascular complications [20].

4.3. Continuous glycemic variables

When FPG, 2hPG, and HbA1c levels were compared between the two groups in the entire cohort, only small differences in FPG favoring vitamin D were noted during follow-up. Although the difference in change from baseline in FPG between vitamin D and placebo in the overall cohort was small (0.80 mg/dL), in the subgroup of participants with baseline 25(OH)D level <12 ng/mL (n = 103), the difference in change from baseline in FPG was larger (4.32 mg/dL). For a dichotomous outcome, such as diabetes, large declines in blood glucose levels are not needed to appreciate large benefits in incident disease. For example, in the DPP study, a reduction in FPG of 6 mg/dL and a reduction of 0.15% in HbA1c between lifestyle and placebo groups resulted in a 60% reduction in the risk of new-onset diabetes, confirming that small changes in glycemia translate to a large difference in incident disease. In a post-hoc analysis of D2d, among participants with baseline 25(OH)D level <12 ng/mL, we previously reported a 60% reduction in the risk of new onset diabetes with vitamin D compared to placebo [12], which was accompanied by a significant improvement in the disposition index, an estimate of pancreatic beta cell function [29], suggesting that people with prediabetes and vitamin D deficiency would benefit the most from vitamin D. Changes in 2hPG and HbA1c were not significantly different over time. This could be due to the large variability in the measurement of 2hPG compared to FPG; the wide variability in HbA1c and its limited ability to capture glycemia; or it may indicate that vitamin D may not have a large effect on post-prandial glycemia, which influences HbA1c.

4.4. Strengths and limitations

Beyond its randomized, double-blind, placebo-controlled study design, the parent D2d study has several strengths, including the large sample size, long duration of treatment, use of a high-dose (4000 IU), daily vitamin D, and baseline 25(OH)D that is representative of the US adult population. Given that many factors can influence glycemia and confound results (e.g., use of diabetes medications before the diagnosis of diabetes), we followed a per-protocol analysis, which minimizes post-randomization confounding, and is more likely to uncover effects of the intervention. The D2d study also utilized all three ADA glycemic criteria currently used to screen for and diagnose prediabetes and diabetes.

For this secondary analysis, a limitation was that the NGR analyses were not prespecified; however, we used definitions that are internally consistent (NGR-1) and consistent with what other studies have used (NGR-2). Another limitation was the relatively short duration of the trial. It is also possible that we would have seen a larger benefit with vitamin D if applied earlier in the natural history of type 2 diabetes given that disturbances in physiology are evident for a decade or more before hyperglycemia becomes clinically apparent [32,33]. Finally, D2d was not designed or powered to evaluate safety, especially because the study may have excluded people who may be at risk for adverse events.

4.5. Conclusions

In summary, among participants who were adherent to the D2d study protocol during follow-up, vitamin D at 4000 IU per day, when compared to placebo, lowered risk of developing type 2 diabetes and increased likelihood of regression to NGR at the end of the study. Vitamin D had a small beneficial effect on change in FPG, especially among participants with a low blood 25(OH)D level but did not have an effect on change in HbA1c or 2hPG.

Guarantor Statement
DSH and JN take full responsibility for the contents of the article.

Prior Publication

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Data Sharing Statement

Datasets generated and/or analyzed during the current study and the associated data dictionary are not publicly available but are available from the D2d Coordinating Center at Tufts Medical Center on reasonable request by bona fide researchers after acceptance for publication. Protocol synopsis, contact details, publications, and the process for collaboration and data requests can be found on the website (d2dstudy.org).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2023.110792.

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