

Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers^{1–4}

Armin Zittermann, Sabine Frisch, Heiner K Berthold, Christian Götting, Joachim Kuhn, Knut Kleesiek, Peter Stehle, Heinrich Koertke, and Reiner Koerfer

ABSTRACT

Background: High blood concentrations of parathyroid hormone and low concentrations of the vitamin D metabolites 25-hydroxyvitamin D [25(OH)D] and calcitriol are considered new cardiovascular disease risk markers. However, there is also evidence that calcitriol increases lipogenesis and decreases lipolysis.

Objective: We investigated the effect of vitamin D on weight loss and traditional and nontraditional cardiovascular disease risk markers in overweight subjects.

Design: Healthy overweight subjects ($n = 200$) with mean 25(OH)D concentrations of 30 nmol/L (12 ng/mL) received vitamin D (83 $\mu\text{g}/\text{d}$) or placebo in a double-blind manner for 12 mo while participating in a weight-reduction program.

Results: Weight loss was not affected significantly by vitamin D supplementation (-5.7 ± 5.8 kg) or placebo (-6.4 ± 5.6 kg). However, mean 25(OH)D and calcitriol concentrations increased by 55.5 nmol/L and 40.0 pmol/L, respectively, in the vitamin D group but by only 11.8 nmol/L and 9.3 pmol/L, respectively, in the placebo group ($P < 0.001$), whereas a more pronounced decrease occurred in the vitamin D group than in the placebo group in blood concentrations of parathyroid hormone (-26.5% compared with -18.7% ; $P = 0.014$), triglycerides (-13.5% compared with $+3.0\%$; $P < 0.001$), and the inflammation marker tumor necrosis factor- α (-10.2% compared with -3.2% ; $P = 0.049$). The beneficial biochemical effects were independent of the loss in body weight, fat mass, and sex. However, compared with placebo, vitamin D supplementation also increased LDL-cholesterol concentrations ($+5.4\%$ compared with -2.5% ; $P < 0.001$).

Conclusions: The results indicate that a vitamin D supplement of 83 $\mu\text{g}/\text{d}$ does not adversely affect weight loss and is able to significantly improve several cardiovascular disease risk markers in overweight subjects with inadequate vitamin D status participating in a weight-reduction program. This trial was registered at clinicaltrials.gov as NCT00493012. *Am J Clin Nutr* 2009;89:1321–7.

INTRODUCTION

The burden of overweight [body mass index (BMI; in kg/m^2) ≥ 25] and obesity (BMI ≥ 30) has dramatically increased during the past several decades (1). The World Health Organization estimated that 1.6 billion adults were overweight and 400 million adults were obese worldwide in 2006 (2). In the United States, 2 in 3 adults have a BMI ≥ 25 (3).

Obesity is often associated with cardiovascular disease risk factors such as hypertension, dyslipoproteinemia, decreased glucose tolerance or diabetes, and elevated inflammation markers, which can lead to enhanced cardiovascular disease mortality (4–6). Accumulating evidence suggests that altered vitamin D homeostasis may also contribute to an increased cardiovascular disease risk in obese subjects. The major observation involves the association between low 25-hydroxyvitamin D [25(OH)D; <43 nmol/L] and obesity (7); 25(OH)D concentrations <33 – 37.5 nmol/L are independently related to a higher risk of myocardial infarction, cardiovascular mortality, and all-cause mortality than are 25(OH)D concentrations >71 – 75 nmol/L (8, 9). In addition, low 25(OH)D concentrations are predictive of elevated concentrations of parathyroid hormone (PTH) (10)—another biochemical variable that is related to cardiovascular disease (11). Obese subjects also have low serum concentrations of the vitamin D hormone calcitriol (12, 13). Calcitriol exerts various beneficial effects in cardiomyocytes (14) and in the vasculature (15). Circulating calcitriol concentrations <60 pmol/L are independently associated with poor clinical outcomes in patients with a high risk of cardiovascular mortality (9, 16, 17). Combined, the available data indicate that inadequate vitamin D status must be a nontraditional cardiovascular disease risk factor. However, it has also been shown in animal experiments that calcitriol increases lipogenesis and decreases lipolysis (18, 19), an effect that probably contributes to obesity.

¹ From the Klinik für Thorax- und Kardiovaskularchirurgie (AZ, SF, HK, and RK) and Institut für Laboratoriums- und Transfusionsmedizin (CG, JK, and KK), Herzzentrum NRW, Ruhr Universität Bochum, Bad Oeynhausen, Germany; Charité-Universitätsmedizin Berlin, Interdisziplinäres Stoffwechsel-Centrum; Lipidambulanz, Lipidapherese und Ernährungsmedizin Berlin, Germany (HKB); and Institut für Ernährungs- und Lebensmittelwissenschaften, Universität Bonn, Bonn, Germany (PS).

² AZ and SF contributed equally to this work.

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⁴ Reprints not available. Address correspondence to A Zittermann, Klinik für Thorax- und Kardiovaskularchirurgie, Herzzentrum Nordrhein-Westfalen, Georgstraße 11, 32545 Bad Oeynhausen, Germany. E-mail: azittermann@hdz-nrw.de.

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Few intervention studies have investigated the effects of vitamin D in combination with calcium on cardiovascular disease risk markers such as weight gain (20), disturbed glucose tolerance (21), and lipid profiles (22). However, the daily amount of vitamin D supplementation was only 10 to 17.5 μg , which is much lower than the vitamin D intake considered to be appropriate for adults (23, 24). Both the low amounts of the vitamin D supplements and the combination with a calcium supplement make it difficult to assess possible beneficial vitamin D effects from these earlier studies. The purpose of the present study, thus, was to investigate whether a daily vitamin D supplement of 83 μg affects weight loss and cardiovascular disease risk markers in overweight subjects participating in a weight-reduction program.

SUBJECTS AND METHODS

Participants

We conducted the study between December 2005 and November 2007 at the Herzzentrum Nordrhein-Westfalen, Bad Oeynhausen, Germany. Recruitment began in November 2005 via advertisements in local newspapers and information sheets provided to different local health insurance companies and was terminated in November 2006. The criteria for eligibility were an age of 18–70 y and a BMI (calculated as weight in kilograms divided by the square of the height in meters) >27 . We excluded patients with a history of myocardial infarction, angina pectoris, heart valve disease, cholelithiasis, urolithiasis, insulin-dependent diabetes mellitus, and pacemaker implantation. Moreover, pregnancy, lactation, vegetarianism, and intake of vitamin D supplements were the exclusion criteria. Of 298 persons who were interested in attending the study, 16 had to be excluded after a first screening by phone because they did not meet the inclusion criteria (**Figure 1**). Seventy-six persons refused to participate before study entry, and 6 other persons were excluded during the baseline investigation. Of the 200 subjects who entered the study, 62 (31%) were men and 87 (43.5%) were smokers. All

participants gave written informed consent to the study procedures, which were approved by the Ethics Committee of the Ruhr-University Bochum, Germany.

Study design

At study entry, all participants were randomly assigned in a double-blind manner from computer-generated random number lists; 100 patients were assigned to the vitamin D group and 100 patients to the placebo group. The vitamin D group had to take 5 drops daily of an oily vitamin D preparation (Vigantol oil; Merck, Darmstadt, Germany). This dose is equivalent to an amount of 83.3 μg (3332 IU) cholecalciferol. Compliance was assessed by measuring 25(OH)D concentrations. The placebo group had to take 5 drops daily of a vitamin D-free oil (Migliol oil; Merck). The treatment was conducted for 12 mo. The weight-reduction program consisted of weekly nutrition education and dietary counseling by phone consultations with a nutritionist during the first 6 study months. Compliance with the phone consultations was 93%. In both study groups, standardized nutrition education programs were used. The phone consultation lasted 5–30 min. This regular weekly support was stopped after the first 6 mo. At baseline and after 12 mo, we measured body weight and height in underwear on a calibrated electronic clinical scale (model 920; Seca GmbH & Co, KG, Hamburg, Germany; tolerance ± 0.2 kg) and waist circumference by standard procedures using a 150-cm anthropometric measuring tape. We assessed body composition by bioelectrical impedance analysis (Multifrequency Analyzer Nutrigard M; Data Input GmbH, Darmstadt, Germany). Blood pressure was manually determined with a cuff and stethoscope.

We assessed energy and nutrient intakes using a 3-d validated food record (25). The metabolic rate was calculated by multiplying the time of exercise by the respective metabolic equivalent task (MET) using adequate schedules to determine METs (26, 27) and with the respective weight. The amount of daily physical activity was assessed by using a standardized, validated questionnaire (28). Blood specimens were collected from the antecubital vein of each patient after they fasted overnight for ≥ 12 h. After centrifugation at room temperature for 20 min ($1500 \times g$), aliquots of the serum samples were frozen consecutively and stored at -80°C until analyzed.

We considered weight loss and loss of fat mass as primary endpoints. Calcitropic hormones and cardiovascular disease risk markers such as blood pressure, blood lipids, proinflammatory cytokines, and glucose metabolism were considered secondary endpoints.

Biochemical measurements

We measured 25(OH)D by radioimmunoassay (DiaSorin, Stillwater, MN) and the vitamin D hormone calcitriol using a competitive enzyme-linked immunosorbent assay (ELISA) (Immundiagnostik, Bensheim, Germany) after solid-phase extraction. Intra- and interassay CVs for the 2 vitamin D metabolites were $<7.0\%$ and $<9.0\%$, respectively. The DiaSorin assay consists of a 2-step procedure. The first procedure involves the rapid extraction of 25OHD and other hydroxylated vitamin D metabolites from 50 μL serum with acetonitrile. After extraction, the treated sample is then assayed by using an equilibrium

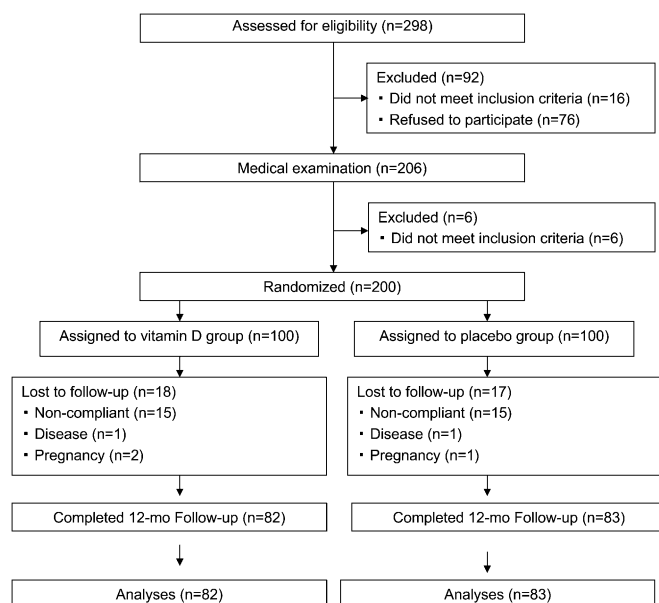


FIGURE 1. Flow of participants throughout the trial.

radioimmunoassay procedure. Cross-reactivity of the assay is 100% for 25-hydroxyvitamin D₃ and 100% for 25-hydroxyvitamin D₂. Generally accepted cutoffs for 25(OH)D are <25 nmol/L (<10 ng/mL) for deficiency, 25–49.9 nmol/L (10–19.9 ng/mL) for insufficiency, 50–74.9 nmol/L (20–29.9 ng/mL) for borderline status, and ≥75 nmol/L (>30 ng/mL) for normal status. The calcitriol assay consists of a 3-step procedure. The first procedure involves an extraction of calcitriol with a chromabond column. The eluate is then dripped from the chromabond column directly on an untreated and dry silica cartridge. After several purification steps, the calcitriol fraction is eluted and evaporated under a nitrogen stream. Then, the sample is dissolved in ethanol and assayed by using the aforementioned ELISA kit. Cross-reactivity for 1,25-dihydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₂ is 100% and 41%, respectively. We used highly sensitive ELISA kits to analyze plasma concentrations of interleukin-6 (IL-6; R&D, Minneapolis, MN) and proinsulin (IBL, Hamburg, Germany). Intra- and interassay CVs were <10.0%. We measured triglycerides, LDL cholesterol, HDL cholesterol, calcium, glucose, and high-sensitive C-reactive protein by using the Architect autoanalyzer (Abbott, Wiesbaden, Germany); glycated hemoglobin (Hb A_{1c}) by using the autoanalyzer HA-8160 (Menarini Diagnostics, Berlin, Germany); and tumor necrosis factor- α (TNF- α) and intact PTH by Immulite (DPC, Bad Nauheim, Germany).

Statistics

Categorical variables are expressed as percentage proportions and continuous variables as means \pm SDs. Because diastolic and systolic blood pressure, and several biochemical variables, such as glucose, Hb A_{1c}, proinsulin, triglycerides, HDL cholesterol, PTH, C-reactive protein, IL-6, TNF- α , 25(OH)D, and calcitriol, were nonnormally distributed, as tested by the Kolmogorov-

Smirnov test, these data were normalized by logarithmic transformation. We used the unpaired *t* test to compare continuous values of the study groups at baseline. A 2-factor repeated-measures analysis of variance was used to assess time effects and to analyze time \times treatment (vitamin D or placebo) interaction effects on all dependent variables. A 2-factor analysis of covariance was used to compare dependent variables between the vitamin D and placebo groups with change in body weight, fat mass, waist circumference, and sex as covariates. *P* values <0.05 were considered statistically significant. We used the statistical program SPSS (version 14; SPSS Inc, Chicago, IL) to perform the analyses.

RESULTS

Of the 200 participants, 35 subjects were lost to follow-up (Figure 1): 30 patients were unwilling to participate in the 12-mo examination for personal reasons (*n* = 7) or were non-compliant with the weight-reduction program (*n* = 23); 2 other patients (1 from the vitamin D group and 1 from the placebo group) terminated the study prematurely because of newly diagnosed Guillain-Barré syndrome and a malignancy requiring chemopreventive therapy, respectively; and 3 participants (2 from the vitamin D group and 1 from the placebo group) became pregnant. Thus, the results of 165 patients (82.5%) were available for data evaluation. The number of dropouts did not differ between study groups (*P* > 0.05). Characteristics of the remaining 165 participants are given in **Table 1**. Baseline physical characteristics were similar in the vitamin D and placebo groups. Moreover, there was a similar decrease in energy and macronutrient intakes between months 0 and 12 in both groups, whereas dietary calcium and vitamin D intakes did not decrease during the weight-loss intervention. There were significant time effects between months 0 and 12 in the primary endpoints body

TABLE 1

Characteristics of obese subjects who received vitamin D supplements (83 μ g/d) or placebo before and after the weight-loss intervention¹

Characteristics	Vitamin D group (<i>n</i> = 82)			Placebo group (<i>n</i> = 83)			<i>P</i> ²		
	Month 0	Month 12	Change	Month 0	Month 12	Change	Time	Treatment	Time \times treatment interaction
Age (y)	47.4 \pm 10.3 ³	—	—	48.8 \pm 10.1	—	—	—	—	—
Men (%)	37.8	—	—	27.7	—	—	—	—	—
Body weight (kg)	101.9 \pm 16.1	96.2 \pm 17.4	-5.7 \pm 5.8	96.1 \pm 15.0	89.7 \pm 14.5	-6.4 \pm 5.6	<0.0001	<0.001	0.248
BMI (kg/m ²)	33.7 \pm 4.1	31.8 \pm 4.3	-2.0 \pm 2.0	33.0 \pm 4.3	30.9 \pm 4.6	-2.2 \pm 1.9	<0.0001	0.059	0.347
Fat mass (kg)	40.1 \pm 10.2	35.9 \pm 11.1	-4.1 \pm 4.6	38.5 \pm 9.9	33.5 \pm 9.5	-4.9 \pm 4.9	<0.0001	0.094	0.140
Waist circumference (cm)	110.2 \pm 11.1	103.7 \pm 15.5	-6.5 \pm 9.6	107.6 \pm 11.0	100.1 \pm 12.2	-7.5 \pm 5.8	<0.0001	0.022	0.436
Energy expenditure (kcal \cdot kg ⁻¹ \cdot d ⁻¹)	32.1 \pm 6.0	34.0 \pm 6.7	-1.8 \pm 3.1	31.1 \pm 4.8	32.6 \pm 4.5	-2.0 \pm 3.5	<0.001	0.492	0.806
Energy intake (kcal/d)	2120 \pm 590	1765 \pm 622	-363 \pm 645	2226 \pm 790	1678 \pm 568	-538 \pm 646	<0.0001	0.685	0.155
Carbohydrate intake (g/d)	236 \pm 78	202 \pm 88	-37 \pm 90	250 \pm 95	196 \pm 71	-53 \pm 77	<0.0001	0.903	0.307
Fat intake (g/d)	83 \pm 30	62 \pm 26	-20 \pm 33	88 \pm 39	57 \pm 29	-28 \pm 32	<0.0001	0.420	0.184
Protein intake (g/d)	85 \pm 26	79 \pm 28	-8 \pm 27	87 \pm 33	75 \pm 32	-11 \pm 31	<0.001	0.372	0.591
Alcohol intake (g/d)	11 \pm 14	9 \pm 11	-2 \pm 12	10 \pm 14	8 \pm 12	-3 \pm 12	0.018	0.444	0.411
Dietary calcium intake (mg/d)	967 \pm 387	1133 \pm 428	142 \pm 444	1023 \pm 413	1059 \pm 490	44 \pm 468	0.033	0.669	0.250
Dietary vitamin D intake (μ g/d)	2.0 \pm 1.9	2.4 \pm 3.9	0.4 \pm 4.4	2.0 \pm 2.4	1.9 \pm 2.8	-0.1 \pm 3.5	0.586	0.409	0.705

¹ No significant differences from month 0 were observed between groups, *P* > 0.05 (unpaired *t* test).

² A 2-factor repeated-measures ANOVA was used to assess time effects and to analyze the time \times treatment (vitamin D or placebo) interaction effects on all dependent variables.

³ Mean \pm SD (all such values).

weight and fat mass and in BMI. In both study groups, weight loss was associated with a significant decrease in waist circumference. However, no significant differences in changes in these variables were observed between groups.

Blood pressure and biochemical variables were also comparable between groups at baseline. Significant time effects between months 0 and 12 were observed for calcitropic hormones, blood pressure, indicators of glucose metabolism, lipid variables, and proinflammatory cytokines (Table 2). There were also significant time \times treatment interaction effects found for 25(OH)D, calcitriol, PTH, triglycerides, LDL cholesterol, and TNF- α . Mean 25(OH)D concentrations increased by 55.5 nmol/L (22.2 ng/mL) in the vitamin D group but only by 11.8 nmol/L (4.7 ng/mL) in the placebo group. The percentage of patients who had 25(OH)D concentrations <25 nmol/L (10 ng/mL), 50 nmol/L (20 ng/mL), and 75 nmol/L (30 ng/mL), respectively, decreased in the vitamin D group from 58.5%, 89.0%, and 97.6%, respectively, at baseline to 7.6%, 19.8%, and 54.3%, respectively, after 12 mo. In the placebo group, the corresponding values were 66.3%, 83.1%, and 96.4%, respectively, at baseline and 41.0%, 77.1%, and 92.8%, respectively, after 12 mo. One patient in the placebo group and 3 patients in the vitamin D group had 25(OH)D concentrations >250 nmol/L (100 ng/mL) after 12 mo (300 nmol/L (120 ng/mL), 263 nmol/L (105.2 ng/mL), 295 nmol/L (118 ng/mL), and 348 nmol/L (139.2 ng/mL). All 4 patients were normocalcemic (serum calcium <2.7 mmol/L). In addition, all other patients in the vitamin D group were normocalcemic.

Mean concentrations of calcitriol increased by 46% in the vitamin D group, but only by 10% in the placebo group. In addition, combined vitamin D supplementation and weight loss

resulted in a more pronounced decrease in serum concentrations of PTH (-26.5% compared with -18.7%), triglycerides (-13.5% compared with +3.0%), and TNF- α (-10.2% compared with -3.2%) than did weight loss alone. In contrast with weight loss alone, combined vitamin D supplementation and weight loss resulted in an increase in LDL cholesterol. The significant differences in the increase in circulating 25(OH)D, calcitriol and LDL-cholesterol concentrations and the decrease in PTH, triglycerides, and TNF- α between study groups remained after adjustments were made for changes in fat mass and waist circumference. Moreover, adjustment for sex did not alter the vitamin D effects on calcitriol, LDL cholesterol, PTH, triglycerides, and TNF- α .

DISCUSSION

This study showed that a daily vitamin D supplement of 83.3 μ g beneficially influences some traditional and nontraditional cardiovascular disease risk markers but has no undesirable effect on weight loss in overweight and obese subjects. The beneficial biochemical effects were independent of the losses in body weight, fat mass, and fat mass in the abdominal region and of sex. Vitamin D supplementation also increased LDL-cholesterol concentrations.

Our results of a vitamin D-mediated decrease in serum triglycerides support earlier nonrandomized results from the third National Health and Nutrition Examination Survey (29). In that earlier trial, the adjusted prevalence of high serum triglycerides was significantly higher in the first than in the fourth quartile of serum 25(OH)D concentrations (<52.5 and >92.5 nmol/L). Our

TABLE 2

Cardiovascular disease risk markers of obese subjects who received vitamin D supplements (83 μ g/d) or placebo before and after the weight-loss intervention¹

Characteristics	Vitamin D group (n = 82)			Placebo group (n = 83)			P ²		
	Month 0	Month 12	Change	Month 0	Month 12	Change	Time	Treatment	Time \times treatment interaction
Diastolic blood pressure (mm Hg)	86 \pm 8 ³	83 \pm 8	-3 \pm 9	86 \pm 9	83 \pm 9	-3 \pm 10	<0.0001	0.532	0.953
Systolic blood pressure (mm Hg)	128 \pm 15	124 \pm 14	-4 \pm 16	128 \pm 14	125 \pm 16	-3 \pm 16	<0.0001	0.350	0.658
25(OH)D (nmol/L)	30.0 \pm 17.5	85.5 \pm 57.5	55.5 \pm 55.8	30.3 \pm 20.1	42.0 \pm 35.0	11.8 \pm 36.3	<0.0001	<0.001	<0.001 ⁴
Calcitriol (pmol/L)	87.0 \pm 33.8	127.0 \pm 87.5	40.0 \pm 95.0	89.0 \pm 40.8	98.3 \pm 48.8	9.3 \pm 57.0	<0.0001	<0.001	<0.001 ⁴
PTH (pmol/L)	4.69 \pm 3.25	3.39 \pm 2.03	-1.24 \pm 2.18	4.83 \pm 2.83	3.94 \pm 1.64	-0.91 \pm 2.55	<0.0001	0.033	0.014 ⁴
Calcium (mmol/L)	2.36 \pm 0.08	2.38 \pm 0.10	0.02 \pm 0.09	2.38 \pm 0.10	2.40 \pm 0.25	0.02 \pm 0.24	0.013	0.193	0.663
Triglycerides (mmol/L)	1.43 \pm 0.68	1.23 \pm 0.50	-0.19 \pm 0.54	1.31 \pm 0.57	1.35 \pm 0.74	0.03 \pm 0.50	0.006	0.706	<0.001 ⁴
LDL cholesterol (mmol/L)	3.52 \pm 0.93	3.70 \pm 1.04	0.19 \pm 1.03	3.65 \pm 0.78	3.57 \pm 0.91	-0.09 \pm 0.64	0.166	0.946	<0.001 ⁴
HDL cholesterol (mmol/L)	1.46 \pm 0.38	1.44 \pm 0.36	-0.02 \pm 0.22	1.51 \pm 0.37	1.46 \pm 0.40	-0.04 \pm 0.20	0.003	0.406	0.333
C-reactive peptide (mg/L)	0.35 \pm 0.32	0.32 \pm 0.42	-0.03 \pm 0.46	0.44 \pm 0.62	0.40 \pm 0.70	-0.05 \pm 0.68	<0.0001	0.995	0.481
Tumor necrosis factor- α (pg/mL)	7.84 \pm 3.15	7.04 \pm 2.25	-0.80 \pm 2.5	8.12 \pm 3.43	7.90 \pm 2.80	-0.26 \pm 2.9	0.005	0.092	0.049 ⁴
Interleukin-6 (pg/mL)	8.9 \pm 15.2	5.4 \pm 4.5	-3.5 \pm 14.0	7.8 \pm 12.3	6.0 \pm 5.4	-1.9 \pm 10.9	<0.0001	0.701	0.121
Glucose (mmol/L)	5.67 \pm 0.78	5.44 \pm 0.61	-0.21 \pm 0.51	5.67 \pm 1.17	5.39 \pm 0.72	-0.27 \pm 0.82	<0.0001	0.489	0.385
Hb A _{1c} (%)	5.62 \pm 0.40	5.37 \pm 0.30	-0.25 \pm 0.23	5.66 \pm 0.57	5.42 \pm 0.44	-0.25 \pm 0.23	<0.0001	0.400	0.962
Proinsulin (pmol/L)	11.5 \pm 15.0	7.0 \pm 4.8	-4.6 \pm 14.9	10.0 \pm 10.5	5.9 \pm 3.3	-4.1 \pm 9.9	<0.0001	0.119	0.746

¹ 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; Hb A_{1c}, glycated hemoglobin. To convert 25(OH)D values to ng/mL, divide by 2.5. To convert calcitriol values to pg/mL, divide by 2.6. To convert PTH values to pg/mL, divide by 0.11. No significant differences from month 0 were observed between groups, $P > 0.05$ (unpaired t test).

² A 2-factor repeated-measures ANOVA was used to assess time effects and to analyze the time \times treatment (vitamin D or placebo) interaction effects on all dependent variables.

³ Mean \pm SD (all such values).

⁴ Differences between groups remained statistically significant ($P < 0.05$) after adjustment for fat mass, waist circumference, and sex.

results are also in line with data in overweight and obese subjects who received a combined daily supplement of 1200 mg Ca and 10 μ g vitamin D (22). In that study, triglyceride concentrations decreased by 10% in the treatment group and remained constant in the placebo group. It was assumed that an increased calcium intake can reduce serum triglycerides by decreasing hepatic triglyceride formation or secretion via an effect on hepatocellular calcium (30). Vitamin D increases intestinal calcium absorption (31) and thus the amount of absorbed calcium. Therefore, an effect similar to oral calcium supplementation (increased amount of absorbed calcium) might explain the vitamin D-mediated reduction in serum triglycerides in our study. An alternative or additional explanation is a suppressive effect of vitamin D on serum PTH concentrations. Because elevated PTH concentrations are accompanied by a decrease in plasma postheparin lipolytic activity (32), the reduction in serum PTH in our study may have decreased serum triglycerides via increased peripheral removal.

The differences in the PTH decline between the vitamin D and placebo groups was explained by the more pronounced increase in 25(OH)D in the vitamin D group than in the placebo group. Results are in line with the fact that PTH decreases degeneratively with increasing 25(OH)D concentrations (33). It is noteworthy that PTH should be considered a nontraditional cardiovascular disease risk factor. Patients with elevated PTH concentrations due to primary or secondary hyperparathyroidism are at higher risk of cardiovascular morbidity and mortality (34–36). Serum PTH was an independent predictor of all-cause mortality in a cohort of frail elderly people, in whom 50% of the deaths were due to cardiovascular reasons (37). The Tromso Study showed that already slightly elevated PTH concentrations (>3.50 pmol/L in men and >3.30 pmol/L in women) are a risk factor for coronary heart disease in the general population (11). Thus, after vitamin D supplementation, most of our overweight subjects had a PTH concentration in the range associated with a low cardiovascular disease risk.

Recent studies indicate that serum 25(OH)D itself is an independent predictor of cardiovascular disease morbidity and mortality. Concentrations of 25(OH)D <37.5 nmol/L, as found in most of our study participants at baseline, were associated with a relative risk of 2.09 for myocardial infarction compared with 25(OH)D concentrations >75 nmol/L (8). In 3258 consecutive male and female patients scheduled for coronary angiography (9), multivariate-adjusted hazard ratios were greater in patients in the lower two 25(OH)D quartiles (medians: 19.0 and 33.3 nmol/L) for cardiovascular mortality (hazard ratio: 2.08 and 1.82, respectively) than in patients in the highest 25(OH)D quartile (median: 71.0 nmol/L). In our study, even the daily supplement of 83 μ g did not result in serum 25(OH)D concentrations >75 nmol/L in all supplemented patients. Aloia et al (24) recently showed that, in addition to the dietary vitamin D intake, a daily vitamin D supplement of 115 μ g is necessary for most participants to achieve a concentration of 75 to 220 nmol/L if they have mean baseline 25(OH)D concentrations of 35 to 41 nmol/L. Thus, it appears that a dose of vitamin D >83 μ g/d must be administered to obese subjects to achieve adequate 25(OH)D concentrations in almost all subjects.

Some preventive effects of vitamin D on cardiovascular disease risk may also be related to an increase in circulating calcitriol concentrations. Accumulating evidence indicates that

calcitriol exerts important physiologic effects on cardiomyocytes, vascular smooth muscle cells, and the vascular endothelium (15). Several studies have related low circulating calcitriol concentrations (<60 pmol/L) to enhanced cardiovascular and all-cause mortality (9, 16, 17). Our results of a vitamin D-induced increase in circulating calcitriol concentrations are in line with the assumption that a substrate-dependent reduction in circulating calcitriol concentrations may occur if the serum 25(OH)D concentration falls below 30–40 nmol/L (10). Interestingly, physically active subjects do not only have a reduced risk of cardiovascular disease but also have enhanced 25(OH)D and calcitriol concentrations (38), whereas immobilization results in low vitamin D metabolite concentrations (39).

High TNF- α concentrations are a risk factor for congestive heart failure and coronary heart disease mortality (40, 41). There is also evidence that the proinflammatory cytokine TNF- α contributes to atherosclerosis (42). The present data support earlier results of our study group of a vitamin D-induced suppression of TNF- α concentrations (43). Because TNF- α can obviously suppress circulating calcitriol concentrations (44), high 25(OH)D concentrations, as achieved by vitamin D supplementation in our study, seems to be necessary to maintain adequate calcitriol concentrations and to protect subjects from a vicious circle of low calcitriol and high TNF- α .

Whereas weight loss alone had no effect on LDL cholesterol in our study, weight loss in combination with vitamin D increased LDL-cholesterol concentrations significantly. One explanation for this effect is a vitamin D-induced increase in intestinal calcium absorption (31), which may have lowered fecal calcium excretion. A lower calcium content in the gut can reduce the formation of insoluble calcium-fatty soaps (45, 46) and can thus reduce the fecal fatty acid content. An increased absorption of fat, especially saturated fat, would also increase serum LDL-cholesterol concentrations. In addition, a lower calcium content in the gut reduces the calcium binding of bile acids (47), decreases the conversion of cholesterol to bile acids, and thus decreases cholesterol excretion (48). In our study participants, the mean daily calcium intake during the intervention was within the adequate daily intake for men and women aged 19 through 50 y of 1000 mg (Table 1) and slightly below the adequate daily intake for men and women aged 51 through 70 y of 1200 mg (49). A higher calcium intake may probably prevent the vitamin D-induced increase in LDL cholesterol. This assumption is in line with the fact that, in the aforementioned study in overweight or obese women with a daily calcium intake <800 mg, the daily supplement of 1200 mg Ca and 10 μ g vitamin D resulted in a significant decrease in LDL cholesterol compared with the placebo (22). However, it should also be considered that such a high calcium intake bears the risk of down-regulation of circulating calcitriol concentrations, which is not desirable (50). In addition, it is noteworthy that the dietary-heart-cholesterol hypothesis has recently been questioned. The unexpected cardiovascular benefits of statins, which are very potent cholesterol-lowering agents, may in part be explained by their capability to activate the vitamin D receptor (51).

Vitamin D supplementation had no effect on glucose metabolism in our study participants. Note that diabetes mellitus was an exclusion criteria. Recently, data from an ancillary analysis using existing data in archived samples from a completed double-blind, parallel-group, randomized trial of the effect of calcium (500 mg)

and vitamin D (17.5 μg) in white adults were published (52). This analysis also showed no difference for the participants with normal fasting glucose in the change in fasting plasma glucose or insulin sensitivity at 3 y between treated and placebo groups.

Our data do not support earlier results of a reduction in blood pressure by vitamin D (53). However, our study was limited in that blood pressure was measured only once at the beginning and at the end of the intervention. Because blood pressure shows markedly circadian variations, we cannot rule out that the method we used was inappropriate to detect small but significant changes in systolic or diastolic blood pressure.

In summary, our data indicate that a daily vitamin D supplement of 83 μg does not adversely affect weight loss and is able to significantly improve several cardiovascular disease risk markers in overweight subjects with inadequate vitamin D status participating in a weight-reduction program.

The authors' responsibilities were as follows—AZ: study design, data interpretation, writing of the manuscript, and final approval of the manuscript; SF: study design, recruitment of patients, data collection, review of the original data and their compilation, and final approval of the manuscript; HKB and HK: study design, critical revision of the manuscript for important intellectual content, and final approval of the manuscript; CG: data analysis and interpretation, critical revision of the manuscript for important intellectual content, and final approval of the manuscript; JK: data analysis, manuscript revision, and final approval of the manuscript; KK: data analysis, critical revision of the manuscript for important intellectual content, and final approval of the manuscript; and PS and RK: data interpretation, critical revision of the manuscript for important intellectual content, and final approval of the manuscript. None of the authors had a conflict of interest.

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