Vitamin D3 Effects on Lipids Differ in Statin and Non-Statin-Treated Humans: Superiority of Free 25-OH D Levels in Detecting Relationships

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Context: Inverse associations between 25-OH vitamin D levels and cardiovascular morbidity and mortality have been reported.

Objectives: Our goals were to 1) investigate effects of correcting inadequate D status on lipids, 2) determine whether free 25-OH D is better correlated with lipids than total 25-OH D.

Design: A randomized, double-blind placebo-controlled trial was performed.

Setting: Participants resided in the general community.

Participants: Adults with inadequate D status were randomized to D3: 14 men, 12 women, age 60 ± 8 years (mean ± SD) or placebo: 12 men, 11 women: 59 ±12 years.

Intervention: Responses to 12-week oral vitamin D3 titrated (1000–3000 IU/d) to achieve 25-OH D levels ≥25 ng/mL were compared to placebo.

Main Outcome Measures: Measurements were 25-OH D (tandem mass spectometry), free 25-OH D (direct immunoassay), lipids (directly measured triglyceride, cholesterol, and subfractions; plant sterols and cholesterol synthesis precursors), and safety labs before and after 6 and 12 weeks D3 or placebo. Data were analyzed by repeated measures ANOVA and linear regression.

Results: Vitamin D3 was titrated to 1000 IU/d in 15/26 (58%), to 2000 IU/d in 10, and 3000 IU/d in one patient. D3 had no effect on cholesterol or cholesterol subfractions except for trends for decreases in atorvastatin-treated patients (cholesterol, $P = .08$; low-density lipoprotein [LDL] cholesterol, $P = .05$). Decreased campesterol concentrations ($P = .05$) were seen with D3 but not placebo in statin-treated patients. Relationships between total 25-OH D and lipids were not detected, but inverse linear relationships were detected between free 25-OH D and triglycerides ($P = .03$ for all participants [n = 49], $P = .03$ in all statin-treated [n = 19], and $P = .0009$ in atorvastatin-treated [n = 11]), and between free 25-OH D and LDL cholesterol ($P = .08$ overall, $P = .02$ in all statin-treated, and $P = .03$ for atorvastatin-treated), and total cholesterol ($P = .09$ overall; $P = .04$ for all statin-treated, and $P = .05$ for atorvastatin-treated).

Conclusions: Vitamin D lipid-lowering effects appear limited to statin-treated patients and are likely due to decreased cholesterol absorption. Relationships between lipids and D metabolites were only detected when free 25-OH D was measured, suggesting the superiority of determining free 25-OH D levels compared to total 25-OH vitamin D levels when analyzing biologic responses. **(*J Clin Endocrinol Metab* 98: 4400–4409, 2013)**

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Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; GC, gas chromatography; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low-density lipoprotein; LPa, lipoprotein a; NIST, National Institutes of Standards and Technology; ODS, Office of Dietary Supplements; VLDL, very low-density lipoprotein.

A role for vitamin D in the atherosclerotic process has been hypothesized as serum vitamin D levels have been inversely correlated with the degree of coronary artery calcification as determined by cardiac computed tomography, prevalence of hypertension, diabetes, obesity, high serum triglyceride levels, and cardiovascular and overall mortality (1–4). The underlying mechanism of such benefits has not been established.

We previously investigated the effects of oral vitamin D supplementation (800 IU/d: 400 IU D3 and 400 IU D2 as part of multivitamins and combined with calcium) in patients receiving atorvastatin in an open-label crossover study (5). Although not an entry criteria for our study, most participants were vitamin D deficient in the absence of supplementation. We hypothesized that CYP3A-metabolized atorvastatin concentrations would decrease during vitamin D supplementation and that cholesterol levels would increase. We found the hypothesized decrease in atorvastatin concentrations during vitamin supplementation but unexpectedly low-density lipoprotein (LDL) cholesterol and total cholesterol were significantly lower during combined atorvastatin and vitamin D administration despite lower atorvastatin concentrations. The findings suggested a vitamin D effect on cholesterol metabolism or transport.

Strong cross-sectional associations between higher 25-OH vitamin D levels and lower total cholesterol, lower LDL cholesterol, higher high-density lipoprotein (HDL) cholesterol, and lower triglycerides have been reported from a large community database with a longitudinal analysis that showed increasing 25-OH D levels affected total and HDL cholesterol levels but not LDL cholesterol and triglyceride levels (6). Prospective data on effects of vitamin D on lipids are limited with differing results in those that have been reported (7–14). Vitamin D has been reported to lower triglyceride levels and to a lesser extent cholesterol concentrations as well as having no effect on these measures in small studies that largely lacked blinding or placebo groups (7–14). The studies used relatively low doses of oral vitamin D3 (300–1000 IU/d), oral 1a,25-$(OH)_2$ vitamin D, or iv 1a,25-$(OH)_2$ vitamin D for relatively short periods of time and only one assessed circulating vitamin D responses (with nonstandardized assays). A more recent study of weekly 50 000 IU D3 for 8 weeks in middle-aged men and women with baseline vitamin D deficiency and at risk of cardiovascular disease reported no significant effects on total cholesterol, LDL cholesterol, small LDL particle number, HDL cholesterol, or triglycerides with mean 25-OH vitamin D levels of 43 ± 12.3 ng/mL. Increases in calcium were positively related to LDL cholesterol and inversely related to serum parathyroid hormone (15). These data suggest a potentially negative clinical effect of vitamin D supplementation on LDL cholesterol.

Our primary goal in the current investigation was to determine effects of individual D3 dose titration with daily oral D3 to correct vitamin D inadequate states on lipid profiles. A secondary goal was to determine the relationship between free 25-OH vitamin D and lipid concentrations.

Methods

Overall design

This was a 12-week double-blind placebo-controlled dose titration study of the effects of D3 on lipid levels.

Subject selection

Subjects were vitamin D–deficient (see Subject enrollment) adults able to provide informed consent, able to take oral noncrushed pills or capsules, clinically stable (no changes in medications/diagnoses within a month, or hospitalization within 6 months), no severe renal disease (estimated glomerular filtration rate [eGFR] $\geq$28 mL/min/m^2), no hypercalcemia or history of hypercalcemia, no history of recent renal stones or sarcoid, no history of osteoporotic fracture, or uncontrolled thyroid or parathyroid disorder, or intestinal bypass surgery or resection of small bowel, granulomatous disease, active malignancy other than nonmelanoma skin cancer, detectable viral load (if HIV-infected), hematocrit $<$30% for women or $<$34% for men, or contraindications or allergy to vitamin D.

Subject enrollment

After informed consent was obtained for the protocol approved by the University of California, San Francisco Committee on Human Research, demographic and medical data were collected, including age, sex, race, height, weight, body mass index (BMI), renal status (eGFR), and disease states (bone disease [osteopenia, osteoporosis, fractures], cardiovascular disease, diabetes, thyroid, cancer, gastric resection, history of malabsorption disease), concomitant medications, nutraceuticals, and sun exposure assessed with a sunshine questionnaire (16). Blood was drawn on two baseline occasions at least 1 week apart to confirm vitamin D deficiency (25-OH vitamin D levels $<$25 ng/mL; Mayo Clinical Laboratories, http://www.mayomedicallaboratories.com/). Additional tests included creatinine, calcium, phosphorus, glycosylated hemoglobin (HbA_{1c}) in persons with diabetes, HIV viral load (in HIV-positive subjects taking protease inhibitors), and fasting complete lipid profile, LDL and HDL subfractions, very low-density lipoprotein (VLDL) and/or lipoprotein a (LPa) concentrations, and triglycerides (VAP test; Atherotech Diagnostics Lab, http://www.atherotech.com). Enrollment was concentrated in spring and summer (84% of participants).

Randomization, study conduct, and vitamin D dose titration

Subjects were randomized to vitamin D3 1000 IU/d (1 capsule) or matching placebo, stratified by sex, and instructed to take capsules with the fattiest meal of the day. Subjects with

severe vitamin D deficiency (<10 ng/mL) were not randomized but could be assigned to 1000 IU/d D3. Subjects were interviewed every 2 weeks for health status (illnesses, physician visits, medication changes, self-reported health status, side effects), and study-related dispensing. After 6 and 12 weeks, fasting blood samples were obtained for 25-OH vitamin D, calcium, lipids, weight, and blood pressure. Dosing was 1) Vitamin D3 group a) with baseline 25-OH vitamin D concentrations of ≤20 ng/mL; doses were increased to 2000 IU/d (two 1000 IU capsules) if 25-OH D concentrations at week 6 were <25 ng/mL. Doses remained at 1000 IU/d (one 1000 IU and one placebo capsule) for weeks 7 to 12 if measured 25 OH-D was ≥25 ng/mL. b) For those with baseline 25-OH vitamin D concentrations of 21–25 ng/mL, D3 dose was increased to 2000 IU/d if 25-OH vitamin D level was not increased at least 25% over baseline after 6 weeks or remained at 1000 IU/d (plus matching placebo) for weeks 7 to 12 if 25-OH D increased greater than 25% of baseline. After 12 weeks, if target 25-OH D levels were not reached, subjects could enroll in a 6-week open-label extension and receive 3000 IU/d. 2) Placebo group. Subjects randomized to placebo received one placebo capsule during weeks 1 to 6 and two placebo capsules for weeks 7 to 12.

Vitamin D formulations

D3 (cholecalciferol) and matching placebo were obtained from Bio-Tech Pharmacal, Inc (www.Bio-Tech-Pharm.com) with validated vitamin D content at formulation, and after 1 and 2 years. Independent content analysis was performed before dispensing and every 12 weeks thereafter (Tai C. Chen, PhD, CTSI, Boston University).

Testing Procedures/Measurements

25-OH Vitamin D3 + D2 measurements were determined by Clinical Laboratory Improvements Amendment certified liquid chromatography tandem mass spectrometry at Mayo Clinical Laboratories with participation in Office of Dietary Supplements-funded National Institutes of Standards and Technology (NIST) quality assurance program for analysis of vitamin D metabolites in human serum. The assay has ~10% coefficient of variation at levels <10 ng/mL. Internal standard is NIST reference standard.

Free (unbound) 25-OH vitamin D concentrations were determined by immunoassay (Future Diagnostics BV, http://www.future-diagnostics.nl/) (17).

D3 capsule content

Analyses of capsule content were performed by HPLC after saponification and correction of the yield according to recovery of added external D3 (NIST) standard (Tai C. Chen, PhD, CTSI, Boston University) (18, 19).

Dietary vitamin D intake

Dietary vitamin D and cholesterol intake were estimated by Block 2005 Food Frequency l questionnaire (NutritionQuest) (20).

Lipid and cholesterol subfraction concentrations

Total and cholesterol-r(real), LDL and subfractions, HDL and subfractions, VLDL and/or LPa concentrations, and triglycerides were determined (Atherotech Diagnostics Lab, http://www.atherotech.com) (21).

Total plasma cholesterol precursor lathosterol and the plant sterol campesterol were determined by gas chromatography (GC)-mass spectrometry-selected ion monitoring using epicoprostanol as internal standard. Cholesterol was measured from the same plasma sample by GC-flame ionization detection using 5α-cholestane as internal standard (22, 23).

Safety measures

Calcium, phosphorus, creatinine, and HbA_{1c} testing were performed by Quest Diagnostics (http://www.questdiagnostics.com). HIV viral copy number was performed in clinical laboratories of San Francisco General Hospital (http://labmed.ucsf.edu/sfghlab/).

Other Measurements

Weight and height were measured using a balance beam scale. BMI was calculated (weight [kg] divided by height^2 [M]). Blood pressure and heart rate were measured using automated aneroid devices.

Adherence was determined from pill counts.

Statistical design and data analysis

Demographic and baseline characteristics of groups are presented as mean ± SD and compared using ANOVA or Kruskal-Wallis for continuous variables, as appropriate, and χ^2 tests for categorical variables. Effects of D3 compared to placebo were determined by repeated measures ANOVA. A priori sample size estimates for the main effect (vitamin D vs placebo) were based on our prior report (5) with an effect size of 0.3, intracluster correlations of 0.5, and analysis by repeated measures ANOVA with $\alpha = .095$, $\beta = .8$, yielding an estimated total sample of 58. Relationships between total or free unbound 25-OH vitamin D and lipid concentrations were tested by linear regression.

ClinicalTrials.gov

The study was registered with http://clinicaltrials.gov as NCT 00723385.

Results

Subjects

Fifty-six subjects had total 25-OH D levels <25 ng/mL on initial screening with two ineligible due to 25-OH D concentrations ≥25 ng/mL on repeat baseline measurement. Fifty-four subjects underwent randomization stratified by sex and season; there was one subject dropout due to physical relocation and four were removed due to study nonadherence (sunburn at visits, stated nonadherence with capsule intake, stated significant change in diet). Data were analyzed for 49 subjects (a priori power calculations for this sample size estimates power of avoiding a type II error of 0.7). Twenty-six received vitamin D3 (14 men and 12 women; 19 Caucasian, 5 African American, 2 Asian) and 23 received placebo (12 men and 11 women; 15 Caucasian, 6 African American, 1 Asian, 1 other). Subject characteristics are summarized in Table 1. The D3 and placebo group were similar in age, weight, medical status,

Table 1. Subject Characteristics at Baseline

	Vitamin D3 (n = 26)	Placebo (n = 23)	Between Group Difference
Age, y	60 ± 8	59 ± 12	ns
Weight, kg	88.4 ± 24.4	82.7 ± 19	ns
Height, cm	169.4 ± 9.1	165.2 ± 9.4	ns
BMI, kg/m^2	30.7 ± 7.6	30.4 ± 7.4	ns
Creatinine, mg/dL	1.0 ± 0.4	0.9 ± 0.2	ns
eGFR, mL/min m^{2a}	78 ± 22	82 ± 20	ns
Charlson Comorbidity Score	3.4 ± 2.5	3.4 ± 4.1	ns
Diabetes	4	6	ns
Hypertension	11	14	ns
Coronary artery disease	4	4	ns
Heart failure	3	2	ns
Number of daily medications	5.2 ± 5.1	5 ± 5.5	ns
Receiving HMG-CoA reductase inhibitor, n	9	10	ns
Vitamin D from diet, IU/d	111 ± 74	128 ± 74	ns
Estimated sun exposure/d, h	2.9 ± 2.7	2.9 ± 2.7	ns
Calcium, mg/dL	9.4 ± 0.4	9.5 ± 0.4	ns
Serum 25-OH Vitamin D, ng/mL	16.2 ± 4.5	16.7 ± 4.4	ns
Cigarette smoker	2	4	
Alcohol drinker	17	10	
Birth control pills	1	0	
Diuretic	7	4	

Data are mean ± SD. ns, no significant difference.

[a] Modification of diet in renal disease formula.

renal status, estimated dietary intake of D, estimated sun exposure, and baseline 25-OH vitamin D levels. There were few smokers (two in the vitamin D group reporting smoking about one-half pack per day and 4 in the placebo group reported smoking about one pack per day) and more subjects reported alcohol intake in the vitamin D3 group. Of the 17 in the D3 group reporting alcohol consumption, 5 consumed it daily and 12 less frequently (8 on a weekly basis, 3 on a monthly basis, and 1 annually). For the 10 placebo group subjects reporting alcohol consumption, consumption was daily in 2 and less frequently in 10 (weekly in 4, monthly in 4, and once yearly in 2). One woman in the D3 group took birth control pills (estrogen and progesterone). No other sex hormones were taken. Other medications taken by more than two subjects per group were: 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (D3 group n = 9, placebo n = 10; more frequent in persons with diabetes than nondiabetics [$P < .001$]), diuretics (D3 n = 7, placebo n = 4; more frequent with hypertension [$P < .001$]), angiotensin-converting-enzyme inhibitors (D3 n = 4, placebo n = 7); aspirin (D3 n = 5, placebo = 6), nonsteroidal anti-inflammatory drugs (D3 n = 4, placebo = 1), thyroid (D3 n = 4, placebo n = 5), insulin (D3 n = 3, placebo n = 1), proton pump inhibitors (D3 n = 3, placebo n = 3).

Study capsule content

Mean content of capsules during the period subjects received capsules was 917 ± 168 IU per capsule.

Vitamin D3 doses and resultant 25-OH vitamin D concentrations (see Table 2)

Final vitamin D3 doses were 1000 IU/d (25 μg/d) in 15/26 (58%), 2000 IU/d (50 μg/d) in 10 (38%), and 3000 IU/d (75 μg/d) in 1 participant. Dietary vitamin D intake did not differ between groups titrated to 1000 or 2000 IU/d (110 ± 71 and 84 ± 28 IU/d, respectively). The D3 dose to achieve target 25-OH D levels was positively related to weight ($P < .03$) and BMI ($P < .008$), and higher in subjects with hypertension ($P < .05$) and/or receiving diuretics ($P < .06$). Diuretics were used in all but one subject with hypertension so effects of hypertension or diuretic use could not be distinguished. No effect of age, diabetes, statin intake, thyroid disease, heart failure, angiotensin-converting-enzyme inhibitors, aspirin or nonsteroidal anti-inflammatory drugs, smoking status, alcohol intake, or season of enrollment and participation was detected on dose requirements to reach target 25-OH vitamin D concentrations.

At study end, 25-OH vitamin D concentrations were about double baseline values in the vitamin D3 group (from 16.2 ± 4.5 to 32.7 ± 6.2 ng/mL, $P < .0001$) and unchanged in the placebo group (from 16.7 ± 4.4 ng/mL at entry to 17.9 ± 8.3 at study end). Free 25-OH vitamin D levels at baseline ranged from 1.7 to 4.5 pg/mL and were undetectable in one participant. Free 25-OH levels increased with D3 to a mean of 5.7 ± 1.1 pg/mL ($P < .0001$; range 1.6 to 7.9 pg/mL) at study end and were unchanged

Table 2. Responses to Vitamin D3 or Placebo

	Baseline	Mid Study	Study End	Between Group Differences in Responses[a]
Total 25-OH vitamin D, ng/mL				
Vitamin D3 group	16.2 ± 4.5	28.2 ± 6.1	32.7 ± 6.2	$P < .0001$
Placebo group	16.7 ± 4.4	18.2 ± 7.1	17.9 ± 8.3	
Free 25-OH vitamin D3, pg/mL				
Vitamin D3 group	2.9 ± 0.9	4.7 ± 1.1	5.7 ± 1.1	$P < .0001$
Placebo group	2.9 ± 0.7	3.2 ± 0.8	3.2 ± 1	
25-OH Vitamin D2, ng/mL				
Vitamin D3 group	0.4 ± 1.8	0.04 ± 0.01	0.5 ± 2.1	ns
Placebo group	0.5 ± 2.2	0.8 ± 2.4	1.2 ± 2.9	
Calcium, mg/dL				
Vitamin D3 group	9.4 ± 0.4	9.4 ± 0.4	9.3 ± 0.4	ns
Placebo group	9.4 ± 0.4	9.3 ± 0.5	9.3 ± 0.4	

Data are mean ± SD. ns, no significant difference.

[a] Repeated measures ANOVA.

with placebo (see Table 2). 25-OH vitamin D2 levels were detectable in four subjects at baseline (three assigned to D3 and one assigned to placebo) and two at study end (both assigned to placebo).

Lipid responses by group assignment

Vitamin D3 did not significantly affect lipids or cholesterol subfractions of LDL and HDL, intermediate density lipoproteins, LDL bound to C-reactive protein, or remnant fractions. Total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, LPa and triglyceride concentrations for vitamin D3, and placebo are shown in Supplemental Tables 1 and 2, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. As results differed from our observation in patients receiving vitamin D with atorvastatin, responses of atorvastatin-treated participants (D3 group n = 5 receiving 45 ± 25 mg/d; placebo, n = 6 receiving 43 ± 27 mg/d) were analyzed. Lipid concentrations were lower in patients receiving atorvastatin compared to those not receiving atorvastatin throughout (repeated measures ANOVA, $P < .001$). Vitamin D3 in the atorvastatin-treated patients lowered total cholesterol 12% ($P = .06$) and LDL cholesterol 14% ($P = .05$) compared to placebo (Figure 1).

To investigate potential underlying mechanisms for decreases in LDL cholesterol, surrogate serum markers of cholesterol absorption (campesterol) and endogenous whole body cholesterol biosynthesis (lathosterol) were examined in the statin-treated participants (n = 21, 19 completed the study and 2 with baseline and midstudy data) and a subset (n = 6) of non-statin-treated participants for whom serum were available. Results are presented in Figure 2 and Supplemental Table 3. Campesterol concentrations were lower after D3 administration in statin-treated patients compared to baseline (from 0.710 ± 0.41 to 0.582 ± 0.31) while unchanged in the statin-treated group that received placebo (0.408 ± 0.186 and 0.408 ± 0.173, $P = .05$ for the D3 and campesterol interaction, repeated measures ANOVA). The decrease in the ratio of campesterol to cholesterol in the D3 treated compared to placebo did not reach significance ($P = .12$). No D3 vs placebo treatment effects were detected on lathosterol concentrations. In D3-treated subjects not receiving statins, lathosterol concentrations were higher than statin-treated subjects ($P = .001$) but neither lathosterol nor

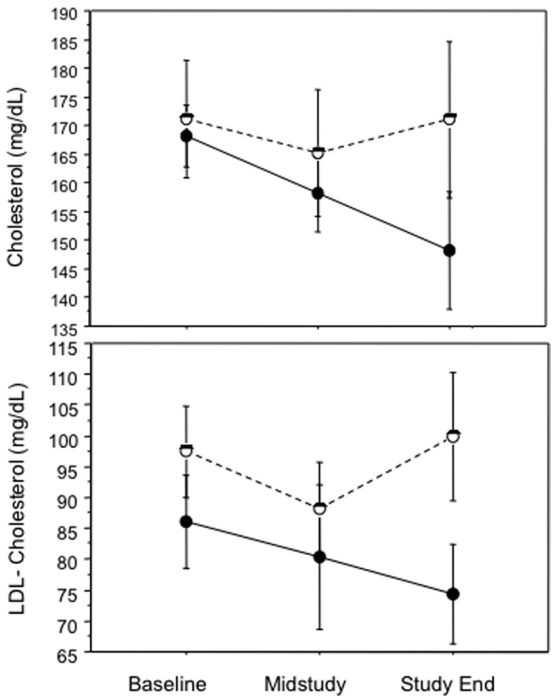

Figure 1. Mean (± SE) cholesterol responses to daily oral vitamin D3 administration (*solid circles connected by solid lines*) or placebo (*open circles connected by dashed lines*) at baseline, after at least 6 weeks (mid-study), and at least 12 weeks (study end) in atorvastatin-treated participants (*upper panel*) and responses of LDL cholesterol (*lower panel*). Differences in responses were significant ($P < .05$).

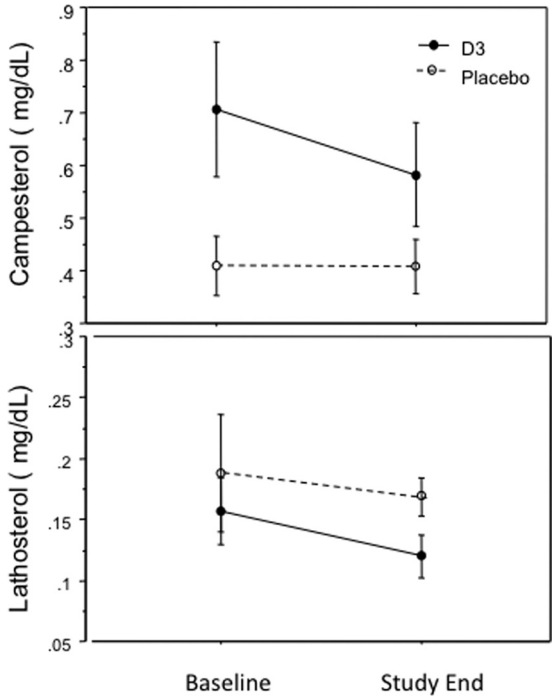

Figure 2. Mean (± SE) campesterol responses (*upper panel*) and lathosterol responses (*lower panel*) to daily oral vitamin D3 administration (*solid circles connected by solid lines*) or placebo (*open circles connected by dashed lines*) at baseline and study end for statin-treated participants are presented. Differences in campesterol responses between the D3-treated vs placebo-treated approached significance ($P = .05$).

campesterol concentrations were affected by vitamin D3 administration.

Relationships between total or free 25-OH vitamin D and lipids

No relationships were detected between circulating total 25-OH vitamin D levels and lipid levels (triglyceride [$P = .62$], cholesterol [$P = .67$], LDL cholesterol [$P = .52$], or HDL cholesterol [$P = .76$]). In contrast, relationships were detected between free 25-OH vitamin D concentrations and lipid parameters with D3 administration but not with placebo administration. Relationships for total cholesterol, LDL cholesterol, and triglycerides and free 25-OH concentrations at baseline and at study end for the placebo and vitamin D groups are presented in Figure 3. No significant relationships were detected at baseline but after vitamin D3 administration, inverse relationships for LDL cholesterol and free 25-OH vitamin D were significant ($P = .009$) and approached significance for total cholesterol ($P = .07$). No relationships between free 25-OH vitamin D and lipids were detected in the placebo group.

Relationships between free 25-OH vitamin D levels and total and LDL cholesterol and triglycerides were also examined for statin-treated participants. Individual data are shown in Figure 4. As more participants randomized to D3 received atorvastatin than other statins (none on pravastatin, and one on lovastatin and two on simvastatin as randomization was not stratified by statin), relationships were largely determined by atorvastatin participants. Inverse relationships were significant for free 25-OH vitamin D and triglycerides (atorvastatin users, $P = .0009$, all statin users, $P = .03$), total cholesterol (atorvastatin users, $P = .05$; all statin users, $P = .04$), and for LDL cholesterol (atorvastatin users, $P = .03$, all statin users, $P = .02$). For HDL cholesterol, no relationships were detected for all statin-treated (0.186), with a possible trend for a positive relationship in atorvastatin-treated subjects ($r^2 = 0.145$, $P = .07$).

Safety measures

Calcium concentrations did not change with D3 or placebo (Table 2) nor did phosphorus or creatinine change (data not shown). HbA_{1c} in persons with diabetes (n = 4 D3 group, n = 5 placebo) was not altered during the study. Five HIV-infected patients with undetectable viral load on protease inhibitors had viral load undetectable throughout except for a blip at midstudy (60 detectable copies) in one with return to nondetectable at 12 weeks.

Vital signs

Weight and heart rate were unchanged during the study. Systolic blood pressure decreased slightly but significantly ($P < .04$) for both the placebo and the vitamin D group: from 128 ± 21 mmHg to 123 ± 15 after 6 weeks and 124.8 ± 22 mmHg in the D3 group and from 125 ± 15 mmHg to 118 ± 14 mmHg after 6 weeks and 124 ± 17 mmHg after 12 weeks in the placebo group.

Adherence

Mean adherence was 98 ± 6% in the vitamin D3 group and 97 ± 7% in the placebo group.

Discussion

Vitamin D plays a role in the regulation of hundreds of genes involved in bone and mineral metabolism, the renin-angiotensin-aldosterone system, the immunologic system, the cardiovascular system, muscle metabolism and strength, cellular proliferation and differentiation, and survival of cells in disorders such as cancer (24–26). With increased recognition of the role of vitamin D in health and disease has come the incentive to assure adequate vitamin D status in people.

We investigated effects of administering vitamin D3 to correct vitamin D inadequacy on lipid concentrations. Based on our prior observations that LDL cholesterol and total cholesterol were significantly lower during combined atorvastatin and vitamin D administration compared to

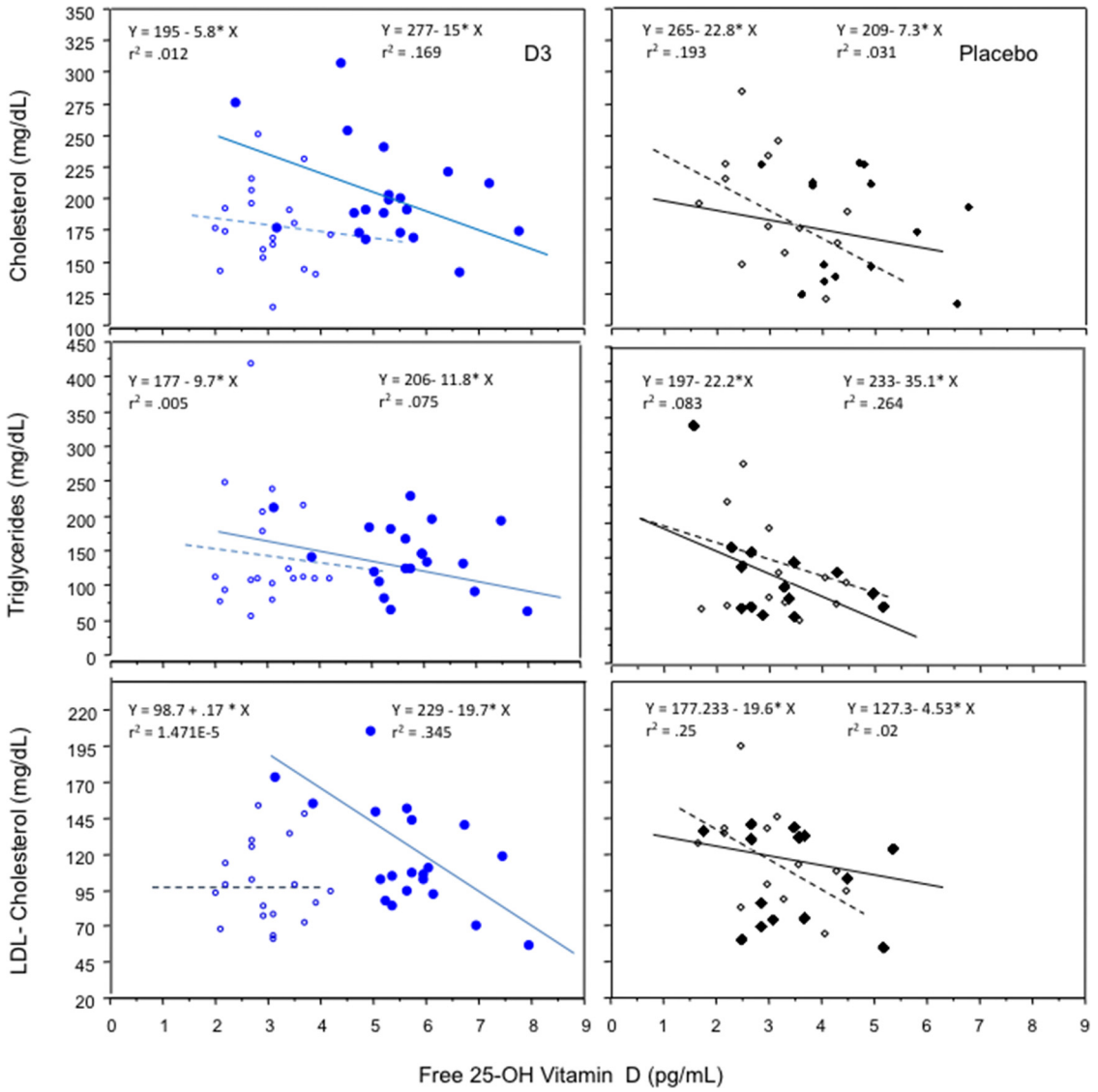

Figure 3. Relationships between free 25-OH vitamin D concentrations and cholesterol (total), triglycerides, and LDL cholesterol are presented for the D3-supplemented group at baseline (*open circles* and *dotted lines*) and at the end of supplementation (*closed circles* and *solid lines*) (*left panels*) and for the placebo group at baseline (*open triangles* and *dotted lines*) and at the end of the study (*closed triangles* and *solid lines*) (*right panels*). Regression results are presented with baseline values above the 0 point and relationships at study end toward the right of each panel. Significant relationships existed after D3 supplementation for LDL cholesterol ($P < .007$) and approached significance for total cholesterol ($P < .08$). At no time points were significant relationships detected in the placebo-treated subjects, likely due to the narrow range of free 25-OH D concentrations. No significant relationships at any time were detected for total 25-OH vitamin D concentrations and lipids (data not shown).

atorvastatin without D supplementation, we hypothesized that cholesterol concentrations would be lower after vitamin D supplementation. We chose a dose titration to a target 25-OH vitamin D concentration strategy and began titration with 1000 IU/d vitamin D3 as previously 800 IU/d vitamin D did not achieve 25-OH D concentrations over 20 ng/mL in one-third of subjects (5). In this study, three participants (of 26 assigned to D3) did not increase 25-OH D concentrations above 20 ng/mL with 1000 IU/d D3 and slightly over one-third of subjects did not achieve concentrations greater than 25 ng/mL. With 2000 IU/d, all but one very obese subject achieved 25-OH D concentrations greater than 25 ng/mL.

We detected no effect of vitamin D3 supplementation and increased total circulating 25-OH vitamin D concentrations on cholesterol, cholesterol subfractions, or triglyceride measurements in the total group of subjects randomized to vitamin D3 compared to those randomized to placebo. These results are in agreement with conclusions from a recent meta-analysis of randomized studies of effects of varying doses of vitamin D on lipids (27). The randomized double-blind placebo-controlled studies included in the meta-analysis excluded patients receiving statins leaving the question of the effects of vitamin D supplementation in the large number of people receiving statins with inadequate vitamin D status unanswered (28).

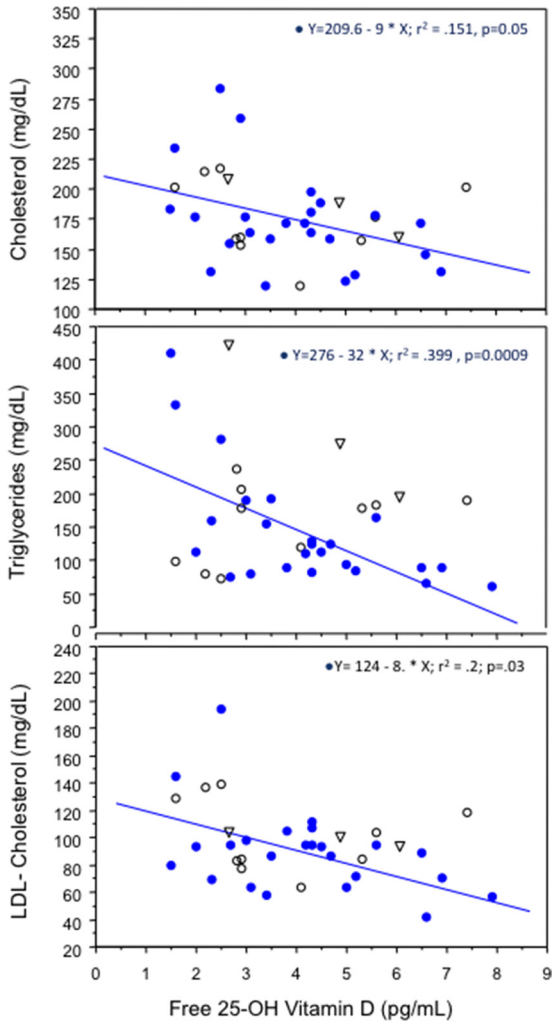

Figure 4. Relationships between circulating free 25-OH vitamin D concentrations and triglycerides, LDL cholesterol, and total cholesterol are shown for the HMG-CoA reductase inhibitor (statin) -treated participants: atorvastatin data (*solid circles*), simvasatin (*open circles*), and lovastatin (*inverted triangles*). Significant inverse relationships were detected between free 25-OH vitamin D concentrations and triglycerides, LDL cholesterol, and cholesterol concentrations (results are presented within the figures for atorvastatin-treated participants; see text for additional details).

Importantly, we found a lowering of LDL cholesterol and total cholesterol in patients receiving statins with correction of vitamin D inadequacy and identified a potential mechanism for lipid lowering in these statin-treated patients. The decreases in LDL cholesterol in atorvastatin-treated patients with baseline vitamin D inadequacy were of the same magnitude of 12% to 14% as our results in a different group of atorvastatin-treated patients (5). Potential explanations include vitamin D or vitamin D metabolite direct effects on cholesterol metabolism— either decreases in absorption or decreases in endogenous cholesterol synthesis. In vitro, vitamin D has been reported to inhibit HMG-CoA reductase in cultured human skin fibroblasts, transformed human liver cells and mouse peritoneal macrophages, and lanosterol-14-α demethylase (CYP51A1) involved in cholesterol biotransformation (29). The lack of effect of vitamin D3 to lower cholesterol in non-statin-treated participants in our studies and those of others argues against a clinically relevant inhibition of HMG-CoA reductase or cholesterol biotransformation.

In humans, although statins inhibit cholesterol synthesis, they also up-regulate cholesterol absorption (30, 31). Cholesterol balance studies of cholesterol synthesis and intestinal cholesterol absorption have demonstrated that the plasma sterol campesterol can serve as a marker of fractional cholesterol absorption and the plasma sterol lathosterol as a surrogate serum marker of endogenous whole body cholesterol biosynthesis (32). We found campesterol concentrations decreased in statin-treated participants after vitamin D3 administration compared to statin-treated participants receiving placebo that had no changes in campesterol concentrations. Furthermore, no effect of vitamin D3 administration on campesterol concentrations was seen in non-statin-treated participants. We also failed to find any effect of vitamin D3 on lathosterol concentrations compared to placebo administration. The lack of changes in lathosterol argue against a decrease in cholesterol synthesis either by direct effects or due to indirect vitamin D immunomodulatory and cytokine suppressive effects (33). The data suggest that decreased cholesterol absorption contributed to reduced LDL and total cholesterol concentrations in response to vitamin D in the statin-treated participants. Inhibition of cholesterol absorption would be predicted to be greatest in atorvastatin-treated participants as atorvastatin has greater effects on increasing cholesterol absorption compared to simvastatin (or rosuvastatin) (30, 31). A vitamin D3 effect to decrease cholesterol absorption might also have been undetected in people not receiving lipid-lowering therapy as dietary absorption contributes less to circulating cholesterol levels than endogenous production. It is also plausible that D3 effects on lipids might only be seen in patients with underlying abnormal lipid metabolism or metabolic disorders such as hypercholesterolemia or diabetes.

A unique aspect of our work is that we analyzed responses of lipid parameters in relation to circulating free as well as total 25-OH vitamin D concentrations, and in subgroups receiving or not receiving statins. We failed to detect relationships between total 25-OH vitamin D concentrations and lipids or lipid subfractions. Variation in vitamin D binding protein levels and properties affect levels of the free fraction of circulating 25-OH vitamin D available to be converted to the active 1α,25 $(OH)_2$ vitamin D moiety (34–38). If vitamin D actions are similar to that of other hormones such as testosterone, free concentrations may be more relevant to biologic responses. In

support of this hypothesis, it has been recently reported that free 25-OH vitamin D concentrations are more closely related to intact parathyroid hormone (iPTH) concentrations than total 25-OH vitamin D circulating concentrations in renal failure patients (39). At baseline, free 25-OH vitamin D concentrations were low and not detectable in some subjects. With D3 supplementation, free 25-OH vitamin D concentrations increased, and inverse relationships between free 25-OH vitamin D concentrations and triglycerides, cholesterol, and LDL cholesterol became apparent. These clinically favorable trends were seen in the vitamin D–treated group as a whole and were significant in the statin-treated participants, especially for triglycerides in atorvastatin-treated subjects. Effects may have been more apparent in atorvastatin-treated subjects as there were more participants on atorvastatin than other statins, or because persons with diabetes that are more likely to have abnormalities in lipid and triglyceride metabolism received atorvastatin than other statins. These data indicate the potential utility of free 25-OH vitamin D measurements when assessing biologic actions in vivo.

There are potential limitations to our study. The duration was relatively short, although adequate to reach steady-state circulating 25-OH vitamin D levels (40) and we did not study lower dosages previously shown to be inadequate, or higher weekly replacement regimens used in severe vitamin D deficiency. The study could not fully investigate the full spectrum of lipid effects in the statin-treated subgroup nor did we investigate individual statins prospectively. Circulating vitamin 1,α25-(OH_2) D levels were not measured because changes in circulating concentrations are not seen during vitamin D supplementation despite changes in 25-OH vitamin D and may not reflect conversion to active 1,25 OH_2 D in tissues. Results were obtained from subjects with limited and constant sun exposure representative of most adults using sun protection but may not reflect requirements of those with significant sunshine or UV exposure. Extrapolation to recommendations for commercial formulations may not be exact as commercial preparations may not have its labeled content.

In summary, our data support the existence of an interaction of vitamin D3 with lipids in HMG-CoA reductase (statin) –treated patients in directions that would be clinically favorable and that a contributing underlying mechanism is likely to be reduced cholesterol absorption. The data further suggest that this interaction is better demonstrated with free 25-OH vitamin D measurements than with total 25-OH vitamin D measurements. The results warrant further study.

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