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Journal

The American journal of clinical nutrition, 105(5)

ISSN

0002-9165

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Publication Date

2017-05-01

DOI

10.3945/ajcn.116.150367

Peer reviewed

See corresponding editorial on page 1031.

A randomized clinical trial in vitamin D–deficient adults comparing replenishment with oral vitamin D_3 with narrow-band UV type B light: effects on cholesterol and the transcriptional profiles of skin and blood[1,2]

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ABSTRACT

Background: Vitamin D deficiency, defined as a serum 25-hydroxyvitamin D [25(OH)D] concentration <20 ng/mL, is correlated with a more atherogenic lipid profile. However, oral vitamin D supplementation does not lower LDL-cholesterol concentrations or raise HDL-cholesterol concentrations. This uncoupling between association and causation may result from a failure of oral vitamin D to mimic the effect of dermally synthesized vitamin D in response to ultraviolet type B (UVB) light.

Objective: We tested the hypothesis that, in vitamin D–deficient adults, the replenishment of vitamin D with UVB exposure would lower LDL-cholesterol concentrations compared with the effect of oral vitamin D_3 supplementation.

Design: We performed a randomized clinical trial in vitamin D–deficient adults and compared vitamin D replenishment between subjects who received oral vitamin D_3 (n = 60) and those who received narrow-band UVB exposure (n = 58) ≤6 mo.

Results: There was no difference in the change from baseline LDL-cholesterol concentrations between oral vitamin D_3 and UVB groups (difference in median of oral vitamin D_3 minus that of UVB: 1.5 mg/dL; 95% CI: −5.0, 7.0 mg/dL). There were also no differences within groups or between groups for changes in total or HDL cholesterol or triglycerides. Transcriptional profiling of skin and blood, however, revealed significant upregulation of immune pathway signaling with oral vitamin D_3 but significant downregulation with UVB.

Conclusions: Correcting vitamin D deficiency with either oral vitamin D_3 or UVB does not improve the lipid profile. Beyond cholesterol, these 2 modalities of raising 25(OH)D have disparate effects on gene transcription. This trial was registered at clinicaltrials.gov as NCT01688102. *Am J Clin Nutr* 2017;105:1230–8.

Keywords: cholesterol, gene transcription, oxysterol, UV light, vitamin D, 25-hydroxycholesterol

INTRODUCTION

Vitamin D deficiency is highly prevalent and is associated with increased risks of a wide spectrum of diseases (1). Vitamin D has a well-established role in mineral metabolism and the promotion of bone health through the regulation of calcium, phosphorus, and parathyroid hormone (PTH)[7] homeostasis (2). Aside from bone health, extant clinical trial data have been conflicted regarding the benefits of supplementation for other diseases (3, 4). Despite this divergence, vitamin D repletion is a common clinical practice. With the use of the serum concentration of 25-hydroxyvitamin D [25(OH)D] as the accepted metric of vitamin D status, a common threshold for deficiency is a 25(OH)D concentration <20 ng/mL, which is consistent with the National Academies Health and Medicine Division's comprehensive review of clinical data of vitamin D and health outcomes (5).

We previously studied the relation between dyslipidemia and vitamin D status. On the basis of >100,000 records from a large clinical database, patients with 25(OH)D concentrations <20 ng/mL compared with those whose concentrations were >30 ng/mL had a more atherogenic lipid profile (i.e., higher LDL cholesterol, lower HDL cholesterol, and higher triglycerides) (6). However, from this same cohort, patients with 25(OH)D concentrations <20 ng/mL that were subsequently repleted to >30 ng/mL

[1] Supported by the Doris Duke Charitable Foundation (clinical scientist development award); the Sackler Center for Biomedicine and Nutrition Research at The Rockefeller University; the National Center for Advancing Translational Sciences (grant UL1TR001866), the NIH Clinical and Translational Science Award program; and the Center for Basic and Translational Research on Disorders of the Digestive System through the generosity of the Leona M and Harry B Helmsley Charitable Trust.

[2] Supplemental Tables 1–3 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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[7] Abbreviations used: NB-UVB, narrow-band UV type B; PTH, parathyroid hormone; RNA-seq, RNA sequencing; UVB, UV type B; 25(OH)D, 25-hydroxyvitamin D.

Received December 2, 2016. Accepted for publication January 30, 2017. First published online February 22, 2017; doi: 10.3945/ajcn.116.150367.

showed no improvement in the lipid profile. We corroborated these findings in a randomized, placebo-controlled trial of vitamin D–deficient subjects in which oral vitamin D_3 failed to improve the lipid profile despite large increases in serum 25(OH)D concentrations (7).

A plausible explanation for the uncoupling of epidemiologic and intervention-based data for vitamin D therapy and cholesterol may be due to the route of vitamin D repletion. Without fortification, foods contain scant amounts of vitamin D, and the dominant natural source of vitamin D for humans is from dermal synthesis in response to UV type B (UVB) radiation. Through photolysis in the upper layers of the skin, UVB ($\lambda = 280$–315 nm) exposure converts 7-dehydrocholesterol into pre–vitamin D, which spontaneously isomerizes to vitamin D_3. Several clinical studies have established the efficacy of the use of UVB for raising 25(OH)D concentrations (8–10).

UVB-derived vitamin D may have different metabolic effects than those of oral vitamin D. Indeed, oral vitamin D undergoes metabolism in the intestine and liver, which are the dominant sites of lipoprotein synthesis. Thus, as inferred by the cross-sectional association of vitamin D deficiency and increased LDL-cholesterol concentrations, the failure of oral vitamin D replenishment to lower LDL cholesterol may be due to different metabolic effects of UVB replenishment. To evaluate this possibility, we conducted a randomized trial in vitamin D–deficient adults to compare replenishment with oral vitamin D_3 supplementation with UVB exposure on the lipid profile.

METHODS

Study design

This study was a 6-mo, randomized, parallel-intervention study. Eligible participants were grouped in blocks of 6 with a random one-to-one allocation. Random assignments and intervention assignments were performed by a Rockefeller University Hospital pharmacist who had no contact with the participants. Subjects were selected to either receive oral vitamin D_3 (oral vitamin D_3 group) or UVB exposure (UVB group). All visits took place at the Rockefeller University Hospital between September 2012 and July 2015. The study was approved by Rockefeller University's Institutional Review Board. This trial was registered at clinicaltrials.gov as NCT01688102.

Subjects

Men and women between the ages of 18 and 70 y were recruited. Potential subjects underwent a telephone prescreen, which was followed by a nonfasting screening visit at which serum cholesterol and 25(OH)D concentrations were measured. Potential subjects were also shown the UVB phototherapy unit and were asked to confirm their willingness to participate irrespective of the group assignment. A medical history was obtained, including the use of current medications and height and weight measurements. The inclusion criterion was a 25(OH)D concentration <20 ng/mL. Exclusion criteria were as follows: a history of melanoma or nonmelanoma skin cancer; intentional UV exposure (e.g., tanning bed use) in the 2 wk before enrollment or planned use during the study period; active pregnancy; use of >400 IU vitamin D/d; a serum calcium concentration >10.5 mg/dL, phosphorus concentration >5.5 mg/dL, PTH concentration <12 pg/mL, or LDL-cholesterol concentration >190 mg/dL; a change in the dose of statin, fibrate, niacin, or ezetimibe ≤ 1 mo of enrollment; or an estimated glomerular filtration rate <30 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}.

Intervention

For the oral vitamin D_3 group, subjects were provided with an 8-wk supply of vitamin D_3 (BTR Group Inc.) and were instructed to take 50,000 IU/wk as 5 capsules of 10,000 IU taken at the same time and to record doses in a medication log, which was reviewed along with pill counts to determine compliance. Serum concentrations of 25(OH)D were checked monthly, and after the first 2 mo, subjects received an additional 50,000 IU before the next 25(OH)D assessment if their serum concentration of 25(OH)D was <35 ng/mL. For the UVB group, subjects received the treatment according to their Fitzpatrick skin type [from type I (very fair, burns easily) to type 6 (very dark, never burns)] with the use of a phototherapy unit (Daavlin) that was fitted with 37 narrow-band UV type B (NB-UVB) TL100W tubes (Philips) (11). The irradiance of the phototherapy unit was set to 2.5 mW/m^2 and was checked weekly with the use of a radiometer. Subjects were unclothed with the exception of undergarments and were provided with goggles and a shield to prevent any eye or facial exposure but otherwise received whole-body radiation. The initial dose according to skin type was as follows: skin types I and II, 45 s; skin types III and IV, 60 s; and skin types V and VI, 75 s. Subjects received a mean of 2 treatments/wk for 8 wk. Thereafter, serum concentrations of 25(OH)D were checked monthly, and subjects received an additional 4 NB-UVB treatments before the next 25(OH)D assessment if their serum concentration of 25(OH)D was <35 ng/mL. The duration of exposure increased by 10% with each subsequent treatment as tolerated, which was similar to the protocols that have been used for psoriasis phototherapy (12). The maximum exposure time was limited to 4.5 min. For subjects who experienced an adverse event that was related to NB-UVB (e.g., skin erythema), treatment was held until symptoms resolved, and the treatment was resumed at a lower dose.

Endpoints

The primary endpoint was the change in LDL-cholesterol concentrations between the initial visit and the final visit. For subjects who dropped out of the study between 2 and 6 mo, the last available measurement was used. The sample-size calculation was based on our previous randomized trial that showed an increase in the LDL-cholesterol concentration of 4 mg/dL after oral vitamin D therapy compared with after placebo intake (7). Our epidemiologic study showed that, in cross-section, individuals with 25(OH)D concentrations >30 ng/mL had an LDL-cholesterol concentration that was 5 mg/dL lower than that of individuals with 25(OH)D concentrations <20 mg/dL (6). Therefore, we estimated an effect size of 9 mg/dL between the oral vitamin D and UV groups. We determined that a sample size of 60 subjects/group would provide 80% power to detect a difference with a 1-tailed $\alpha < 0.05$.

Laboratory testing was performed by the Memorial Sloan-Kettering Cancer Center clinical laboratory concurrent with study visits with the exception of 25-hydroxycholesterol measurements. 25(OH)D concentrations were determined with the use of the

LIASON automated chemiluminescent immunoassay (DiaSorin). Lipid profiles were measured with the use of an enzyme-based platform (Siemens) on which LDL was calculated in accordance with the Friedewald equation (13). 25-Hydroxycholesterol concentrations were determined via the Lipid Metabolites and Pathways Strategy Lipidomics Core Facility at the University of California San Diego as previously described (14). Intergroup comparisons were based on samples with detectable concentrations of 25-hydroxycholesterol ($n = 46$ for oral vitamin D_3; $n = 39$ for UVB). Participants were given the option to undergo a skin biopsy, and 16 participants ($n = 9$ in the oral vitamin D_3 group; $n = 7$ in the UVB group) elected to do so.

Transcriptional analyses

Blood was collected into RNA PAXgene tubes (PreAnalytix) at baseline and the 2-mo visits. RNA was subsequently extracted with the use of the PAXgene Blood RNA Kit (PreAnalytix), and nucleic acid integrity was assessed with an Agilent Bioanalyzer. The RNA integrity number ranged from 6.9 to 9.2 with a mean value of 8.5. Total RNA was submitted to the New York Genome Center where RNA sequencing (RNA-seq) was performed in a 2×50-bp paired read format with 6 samples per lane with the use of a HiSeq 2500 sequencer (Illumina). Skin tissue was obtained with the use of a 6-mm skin-punch biopsy at baseline and the 2-mo visits. In patients who received UVB treatment, biopsies were taken from sites that were exposed to phototherapy. The biopsy site was determined according to cosmetic considerations but was generally taken from the lower abdomen or upper thigh with the second biopsy site being contralateral to the first biopsy site. RNA was extracted and hybridized as previously described (15). A microarray analysis was used to be compatible with previous validated studies of the skin transcriptome. All subjects who underwent blood or skin transcriptional profiling were part of the larger study.

For RNA-seq data, fastq files were aligned to the Genome Reference Consortium Human genome build 37 with the use of a STAR v2.3 (GitHub) aligner with default variables (16). Alignment results were evaluated with the use of the RNA-SeQC v1.17 program (Broad Institute) to ensure that all the samples had a consistent alignment rate and no obvious 5′ or 3′ bias (17). Aligned reads were summarized with the use of featureCounts v1.5.0 software (Bioconductor; bioconductor.org) and the gene model from Ensemble at the gene level (18). The DESeq2 v.1.2.10 program (Bioconductor) was applied to normalize and estimate the fold change with the use of a negative binomial distribution (19).

Microarray data were analyzed through Bioconductor packages. Specifically, CEL files were read into the R system through the Bioconductor package affy. Limma software v3.22.7 (Bioconductor) was used to estimate the fold change after robust multi-array average normalization (20).

A gene set enrichment analysis (GSEA, v2.2; Broad Institute) was performed with the use of the preranked method (21). Gene ranks for both RNA-seq and microarrays were determined by a score that was equal to the sign of the log fold change that was multiplied by the inverse of the adjusted P value. The analysis was performed with the use of the javaGSEA desktop application with the following variables: gene sets database: hallmark; permutations: $n = 1000$; enrichment statistic: classic; maximum gene set size: 5000; minimum gene set size: 15; and normalization mode: meandiv.

Statistical analysis

For nontranscriptional data, comparisons within and between groups were made with the use of the 2-tailed Mann-Whitney U test. For intergroup differences, 95% CIs were also calculated. $P < 0.05$ was considered significant. Statistical analyses were conducted with the use of Tibco S+ v8.2 and GraphPad Prism v6.07 software.

RESULTS

The study participant flowchart is shown in **Figure 1**. Subjects were recruited through Rockefeller University's centralized recruitment core facility, and 282 individuals presented for screening visits (22). On the basis of serum 25(OH)D concentrations, 117 individuals were excluded. In addition, after the study procedures were explained, 17 individuals declined to participate. The remaining 148 vitamin D–deficient subjects were randomly assigned to receive either oral vitamin D_3 (oral vitamin D_3 group; $n = 73$) or NB-UVB (UVB group; $n = 75$). Of subjects who were selected to receive oral vitamin D_3, 5 individuals were lost to follow-up, and 2 individuals withdrew consent before receiving any treatment doses. Of the remaining 66 subjects, 44 subjects in the oral vitamin D_3 group completed the full 6 mo of treatment. However, 60 subjects completed $\geq$2 mo of therapy, which was the prespecified threshold for inclusion in the analysis. Of subjects who were randomly assigned to the UVB group, 1 individual was lost to follow-up, and 5 individuals withdrew consent before receiving any treatment. Of the remaining 69 subjects, 40 subjects in the UVB group completed the full 6 mo of treatment, with 58 subjects completing $\geq$2 mo of therapy. The mean durations of treatment and follow-up were similar between groups of 5.4 mo of oral vitamin D_3 and 5.1 mo of UVB. The most frequent adverse events in the oral vitamin D_3 group were gastrointestinal distress (10% for oral vitamin D_3 compared with 3% for UVB) and constipation (7% for oral vitamin D_3 compared with 2% for UVB). The majority of participants in the UVB group tolerated the radiation without incident, although other subjects experienced adverse events that were related to skin erythema (21% for UVB compared with 0% for oral vitamin D_3) and skin irritation (14% for UVB compared with 2% for oral vitamin D_3).

Baseline characteristics of subjects who were analyzed in the 2 groups are shown in **Table 1**. Compared with subjects who received UVB, the oral vitamin D group was significantly older, had lower triglycerides, and showed a trend toward fewer persons with Fitzpatrick skin type III or IV and more persons with type V or VI. Three participants in the oral vitamin D_3 group were receiving statin treatment for dyslipidemia, and no participants in the UVB group were receiving statin therapy.

Both modalities were effective at correcting vitamin D deficiency (**Figure 2**). After 2 mo of 50,000 IU oral vitamin D_3/wk, mean 25(OH)D concentrations increased by 33 ng/mL from baseline to a final concentration of 47 ng/mL ($P < 0.0001$). After 2 mo in the UVB group, mean 25(OH)D concentrations were 27 ng/mL (a 14-ng/mL increase; $P < 0.0001$) but were significantly lower than in the oral vitamin D_3 group ($P < 0.0001$). During the maintenance period (months 2–6), subjects received

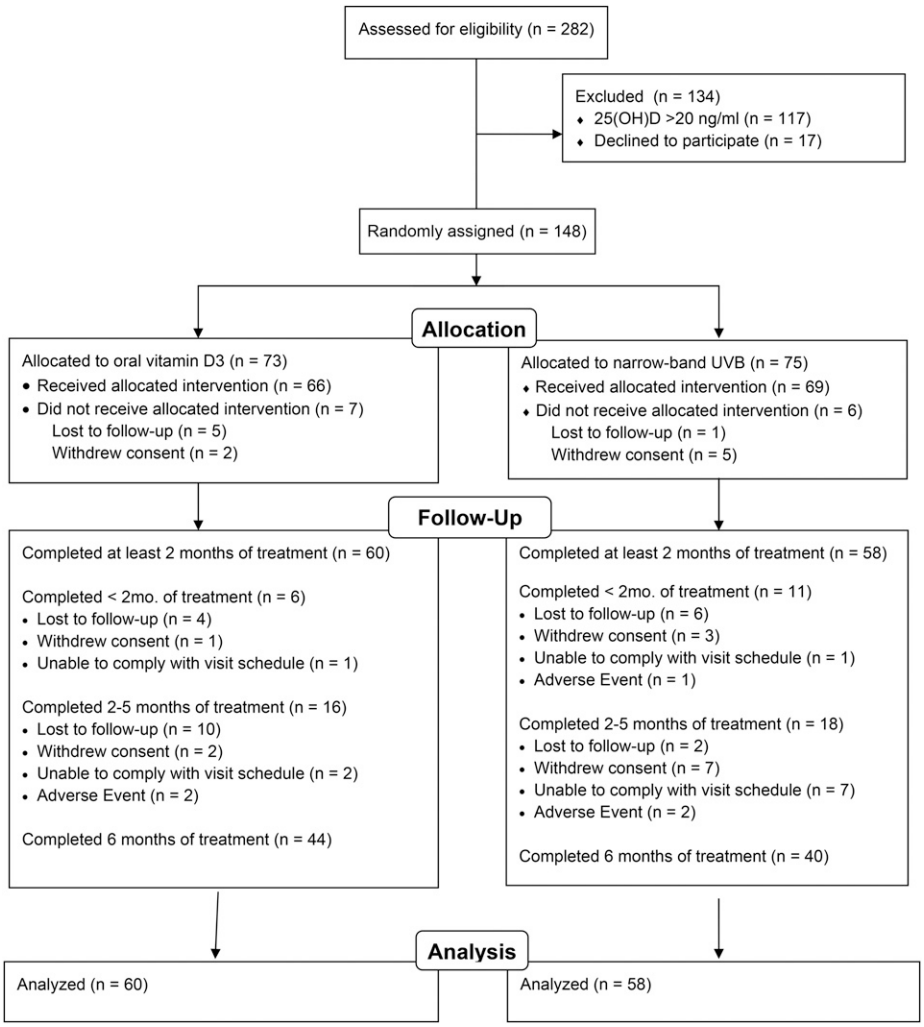

FIGURE 1 Study participant flow. Potential subjects were screened for eligibility, and a 25(OH)D concentration >20 ng/mL was the most common reason for exclusion. Eligible subjects were randomly assigned to receive either 50,000 IU oral vitamin D_3/wk for 8 wk, which was followed by supplemental doses of 50,000 IU/mo if 25(OH)D concentrations were <35 ng/mL, or a narrow-band UVB treatment delivered 2 times/wk for 8 wk with the dose adjusted on the basis of skin pigmentation with supplemental doses of 4 treatments/mo if 25(OH)D concentrations were <35 ng/mL. Participants were followed for ≤6 mo, and subjects with ≥2 mo of follow-up data were included in the primary analysis. UVB, UV type B; 25(OH)D, 25-hydroxyvitamin D.

treatment only if 25(OH)D concentrations were <35 ng/mL although the vast majority of subjects in both groups required supplemental treatment (98% in the UVB group; 92% in the for oral vitamin D_3 group). In this maintenance phase, changes in 25(OH)D concentrations from baseline were similar in the oral vitamin D_3 and UVB groups. Serum calcium and phosphorus concentrations remained stable for the duration of the study and were not clinically changed from baseline (**Figure 3**A, B). As expected, PTH values declined significantly after the acute phase of therapy [−7 pg/mL in both groups ($P < 0.001$ for oral vitamin D_3; $P < 0.01$ for UVB)] and were lower than baseline values for the duration of treatment (Figure 3C).

For the primary endpoint of the change in LDL cholesterol from baseline to the end of participation, there was no significant difference between oral vitamin D_3 and UVB groups (**Table 2**). There were also no significant differences in other components of the lipid profile. There were no differences within or between groups for LDL- and HDL-cholesterol concentrations after the initial repletion phase (through 2 mo) or during the maintenance phase (**Figure 4**).

As a steroid hormone, vitamin D regulates the expression of a broad array of genes by binding to the vitamin D–receptor transcription factor (23). Therefore, we determined the effect of oral vitamin D_3 and UVB on the transcriptional profile of peripheral blood and skin (characteristics of these participant subgroups are shown in **Supplemental Table 1**). RNA-seq was used for peripheral blood samples, and data were subsequently analyzed with the use of a gene set enrichment analysis to determine changes in signaling pathways. Gene sets that were curated on the basis of canonical vitamin D–receptor binding sequences were significantly affected by oral vitamin D_3 and UVB treatments in both the blood and skin (**Table 3**). To explore other well-established signaling pathways, we used the collection of hallmark gene sets in the GSEA program, which included 50 defined biological processes (24). Several gene sets showed similar directional changes with oral vitamin D_3 and UVB (**Supplemental Tables 2** and **3**). However, interferon-α and interferon-γ response gene sets were significantly upregulated with oral vitamin D_3 and were significantly downregulated with UVB (Table 3). This pattern was consistent for both blood and skin.

TABLE 1
Baseline characteristics[1]

	Oral vitamin D_3 group (n = 60)	UVB group (n = 58)	P
Age, y	44.7 ± 12.5	39.2 ± 12.3	0.02
Women, %	53	57	0.70
BMI, kg/m^2	28.8 ± 6.8	29.4 ± 6.9	0.61
Fitzpatrick skin type, %			0.07
I or II	10	10	
III or IV	27	47	
V or VI	63	43	
25(OH)D, ng/mL	14 ± 4	13 ± 4	0.30
Calcium, mg/dL	9.1 ± 0.3	9.1 ± 0.4	0.63
Phosphorus, mg/dL	3.4 ± 0.5	3.7 ± 0.9	0.11
PTH, pg/mL	54 ± 24	56 ± 34	0.73
Total cholesterol, mg/dL	171 ± 27	179 ± 33	0.14
HDL cholesterol, mg/dL	59 ± 15	56 ± 17	0.36
LDL cholesterol, mg/dL	97 ± 26	104 ± 26	0.17
Triglycerides, mg/dL	74 ± 36	94 ± 53	0.02
hs-CRP, mg/L	3.0 ± 4.2	3.7 ± 4.6	0.44

[1] Values are means ± SDs unless otherwise noted. Intergroup comparisons were made with the use of the Mann-Whitney U test. hs-CRP, high-sensitivity C-reactive protein; PTH, parathyroid hormone; UVB, UV type B; 25(OH)D, 25-hydroxyvitamin D.

We measured serum oxysterol concentrations as a proof of concept that transcriptionally active metabolites, aside from vitamin D, are produced by dermal UVB exposure and detected in the circulation. Mean serum concentrations of 25-hydroxycholesterol rose in UVB-treated individuals after 2 mo of treatment but fell in the oral vitamin D_3 group (1.5 ± 9.3 ng/mL in the UVB group compared with −2.6 ± 10.2 ng/mL in the oral vitamin D_3 group; P-UVB compared with oral vitamin D_3 < 0.05).

DISCUSSION

We conducted a randomized controlled trial to determine whether vitamin D repletion through UVB radiation had a distinct metabolic effect compared with that with intake of oral vitamin D_3. Both methods significantly raised serum 25(OH)D concentrations into an acceptable range, thereby effectively doubling pretreatment values. However, UVB treatment over a 6-mo period did not improve LDL-cholesterol concentrations or other lipid variables, which was similar to the effect of oral vitamin D. This result is congruent with previous studies in which increases in 25(OH)D concentrations failed to change the lipid profile despite strong cross-sectional associations between these variables. Thus, our study further supports the notion that correcting vitamin D deficiency does not improve the lipid profile and has addressed the mode of repletion as a potential confounding variable. Nevertheless, these results do not answer why lipid variables and 25(OH)D are so strongly associated with each other.

Although we controlled for several variables, there are limitations to our study. We did not include a placebo group, and thus, it is conceivable that UVB or oral vitamin D may have had a significant effect on lipids in comparison with the effect of no treatment alone. We used weekly doses of oral vitamin D_3 to improve compliance, although daily doses may have had different effects on lipid metabolism, particularly regarding the cholecalciferol-mediated effects that were independent of 25(OH)D. In addition, supplemental doses of UVB and oral vitamin D were required for nearly all participants to maintain their 25(OH)D concentrations. However, some subjects required more doses than other subjects did, and therefore, the total dose varied in participants. This analysis was further complicated by the absence of the monitoring of environmental UV exposure. In our previous study of vitamin D–deficient subjects, there was no change in 25(OH)D concentrations in placebo-treated participants. We did not track physical activity or diet throughout the study, and both factors may have influenced the lipid profile. However, we monitored weight, and there was no intergroup or intragroup change in BMI. Also, our power calculation was based on an effect size of a change in the LDL-cholesterol concentration of 9 mg/dL. This threshold was chosen on the basis of 2 previous studies, one of which was a large observational study that identified a 5-mg/dL-higher LDL-cholesterol concentration in individuals with 25(OH)D concentrations <20 ng/mL compared with individuals whose concentrations were >30 ng/mL, and the other of which was small randomized trial of vitamin D repletion that showed a nonsignificant 4 mg/dL increase in LDL cholesterol after repletion compared with after placebo intake. If we chose an effect size of 5 mg/dL, a larger study population would have been required for adequate power.

A survey of transcriptional activity in blood and skin showed that oral vitamin D and UVB exposures have overlapping although distinct biologic effects. Interferon signaling was consistently upregulated with oral vitamin D and downregulated with UVB. More generally, several other immune signaling pathways were downregulated in blood after UVB treatment including complement, inflammatory-response, and IL-6 signaling pathways; meanwhile complement signaling and IL-6 signaling were upregulated with oral vitamin D in the skin. Therefore, although some measures, such as declines in serum PTH, may be independent of the mode of repleting 25(OH)D concentrations, other physiologic responses behave diametrically. Dermally synthesized vitamin D and oral vitamin D may differ in terms of tissue exposure. Vitamin D that is synthesized in the epidermis may have a much greater influence over skin immune cells, whereas

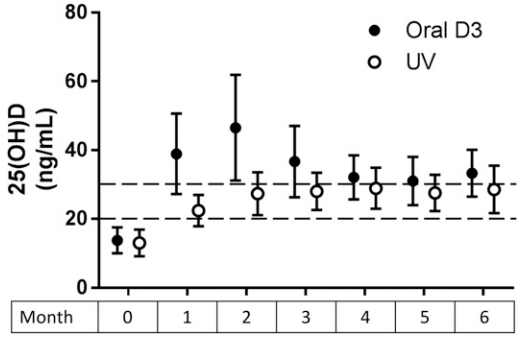

FIGURE 2 Mean ± SD longitudinal 25(OH)D concentrations by month after treatment with either oral D3 or UV. Dashed lines indicate common clinical thresholds of vitamin D status with <20 ng/mL indicating vitamin D deficiency and >30 ng/mL indicating replete. The number of subjects in each group over time is also shown. 25(OH)D concentrations rose significantly with both modalities. After 2 mo of active repletion, the oral D3 group had higher 25(OH)D concentrations than the UV group did, although after the maintenance phase, there was no difference between groups in terms of the change from baseline 25(OH)D concentrations (per-protocol analysis). In the oral D3 group, n = 60 at months 0–2, n = 57 at month 3, n = 53 at month 4, n = 51 at month 5, and n = 44 at month 6. In the UV group, n = 58 at months 0–2, n = 53 at month 3, n = 46 at month 4, n = 42 at month 5, and n = 40 at month 6. D3, vitamin D_3; UV, narrow-band UV type B; 25(OH)D, 25-hydroxyvitamin D.

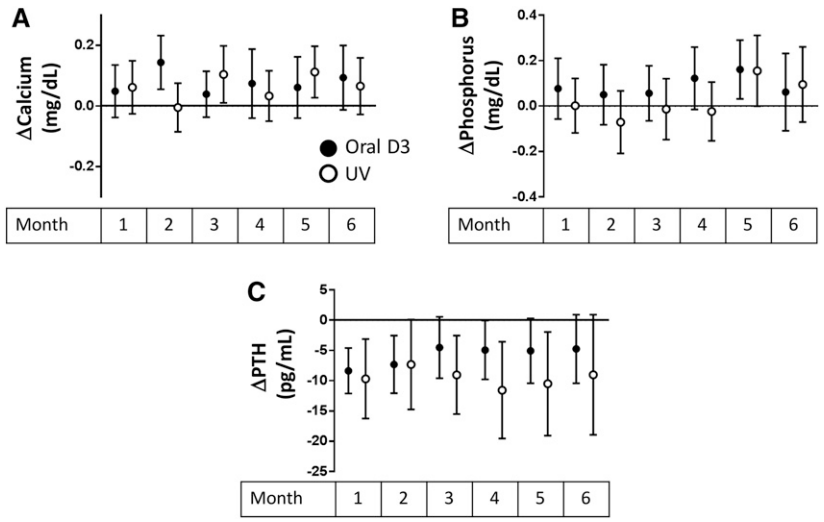

FIGURE 3 Mean ± SD longitudinal changes by month after treatment with either oral D3 or UV for serum calcium concentrations (A), serum phosphorus concentrations (B), and serum PTH concentration (C). Neither calcium nor phosphorus concentrations changed from baseline values either during the active repletion phase or the maintenance phase. However, PTH concentrations showed a significant, early, and sustained decline from baseline with either modality of vitamin D repletion (per-protocol analysis). In the oral D3 group, $n = 60$ at months 0–2, $n = 57$ at month 3, $n = 53$ at month 4, $n = 51$ at month 5, and $n = 44$ at month 6. In the UV group, $n = 58$ at months 0–2, $n = 53$ at month 3, $n = 46$ at month 4, $n = 42$ at month 5, and $n = 40$ at month 6. D3, vitamin D_3; PTH, parathyroid hormone; UV, narrow-band UV type B.

oral vitamin D would likely have a stronger effect on enterohepatic metabolism. Another plausible explanation for the disparate actions of the 2 modes of raising 25(OH)D concentrations is that UVB exposure generates bioactive molecules that, similar to vitamin D, can exert systemic effects. Oral vitamin D does not mimic this activity. For example, UV can convert *trans*-urocanic acid to *cis*-urocanic acid, which is an immunosuppressant (25). In a targeted approach that was focused on oxysterols, we detected a significant increase in serum concentrations of the liver X receptor agonist 25-hydroxycholesterol in UVB-treated compared with oral vitamin D-treated subjects. Indeed, 25-hydroxycholesterol and other oxysterols are produced by keratinocytes in response to UVB (26). Therefore, multiple mechanisms may differentiate oral vitamin D from UV exposure.

The carcinogenic potential of UV exposure mandates that chronic phototherapy provides a benefit that significantly outweighs risk. Although excessive exposure results in erythema, inflammation, and malignancy, suberythemal doses of UVB have been used safely as therapy for dermatoses (27, 28). The standard of care for such diseases is NB-UVB, which is restricted to a spectrum of 305–315 nm, thereby avoiding emissions at shorter, more damaging wavelengths. NB-UVB has been used extensively for decades, and a long-term follow-up of large patient cohorts who were receiving this therapy has not shown an increased incidence of malignancy (28, 29). Some studies have challenged the assumption of linear risk with increasing UV radiation and reported a lower incidence and mortality for several cancers in populations with greater exposure to solar radiation (30, 31). Regardless, it is impractical for UV phototherapy to be implemented on a population level. As our trial showed, even with careful monitoring, skin erythema and dryness were common adverse events. In addition, to sustain 25(OH)D concentrations, exposure would be required ≥2 times/wk. In all, the goal of using UV would not be to replace oral vitamin D therapy with UV phototherapy; rather, UV can be used as an effective research tool to identify potential mediators of health.

Although NB-UVB did not improve the lipid profile, broadening the light spectrum of phototherapy beyond NB-UVB more

TABLE 2
Changes in the lipid profile[1]

	Change in oral vitamin D_3 group	*P*	Change in UVB group	*P*	Intergroup difference (Δoral vitamin D_3 – ΔUVB)[2]	*P*
Total cholesterol, mg/dL	4.1 ± 22.0[3]	0.16	2.0 ± 18.8	0.29	3.0 (−5.0, 9.0)	0.63
LDL cholesterol, mg/dL	3.2 ± 18.4	0.24	1.4 ± 17.8	0.79	1.5 (−5.0, 7.0)	0.73
HDL cholesterol, mg/dL	0.2 ± 8.1	0.84	0.3 ± 8.4	0.93	1.0 (−3.0, 3.0)	0.83
Triglycerides, mg/dL	2.6 ± 35.4	0.57	1.7 ± 42.0	0.76	−5.0 (−12.0, 7.0)	0.53

[1] Changes in lipid variables after the active repletion phase were determined according to the baseline and final-visit values after treatment with oral vitamin D_3 or UVB. For subjects who completed the active repletion phase (2 mo) but did not complete the entire 6 mo of therapy, the last observation was carried forward (intention-to-treat analysis). $n = 60$ in the oral vitamin D_3 group, and $n = 58$ in the UVB group. There were no significant intragroup or intergroup differences for any lipid variable. Comparisons were made with the use of the Mann-Whitney *U* test. UVB, UV type B.

[2] All values are medians (95% CIs). The 95% CI values represent differences in medians between the oral vitamin D_3 group ($n = 60$) and the UVB group ($n = 58$) groups.

[3] Mean ± SD (all such values).

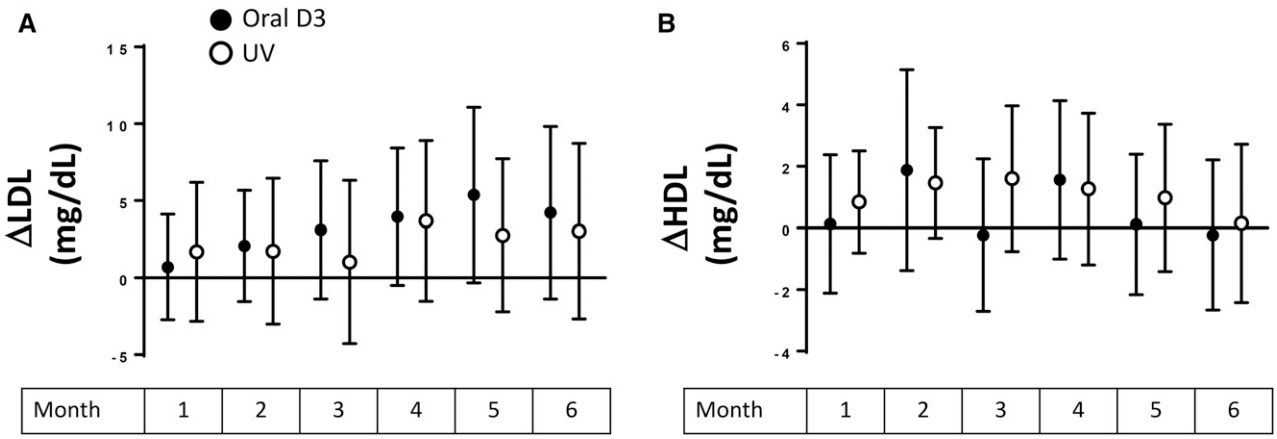

FIGURE 4 Mean ± SD longitudinal changes by month after treatment with either oral D3 or UV for serum LDL concentrations (A) and serum HDL concentrations (B). Neither LDL nor HDL cholesterol concentrations changed from baseline values for either group throughout the duration of treatment (per-protocol analysis). In the oral D3 group, $n = 60$ at months 0–2, $n = 57$ at month 3, $n = 53$ at month 4, $n = 51$ at month 5, and $n = 44$ at month 6. In the UV group, $n = 58$ at months 0–2, $n = 53$ at month 3, $n = 46$ at month 4, $n = 42$ at month 5, and $n = 40$ at month 6. D3, vitamin D_3; UV, narrow-band UV type B.

closely mimics exposure to sunlight. Indeed, previous studies have shown an inverse correlation between 25(OH)D concentrations and LDL cholesterol with an independent effect of solar UV radiation (32). To this end, additional studies are required to identify metabolites that are produced throughout the spectrum of natural light. For example, UV type A radiation can lower blood pressure by mobilizing epidermal nitric oxide into the circulation, which may explain why higher 25(OH)D concentrations that are caused by solar radiation are associated with better cardiovascular health (33). Metabolites such as nitric oxide have activities that may have been erroneously ascribed to vitamin D because of the association of 25(OH)D concentrations with UV exposure. The study of the effects of solar spectrum-light exposure may help resolve the paradox of why correcting vitamin D deficiency does not improve many of the same conditions with which the deficiency itself is so strongly associated.

25(OH)D concentrations have been repeatedly associated with health and disease. Vitamin D deficiency is associated with higher risks of malignancy, cardiovascular disease, and overall mortality, although the underlying mechanisms are unclear and no causal relation has been established (34, 35). Earlier clinical trials, such as the Women's Health Initiative, were decidedly negative (36–38) although insufficient doses of vitamin D and the co-administration of calcium may have confounded the results. However, a series of recent studies that have used higher concentrations of vitamin D without calcium have continued to show a lack of a benefit (39–45).

In conclusion, in the era of evidence-based medicine, it is difficult to overlook the shortfall in the efficacy of vitamin D supplementation. A logical step toward reconciling the association of higher 25(OH)D concentrations with better health that is not reproduced by supplementation is to entertain the hypothesis that the association is noncausal but an epiphenomenon of exposure to sunlight including UV. Perhaps the beneficial effects of vitamin D on health are not due to vitamin D itself but to the sunlight required to generate the vitamin D. By studying the comparative effect of vitamin D repletion through oral vitamin D

TABLE 3
GSEA of canonical VDR-dependent and GSEA hallmark immune-pathway gene sets[1]

	Peripheral blood				Skin			
	Oral vitamin D_3		UVB		Oral vitamin D_3		UVB	
Gene set	NES	FDR q	NES	FDR q	NES	FDR q	NES	FDR q
V$VDR_Q3	−2.55[2]	0.001[2]	−1.64[2]	0.050[2]	2.14[2]	0.005[2]	2.54[2]	<0.001[2]
V$VDR_Q6	−1.78[2]	0.037[2]	−1.89[2]	0.022[2]	2.67[2]	<0.001[2]	2.28[2]	0.002[2]
V$DR3_Q4	−1.82[2]	0.032[2]	−1.54	0.085	1.96[2]	0.012[2]	2.16[2]	0.004[2]
Hallmark immune gene sets								
Allograft rejection	0.96	0.491	−2.83[2]	<0.001[2]	1.03	0.416	−1.40	0.255
Coagulation	−0.80	0.785	−1.34	0.169	1.20	0.252	0.71	0.855
Complement	1.11	0.382	−3.03[2]	<0.001[2]	1.89[2]	0.018[2]	1.46	0.115
IFN-α response	4.14[2]	<0.001[2]	−4.05[2]	<0.001[2]	2.08[2]	0.006[2]	−2.43[2]	0.003[2]
IFN-γ response	3.41[2]	<0.001[2]	−4.86[2]	<0.001[2]	2.12[2]	0.004[2]	−2.00[2]	0.006[2]
IL6-JAK/STAT3 signaling	−1.06	0.488	−1.97[2]	0.015[2]	1.73[2]	0.037[2]	1.36	0.362
Inflammatory response	−1.07	0.491	−2.29[2]	0.003[2]	1.45	0.104	−1.23	0.363

[1] Data were stratified by tissue and treatment. Gene sets were based on the Molecular Signatures Database (21, 24). For peripheral blood analyses, $n = 15$ for oral vitamin D_3, and $n = 13$ for UV. For skin analyses, $n = 9$ for oral vitamin D_3, and $n = 7$ for UV. FDR, false-discovery rate; GSEA, gene set enrichment analysis; IFN, interferon; NES, normalized enrichment score; UVB, UV type B; VDR, vitamin D receptor.

[2] Significant, $P < 0.05$.

and UVB exposure, we show these modalities to be nonsynonymous and highlight the potential to identify systemic bioactive metabolites to complement the activity of vitamin D.

The authors' responsibilities were as follows—MPP, YL, and JLB: analyzed and interpreted the data; MPP, JK, RH, KD, PG, MMS-W, TR, DJK, IB, and MAL: conducted the research; MPP, MAL, and JLB: designed the research; MPP and JLB: prepared the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to this study.

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