Safety and tolerability of high-dose daily vitamin D₃ supplementation in the vitamin D and type 2 diabetes (D2d) study—a randomized trial in persons with prediabetes

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BACKGROUND/OBJECTIVES: Routine use of vitamin D supplements has increased substantially in the United States. However, the safety and tolerability of long-term use of high-dose vitamin D are not known. We assessed the safety and tolerability of high-dose, daily vitamin D₃ in the vitamin D and type 2 diabetes (D2d) study.

SUBJECTS/METHODS: In total, 2423 overweight/obese persons with prediabetes were randomized in a double-blind manner to either 4000 IU of vitamin D₃ (the tolerable upper intake level for adults by the National Academy of Medicine) taken daily or matching placebo. All participants were included in this analysis. Incident adverse events (AE) were ascertained 4 times a year at in-person visits (twice a year) and interim remote encounters (twice a year) and were defined as untoward or unfavorable medical occurrences. Serious adverse events (SAE) included death, life-threatening events, and hospitalizations.

RESULTS: A total of 8304 AEs occurred during 3 years of follow-up and were less frequent in the vitamin D group compared to placebo (Incidence Rate Ratio [IRR] = 0.94; 95% Confidence Interval [CI] 0.90, 0.98). The overall frequency of protocol-specified AEs of interest, which included nephrolithiasis, hypercalcemia, hypercalciuria, or low estimated glomerular filtration rate, was low and did not differ by group. There were no significant between-group differences in total AEs (IRR = 0.96 (0.81, 1.14)).

CONCLUSION: Vitamin D₃ supplementation at 4000 IU per day was safe and well tolerated among overweight/obese participants at high risk for diabetes who were appropriately monitored for safety. In this population, this dose of vitamin D₃ did not increase risk of AEs or SAEs, including those previously associated with vitamin D such as hypercalcemia, hypercalciuria, or nephrolithiasis.

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INTRODUCTION

There has been substantial interest in vitamin D and its potential role in prevention of a number of chronic diseases, including diabetes, cardiovascular disease, and cancer [1–3]. Recently, the focus of vitamin D research has turned to randomized controlled trials of vitamin D at doses higher than typically recommended compared to placebo in persons who were generally considered to be vitamin D sufficient by current guidelines [4–9]. However, there is insufficient evidence regarding the safety and tolerability of vitamin D supplementation at these higher doses [10, 11]. At the same time, routine use of vitamin D supplements, especially at doses higher than are typically recommended in guidelines, has increased substantially in the United States, despite insufficient data regarding potential safety issues or side effects with longer term use at these higher doses [12].

The Vitamin D and Type 2 Diabetes (D2d) study was a randomized clinical trial of vitamin D₃ supplementation at a dose of 4000 IU per day compared to placebo among overweight/obese participants who were at high risk for type 2 diabetes [6]. In this pre-specified analysis, we examined the safety and tolerability of vitamin D₃ supplementation in the D2d study, which tested the vitamin D dose that is considered the tolerable upper intake level (UL) for adults by the National Academy of Medicine [13].

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SUBJECTS AND METHODS

Trial design overview

The D2d study was a randomized, double-blind, placebo-controlled clinical trial conducted to evaluate the safety and efficacy of oral vitamin D3 for diabetes prevention in adults at high risk for type 2 diabetes [5]. The study involved collaboration among 22 academic medical centers in the United States (d2dstudy.org/sites) [5]. The trial protocol is available at D2dstudy.org. A sponsor-appointed data and safety monitoring board approved the protocol and provided independent study monitoring. The institutional review board at each clinical site also approved the protocol, and all participants provided written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The statistical analysis and vouches for its accuracy.

Participants

Eligible participants met at least two of three glycemic criteria for prediabetes as defined by the 2010 American Diabetes Association (ADA) guidelines. Other inclusion criteria were age greater than or equal to 30 years (25 years for American Indians, Alaska Natives, Native Hawaiians, or other Pacific Islanders) and body mass index (BMI) of 24–42 kg/m² (22.5–42 kg/m² for Asian Americans) [14]. A low serum 25-hydroxyvitamin D (25(OH)D) concentration was not an inclusion criterion. Key exclusion criteria included use of diabetes or weight-loss medications or a history of hyperparathyroidism, nephrolithiasis, hypercalcemia, chronic kidney disease (defined as estimated glomerular filtration rate [eGFR] <50 mL/min/1.73 m²), calcium-to-creatinine ratio greater than 0.275 at baseline, or bariatric surgery. Persons were also excluded for use of supplements containing total doses of vitamin D higher than 1000 IU/day or total calcium higher than 600 mg/day. A complete list of exclusion criteria has been published and the recruitment process described previously [5, 6, 15].

Intervention and Procedures

Participants were randomized in a 1:1 allocation ratio to take once-daily, a soft-gel containing either 4000 IU of vitamin D₃ (cholecalciferol) or matching placebo, with stratification by site, BMI (<30 or ≥30 kg/m²), and race (White or non-White). Participants and all study staff were blinded to treatment assignment. Participants were asked to limit the use of outside-of-study vitamin D to 1000 IU per day from all supplements. To optimize safety, participants were also asked to limit calcium supplements to 600 mg per day.

In-person follow-up visits occurred at month 3, month 6, and twice per year thereafter. Midway between the in-person visits starting after month 6, an audio message (phone or email) took place. All visits were designed to promote retention, encourage adherence, and assess for diabetes outside of the study, tolerability of study pills, occurrence of adverse events, and personal use of vitamin D supplements higher than allowed by study protocol. Participants were monitored for adverse events (AEs) including those previously associated with vitamin D supplementation. Any event not attributed to the participant was referred to visits and interim encounters 4 times a year in a similar manner in both groups. The protocol outlined in detail the safety parameters for which the study trial pills should be discontinued (e.g., nephrolithiasis, hypercalcemia, low eGFR, etc.) [6].

Vitamin D content of study trial pills was analyzed for each production lot at bottling for the vitamin D₃ pills as well as for placebo pills to confirm they were free of vitamin D. Acceptable vitamin D₃ content for the active vitamin D pill was pre-defined as 80–120% of the 4000 IU planned dosage.

Outcomes

The primary outcome of the D2d study was time to incident diabetes [14]. Participants who met the primary outcome of diabetes remained on the study pills and continued to be followed for safety and additional outcomes. The primary results have been previously published [6].

Adverse events were ascertained at each participant contact by study staff. At these encounters, each participant was asked if they had experienced any changes to their health or had sought medical care since last contact. If the participant responded affirmatively, study staff collected information on the health change or reason for and timing of medical care including diagnostic tests, diagnosis, and treatment. Study staff also reviewed with participants previously reported ongoing AEs to determine if the event had resolved.

An AE was defined as any untoward or unfavorable and unintended medical occurrence (including symptom, physical sign, laboratory finding, or disease) observed in or experienced by a participant, whether or not it was considered study related. A serious adverse event (SAE) was defined as any AE that resulted in death, a life-threatening event, a new or significantly permanent or persistent disability or incapacity, a congenital anomaly or birth defect, or any other significant hazard that, based upon appropriate medical judgment by the investigators, may have jeopardized the participant's health and may have required medical or surgical intervention to prevent one of the outcomes listed in this definition. For all SAEs reported by the sites, medical records were collected and reviewed by the study's Safety and Outcomes Subcommittees, composed of D2d investigators who were blinded to the participant's assignment.

Key protocol-specified AEs of interest included hypercalcemia, hypercalcuria, low eGFR and nephrolithiasis. Follow-up serum calcium (assessed at each site's local laboratory), the urine calcium-to-creatinine ratio (assessed at the central laboratory), and serum creatinine (assessed at each site's local laboratory) to estimate GFR (calculated centrally) were measured at month 3, and annually thereafter. If serum calcium value (uncorrected for albumin concentration) was greater than the site's clinical laboratory upper level of normal and less than or equal to the upper level of normal plus 1 mg/dL, participants were queried about calcium intake and supplements and medications (e.g., use of hydrochlorothiazide) and educated about the use of calcium supplements. Testing was repeated within 6 weeks. If the repeat serum calcium value was greater than the site's clinical laboratory upper level of normal, the participant was confirmed to have met the outcome of hypercalcemia; study pills were stopped, and the participant was referred to their health care provider. If the first measurement of calcium was greater than the site's clinical laboratory upper level of normal plus 1 mg/dL, no repeat testing was required and the participant was considered to have met the outcome of hypercalcemia; study pills were stopped, and the participant was referred to their health care provider. Regarding the adverse event of low eGFR, if eGFR value was greater than 30 and less than or equal to 40 mL/min/1.73 m², testing was repeated within 4 weeks. If repeat eGFR measurement was equal to or less than 30 mL/min/1.73 m², the participant was confirmed to have met the outcome of low eGFR; study pills were stopped, and the participant was referred to their health care provider. The 0.375 cutoff was chosen because it represents the calcium-to-creatinine ratio in a random spot urine specimen that corresponds to a 24-hour urine calcium of 40 mg/g creatinine, which is the reference range for renal insufficiency.

Participants were asked to contact site staff to report the occurrence of a kidney stone and were additionally specifically queried about kidney stones at each contact (phone or in-person visit). All reports of kidney stone were included in the nephrolithiasis outcome and participants reporting a kidney stone were instructed to discontinue study pills. If available, medical records related to nephrolithiasis were collected and then adjudicated by the study's safety and outcomes subcommittee. For all of the above key protocol-specified AEs of interest where study pills were stopped per protocol, the pills were discontinued without unmasking participants or study staff, and participants continued in the study and completed all subsequent planned visits and measurements including collection of the primary outcome of diabetes and safety assessment.

The D2d study did not specifically query the participants regarding falls using a validated questionnaire, but injuries and musculoskeletal events were self-reported by participants and were included in the overall assessment of safety.

Additional protocol-specified AEs of interest reported in this analysis were per protocol, defined as any AE that resulted to the study pills and included polyuria, nausea, vomiting, poor appetite, metallic taste, hyperphosphatemia, anemia, weakness, fatigue, insomnia, and headache (all self-reported). Participants could request to discontinue study pills at any point and for any reason. Participants who discontinued the study pills regardless of reason (AE or personal choice) were followed for the efficacy outcome per intent-to-treat principle.
Laboratory testing
Serum calcium and creatinine were analyzed locally at each site, and eGFR was calculated centrally using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation adjusted for race, as the formula was commonly applied when the study was conducted [16]. Other blood and urine specimens (including for calcium-to-creatinine ratio) were shipped to the central laboratory at baseline and during follow up. Serum total 25(OH)D, which includes total 25(OH)D3 and total 25(OH)D2, from stored frozen fasting serum samples from the baseline and annual visits, was measured by liquid chromatography-tandem mass spectrometry with calibrators that are traceable to the National Bureau of Standards and Technology and validated by quarterly proficiency testing program administered by the Vitamin D External Quality Assessment scheme (DEQAS, United Kingdom) [17, 18]. The D2d study did not measure free 25(OH)D3 or free 25(OH)D2 levels.

Statistical analyses
The sample size for the parent study was determined based on a target of 508 diabetes outcome events. The rationale has been previously published [5]. The frequency of AEs was evaluated in intention-to-treat analyses that compared groups defined by the randomization procedure and included all participants irrespective of adherence to assigned treatment or the protocol. All events were considered to occur independently, and no adjustment has been made for multiple events occurring in the same person. Incidence rates of AEs or differences in proportions of AEs were compared between the two groups. No adjustments were made for multiple comparisons.

RESULTS
From October 2013 through February 2017, 7133 people were screened, and 2423 were randomly assigned to vitamin D3 (n = 1211) or placebo (n = 1212), forming the intention-to-treat population (Fig. 1) [6]. Of those randomized, 45% of participants were women; 33% were of a non-White race; and 9% were of Hispanic ethnicity (Table 1) [19]. Mean age was 60.0 years; body-mass index, 32.1 kg/m²; and HbA1c, 5.9% (48 mmol/mol). Mean baseline serum 25(OH)D concentration was 28.0 ng/mL (68.8 nmol/L) with 78.3% of participants having a concentration equal to or greater than 20 ng/mL (49.1 nmol/L). There were no statistically significant differences in baseline characteristics by treatment assignment.

The last study encounter was in December 2018 and the trial was stopped when the number of prespecified diabetes events had occurred per protocol. The overall median follow-up was 3.0 years (vitamin D3 3.0 [interquartile range, 2.0–3.6] years; placebo 2.9 [interquartile range, 2.0–3.5] years) and 99.1% of the cohort (1201 vitamin D3 and 1199 placebo group) contributed follow-up data.

The overall frequency of protocol-specified AEs of interest was low, with no significant between-group differences in the incidence of the first occurrence of the following protocol-specified adverse
events of interest: hypercalcemia, hypercalciuria, hyperphosphatemia, low eGFR, metallic taste, fatigue / weakness, insomnia, polyuria, or nephrolithiasis (Table 2). There were 36 cases of participants with new-onset hypercalcemia on initial testing; on repeat testing, only 10 cases were confirmed, 6 in the vitamin D3 and 4 in the placebo group (incidence rate ratio [IRR] for vitamin D3 vs. placebo = 1.49; 95% CI 0.42, 5.27). There were 21 participants with new-onset hypercalciuria on initial testing; on repeat testing,

| Characteristic | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|---------------|--------------------|------------------------|--------------------|
| 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. (%)b|                    |                        |                    |
| 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 (2.3)           |
| Hispanic or Latino Ethnicity, no. (%)b| | 225 (9.3) | 120 (9.9) | 105 (8.7) |
| Body-mass index, kg/m² | 32.1 ± 4.5 | 32.0 ± 4.5 | 32.1 ± 4.4 |

Health history

Medical conditions, no. (%)

| Medical conditions | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|--------------------|--------------------|------------------------|--------------------|
| Hypercholesterolemia | 1346 (55.6) | 661 (54.6) | 685 (56.5) |
| Cancerc            | 262 (10.8)        | 126 (10.4)             | 136 (11.2)         |
| Cardiovascular diseased | 305 (12.6) | 106 (9.8) | 199 (14.9) |
| Hypertension       | 1297 (53.5)       | 622 (51.4)             | 675 (55.7)         |

Dietary supplements*e

Vitamin D

| Participants taking vitamin D supplements, no. (%) | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|----------------------------------------------------|--------------------|------------------------|--------------------|
| 1037 (42.8)                                        | 508 (41.9)         | 529 (43.6)             |
| Vitamin D intake among participants, IU/dayf      | 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. (%) | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-------------------------------------------------|--------------------|------------------------|--------------------|
| 804 (33.2)                                      | 385 (31.8)        | 419 (34.6)             |
| Calcium intake among participants, mg/dayf      | 103 ± 176          | 100 ± 175              | 107 ± 176          |
| Calcium intake among participants using supplements, mg/day | 312 ± 167 | 316 ± 168 | 308 ± 166 |

Laboratory

Serum 25-hydroxyvitamin D

| Mean, ng/mL | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-------------|--------------------|------------------------|--------------------|
| 28.0 ± 10.2 | 27.7 ± 10.2        | 28.2 ± 10.1            |

Distribution, no. (%)

| <12 ng/mL | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-----------|--------------------|------------------------|--------------------|
| 103 (4.3) | 60 (5.0)           | 43 (3.6)               |

| 12–19 ng/mL | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-------------|--------------------|------------------------|--------------------|
| 422 (17.4) | 216 (17.8)         | 206 (17.0)             |

| 20–29 ng/mL | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-------------|--------------------|------------------------|--------------------|
| 876 (36.2) | 453 (37.4)         | 423 (34.9)             |

| ≥30 ng/mL | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-----------|--------------------|------------------------|--------------------|
| 1021 (42.2) | 482 (39.8)        | 539 (44.5)             |

| Serum calcium, mg/dL | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|----------------------|--------------------|------------------------|--------------------|
| 9.41 ± 0.37          | 9.40 ± 0.37        | 9.41 ± 0.38            |

| Estimated glomerular filtration rate, mL/min/1.73 m²g | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|------------------------------------------------------|--------------------|------------------------|--------------------|
| 87.1 ± 15.7                                          | 87.5 ± 15.6        | 86.7 ± 15.9            |

| Fasting urine calcium-creatinine ratio | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|---------------------------------------|--------------------|------------------------|--------------------|
| 0.09 ± 0.06                            | 0.09 ± 0.06        | 0.08 ± 0.06            |

| Hematocrit, %h | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|----------------|--------------------|------------------------|--------------------|
| 42.8 ± 3.5     | 42.8 ± 3.4         | 42.8 ± 3.5             |

*aPlus-minus values are means ± SD. Percentages may not add up to 100 because of rounding. To convert 25-hydroxyvitamin D from ng/mL to nmol/L, multiply by 2.456; to convert vitamin D intake from IU to mcg, divide by 40.

bRace and ethnicity were reported by the participant. The category “other” includes American Indian or Alaska Native; Native Hawaiian or other Pacific Islander; or other race. Ethnicity includes any race.

Cancer (except for basal cell skin cancer) within 5 years of randomization was an exclusion criterion. Prostate cancer or well-differentiated thyroid cancer not expected to require treatment over the next 4 years were not exclusions. Persons with history of squamous cell cancer of the skin, which was completely excised and with no evidence of metastases, were eligible.

dCardiovascular disease included: arrhythmias, chest pain, congestive heart failure, coronary artery disease, CABG/PCI, myocardial infarction, palpitations, peripheral vascular disease.

eData on vitamin D and calcium intake are derived from a question about supplements, including multivitamins and high-dose prescribed doses. Participants were allowed to take, from supplements, up to 1000 IU/day of vitamin D and 600 mg/day of calcium. Dietary intake of vitamin D and calcium was not limited.

fValue shown is among all participants regardless of whether they reported use of supplements or not.

gBased on the Chronic Kidney Disease Epidemiology Collaboration equation.

hIndividuals were excluded if they had anemia at screening visit defined as hematocrit <32% for women, <36% for men.
only 2 cases were confirmed, 1 in each group (IRR for vitamin D3 vs. placebo = 0.99; 95% CI 0.96, 1.02). There were 3 cases of confirmed low eGFR, 1 in the vitamin D3 group and 2 in the placebo group (IRR for vitamin D3 vs. placebo = 0.50; 95% CI 0.04, 5.47). There were 52 self-reported cases of nephrolithiasis, 28 in the vitamin D3 and 24 in the placebo group (IRR for vitamin D3 vs. placebo = 1.16; 95% CI 0.67, 2.00) (Table 3). While the number of adverse events related to nausea/vomiting or poor appetite was low (n = 29), there were more cases reported among persons taking vitamin D3 compared to placebo, 20 vs 9 respectively (IRR for vitamin D3 vs. placebo = 2.20; 95% CI 1.00, 4.84). Supplementary Table 1 provides additional information on the total frequency of protocol-specified AEs of interest among persons who were taking their study pills at the time of the event.

A total of 8304 AEs occurred during follow-up. The incidence rate of total AEs was lower in the vitamin D3 group (4039; 116.1 events per 100 person-years) compared to the placebo group (4265; 123.6 events per 100 person-years) (IRR = 0.94; 95% CI 0.90, 0.98) (Table 3). A total of 529 SAES occurred during follow-up. The incidence rate of SAES was not different between the vitamin D3 (260 events; 7.47 per 100 person-years) and placebo groups (269; 7.80 per 100 person-years) (IRR = 0.96; 95% CI 0.81, 1.14). The majority of SAES were for hospitalization and there was no statistically significant difference among the treatment groups (IRR = 0.94; 95% CI 0.79, 1.12) (Table 3). Supplementary Tables 2, 3, and 4 provide additional data for total AEs and SAES by treatment group using an organ classification. The vitamin D3 group had fewer AEs and SAES for injury and musculoskeletal events.

Adherence to the intervention was high (84.1% of prescribed pills were taken) and a similar proportion of participants in the vitamin D3 group (12.1%) and placebo group (10.3%) stopped trial pills (difference, 1.8 percentage points; 95% CI, −0.7, 4.3). There was no significant difference between the proportions of participants who stopped study pills for any reason, including due to AEs or due to participant choice (17.5% in the vitamin D3 group vs. 16.0% in the placebo group). There was no significant difference between the proportions of participants who stopped study pills due to an AE: overall, 58 (4.8%) participants in the vitamin D3 group stopped trial pills due to an AE, including abnormal safety labs, compared to 46 (3.8%) in the placebo group (difference in proportions for vitamin D vs. placebo, 0.9% [95% CI, −0.6, 2.6%]) (Table 4).

**DISCUSSION**

In this multi-center, randomized, double-blind, placebo-controlled trial among overweight/obese persons at high risk of type 2 diabetes not selected for vitamin D insufficiency and who were screened and routinely monitored for safety, compared to placebo, oral vitamin D3 supplementation at a dose of 4000 IU per day (considered the UL for adults by the National Academy of Medicine) [13] was well-tolerated and did not result in an increased risk of AEs or SAES, including side effects typically linked to vitamin D, such as hypercalcemia, nephrolithiasis, or hypercalcuria. This finding of no increased risk of AE or SAE in the vitamin D3 group is reassuring given that the majority of D2d participants began the trial with concentrations of serum 25(OH)D considered sufficient for healthy adults [6].

These data from the D2d study suggest that in similar populations of people who are overweight/obese and with prediabetes, the UL for safety and tolerability of vitamin D3 may be higher than previously established. The dose of vitamin D3 of 4000 IU per day used in the D2d study is the National Academy of Medicine recommended UL for persons over 8 years of age [13], and D2d participants were allowed to take up to 1000 IU of vitamin D3 on their own, for a maximum total dose of vitamin D of 5000 IU daily from supplements. The UL was established by the National Academy of Medicine in 2011 based on a synthesis of data indicating that a dose of 4000 IU was unlikely to cause hypercalcemia [20]. The National Academy of Medicine also chose a UL dose that would maintain a serum 25(OH)D concentration lower than 50–75 ng/mL (125–150 nmol/L), a concentration that was previously thought to be associated with beneficial outcomes. However, the benefit-risk ratio may be different in populations that vary by BMI, skin complexion, or when a trial attempts to achieve higher 25(OH)D concentrations. Future trials are warranted to test the efficacy-safety ratio of higher doses of vitamin D supplementation that aim to achieve higher 25(OH)D levels in specific populations at-risk for specific conditions, such as diabetes, osteoporosis, cancer etc.

The dose of 4000 IU daily of vitamin D3 in D2d is a higher dose than administered in other recently completed clinical trials. For example, Lappe et al tested a 2000 IU daily dose of vitamin D3 and administered calcium supplements in addition to the vitamin D3 and D2d participants were allowed to take up to 1000 IU of vitamin D3 on their own, for a maximum total dose of vitamin D of 5000 IU daily from supplements. The UL was established by the National Academy of Medicine in 2011 based on a synthesis of data indicating that a dose of 4000 IU was unlikely to cause hypercalcemia [20]. The National Academy of Medicine also chose a UL dose that would maintain a serum 25(OH)D concentration lower than 50–75 ng/mL (125–150 nmol/L), a concentration that was previously thought to be associated with beneficial outcomes. However, the benefit-risk ratio may be different in populations that vary by BMI, skin complexion, or when a trial attempts to achieve higher 25(OH)D concentrations. Future trials are warranted to test the efficacy-safety ratio of higher doses of vitamin D supplementation that aim to achieve higher 25(OH)D levels in specific populations at-risk for specific conditions, such as diabetes, osteoporosis, cancer etc.
Safety labs

Calcium

Calcium

of-study encounter

Did not complete end-study encounter

Participant decision

170
92
78

6

Serious adverse event

260
7.47
269
7.80
0.96 (0.81, 1.14)

Adverse event

4039
116.1
4265
123.6
0.94 (0.90, 0.98)

Within-study laboratory evaluation (confirmation with repeated testing)

Hypercalcemia

6
0.17
4
0.12
1.49 (0.42, 5.27)

Hypercalciuria

1
0.03
1
0.03
0.99 (0.06, 15.86)

Low estimated glomerular filtration rate

1
0.03
2
0.06
0.50 (0.04, 5.47)

Self-reported

Nephrolithiasis

28
0.80
24
0.70
1.16 (0.67, 2.00)

Hypercalcemia was defined as serum calcium (uncorrected for albumin concentration) higher than the upper limit of the normal range for the clinical laboratory at each clinical site; hypercalciuria was defined as fasting morning urine calcium-creatinine ratio over 0.375 measured by the central laboratory; low estimated glomerular filtration rate was defined as equal to or lower than 30 mL per min per 1.73 m² of body-surface based on serum creatinine measured at each clinical site's clinical laboratory using the Chronic Kidney Disease Epidemiology Collaboration equation. Unless a specified threshold was reached, hypercalcemia, hypercalciuria and low estimated glomerular filtration rate required confirmation (see “Methods”). Table includes events in all participants who underwent randomization regardless of adherence; analyses censored at death, withdrawal, or end-of-study encounter (visit or phone call).

| Reason for permanent discontinuation of study pills, n | Overall (n = 2423) | Vitamin D3 (n = 1211) | Placebo (n = 1212) |
|-------------------------------------------------------|---------------------|-----------------------|-------------------|
| withdrew consent while on study pills                 | 35                  | 18                    | 17                |
| Adverse event                                         | 89                  | 50                    | 39                |
| Nephrolithiasan                                      | 46                  | 26                    | 20                |
| Other adverse event of major interest                 | 36                  | 20                    | 16                |
| Death                                                | 7                   | 4                     | 3                 |
| Safety labs—high serum calcium                        | 10                  | 6                     | 4                 |
| Safety labs—high urine calcium                        | 3                   | 1                     | 2                 |
| Safety labs—low GFR                                   | 2                   | 1                     | 1                 |
| Participant decision                                 | 170                 | 92                    | 78                |
| Other reason                                          | 9                   | 4                     | 5                 |
| Did not complete end-of-study encounter               | 87                  | 40                    | 47                |

*Reasons for permanent discontinuation are mutually exclusive.

*Nephrolithiasis diagnosed by either a study physician or physician outside of D2d based on clinical, radiologic findings, or both. All cases—except 5—were classified as “possibly” or “probably related” to study pills.

*Other adverse event that led to discontinuation of study pills at the discretion of the site study physician or participant decision.

*Does not include 4 participants who stopped reason and died at a later date.

*Participant requested discontinuation of study pills for any other reason other than an adverse event.

*For participant who did not complete the end-of-study encounter, the study pill discontinuation date is the date of the last encounter that was completed.

There are trials with vitamin D supplementation that have reported other adverse events that were not assessed in the D2d study. A Canadian study found that, among healthy older adults (ages 55 to 70 years), vitamin D3 for 3 years at 4000 IU or 10,000 IU per day compared with 400 IU per day, resulted in statistically significant lower radial bone mineral density (BMD) as measured by high resolution peripheral quantitative computed tomography, but there were no significant differences in bone strength [25]. The D2d study did not assess BMD and thus cannot contribute information about the effect of the 4000 IU per day on BMD and bone strength in overweight/obese people with prediabetes. A trial conducted in Switzerland tested vitamin D3 at 60,000 IU per month vs. 24,000 IU per month and reported increased risk of falls with the higher dose [26]. The D2d study did not specifically query the participants regarding falls using a validated questionnaire; however the vitamin D3 had fewer AEs and SAEs for injury or musculoskeletal events than the placebo group (Supplementary Tables 2 and 4).

D2d is the first trial to assess the safety of vitamin D3 given at the tolerable upper intake level for adults by the National Academy of Medicine in a population of US adults with overweight/obesity and prediabetes. The D2d study has several additional strengths, including a large diverse group of participants who were at low risk for safety concerns related to vitamin D but at high risk for diabetes. The vitamin D3 dose of 4000 IU per day was selected to balance safety and efficacy and resulted in, on average, large differences in serum 25(OH)D concentrations between the vitamin D3 and placebo groups (54.3 ng/mL vs. 28.8 ng/mL, respectively, at month 24) [6]. Use of a placebo to blind investigators, staff, and participants to treatment assignment minimized ascertainment bias of adverse events, and careful attention to protocol fidelity resulted in a rigorously conducted clinical trial. Adverse events were collected frequently and in a similar way in both groups to reduce ascertainment bias. All serious adverse events and cases of
nephrolithiasis were adjudicated by study investigators blinded to treatment assignment.

There are several considerations to put interpretation of our findings in context. As in all vitamin D trials, participants were excluded if they had a condition (e.g., high baseline serum calcium, etc.) that would have increased their risk for vitamin D-associated adverse events, and this may have reduced the occurrence of AEs compared to the general population taking vitamin D. The median time of follow-up in the D2d study was three years, and our findings may not extrapolate to longer term use of 4000 IU per day of vitamin D3. Thus, the risk of vitamin D-associated AEs and SAEs may be greater among persons who are at higher risk, who take the supplement for longer periods of time, or who have been less carefully screened and monitored. Finally, the D2d study did not assess whether participants had a CYP24A1 mutation or other mutations in the vitamin D pathway, so we are unable to provide pharmacogenomic information.

CONCLUSION

High-dose daily vitamin D3 supplementation at a dose of 4000 IU daily (considered the tolerable upper intake level for adults by the National Academy of Medicine) was safe and well tolerated among overweight/obese participants with prediabetes who were screened for risk of AEs and monitored for safety; and use of this supplement did not increase risk of AEs or SAEs, including side effects that have been previously associated with vitamin D.

DATA AVAILABILITY

Datasets generated and analyzed during the current study and the associated data dictionary and code are not publicly available. Requests for datasets analyzed and code utilized in the current study can be made after acceptance for publication by bona fide researchers by submitting a request to the D2d Publications Committee. 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).

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AUTHOR CONTRIBUTIONS

All authors (KCJ, KLM, ALP, LSP, EMV, JN, PRS, DR, SM, RC) are responsible for the design and final content of the manuscript. JN performed the statistical analysis. KCJ and RC are responsible for drafting the manuscript and AP, KLM, ALP, LSP, EMV, JN, PRS, DR, SM, RC are responsible for review and substantive revisions of the manuscript. All authors (KCJ, AP, KLM, ALP, LSP, EMV, JN, PRS, DR, SM, RC) have read and approved the final version of the manuscript.

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COMPETING INTERESTS
The following authors report no conflicts of interest Karen Johnson, Anastassios Pittas, Karen Margolis, Anne Peters, Ellen Vickery, Jason Nelson, Patricia Sheehan, David Rebousini, Saul Malozowski, Ranee Chatterjee Lawrence Phillips reports the following: Grant Support: Supported in part by VA awards CSP #2008, I01 CX001899, I01 CX001737, and HS86D IIR 07–138, NIH awards R21 DK099716, R18 DK066204, R03 AI133172, R21 AI156161, U01 DK091958, U01 DK89246, U11 TR002378, and a Cystic Fibrosis Foundation award PHILLI1210. Disclosure Statement: Dr. Phillips declares that there is no duality of interest associated with this manuscript. With regard to potential conflicts of interest, Dr. Phillips has served on Scientific Advisory Boards for Boehringer Ingelheim and Janssen, and has or had research support from Merck, Pfizer, Eli Lilly, Novo Nordisk, Sanofi, PhaseBio, Roche, AbbVie, Vascular Pharmaceuticals, Janssen, Glaxo SmithKline, and the Cystic Fibrosis Foundation. Dr. Phillips is also a cofounder and Officer and Board member and stockholder of a company, Diasys, Inc., which markets software aimed to help improve diabetes management. Anne Peters reports the following: Dr. Peters has served on scientific advisory boards for: Abbott Diabetes Care, Biorad, Eli Lilly and Company, MannKind, Medscape, Merck, Novo Nordisk, Zealand Research Support: Dexcom, vTv Therapeutics, Abbott Diabetes Care Stock Options: Omada Health, Teladoc.

ETHICAL APPROVAL
A sponsor-appointed data and safety monitoring board approved the protocol and provided independent study monitoring. The institutional review board at each clinical site also approved the protocol, and all participants provided written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice.

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