No Effect of High-Dose Vitamin D Treatment on β-Cell Function, Insulin Sensitivity, or Glucose Homeostasis in Subjects With Abnormal Glucose Tolerance: A Randomized Clinical Trial

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OBJECTIVE

There has been conflicting evidence regarding the potential role of vitamin D in glucose homeostasis. This study was designed to investigate the effect of high-dose vitamin D3 treatment on β-cell function, insulin sensitivity, and glucose tolerance in subjects with prediabetes or diet-treated type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects (*n* = 44) were randomized to 30,000 IU vitamin D3 once weekly or placebo for 8 weeks. Hyperglycemic clamp assessed first-phase (0–12 min) and second-phase (12–120 min) insulin response, insulin sensitivity, and disposition index (DI). An oral glucose tolerance test assessed glucose tolerance and glycosylated hemoglobin assessed glycemic control.

RESULTS

A total of 21 (vitamin D) and 22 (placebo) subjects completed the study, respectively. Season-adjusted 25-OH-vitamin D [25(OH)D] levels were doubled in the active treated group (43–82 nmol/L). No effect of vitamin D treatment, compared with placebo, was seen on first-phase or second-phase insulin secretion. There were no group differences in insulin sensitivity, DI, or any measures of glycemic control. No hypercalcemia or other adverse effects of vitamin D treatment were seen compared with placebo. Subgroup analyses of those with the lowest basal and greatest increase in 25(OH)D levels did not change these results.

CONCLUSIONS

This study gives no support for any substantial effect of high-dose vitamin D treatment for 8 weeks in prediabetes or diet-treated type 2 diabetes on β-cell function, insulin sensitivity, or glycemic control.

Evidence suggesting that vitamin D has an important role in glucose homeostasis has accumulated during the last three decades. A meta-analysis concluded that observational studies have demonstrated a relatively consistent association between low vitamin D serum levels and prevalence of type 2 diabetes or metabolic syndrome (1). Moreover, cohort studies have shown an inverse association between baseline

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serum 25-OH-vitamin D [25(OH)D] and prospective risk of type 2 diabetes, hyperglycemia, and insulin resistance (2,3). We demonstrated in a Swedish cohort that low 25(OH)D levels were predicting type 2 diabetes risk over 10 years in subjects with prediabetes, but not with normal glucose tolerance at baseline (4). Recently, a meta-analysis confirmed this association (5).

These epidemiological data have evoked human studies investigating if intervention with vitamin D could improve β-cell function and/or insulin sensitivity. As to β-cell function, some studies have shown beneficial effects (6–9), whereas some have not (10–13). Only two studies used a more sophisticated method for assessment (intravenous glucose tolerance test) (8,10). Regarding insulin sensitivity, a few studies have reported an improvement in insulin sensitivity (13,14), but several did not (8,12,15–19).

To summarize, there has been conflicting evidence of the potential role of vitamin D in this field. We therefore performed a randomized, placebo-controlled trial to assess the effect of 8 weeks of high-dose vitamin D treatment, in people with prediabetes or diet-treated type 2 diabetes, on β-cell function, insulin sensitivity, and glucose tolerance. The gold standard hyperglycemic clamp method (20) was used to assess β-cell function and insulin sensitivity.

RESEARCH DESIGN AND METHODS

Trial Design

The trial was a randomized 1:1, parallel-group, double-blind, placebo-controlled study with an 8-week intervention period. Two study centers in Stockholm were used. The Regional Human Ethics Review Board in Stockholm approved the study protocol.

The study design included a screening visit (−1 to 3 weeks), a randomization visit (0 weeks), a half-time visit (+4 weeks), and two study-end visits (+8 weeks and +8 weeks + 1–3 days). In between these visits, telephone contacts were performed weekly to assess safety and optimize compliance.

Participants

Subjects were recruited from the Stockholm Diabetes Prevention Program (SDPP), a prospective cohort study in Stockholm (21). Subjects who at follow-up in the years 2003–2006 were categorized as having prediabetes, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or IFG and IGT according to an oral glucose tolerance test (OGTT) were contacted by phone. Those who were thought to meet eligibility criteria were invited to a screening visit. Written informed consent was obtained from all participants at the screening visit. They were instructed to maintain their diet and exercise habits during the study period.

Inclusion criteria were as follows: *1*) IFG, IGT, IFG + IGT, or drug-naïve diabetes at the screening OGTT (IFG = fasting plasma glucose [p-glucose] 6.1–6.9 mmol/L; IGT = 2-h p-glucose 7.8–11.0 mmol/L; diabetes = fasting p-glucose ≥7.0 mmol/L and/or 2-h p-glucose ≥11.1 mmol/L) (22); *2*) age ≥45 and ≤75 years, female or male; *3*) BMI ≤32 kg/m^2; *4*) glycosylated hemoglobin (HbA_{1c}) ≤7.9% (63 mmol/mol); *5*) fasting p-glucose <9 mmol/L; *6*) serum 25(OH)D <75 nmol/L (below normal lab reference); and *7*) able and willing to perform tests and examinations specified in the protocol.

Exclusion criteria included the following: *1*) antidiabetic medication of any kind; *2*) anticipated change of concomitant medication that may interfere with glucose metabolism, such as systemic corticosteroids, nonselective β-blockers, monoamine oxidase inhibitors, and anabolic steroids; *3*) treatment with any vitamin D preparation; *4*) regular sunbathing in solarium; *5*) hypercalcemia at screening, defined as ionized s-calcium >1.35 mmol/L; *6*) hyperphosphatemia at screening, defined as s-phosphate >1.5 mmol/L; *7*) sarcoidosis or other granulomatous disease; *8*) treatment with phenytoin, barbiturates, rifampicin, isoniazid, cardiac glycosides, orlistat, or colestyramin (known to interfere with vitamin D metabolism); *9*) impaired hepatic function, defined as alanine aminotransferase (ALT) three or more times the upper reference limit; *10*) impaired renal function, defined as s-creatinine >133 μmol/L for males and >115 μmol/L for females; *11*) cardiac disease, defined as *a*) unstable angina pectoris, *b*) myocardial infarction within the last 6 months, or *c*) congestive heart failure New York Heart Association class III and IV; *12*) cerebral stroke within the last 6 months; *13*) uncontrolled treated/untreated hypertension (systolic blood pressure ≥180 mmHg and/or diastolic blood pressure ≥110 mmHg); *14*) cancer (except basal cell skin cancer or squamous cell skin cancer); *15*) females of childbearing potential who were pregnant, breast-feeding, or intended to become pregnant or were not using adequate contraceptive methods; *16*) known or suspected abuse of alcohol or narcotics; or *17*) mental incapacity, unwillingness, or language barrier precluding adequate understanding or cooperation.

Interventions

Study participants were randomly assigned to receive *1*) vitamin D3 (cholecalciferol, Vigantol Oil), 30,000 IU (1.5 mL/45 drops) given orally once weekly or *2*) matching placebo oil (1.5 mL/45 drops) given orally once weekly. Merck KGaA (Darmstadt, Germany) manufactured vitamin D3 and matching placebo.

The first dose was given at the randomization visit, after study assessments. The fifth dose was given at the half-time visit, and the subject at home took the remaining six doses. Study drug bottles were brought to the study center at the half-time visit and at the last visit by each participant to measure remaining study drug for compliance assessment.

Outcomes

The primary end point was to test whether there was a difference in relative change in first-phase serum insulin secretion (0–12 min; $ISec_{0–12}$) at the hyperglycemic clamp investigation at study end compared with baseline between the two groups.

Secondary end points were included to test whether there was a difference in relative change in second-phase insulin secretion (12–120 min; $ISec_{12–120}$), insulin sensitivity, and disposition index (DI) during the hyperglycemic clamp, change in glucose tolerance assessed by an OGTT, change in fasting p-glucose and HbA_{1c}, and change in blood lipids between the two groups. Secondary end points also included assessing change in 25(OH)D serum levels and safety end points as incidence of hypercalcemia and adverse events (AEs).

Assessments

All assessments were performed at the randomization visit and at the study-end visits if not stated otherwise.

OGTT

A sample for fasting p-glucose was obtained (0 min). Glucose (75 g) in water was then given orally. P-glucose was obtained at 30 and 120 min after the glucose administration. The baseline investigation was performed at the screening visit and the second investigation at the first of the two study-end visits.

Hyperglycemic Clamp

Glucose 200 mg/mL was infused intravenously at a variable rate to increase p-glucose to a target value of 6.9 mmol/L above the fasting glucose value during 120 min. For the first 14 min, the infusion rate was given in a stepwise manner and based on estimated body surface. From minute 14 until the end of the investigation, the infusion rate was based on preset computer calculations as described previously (20). Arterialized blood samples for glucose and insulin measurement were obtained from a retrograde inserted venous injection needle on the dorsal side of the heated hand and taken every 2 min from minute 0 to 14. Thereafter, glucose was measured every 5 min and samples for insulin measurement were obtained every 10 min until the end of the investigation. Total volume of infused glucose was recorded from the drop counter every 5–10 min to calculate glucose infusion rate (GIR). Urine was collected during the investigation for measurement of urinary glucose excretion. The second investigation was performed at the second of the two study-end visits.

Laboratory

Glucose was measured by a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH) and insulin by an in-house radioimmunoassay method with inter- and intra-assay coefficient of variation of 11.5–16.9% and 5.8–8.4%, respectively. HbA_{1c} was analyzed by high-performance liquid chromatography (Bio-Rad), 25(OH)D by competitive chemical immunoluminescence (LIAISON, intra- and interassay coefficient of variation of 5 and 8–11%, respectively), blood lipids and urine glucose by enzymatic reaction (Beckman Coulter), apolipoproteins (ApoB and ApoA1) by immunochemistry (Beckman Coulter), and free calcium by potentiometry (ABL800 FLEX). The baseline biochemistry was performed at the screening visit.

Other Assessments

A Tanita Body Composition Analyzer (Tokyo, Japan) was used to measure weight and bioimpedance, estimating total body fat and fat-free mass. Step count was measured by a regular step counter during the 7 days preceding the randomization visit and study end visits.

Safety

AEs were collected and recorded at all contacts with the subjects after the first dose of study medication until the last telephone contact. A clinical examination was performed at the randomization and study-end visits. Any difference from baseline was recorded. All biochemistry needed for assessment of eligibility was remeasured at study end. Aside from these, a blood count was performed at the screening visit and at study end. Heart rate and blood pressure were obtained at the half-time visit, as well as a blood sample for free serum calcium.

Calculations

$ISec_{0-12}$ was calculated as area under the curve of serum insulin during minutes 0–12 of the hyperglycemic clamp by the trapezoidal rule and was adjusted for the basal insulin level. The result was adjusted for relative random fluctuation in glucose increase minutes 0–12, calculated as area under the curve in p-glucose above the basal level, between the baseline and study-end investigations (23). $ISec_{12-120}$ was calculated accordingly during minutes 12–120 but was not adjusted for glucose stimuli as the glucose levels were very stable during this period. The basal insulin level was calculated as the mean of insulin measurements at minute −10 and minute 0. An investigator, blinded to the treatment allocation, evaluated the fluctuation of these two values and decided if a third measurement obtained at minute −15 should be incorporated in the mean calculation. Insulin sensitivity (GIR/mean insulin level [I]) was calculated during the last 30 min of the clamp investigation as (GIR – urinary glucose excretion rate)/fat-free mass/mean insulin concentration × 100. This measure of insulin sensitivity has been shown to be highly correlated (r = 0.84–0.86) to results obtained by the hyperinsulinemic-euglycemic clamp (20,24). The DI, a measure of β-cell function in relation to insulin sensitivity, was calculated as ISec × GIR/I during minutes 0–12 and 12–120, respectively. 25(OH)D levels were season-adjusted as described previously in the SDPP cohort (4). In short, the period of the year for the measurement was divided in four quarters: November–January (Q1), February–April (Q2), May–July (Q3), and August–October (Q4). Q1 was set at reference (no correction) and a correction value in nmol/L was added or subtracted to the measurements value according to Q2 + 6.7, Q3 – 1.9, and Q4 – 13.7. The adjusted values are given throughout the article.

Sample Size

The current study is considered as a pilot study. No similar study was found that could provide data for a formal power calculation.

Randomization

A simple random sampling was performed, creating a list including numbers 1–44 and active/placebo treatment in a 1:1 ratio. An independent statistician created the list by software STATISTICA version 10 (StatSoft, 2011). The study drug and matching placebo were marked with the randomization numbers on identical bottles accordingly and independently by the study pharmacy (APL). Study subjects were allocated a randomization number consecutively as they arrived to the randomization visit (e.g., the first subject was given randomization number 1, etc.). They were then provided the corresponding marked study medication. No blocking or stratification was performed.

Blinding

Study participants, study personnel, and investigators were blinded to the allocated study intervention. The randomization list was kept in a locked cabinet, only accessible by a study nurse not involved in the study. All data collection and its recording in the database were made before the randomization code was broken. Vitamin D3 and matching placebo oil were delivered in identical dark bottles directly to the study pharmacy.

Statistical Methods

Due to the relatively small sample size, data are presented as medians (interquartile range). The Mann-Whitney U test was used to assess continuous variables between groups. Relative changes were used to adjust for baseline differences. The two-tailed Fisher exact test was used for categorical variables. The Wilcoxon matched pairs signed

rank test was used to test within-group effects. For additional analyses, correlations were calculated by the nonparametric Spearman rank correlation test. A P value <0.05 was regarded as significant. STATISTICA version 10 was used for the analyses.

RESULTS

Participant Flow

Out of 68 subjects screened for eligibility, 23 did not meet inclusion/exclusion criteria and 1 subject declined to participate (Fig. 1). A total of 44 subjects were randomized, all of white ethnicity. One subject in the vitamin D group was excluded per protocol from the study due to initiation of oral corticosteroid treatment for a relapse in her ulcerous colitis disease. Thus, 43 subjects were available for the intent-to-treat analysis. The total study period was from February 2012 to May 2013.

Baseline Data

Baseline characteristics are shown in Table 1. Overall the study population consisted of 47% females and had a median age of 67.3 years. BMI was 28.5 kg/m^2 and waist circumference 101 cm. Hypertension was treated in 58% of the study population and dyslipidemia in 35%. HbA_{1c} was similar in the two groups: vitamin D group 6.2% (6.0–6.4) (43 mmol/mol [38–44]) and placebo group 6.1% (5.6–6.2) (44 mmol/mol [42–46]). Season-adjusted 25(OH)D levels were also similar in the two groups: 43 nmol/L (36–50) and 43 nmol/L (37–54), respectively, in the vitamin D and placebo groups. Twelve subjects were categorized as having diabetes, of which five and seven individuals were randomized to vitamin D and placebo, respectively. Comparative baseline data for these subjects are presented in Supplementary Table 3. When assessing men and women separately, there were no significant group differences at baseline in these variables, except a slightly higher 25(OH)D level among men in the placebo group (data not shown).

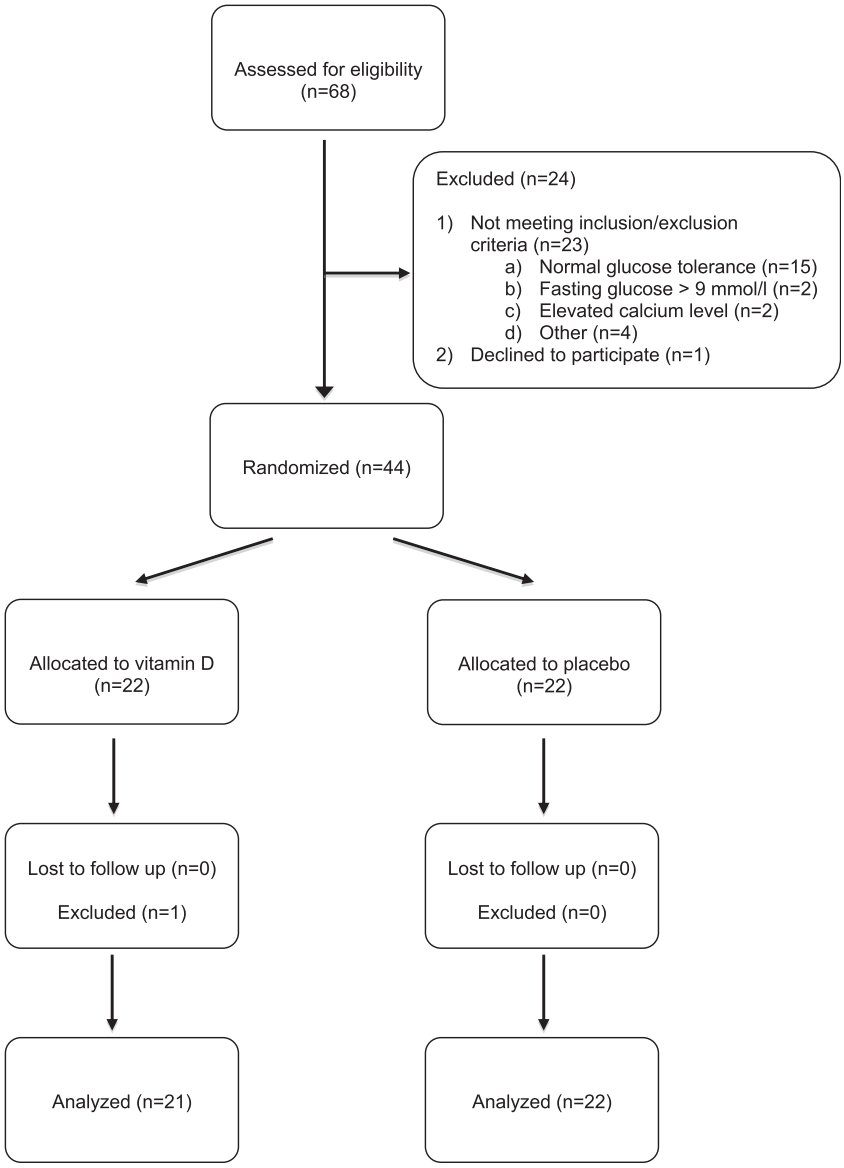

Figure 1—Flowchart. One subject was excluded in the vitamin D group due to initiation of oral corticosteroid treatment.

Outcomes

Effects of vitamin D and placebo are presented in Table 2. The 25(OH)D level was doubled in the vitamin D group (+42 nmol/L [32–50]) and remained unchanged in the placebo group (0 nmol/L [−7 to 11]).

As to the primary end point $ISec_{0–12}$, there was a tendency to an increase in the vitamin D group (+44 mU · L^{-1} · min [−3 to 63]) and a significant increase in the placebo group (+45 mU · L^{-1} · min [−5 to 136]), with no difference between the two groups (P = 0.45). No changes in $ISec_{12–120}$ or insulin sensitivity (GIR/I) within or between the groups were seen. First-phase DI ($DI_{0–12}$) increased in both groups, with no group difference in concordance with $ISec_{0–12}$. Second-phase DI ($DI_{12–120}$) showed a tendency to increase in the vitamin D group (+4,608 mg · kg^{-1} · 100 [−2,345 to 12,360]; P = 0.06), but there was no statistical difference toward placebo (P = 0.95).

Assessments of body composition did not alter except a small increase in percent fat mass in the vitamin D group, accompanied by a similar decrease in fat-free mass, these changes being significant in group comparison. There was a tendency toward a small reduction in median HbA_{1c} in the vitamin D group of 0.1% (−0.3 to 0.1) (1 mmol/mol [−3–1]) (P = 0.06), but with no significant difference versus placebo (P = 0.84). No other measurements of glycemia showed any changes. Glycemic tolerance category improved slightly in both groups, with no difference between the groups. There was a decrease of parathyroid hormone (PTH) in the vitamin D group

Table 1—Baseline characteristics

	Overall	Vitamin D	Placebo
n (females/males)	43 (20/23)	21 (9/12)	22 (11/11)
Age (years)	67.3 (64.0–68.5)	67.6 (63.4–68.8)	67.0 (64.7–68.5)
n glycemic tolerance category at OGTT (IFG/IGT/IFG + IGT/diabetes)	10/10/11/12	7/5/4/5	3/5/7/7
n treated for hypertension/dyslipidemia	25/15	12/5	13/10
BMI (kg/m^2)	28.5 (25.7–29.8)	28.3 (24.5–29.4)	28.6 (26.4–29.9)
Waist (cm)	101 (95–107)	100 (94–106)	101 (95–112)
Fat mass (%)	30.5 (24.4–40.8)	30.0 (22.4–41.1)	33.0 (29.2–37.0)
Fat-free mass (kg)	55.8 (44.1–68.4)	58.6 (45.8–68.7)	54.5 (44.0–67.3)
HbA_{1c} (%) (mmol/mol)	6.2 (5.9–6.3) 44 (41–45)	6.1 (5.6–6.2) 43 (38–44)	6.2 (6.0–6.4) 44 (42–46)
Fasting p-glucose (mmol/L)	6.3 (6.0–6.6)	6.3 (5.5–6.6)	6.3 (6.1–6.6)
2-h p-glucose OGTT (mmol/L)	9.4 (7.5–10.3)	9.3 (7.1–9.7)	9.7 (8.2–10.3)
P-glucose OGTT* (mmol · L^{-1} · min)	387 (281–442)	386 (237–440)	410 (309–442)
25(OH)D (nmol/L)	47 (36–55)	42 (35–55)	47 (42–53)
25(OH)D (nmol/L) adjusted†	43 (36–54)	43 (36–50)	43 (37–54)
Free calcium mmol/L	1.22 (1.20–1.25)	1.22 (1.19–1.25)	1.23 (1.21–1.24)
PTH (ng/L)	59 (46–67)	62 (50–72)	55 (42–63)
Triglycerides (mmol/L)	1.3 (0.9–1.8)	1.3 (0.9–1.6)	1.4 (1.0–2.2)
Cholesterol (mmol/L)	5.4 (4.5–5.7)	5.0 (4.4–5.7)	5.5 (5.0–5.7)
LDL (mmol/L)	3.3 (2.7–3.8)	3.0 (2.7–3.8)	3.5 (3.1–3.8)
HDL (mmol/L)	1.2 (1.1–1.6)	1.3 (1.1–1.6)	1.2 (1.0–1.6)
ApoB/ApoA1	0.6 (0.5–0.8)	0.6 (0.5–0.7)	0.7 (0.5–0.8)
Step count/day	6,300 (4,359–8,898)	5,645 (4,277–8,316)	7,099 (4,414–9,376)
$ISec_{0–12}$ (mU · L^{-1} · min)	168 (84–319)	168 (122–231)	137 (51–363)
$ISec_{12–120}$ (mU · L^{-1} · min)	4,861 (2,865–7,204)	5,689 (2,865–8,118)	4,509 (3,000–6,508)
GIR/I (mg · min^{-1} · kg^{-1} · mU^{-1} · L · 100)	6.6 (4.6–11.3)	6.7 (4.7–11.4)	5.9 (4.6–9.7)
$DI_{0–12}$ (mg · kg^{-1} · 100)	1,303 (650–2,252)	1,449 (986–1,922)	1,113 (389–2,324)
$DI_{12–120}$ (mg · kg^{-1} · 100)	33,150 (23,528–42,144)	34,464 (31,500–42,144)	29,157 (22,429–40,285)

Continuous data are medians (interquartile range). *Area under the curve. †Season adjusted.

(−8 ng/L [−18 to −2]), showing a tendency in group comparison (P = 0.07). Triglycerides decreased nearly significantly in the placebo group, a change being significant versus the vitamin D–treated group (P = 0.02). Cholesterol and LDL decreased slightly in both groups, with no difference between the groups. Step count did not change during the study.

Ancillary Analyses

There was a tendency to a negative correlation between basal 25(OH)D levels and change in 25(OH)D levels in the active treated group (r = −0.38, P = 0.09) (Fig. 2*A*). To explore if subjects with the lowest basal 25(OH)D levels were those who might benefit from treatment, a correlation of change in 25(OH)D levels and fold change in $ISec_{0–12}$ was performed. No such association was seen (r = −0.22, P = 0.34) (Fig. 2*B*) and using fold change in 25(OH)D levels gave a similar result (data not shown). Further analyses in the vitamin D group, where the subjects were divided in two groups according to their basal 25(OH)D levels (<50 nmol/L or ≥50 nmol/L), showed no significant effect on fold change $ISec_{0–12}$ (P = 0.74) (Fig. 2*C*). To evaluate if 25(OH)D levels at study end had an impact, a correlation of final 25(OH)D levels and fold change $ISec_{0–12}$ was performed in the vitamin D group, which did not show any association (Fig. 2*D*). No other outcomes were correlated to delta values of 25(OH)D level in the active treated group (data not shown). Further, there were no associations between sex and any of the outcomes, and omitting subjects with diabetes according to the baseline OGTT did not alter the results (data not shown).

Compliance and Harms

Measure of retrieved study drug did not show any significant deviation in any subject. No events of hypercalcemia occurred in the study. There were 26 recorded AEs in 20 subjects, 10 in the vitamin D group and 16 in the placebo group (Supplementary Table 4). Intensity was graded as "severe" in two AEs, both fractures of the radius (one vitamin D and one placebo group). Otherwise intensity was estimated as mild to moderate. One AE had a probable causality to study medication, diarrhea after medication intake (placebo group). Otherwise causality was judged as unlikely for all AEs. No serious AEs occurred.

CONCLUSIONS

The current study showed no effect of high-dose vitamin D3 treatment during 8 weeks in comparison with placebo, in subjects with mild abnormal glucose regulation, on $ISec_{0–12}$ measured by hyperglycemic clamp. Nor could we see any effects on $ISec_{12–120}$, insulin

Table 2—Effects of intervention

	Vitamin D			Placebo			Between-group effect
	Baseline	Δ study end	*P**	Baseline	Δ study end	*P**	*P*†
BMI (kg/m^2)	28.3 (24.5–29.4)	−0.1 (−0.4 to 0.4)	0.63	28.6 (26.4–29.9)	0.0 (−0.1 to 0.3)	0.28	0.39
Waist (cm)	100 (94–106)	0 (−1 to 2)	0.92	101 (95–112)	0 (−1 to 1)	0.44	0.88
Fat mass (%)	30.0 (22.4–41.1)	+0.6 (−0.3 to 1.9)	0.03	33.0 (29.2–37.0)	−0.4 (−0.9 to 0.6)	0.49	0.047
Fat-free mass (kg)	58.6 (45.8–68.7)	−0.6 (−1.4 to 0.2)	0.03	54.5 (44.0–67.3)	+0.3 (−0.8 to 1.2)	0.28	0.02
HbA_{1c} (%) (mmol/mol)	6.1 (5.6–6.2) 43 (38–44)	−0.1 (−0.3 to 0.1) −1 (−3 to 1)	0.06	6.2 (6.0–6.4) 44 (42–46)	−0.1 (−0.3 to 0.1) −1 (−3 to 1)	0.11	0.84
Fasting p-glucose (mmol/L)	6.3 (5.5–6.6)	−0.1 (−0.5 to 0.4)	0.58	6.3 (6.1–6.6)	0.0 (−0.3 to 0.4)	0.94	0.78
2-h p-glucose OGTT (mmol/L)	9.3 (7.1–9.7)	−0.5 (−1.4 to 1.0)	0.96	9.7 (8.2–10.3)	−0.9 (−1.5 to 1.8)	0.95	0.62
P-glucose OGTT‡ (mmol · L^{-1} · min)	386 (237–440)	−36 (−125 to 132)	0.88	410 (309–442)	−9 (−131 to 81)	0.43	0.69
Glycemic tolerance category (better/worse/unchanged)		6/4/11			5/2/15		0.74§
25(OH)D (nmol/L)	42 (35–55)	+41 (27–50)	<0.001	47 (42–53)	−1 (−3 to 5)	0.90	<0.001
25(OH)D (nmol/L) adjusted#	43 (36–50)	+42 (32–50)	<0.001	43 (37–54)	0 (−7 to 11)	0.53	<0.001
Free calcium (mmol/L)	1.22 (1.19–1.25)	0.00 (−0.01 to 0.01)	0.52	1.23 (1.21–1.24)	−0.01 (−0.02 to 0.00)	0.047	0.10
PTH (ng/L)	62 (50–72)	−8 (−18 to −2)	0.001	55 (42–63)	−3 (−8 to 0)	0.25	0.07
Triglycerides (mmol/L)	1.3 (0.9–1.6)	+0.1 (0.0–0.2)	0.16	1.4 (1.0–2.2)	−0.2 (−0.5 to 0.1)	0.06	0.02
Cholesterol (mmol/L)	5.0 (4.4–5.7)	−0.1 (−0.5 to 0.1)	0.07	5.5 (5.0–5.7)	−0.4 (−0.8 to 0.0)	0.004	0.14
LDL (mmol/L)	3.0 (2.7–3.8)	−0.1 (−1.0 to 0.5)	0.04	3.5 (3.1–3.8)	−0.2 (−1.4 to 0.7)	0.005	0.74
HDL (mmol/L)	1.3 (1.1–1.6)	0.0 (−0.1 to 0.1)	0.44	1.2 (1.0–1.6)	−0.1 (−0.2 to 0.1)	0.34	0.48
ApoB/ApoA1	0.6 (0.5–0.7)	0.0 (0.0–0.1)	0.27	0.7 (0.5–0.8)	0.0 (0.0–0.1)	0.07	0.69
Step count/day	5,645 (4,277–8,316)	−783 (−1,535 to 640)	0.18	7,099 (4,414–9,376)	−572 (−2,832 to 1,441)	0.37	0.91
$ISec_{0–12}$ (mU · L^{-1} · min)	168 (122–231)	+44 (−3 to 63)	0.06	137 (51–363)	+45 (−5 to 136)	0.02	0.45
$ISec_{12–120}$ (mU · L^{-1} · min)	5,689 (2,865–8,118)	+547 (−1,053 to 960)	0.79	4,509 (3,000–6,508)	+56 (−934 to 1,006)	0.51	0.84
GIR/I (mg · min^{-1} · kg^{-1} · mU^{-1} · L · 100)	6.7 (4.7–11.4)	+0.3 (−0.3 to 1.4)	0.09	5.9 (4.6–9.7)	+0.5 (−0.5 to 1.8)	0.25	0.59
$DI_{0–12}$ (mg · kg^{-1} · 100)	1,449 (986–1,922)	+512 (64–1,082)	0.005	1,113 (389–2,324)	+260 (−7 to 963)	0.006	0.66
$DI_{12–120}$ (mg · kg^{-1} · 100)	34,464 (31,500–42,144)	+4,608 (−2,345 to 12,360)	0.06	29,157 (22,429–40,285)	+4,210 (−4,457–12,524)	0.21	0.95

Continuous data are medians (interquartile range). *Wilcoxon matched pairs signed rank test. †Mann-Whitney *U* test on relative changes. ‡Area under the curve. §Fisher exact test. #Season adjusted.

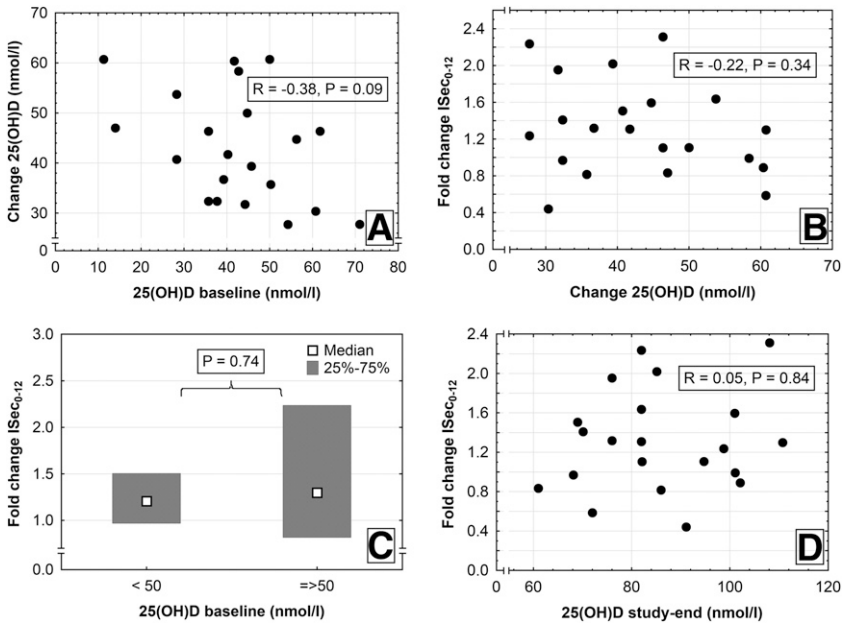

Figure 2—*A–D*: Ancillary analyses in the vitamin D group only. *R* and *P* values for correlations were calculated by Spearman rank correlation test. Mann-Whitney *U* test was used for group comparison (*C*).

sensitivity, or DI or in different measures of glycemic control.

The small changes in HbA_{1c} and blood lipids could be due to a study participation effect, since improvements mostly were seen in both intervention groups. The high-dose vitamin D treatment was well tolerated, with no increased risk of harm compared with the placebo.

Our findings are in accordance with some recent studies. Davidson et al. (25) investigated, in subjects with prediabetes and mean 25(OH)D level of 54 nmol/L, if a very high vitamin D3 dose (88,865 IU/week) during 1 year affected glucose tolerance or insulin secretion and insulin sensitivity assessed by several OGTT-based indices. In 109 randomized subjects, no effects in comparison with placebo were found, except a small decrease in HbA_{1c} of 0.2% (2 mmol/mol) in the vitamin D group (25). Further, 1-year data from an ongoing 5-year trial has been published, where subjects with prediabetes are treated with vitamin D3 20,000 IU/week or placebo. In 484 analyzed participants, an increase in 25(OH)D levels from 60 to 106 nmol/L in the active treated group did not have any effect on glucose metabolism or insulin sensitivity, assessed by HOMA-IR and QUICKI (26). Subgroup analyses of subjects with lower baseline 25(OH)D levels did not change these results. The above two studies used fasting measurements or OGTT-based methods of assessing insulin secretion and insulin sensitivity, in comparison with the clamp technique in our study. Finally, intervention with 50,000 IU vitamin D3/month did not improve glycemia in mild type 2 diabetes after 6 months, nor in subjects with baseline 25(OH)D levels <50 nmol/L (27).

As opposed to these studies, Mitri et al. (28) found that treatment with 2,000 IU vitamin D3/day for 16 weeks improved $ISec_{0-12}$ and DI in comparison with placebo. The method for assessment was a frequently sampled intravenous glucose tolerance test and minimal model analysis, used in 88 subjects with glucose intolerance or early diabetes (28). This study also investigated calcium supplementation (800 mg/day), which did not affect the outcomes.

When exploring the data if subjects who increased their 25(OH)D level the most might have benefitted from treatment, we could not see any evidence for that notion. No correlations were seen between the outcomes and increase in 25(OH)D level in the vitamin D group. In addition, the baseline level of 25(OH)D did not seem to have an impact. Further, if there exists a threshold effect of 25(OH)D level on glycemic effects, only subjects who exceed this threshold would show any change. Sorkin et al. (29) performed a cross-sectional study supporting this theory. In 239 overweight postmenopausal women, a threshold effect was seen for glucose tolerance and insulin resistance (HOMA-IR) at a 25(OH)D level of 65 nmol/L (29). However, when we correlated $ISec_{0-12}$ to 25(OH)D levels at study end in our study, we could not see any association. All but one subject in the vitamin D group had a basal 25(OH)D level <65 nmol/L, and all but one subject increased their level to >65 nmol at study end. Therefore, we should have been able to detect a threshold around 65 nmol/L.

The current study sample was rather small, which could have caused a problem of power. When planning this study, no previous study was found to constitute a base for a power calculation. A post hoc power analysis showed a potential to detect a relative difference in $ISec_{0-12}$ of 65%, with 80% power and $\alpha = 0.05$. This can be compared with a relative difference of 80% in first-phase C-peptide secretion between normal glucose tolerance and IFG/IGT in a recent study (30). We were able to use gold standard technique for estimation of insulin secretion and insulin sensitivity (hyperglycemic clamp), which to our knowledge has not been used before in this field of interest. The intervention period of 8 weeks could also be considered as rather short to fully explore the effect of long-time exposure of vitamin D. On the other hand, subjects lost to follow-up were minimal and change in lifestyle factors affecting glucose metabolism could be kept low. A decrease in PTH was seen in the vitamin D group, which could be regarded as a proxy of a metabolic effect of the treatment. The results cannot be generalized to, for example, young individuals or subjects with pronounced obesity.

A strength is also that we adjusted for seasonal changes in serum 25(OH)D levels. When unadjusted 25(OH)D levels first were used, it seemed like subjects with the highest basal levels (and the smallest increase) benefitted from treatment in terms of $ISec_{0-12}$. This could in turn indicate a threshold effect. However, this was erroneous as the correlation vanished when season-adjusted values were applied. In the above-mentioned studies, Davidson et al. (25) did not season-adjust their 25(OH)D

levels, which were the basis for inclusion and a subgroup analysis. Sollid et al. (26) did not season-adjust their levels either, which were used to define subgroups of more pronounced lack of vitamin D. In the study by Mitri et al. (28), their outcomes were adjusted for time of year of study entry but not for actual adjusted 25(OH)D levels. According to the findings of our study, we see no implication for vitamin D treatment to affect glucose homeostasis in subjects with abnormal glucose tolerance.

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Merck KGaA reviewed the manuscript but the views and opinions described solely represent those of the authors.

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