Effect of vitamin D replacement on indexes of insulin resistance in overweight elderly individuals: a randomized controlled trial[1,2]

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ABSTRACT

Background: It is unclear whether and at what dose vitamin D supplementation affects insulin resistance (IR).

Objective: We sought to investigate whether vitamin D at doses higher than currently recommended decreases indexes of IR in an ambulatory population of overweight elderly subjects.

Design: This double-blind, randomized, controlled multicenter trial enrolled 257 elderly overweight individuals aged ≥65 y with baseline 25-hydroxyvitamin D [25(OH)D] concentrations between 10 and 30 ng/mL. All subjects received 1000 mg calcium citrate/d, with vitamin D administered weekly at an equivalent dose of 600 or 3750 IU/d. The homeostasis model assessment (HOMA) of IR index at 1 y was the primary outcome. We also assessed the McAuley index.

Results: In total, 222 subjects (55% women) with a mean ± SD age and body mass index (BMI; in kg/m^2) of 71 ± 4 y and 30 ± 4, respectively, completed the study. Subjects' baseline characteristics, including IR indexes, were similar across groups: 69% had prediabetes, 54% had hypertension (47% were taking antihypertensive medications), and 60% had hyperlipidemia, nearly half of whom were receiving lipid-lowering drugs. At 1 y, mean ± SD serum 25(OH)D increased from 20 ± 7 to 26 ± 7 ng/mL in the low-dose arm ($P < 0.0001$) and from 21 ± 8 to 36 ± 10 ng/mL in the high-dose arm ($P < 0.001$). Median HOMA-IR indexes did not change compared with baseline concentrations and were similar in the high- [2.2 (IQR: 1.5, 2.9)] and low-dose [2.3 (IQR: 1.6, 3.3] treatment groups. Adjusted analyses showed that HOMA-IR was predicted by the baseline HOMA index and BMI but not by vitamin D dose, baseline serum 25(OH)D, or change in 25(OH)D.

Conclusion: Vitamin D_3 at 3750 IU/d did not improve HOMA-IR compared with the Institute of Medicine Recommended Dietary Allowance of 600 IU/d in elderly overweight individuals. This trial was registered at clinicaltrials.gov as NCT01315366. *Am J Clin Nutr* 2016;104:315–23.

Keywords: HOMA, insulin resistance, prediabetes, IOM RDA, high-dose vitamin D

INTRODUCTION

Although the beneficial effect of vitamin D on musculoskeletal parameters in a growing skeleton and in the elderly if coadministered with calcium is undisputed (1–4), its beneficial effect on other health outcomes is becoming increasingly questionable (2, 5). Indeed, although the list of chronic noncommunicable diseases associated with low serum 25-hydroxyvitamin D [25(OH)D][11] concentrations keeps expanding (1, 6), the evidence for a causal relation is increasingly lacking (2, 7, 8).

Abdominal obesity, hyperglycemia, dyslipidemia, and hypertension, core traits of the metabolic syndrome phenotype, account in large part for the rising tide in noncommunicable diseases, the leading cause of death worldwide (9, 10). However, whereas association studies evaluating the relation between 25(OH)D concentrations and cardiometabolic outcomes have yielded positive results, those from meta-analyses of randomized trials have been negative (11–14).

Despite its sunny climate, the Middle East registers some of the highest rates of low vitamin D worldwide, and Lebanon is no exception (15, 16). This region also has some of the highest obesity rates and the greatest relative increase in diabetes prevalence, with proportions reaching 20% in Bahrain, Saudi Arabia, and the United Arab Emirates (17).

[1] Supported by governmental and institutional funds from the American University of Beirut, St. Joseph University, the Lebanese Council for National Scientific Research, and in part by NIH Fogary International Center and Office of Dietary Supplements grant 3D43TW009-118-03W1. Assays performed outside the American University of Beirut were supported in part by institutional grants from the Mayo Clinic and Odense University.

[2] Supplemental Figure 1, Supplemental Tables 1 and 2, and Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[11] Abbreviations used: AUBMC, American University of Beirut Medical Center; DSMB, data safety monitoring board; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; IOM, Institute of Medicine; IR, insulin resistance; LC-MS, liquid chromatography–mass spectroscopy; PTH, parathyroid hormone; RDA, Recommended Dietary Allowance; SAE, serious adverse event; 25(OH)D, 25-hydroxyvitamin D.

Received February 10, 2016. Accepted for publication June 8, 2016.
First published online July 13, 2016; doi: 10.3945/ajcn.116.132589.

The objective of this trial was to investigate whether vitamin D administered at doses higher than currently recommended would decrease indexes of insulin resistance (IR) in nondiabetic, overweight, elderly ambulatory subjects. The indexes chosen were HOMA-IR and the McAuley index (18–20). Both indexes have been validated in terms of sensitivity and specificity in the general population against established but challenging-to-implement homeostatic studies (18–20). HOMA-IR is one of the most commonly used indexes in the literature, and its calculation includes fasting insulin and glucose concentrations. The McAuley index incorporates fasting serum insulin and triglyceride concentrations. These indexes are somewhat complementary because they use a common (insulin) but also different (glucose and triglycerides) variables in their formulas, and both have been used in the literature (20).

METHODS

Study design

We used a double-blind, randomized controlled trial (NCT01315366) conducted at the American University of Beirut Medical Center (AUBMC), St. Joseph University Hospital, and Rafic Hariri Governmental University Hospital to compare treatment with low- and high-dose vitamin D. Recruitment, prescreening, and screening procedures were performed at all centers between January 2011 and July 2013, whereas enrollment and protocol implementation took place at AUBMC and ended July 2014.

Study drug

All subjects received 4 tablets of calcium citrate (Citracal D; 250 mg elemental Ca and 125 IU vitamin D_3/tablet; Europharm) taken as 2 tablets 2 times/d, resulting in a total expected active drug received of 1000 mg Ca and 500 IU vitamin D. In addition, patients received 2 pills to be taken 1 time/wk that were identical in shape, color, size, smell, and taste of either placebo (low-dose group) or 10,000 IU vitamin D/tablet (Euro D; 10,000 IU/tablet; high-dose group) (Europharm). Based on a certificate of analysis provided by Europharm to the Canadian regulatory agencies obtained on all trial lots, the mean vitamin D actual content of the calcium citrate/D and Euro D tablets was 150 and 11,000 IU, respectively. Therefore, the total daily intake of vitamin D in the low- and high-dose groups was equivalent to 600 and 3750 IU/d, respectively. Study drugs were stored and dispensed to subjects in identical boxes at the AUBMC central pharmacy.

The randomization and allocation sequence was implemented by the senior pharmacist at AUBMC, with stratification by center and sex. Allocation was based on a simple randomization approach and assigned by matching subjects' baseline serial identification numbers (odd compared with even serial study identification numbers) with a preassigned treatment code (high- compared with low-dose vitamin D). Randomization was concealed, and boxes were sequentially numbered per the random allocation list. All study team members and participants were blinded to drug assignment until the trial was completed and the data were entered.

The institutional review board at each center approved the protocol, and all participants provided written informed consent. An external data safety monitoring board (DSMB) was formed before the trial started to monitor safety and review the final protocol. The institutional review board and DSMB received reports on all serious adverse events (SAEs) within 24 h of their occurrence. An increase in serum calcium or creatinine was reported within 1 wk of occurrence and was managed based on an algorithm used in a similar trial (21) (**Supplemental Figure 1**).

Participants

Elderly (≥65 y), overweight [BMI (in kg/m^2) >25], ambulatory subjects with a 25(OH)D between 10 and 30 ng/mL at screening were recruited through the outpatient departments, clinics, and advertisements posted at 3 major teaching hospitals and health dispensaries of the Ministry of Social Affairs from the greater Beirut area. Subjects with prediabetes who were not receiving any medications were included. Exclusion criteria were patients with prediabetes [fasting blood sugar (FBS) between 100 and 125 mg/dL or glycated hemoglobin (HbA1c) between 5.7% and 6.4%)] taking oral hypoglycemic drugs, diabetes (FBS ≥126 mg/dL or HbA1c ≥6.5%), severe chronic diseases, or major organ failure, which included severe heart failure (stages III and IV), liver failure and cirrhosis, kidney failure (estimated glomerular filtration rate, <30 mL/min), cancer, and autoimmune diseases. Subjects were also excluded if they had conditions (or were on medications) known to affect bone metabolism, osteomalacia, a history of kidney stones, fragility fractures, or a 10-y fracture risk for major osteoporotic fractures >10% based on the FRAX Lebanon risk calculator (https://www.shef.ac.uk/FRAX/tool.jsp).

Study visits and measurements

Enrolled subjects came in for visits every 3 mo, during which height, weight, and vital signs were measured; questionnaires were administered; study drug bottles were returned; and refills were provided. Subjects were also contacted by phone every 2 wk to reinforce compliance with the study drug. Information on adverse events, intake of medications, and study drug pill counts were obtained at each visit (0, 3, 6, and 12 mo). Compliance was defined as percentage of full dose with the use of pill count [(total number of study drug pills taken/total number of pills provided for time intervals between study visits) × 100].

Routine chemistries and calciotropic hormones were assayed at 0, 3, 6, and 12 mo, whereas insulin and C-peptide were assayed only at baseline and 12 mo (study end). Blood samples were allowed to clot for 30 min, centrifuged for 20 min, g-force 3500 rpm/rcf, and immediately processed for routine studies or stored at −20°C within 2 h and then at −80°C depending on the assay. Serum 25(OH)D was run at the Mayo Clinic with the use of liquid chromatography–mass spectroscopy (LC-MS). Hormonal assays were run in batches, and samples for hormones drawn at serial time points were included within the same assay. Glucose was measured by the hexokinase method with the use of the Roche cobas 6000 analyzer. Interassay CVs were 1.6% and 1.8% at 88 and 292 mg/dL, respectively.

Serum insulin and C-peptide were measured with the use of Roche cobas immunoassay platforms. Intra- and interassay CVs were 1.1–1.4% and 3.5–3.7%, respectively, for serum insulin

and 1.3–4.6% and 1.8–5.0% for C-peptide. Details on other assays are provided in **Supplemental Methods**.

Study outcomes

The HOMA-IR index (18, 19) was 1 of 2 primary outcome measures used for the trial; the other was bone mineral density. HOMA-IR was chosen as a primary outcome because of the studies available that have used this index to explore the impact of vitamin D on insulin sensitivity (22).

$$\text{HOMAIR} = \text{glucose}\left(\frac{\text{ng}}{\text{dL}}\right) \times \text{insulin}\left(\mu\frac{\text{U}}{\text{mL}}\right)/405 \qquad (1)$$

We derived a complementary assessment of IR based on the McAuley equation (20).

$$\text{McAuley IR} = e^{2.63 - 0.28\ln\left[\text{insulin}\left(\mu\frac{\text{U}}{\text{mL}}\right)\right] - 0.31\ln\left[\text{triglycerides}\left(\frac{\text{mmol}}{\text{L}}\right)\right]} \qquad (2)$$

The conversions for the McAuley equation are as follows: insulin, 1 mU/mL = 7.175 pmol/L; triglycerides, 1 mg/dL = 0.0113 mmol/L (23).

The effect of vitamin D replacement on indexes of IR was compared within and across the 2 treatment doses and in prespecified subgroup analyses: sex subgroups and baseline vitamin D deficiency status based on 25(OH)D and parathyroid hormone (PTH) thresholds of 20 ng/mL and 76 pg/mL, respectively [as opposed to 25(OH)D <20 ng/mL − PTH <76 pg/mL and 25(OH)D >20 ng/mL]. As validated by prospective bone loss in a cohort of elderly individuals (24), this grouping approach was shown to be superior to 25(OH)D only. We also implemented post hoc subgroup analyses by baseline glucose tolerance status (impaired fasting glucose or prediabetes at study entry) and in subjects with 25(OH)D <20 ng/mL at study entry.

Sample size calculation

We calculated the sample size based on post hoc analyses from a calcium vitamin D trial conducted in overweight elderly Caucasian subjects (22). Based on an anticipated between-arms mean ± SD difference in the HOMA-IR index of 0.9 ± 2, a power of 80%, and a significance level of 2.5% (instead of 5% to take into account the second primary outcome of bone mineral density), the total sample size needed was calculated at 222 subjects (111/arm). Allowing for a possible estimated dropout rate of 30%, 257 subjects were enrolled into the study.

Statistical analyses

Given the nature of the primary outcome, HOMA-IR at 12 mo, and the fact that variables to calculate HOMA were only obtained at baseline and 1 y, the planned intention-to-treat analysis was equivalent to a per-protocol analysis. We used descriptive statistics, parametric (paired and independent *t* tests) and nonparametric tests, and ANOVA to test for time trends between and within (repeated-measures ANOVA) treatment arms as indicated. Results are expressed as means ± SDs for normally distributed variables and medians (25th and 75th quartiles) for nonnormally distributed variables. Normal distribution was evaluated by visually inspecting histograms and stem leaf plots, and log transformation was performed when appropriate. We implemented regression models to further investigate the impact of vitamin D treatment on IR indexes after adjusting for several baseline values and for clinically relevant predictors: age, baseline BMI, IR indexes or glucose tolerance status, statin use, and vitamin D status at study entry. SPSS version 22.0 (IBM) and SigmaPlot version 12.0 (Systat Software Inc.) were used. $P < 0.05$ was considered statistically significant.

RESULTS

Subjects and baseline characteristics

In total, 129 elderly subjects were randomly assigned to receive high-dose vitamin D and 128 to receive low-dose vitamin D; 35 subjects (14%) did not complete the study, and no outcome data were available after study discontinuation (**Figure 1**). Patient characteristics, baseline data, and comorbidity in the 222 subjects did not differ from the original randomized group (data not shown). These were also similar for the low- (n = 112) compared with high-dose (n = 110) vitamin D arms (**Table 1**; all comparisons nonsignificant). Subjects had a median age of 70 y and a BMI of 29.2, and 55% were women. Overall, 77% had ≥1 comorbidity, and 69% met the definition of prediabetes but were not taking oral hypoglycemic drugs. Baseline mean serum 25(OH)D concentration (low compared with high: 20.0 ± 7.0 and 20.9 ± 8.2 ng/mL), and indexes of IR, reported as medians, were for HOMA 2.2 (IQR: 1.6, 3) compared with HOMA 2 (IQR: 1.3, 2.6). All other biochemical and hormone concentrations were similar across treatment arms.

Response to vitamin D supplementation

Compliance with the study drug was >90% for both calcium and vitamin D pills, overall, and by study visit for both treatment arms. There were no changes in mean BMI by treatment allocation at intermediate visits or study completion (data not shown).

Serum 25(OH)D concentrations at 12 mo

Compared with baseline concentrations, mean serum 25(OH)D at 1 y increased in the high- (20.9 ± 8.2 to 36 ± 9.7 ng/mL; $P <$ 0.0001) and low-dose (20 ± 7 to 25.9 ± 6.9 ng/mL; $P < 0.0001$) groups, although more substantially for the high-dose group (**Table 2**). One-year 25(OH)D concentrations ≥20 ng/mL were achieved in a larger proportion of subjects in the high-dose group (98%) than the low-dose group (83%) ($P < 0.0001$).

Indexes of fuel metabolism at 12 mo

After 12 mo of treatment, the HbA1c, insulin, C-peptide, and lipid concentrations did not differ between the low- and high-dose groups (Table 2). Whereas fasting serum hemoglobin A1C, triglycerides, and insulin concentrations did not change, serum fasting glucose increased between 0 and 12 mo (**Supplemental Table 1**, Table 2), and C-peptide concentrations decreased between 0 and 12 mo (Table 2), similarly and significantly, in both groups. Two-factor ANOVA showed no significant difference between the 2 groups in the changes over time for FBS, HbA1C, and triglycerides (Supplemental Table 1).

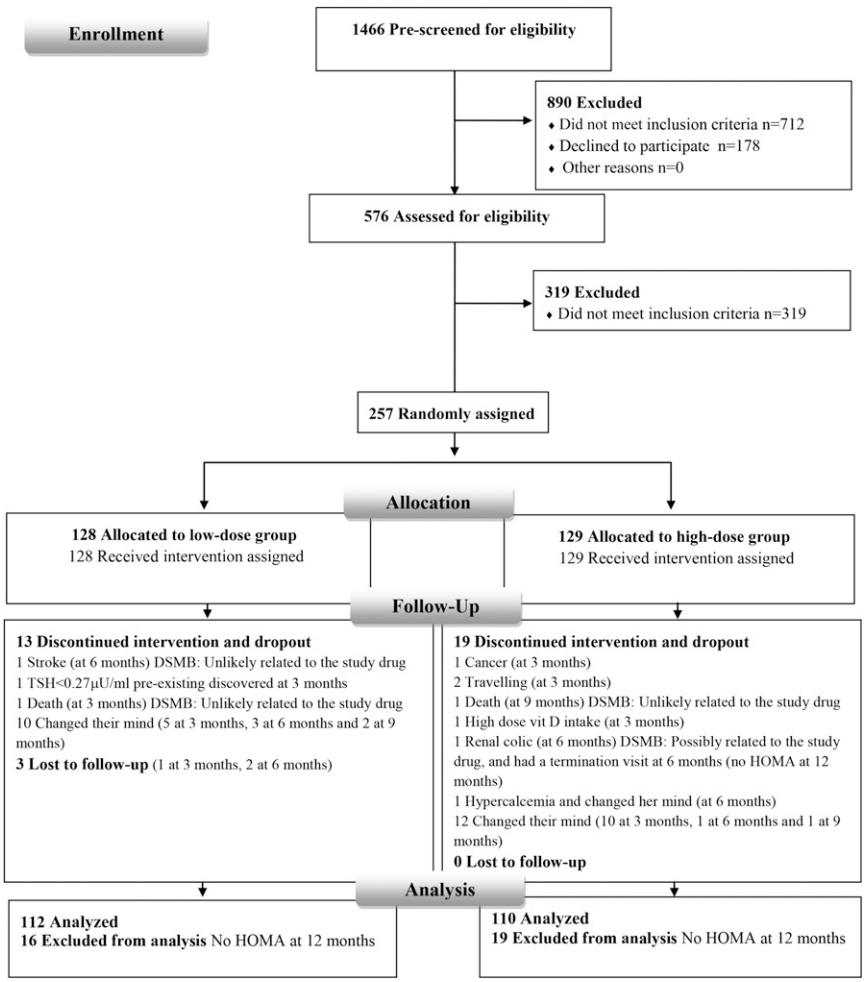

FIGURE 1 CONSORT flow diagram detailing participant recruitment and retention status from prescreening to study completion. CONSORT, Consolidated Standards of Reporting Trials; DSMB, Data and Safety Monitoring Board; TSH, thyrotropin; vit D, vitamin D.

Indexes of IR at 12 mo

After 12 mo of treatment, the HOMA-IR, lnHOMA, and McAuley indexes did not differ between the low- and high-dose supplementation groups (Table 2). Additional prespecified subgroup analyses showed no significant difference in indexes of IR at 12 mo between low- and high-dose groups in men [low-dose lnHOMA: 0.66 ± 0.49 (n = 50); high-dose lnHOMA: 0.74 ± 0.48 (n = 47)] and women [low-dose lnHOMA: 0.84 ± 0.63 (n = 57); high-dose lnHOMA: 0.91 ± 0.54 (n = 58)]. Similarly, there was no difference in lnHOMA at 12 mo between the low- and high-dose groups in subjects with <20 ng 25(OH)D/mL and >76 pg PTH/mL [low-dose lnHOMA: 0.81 ± 0.64 (n = 16); high-dose lnHOMA: 1.00 ± 0.46 (n = 18)].

Predictors of IR indexes at 12 mo

Pooling both treatment groups, lnHOMA-IR at 12 mo was associated with baseline BMI (R^2 = 0.145; P < 0.0001), baseline lnHOMA-IR (R^2 = 0.421; P < 0.0001), and prediabetes at baseline (R^2 = 0.043; P = 0.005). Baseline 25(OH)D, 12-mo 25(OH)D, change in 25(OH)D, age, statin use, vitamin D-PTH subgroup at study entry, and vitamin D dose were not associated with lnHOMA-IR at 12 mo (**Supplemental Table 2**). Similar patterns were found for the McAuley index except for prediabetes (Supplemental Table 2).

Multivariate regression analyses showed that, after adjusting for BMI and baseline lnHOMA-IR or McAuley index, treatment group was not a considerable predictor of 1-y lnHOMA-IR and McAuley index (**Table 3**, model 1). Similar results were obtained after adjusting for BMI and the presence of prediabetes at entry (Table 3, model 2). Further adjustment for important determinants of IR such as age and statin use led to almost identical results, whether all forced in the model at once (Table 3, model 3) or in a stepwise model (Table 3, model 4). These results were essentially replicated when the McAuley index was used (Table 3, models 1–4).

Post hoc analyses

There was no beneficial effect of high-dose vitamin D on indexes of IR at 12 mo in the subgroup of subjects who had baseline 25(OH)D concentrations <20 ng/mL; ΔlnHOMA-IR in the low-dose group (mean ± SE) was Δ = 0.13 ± 0.07, (n = 57), and in the high-dose group was Δ = 0.05 ± 0.06, (n = 55). Similarly, there was no significant difference at 12 mo in the subgroup of subjects with impaired fasting glucose at study entry, low-dose group (Δ = 0.10 ± 0.12), and high-dose group (Δ = −0.09 ± 0.08). There was also no significant difference in

TABLE 1
Baseline clinical and biochemical characteristics of the study cohort overall and by vitamin D dose allocation[1]

	Overall (n = 222)	Low dose (n = 112)	High dose (n = 110)
Clinical characteristics			
Sex, n			
Women	123	60	63
Men	99	52	47
Age, y	70 (67, 74)[2]	70 (67, 73.5)	71 (67, 74)
BMI, kg/m^2	29.2 (26.8, 32.4)	28.5 (26.1, 32.3)	30.1 (27.1, 32.6)
Calcium and vitamin D supplement, n (%)	22 (10)	11 (10)	11 (10)
Dietary calcium intake, mg/d	389 (260, 560)	398 (288, 569)	373 (239, 535)
Comorbidities, n (%)			
Prediabetes[3]	153 (69)	75 (67)	78 (71)
Hypertensive (on physical exam)	120 (54)	56 (50)	64 (58)
Taking antihypertensive drugs	105 (47)	49 (44)	56 (51)
Hypercholesterolemia	142 (64)	71 (63)	71 (65)
Taking lipid-lowering drugs	66 (30)	34 (30)	32 (29)
Subjects with comorbidities,[4] n (%)	170 (77)	85 (76)	85 (77)
1	68 (31)	35 (31)	33 (30)
2	81 (36)	42 (38)	39 (35)
≥3	21 (9)	8 (7)	13 (12)
Biochemical variables			
Serum 25(OH)D, ng/mL	20.4 ± 7.4[5]	20.0 ± 7	20.9 ± 8.2
Calcium, mg/dL	9.5 ± 0.4	9.4 ± 0.4	9.5 ± 0.4
Creatinine, mg/dL	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Phosphorus, mg/dL	3.4 ± 0.5	3.4 ± 0.5	3.4 ± 0.4
GFR[6]	85.6 (73.6, 91.3)	85.9 (73.5, 91.7)	85.2 (73.6, 90.5)
Parathyroid hormone, pg/mL	63 (45, 82)	63 (47, 78)	62 (42, 83)

[1]Independent t tests were used for continuous normally distributed variables and Mann-Whitney U tests for skewed variables. Chi-square tests were used for categorical variables at baseline low compared with high dose. CKD-EPI, Chronic Kidney Disease Epidemiology; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; 25(OH)D, 25-hydroxyitamin D.
[2]Median; IQR in parentheses (all such values).
[3]Prediabetes was defined as a fasting blood sugar between 100 and 125 mg/dL or HbA1c between 5.7% and 6.4%
[4]Includes cardiovascular disease, coronary artery disease, congestive heart failure, hypertension, and hypercholesterolemia.
[5]Mean ± SD (all such values).
[6]Estimated with the use of the CKD-EPI equation.

subjects with prediabetes, low dose (Δ = 0.1 ± 0.06), and high dose (Δ = 0.01 ± 0.05) or in normal subjects at entry, low dose (Δ= 0.05 ± 0.07), and high dose (Δ = 0.13 ± 0.07) (**Figure 2**).

Univariate analyses showed that fasting blood glucose at 12 mo significantly correlated with baseline fasting blood glucose (R^2 = 24%; $P < 0.01$) and baseline HOMA-IR (R^2 = 2.9%; P = 0.012) but not with age, BMI, baseline 25(OH)D concentration, vitamin D status, vitamin D dose, or statin use. Baseline prediabetes predicted 12-mo fasting blood sugar ($P < 0.0001$). Only baseline fasting blood glucose predicted 12-mo fasting glucose by multivariate analysis. Overall, 9.5% of subjects developed frank diabetes at 12 mo, 12.7% in the high-dose group compared with 6.3% in the low-dose group (P = 0.099).

Subject retention and adverse events

A total of 35 subjects, 14% of the original randomized cohort, did not complete the study. The dropout rate was similar in both groups and resulted in large part from patients having changed their mind about participating in the study (Figure 1). In all, 2 patients died—1 in each treatment group. The first, from the low-dose group, was a man aged 79 y with hypertension, receiving lipid-lowering therapy, and with a previous upper-extremity deep venous thrombosis who was admitted with a myocardial infarction. The second, from the high-dose group, was a man aged 83 y with a negative risk profile who had sudden cardiac arrest. The DSMB unanimously adjudicated both deaths as unlikely to be related to the study drug. One subject in the low-dose group with a prior history of hypertension and hyperlipidemia had a stroke while being treated. One subject in the high-dose group developed a renal colic and underwent lithotripsy, although no stone was clearly identified on scans. The latter SAE was deemed possibly related to the study drug given that no prior history of kidney stones was documented, and the lack of prior X-rays prevented assessing for the presence of a pre-existing stone disease, if any.

The study recorded a total of 11 SAEs, 6 in the low-dose and 5 in the high-dose group. In addition to the events reported above, one of each of the following SAEs occurred in one patient each: thrombophlebitis, glaucoma, hemorrhoids, and disc surgery in the low-dose group and high blood pressure, retinal detachment, and arthroscopic surgery in the high-dose group. One subject in the high-dose group had a calcium concentration of 10.6 mg/dL and a 25(OH)D concentration of 30 ng/mL at 6 mo and decided to drop out. One subject in the low-dose group had a creatinine of 1.4 mg/dL and was managed per algorithm. His creatinine concentration at follow-up was 1.3 mg/dL (back to baseline value), and he completed the study.

TABLE 2
Serum 25(OH)D, indexes of fuel metabolism, and insulin resistance by study group at baseline and 12 mo[1]

	Low-dose vitamin D (n = 112)		High-dose vitamin D (n = 110)	
	Baseline	12 mo	Baseline	12 mo
Serum 25(OH)D,[2] ng/mL	20.0 ± 7[3]	25.9 ± 6.9*	20.9 ± 8.2	36.0 ± 9.7*‡
Fuel metabolism				
FBS, mg/dL	94 ± 9	97 ± 13*	94 ± 10	100 ± 12*
HbA1c, %	5.8 ± 0.3	5.8 ± 0.4	5.8 ± 0.3	5.8 ± 0.4
Triglycerides, mg/mL	134 ± 67	132 ± 62	134 ± 66	140 ± 76
Insulin, pmol/L	66 (48, 91)[4]	67 (50, 97)	61.5 (43, 79)	64.5 (47.5, 84)
C-peptide, pmol/L	736 (547, 1019)	645 (425, 857)*	670 (529, 897)	603 (417, 805)*
Total cholesterol, mg/dL	207 ± 41	201 ± 34*	208 ± 41	196 ± 38*
HDL, mg/dL	53 ± 15	52 ± 15	49 ± 12	49 ± 12
LDL, mg/dL	131 ± 40	122 ± 29*	133 ± 36	120 ± 33*
Indexes of insulin resistance				
HOMA[5]	2.2 (1.6, 3)	2.3 (1.6, 3.3)	2 (1.3, 2.6)	2.2 (1.5, 2.9)
lnHOMA	0.6 ± 0.5	0.7 ± 0.5	0.7 ± 0.5	0.8 ± 0.5
McAuley[6]	7.1 ± 1.6	6.9 ± 1.4	6.8 ± 1.4	6.8 ± 1.5

[1]Independent t tests were used for continuous normally distributed variables and Mann-Whitney U test for skewed variables at 12 mo for low- and high-dose comparisons. There were no significant differences between the 2 treatment groups at baseline. ‡$P < 0.05$ between the 2 treatment groups at 12 mo. *$P < 0.05$ within groups comparing mean baseline and 12-mo concentrations. FBS, fasting blood sugar; HbA1c, glycated hemoglobin; 25(OH)D, 25-hydroxyitamin D.

[2]$P < 0.0001$ for low- and high-dose comparison.

[3]Mean ± SD (all such values).

[4]Median; IQR in parentheses (all such values).

[5]HOMA: [glucose (mg/dL) × insulin (μU/mL)]/405 (19).

[6]McAuley's index: exp{2.63 − 0.28 ln [insulin (μU/mL)] − 0.31 ln [triglycerides (mmol/L)]} (20). Conversions: insulin, 1 mU/mL = 7.175 pmol/L; triglycerides, 1 mg/dL = 0.0113 mmol/L (23).

DISCUSSION

In this double-blind controlled trial, high-dose vitamin D at a dose equivalent to 3750 IU/d did not improve indexes of insulin resistance compared with the Institute of Medicine (IOM) Recommended Dietary Allowance (RDA) of 600 IU/d in elderly overweight individuals.

The negative findings for any effect of high-dose vitamin D on insulin resistance compared with the lower dose were consistent across the 2 indexes of insulin resistance. The only predictors for these indexes at 1 y were BMI, the corresponding baseline IR concentration, and glucose tolerance status (prediabetes) at study entry. Although subjects in the 2 treatment groups were matched for all baseline characteristics, glucose tolerance status, and comorbidities, we confirmed the robustness of these negative findings in the regression analyses.

At the time that the trial was launched, the evidence for a beneficial effect of vitamin D on insulin sensitivity/resistance was scarce to our knowledge (22, 25, 26). Although one recent study (27) from India has demonstrated a positive effect of vitamin D at a high dose on diabetes progression in young prediabetic subjects (n = 55), several other studies with negative findings have been reported as well (28–36). Most suffered from ≥1 limitation, such as being conducted in younger or lower-risk individuals, having a small sample size and short study duration, not using IR as a primary outcome, being based on interim analyses, or using lower doses of vitamin D and methods other than LC-MS to measure serum 25(OH)D concentrations. Similarly, several recent meta-analyses of randomized trials, excluding those that were conducted in diabetic subjects or pregnant women, have also yielded negative results. These were also importantly limited by the large heterogeneity noted between studies and the short follow-up of studies considered (12–14, 37–39).

The decrease in cholesterol and LDL concentrations at 12 mo in both arms is interesting and unexplained by changes in medication/statin use or weight. It has been previously documented in other studies, both in pre- and postmenopausal women (40–42) taking vitamin D or a calcium vitamin D combination. It was proposed to be because of an interference with intestinal lipid absorption (41). However, in view of the lack of a placebo arm, definitive conclusions cannot be drawn.

Our negative trial results cannot be explained by a low study power, low compliance with the study drug, or low 25(OH)D concentrations achieved. Indeed, our post hoc power analyses revealed a power of 90% in view of the lower-than-anticipated dropout rate. The compliance with both calcium and vitamin D was very high, and a large proportion of subjects (98% in the high-dose group and 83% in the low-dose group) achieved the IOM-recommended desirable concentration of 20 ng/mL. The lower-than-expected increment in mean serum 25(OH)D concentration of 0.4 ng vitamin D/100 U in the high-dose group may be explained by low absorption due to aging, calcium, concomitant drugs, or possible modulation by vitamin hydroxylation polymorphisms (43, 44). It is unlikely to be because of a weekly as opposed to a daily dosing regimen, because mean serum 25(OH)D concentrations achieved with equivalent weekly versus daily doses of vitamin D were shown to be comparable in elderly individuals (45). It could be argued that higher vitamin D doses and/or 25(OH)D concentrations are needed to affect indexes of insulin resistance. However, although positive results were noted in post hoc analyses in subjects with abnormal fasting glucose who achieved a mean serum 25(OH)D of 45 ng/mL on 700 IU vitamin D/d (22) and in

TABLE 3
Multivariate analysis for predictors of indexes of insulin resistance at 12 mo

Dependent variables and predictors	β	P
Model 1[1]		
lnHOMA index		
BMI at baseline	0.020	0.004
Concentration at baseline	0.559	<0.0001
High to low dose	0.000	
McAuley index		
BMI at baseline	−0.038	0.035
Concentration at baseline	0.647	<0.0001
High to low dose	−0.090	
Model 2[2]		
lnHOMA index		
BMI at baseline	0.043	<0.0001
Prediabetes at baseline	0.181	0.012
High to low dose	−0.040	
McAuley index		
BMI at baseline	−0.119	<0.0001
Prediabetes at baseline	−0.089	
High to low dose	0.031	
Model 3[3]		
lnHOMA index		
BMI at baseline	0.020	0.004
Concentration at baseline	0.571	<0.0001
High to low dose	0.001	
McAuley index		
BMI at baseline	−0.037	0.041
Concentration at baseline	0.658	<0.0001
High to low dose	−0.094	
Model 4[4]		
lnHOMA index		
BMI at baseline	0.020	0.004
Concentration at baseline	0.559	<0.0001
McAuley index		
BMI at baseline	−0.038	0.036
Concentration at baseline	0.645	<0.0001

[1]Adjusted for BMI (in kg/m^2) index of insulin resistance and vitamin D dose.
[2]Adjusted for BMI, prediabetes, and vitamin D dose.
[3]Adjusted as for model 1 and for age and statin use.
[4]Adjusted as for model 3 but covariates entered stepwise.

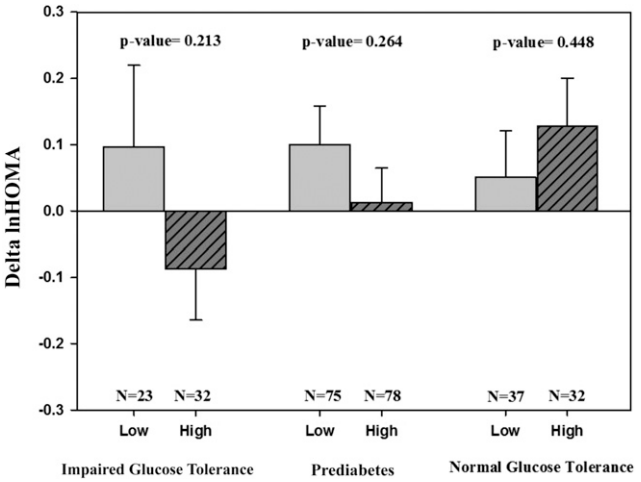

FIGURE 2 Subgroup analyses on the ΔlnHOMA by glucose tolerance status at entry (means ± SEMs). Impaired glucose tolerance: fasting blood sugar, >100 mg/dL; prediabetes: fasting blood sugar, 100–125 mg/dL or HbA1c 5.7–6.4%; and normal glucose tolerance: fasting blood sugar, <100 mg/dL or HbA1c <5.7%. P = independent t test for ΔlnHOMA (low compared with high dose). HbA1c, glycated hemoglobin.

another study of subjects at risk for developing diabetes reaching a concentration of 30.6 ng/mL on 2000 IU/d (25), mean 25(OH)D concentrations between 42 and 70 ng/mL were achieved in 3 other negative trials with the use of daily equivalent vitamin D doses of 2850, 5700, and 12,000 IU in high-risk individuals (28, 33, 46).

Potential limitations of our study include the lack of a placebo, the fact that only half of the subjects had serum 25(OH)D concentrations <20 ng/mL, the use of surrogate markers of insulin resistance rather than an insulin clamp, and the intake of antihyperlipidemic medications, mostly statins (drugs known to affect glucose homeostasis). We considered a study design with a placebo arm to be unethical in a high-risk population and were able to benchmark our high-dose against the latest IOM RDA, an important comparison. Subgroup analyses in subjects with frankly low 25(OH)D concentrations also led to essentially similar negative findings. The 2 indexes of insulin resistance used in our trial have been shown to correlate with insulin sensitivity when validated against an intravenous glucose tolerance/insulin clamp in healthy individuals, with sensitivities of 65–81%, and specificities of 87–91% (18). Finally, the proportion of subjects on statins were matched in both study groups and unchanged at 1 y, and statin use was not an important predictor of insulin resistance, both on univariate and multivariate analyses.

Our study has several strengths, including its double-blind nature, an adequate power with a large number of high-risk elderly participants, the large vitamin D dose administered over 1 y, and the use of LC-MS for serum 25(OH)D measurements. Interestingly, we noted a positive trend for a beneficial effect of high-dose vitamin D compared with 600 IU/d on changes in HOMA-IR in the subgroup of subjects with impaired fasting glucose at baseline, similar to the trend observed in 2 other studies (22, 34), observations that were not noted on primary outcomes but certainly deserving of further evaluation. They do, however, run counter to the observed higher incidence of diabetes at 1 y in the high-dose group as opposed to the low-dose group in the overall study. The increase in mean FBS and decrease in C-peptide probably reflect the natural progress of glucose tolerance in overweight elderly individuals. It is hoped that the 2 ongoing large multicenter trials D2d (Vitamin D and Type 2 Diabetes) and VITAL (Vitamin D and Omega-3) will shed definite light on the putative beneficial effect of high-dose vitamin D on glucose metabolism (47, 48).

In conclusion, vitamin D at a dose equivalent to 3750 IU/d did not improve indexes of insulin resistance compared with the IOM RDA of 600 IU/d in high-risk, overweight, elderly subjects after 1 y of treatment. Our study adds to the increasing body of evidence on this topic and provides the basis to recommend against the use of doses of vitamin D that exceed the current RDA with the aim of decreasing IR in high-risk individuals.

We thank Ulfat Usta, Heike Bischoff-Ferrari, Christopher Gallagher, and Reinhold Vieth for their time and dedication.

The authors' responsibilities were as follows—GE-HF, RB, RHH, and ZM: conceived the trial and designed the study; RB, GH, MR, and MH: recruited the subjects; AA, MR, and MH: executed the trial; GE-HF, RHH, MR, RJS, MK, MH, RTD, and M-FK: entered the data and conducted the analyses; GE-HF, RHH, and MR: wrote the manuscript; and all authors: read

and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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