No Effect of High-Dose Vitamin D Supplementation on Glycemic Status or Cardiovascular Risk Factors in Subjects With Prediabetes

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OBJECTIVE

In observational studies, low serum 25-hydroxyvitamin D [25(OH)D] concentrations have been associated with insulin resistance and other risk factors for cardiovascular disease.

RESEARCH DESIGN AND METHODS

We present 1-year data from an ongoing 5-year trial in 511 individuals with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) randomly assigned to 20,000 IU/week vitamin D3 or placebo. An oral glucose tolerance test was performed at baseline and after 1 year.

RESULTS

Mean baseline serum 25(OH)D was 59.9 nmol/L and 61.1 nmol/L in the vitamin D and placebo groups, respectively, and increased by 45.8 nmol/L and 3.4 nmol/L, respectively. With adjustment for baseline concentrations, no differences in measures of glucose metabolism, insulin secretion or sensitivity, blood pressure, or hs-CRP were found after 1 year. There was a slight, but significant decrease in total and LDL cholesterol in the vitamin D group compared with the placebo group, but as there was also a decrease in HDL cholesterol, the change in the total/HDL cholesterol ratio did not differ significantly. Only analyzing subjects with 25(OH)D <50 nmol/L did not change the results.

CONCLUSIONS

This study shows that vitamin D supplementation does not improve glycemic indices, blood pressure, or lipid status in subjects with IFG and/or IGT.

The number of people with type 2 diabetes has doubled in the past 30 years, and it is estimated that >360 million people worldwide have type 2 diabetes (1). Type 2 diabetes is associated not only with obesity but also with hypertension and hyperlipidemia and, subsequently, cardiovascular disease (2). Thus, the World Health Organization projects that diabetes will be the seventh leading cause of death in 2030 (3).

Type 2 diabetes develops through a prediabetic stage with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (4). Intervention in the prediabetic stage with changes in lifestyle and/or with medications may prevent progression to type 2 diabetes, as has been demonstrated in several clinical trials (5–7). Bariatric surgery resulting in weight loss may also prevent the development of type 2

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diabetes (8). However, in clinical practice, changes in lifestyle are difficult to implement, and pharmacological intervention as well as surgery may be expensive and have unforeseen side effects (9,10). Other therapeutic options, therefore, are needed, and supplementation with vitamin D has been suggested as one such alternative (11).

Humans either obtain vitamin D through diet (fatty fish, cod liver oil, or vitamin D supplementation) or produce it endogenously in the skin from sun exposure. The main role of vitamin D is maintenance of mineral homeostasis and bone health (12). However, the vitamin D receptor (VDR) is also found in numerous extraskeletal tissues, and low concentrations of serum 25-hydroxyvitamin D [25(OH)D] have been associated with increased plasma glucose and insulin resistance, increased BMI and blood pressure, and an unfavorable lipid profile (13,14). It is therefore no surprise that a low serum 25(OH)D concentration is a strong predictor of future type 2 diabetes and cardiovascular disease (15,16). However, whether this reflects a causal relationship or merely an association is not known. The direction of the association is also uncertain, and it may well be that the low 25(OH)D concentrations result from a disease-associated unhealthy lifestyle. To answer these questions, randomized clinical trials (RCTs) must be performed.

Several RCTs with vitamin D supplementation in subjects with obesity, prediabetes, and type 2 diabetes have been reported, but the results have not been conclusive (17,18). We present 1 year of glucose, lipid, and blood pressure data from an ongoing 5-year intervention trial comparing high-dose vitamin D supplementation with placebo in 511 subjects with prediabetes.

RESEARCH DESIGN AND METHODS

Study Design

The 1-year results are part of an ongoing 5-year RCT of vitamin D in subjects with prediabetes. The inclusion criteria were age 21–80 years and IFG (fasting glucose >6.0 mmol/L and <7.0 mmol/L) and/or IGT (2-h glucose >7.7 mmol/L and <11.1 mmol/L on oral glucose tolerance test [OGTT] with 75 g glucose combined with a fasting glucose <7.0 mmol/L) (19).

Subjects with primary hyperparathyroidism, sarcoidosis, or other granulomatous disorders; urolithiasis; cancer in the past 5 years; allergies to nuts; unstable angina pectoris or acute myocardial infarction or stroke in the past year; or reduced kidney function with creatinine >125 μmol/L in men and 105 μmol/L in women were excluded. For women, additional exclusion criteria were pregnancy, lactation, and fertile age and no use of contraception.

Subjects were principally recruited from the Tromsø Study, a longitudinal, population-based, multipurpose study with cardiovascular disease as the main focus (20). In the sixth survey in 2007–2008, 19,762 subjects were invited, 12,984 attended, and glycated hemoglobin (HbA_{1c}) was measured in 12,769. All subjects without known diabetes and with HbA_{1c} in the range of 5.8–6.9% (39.9–51.9 mmol/mol) (HbA_{1c} as a diagnostic criterion for diabetes was not established at the time of study start in 2008) as well as a random sample of subjects with lower HbA_{1c} values were invited to undergo an OGTT. Among the 4,393 subjects invited, 3,476 completed the OGTT, and 713 had IFG and/or IGT (21). In addition, a few subjects were recruited based on OGTTs performed at the outpatient clinic at the University Hospital of North Norway, on follow-up OGTTs performed in former participants from a vitamin D and obesity study from 2005–2007 (22), and on OGTTs performed after elevated fasting blood glucose values were found in subjects in the Renal Iohexol-clearance Survey (RENIS) (23). All subjects were included within 1–2 weeks after their OGTT.

Eligible subjects were invited by letter, and those who responded positively were invited to the first visit at the Clinical Research Unit of the University Hospital of North Norway. At the same time, the hospital's pharmacy assigned each subject the next randomization number on the randomization list. The randomization list was computer generated by the randomization unit of the hospital's research department in a 1:1 ratio between vitamin D and placebo, and a copy was kept at the hospital's pharmacy. The code was only known to the pharmacy and the research department, and everyone else was blinded. The randomization was not stratified.

At the first visit, further information about the study was given, a written consent form signed, a brief clinical examination performed, and oral and written information about physical activity, healthy food habits, and the importance of weight loss (if needed) given. The medical history, including smoking habits and use of calcium and vitamin D supplements, was recorded. Height and weight were measured while the subjects wore light clothing and no shoes. BMI was calculated as weight (kg) divided by height squared (m^2). Blood pressure was measured three times with an automatic device (A&D Medical, Tokyo, Japan), and the mean of the second and third measurements was used in the statistical analyses. The study medication was cholecalciferol (vitamin D3 20,000 IU [Dekristol; Mibe, Jena, Germany]) capsules or placebo delivered as identical-looking capsules containing arachis oil (Hasco-Lek, Wroclaw, Poland) for 6 months; one capsule was to be taken each week. The subjects were not allowed to take vitamin D supplements (including cod liver oil) exceeding 400 IU/day. Also at the first visit, nonfasting blood samples were drawn. Fasting blood samples for glucose, insulin, and lipids had been collected at the previous OGTT.

The next visit was after 6 months for return of unused study medication, supply of new study medication, measurement of serum calcium and creatinine levels, and registration of adverse effects. After 12 months, a new OGTT was performed together with the same examinations as at the first visit. Unused medication was returned and counted. All participants were informed about the risk and symptoms of hypercalcemia, and if experiencing such symptoms, they were instructed to contact the Clinical Research Unit.

Serum glucose, insulin, C-peptide, total cholesterol (TC), triglycerides, HDL cholesterol, LDL cholesterol, apolipoprotein A1, apolipoprotein B, calcium, parathyroid hormone (PTH), hs-CRP, and HbA_{1c} were measured as previously described (24). Estimates of insulin sensitivity were calculated with HOMA-IR ([insulin (pmol/L) × glucose (mmol/L)]/135) (25) and QUICKI (1 / [log insulin (mU/mL) + log glucose (mg/dL)]) (26). Serum concentrations of 25(OH)D were measured by in-house liquid chromatography–tandem mass spectrometry (Supplementary Data).

To keep all investigators blinded, all data were sent directly to the hospital's

research department, where the data files were merged and coupled to the randomization code. The research department sent the final file without personal identification information to the principal investigators (S.T.S. and R.J.).

Statistical Analysis

Normal distribution was evaluated by visual inspection of histograms and by kurtosis and skewness, and log-transformation was performed before statistical analyses, where appropriate. At baseline, relations between serum 25(OH)D and glucose metabolism, blood pressure, serum lipids, and hs-CRP were evaluated for trend across groups with serum 25(OH)D concentrations <30 nmol/L, 30–49 nmol/L, 50–74 nmol/L, and >74 nmol/L by using linear regression, with sex, age, and BMI as covariates, or χ^2 linear-by-linear association. At baseline, the vitamin D and placebo groups were compared with Student *t* test for continuous variables and χ^2 test for categorical variables. The effects of vitamin D and placebo on the outcome variables were evaluated with regression models adjusting for baseline values (27). These analyses were performed both per protocol and as intention to treat (last observation carried forward). No significant interactions were found between treatment group and sex regarding the outcome variables HOMA-IR, systolic blood pressure, TC, and hs-CRP; therefore, the data are presented for both sexes together. The compliance rate was calculated as the ratio between capsules used and capsules supplied for that time period.

Data are presented as mean ± SD for normally distributed variables and as median (2.5th, 97.5th percentiles) for nonnormally distributed variables unless otherwise specified. Level of significance was set at $P < 0.05$ (two-tailed). All statistical analyses were performed using IBM SPSS version 21 software.

Power Calculation

The main end point for the 5-year intervention is development of type 2 diabetes (defined as fasting glucose >6.9 mmol/L or 2-h glucose >11.0 mmol/L at OGTT) and the null hypothesis that this development would be equal in the two groups. The power calculation was based on the assumptions that an equal number would be included in each group; that 10% in the placebo group would develop type 2 diabetes annually; that supplementation with vitamin D would reduce the development of type 2 diabetes in 30% during the 5-year intervention; that the dropout rate would be 20% and equal in both groups; and that the study would last for 5 years. For the study to have a power of 0.80 with an α of 0.05 and a β of 0.20, 505 subjects with IFG and/or IGT had to be included. A power calculation for the 1-year evaluation of glucose metabolism, blood pressure, lipids, and hs-CRP was not performed before the start of the study.

Ethics

The study was approved by the Norwegian Medicines Agency and by the Regional Committee for Medical Research Ethics. The trial is registered at ClinicalTrials.gov (NCT00685594).

RESULTS

Study Flow

The study flow is shown in Fig. 1. Seven hundred forty-three subjects with IFG and/or IGT were invited to participate, and 556 accepted the invitation and were allocated a randomization number. Twenty-two subjects allocated to vitamin D and 23 allocated to placebo were excluded at the baseline visit (2 did not

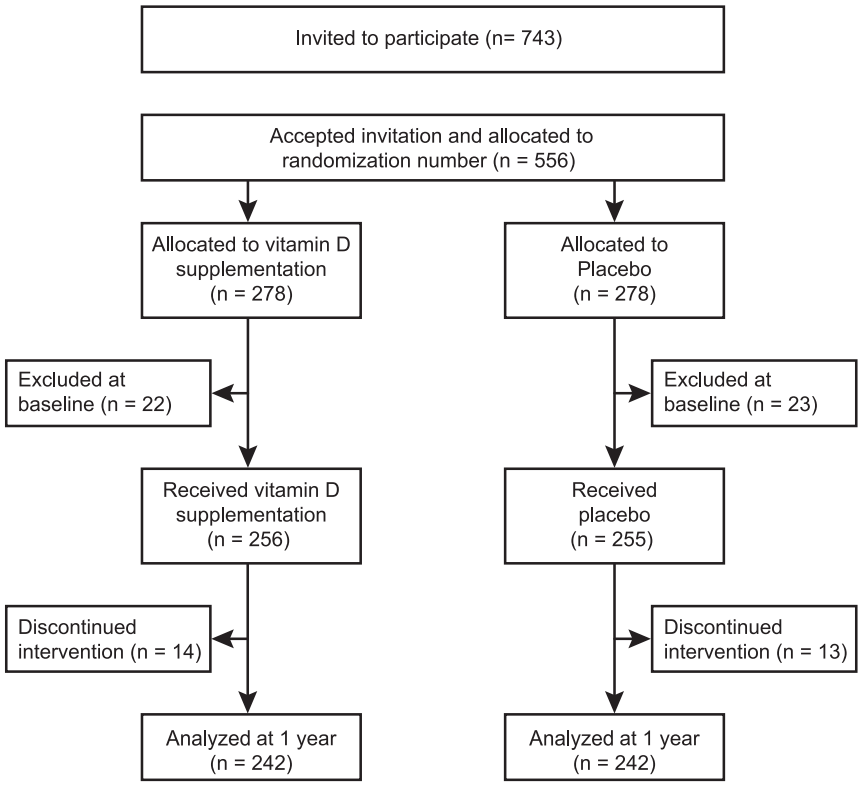

Figure 1—Flowchart of the study.

meet the inclusion criteria, and 43 had one or more exclusion criteria), leaving 511 who received the study medication (256 vitamin D and 255 placebo). In the vitamin D group, 127 subjects had isolated IFG, 79 isolated IGT, and 50 both IFG and IGT; the corresponding figures in the placebo group were 127, 89, and 39, respectively (Table 1). Thirteen subjects in the vitamin D group and 14 in the placebo group dropped out during the first 12 months. Thus, 242 subjects in both groups completed the 1-year visit. At 6 months, one subject in the vitamin D group had a serum calcium level of 2.63 mmol/L and was excluded from the trial; after 5 months the value had normalized.

Evaluation of Baseline Levels

The baseline characteristics of the 511 subjects together and by serum 25(OH)D concentration group are shown in Table 2. With increasing serum 25(OH)D concentration, there was a significant decrease in BMI, fasting and 2-h glucose, fasting insulin and 2-h insulin, HbA_{1c}, and HOMA-IR and an increase in QUICKI, HDL cholesterol, apolipoprotein A1, and apolipoprotein B.

Effect of the Intervention

At baseline, there were no significant differences between the vitamin D and placebo groups (Table 3). The baseline values in the 14 subjects in the vitamin D group and 13 subjects in the placebo group who discontinued before the 1-year visit did not differ significantly on any of the outcome variables (data not shown). The analyses, therefore, are presented as per protocol. (Analyses on an intention-to-treat basis did not change any of the results significantly.) The compliance rates during the first and last 6 months were 0.86 and 0.84 in the vitamin D group and 0.86 and 0.83 in the placebo group.

In subjects who received vitamin D, there was an increase in serum 25(OH)D of 45.8 $\pm$ 24.2 nmol/L compared with 3.4 $\pm$ 16.9 nmol/L in the placebo group ($P < 0.001$) and a decrease in serum PTH of 0.5 pmol/L compared with an increase of 0.2 pmol/L in the placebo group ($P < 0.001$). When adjusting for baseline values, after 1-year, there were no significant differences between those given vitamin D and those given placebo in any of the glycemic indices, blood pressures, or hs-CRP levels. There was a slight reduction in TC of 0.16 mmol/L and LDL cholesterol of 0.17 mmol/L in the vitamin D group compared with the placebo group ($P < 0.05$). However, there was also a decrease in HDL cholesterol, so the change in the TC/HDL cholesterol ratio did not differ significantly (Table 3).

Among the 242 subjects in the vitamin D group who underwent the OGTT at the 1-year visit, 68 had normal glucose values, 133 IFG and/or IGT, and 39 fasting glucose >6.9 mmol/L and/or 2-h glucose >11.0 mmol/L; the corresponding numbers in the placebo group were 68, 133, and 41 (Table 1).

Subgroup Analyses

A separate analysis was performed in the 87 subjects in the vitamin D group and the 83 subjects in the placebo group who at baseline had serum 25(OH)D concentrations <50 nmol/L and did not show significant differences in change from baseline between the two groups (Supplementary Table 1). The same was seen when separately analyzing the 37 subjects in the vitamin D group and the 35 subjects in the placebo group with serum 25(OH)D <40 nmol/L at baseline (data not shown). Furthermore, if only the 62 subjects in the vitamin D group who had baseline 25(OH)D <50 nmol/L and who at 12 months had serum 25(OH)D ≥75 nmol/L and the 51 subjects in the placebo group who had both baseline and 12-month 25(OH)D <50 nmol/L were included, there still were no significant differences in change in any of the outcome parameters after 12 months (Supplementary Table 2). Inclusion of change in serum 25(OH)D did not alter these results (data not shown).

Subgroup analyses were also performed regarding blood pressure for subjects not taking blood pressure medication, those on stable blood pressure medication, and those with baseline hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg) and did not show significant differences in blood pressure change between the two groups after 1 year (Supplementary Tables 3 and 4).

Similar subgroup analyses were performed for serum lipids in subjects not taking lipid medication, those on stable lipid medication, and those with baseline hyperlipidemia (serum TC >7.8 mmol/L and/or LDL cholesterol >4.9 mmol/L). For subjects not using statins,

Table 1—Glycemic status at baseline and after 12 months in the vitamin D and the placebo groups

		Glycemic status after 1 year						
						Type 2 diabetes		
Glycemic status at baseline	Dropouts	Normal	Isolated IFG	Isolated IGT	Both IFG and IGT	Fasting glucose >6.9 mmol/L	2-h glucose >11.0 mol/L	Fasting glucose >6.9 mmol/L and 2-h glucose >11.0 mmol/L
Vitamin D group								
Isolated IFG (*n* = 127)	6	41	37	12	17	9	1	4
Isolated IGT (*n* = 79)*	8	18	5	34	7	0	5	0
Both IFG and IGT (*n* = 50)	0	9	5	5	11	7	10	3
Placebo group								
Isolated IFG (*n* = 127)	7	38	41	5	23	8	2	3
Isolated IGT (*n* = 89)	5	27	4	35	8	1	7	2
Both IFG and IGT (*n* = 39)	1	3	5	3	9	2	7	9

*Two subjects in the vitamin D group with isolated IGT at baseline had only fasting values (normal) at 12 months, with 2-h values from the OGTT missing.

Table 2—Baseline characteristics of all the subjects and in relation to serum 25(OH)D concentrations

	All subjects (*n* = 511)	Serum 25(OH)D <30 nmol/L (*n* = 9)	Serum 25(OH)D 30–49 nmol/L (*n* = 151)	Serum 25(OH)D 50–74 nmol/L (*n* = 228)	Serum 25(OH)D >74 nmol/L (*n* = 111)
Male sex	314 (61.4)	13 (68.4)	101 (66.9)	148 (64.9)	52 (46.8)
Age (years)	62.1 ± 8.7	56.9 ± 9.8	60.3 ± 9.1	63.0 ± 8.4	63.6 ± 8.5*
BMI (kg/m^2)	29.9 ± 4.3	30.3 ± 4.2	30.7 ± 4.1	29.8 ± 4.1	29.1 ± 4.7*
Systolic blood pressure (mmHg)	135.4 ± 16.8	135.3 ± 15.9	134.7 ± 15.2	136.1 ± 16.8	135.5 ± 19.1
Diastolic blood pressure (mmHg)	83.2 ± 10.1	88.4 ± 11.1	83.5 ± 10.6	82.8 ± 9.8	82.9 ± 9.7
OGTT					
Fasting serum glucose (mmol/L)	6.10 ± 0.48	6.30 ± 0.44	6.13 ± 0.46	6.10 ± 0.48	6.02 ± 0.51*
2-h serum glucose (mmol/L)	7.33 ± 1.98	7.65 ± 1.98	7.36 ± 1.97	7.33 ± 2.01	7.22 ± 1.98*
Fasting serum insulin (pmol/L)	93 (88, 98)‡	103 (84, 122)	106 (95, 117)	95 (87, 104)	69 (63, 76)†
2-h serum insulin (pmol/L)	576 (531, 621)§	658 (486, 830)	631 (550, 713)	604 (528, 680)	433 (365, 502)†
Fasting serum C-peptide (pmol/L)	1,088 ± 371‖	1,172 ± 282	1,149 ± 375	1,095 ± 404	981 ± 281†
HbA_{1c} (%)	5.97 ± 0.31¶	6.23 ± 0.49	6.00 ± 0.29	5.95 ± 0.30	5.92 ± 0.31*
HbA_{1c} (mmol/mol)	41.8 ± 3.4	44.6 ± 5.3	42.1 ± 3.2	41.6 ± 3.2	41.3 ± 3.4*
HOMA-IR	4.25 (4.00, 4.50)	4.87 (3.94, 5.80)	4.85 (4.33, 5.38)	4.35 (3.94, 4.76)	3.12 (2.82, 3.42)†
QUICKI	0.33 (0.32, 0.33)	0.31 (0.30, 0.33)	0.32 (0.31, 0.32)	0.33 (0.32, 0.33)	0.34 (0.33, 0.35)*
Serum 25(OH)D (nmol/L)	60.5 ± 21.6‡	24.4 ± 3.8	41.5 ± 5.1	61.0 ± 7.2	91.4 ± 17.8
Serum calcium (mmol/L)	2.31 ± 0.08‖	2.32 ± 0.07	2.30 ± 0.08	2.31 ± 0.08	2.31 ± 0.08
Serum PTH (pmol/L)	5.7 (5.5, 5.9)¶	7.3 (5.3, 9.3)	6.2 (5.8, 6.6)	5.6 (5.3, 5.9)	5.0 (4.7, 5.4)*
Serum creatinine (μmol/L)	69.6 ± 13.7	66.6 ± 9.8	69.6 ± 13.6	70.0 ± 13.4	69.6 ± 15.3
Serum hs-CRP (mg/L)	3.91 ± 11.00	2.10 ± 1.57	4.22 ± 11.00	4.47 ± 13.7	2.71 ± 2.54
Serum TC (mmol/L)	5.76 ± 1.07	5.93 ± 0.97	5.49 ± 1.09	5.78 ± 1.02	6.06 ± 1.07
Serum LDL cholesterol (mmol/L)	3.73 ± 0.93‖	3.81 ± 0.79	3.51 ± 0.99	3.76 ± 0.88	3.93 ± 0.90
Serum HDL cholesterol (mmol/L)	1.37 ± 0.36	1.19 ± 0.32	1.30 ± 0.32	1.37 ± 0.37	1.48 ± 0.36*
Ratio TC/HDL cholesterol	4.5 ± 1.3	5.3 ± 1.4	4.5 ± 1.5	4.5 ± 1.3	4.3 ± 1.0
Serum triglycerides (mmol/L)	1.64 (1.55, 1.73)	2.32 (1.64, 2.99)	1.72 (1.53, 1.91)	1.66 (1.52, 1.80)	1.39 (1.28, 1.50)
Serum apolipoprotein A1 (mmol/L)	1.53 ± 0.29#	1.38 ± 0.24	1.49 ± 0.34	1.54 ± 0.26	1.61 ± 0.26*
Serum apolipoprotein B (mmol/L)	1.09 ± 0.24#	1.13 ± 0.22	1.04 ± 0.26	1.10 ± 0.23	1.14 ± 0.24*
Smoking	96 (19.8)**	4 (22.2)	34 (25.0)	39 (17.3)	19 (18.4)
Antihypertensive drug use	233 (45.6)††	10 (52.6)	69 (45.7)	103 (45.2)	50 (45.0)
Statin use	132 (25.8)††	6 (31.6)	49 (32.5)	54 (23.7)	23 (20.7)
Vitamin D supplement use‡‡	168 (33.5)§§	5 (27.8)	39 (26.7)	87 (38.3)	37 (34.3)

Data are *n* (%), mean ± SD, and median (2.5th, 97.5th percentiles). *$P < 0.001$. †$P < 0.05$; linear trend across the four 25(OH)D groups with age, sex, and BMI as covariates where appropriate. ‡Data missing for three subjects. §Data missing for four subjects. ‖Data missing for one subject. ¶Data missing for five subjects. #Data missing for 11 subjects. **Data missing for 27 subjects. ††Data missing for two subjects. ‡‡Including cod liver oil. §§Data missing for 10 subjects.

there was a slight, but significant decrease in TC and LDL cholesterol compared with the placebo group but not in the TC/HDL cholesterol ratio. For subjects not using or on stable lipid medication and with baseline serum TC >7.8 mmol/L and/or baseline serum LDL cholesterol >4.9 mmol/L, there was a slight, but significant increase in triglycerides compared with the placebo group (Supplementary Tables 5 and 6).

Dividing the cohort according to baseline fasting glucose, baseline 2-h glucose, and HbA_{1c} (>50th percentile or <50th percentile) did not reveal any consistent significant effects of the vitamin D supplementation on any of the outcomes measured (data not shown). Furthermore, no interactions were found between use of statins and randomization status regarding any of the outcomes (data not shown).

CONCLUSIONS

We found that in subjects with prediabetes, supplementation with 20,000 IU/week vitamin D was no better than placebo regarding measures of glucose metabolism, blood pressure, and hs-CRP level and had no clinically relevant effect on serum lipids. On the other hand, no major adverse effects were seen.

There are many indications for a relationship between vitamin D and glucose metabolism, with a number of observational studies having shown an inverse association between serum 25(OH)D concentrations and insulin resistance (13,15). The VDR is found in cells important for glucose metabolism, like the pancreatic β-cells and adipocytes (28), and β-cells have the enzymes necessary for the conversion of 25(OH)D to its active form 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (29). In animal models, severe vitamin D deficiency results in reduced insulin secretion (30), and $1,25(OH)_2D$ has been reported to stimulate the expression of insulin receptors in

Table 3—Baseline values and change from baseline after 12 months (12-month value − baseline value) in the vitamin D and the placebo groups and difference in change between the two groups (change in vitamin D group − change in placebo group) adjusted for baseline level

	Baseline value		Change from baseline after 12 months		Difference in change between vitamin D and placebo groups‖
	Vitamin D group (*n* = 256)	Placebo group (*n* = 255)	Vitamin D group (*n* = 242)	Placebo group (*n* = 242)	
Male sex	161 (62.9)	153 (60.0)			
Age (years)	62.3 ± 8.1	61.9 ± 9.2			
BMI (kg/m^2)	30.1 ± 4.1	29.8 ± 4.4	−0.0 ± 1.0	−0.0 ± 1.1	0.0 (−0.2, 0.2)
Systolic blood pressure (mmHg)‡	135.1 ± 16.8	135.8 ± 16.9	−2.9 ± 13.7	−3.5 ± 15.0	−0.5 (−2.7, 1.8)
Diastolic blood pressure (mmHg)‡	83.6 ± 10.7	82.8 ± 9.5	−4.6 ± 8.9	−4.6 ± 9.4	−0.1 (−1.5, 1.3)
OGTT					
Fasting serum glucose (mmol/L)	6.12 ± 0.47	6.08 ± 0.50	−0.07 ± 0.54	0.02 ± 0.63	−0.08 (−0.18, 0.03)
2-h serum glucose (mmol/L)	7.26 ± 2.11	7.40 ± 1.84	0.71 ± 2.41	0.62 ± 2.27	0.01 (−0.39, 4.40)
Fasting serum insulin (pmol/L)	93 (86, 99)	94 (85, 102)	14 (9, 19)	15 (9, 22)	−1 (−9, 7)
2-h serum insulin (pmol/L)	570 (506, 634)	582 (519, 645)	114 (47, 180)	74 (17, 132)	36 (−51, 123)
Fasting serum C-peptide (pmol/L)	1,095 ± 345	1,081 ± 397	41 ± 231	36 ± 287	7 (−38, 52)
HbA_{1c} (%)	5.98 ± 0.28	5.97 ± 0.34	0.12 ± 0.29	0.13 ± 0.33	−0.00 (−0.06, 0.05)
HbA_{1c} (mmol/mol)	41.8 ± 3.1	41.7 ± 3.7	1.3 ± 3.2	1.4 ± 3.6	−0.02 (−0.6, 0.6)
HOMA-IR	4.24 (3.94, 4.54)	4.24 (3.87, 4.62)	0.63 (0.35, 0.90)	0.76 (0.40, 1.13)	0.03 (−0.26, 0.31)
QUICKI	0.33 (0.32, 0.33)	0.33 (0.32, 0.33)	−0.01 (−0.01, −0.00)	−0.01 (−0.01, −0.01)	0.00 (−0.00, 0.00)
Serum 25(OH)D (nmol/L)	59.9 ± 21.9	61.1 ± 21.2	45.8 ± 24.2	3.4 ± 16.9	41.9 (38.3, 45.5)*
Serum calcium (mmol/L)	2.31 ± 0.08	2.31 ± 0.08	−0.04 ± 0.08	−0.05 ± 0.08	0.01 (−0.00, 0.02)
Serum PTH (pmol/L)	5.8 (5.5, 6.1)	5.6 (5.3, 5.9)	−0.5 (−0.7, −0.3)	0.2 (0.0, 0.4)	−0.6 (−0.9, −0.4)*
Serum creatinine (μmol/L)	69.7 ± 13.6	69.5 ± 13.9	0.1 ± 0.8	−0.4 ± 7.2	0.5 (−0.8, 1.7)
Serum hs-CRP (mg/L)	3.28 ± 5.37	4.55 ± 14.60	2.67 ± 3.22	3.06 ± 4.06	−0.27 (−0.89, 0.35)
Serum TC (mmol/L)§	5.72 ± 1.06	5.80 ± 1.08	−0.11 ± 0.79	−0.03 ± 0.85	−0.16 (−0.29, −0.03)†
Serum LDL cholesterol (mmol/L)§	3.72 ± 0.93	3.73 ± 0.92	−0.11 ± 0.70	0.09 ± 0.66	−0.17 (−0.28, −0.06)†
Serum HDL cholesterol (mmol/L)§	1.36 ± 0.34	1.38 ± 0.37	−0.04 ± 0.20	−0.02 ± 0.21	−0.03 (−0.07, 0.00)
Ratio TC/HDL cholesterol	4.4 ± 1.3	4.5 ± 1.4	0.15 ± 1.3	0.05 ± 0.9	0.10 (−0.10, 0.30)
Serum triglycerides (mmol/L)§	1.62 (1.51, 1.72)	1.67 (1.52, 1.82)	0.03 (−0.05, 0.12)	−0.10 (−0.23, 0.03)	0.11 (−0.03, 0.24)
Serum apolipoprotein A1 (mmol/L)§	1.53 ± 0.25	1.54 ± 0.32	−0.10 ± 0.16	−0.09 ± 0.27	−0.03 (−0.06, 0.01)
Serum apolipoprotein B (mmol/L)§	1.09 ± 0.24	1.09 ± 0.24	−0.06 ± 0.18	−0.06 ± 0.17	−0.02 (−0.05, 0.01)

Data are *n* (%), mean ± SD, and median (2.5th, 97.5th percentiles). *$P < 0.001$. †$P < 0.05$; general linear regression models adjusting for baseline value. ‡Eighteen subjects with changes in antihypertensive medication during the 12 months were excluded from the analyses of changes in blood pressure and heart rate. §Twenty-four subjects with changes in statin medication during the 12 months were excluded from the analyses of changes in lipids. ‖Adjusted for baseline level (mean [2.5th, 97.5th percentiles]). A positive value means that there was an increase in the vitamin D group vs. the placebo group.

peripheral tissues (31) and may thereby increase insulin sensitivity. Furthermore, in mouse pancreatic islet cells, hypovitaminosis D increases renin-angiotensin system gene expression (32). Upon renin-angiotensin system inhibition, the islets' function improved in mice with diabetes, thus leading to a hypothesis that vitamin D supplementation might improve islet cell function before too much damage occurs in the islet cells (32).

To establish causal relationships, however, one must rely on intervention studies. There is a general agreement that in subjects with normal glucose tolerance vitamin D supplementation has no beneficial effect on insulin resistance (33). The majority of trials in subjects with type 2 diabetes have also not proven an effect of vitamin D supplementation on glucose metabolism (33–35). Of special interest is a recently published study in subjects with vitamin D deficiency (mean baseline serum 25(OH)D concentration 29.7 nmol/L) (36). A few trials in subjects with Iranian ethnicity and type 2 diabetes have suggested, on the other hand, a positive effect of vitamin D supplementation on glycemic outcomes; whether this is due to ethnicity or sociocultural differences remains unknown (37). In subjects with obesity or prediabetes, where an effect on glucose metabolism presumably would be more easily detected, the result is even more uncertain (17,18,38). However, it should be emphasized that in the study by von Hurst et al. (17), where subjects had a very low median baseline serum 25(OH)D concentration of 21 nmol/L, there was a highly significant improvement in insulin sensitivity after vitamin D supplementation.

Recently, in a study of 117 subjects with prediabetes with mean baseline serum 25(OH)D levels of 55 nmol/L, Davidson et al. (39) found no effect on insulin secretion, insulin resistance, or measures of glycemia after 1 year of high-dose vitamin D supplementation (mean dose 88,865 IU/week) compared with placebo, and in a study of 45 subjects with prediabetes, vitamin D supplementation had no effect on insulin sensitivity (40). Of note, the same was seen in a subanalysis of the 28 subjects who had baseline serum 25(OH)D $<$50 nmol/L (39). The present results are very similar, wherein at baseline, the subjects had a mean serum 25(OH)D of 60.5 nmol/L. The increase in serum 25(OH)D in those given vitamin D was substantial (45.8 nmol/L), but no significant effects or trends were seen on any measure of insulin or glucose metabolism. Because our hypothesis was that vitamin D would have a positive effect and that this obviously would be most easily detected in those with vitamin D deficiency, we also analyzed separately those with baseline serum 25(OH)D $<$50 nmol/L (n = 170) and those with baseline serum 25(OH)D $<$40 nmol/L (n = 72). We found no significant effects of the vitamin D supplementation or any S-shaped curves for the responses.

Although the current study is intended to last for 5 years with the main end point being the development of type 2 diabetes, it is also noteworthy that in this 1-year analysis the vitamin D and placebo groups were almost identical regarding change in glycemic status. These findings are also similar to those in Davidson et al. (39), but because type 2 diabetes develops slowly and small effects on glucose metabolism might accumulate over time, a 1-year intervention might be too short to draw firm conclusions. In line with this, Afzal et al. (41) performed a Mendelian randomization study in 96,423 subjects where genetic variation in the DHCR7 gene was associated with risk of type 2 diabetes; thus, as pointed out in a commentary by Pilz et al. (42), lifelong endogenous vitamin D is possibly needed for the prevention of diabetes.

Prediabetes as well as type 2 diabetes are associated with hypertension (15), an unfavorable lipid profile (14,15), and increased markers of inflammation (35). These cardiovascular disease risk factors are also associated with vitamin D deficiency (15,34,35) and may, from a therapeutic point of view, be more important than hyperglycemia. For blood pressure and the cardiovascular system, an effect of vitamin D could be expected. VDRs are present in vascular smooth muscle, endothelium, and cardiomyocytes (28), and vitamin D appears to inhibit the synthesis of renin in juxtaglomerular cells (43) and may thereby reduce blood pressure. As anticipated, cross-sectional studies show an inverse association between serum 25(OH)D concentrations and blood pressure, and prospective studies have found the same (44). However, vitamin D supplementation in most, but not all, studies has been ineffective in reducing blood pressure (44,45). This may be due to inclusion of subjects with fairly normal blood pressure and adequate vitamin D status. Thus, in a study of 130 hypertensive patients given 3,000 IU vitamin D over 20 weeks, a significant reduction in 24-h ambulatory blood pressure of 4 mmHg versus those given placebo was only seen when vitamin D–insufficient individuals were analyzed separately (46). In the present study, however, we found no effect of vitamin D supplementation on blood pressure in all subjects together (n = 466), only those with vitamin D deficiency (n = 161), only those with hypertension (n = 211), and only those with both hypertension and vitamin D deficiency (n = 63).

Similar to blood pressure, observational studies indicate that low 25(OH)D concentrations are associated with an unfavorable lipid profile, whereas RCTs have not been able to show a positive effect by vitamin D supplementation (14). However, vitamin D combined with calcium appears to lower LDL cholesterol (47). In the present study, there was a significant reduction in TC and LDL cholesterol in the vitamin D group compared with the placebo group, but because there also was a reduction in HDL cholesterol, the benefit is questionable. Furthermore, the effect was marginal, and if correction for testing multiple lipid parameters had been performed, it would not have been statistically significant. Also similar to blood pressure, subgroup analyses based on baseline serum 25(OH)D and lipid status did not reveal effects not seen in the total cohort. Finally, we observed no effect of vitamin D supplementation on the inflammation marker hs-CRP in the total cohort or in subgroups.

One obvious short-coming of the current study is that the power calculation was made regarding prevention of type 2 diabetes during a 5-year intervention, and over time, even small changes may accumulate and end up being of clinical significance. However, if doing a post hoc power analysis and assuming that the differences found in this cohort at baseline between those with low versus high serum 25(OH)D concentrations ($\sim$0.2% for HbA_{1c}, $\sim$0.15 mmol/L for

HDL cholesterol, and ~1 mmHg for systolic blood pressure) are correct and are what can be maximally improved by vitamin D supplementation in subjects with prediabetes and vitamin D deficiency, one would need to include, respectively, 120, 160, and >2,000 subjects with serum 25(OH)D <50 nmol/L in RCTs for a power of 0.8 and a significance level of 0.05. Accordingly, we had the power to determine such effects for HbA_{1c} and HDL cholesterol levels but not for blood pressure. However, it is highly unlikely that these baseline differences were only a result of the 25(OH)D concentrations, and what could realistically be achieved was probably much less. These small differences at baseline also illustrate that vitamin D probably is not a major determinant for glucose metabolism, blood pressure, and lipid levels in subjects with prediabetes. Another shortcoming is that the present trial is based on the pharmaceutical drug model, and subjects had a mean 25(OH)D concentration of 60.5 nmol/L. If instead the nutrient response curve described by Heaney (48) was taken into account, another conclusion might have been made. Only nine subjects in the present trial had very low 25(OH)D concentrations (<25 nmol/L) at baseline thus far, which is too few for adequate power to detect the predicted changes in glycemia at 1 year. Furthermore, it should be considered that early supplementation, before marked dysglycemia has developed, may have a better effect. At present, several large trials of vitamin D supplementation are looking at diverse health outcomes, but unfortunately, vitamin D deficiency has not been an inclusion criterion in these studies. In light of evidence suggesting a measurable benefit only in subjects with low vitamin D concentrations, at least regarding muscle function (49), these trials may not be able to finally settle the question of the need for vitamin D supplementation (50).

Despite these shortcomings, the present study has considerable strength and importance because it is, to our knowledge, the largest RCT by far on vitamin D supplementation in subjects with prediabetes. The size of this study has enabled us to complete subanalyses of various combinations of baseline risk factors.

In conclusion, there is no doubt that vitamin D is of vital importance for skeletal health (12). However, for glucose metabolism, blood pressure, and lipid levels, it is very difficult to show a beneficial effect of vitamin D supplementation, even in those with fairly low serum 25(OH)D concentrations. This does not mean that vitamin D is without importance for glucose regulation and cardiovascular health, but it does mean that the conventional definition of vitamin D deficiency as serum 25(OH)D <50 nmol/L in this regard is not relevant.

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