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Vitamin D supplementation vs. placebo and incident type 2 diabetes in an ancillary study of the randomized Vitamin D and Omega-3 Trial

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Observational and experimental evidence suggests that vitamin D plays a role in type 2 diabetes (T2D). However, prior randomized supplementation trials are limited to high-risk patients with prediabetes. Here we aim to evaluate whether vitamin D supplementation reduces risk of T2D in a general population of older US adults. The study design is an ancillary analysis (VITAL-T2D) of The Vitamin D and Omega-3 Trial (VITAL), a completed randomized, double-blind, placebo-controlled 2 × 2 trial of daily vitamin D_3 (cholecalciferol; 2000 IU/day) and omega-3 fatty acids (1 g/day) for the primary prevention of cancer and cardiovascular disease. We also conducted a systematic review and meta-analysis of vitamin D trial (≥1000 IU/d cholecalciferol) vs. placebo and T2D risk. We analyzed 22,220 adults with mean age 67.2 years (SD = 7.1) without T2D at enrollment (2011 to 2014), randomized to vitamin D_3 or placebo. Mean body mass index (BMI) was 27.5 kg/m^2 (SD = 5.3), with 51% female and 17% Black race/ethnicity. A subcohort (n = 911) attended in-person visits at baseline and 2 years for glycemic trait analyses. Our meta-analysis included 3 additional trials (5205 participants; 936 T2D cases). The primary outcome for the VITAL-T2D is intention-to-treat effect of vitamin D vs. placebo for incident T2D. T2D incidence (cases/1000py) at median follow-up of 5.3 y was 3.98 for vitamin D and 4.37 for placebo (hazard ratio [HR] = 0.91; 95% confidence interval [CI] = 0.76, 1.09). Results did not differ by age, sex, BMI, or baseline 25-hydroxyvitamin D, and vitamin D had no effect on glycemic traits at 2 years. Meta-analysis of 4 trials (n = 5205; 936 T2D cases) obtained HR = 0.89 (CI = 0.80, 0.99). In conclusion, Vitamin D supplementation did not reduce T2D in older US adults, but a modest reduction was observed when meta-analyzed with prior trials. **Trial Registration**: ClinicalTrials.gov #NCT01633177. **Systematic Review Registration**: PROSPERO #CRD42019147562.

The incidence of type 2 diabetes (T2D) has escalated in recent decades, now affecting nearly 10% of the global population[1,2]. Although dietary factors, including excessive intake of refined grains and processed meats, weight gain, and lack of physical activity are major determinants of T2D, some nutrients such as vitamin D have been implicated in potentially reducing T2D risk. Geographical differences in sun exposure, as a proxy of between-population variability in serum 25-hydroxyvitamin D (25[OH]D) levels, align with differences in T2D

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incidence rates. These observations are supported by several prospective cohort studies indicating individuals with incrementally lower serum vitamin D have higher insulin resistance, β-cell dysfunction[3,4], and T2D[5] compared with those who have adequate 25(OH)D levels. Experimental evidence[6] and short-term supplementation trials in humans[7,8] are mixed but suggest biologically plausible mechanisms of a causal role for vitamin D in reducing T2D, including improved β-cell function and insulin secretion.

Despite accumulating evidence relating vitamin D status or supplementation with T2D, findings from randomized controlled trials (RCTs) are inconsistent. Potential explanations for discrepancies between RCTs and the epidemiologic evidence include the potential for bias in the observational literature from lifestyle correlates of vitamin D exposure, and limitations of previous RCTs in targeting the enrollment of only high-risk populations, having relatively short-term follow-up, and inability to stratify participants by their baseline serum 25(OH)D[9]. Nonetheless, 3 recent placebo-controlled trials enrolling patients with pre-diabetes were suggestive of a benefit of 20,000 IU/week to 4000 IU/day of vitamin D_3 or 0.75 μg/day of eldecalcitol, a vitamin D analog[10–12], and compelling subgroup findings support an emerging hypothesis of a differential response to vitamin D supplementation by baseline body weight (blunted response among those with higher body mass index [BMI]) that warrants further investigation[11–13].

Here, we show that vitamin D supplementation does not prevent T2D in a general population of older US adults in the Vitamin D and Omega-3 Trial (VITAL), a completed RCT of high-dose vitamin D_3 vs. placebo for primary prevention of cancer and cardiovascular disease[13]. We also investigated the plausible interaction of vitamin D with T2D risk by baseline BMI and other factors. As previously reported, there was no main effect of randomized vitamin D_3 vs. placebo for the primary prevention of total cancer (hazard ratio [HR] = 0.96, 95% confidence interval [CI] = 0.88, 1.06) or cardiovascular disease (0.97 [0.85-1.12])[13]. The current analysis included VITAL participants without T2D at baseline followed for incident T2D. We also evaluated glycemic trait outcomes in a subcohort attending in-person visits at baseline and 2-year follow-up. Finally, we synthesized the state-of-the-art evidence for moderate-to-high dose vitamin D_3 supplementation and T2D risk with an updated meta-analysis of 4 trials.

Results

Among 22,220 VITAL-T2D participants without self-reported T2D at baseline, baseline mean age and BMI were 67.2 years (SD = 7.1) and 27.5 kg/m^2 (SD = 5.3), respectively, and 51% were self-reported female. Seventy-two percent reported non-Hispanic White race/ethnicity, with 17% Black, and 3.6% Hispanic non-African American. These and other demographic and health characteristics were balanced between randomized treatment groups (Table 1). In both treatment groups, 44% of participants reported taking dietary supplements containing ≤800 IU/d of vitamin D prior to enrollment (those taking >800 IU/d were excluded). Prevalence of serum 25(OH)D < 20 ng/ml at enrollment was 11% and 12% among the vitamin D and placebo groups, respectively, among the subset of participants providing baseline blood samples.

There were 484 incident T2D cases confirmed over a median = 5.3 y follow-up. The incidence of T2D was 3.98 cases/1000 PY among active vitamin D and 4.37 cases/1000 PY among placebo (Fig. 1). There was no overall effect of randomized vitamin D vs. placebo on the risk of T2D (ITT HR = 0.91 [0.76, 1.09]; *p*-value = 0.31) (Table 2). There was no statistical effect modification for vitamin D efficacy by age, self-reported sex, BMI, serum 25(OH)D levels, region, or omega-3 randomization assignment (Table 3). We did observe a global interaction for vitamin D vs. placebo by race/ethnicity with a lower HR among Black participants (p-interaction=0.03); however, no stratum-specific estimates were statistically significant, and multiple tests were performed for these secondary analyses.

Table 1 | Baseline characteristics of VITAL-T2D participants randomized to vitamin D vs. placebo

VITAL-T2D Cohort	Placebo (n = 11,158)	Vitamin D (n = 11,062)
Baseline characteristics, *n* (%)		
Age, mean (SD), yr	67.2 (7.1)	67.2 (7.1)
<65.0	4214 (37.8)	4173 (37.7)
≥65.0	6944 (62.2)	6889 (62.3)
Female sex[a]	5615 (50.3)	5600 (50.6)
Body mass index[b], mean (SD), kg/m^2	27.5 (5.3)	27.5 (5.2)
<25.0	3739 (34.3)	3657 (33.8)
25.0 to 30.0	4499 (41.2)	4460 (41.2)
≥30.0	2672 (24.5)	2703 (25.0)
Race/ethnicity[a]		
Non-Hispanic White	8073 (72.4)	8048 (72.8)
Black	1947 (17.4)	1905 (17.2)
Hispanic	397 (3.6)	406 (3.7)
Asian or Pacific Islander	160 (1.4)	146 (1.3)
Native American or Alaskan Native	98 (0.9)	94 (0.8)
Other or unknown	230 (2.1)	216 (2.0)
US region		
West	2620 (23.5)	2542 (23.0)
Midwest	2357 (21.1)	2350 (21.2)
Southeast	3075 (27.6)	3044 (27.5)
Northeast	3105 (27.8)	3126 (28.3)
Hypertension treated with medication	5293 (47.4)	5147 (46.5)
Cholesterol-lowering medication use	3633 (32.6)	3636 (32.9)
Vitamin D supplement use ≤800 ID/d[c]	4880 (43.7)	4845 (43.8)
Serum 25-hydoxyvitamin D, median (IQR), ng/ml	31 (25—37)	31 (25—37)
<20 ng/ml	838 (12.2)	765 (11.3)
Randomized to active omega-3 group	5572 (49.9)	5519 (49.9)

[a]Race, ethnicity, and sex were reported by the participants.
[b]Body mass index is weight in kilograms divided by square of height in meters.
[c] Participants using dietary supplements containing >800 IU/d vitamin D were ineligible; *VITAL* Vitamin D and Omega-3 Trial, *T2D* type 2 diabetes, *SD* standard deviation.

Glycemic trait outcomes

There were 911 VITAL-CTSC participants without T2D at baseline and eligible for analyses (placebo *n* = 465, vitamin D *n* = 446). Of these, 847 (93.0%; placebo *n* = 436, vitamin D *n* = 411) also provided blood samples at the follow-up visit at 2 years. At baseline, the median (interquartile range) concentrations for all VITAL-CTSC participants for red blood cell HbA1c = 5.3% (5.1-5.5), fasting glucose = 97.0 mg/dl (92.0–104.0), and fasting insulin=7.1 uIU/ml (4.7–11.0). For the derived indices of insulin resistance, median (IQR) were Matsuda ISI = 5.1 (3.2–8.0), HOMA-IR = 1.7 (1.1, 2.8), and HOMA-β = 4.2 (3.0,6.1). These traits were similar between the placebo and vitamin D treatment groups at baseline, and there were no statistically significant differences at 2 years by treatment group (Table 4). Briefly, neither group had statistically significant changes in HbA1c, HOMA-IR, or HOMA-β. The adjusted mean (95% CI) percent change in Matsuda ISI decreased at 2 years for the placebo group (mean [95% CI] percent change at 2 years = −4.1% [-7.4, −0.6]), but this modest decline in glucose tolerance was not significantly different from the borderline decrease in the vitamin D treatment group (−3.4% [−6.9, 0.2]; p-interaction = 0.80). For the vitamin D treatment group, glucose levels during the OGTT decreased at 120 minutes by -3.0% (−5.4, -0.6) and the overall glucose

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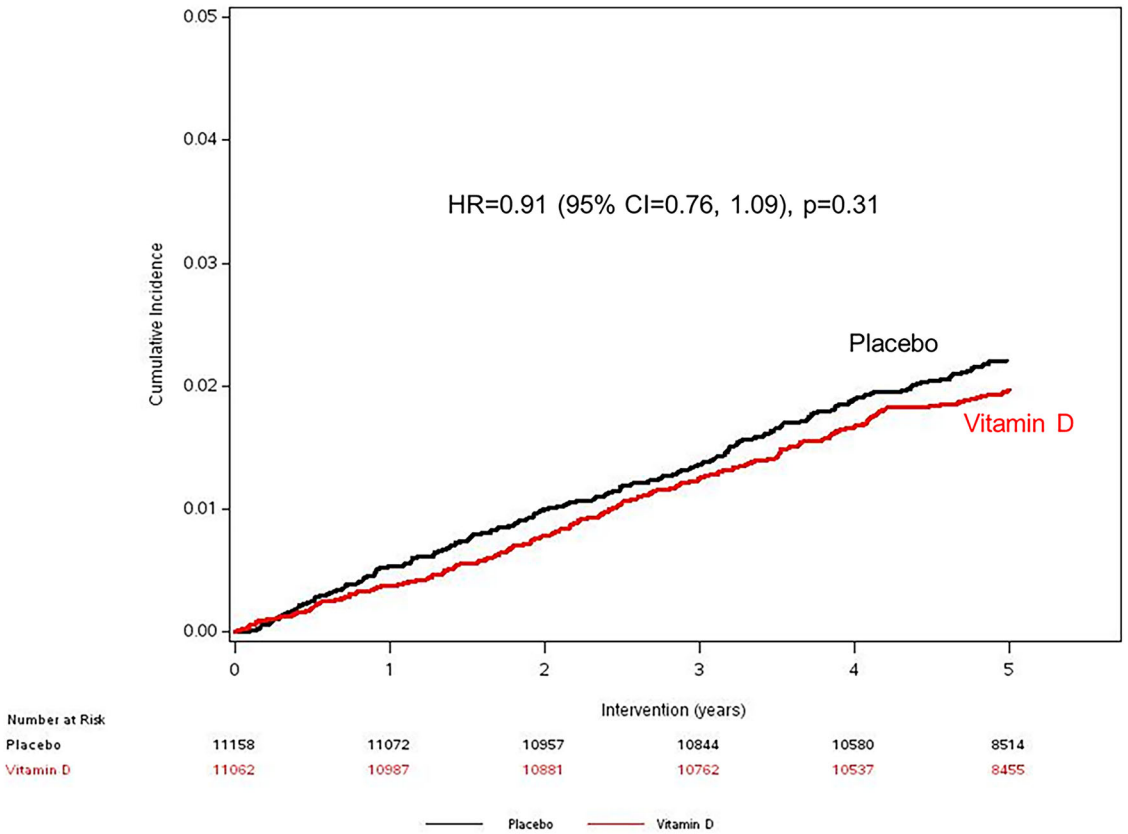

Fig. 1 | Cumulative incidence of T2D by randomized vitamin D vs. placebo in VITAL-T2D. The cumulative incidence plot and intention-to-treat hazard ratio and 95% confidence interval of T2D by randomized vitamin D vs. placebo were calculated using a Cox proportional hazards regression model adjusted for age at baseline, sex, and randomized omega-3 treatment group (active vs. placebo) using two-sided hypothesis tests. Source data are provided as a Source Data file. HR hazard ratio CI 95% confidence interval.

Table 2 | Effect of randomization to vitamin D vs. placebo on T2D incidence in VITAL-T2D

Treatment group	T2D cases, No. (%)	T2D incident rate	HR (95% CI)	*P*-value
Placebo	254 (2.27%)	4.37 cases/1000 PY	Reference	
Vitamin D	230 (2.08%)	3.98 cases/1000 PY	0.91 (0.76, 1.09)	0.31

Intention-to-treat Cox proportional hazards regression model is adjusted for age at baseline randomization, self-reported sex, and omega-3 treatment group assignment; two-sided *P*-value; *T2D* type 2 diabetes; *PY* person years, *CI* confidence interval.

AUC by −1.4% (−2.7, −0.1), but the treatment effect vs. placebo was not statistically significant ($p = 0.12$ and $p = 0.13$, respectively). Insulin response during the OGTT worsened for both placebo and vitamin D at 2 years, including increases in insulin concentrations at 120 minutes (9.1% [3.0, 15.6] and 7.2% [1.0, 13.8], respectively) and the OGTT insulin AUC (5.5% [1.8, 9.4] and 4.3% [0.5, 8.3], respectively). In our exploratory analyses stratifying by baseline demographic and T2D risk factors (Supplemental Table 1), the effects of vitamin D vs. placebo for all glycemic biomarker outcome at 2 years were consistent across strata (all p-interaction>0.05).

Meta-analysis of RCTs

Our systematic literature review identified 3 RCTs meeting our eligibility criteria, including the Tromso Study[11], Vitamin D and Type 2 Diabetes (D2d)[12], and Finnish Vitamin D

Trial (FIND) trials (Supplementary Fig. 1). The RCTs tested vitamin D3 for the primary prevention of major chronic disease, with T2D as a specified primary[11,12] or tertiary[14] outcome. Briefly, Tromso enrolled 511 adults with prediabetes from 2007 to 2008 to test the effects of 20,000 IU/week of cholecalciferol over 5 years[11]. Similarly, in D2d, 2423 adults with prediabetes and overweight or obesity were randomized from 2013 to 2017 to compare the effects of 4000 IU/day of cholecalciferol vs. placebo, with a median follow-up of 2.5 years. FIND investigators randomized 2271 generally healthy older adults to placebo vs. 1600 IU/day vs. 3200 IU/day vitamin D3 for a median 4.2 years, for the primary prevention of CVD[14]. The meta-analyzed estimate of VITAL with the other 3 RCTs indicated randomized vitamin D led to a 11% lower risk of T2D vs. placebo (pooled HR = 0.89 [0.80-0.99]; $p = 0.035$; $I^2 = 0\%$) (Supplementary Fig. 2). D2d and FIND also reported results stratified by BMI, and when combined with the VITAL-T2D in our meta-analysis there was a suggested dose-response of lower T2D for vitamin D vs. placebo across BMI strata, with BMI < 25.0 kg/m^2 HR = 0.57 (0.32, 1.02), BMI 25.0-29.0 kg/m^2 HR = 0.80 (0.65, 0.97), and BMI ≥ 30.0 kg/m^2 HR = 0.96 (0.83, 1.11); however, the statistical interaction by BMI category was not significant ($p = 0.10$).

Sensitivity analyses

The rate of adherence, defined as taking at least two-thirds of capsules, was 91.5% and 89.2% for placebo and 91.7% and 90.4% for vitamin D at 2 years and 5 years, respectively. Initiating non-study vitamin D (>800 IU/d) was 5.4% and 10.5% in the placebo group and 3.7% and 6.1% in the vitamin D group at 2 years and 5 years, respectively. In analyses censoring at non-adherence, the estimated effect of vitamin D vs. placebo was similar, with HR = 0.88 (0.72-1.07).

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Table 3 | Effect of randomization to vitamin D vs. placebo on T2D incidence, by baseline demographic and risk factor status

Baseline risk factors	Placebo T2D cases, no. (rate per 1000 PY)	Vitamin D T2D cases, no. (rate per 1000 PY)	Treatment effect HR (95% CI)	Interaction *p*-value
Age				
<65 years	117 (5.45)	116 (5.44)	1.00 (0.77, 1.29)	0.34
≥65 years	137 (3.73)	114 (3.13)	0.84 (0.65, 1.08)	
Self-reported sex				
Male	131 (4.52)	133 (4.66)	1.03 (0.81, 1.31)	0.15
Female	123 (4.21)	97 (3.32)	0.79 (0.61, 1.03)	
Total blood 25(OH)D				
<20 ng/ml	32 (7.47)	34 (8.67)	1.17 (0.72, 1.89)	0.44
≥20 ng/ml	113 (3.55)	104 (3.28)	0.93 (0.71, 1.21)	
Race/ethnicity				
Non-Hispanic White	93 (3.33)	93 (3.34)	1.00 (0.75, 1.34)	0.03
Black	38 (8.32)	23 (5.22)	0.63 (0.37, 1.06)	
Other or unknown	9 (3.16)	17 (6.8)	2.16 (0.96, 4.85)	
Geographic US region				
West	39 (4.39)	33 (3.8)	0.87 (0.55, 1.38)	0.88
Midwest	34 (4.28)	30 (3.78)	0.89 (0.54, 1.45)	
Southeast	37 (3.75)	39 (4.1)	1.10 (0.70, 1.72)	
Northeast	30 (3.47)	31 (3.59)	1.03 (0.63, 1.71)	
Body mass index, kg/m^2				
<30.0	71 (2.64)	56 (2.14)	0.81 (0.57, 1.15)	0.28
≥30.0	66 (8.58)	71 (9.06)	1.06 (0.76, 1.48)	
Omega-3 treatment group				
Placebo	60 (3.47)	60 (3.51)	1.01 (0.71, 1.45)	0.59
Active	77 (4.47)	67 (3.96)	0.89 (0.64, 1.23)	

Intention-to-treat Cox proportional hazards regression model is adjusted for age at baseline randomization, self-reported sex, and omega-3 treatment group assignment; two-sided *P*-value; *T2D* type 2 diabetes, *PY* person years, *CI* confidence interval.

Discussion

In this large, general population trial among diverse older Americans without T2D at baseline, we did not observe an effect of 2000 IU/day vitamin D_3 vs. placebo on the incidence of T2D over 5.3 years. However, in our pre-registered meta-analysis among 25,154 total trial participants there was a statistically significant 11% lower T2D risk with vitamin D vs. placebo overall, and a 25% lower T2D risk among participants without obesity (BMI < 30 kg/m^2). Our findings among the VITAL-CTSC subcohort with repeated blood collections and OGTTs did not indicate any significant differences with vitamin D vs. placebo for indices of insulin sensitivity, glucose tolerance, or β-cell function (Matsuda ISI, HOMA-IR, HOMA, β), glucose or insulin responses, or Hba1c after 2 years of supplementation.

Maintaining sufficient vitamin D blood levels is important for calcium metabolism and bone health, although the minimum thresholds to optimize these endpoints are debated and possibly vary by the status of other risk factors[15]. Vitamin D is a fat-soluble vitamin formed endogenously in humans with exposure to ultraviolet sunlight triggering biosynthesis of vitamin D_3 (cholecalciferol) in the skin. It is also derived from diet in the vitamin D_3 and vitamin D_2 forms, including fatty fish and some dark green vegetables, and several commercial products such as dairy, are fortified with vitamin D in the US and elsewhere. US adults are recommended to intake 600-800 IU/day[16], while higher levels of 1500-2000 IU/day have been recommended by the Endocrine Society[17]. Recommendations for higher vitamin D intakes, including through use of dietary supplements, above and beyond maintaining blood levels sufficient for bone health are debated, largely due to an incomplete understanding of vitamin D's role in preventing chronic disease outcomes, including T2D. Despite dietary recommendations and fortification programs, vitamin D status in the general US population remains suboptimal. From nationally representative data, it is estimated that approximately one-quarter of US adults have inadequate or deficient blood levels of serum 25(OH)D below 20 ng/ml (50 nmol/L)[18]. Thus, establishing the benefits, or lack thereof, of vitamin D supplementation in the context of a general population setting is warranted.

The VITAL-T2D results and our meta-analysis with 3 additional high-dose cholecalciferol RCTs with long-term follow-up demonstrate that vitamin D is related to a 11% lower risk of T2D vs. placebo controls. Notably, although all the RCTs were consistently suggestive of a benefit with no heterogeneity between trials' effect estimates, none achieved statistical significance individually. A previous meta-analysis of RCTs among prediabetes patients reported a modest but statistically significant 3% reduction in absolute risk of T2D at 3 y of vitamin D supplementation (daily or weekly cholecalciferol or eldecalciferol formulations) vs. placebo[8]. Most prior longer-term RCTs, however, exclusively enrolled patients with prediabetes and other higher-risk clinical populations, underscoring the previous gap in the potential role of vitamin D supplementation in the general population.

In our prospectively registered subgroup meta-analysis, we observed a 20% reduction in T2D with vitamin D vs. placebo among those with BMI in the range of overweight (BMI 25.0–29.9 kg/m^2) and a non-significant 63% lower risk among normal BMI (<25.0 kg/m^2). In striking contrast, there was no effect of vitamin D vs. placebo among BMI ≥ 30 kg/m^2. Previous meta-analyses of RCTs also suggested a modifying association of body weight, reporting benefits among lower BMI subgroups vs. those with obesity[7,8]. There are divergent hypotheses for why vitamin D would be less effective for the prevention of T2D and other disease endpoints at a higher BMI. One hypothesis is that that greater adiposity volume increases the capacity for vitamin D storage, leading to excessive sequestration and reduced levels in circulation[19,20]. Alternatively, vitamin D metabolism may be impaired by obesity's interference with hepatic enzyme activity, resulting in inefficient or downregulated conversion to 25(OH)D or its active form (1,25 dihydroxy vitamin D)[21]. The latter hypothesis aligns with prior findings among VITAL participants for a blunted response to vitamin D supplementation, indicated by diminished increases in blood 25(OH)D and circulating markers of vitamin D metabolism and activity in individuals with vs. without obesity[22]. Further, if effect modification by BMI was simply a matter of increased vitamin D sequestration in adipose tissue, then increasing the supplement dose should lead to higher circulating 25(OH)D to achieve a similar efficacy for patients with obesity. However, RCTs with substantially higher supplement doses than VITAL, including the D2d and FIND trials, do not corroborate this hypothesis; in D2d, despite supplementation with 4000 IU/D vitamin D, the notable interaction by BMI persisted, with no T2D risk reduction among BMI ≥ 30 kg/m^2 (HR = 0.97; 0.80, 1.17), compared to a HR of 0.71 (95% CI = 0.53, 0.95) in those with BMI < 30 kg/m^2[12]. Further research for the biology underlying an interaction of vitamin D effectiveness by body weight is warranted.

Several prior RCTs of vitamin D supplementation provide conflicting evidence and on average, indicate no effect on intermediate biomarkers of glycemic and insulin homeostasis. A previous meta-analysis combined RCTs with various doses, formulations, and comparator interventions and reported no treatment effect for vitamin D on fasting glucose, HbA1c, or HOMA-IR levels after 4 to 52 weeks intervention[23]. We also did not observe an effect of 2000 IU/d of cholecalciferol vs. placebo on these biomarkers after 2 years of treatment. In the placebo group, there was a decline in insulin sensitivity

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Table 4 | Effect of randomization to vitamin D vs. placebo on biomarkers of insulin sensitivity, beta-cell function, and glycemic control at 2 years in VITAL-T2D CTSC subcohort

	Placebo (*n* = 465)		Vitamin D (*n* = 446)		Treatment Effect
	Baseline Mean (95% CI)	% change Mean (95% CI)	Baseline Mean (95% CI)	% change Mean (95% CI)	*P*-value
Matsuda ISI	4.8 (4.5, 5.1)	−4.1% (−7.4, −0.6)	5.1 (4.8, 5.4)	−3.4% (−6.9, 0.2)	0.80
HOMA-IR, ng/ml	1.8 (1.7, 2.0)	2.4% (−1.7, 6.7)	1.7 (1.6, 1.8)	3.9% (−0.3, 8.4)	0.62
HOMA-β, ng/ml	4.4 (4.2, 4.6)	0.4% (−3, 4.0)	4.3 (4.1, 4.5)	0.7% (−2.8, 4.4)	0.91
Hba1c, %	5.3 (5.3, 5.3)	0.3% (0.0, 0.7)	5.3 (5.3, 5.3)	0.3% (0.0, 0.7)	0.98
Glucose 0 min, mg/dl	98.3 (97.5, 99.1)	0.5% (−0.2, 1.2)	97.2 (96.4, 98.0)	0.8% (0.0, 1.5)	0.61
Glucose 30 min, mg/dl	150.7 (148.4, 153.0)	0.0% (−1.5, 1.4)	149.1 (146.8, 151.5)	−0.7% (−2.2, 0.9)	0.56
Glucose 120 min, mg/dl	116.2 (113.0, 119.4)	−0.4% (−2.7, 2.0)	117.3 (114.0, 120.6)	−3.0% (−5.4, −0.6)	0.12
Glucose OGTT AUC	265.2 (260.8, 269.6)	0.0% (−1.3, 1.2)	263.5 (259.1, 268.0)	−1.4% (−2.7, −0.1)	0.13
Insulin 0 min, uIU/ml	7.6 (7.2, 8.0)	2.1% (−1.6, 6.0)	7.2 (6.8, 7.6)	3.4% (−0.5, 7.5)	0.65
Insulin 30 min, uIU/ml	54.1 (51.2, 57.1)	4.3% (0.1, 8.6)	51.9 (49.1, 54.9)	2.9% (−1.3, 7.4)	0.66
Insulin 120 min, uIU/ml	47.7 (44.3, 51.3)	9.1% (3.0, 15.6)	46.3 (43.0, 50.0)	7.2% (1.0, 13.8)	0.67
Insulin OGTT AUC	98.4 (93.3, 103.8)	5.5% (1.8, 9.4)	94.0 (89.0, 99.3)	4.3% (0.5, 8.3)	0.66

Baseline means (adjusted geometric means and 95% confidence intervals) at baseline, mean percent change at 2 years, and two-sided *p*-value for treatment effect, are calculated from multivariable-adjusted linear mixed models with log-transformed biomarkers as the dependent variable for the effect of vitamin D vs. placebo over time, adjusted for baseline age, self-reported sex, and randomized omega-3 treatment group. The number of participants with repeated biomarkers at 2 years follow-up were 436 (93.8%) among placebo and 411 (92.2%) among the vitamin D groups. *CTSC* clinical trial subcohort, *CI* confidence interval, *ISI* insulin sensitivity index, *HOMA-IR* insulin resistance index, *HOMA-β* beta cell function index, *OGTT* oral glucose tolerance test, *min* minutes, *AUC* area under the curve.

over time characterized by the Matsuda ISI, paralleling the increase in post-load insulin, and this did not occur for the vitamin D group. However, treatment group differences were not statistically significant. Our biomarker analyses further stratified by baseline characteristics did not indicate any statistically significant interactions of vitamin D with age, self-reported sex, omega-3 treatment group, race/ethnicity, or low vitamin D blood levels. Further, despite the suggested protective effect of vitamin D vs. placebo among BMI < 30 in the combined RCT meta-analysis, there were no statistically significant differences in glycemic trait outcomes by BMI. Collectively, while our findings suggest that the potential efficacy of vitamin D supplementation for T2D prevention, there is minimal impact of vitamin D vs. placebo on related glycemic traits after 2 years.

Findings from VITAL for other clinical endpoints indicate a modifying effect of BMI on the efficacy of vitamin D supplementation vs. placebo for reducing cancer incidence[3] and mortality[6], and incidence of autoimmune diseases[7], with benefits limited to participants without obesity at enrollment. Moreover, vitamin D at levels above current clinical thresholds for deficiency may support glycemic health and/or protect against pancreatic β-cell destruction through several biological processes previously linked to vitamin D, including immunemodulation through blockade of dendritic cell differentiation, inhibition of lymphocyte proliferation, and suppression of local or systemic inflammation[24]. Previously published in the VITAL-CTSC, there was a significant 19% reduction in high sensitivity C-reactive protein with vitamin D that was sustained at 4 y[25]. An effect was not observed for other biomarkers of inflammation, interleukin-6, interleukin-10, or tumor necrosis factor-α. Thus, it is plausible that vitamin D has systemic and broad-spectrum effects on immune function and other pathways that underlie potential benefits for several health outcomes and that may be modified by adiposity.

The strengths of this pre-specified ancillary analysis include leveraging the large VITAL trial with a diverse US population, high participant retention over long-term follow-up, and confirmed T2D outcome ascertainment. Limitations of our study include the enrollment of participants without selection for low serum 25(OH)D levels, resulting in the majority of participants with levels above 20 ng/ml at baseline, as is true for most vitamin D trials[23]. However, subgroup analyses did not suggest effects of vitamin D vs. placebo for T2D differed among participants with <20 ng/ml at baseline. The incidence of T2D was lower than predicted for the ages enrolle,d modestly impacting statistical power. It is also unknown whether our findings are generalizable to other age and demographic subgroups. Longer follow-up may be informative, especially given the latency of clinical T2D onset, and observational follow-up of VITAL participants is ongoing in an open-label extension study.

In conclusion, vitamin D supplementation at 2000 IU/d over 5.3 years did not reduce T2D compared with placebo in a general population of older US adults not preselected for prediabetes or vitamin D deficiency. In an updated meta-analysis including 4 RCTs (2 conducted in patients with prediabetes), there was an overall combined 11% reduction in T2D with vitamin D supplementation vs. placebo; however, there was no effect of vitamin D vs. placebo in VITAL on intermediate glycemic traits after 2 years. Our findings do not support routine vitamin D supplementation for the prevention of T2D among older adults in the general population.

Methods

The study complies with all relevant ethical regulations and was approved by the institutional review board of the Brigham and Women's Hospital (BWH) and monitored by an external data and safety monitoring board, and all participants provided written informed consent.

VITAL-T2D population

VITAL-T2D is a funded ancillary study (NCT01633177) of the primary VITAL study (NCT01169259), initiated before the onset of trial enrollment. The VITAL rationale and study protocol are published elsewhere[26]; briefly, the investigators conducted a randomized, double blind, placebo-controlled, two-by-two factorial trial of daily supplementation with vitamin D_3 (2000 IU/day cholecalciferol) and marine omega-3s (460 mg/d eicosapentaenoic acid; 380 mg/d docosahexaenoic acid). There were 25,871 eligible US men ≥50 and women ≥55 years old randomized from November 11, 2011 to March 27, 2014, without a history of invasive cancer (except skin) or cardiovascular disease. During the run-in period, 16,956 participants contributed blood samples, from which serum total 25[OH]D has been previously measured (Supplemental Methods). Randomization was computer generated within self-reported sex, race, and 5-year age groups in blocks, and participants were mailed blinded capsules in calendar packs. Participant demographics and health status were ascertained

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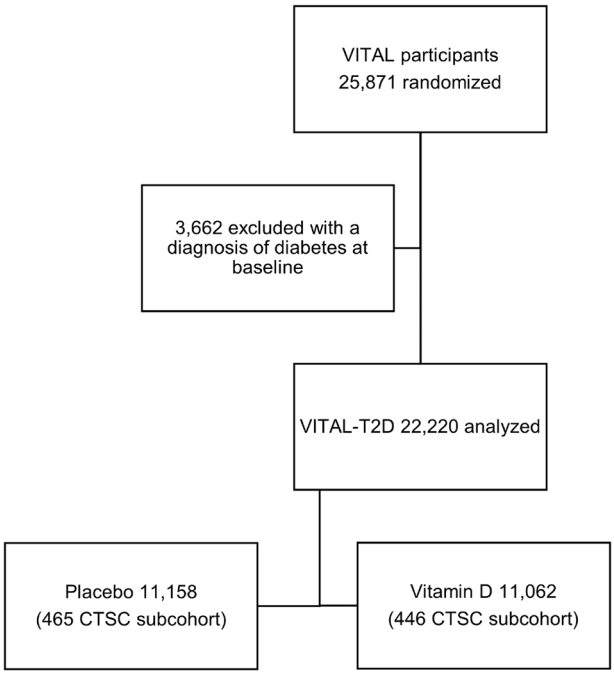

Fig. 2 | Randomization and eligibility of VITAL-T2D participants. This shows the enrollment and inclusion of participants eligible for analysis in the VITAL-T2D ancillary study.

via mailed questionnaire at baseline, 6 months, 1 year, and annually thereafter.

There were 22,200 participants eligible for the current analysis after excluding participants reporting a diagnosis of T2D at baseline randomization (Fig. 2). The participant response to the follow-up questionnaires in this analytic population were 90.1% for the active vitamin D treatment group and 89.4% for the placebo group at Year 5, indicating excellent retention and minimal loss-to-follow-up. Based on the age-specific incidence of T2D in the US general population among non-Hispanic whites and Blacks in 2008–2009, we estimated an incidence of 2.7% and 80% power to detect a 10% reduction in diabetes during 5 years of treatment at a 2-sided significance of 5%.

Ascertainment of incident T2D

Participants reporting a new diagnosis of T2D on the follow-up questionnaires were sent a supplemental questionnaire to collect additional information on the date of diagnosis, diagnostic glycemic values, symptoms, and medications. With written permission, we contacted participants' healthcare providers to provide medical records and/or complete a questionnaire related to the diagnosis. The response rate for follow-up information was 93.4% of self-reported cases. Cases were confirmed based on the 2003 American Diabetes Association diagnostic criteria by a study physician blinded to randomized treatment assignment[27] and were determined unlikely to be new onset type 1 diabetes, given the age of the population. Only clinician-confirmed cases were included in our analyses.

Clinical subcohort

Our prespecified secondary outcomes included estimating the effects of vitamin D vs. placebo on biomarkers of glucose tolerance among a subset of VITAL participants with repeated in-person clinic visits and biospecimen collections at baseline and 2 years. Briefly, 1054 VITAL study participants living in the Boston area attended repeated health assessments at the BWH Clinical and Translational Science Center (CTSC) at baseline prior to randomization and again at 2 years[19]. The visits included a standard physical exam, vital signs, anthropometrics, biospecimen collections, and a 75 g 2-hour oral glucose tolerance test (OGTT) with draws at fasting (0 minutes), 30 minutes, and 120 minutes post glucose load. Per the VITAL-CTSC visit protocol, participants who reported a T2D diagnosis were not administered an OGTT.

Blood samples were collected and processed on-site and stored at the BWH Division of Preventive Medicine biorepository in vapor phase liquid nitrogen (-150 °C) until they were thawed and aliquoted. Serum and plasma samples were shipped blinded to collection timepoint and treatment group, and assayed to quantify HbA1c, plasma glucose, and plasma insulin levels at Boston Children's Hospital, Boston, MA (Supplemental Methods). We calculated the Matsuda insulin sensitivity index (ISI) from the fasting, 30 minute, and 120 minute glucose and insulin measures, among participants completing the OGTT[20]. The Matsuda ISI reflects the degree of insulin resistance of peripheral and hepatic tissues in a postabsorptive state, with a higher value indicating more favorable insulin sensitivity. Fasting glucose and insulin were used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of beta-cell function (HOMA-β) (Supplemental Methods)[28].

Statistical analyses

Baseline characteristics, demographics, and medication use were obtained from self-reported questionnaires at time of enrollment and prior to randomization, and height and weight were used to derive BMI (kg/m^2). Follow-up time was from the date of randomization to the date of T2D diagnosis, death, or December 31, 2017, whichever came first. We calculated T2D incidence and rates per 1000 person-years (PY) by randomized vitamin D vs. placebo treatment group assignment. We calculated the intention-to-treat (ITT) HR and 95% CI for vitamin D vs. placebo with incident T2D risk using a Cox proportional hazards regression model adjusted for age at baseline, self-reported sex, and randomized omega-3 treatment group (active vs. placebo). We conducted prespecified stratified analyses by baseline obesity (BMI above/below 30 kg/m^2), age (above/below 65 years old), self-reported sex, serum 25(OH)D level (above/below 20 ng/ml), race/ethnicity (non-Hispanic White, Black, Other), region (West, Midwest, Southeast, Northeast), and omega-3 treatment group. Stratified Cox models were used to calculate HRs and 95% CIs of vitamin D vs. placebo and T2D risk by levels of effect modifiers. We added multiplicative interaction terms of the effect modifier by treatment group to the main effects models to estimate the *p*-values for interaction. In a prespecified sensitivity analysis, we estimated the per-protocol effect using the Cox model above and censoring participants at protocol non-compliance, defined as taking <66.6% of study pills or initiating non-study vitamin D supplements (>800 IU/day).

For the repeated biomarkers analyses at baseline and 2 years, we excluded participants with self-reported or biochemical T2D (fasting glucose ≥126 mg/dl or Hba1c ≥ 6.5%). All participants eligible at baseline were included in the repeated measures analyses regardless of returning for the follow-up visit. We excluded glucose and insulin values from the analyses if the participant was non-fasted at blood draw. Distributions of the biomarkers and indices were skewed and log-transformed to improve normality for analysis. We calculated treatment group-specific adjusted least-squared geometric means and 95% CIs at baseline and 2 years using linear mixed effects models with maximum likelihood estimation of the covariance parameters and an unstructured covariance matrix, with fixed effects for randomized treatment group, timepoint, and interaction between treatment group and time. Models were adjusted for baseline age, self-reported sex, and omega-3 assignment. We calculated the treatment effect as the mean percent change difference and 95% CIs between vitamin D vs. placebo groups from these models. We repeated the models for glycemic traits stratifying by the same baseline characteristics as described above for T2D incidence and evaluated effect modification using the three-way interaction of time, treatment, and baseline characteristics.

Pre-registered meta-analysis of RCTs

We additionally conducted a prospectively registered systematic literature search and meta-analysis of RCTs reporting effects of randomized moderate to high dose vitamin D vs. placebo on incident T2D risk (PROSPERO Registration: CRD42019147562). Briefly, our protocol and search strategy for PubMed (http://www.ncbi.nlm.nih.gov/pubm), EMBASE, and Cochrane central register were developed to identify double-blind placebo-controlled RCTs published through December 4, 2024, reporting the effects of cholecalciferol vitamin D supplementation (≥1000 IU/day) vs. placebo on incident T2D risk in adults[29]. We limited eligibility to interventions randomizing ≥100 participants and ≥2 years treatment duration (≥1000 IU/d). Abstract and full text screening, data extraction, and Jadad trial quality assessment were conducted independently by two investigators (DKT and EKD), and any conflicts were adjudicated by a third reviewer (ADP) as needed.

We meta-analyzed a pooled ITT HR and CI using a random effects model with Der Simonian-Laird inverse-variance weighting[21]. We assessed between-study heterogeneity from the Mantel-Haenszel model and I^2 values with $I^2 > 50\%$ suggesting moderate heterogeneity. Where reported, we also meta-analyzed HRs and CIs stratified by baseline BMI, given prior meta-analyses have suggested a modifying effect of obesity

Meta-analyses were performed with STATA version 13 (StataCorp LP). All other analyses were conducted in SAS Software version 9.4 (SAS Institute Inc, Cary, NC). Statistical tests were 2-sided with alpha=0.05 and no adjustment for multiple testing as planned.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Source data are provided as a Source Data file. We will provide individual participant data that underlie the results reported in this article, including text, tables, figures, and appendices, after deidentification. We will also provide the relevant data dictionaries and study protocol beginning after publication. Non-study investigators may make their request to achieve aims in the approved proposal by completing the Data Request Form and a Memorandum of Understanding with the Brigham and Women's Hospital, in accordance with the study participants' informed consent. Proposals should be directed to https://www.vitalstudy.org/Investigators.html. To gain access, data requesters will need to sign a data access agreement. Data are available for 5 years at a third-party website. (https://data.harvard.edu/dataverse) Source data are provided with this paper.

Code availability

The SAS code developed to conduct the statistical analyses reported in this manuscript are available at the following link: https://codeocean.com/capsule/5874806/tree[30].

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Author contributions

Deirdre K Tobias performed the statistical analyses, developed and preregistered the systematic review protocol, conducted the systematic review and meta-analysis, and participated in the drafting of the manuscript. Aruna D. Pradhan obtained funding and was PI of VITAL-T2D, developed the protocol and statistical analysis plan, participated in the development of the systematic review protocol, drafted the manuscript, and contributed to the interpretation of results and manuscript review. Edward K. Duran participated in the development of the systematic review protocol, conducted the systematic review, and contributed to the manuscript review. Chunying Li coordinated the datasets and initial data analyses, and contributed to the manuscript review. Yiqing Song obtained funding and was co-PI of VITAL-T2D, developed the protocol and statistical analysis plan, and contributed to the manuscript review. Julie E. Buring obtained funding and was co-PI of VITAL and contributed to the development of the protocol and manuscript review. Nancy R. Cook contributed to the protocol and statistical analysis plan, assisted with the statistical analyses, and contributed to the interpretation of results and manuscript review. Samia Mora obtained funding for T2D-related biomarkers, contributed to the protocol development, and contributed to the interpretation of results and manuscript review. JoAnn E. Manson obtained funding and is PI of VITAL and contributed to the development of the protocol, interpretation of results and manuscript review.

Competing interests

The authors declare no competing interests.

Additional information

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