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Effects of vitamin D supplementation on markers for cardiovascular disease and type 2 diabetes: an individual participant data meta-analysis of randomized controlled trials

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ABSTRACT

Background: Evidence from randomized controlled trials (RCTs) for the causal role of vitamin D on noncommunicable disease outcomes is inconclusive.

Objective: The aim of this study was to investigate whether there are beneficial or harmful effects of cholecalciferol (vitamin D₃) supplementation according to subgroups of remeasured serum 25-hydroxyvitamin D [25(OH)D] on cardiovascular and glucometabolic surrogate markers with the use of individual participant data (IPD) meta-analysis of RCTs.

Design: Twelve RCTs (16 wk to 1 y of follow-up) were included. For standardization, 25(OH)D concentrations for all participants ($n = 2994$) at baseline and postintervention were remeasured in bio-banked serum samples with the use of a certified liquid chromatography–tandem mass spectrometry method traceable to a reference measurement procedure. IPD meta-analyses were performed according to subgroups of remeasured 25(OH)D. Main outcomes were blood pressure and glycated hemoglobin (HbA1c). Secondary outcomes were LDL, HDL, and total cholesterol and triglycerides; parathyroid hormone (PTH); fasting glucose, insulin, and C-peptide; and 2-h glucose. In secondary analyses, other potential effect modifiers were studied.

Results: Remeasurement of 25(OH)D resulted in a lower mean 25(OH)D concentration in 10 of 12 RCTs. Vitamin D supplementation had no effect on the main outcomes of blood pressure and HbA1c. Supplementation resulted in 10–20% lower PTH concentrations, irrespective of the 25(OH)D subgroups. The subgroup analyses according to achieved 25(OH)D concentrations showed a significant decrease in LDL-cholesterol concentrations after vitamin D supplementation in 25(OH)D subgroups with <75, <100, and

<125 nmol of -0.10 mmol/L (95% CI: -0.20 , -0.00 mmol/L), -0.10 mmol/L (95% CI: -0.18 , -0.02 mmol/L), and -0.07 mmol/L (95% CI: -0.14 , -0.00 mmol/L), respectively. Patient features that modified the treatment effect could not be identified.

Conclusions: For the main outcomes of blood pressure and HbA1c, the data support no benefit for vitamin D supplementation. For the secondary outcomes, in addition to its effect on PTH, we observed indications for a beneficial effect of vitamin D supplementation only on LDL cholesterol, which warrants further investigation. This trial was registered at www.clinicaltrials.gov as NCT02551835. *Am J Clin Nutr* 2018;107:1043–1053.

Keywords: individual participant meta-analysis, vitamin D, randomized controlled trials, subgroups, cardiovascular disease, type 2 diabetes, ODIN, remeasured 25-hydroxyvitamin D

INTRODUCTION

Vitamin D plays a central role in the absorption of calcium and in bone health. In addition, numerous observational studies have shown associations of low 25-hydroxyvitamin D [25(OH)D] concentrations with nonskeletal outcomes (1), and that persons with low 25(OH)D status (<30 nmol/L) have the highest all-cause and cardiovascular mortality risk (2–5). However, evidence for causality from randomized controlled trials (RCTs) for cardiometabolic outcomes is inconclusive or negative (6).

Previous results from meta-analyses among RCTs showed inconsistent or null effects of vitamin D supplementation on cardiovascular disease (CVD) incidence, CVD risk factors, or

glycemic outcomes (7–13). Most previous and ongoing RCTs did not include participants based on their vitamin D status (14), resulting in relatively high baseline values and low numbers of deficient people included, with a subsequent null effect of vitamin D supplementation. In addition, there are limitations to most meta-analyses, including analytical variability in 25(OH)D data arising from different methods of analysis (15) and limited attention to subgroups defined on the basis of vitamin D status at enrollment and conclusion of the interventions.

On the other hand, adverse effects have been reported in both observational and interventional studies, including an increased mortality risk in those with high concentrations of 25(OH)D (16–19), although definite evidence is lacking. In addition, when the serum 25(OH)D data are standardized, the shape of the curve may change (19, 20). Harmful effects have also been shown in RCTs that observed an increased risk for falls after a monthly dose of 60,000 IU vitamin D (21), or falls and fractures after an annual dose of 500,000 IU vitamin D (22). Studying the effect of vitamin D supplementation according to 25(OH)D status, particularly those with deficient baseline 25(OH)D concentrations or high post-treatment 25(OH)D concentrations, might provide more insight into potential beneficial or harmful effects.

Importantly, differences in assay and laboratory methods have a significant impact on 25(OH)D concentrations, and therefore assay variability makes pooling of 25(OH)D results from different RCTs in meta-analyses problematic (23). Re-analyses of serum 25(OH)D in bio-banked samples from high-quality completed RCTs at baseline and post-treatment by a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method that is traceable to a reference measurement procedure would considerably decrease the variability inherent in meta-analyses by ensuring that 25(OH)D data are not confounded by well-established method-related variability.

In this meta-analysis that used individual participant data (IPD) of existing RCTs in which serum 25(OH)D was re-analyzed by a centralized LC-MS/MS platform, we aimed to study subgroup effects of vitamin D supplementation among persons with low baseline 25(OH)D concentrations and high post-treatment 25(OH)D concentrations (applying various thresholds) on cardiovascular and glucometabolic surrogate markers. A secondary aim was to identify other patient features that are related to the treatment effects.

This project has received funding from the European Union's Seventh Framework Program (FP7/2007-2013) under grant agreement 613977 [ODIN ("Food-based solutions for eradication of vitamin D deficiency and health promotion throughout the life cycle")]. The funders did not have any role in the implementation of this study.

Supplemental Materials 1 and 2, Supplemental Figure 1, and Supplemental Tables 1–6 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: CVD, cardiovascular disease; DM, diabetes mellitus; HbA1c, glycated hemoglobin; IPD, individual participant data; LC-MS/MS, liquid chromatography–tandem mass spectrometry; PTH, parathyroid hormone; RCT, randomized controlled trial; SNP, single nucleotide polymorphism; UCC, University College Cork; 25(OH)D, 25-hydroxyvitamin D.

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METHODS

Study selection

This work is part of the European Commission–funded integrated project "Food-based solutions for eradication of vitamin D deficiency and health promotion throughout the life cycle" (ODIN). For the current collaborative IPD meta-analysis we established a consortium of European-based researchers with RCT data on the effect of vitamin D supplementation on nonskeletal outcomes. RCTs were considered for selection if bio-banked sera were available in order to remeasure 25(OH)D concentrations by the CDC-certified LC-MS/MS method at University College Cork (UCC). In addition, studies were selected if data were available on the outcomes. Trials in pregnant women or children or performed in patient populations were excluded. This IPD-level meta-analysis is registered at clinicaltrials.gov as NCT02551835.

Study details

Twelve previously published RCTs were selected for inclusion, and included the studies by Chel et al. and Oosterwerff et al., and Paravit; the Styrian Vitamin D Hypertension Trial; Tromsø BMD; Tromsø Clamp; Tromsø Depression; Tromsø IGT; Tromsø Obesity; UCC RCT1 (UCC1) and UCC RCT2 (UCC2); and the study by Wicherts et al. (24–36), details of which are summarized in **Table 1** and **Supplemental Material 1**. The participants of all studies provided written informed consent. All of the studies were in accordance with the Declaration of Helsinki.

Study quality

The risk of bias of the selected RCTs was assessed by using the Cochrane Collaboration's tool for assessing risk of bias (37). Information was derived from the published articles, or provided by the authors if information was not described in the publications. If the criteria for a high-quality study could not be met in >1 domain, the study was regarded as having a considerable risk of bias.

Intervention

Vitamin D₃ supplementation (either vitamin D₃ alone or vitamin D₃ plus calcium) was compared with a comparator (either placebo, placebo plus calcium, or sunlight advice) (**Table 1**). Four studies examined different doses of vitamin D and had therefore >1 treatment group and a single comparator group [Tromsø Obesity, UCC1, UCC2, and Wicherts et al. (33–36)]. Because participants in these control groups contributed information to >1 effect size when pooling the data, only the treatment group with the highest dose was compared with the comparator.

Outcomes

Outcomes were considered at baseline and at 1 follow-up time point (i.e., the end of the intervention period). CVD-related outcomes included systolic and diastolic blood pressure (main outcomes), total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and parathyroid hormone (PTH). Diabetes mellitus (DM)–related outcomes included glycated hemoglobin (HbA1c);

TABLE 1
Main study characteristics of the 12 included studies, with data from 2994 participants¹

| Study (ref) | Country | Year of study completion | Total study population, <i>n</i> | Population | Duration of intervention | Vitamin D intervention | Comparator |
|---|-------------|--------------------------|----------------------------------|---|--------------------------|--|-------------------------------|
| Chel et al. (24) | Netherlands | 2006 | 338 | Nursing home residents; aged >70 y | 4 mo | 600 IU/d or 4200 IU/wk or 18,000 IU/mo | Placebo |
| Oosterwerff et al. (25) | Netherlands | 2011 | 130 | Non-Western immigrants; prediabetic; 25(OH)D <50 nmol/L | 16 wk | 1200 IU/d + 500 mg Ca/d | Placebo + 500 mg Ca/d |
| Paravit (26, 27) | Denmark | 2012 | 52 | BMI (kg/m ²) >30; 25(OH)D <50 nmol/L | 6 mo | 7000 IU/d | Placebo |
| Styrian Vitamin D Hypertension Trial (28) | Austria | 2014 | 200 | History of arterial hypertension; 25(OH)D <75 nmol/L | 8 wk | 2800 IU/d | Placebo |
| Tromsø BMD (29) | Norway | 2010 | 297 | Women; BMD T score ≤ -2.0 | 1 y | 20,000 IU 2 times/wk + 800 IU/d + 1000 mg Ca/d | Placebo 2 times/wk + 800 IU/d |
| Tromsø Clamp (30) | Norway | 2010 | 104 | 25(OH)D <42 nmol/L | 6 mo | 20,000 IU 2 times/wk | Placebo |
| Tromsø Depression (31) | Norway | 2010 | 243 | 25(OH)D <55 nmol/L | 6 mo | 20,000 IU 2 times/wk | Placebo |
| Tromsø IGT (32) | Norway | 2016 ² | 511 | IFG and/or IGT | 1 y | 20,000 IU/wk | Placebo |
| Tromsø Obesity (33) | Norway | 2007 | 445 | BMI: 28-47 | 1 y | 20,000 or 40,000 IU/wk + 500 mg Ca/d | Placebo + 500 mg Ca/d |
| UCC1 (34) | Ireland | 2008 | 225 | Age ≥64 y | 22 wk | 0 or 200 or 400 or 600 IU/d | Placebo |
| UCC2 (35) | Ireland | 2007 | 238 | Age 20-40 y | 22 wk | 0 or 200 or 400 or 600 IU/d | Placebo |
| Wicherts et al. (36) | Netherlands | 2005 | 211 | Non-Western immigrants; 25(OH)D <25 nmol/L | 6 mo | 800 IU/d or 100,000 IU/3 mo | Sunlight advice |

¹For conversion of 25(OH)D from nmol/L to ng/mL, divide by 2.496. BMD, bone mineral density; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; ref, reference; UCC, University College Cork; 25(OH)D, 25-hydroxyvitamin D.

²The 5-y follow-up of the study is planned to be completed in 2016. Data from the 1-y follow-up were used.

main outcome), fasting glucose, fasting insulin, C-peptide, and 2-h postload glucose. In case of duplicate measurements, the mean value of the measurements was used.

Remeasured 25(OH)D concentrations

The remeasurement of 25(OH)D has been done in bio-banked sera (baseline and follow-up) from participants of all 12 RCTs. Serum samples were shipped to the Cork Center of Vitamin D and Nutrition Research at UCC (Ireland), and total 25(OH)D [i.e., 25(OH)D₂ plus 25(OH)D₃] concentrations were analyzed using their CDC-certified LC-MS/MS method, which is traceable to the higher-order Reference Measurement Procedure at the National Institute of Standards and Technology in the United States (23, 38).

For some participants, bio-banked serum was not available, resulting in missing values on remeasured 25(OH)D. If >5% of the remeasured 25(OH)D concentrations were missing in an RCT, the missing values were imputed for that RCT (39). This only applied to the Tromsø Obesity study (16.1% missing). A regression equation of the original and the remeasured 25(OH)D concentrations was developed in the Tromsø Obesity data set, according to a procedure that was consistent with the procedure that was used for the development of a regression equation as performed during standardization of 25(OH)D concentrations in cohort studies (23, 38, 40). Missing values of remeasured 25(OH)D in the Tromsø Obesity data set were subsequently calculated from the original 25(OH)D concentrations. More details on the development of the regression equation are described in **Supplemental Material 2**.

Cutoffs for serum 25(OH)D concentrations were 30, 50, and 75 nmol/L at baseline and 75, 100, and 125 nmol/L at follow-up.

Confounders and effect modifiers

Confounders included age (years), sex (male or female), BMI (kg/m²), current smoking (yes or no), estimated glomerular filtration rate (mL · min⁻¹ · 1.73 m⁻²), and serum calcium (millimoles per liter).

A high baseline PTH concentration (≥ 7.2 pmol/L; the high-est quartile) in combination with a low baseline 25(OH)D concentration (<50 nmol/L) was prespecified as a candidate effect modifier. Other potential effect modifiers included age, sex, BMI, estimated glomerular filtration rate, serum calcium, dose of vitamin D supplementation, calcium supplementation, baseline values of the outcome marker, and single nucleotide polymorphisms (SNPs) of the vitamin D metabolism pathway. The selection of SNPs was made according to the availability of SNPs and included SNPs from the vitamin D receptor gene [rs1544410 (*Bsm-I*), rs3782905, rs731236 (*Taq-I*), rs7975232 (*Apa-I*), rs2228570 (*Fok-I*), and rs11568820]; the GC gene (rs4588, rs7041, rs2282679, and rs2298850) responsible for binding and transportation of vitamin D metabolites in the circulation; the DHCR7/NADSYN1 gene (rs3829251) responsible for the availability of vitamin D precursor 7-DHC in the skin; the CYP2R1 gene (rs10741657) involved in the conversion of vitamin D into 25(OH)D in the liver; the CYP24A1 gene (rs6013897) involved in the degradation of 25(OH)D; and SNPs related to calcium metabolism (rs1697421), calcium-sensing receptor (rs17251221), and 1,25-dihydroxyvitamin D (rs6680429).

Statistical analyses

Baseline summary statistics per study as well as original and remeasured 25(OH)D distributions were described. The outcomes of triglycerides, PTH, HbA1c, fasting glucose, and fasting insulin were not normally distributed and were log-transformed in order to reach near-normal distributions. Hardy-Weinberg equilibrium of the genotype distributions was calculated with standard procedures of chi-square analyses.

Between-study heterogeneity was considered with the use of the I^2 statistic (the proportion of the total variance that can be explained by heterogeneity, 0–100%), derived from ordinary 2-step meta-analysis. I^2 of 0% indicates no heterogeneity, I^2 of 25% indicates low heterogeneity, I^2 of 50% indicates moderate heterogeneity, and I^2 of 75% indicates high heterogeneity. Heterogeneity was further explored by examining potential effect modifiers, as described below, and by determining the random effects of the linear mixed models of the primary analyses.

Unpaired *t* tests were used to compare the change in remeasured 25(OH)D concentrations from baseline to follow-up between the vitamin D and the comparator group in each study. Linear mixed models, with study added as a random effect, were used to examine the effect of vitamin D supplementation in subgroups of remeasured 25(OH)D concentrations. Likelihood ratio tests were used to determine the best models (random intercept, random slope, or both). Baseline subgroups compared persons with 25(OH)D concentrations at baseline below and above the cutoffs (i.e., 30, 50, and 75 nmol/L) in the vitamin D group with persons with the same 25(OH)D concentrations in the comparator group. Follow-up subgroups compared persons with an achieved 25(OH)D response below and above the cutoffs of 75, 100, and 125 nmol/L in the vitamin D group with all persons in the comparator group. Adjustments were made for confounding factors. Because linear mixed models make optimal use of the available data, missing data on the outcomes were not imputed. MLwiN version 2.22 (University of Bristol, United Kingdom) was used for the linear mixed models (41). The significance level was set at $\alpha = 0.05$ (2-tailed).

In secondary analyses, the other potential effect modifiers, in addition to 25(OH)D concentrations, were examined. Stratified analyses were performed to examine the modifying effect of high baseline PTH concentrations in combination with low baseline 25(OH)D concentrations. Other potential effect modifiers were first tested by adding an interaction term with the treatment group to the linear mixed model. A *P*-interaction value <0.10 was considered as a justification for stratified analyses. Adjustments for multiple testing (α/n) were made in these secondary analyses, resulting in a significance level of $P < 0.0003$.

If studies with a high risk of bias were identified, sensitivity analyses were performed in which these studies were excluded (sensitivity analyses I). In addition, sensitivity analyses were performed in which the treatment groups were combined in case of >1 treatment group (sensitivity analyses II).

RESULTS

The 12 included RCTs and their main characteristics are presented in **Table 1**. In total, data from 2994 participants were available (**Supplemental Figure 1**). Baseline characteristics per study are presented in **Supplemental Table 1**. The risk of bias was low for most studies (**Supplemental Table 2**); the study by Wicherts

et al. (36) was identified as having a considerable risk of bias due to its open study design.

The mean, SD, median, and 5th, 25th, 75th, and 95th percentiles of serum 25(OH)D, as well as prevalence estimates for serum 25(OH)D concentration below various thresholds, for the original serum 25(OH)D data as well as remeasured 25(OH)D concentration are shown in **Table 2** (per study). In general, the mean 25(OH)D concentration was lower after LC-MS/MS re-measurement, except for the Oosterwerff (+6%) and Paravit (+20%) studies. A higher number of participants with baseline 25(OH)D concentrations of <30, <40, or <50 nmol/L was observed, as well as a lower number of participants who reached a final achieved 25(OH)D concentration of ≥ 75 , ≥ 100 , or ≥ 125 nmol/L.

Heterogeneity

Heterogeneity between the RCTs was moderate to low, indicated by the I^2 statistics <50% for most outcomes. Only the analysis on total cholesterol suggested considerable heterogeneity between the RCTs ($I^2 = 53\%$; $P = 0.02$, Q test). For HDL cholesterol and PTH, I^2 values were 42% and 40%, respectively. In addition, the multilevel models suggested limited heterogeneity, as reflected by the absence of a random slope in the final models: for all outcomes, models that used random intercepts only were indicated as the best models by the likelihood ratio test.

Effect of vitamin D supplementation

In all of the RCTs the mean 25(OH)D concentrations increased significantly from baseline to follow-up in the vitamin D group compared with the comparator group ($P < 0.001$ in all RCTs; **Supplemental Table 3**).

Effects according to 25(OH)D concentrations

Vitamin D supplementation had no effect on the main outcomes of systolic and diastolic blood pressure and HbA1c (**Tables 3** and **4**). A significant effect on PTH was observed, irrespective of baseline 25(OH)D concentrations or achieved response (**Tables 3** and **4**). The analyses according to baseline 25(OH)D concentrations showed that, in the subgroup with baseline 25(OH)D concentrations <30 nmol/L, significantly higher fasting glucose and fasting insulin concentrations were observed after vitamin D supplementation (**Table 3**). The subgroup analyses according to achieved 25(OH)D concentrations showed a significant decrease in LDL cholesterol in subgroups of 25(OH)D concentrations of <75, <100, and <125 nmol/L of 0.10, 0.10, and 0.07 mmol/L, respectively (**Table 4**).

Other subgroup effects

In the subgroup of participants with PTH ≥ 7.2 pmol/L in combination with 25(OH)D <50 nmol/L, 18% lower PTH concentrations (ratio of geometric means: 0.82; 95% CI: 0.77, 0.88) were observed after vitamin D supplementation (**Supplemental Table 4**). The identification of other patient features that were related to the treatment effects was explored by testing the interaction of treatment with the identified candidate effect modifiers. A

justification for stratified analyses was not found for any of the examined patient features (**Supplemental Table 5**).

With respect to genetic factors, the genotype distribution of rs11568820 (vitamin D receptor gene) deviated from Hardy-Weinberg equilibrium ($P < 0.001$) and was therefore excluded from the analyses. None of the remaining SNPs was identified to modify the effect of vitamin D supplementation on the outcomes (**Supplemental Table 6**). The sensitivity analyses resulted in similar findings (data not shown).

DISCUSSION

This IPD meta-analysis on the effect of vitamin D supplementation on markers for CVD and DM is unique, because the use of 25(OH)D values at baseline and post-treatment derived from re-analysis of all samples using a CDC-certified LC-MS/MS method removed the impact of method-related variability. With the use of data from almost 3000 participants from 12 RCTs, vitamin D supplementation resulted in 10–20% lower PTH concentrations. The evidence for an effect of vitamin D supplementation on PTH is undisputed (10, 42), and the current findings further support this. When the achieved response of 25(OH)D remained <75, 100, or 125 nmol/L, vitamin D supplementation resulted in lower LDL-cholesterol concentrations. These findings are supported by lower LDL- as well as total-cholesterol concentrations in subgroups with lower baseline 25(OH)D concentrations, although the latter findings failed to reach significance. Although not all observations concerning LDL and total cholesterol were significant, and residual confounding might have biased the results of achieved responses, the findings might be clinically relevant. No clear beneficial effects on the other chosen surrogate markers for CVD or DM were observed, nor were adverse effects observed among participants with high achieved serum 25(OH)D response. Other patient characteristics, in addition to 25(OH)D concentration, that might be related to treatment effects could not be identified.

Only 1 previous meta-analysis on the effects of vitamin D supplementation on CVD-related outcomes used IPD and was thereby able to accurately study subgroup effects (7). The outcomes of that meta-analysis were systolic and diastolic blood pressure. In line with the current findings, no subgroup effects of baseline 25(OH)D or other factors could be identified in that study, with the limitation that 25(OH)D data were derived from various assays (7). Further evidence from Mendelian randomization trials also did not show causality of genetically reduced 25(OH)D concentrations and myocardial infarction, ischemic heart disease, or coronary artery disease (43, 44).

With respect to LDL cholesterol, both the meta-analyses of Wang et al. (45) and Manousopoulou et al. (8) reported an increase in LDL cholesterol after vitamin D supplementation [3.23 mg/dL ($I^2 = 0\%$) and 0.34 mmol/L ($I^2 = 71.7\%$), respectively]. However, both meta-analyses did not examine 25(OH)D subgroup responses. In a meta-analysis by Elamin et al. (46), vitamin D supplementation had no effect on lipids ($I^2 = 28$ –99% depending on outcome), and there were no indications of subgroup effects, including subgroups of patients with or without vitamin D deficiency (defined by the authors as <50 nmol/L at baseline).

With respect to fasting glucose, small effects were observed in the current meta-analysis, but the effects were inconsistent across

TABLE 2
Distribution of serum 25(OH)D according to the original and remeasured 25(OH)D concentrations per study¹

| | Baseline concentrations (intervention and control group combined) | | | | | | | | | | Achieved post-treatment response (intervention group only) ² | | | | |
|---|---|-------------------|----------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------|---------------|---------------|---|---------------|---------------|----------------|----------------|
| | <i>n</i> | Mean ± SD, nmol/L | Median, nmol/L | Fifth percentile, nmol/L | 25th Per-centile, nmol/L | 75th Per-centile, nmol/L | 95th Per-centile, nmol/L | <30 nmol/L, % | <40 nmol/L, % | <50 nmol/L, % | <i>n</i> | ≥50 nmol/L, % | ≥75 nmol/L, % | ≥100 nmol/L, % | ≥125 nmol/L, % |
| Chel et al. (24) | | | | | | | | | | | | | | | |
| Original | 335 | 24.9 ± 10.9 | 23.7 | 10.3 | 18.2 | 29.4 | 45.1 | 76.7 | 91.3 | 97.3 | 136 | 80.9 | 23.5 | 2.9 | 0.7 |
| LC-MS/MS | 335 | 23.3 ± 11.3 | 20.9 | 10.9 | 16.7 | 26.3 | 42.3 | 82.1 | 93.7 | 97.3 | 136 | 81.0 | 16.1 | 0.7 | 0 |
| Oosterwerff et al. (25) | | | | | | | | | | | | | | | |
| Original | 130 | 23.4 ± 10.7 | 21.5 | 9.0 | 15.0 | 31.3 | 43.9 | 71.5 | 92.3 | 99.2 | 53 | 79.2 | 17.0 | 0 | 0 |
| LC-MS/MS | 130 | 25.3 ± 11.0 | 22.5 | 10.5 | 17.5 | 32.0 | 46.2 | 69.2 | 89.2 | 97.7 | 53 | 81.1 | 15.1 | 0 | 0 |
| Paravit (26, 27) | | | | | | | | | | | | | | | |
| Original | 52 | 34.5 ± 10.4 | 35.0 | 18.7 | 26.3 | 43.8 | 49.4 | 34.6 | 63.5 | 96.2 | 22 | 100 | 95.5 | 63.6 | 37.3 |
| LC-MS/MS | 51 | 42.6 ± 23.0 | 39.0 | 16.1 | 29.6 | 49.4 | 106.6 | 25.5 | 54.9 | 80.4 | 22 | 90.9 | 81.8 | 72.7 | 31.8 |
| Styrian Vitamin D Hypertension Trial (28) | | | | | | | | | | | | | | | |
| Original | 200 | 52.8 ± 14.0 | 54.5 | 29.3 | 43.0 | 64.3 | 73.2 | 7.0 | 22.5 | 37.5 | 93 | 100 | 78.5 | 30.1 | 3.2 |
| LC-MS/MS | 197 | 45.7 ± 13.4 | 45.9 | 22.0 | 36.1 | 55.9 | 67.9 | 13.2 | 34.0 | 55.3 | 93 | 84.9 | 33.3 | 1.1 | 0 |
| Tromsø BMD (29) | | | | | | | | | | | | | | | |
| Original | 295 | 70.9 ± 22.2 | 69.7 | 36.8 | 56.3 | 85.4 | 107.4 | 2.4 | 7.1 | 15.3 | 136 | 100 | 100 | 99.3 | 97.1 |
| LC-MS/MS | 291 | 64.4 ± 20.3 | 65.0 | 31.0 | 50.6 | 77.6 | 97.4 | 3.8 | 11.7 | 23.0 | 136 | 100 | 100 | 97.8 | 91.9 |
| Tromsø Clamp (30) | | | | | | | | | | | | | | | |
| Original | 105 | 40.3 ± 12.8 | 38.6 | 21.0 | 31.0 | 47.4 | 63.0 | 21.9 | 53.3 | 80.0 | 49 | 100 | 100 | 91.8 | 79.6 |
| LC-MS/MS | 105 | 36.7 ± 11.1 | 36.6 | 19.8 | 28.1 | 43.6 | 57.3 | 30.5 | 61.9 | 88.6 | 49 | 100 | 100 | 89.8 | 46.9 |
| Tromsø Depression (31) | | | | | | | | | | | | | | | |
| Original | 241 | 47.3 ± 15.6 | 45.1 | 25.1 | 36.0 | 55.5 | 77.6 | 10.0 | 34.9 | 62.7 | 120 | 100 | 100 | 97.5 | 77.5 |
| LC-MS/MS | 241 | 42.4 ± 13.8 | 40.7 | 23.4 | 32.7 | 50.4 | 59.4 | 16.0 | 49.0 | 73.3 | 120 | 100 | 99.2 | 85.0 | 49.2 |
| Tromsø IGT (32) | | | | | | | | | | | | | | | |
| Original | 509 | 60.5 ± 21.6 | 57.2 | 31.7 | 45.0 | 72.8 | 97.8 | 3.7 | 14.1 | 33.4 | 242 | 98.8 | 87.6 | 57.4 | 20.2 |
| LC-MS/MS | 509 | 56.4 ± 18.6 | 53.6 | 30.0 | 44.1 | 68.4 | 86.7 | 4.9 | 16.8 | 39.9 | 242 | 98.3 | 80.6 | 36.8 | 7.0 |
| Tromsø Obesity (33) | | | | | | | | | | | | | | | |
| Original | 300 | 53.9 ± 16.1 | 53.3 | 27.9 | 42.6 | 64.8 | 81.2 | 6.7 | 19.7 | 40.7 | 116 | 99.1 | 96.6 | 70.7 | 33.6 |
| LC-MS/MS ³ | 300 | 50.6 ± 17.4 | 48.3 | 24.4 | 38.4 | 62.6 | 84.5 | 11.3 | 27.7 | 53.7 | 116 | 98.3 | 94.8 | 80.2 | 46.6 |
| UCCI (34) | | | | | | | | | | | | | | | |
| Original | 103 | 56.5 ± 21.0 | 55.7 | 27.9 | 39.8 | 71.3 | 89.4 | 8.7 | 25.2 | 43.7 | 50 | 90.0 | 44.0 | 18.0 | 2.0 |
| LC-MS/MS | 103 | 50.0 ± 17.3 | 51.6 | 22.4 | 36.9 | 62.4 | 75.0 | 13.6 | 30.1 | 46.6 | 50 | 86.0 | 30.0 | 4.0 | 0 |
| UCC2 (35) | | | | | | | | | | | | | | | |
| Original | 108 | 78.2 ± 31.6 | 71.6 | 32.6 | 57.2 | 90.2 | 146.5 | 1.9 | 6.5 | 17.6 | 52 | 92.3 | 62.3 | 19.6 | 1.9 |
| LC-MS/MS | 108 | 66.7 ± 22.9 | 62.7 | 33.3 | 52.5 | 75.6 | 114.9 | 1.9 | 7.4 | 21.3 | 52 | 76.9 | 19.2 | 0 | 0 |
| Wicherts et al. (36) | | | | | | | | | | | | | | | |
| Original | 130 | 22.5 ± 12.1 | 20.4 | 8.3 | 14.8 | 22.5 | 50.8 | 81.3 | 89.9 | 94.2 | 56 | 41.1 | 7.1 | 1.8 | 0 |
| LC-MS/MS | 127 | 21.2 ± 10.7 | 18.8 | 8.7 | 14.4 | 24.3 | 46.1 | 84.3 | 92.9 | 96.1 | 56 | 37.9 | 6.9 | 0 | 0 |

¹For conversion of 25(OH)D from nmol/L to ng/mL, divide by 2.496. BMD, bone mineral density; IGT, impaired glucose tolerance; LC-MS/MS: liquid chromatography-tandem mass spectrometry; UCC, University College Cork; 25(OH)D, 25-hydroxyvitamin D.

²The intervention group with the highest dose of vitamin D was used in studies with >1 intervention group (i.e., Tromsø Obesity, UCC1, UCC2, and Wicherts et al.). Values indicate percentages of the intervention group with the final achieved concentrations of 25(OH)D.

³Missing data on remeasured 25(OH)D were imputed in the Tromsø Obesity study.

TABLE 3
Participant-data results of the subgroup effects of vitamin D supplementation according to remeasured baseline 25(OH)D concentrations¹

| | Baseline 25(OH)D | | | | | | | | | | | |
|--|----------------------|-----|----------------------|------|-----------------------|-----|-----------------------|-----|-----------------------|------|-------------------------|-----|
| | <30 nmol/L | | ≥30 nmol/L | | <50 nmol/L | | ≥50 nmol/L | | <75 nmol/L | | ≥75 nmol/L | |
| | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n |
| SBP, ² mm Hg | -0.34 (-3.62, 2.94) | 275 | -0.18 (-1.50, 1.14) | 1252 | -0.65 (-2.35, 1.04) | 843 | 0.53 (-1.27, 2.33) | 684 | -0.39 (-1.69, 0.91) | 1400 | 1.24 (-2.49, 4.96) | 127 |
| DBP, ² mm Hg | 0.14 (-1.94, 2.22) | 275 | 0.26 (-0.58, 1.11) | 1252 | -0.53 (-1.62, 0.56) | 843 | 1.18 (0.05, 2.31)* | 684 | 0.05 (-0.77, 0.88) | 1400 | 1.22 (-1.23, 3.67) | 127 |
| Total cholesterol, ² mmol/L | -0.15 (-0.33, 0.02) | 243 | 0.02 (-0.05, 0.09) | 1569 | -0.01 (-0.10, 0.08) | 912 | 0.01 (-0.09, 0.11) | 900 | 0.01 (-0.06, 0.07) | 1599 | -0.08 (-0.28, 0.12) | 213 |
| HDL cholesterol, ² mmol/L | -0.03 (-0.08, 0.03) | 243 | 0.02 (-0.01, 0.04) | 1569 | 0.01 (-0.01, 0.04) | 912 | 0.00 (-0.03, 0.04) | 900 | 0.012 (-0.01, 0.03) | 1599 | -0.00 (-0.07, 0.07) | 213 |
| LDL cholesterol, ² mmol/L | -0.11 (-0.27, 0.04) | 240 | -0.03 (-0.09, 0.04) | 1563 | -0.06 (-0.14, 0.02) | 905 | -0.01 (-0.09, 0.08) | 898 | -0.04 (-0.10, 0.03) | 1590 | -0.08 (-0.27, 0.12) | 213 |
| Triglycerides, ³ mmol/L | 1.01 (0.92, 1.09) | 243 | 1.02 (0.99, 1.06) | 1569 | 1.01 (0.96, 1.06) | 912 | 1.03 (0.98, 1.07) | 900 | 1.02 (0.99, 1.06) | 1599 | 0.99 (0.90, 1.08) | 213 |
| PTH, ³ pmol/L | 0.83 (0.78, 0.88)* | 317 | 0.87 (0.84, 0.89)* | 1575 | 0.84 (0.81, 0.86)* | 993 | 0.89 (0.86, 0.91)* | 899 | 0.85 (0.84, 0.88)* | 1682 | 0.91 (0.85, 0.98)* | 210 |
| HbA1c, ³ % | 0.99 (0.98, 1.01) | 222 | 1.00 (1.00, 1.00) | 1362 | 1.00 (0.99, 1.00) | 829 | 1.00 (1.00, 1.01) | 755 | 1.00 (1.00, 1.00) | 1411 | 0.99 (0.98, 1.01) | 173 |
| Fasting glucose, ³ mmol/L | 1.03 (1.00, 1.06)* | 237 | 0.98 (0.97, 0.99)* | 1120 | 0.99 (0.97, 1.01) | 725 | 0.99 (0.98, 1.01) | 632 | 0.99 (0.98, 1.00)* | 1234 | 0.99 (0.96, 1.03) | 123 |
| Fasting insulin, ³ mU/mL | 1.14 (1.00, 1.28)* | 194 | 0.96 (0.91, 1.02) | 1101 | 0.98 (0.92, 1.05) | 668 | 0.99 (0.92, 1.06) | 627 | 1.00 (0.95, 1.05) | 1172 | 0.85 (0.72, 0.99) | 123 |
| Fasting C-peptide, ² pmol/L | 4.69 (-54.54, 63.92) | 132 | 7.66 (-32.44, 47.75) | 553 | -2.25 (-49.45, 44.95) | 380 | 24.43 (-24.64, 73.51) | 305 | 16.14 (-19.85, 52.13) | 617 | -52.64 (-167.34, 62.06) | 68 |
| 2-h Glucose, ² mmol/L | -0.01 (-0.68, 0.66) | 130 | -0.01 (-0.29, 0.28) | 686 | -0.15 (-0.53, 0.24) | 419 | 0.10 (-0.25, 0.46) | 397 | -0.08 (-0.36, 0.20) | 732 | 0.19 (-0.60, 0.98) | 84 |

¹Results are derived from 1-step linear mixed models with study added as a random effect. Results were adjusted for age, sex, baseline values of the outcome, BMI, serum calcium concentration, and smoking status. * $P < 0.05$. For conversion of 25(OH)D from nmol/L to ng/mL, divide by 2.496. The intervention group with the highest dose of vitamin D was used in studies with >1 intervention group [i.e., Tromsø Obesity (33), UCCI (34), UCC2 (35), and Wicherts et al. (36)]. DBP, diastolic blood pressure; HbA1c: glycated hemoglobin; PTH, parathyroid hormone; SBP, systolic blood pressure; 25(OH)D, 25-hydroxyvitamin D.

²Results represent the regression coefficient β , which is the mean difference in the outcome in the vitamin D group compared with the comparator group.

³Results were back-transformed, and therefore the effect estimate is the ratio of geometric means in the intervention group compared with the comparator group. The geometric mean resembles the median. Example of interpretation: PTH concentrations are 0.8 times higher after vitamin D supplementation compared with concentrations in the comparator group.

TABLE 4
Participant-data results of the subgroup effects of vitamin D supplementation according to remeasured achieved post-treatment 25(OH)D concentrations¹

| | Achieved 25(OH)D | | | | | | | | | | | | | | | | |
|--|-----------------------|------|-----------------------|------------|-----------------------|------|-----------------------|------|-----------------------|-------------|------------------------|------|----------------------|---|----------------------|-------------|--|
| | <75 nmol/L | | | ≥75 nmol/L | | | <100 nmol/L | | | ≥100 nmol/L | | | <125 nmol/L | | | ≥125 nmol/L | |
| | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | N | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n | Effect size (95% CI) | n | |
| SBP, ² mm Hg | 0.06 (-1.82, 1.93) | 1032 | -0.47 (-1.90, 0.97) | 1244 | -0.47 (-1.98, 1.04) | 1235 | -0.06 (1.83, 1.70) | 1041 | -0.29 (-1.62, 1.04) | 1391 | 0.24 (-2.24, 2.72) | 885 | | | | | |
| DBP, ² mm Hg | 0.82 (-0.39, 2.03) | 1033 | -0.13 (-1.02, 0.77) | 1243 | 0.26 (-0.70, 1.22) | 1236 | -0.03 (-1.15, 1.09) | 1040 | 0.19 (-0.66, 1.03) | 1391 | 0.28 (-1.30, 1.86) | 885 | | | | | |
| Total cholesterol, ² mmol/L | -0.09 (-0.20, 0.02) | 1142 | 0.03 (-0.04, 0.11) | 1577 | -0.07 (-0.15, 0.02) | 1350 | 0.05 (-0.04, 0.13) | 1369 | -0.04 (-0.11, 0.04) | 1537 | 0.09 (-0.01, 0.20) | 1182 | | | | | |
| HDL cholesterol, ² mmol/L | -0.01 (-0.04, 0.03) | 1142 | 0.01 (-0.01, 0.04) | 1577 | 0.00 (-0.03, 0.03) | 1350 | 0.01 (-0.01, 0.04) | 1369 | -0.00 (-0.02, 0.02) | 1537 | 0.03 (-0.00, 0.04) | 1182 | | | | | |
| LDL cholesterol, ² mmol/L | -0.10 (-0.20, -0.00)* | 1136 | -0.02 (-0.08, 0.05) | 1572 | -0.10 (-0.18, -0.02)* | 1342 | 0.00 (-0.07, 0.08) | 1366 | -0.07 (-0.14, -0.00)* | 1528 | 0.04 (-0.05, 0.13) | 1180 | | | | | |
| Triglycerides, ³ mmol/L | 1.02 (0.97, 1.07) | 1143 | 1.02 (0.98, 1.05) | 1577 | 1.03 (0.98, 1.07) | 1351 | 1.02 (0.98, 1.05) | 1369 | 1.03 (0.99, 1.07) | 1537 | 0.99 (0.95, 1.04) | 1183 | | | | | |
| PTH, ³ pmol/L | 0.89 (0.85, 0.92)* | 1224 | 0.85 (0.83, 0.87)* | 1608 | 0.89 (0.86, 0.91)* | 1433 | 0.84 (0.81, 0.87)* | 1399 | 0.87 (0.85, 0.90)* | 1619 | 0.84 (0.81, 0.87)* | 1213 | | | | | |
| HbA1c, ³ % | 1.00 (0.99, 1.01) | 948 | 1.00 (1.00, 1.00) | 1431 | 1.00 (0.99, 1.01) | 1131 | 1.00 (0.99, 1.01) | 1248 | 1.00 (0.99, 1.01) | 1312 | 1.00 (0.99, 1.01) | 1067 | | | | | |
| Fasting glucose, ³ mmol/L | 1.00 (0.98, 1.01) | 939 | 0.99 (0.97, 1.00) | 1096 | 0.99 (0.97, 1.00) | 1129 | 0.99 (0.98, 1.01) | 906 | 0.99 (0.98, 1.00) | 1264 | 0.99 (0.96, 1.01) | 771 | | | | | |
| Fasting insulin, ³ mIU/mL | 1.00 (0.93, 1.07) | 883 | 0.98 (0.93, 1.04) | 1060 | 1.00 (0.94, 1.05) | 1070 | 0.96 (0.90, 1.04) | 873 | 0.99 (0.94, 1.04) | 1203 | 0.96 (0.86, 1.06) | 740 | | | | | |
| Fasting C-peptide, ² pmol/L | 11.11 (-46.08, 68.31) | 433 | 14.44 (-23.89, 52.77) | 593 | 6.93 (-34.88, 48.75) | 552 | 19.61 (-27.18, 66.39) | 474 | 12.46 (-23.56, 48.47) | 645 | -12.52 (-92.31, 67.28) | 381 | | | | | |
| 2-h Glucose, ² mmol/L | 0.23 (-0.24, 0.69) | 507 | -0.08 (-0.36, 0.21) | 719 | 0.04 (-0.29, 0.38) | 637 | -0.14 (-0.48, 0.20) | 589 | -0.05 (-0.33, 0.24) | 747 | 0.03 (-0.48, 0.54) | 479 | | | | | |

¹Results are derived from 1-step linear mixed models with study added as a random effect. Results were adjusted for age, sex, baseline values of the outcome, BMI, serum calcium concentration, and smoking status. * $P < 0.05$. For conversion of 25(OH)D from nmol/L to ng/mL, divide by 2.496. Participants in the vitamin D group with the relevant achieved 25(OH)D concentrations were compared with all of the participants in the comparator group. The intervention group with the highest dose of vitamin D was used in studies with > 1 intervention group [i.e., Tromsø Obesity (33), UCC1 (34), UCC2 (35), and Wicherts et al. (36)]. DBP, diastolic blood pressure; HbA1c: glycated hemoglobin; PTH, parathyroid hormone; SBP, systolic blood pressure; 25(OH)D, 25-hydroxyvitamin D.

²Results represent the regression coefficient β , which is the mean difference in the outcome in the vitamin D group compared with the comparator group.

³Results were back-transformed and therefore the effect estimate is the ratio of geometric means in both treatment groups. The geometric mean resembles the median. Example of interpretation: PTH concentrations are 0.8 times higher after vitamin D supplementation compared with concentrations in the comparator group.

the subgroups. Empirical support for an effect on fasting glucose was found among prediabetics in the meta-analysis by Poolsup et al. (9). In the meta-analysis by George et al. (47), subgroup analyses among patients with DM or impaired glucose tolerance showed a small effect on lowering fasting glucose and a small improvement in insulin resistance. However, we did not observe indications of a treatment effect according to baseline glucose concentrations. The current observed effect of increased fasting insulin concentrations in the subgroup of baseline 25(OH)D values <30 nmol/L was not hypothesized and not supported by the effects on insulin in the other subgroups. This result is therefore considered as an isolated observation that should be interpreted in the light of multiple testing with a risk of statistical type 2 errors. Furthermore, there was no effect on HbA1c, which argues for a neutral effect of vitamin D on overall glucose homeostasis.

The current results showed no indication for differences in vitamin D supplementation effects on markers for CVD and DM according to SNPs involved in the vitamin D metabolism pathway. Previously, it was shown that SNPs in the DBP and CYP2R1 genes relate to the effect of vitamin D supplementation on 25(OH)D concentrations: the SNPs that were associated with lowest baseline 25(OH)D concentrations were also associated with the smallest increase in 25(OH)D after supplementation (48). Another study observed that the associations of low 25(OH)D concentrations with major health outcomes may vary according to genetic differences. The rs7968585 SNP of the vitamin D receptor gene was identified as the most promising SNP with respect to major clinical outcomes (49). Unfortunately, this SNP was not available in the current RCTs.

The remeasurement of serum 25(OH)D with the use of a CDC-certified LS-MS/MS method resulted in lower mean concentrations of 25(OH)D in 10 of 12 RCTs. The deviation between original and remeasured values relates to the type of assay used (40). Depending on the RCT, a higher or lower percentage of participants was reclassified into a different 25(OH)D group after remeasurement. This influenced the intervention effects to some extent: when original 25(OH)D values were used, the trend on LDL cholesterol was significant among persons with a 25(OH)D concentration <50 nmol/L, whereas this trend was no longer significant after reclassification (data not shown). This underlines the importance of accurate 25(OH)D measurements, especially in the context of defining serum 25(OH)D thresholds that may be of use in defining Dietary Reference Intakes for vitamin D.

With data from almost 3000 randomized participants we had sufficient power to detect clinically relevant effects. However, low numbers of participants in the subgroups of baseline 25(OH)D <30 nmol/L [even though overall higher than that evident by using the originally analyzed 25(OH)D] limited our ability to study the effects in this subgroup. Other issues that should be taken into consideration include the high number of statistical tests, which increased the risk of false-positive findings. In addition, the selection of RCTs was based on the availability of data in the consortium and was therefore not systematic. The funnel plots we created (plots not shown), however, did not suggest a selection bias. Because we included trials from reasonably healthy volunteers, the results may not be generalizable to specific patient populations. In addition, some RCTs were not designed to study markers for CVD or DM as primary outcomes, and the outcome markers were still subject to interassay variability. The

major strengths of this study include the remeasurement of the 25(OH)D values and the 1-step IPD approach with the ability to study subgroups.

Several large vitamin D supplementation trials on the effect on CVD, respiratory infection, falls, fractures, cancer, stroke, and mortality are currently being undertaken, such as VITAL (Vitamin D and Omega-3 Trial), TIPS (The International Polycap Study 3), FIND (Finnish Vitamin D Trial), and D-Health (50–53). The results of these impressive studies with numbers of participants ranging from 5000 to >25,000 are awaited. Recent findings from the ViDA study, in >5000 persons aged 50–84 y, failed to show significant effects of vitamin D on incident CVD or among persons with baseline 25(OH)D <50 nmol/L (54). Although these trials will report important data, they only included healthy volunteers with relatively high 25(OH)D concentrations, which showed no effect. In this context, we currently have shown that vitamin D supplementation has no impact on CVD or DM surrogate markers in reasonably healthy people. Future research should be conducted in vitamin D-deficient participants. However, this will be difficult due to ethical considerations of assigning deficient participants to placebo. It is hoped that future meta-analyses will allow for further stratified analyses on the basis of different subgroups, including 25(OH)D subgroups and persons at metabolic and CVD risk.

Overall, the findings from the current IPD-level meta-analysis suggest that vitamin D supplementation has a beneficial effect on PTH. The effect on lipids warrants further investigation. We did not find indications for harmful effects among participants with a high achieved response. Subgroup comparisons with the data of the large, ongoing vitamin D RCTs would be very valuable to see whether the findings can be replicated.

The authors' responsibilities were as follows—MK and KDC: designed the research; KGD, GH, and ZS: undertook all of the serum 25(OH)D reanalysis; PL, IAB, RJ, GG, MRG, MG, AT, SP, GE, VG, LW, LR, CTS, and RAD-A: conducted the research; KMAS, MWH, and NMvS: analyzed the data or performed statistical analysis; KMAS: wrote the manuscript and had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors declared a conflict of interest.

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