



# Intratrial Exposure to Vitamin D and New-Onset Diabetes Among Adults With Prediabetes: A Secondary Analysis From the Vitamin D and Type 2 Diabetes (D2d) Study

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## OBJECTIVE

Postrandomization biases may influence the estimate of efficacy of supplemental vitamin D in diabetes prevention trials. In the Vitamin D and Type 2 Diabetes (D2d) study, repeated measures of serum 25-hydroxyvitamin D [25(OH)D] level provided an opportunity to test whether intratrial vitamin D exposure affected diabetes risk and whether the effect was modified by trial assignment (vitamin D vs. placebo).

## RESEARCH DESIGN AND METHODS

The D2d study compared the effect of daily supplementation with 100  $\mu\text{g}$  (4,000 units) of vitamin D<sub>3</sub> versus placebo on new-onset diabetes in adults with prediabetes. Intratrial vitamin D exposure was calculated as the cumulative rolling mean of annual serum 25(OH)D measurements. Hazard ratios for diabetes among participants who had intratrial 25(OH)D levels of <50, 75–99, 100–124, and  $\geq 125$  nmol/L were compared with those with levels of 50–74 nmol/L (the range considered adequate by the National Academy of Medicine) in the entire cohort and by trial assignment.

## RESULTS

There was an interaction of trial assignment with intratrial 25(OH)D level in predicting diabetes risk (interaction  $P = 0.018$ ). The hazard ratio for diabetes for an increase of 25 nmol/L in intratrial 25(OH)D level was 0.75 (95% CI 0.68–0.82) among those assigned to vitamin D and 0.90 (0.80–1.02) among those assigned to placebo. The hazard ratios for diabetes among participants treated with vitamin D who maintained intratrial 25(OH)D levels of 100–124 and  $\geq 125$  nmol/L were 0.48 (0.29–0.80) and 0.29 (0.17–0.50), respectively, compared with those who maintained a level of 50–74 nmol/L.

## CONCLUSIONS

Daily vitamin D supplementation to maintain a serum 25(OH)D level  $\geq 100$  nmol/L is a promising approach to reducing the risk of diabetes in adults with prediabetes.

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Over the past decade, vitamin D has emerged as a possible determinant of risk of type 2 diabetes, and vitamin D supplementation has been hypothesized as a potential intervention to lower diabetes risk (1,2). Observational studies strongly support an association between high blood 25-hydroxyvitamin D [25(OH)D] levels and a lower risk of developing diabetes, especially in people at risk for type 2 diabetes (3). Mechanistic studies provide a biologic plausibility to this hypothesis (4,5). The Vitamin D and Type 2 Diabetes (D2d) study was a randomized placebo-controlled trial conducted to test whether vitamin D supplementation reduces the risk of diabetes among adults at high risk for type 2 diabetes (6). In the intention-to-treat analysis, there was a 12% reduction in new-onset diabetes among participants assigned to vitamin D compared with placebo, but the result was not statistically significant (7).

All large-scale trials are expected to be free of confounding at the start because the randomization process should balance the groups at baseline (8,9). However, biases may emerge during follow-up as a result of nonadherence to the trial intervention, use of rescue medications (e.g., metformin to prevent diabetes), or differential loss to follow-up between participants assigned to the active intervention or placebo. These biases, in turn, lead to postrandomization confounding, which may influence the estimate of treatment efficacy and study power. Vitamin D supplementation trials are especially vulnerable to postrandomization biases given confusing public messages about the needed amount of vitamin D intake, frequent testing of blood 25(OH)D level to assess vitamin D status in the routine clinical setting, replacement with pharmacologic doses of vitamin D, widespread use of over-the-counter vitamin D supplements at variable doses, and seasonal inconsistent cutaneous biosynthesis of vitamin D, among others. These factors have significant potential to influence the estimate of efficacy of the vitamin D intervention in clinical trials. Therefore, while the result from the intention-to-treat analysis in the D2d study estimates the effect by treatment assignment, it may not necessarily estimate the effect of vitamin D supplementation itself (9).

The circulating blood 25(OH)D level in a vitamin D trial reflects exposure to vitamin D from different sources, whether resulting from high adherence to the randomized assignment to vitamin D, out-of-study use of vitamin D supplements, or cutaneous biosynthesis. Repeated measures of blood 25(OH)D levels in the D2d study provided a unique opportunity to test whether intratrial exposure to vitamin D influenced the outcome of interest: new-onset diabetes. We conducted a prespecified secondary analysis to examine whether the intratrial serum 25(OH)D level during the D2d study predicted the development of diabetes. We also assessed whether achieving a higher serum 25(OH)D level among participants assigned to the trial intervention (daily vitamin D supplementation) versus placebo affected risk of developing diabetes differentially.

## RESEARCH DESIGN AND METHODS

### Overview of the D2d Study

The D2d study (ClinicalTrials.gov, NCT01942694) was an event-driven, randomized, double-blind, placebo-controlled clinical trial to test whether daily supplementation with vitamin D<sub>3</sub> lowers diabetes risk in adults at risk for type 2 diabetes (prediabetes) (6). The median follow-up period was 2.5 years. Methods of the D2d study have been reported (6,7) and are briefly summarized below.

Eligible participants met at least two of three glycemic criteria for prediabetes as defined by the 2010 American Diabetes Association (ADA) guidelines: fasting plasma glucose (FPG) 5.6–6.9 mmol/L; plasma glucose 2 h after a 75-g oral glucose load (2hPG) 7.8–11.0 mmol/L; HbA<sub>1c</sub> 5.7–6.4% (39–47 mmol/mol), and not meeting any of the criteria for diabetes (10). Other inclusion criteria were age  $\geq$ 30 years (25 years for American Indians, Alaska Natives, Native Hawaiians, or other Pacific Islanders) and BMI 24–42 kg/m<sup>2</sup> (22.5–42 kg/m<sup>2</sup> for Asian Americans). A low serum 25(OH)D level was not an inclusion criterion. Exclusion criteria included use of diabetes or weight loss medications, hyperparathyroidism, nephrolithiasis, hypercalcemia, and bariatric surgery. Participants were randomized to take either a single softgel that contained 100  $\mu$ g (4,000 units) of vitamin D<sub>3</sub> or matching placebo once daily. To maximize the study's ability to observe a treatment effect, participants

were asked to refrain from using diabetes-specific and/or weight loss medications during the study and to limit the use of outside-of-study vitamin D to 25  $\mu$ g (1,000 units) per day from all supplements, including multivitamins.

The primary outcome of D2d was new-onset diabetes on the basis of glycemic testing. Glycemic status was assessed annually with FPG, HbA<sub>1c</sub>, and 2hPG and semiannually with FPG and HbA<sub>1c</sub>. If at least two of the glycemic measures met the ADA thresholds for diabetes (FPG  $\geq$ 7.0 mmol/L, 2hPG  $\geq$ 11.1 mmol/L, or HbA<sub>1c</sub>  $\geq$ 6.5% [48 mmol/mol]) (10), the participant was considered to have met the diabetes outcome. When only one glycemic measure met the threshold, confirmatory testing was performed. A diagnosis of diabetes made outside of D2d was validated by in-study laboratory testing or adjudicated by an independent clinical outcomes committee.

### The Current Analysis

The purpose of the present analysis was to test whether 1) the intratrial average serum 25(OH)D level, a reflection of exposure to vitamin D during the trial, predicted the development of diabetes in the entire cohort and 2) the approach to achieving a given intratrial serum 25(OH)D level (by daily supplementation with 100  $\mu$ g [4,000 units] per day of vitamin D<sub>3</sub>, as in the intervention group, vs. other means, as in the placebo group) influenced the risk of diabetes. Serum 25(OH)D was measured in stored fasting serum samples from the baseline and month 12, 24, 36, and 48 visits by liquid chromatography-tandem mass spectrometry with calibrators that are traceable to the National Institute of Standards and Technology and validated by a quarterly proficiency testing program administered by the Vitamin D External Quality Assessment scheme (DE-QAS, London, U.K.) (11,12). The coefficient of variation of this assay is 5–8%.

The primary modeling strategy was carried out using intratrial vitamin D exposure as the predictor variable, a cumulative average measure of serum 25(OH)D level during follow-up. For each participant, intratrial vitamin D exposure was calculated as a cumulative annual rolling mean of all available annual serum 25(OH)D values before the occurrence of the primary end point of new-onset diabetes, start of a diabetes or weight loss

medication (before the diagnosis of diabetes), or last follow-up. For example, for a participant diagnosed with diabetes at month 30, the intratrial 25(OH)D level is calculated as follows: (mean [baseline and month 12] + mean [month 12 and month 24])/2. The rationale for including the serum baseline 25(OH)D value in the model is that it takes several months for the serum 25(OH)D level to reach steady state after a change in vitamin D intake (13) and because it is expected to take time for any intervention to have an effect on the pathophysiology of type 2 diabetes. Thus, the intratrial mean vitamin D status in the 1st year of the trial is better estimated as the average of the baseline and month 12 measurements than just the level at month 12.

Participants who stopped taking their trial pills and those who took more than the allowed 25  $\mu$ g (1,000 units) of vitamin D per day outside of the study were included in this analysis. Thus, this modeling captures incomplete adherence to the assigned treatment and use of off-protocol concomitant vitamin D therapies. Covariates in this analysis were assessed at baseline and included site, BMI, race (White, Black, or other), sex, age, usual physical activity, and statin use.

We established categories of participants defined by their intratrial mean 25(OH)D level on the basis of the National Academy of Medicine (formerly the Institute of Medicine)—recommended cutoffs: <30, 30–49, 50–74, 75–124, and  $\geq$ 125 nmol/L (14). The 25(OH)D values were reported without a decimal point (conventional rounding was applied by the laboratory). There were very few participants who maintained an intratrial 25(OH)D level <30 nmol/L; thus, we formed a single category with participants who had an intratrial mean 25(OH)D level <50 nmol/L. The category of 75–124 nmol/L was larger than the other categories; hence we split it into two categories: 75–99 nmol/L and 100–124 nmol/L. We used Cox proportional hazard models to estimate the hazard ratios for diabetes among participants in each intratrial mean 25(OH)D category compared with those in the category of 50–74 nmol/L (the referent, the range considered sufficient by the National Academy of Medicine) (14). We provide unadjusted and adjusted hazard ratios (15). We show results for the entire

cohort (adjusted for D2d treatment assignment only and fully adjusted) and by D2d treatment assignment of vitamin D or placebo (unadjusted and fully adjusted). The proportional hazards assumption was confirmed using Schoenfeld residuals. In a continuous model, D2d treatment assignment (vitamin D vs. placebo) was entered as an effect modifier to test the hypothesis that achieving a given intratrial mean serum 25(OH)D level by daily supplementation (as in the vitamin D intervention) versus other means (as in the placebo) influences risk of diabetes. To assess variability of vitamin D status during follow-up among those assigned to vitamin D versus placebo, we calculated a coefficient of variation for each participant by dividing the SD of all available serum 25(OH)D values after baseline by the average level and multiplying by 100.

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

#### Data and Resource Availability

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (the study's primary funding agency) has established central biosample, genetic, and data repositories for the archival and storage of data and biosamples collected in large, multisite studies funded by NIDDK (such as D2d). The study's coordinating center will coordinate with the NIDDK data repository to prepare the collected data and samples for eventual archiving to the repositories. All samples and data transferred to the repositories will be under the custodianship of the NIDDK and will become available to the public in accordance with standard NIDDK policies.

#### RESULTS

Among the 2,423 randomized participants, 3 were on a diabetes medication at baseline and 1 did not have a serum 25(OH)D measurement at baseline; thus, these participants were excluded from this analysis. An additional 261 were excluded because they developed diabetes ( $n = 106$ ), withdrew ( $n = 49$ ), started a weight loss ( $n = 3$ ) or diabetes ( $n = 9$ ) medication, or died ( $n = 5$ ) before a second serum 25(OH)D measurement

or because blood tests were not available at follow-up annual visits (not drawn [ $n = 2$ ], did not have an in-person follow-up annual visit [ $n = 87$ ]). Included in this analysis are 2,158 participants (1,074 in the vitamin D group and 1,084 in the placebo group) who had a baseline and at least one more follow-up serum 25(OH)D measurement.

Baseline characteristics of participants in the five analyzed intratrial 25(OH)D categories (<50, 50–74, 75–99, 100–124, and  $\geq$ 125 nmol/L) are shown in Table 1. Participants with higher intratrial mean 25(OH)D levels were predominantly White; had, at baseline, higher levels of physical activity and lower BMI; and were more likely to have taken personal vitamin D supplements and statins. Baseline HbA<sub>1c</sub> levels did not differ across the five categories.

As expected, participants in the higher intratrial mean 25(OH)D categories were more likely to have been assigned to vitamin D (the active trial intervention). For example, of those who maintained an intratrial mean 25(OH)D level  $\geq$ 125 nmol/L, 95% had been assigned to vitamin D (Table 1). Of 1,074 participants assigned to treatment with vitamin D, 319 (30%) maintained intratrial mean 25(OH)D levels in the 100–124 nmol/L range, and 430 (40%) maintained 25(OH)D levels  $\geq$ 125 nmol/L. Only 22 participants assigned to placebo achieved an intratrial mean 25(OH)D level  $\geq$ 125 nmol/L.

The hazard ratios for diabetes for each category of intratrial mean 25(OH)D level were determined using the sufficient range of 50–74 nmol/L as the referent. When adjusted for treatment assignment only (Table 2, model 1), hazard ratios for diabetes for the two highest categories were 0.65 (95% CI 0.48–0.89) for the category of 100–124 nmol/L and 0.41 (0.29–0.57) for the category  $\geq$ 125 nmol/L. Fully adjusted hazard ratios for diabetes were 0.57 (0.41–0.79) and 0.35 (0.24–0.50), respectively (Table 2, model 5).

In a continuous interaction model, there was a significant interaction of D2d treatment assignment (vitamin D or placebo) with intratrial mean 25(OH)D level on risk of diabetes ( $P = 0.018$ ). The hazard ratio for diabetes for an increase of 25 nmol/L in intratrial mean 25(OH)D level was 0.75 (95% CI 0.68–0.82) among those assigned to vitamin D and 0.90 (0.80–1.02) among those assigned to

**Table 1—Baseline characteristics of participants in categories defined by intratrial mean serum 25(OH)D level**

Characteristic	Intratrial mean serum 25(OH)D category				
	<50 nmol/L (n = 247)	50–74 nmol/L (n = 456)	75–99 nmol/L (n = 590)	100–124 nmol/L (n = 413)	≥125 nmol/L (n = 452)
Assigned to vitamin D at randomization, n (%)	22 (8.9)	78 (17.1)	225 (38.1)	319 (77.2)	430 (95.1)
Serum 25(OH)D (nmol/L), median (IQR)	40 (32.5–47.5)	62.5 (50–70)	77.5 (62.5–87.5)	75 (57.5–92.5)	86.3 (70–102.5)
Among those assigned to vitamin D	37.5 (30–40)	43.8 (35–52.5)	57.5 (45–70)	67.5 (52.5–80)	85 (67.5–102.5)
Among those assigned to placebo	40 (32.5–47.5)	65 (55–72.5)	85 (77.5–92.5)	105 (97.5–117.5)	122.5 (112.5–150)
Age (years)	55.3 ± 10.5	59.2 ± 10.1	61.4 ± 9.5	60.9 ± 9.5	62.8 ± 8.3
Women, n (%)	94 (38.1)	206 (45.2)	254 (43.1)	173 (41.9)	240 (53.1)
Race, n (%)*					
White	100 (40.5)	302 (66.2)	424 (71.9)	299 (72.4)	330 (73.0)
Black	125 (50.6)	121 (26.5)	111 (18.8)	86 (20.8)	87 (19.2)
Asian	16 (6.5)	21 (4.6)	35 (5.9)	23 (5.6)	22 (4.9)
Other	6 (2.4)	12 (2.6)	20 (3.4)	5 (1.2)	13 (2.9)
Dietary supplement use†					
Vitamin D					
Participants taking vitamin D supplements, n (%)	37 (15.0)	159 (34.9)	306 (51.9)	205 (49.6)	237 (52.4)
Vitamin D intake among all participants (μg/day)‡	2.5 ± 6.6	6.1 ± 9.1	9.3 ± 10.2	9.2 ± 10.1	10.1 ± 10.6
Vitamin D intake among participants using supplements, μg/day	16.9 ± 7.2	17.5 ± 6.3	18.0 ± 6.6	18.6 ± 6.0	19.2 ± 6.2
Calcium					
Participants taking calcium supplements, n (%)	28 (11.3)	135 (29.6)	241 (40.8)	162 (39.2)	175 (38.7)
Calcium intake among all participants (mg/day)‡	33 ± 112	93 ± 171	125 ± 180	126 ± 188	125 ± 191
Calcium intake among participants using supplements (mg/day)	290 ± 192	314 ± 173	307 ± 152	320 ± 168	322 ± 175
BMI (kg/m <sup>2</sup> )	33.5 ± 4.8	32.4 ± 4.3	32.0 ± 4.5	32.0 ± 4.4	30.7 ± 4.2
Physical activity (total MET h/week), median (IQR)	41.9 (17.7–101.1)	52.1 (25.4–124.3)	57.3 (27.1–123.9)	59.5 (25.9–120)	64 (27.9–136.2)
Smoking, n (%)					
Never	150 (60.7)	282 (61.8)	337 (57.1)	243 (58.8)	247 (54.7)
Former	72 (29.2)	138 (30.3)	214 (36.3)	143 (34.6)	187 (41.4)
Current	23 (9.3)	34 (7.5)	30 (5.1)	25 (6.1)	15 (3.3)
Unknown or not reported	2 (0.8)	2 (0.4)	9 (1.5)	2 (0.5)	3 (0.7)
HbA <sub>1c</sub>					
%	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2
mmol/mol	41 ± 2.2	41 ± 2.2	41 ± 2.2	41 ± 2.2	41 ± 2.2
Statin use, n (%)	71 (28.7)	173 (37.9)	270 (45.8)	209 (50.6)	211 (46.7)

Data are mean ± SD unless otherwise indicated. Percentages may not total 100 because of rounding. To convert the values for 25(OH)D to ng/L, divide by 2.496. To convert vitamin D intake to units, multiply by 40. IQR, interquartile range. \*Race was reported by the participant. The category "other" includes Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, or other race. †Data on vitamin D and calcium intake are derived from a question about dietary supplements, including multivitamins and high-dose prescribed doses. Participants were allowed to take supplements up to 25 μg (1,000 units) per day of vitamin D and 600 mg per day of calcium. Dietary intake of vitamin D and calcium was not limited. ‡Value shown is among all participants regardless of whether they reported use of supplements.

placebo. We show the hazard ratios between intratrial mean 25(OH)D level and diabetes risk by D2d treatment assignment (vitamin D or placebo) in Tables 3 and 4. Among participants treated with vitamin D (Table 3), intratrial mean 25(OH)D levels influenced the development of diabetes. In unadjusted analyses, the hazard ratios for diabetes among participants who maintained an intratrial mean 25(OH)D level of 100–124 and ≥125 nmol/L during follow-up were 0.57 (0.36–0.92) and 0.34 (0.21–0.55), respectively, compared with participants who

maintained a 25(OH)D level of 50–74 nmol/L (Table 3, model 1). After full adjustment for potential confounders, the hazard ratios for diabetes were 0.48 (0.29–0.80) and 0.29 (0.17–0.50), respectively (Table 3, model 5). Among participants assigned to placebo (Table 4), there was a pattern of declining risk of diabetes at higher intratrial mean 25(OH)D levels, but the 95% CIs were wide.

Variability in annual serum 25(OH)D levels during the trial was lower in those assigned to vitamin D compared with those assigned to placebo. Specifically,

the mean ± SD coefficient of variation for all available serum 25(OH)D values during follow-up was 10.5 ± 9.1 among participants assigned to vitamin D and 13.0 ± 11.1 among participants assigned to placebo ( $P < 0.001$ ).

## CONCLUSIONS

In the D2d study of adults with prediabetes, trial participants who maintained higher intratrial serum 25(OH)D levels during follow-up had a reduced risk of diabetes, with the greatest risk reduction occurring at intratrial 25(OH)D levels ≥125

**Table 2—Hazard ratios (95% CIs) for new-onset diabetes in categories stratified by intratrial mean serum 25(OH)D level in the entire D2d cohort**

	<50 nmol/L (n = 247)	50–74 nmol/L (n = 456)	75–99 nmol/L (n = 590)	100–124 nmol/L (n = 413)	≥125 nmol/L (n = 452)
Median level	40.0	63.8	86.3	110.0	145.2
Model 1	1.14 (0.84–1.54)	Reference	1.08 (0.84–1.38)	0.65 (0.48–0.89)	0.41 (0.29–0.57)
Model 2	1.24 (0.90–1.70)	Reference	1.01 (0.79–1.30)	0.59 (0.43–0.82)	0.38 (0.27–0.54)
Model 3	1.22 (0.89–1.68)	Reference	1.01 (0.79–1.30)	0.59 (0.43–0.82)	0.39 (0.27–0.55)
Model 4	1.24 (0.90–1.71)	Reference	0.99 (0.77–1.27)	0.58 (0.42–0.81)	0.36 (0.25–0.51)
Model 5	1.25 (0.91–1.72)	Reference	0.98 (0.76–1.26)	0.57 (0.41–0.79)	0.35 (0.24–0.50)

Model 1: adjusted for trial assignment (vitamin D or placebo) only. Model 2: additionally adjusted for site, BMI (at baseline), race (White, Black, other). Model 3: additionally adjusted for sex and age (at baseline). Model 4: additionally adjusted for physical activity (at baseline). Model 5: additionally adjusted for statin use (at baseline). To convert the values for 25(OH)D to ng/L, divide by 2.496.

nmol/L and partial risk reduction at levels of 100–124 nmol/L. The inverse association between higher intratrial 25(OH)D levels and diabetes risk was significant only among trial participants who were assigned to the trial intervention of 100 µg (4,000 units) per day of vitamin D<sub>3</sub>.

The present analysis within a clinical trial allowed for testing the hypothesis that the approach to achieving a given blood 25(OH)D level by daily vitamin D supplementation (as in the D2d intervention group) versus other means (as in the placebo group) influenced the risk of diabetes differentially. There was a strong and statistically significant inverse association of intratrial 25(OH)D concentration with new-onset diabetes in the vitamin D–treated group and a weaker, nonstatistically significant association in the placebo group. Trial participants who were assigned to daily vitamin D supplementation and maintained high intratrial 25(OH)D levels (100–124 and ≥125 nmol/L) had substantial relative reductions in risk of diabetes (52% and 71%, respectively, in fully adjusted models) compared with those who maintained an intratrial 25(OH)D level of 50–74 nmol/L, the referent level by

the National Academy of Medicine. In contrast, among participants assigned to placebo, the pattern of declining risk of diabetes at higher intratrial 25(OH)D levels was not significant. It is not clear why achieving a given 25(OH)D level by daily vitamin D supplementation versus by other means (as in the placebo group) would be important for reducing diabetes risk. However, fluctuating blood 25(OH)D levels are considered nonphysiologic, and continuous and steady exposure to vitamin D is preferred for optimal benefit (16,17). We hypothesize that administration of 100 µg (4,000 units) of vitamin D<sub>3</sub> produced higher 25(OH)D levels that were stable throughout the trial period among participants who received the active intervention, whereas 25(OH)D levels among those who took placebo were variable, depending on timing of out-of-study vitamin D use, sun exposure, and other factors. Our hypothesis is supported by the lower coefficient of variation of serum 25(OH)D levels during follow-up in participants treated with daily vitamin D versus those assigned to placebo.

How might one reconcile the observation that higher intratrial 25(OH)D levels are beneficial with the main, intention-to-treat analysis result in the parent trial of a nonstatistically significant reduction in new-onset diabetes with vitamin D supplementation? Large-scale trials evaluate the efficacy of an intervention because they are expected to be free of confounding at baseline; however, during follow-up, and especially in long-term trials, biases emerge, which may influence the estimate of treatment efficacy (9,18). This is true in vitamin D trials, and there is evidence that postrandomization confounding occurred in D2d, as previously reported (7). During follow-up, more participants assigned to vitamin D than placebo (11.3% vs. 8.9%) stopped trial pills, and more participants assigned to placebo (5.2% vs. 2.6%) reported use of out-of-study vitamin D supplements above the study limit of 25 µg (1,000 units) per day (7). Using the intratrial serum 25(OH)D level rather than the randomized assignment to assess the effect of vitamin D exposure on diabetes risk circumvents these sources of postrandomization confounding. Once

**Table 3—Hazard ratios (95% CIs) for new-onset diabetes in categories stratified by intratrial mean serum 25(OH)D level in D2d study participants assigned to vitamin D**

	<50 nmol/L (n = 22)	50–74 nmol/L (n = 78)	75–99 nmol/L (n = 225)	100–124 nmol/L (n = 319)	≥125 nmol/L (n = 430)
Median level	42.5	66.3	89.4	111.3	145.6
Model 1	0.74 (0.25–2.13)	Reference	0.97 (0.60–1.55)	0.57 (0.36–0.92)	0.34 (0.21–0.55)
Model 2	0.94 (0.31–2.82)	Reference	0.94 (0.57–1.55)	0.53 (0.32–0.87)	0.33 (0.20–0.56)
Model 3	0.95 (0.31–2.85)	Reference	0.91 (0.55–1.51)	0.51 (0.31–0.85)	0.32 (0.19–0.55)
Model 4	0.97 (0.32–2.94)	Reference	0.86 (0.51–1.44)	0.49 (0.29–0.82)	0.29 (0.17–0.51)
Model 5	1.03 (0.34–3.14)	Reference	0.86 (0.51–1.44)	0.48 (0.29–0.80)	0.29 (0.17–0.50)

Model 1: no adjustment. Model 2: adjusted for site, BMI (at baseline), race (White, Black, other). Model 3: additionally adjusted for sex and age (at baseline). Model 4: additionally adjusted for physical activity (at baseline). Model 5: additionally adjusted for statin use (at baseline). To convert the values for 25(OH)D to ng/L, divide by 2.496.

**Table 4—Hazard ratios (95% CIs) for new-onset diabetes in categories stratified by intratrial mean serum 25(OH)D level in D2d study participants assigned to placebo**

	<50 nmol/L (n = 225)	50–74 nmol/L (n = 378)	75–99 nmol/L (n = 265)	100–124 nmol/L (n = 94)	≥125 nmol/L (n = 22)
Median level	40.0	63.8	85.0	108.4	134.1
Model 1	1.20 (0.87–1.66)	Reference	1.09 (0.82–1.46)	0.69 (0.42–1.14)	0.64 (0.24–1.75)
Model 2	1.28 (0.91–1.81)	Reference	1.05 (0.78–1.41)	0.68 (0.41–1.14)	0.60 (0.22–1.67)
Model 3	1.24 (0.89–1.76)	Reference	1.05 (0.78–1.41)	0.69 (0.41–1.16)	0.62 (0.22–1.73)
Model 4	1.23 (0.87–1.75)	Reference	1.04 (0.77–1.39)	0.67 (0.40–1.13)	0.48 (0.15–1.56)
Model 5	1.23 (0.86–1.75)	Reference	1.03 (0.76–1.38)	0.67 (0.40–1.12)	0.47 (0.15–1.52)

Model 1: no adjustment. Model 2: adjusted for site, BMI (at baseline), race (White, Black, other). Model 3: additionally adjusted for sex and age (at baseline). Model 4: additionally adjusted for physical activity (at baseline). Model 5: additionally adjusted for statin use (at baseline). To convert the values for 25(OH)D to ng/L, divide by 2.496.

identifiable sources of postrandomization bias were accounted for, a benefit of achieving higher intratrial levels of 25(OH)D for reducing risk of diabetes became evident.

Corroboration of our findings in other diabetes prevention trials with vitamin D should be encouraged but may be difficult because some trials have tested different vitamin D formulations (19) or used nondaily dosing (20). A treat-to-target trial that aims to keep an intratrial 25(OH)D level >125 nmol/L and that prespecifies an analysis strategy that minimizes postrandomization confounding related to intercurrent events and exposures would be required to confirm these findings; however, such a trial may also encounter similar emergence of confounding after launching. Recently, two meta-analyses that combined aggregate data from trials on vitamin D for diabetes prevention were published. Zhang et al. (21) synthesized results from eight trials (total of 4,896 participants) in people with prediabetes and found that vitamin D supplementation reduced diabetes risk by 11% compared to placebo. All eight trials used a vitamin D dose ≥50 μg (2,000 units) per day, and seven trials that reported such data achieved a blood 25(OH)D level ≥75 nmol/L in participants treated with vitamin D. Barbarawi et al. (22) synthesized results from nine trials (total of 43,559 participants) and reported a reduction in diabetes risk only in trials with people with prediabetes, all of which administered moderate to high vitamin D doses equivalent to ≥25 μg (1,000 units) per day. The observed risk reduction of 11% in the meta-analysis by Zhang et al. was similar to the 12% reduction reported by Barbarawi et al. The results from these meta-analyses,

that high doses of vitamin D supplements are required to reduce diabetes risk among people with prediabetes, are in agreement with our present findings.

In longitudinal observational studies, a consistent association between higher blood 25(OH)D level and lower diabetes risk has been reported, but the range of 25(OH)D values in the studied cohorts is lower than the serum 25(OH)D range achieved in D2d. In the observational studies, the highest category of blood 25(OH)D (conferring lowest risk of diabetes) was in the 62.5–75 nmol/L range and the lowest category (conferring highest risk of diabetes) was in the 25–37.5 nmol/L range. Higher blood 25(OH)D levels have been monotonically associated with a lower diabetes risk (3); however, because observational studies report blood 25(OH)D ranges <100 nmol/L, they do not address whether higher levels are associated with an even lower risk of diabetes. Our findings provide trial-based evidence that circulating blood 25(OH)D levels >100 nmol/L are beneficial for diabetes prevention.

The present analysis tested the impact of sustained higher 25(OH)D levels on diabetes risk, and maximal risk reduction occurred when participants assigned to the vitamin D intervention maintained intratrial serum 25(OH)D levels ≥125 nmol/L, and partial risk reduction occurred at levels of 100–124 nmol/L, suggesting that the blood 25(OH)D levels needed to reduce diabetes risk are considerably higher than those recommended by the National Academy of Medicine for skeletal health of 50–74 nmol/L (14). During the D2d study, among participants assigned to treatment with 100 μg per day of vitamin D, which is the tolerable upper limit set by the National Academy of Medicine (14), 30% maintained

intratrial mean 25(OH)D levels in the 100–124 nmol/L range, and 40% maintained intratrial mean 25(OH)D levels ≥125 nmol/L. Either a higher dose or a greater adherence/persistence with a 100-μg (4,000 units) dose would be required for the others to achieve the 25(OH)D level associated with the greatest reduction in diabetes of 125 nmol/L.

The present analysis retains the strengths of the parent trial, including use of the gold standard assay for 25(OH)D standardized to the National Institute of Standards and Technology, use of the latest ADA glycemic criteria to define prediabetes and diabetes, and ascertainment for diabetes at regular intervals by blood glucose testing using a central laboratory. Additionally, D2d allowed for a detailed examination of diabetes risk at higher intratrial 25(OH)D levels not possible in observational studies or in clinical trials that tested lower doses of vitamin D. One study limitation was the relatively small number of participants with intratrial low 25(OH)D levels (<50 nmol/L), which precluded examination of the impact of persistently low levels of 25(OH)D on diabetes risk. Not surprisingly, there were relatively small numbers of participants assigned to placebo who achieved 25(OH)D levels of ≥100 nmol/L, but despite this, there was a significant interaction of 25(OH)D concentration with treatment group on diabetes incidence, with larger decrements in risk for a 25 nmol/L increment in 25(OH)D in the vitamin D-treated group. Our analysis did not assess safety, which we will address in detail elsewhere (Johnson et al., manuscript in preparation).

In conclusion, daily vitamin D supplementation to reach and sustain 25(OH)D levels of 100–124 and ≥125 nmol/L conveyed a progressively lower risk of

progression to diabetes in adults with prediabetes. Vitamin D supplementation to reach these levels is a promising approach to reducing risk of diabetes in adults at high risk for diabetes.

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**Author Contributions.** B.D.-H., M.A.S., E.S.L., and A.G.P. contributed to the study design. B.D.-H., W.C.K., J.N., E.M.V., and A.G.P. contributed to the data analysis. B.D.-H. and A.G.P. wrote the first manuscript draft. J.N. conducted the analyses. All authors interpreted the data, participated in writing the manuscript, and read and approved the submitted version of the manuscript. J.N. and A.G.P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

## Appendix

### D2d Research Group Collaborators

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