Open Forum Infectious Diseases

MAJOR ARTICLE

Vitamin D Supplementation Does Not Affect Metabolic Changes Seen With ART Initiation

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Background. Insulin resistance and lipid changes are common after antiretroviral therapy (ART) initiation. Observational studies suggest that vitamin D supplementation reduces the risk of developing diabetes and improves lipid profiles.

Methods. This 48-week prospective, randomized, double-blind, placebo-controlled study evaluated high-dose vitamin D3 (4000 IU daily) plus calcium supplementation (1000 mg calcium carbonate daily) in HIV-infected participants initiating ART with efavirenz/emtricitabine/tenofovir (EFV/FTC/TDF). Changes in insulin resistance (as estimated by homeostatic model assessment), fasting lipid profile, and components of the metabolic syndrome were assessed at baseline, 24 weeks, and 48 weeks. Stratified Wilcoxon rank sum tests and stratified normal score tests were used to evaluate differences between treatment arms, stratified by screening 25-OH vitamin D stratum (≤/>20 ng/mL).

Results. A total of 165 participants enrolled: 79 in the vitamin D/calcium (Vit D/Cal) arm and 86 in the placebo arm. Only the placebo arm experienced a modest increase in insulin resistance at week 24 ($P < .001$). While increases in total and high-density lipoprotein cholesterol were significant in both arms at weeks 24 and 48, increases in low-density lipoprotein cholesterol at week 24 were only identified in the placebo arm ($P = .011$). Body mass index remained stable, whereas modest increases in waist circumference were observed in the placebo arm. Metabolic syndrome was present in 19 participants (12%) at baseline and 20 participants (14%) at week 48, without differences between arms.

Conclusions. Vit D/Cal supplementation over 48 weeks did not alter the lipid profile or glucose metabolism experienced with initiation of EFV/FTC/TDF in ART-naïve persons. Vitamin D supplementation is unlikely to be an effective strategy to attenuate metabolic dysregulations with ART initiation.

Keywords. HIV; vitamin D; antiretroviral therapy; insulin resistance; metabolic syndrome.

Metabolic abnormalities, including insulin resistance and dyslipidemia, are common among HIV-infected persons and contribute to the increased risk of cardiovascular disease in this population [1–3]. The initiation of antiretroviral therapy (ART), even with modern ART regimens, increases visceral fat, worsens glycemia, and alters lipid profiles [4, 5]. For example, ART initiation with efavirenz (EFV) has been associated with a less favorable lipid profile compared with other non-nucleoside reverse transcriptase inhibitors (NNRTIs) [5] and a modestly greater increase in fasting glycemia compared with atazanavir/ritonavir [6]. Notably, initiation of modern ART regimens, whether based on protease inhibitors (PIs), NNRTIs, or integrase strand transferase inhibitors (INSTIs), induces similar increases in total cholesterol, total body weight, and fat mass [7–9]. Thus, even with modern ART, the metabolic dysregulations that occur with ART initiation require additional interventions.

Vitamin D deficiency has been associated with dyslipidemia and insulin resistance in both the general population and in HIV-infected patients, although whether the relationship between hypovitaminosis D and metabolic derangements is causal remains unclear [10–12]. The expression of the vitamin D receptor on most nucleated cells in the body suggests that vitamin D serves some regulatory function beyond calcium homeostasis. Potential noncalcitropic functions include lipid regulation or glucose metabolism [13]. Vitamin D supplementation trials examining glucose and lipid outcomes have had mixed results in the general population [12, 14–17], and data from HIV-infected populations are limited. In 1 study, vitamin D supplementation was associated with a decreased incidence

Received 17 May 2017; editorial decision 19 September 2017; accepted 18 October 2017.
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DOI: 10.1093/ofid/ofx210

of type 2 diabetes in an HIV-infected population [18]. In a small randomized 12-week supplementation trial among ART-treated virologically suppressed HIV-infected persons, vitamin D led to modest but significant decrease in total cholesterol with increase in insulin resistance, estimated by homeostatic model assessment [19]. No study, to our knowledge, has examined the effect of vitamin D supplementation in HIV-infected persons on metabolic outcomes during the period of ART initiation.

We have previously shown in a randomized, placebo-controlled trial (ACTG A5280) that daily supplementation with vitamin D/calcium (4000 IU/1000 mg) given to HIV-infected individuals initiating ART with efavirenz/emtricitabine/tenofovir disoproxil fumarate (EFV/FTC/TDF) attenuated the loss in bone mineral density by approximately 50% over 48 weeks [20]. Here, we report the effects of vitamin D/calcium on metabolic outcomes in ACTG A5280. We hypothesized that vitamin D/calcium supplementation would be associated with salutary effects on glucose and lipid metabolism in HIV-infected persons initiating EFV/FTC/TDF.

METHODS

ART-naïve, HIV-infected adults with baseline 25(OH) vitamin D between 10 and 75 ng/mL (≥25 and <188 nmol/L) were eligible to enroll [20]. Participants initiated ART with EFV/FTC/TDF and were randomized to 4000 IU cholecalciferol (vitamin D3) daily plus 500 mg calcium carbonate twice daily or identically matching placebo (Tishcon Corporation, Westbury, NY) for 48 weeks. The institutional review boards of all participating sites approved the study; all participants provided written informed consent. (clinicaltrials.gov Identifier NCT01403051).

Metabolic Assessments

Serum concentrations of glucose and insulin were measured using a Cobas Colorimetric Assay and Human-specific Insulin radioimmunoassay (RIA), respectively, and serum concentrations of 25(OH) D_2 and D_3 were measured by liquid chromatography tandem mass spectrometry at the Irving Institute Biomarkers Core at Columbia University Medical Center (New York, NY). Insulin resistance was assessed by homeostatic model assessment (HOMA-IR) [21]. Fasting lipid profiles were performed at local laboratories in real time; low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation [22]. Daily calcium and vitamin D intake was determined by a 24-hour dietary recall performed at entry. Waist circumference was measured using a standardized protocol [23]. The metabolic syndrome was assessed using the ATPIII/NCEP criteria at each time point and was considered to be present if ≥3 of the following 5 criteria were observed: 1) waist circumference ≥102 cm if male, ≥88 cm if female; 2) triglyceride concentration: ≥150 mg/dL;3) HDL-c concentration <40 mg/dL if male, <50 mg/dL if female; 4) systolic blood pressure (BP) ≥130 mmHg or diastolic BP ≥85 mmHg; 5) fasting glucose: ≥110 mg/dL [24].

Statistical Analysis

All analyses were limited to participants with available data regardless of treatment change/discontinuation. Stratified Wilcoxon rank sum tests were used to evaluate differences between treatment arms for continuous outcomes, stratified by screening 25-OH vitamin D stratum (≤/>20 ng/mL). Wilcoxon signed rank tests were used to evaluate within–treatment arm changes. Stratified normal score tests were used to evaluate differences between treatment arms in categorical responses, stratified by screening 25-OH vitamin D stratum. All statistical tests were 2-sided and interpreted at the 5% nominal level of significance without adjustment for multiple comparisons. SAS version 9.4 and Cytel Proc StatXact package version 9 were used.

RESULTS

The details and disposition of the study population have been previously described [20]. Of the 165 eligible participants included this analysis (79 in the VitD/Cal arm, 86 in the placebo arm), 148 (90%) completed 48 weeks of study treatment. Twenty-five (15%) participants discontinued EFV/FTC/TDF prematurely (13 in VitD/Cal and 12 in placebo).

Baseline Demographics

Baseline characteristics are shown in Table 1. For the overall study population at baseline, the median age was 33 years, 90% male, 37% white non-Hispanic, 33% black non-Hispanic, with a median body mass index (BMI) of 24.4 kg/m^2 (Table 1).

25 OH Vitamin D

At baseline, 25(OH)D levels appear to be balanced between the treatment arms, but they differed over the 48-week study period (Tables 1–2). Increases in 25(OH)D levels were observed in the VitD/Cal arm at 24 and 48 weeks: median concentrations increased 27.5 ng/mL (q1, Q3 = 15.0, 38.0 ng/mL) and 24.2 ng/mL (q1, Q3 = 14.6, 37.8 ng/mL) from baseline to weeks 24 and 48, respectively ($P < .001$ for both) in the VitD/Cal arm. No significant changes in the placebo arm were detected (–0.8 ng/mL [Q1, Q3 = –5.9, 4.9 ng/mL] and 0.6 ng/mL [Q1, Q3 = –6.1, 4.3 ng/mL], respectively).

Lipid Profile

Baseline lipid parameters appear to be balanced between the treatment arms (Table 1). Fasting total and HDL cholesterol levels increased significantly by weeks 24 and 48 for both arms, with no significant differences between the 2 arms (Table 2). In the placebo arm, LDL cholesterol increased at week 24 from baseline but not at week 48. However, triglyceride changes from baseline to week 24 or week 48 were not significant in either

Table 1. Baseline Demographic and Laboratory Parameters

Parameter	Vitamin D/Calcium (n = 79)	Placebo (n = 86)
Age, y	36 (28, 47)	31 (25, 44)
Race/ethnicity		
White non-Hispanic	28 (35%)	33 (38%)
Black non-Hispanic	24 (30%)	30 (35%)
Hispanic	23 (29%)	18 (21%)
Other	4 (6%)	5 (6%)
Male sex	72 (91%)	77 (90%)
BMI, kg/m^2	25.0 (22.5, 28.2)	24.0 (21.7, 27.2)
Presence of metabolic syndrome	7 (9%)	12 (14%)
Plasma HIV RNA, $\log_{10}$ cp/mL	4.5 (4.1, 5.1)	4.5 (4.0, 5.1)
CD4 cell count, cells/mm^3	339 (230, 500)	342 (232, 454)
CD4 cell count ≤ 200 cells/mm^3	17 (22%)	15 (17%)
Estimated daily Ca intake, mg	813 (492, 1303)	811 (365, 1227)
Estimated daily vit D intake, IU	120 (62, 215)	137 (59, 279)
Laboratory parameters		
25-OH vitamin D, ng/mL	28.4 (20.9, 38.5)	26.4 (19.6, 33.0)
Insulin, uIU/mL	5.0 (2.7, 9.8)	4.4 (1.9, 8.4)
Glucose, mg/dL	85 (80, 91)	85 (79, 92)
HOMA-IR	0.97 (0.52, 2.12)	0.94 (0.46, 1.81)
Total chol, mg/dL	161 (138, 182)	156 (135, 184)
HDL-c, mg/dL	43 (34, 50)	42 (33, 49)
LDL-c, mg/dL	95 (80, 112)	89 (72, 115)
Triglycerides, mg/dL	99 (65, 127)	96 (73, 116)

All values are presented as median values with interquartile ranges (Q1, Q3) or number of participants with percentages.

Abbreviations: BMI, body mass index; chol, cholesterol; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, insulin resistance as assessed by homeostatic model assessment; LDL-c, low-density lipoprotein cholesterol.

arm. None of the changes in lipid parameters appeared to differ by treatment arm.

Glucose Metabolism and Measures of Insulin Resistance

Baseline fasting glucose and insulin levels, as well as HOMA-IR values, appear to be balanced between arms (Table 1). Glucose levels were increased modestly at weeks 24 and 48 in both arms (Table 2). Insulin levels increased in the placebo arm but not in the VitD/Cal arm at week 24, while the week 48 changes were not statistically significant for either arm. HOMA-IR increases were modest for both arms only at week 24. Between-arm differences were not significant for glucose, insulin, or HOMA-IR changes at either week 24 or week 48 ($P > .05$ for all).

Body Composition and Metabolic Syndrome

Baseline waist circumference and BMI values appear to be balanced between the 2 arms (Table 1). Waist circumference increased in the placebo arm but not in the VitD/Cal arm at both weeks 24 and 48, but differences between the 2 arms were not significant (Table 2). Changes in BMI were not significant over 48 weeks for either arm. Metabolic syndrome was identified in 19 participants (12%) at baseline and 20 participants (14%) at week 48, with no significant difference between arms. For specific parameters of the metabolic syndrome, low HDL prevalence decreased from a 45% overall prevalence at baseline to 26% at week 48, and elevated TG prevalence increased from a 14% overall prevalence at baseline to 22% at week 48.

CONCLUSIONS

Despite the marked increase in vitamin D levels from the time of ART initiation and benefits regarding bone health, high-dose vitamin D and calcium supplementation did not meaningfully improve relevant metabolic parameters, including glucose metabolism, insulin resistance, lipid profiles, body composition measures, or prevalence of the metabolic syndrome. While the observed modest increases in fasting glucose, insulin resistance, waist circumference, and total and HDL cholesterol observed in the placebo are consistent with previous trials in which participants initiated EFV/FTC/TDF, no consistent effect on these metabolic parameters was observed with supplementation.

While the NNRTI class of HIV medications has been reported to have a more favorable effect on lipids and glucose metabolism than protease inhibitors and thymidine analog NRTIs and has not been associated with an increased risk for atherosclerotic cardiovascular disease, efavirenz has been previously reported to cause increases in cholesterol levels when compared with other NNRTIs [25–28]. One proposed explanation was the effect of efavirenz on vitamin D metabolism. Efavirenz has previously been demonstrated to decrease circulating 25(OH)D levels through induction of CYP3A4 (25-hydroxylase) and CYP24 (24-hydroxylase) enzymes, which catabolize vitamin D3 to inactive metabolites [29]. While previous observational trials have identified an association between efavirenz and vitamin D deficiency, the current study failed to demonstrate a reduction in vitamin D with initiation of an efavirenz-containing ART regimen [30–32]. A large proportion of the study population had 25(OH)D levels <30 ng/mL at entry, yet the participants in the placebo arm had stable 25(OH)D levels throughout the trial. These findings from the placebo arm suggest that efavirenz does not significantly alter vitamin D metabolism. Furthermore, the demonstrable increase in 25(OH)D levels with vitamin D and calcium supplementation does not support earlier observations that efavirenz blunts the effect of vitamin D supplementation [19, 33, 34].

Despite having low vitamin D levels throughout the study, participants in the placebo arm had only modest changes in the metabolic parameters measured. Additionally, the VitD/Cal arm did not experience any metabolic benefits when compared with the placebo arm: changes in glucose, insulin, and HOMA-IR levels were similar to placebo. Parameters of the metabolic syndrome were also altered in similar fashion for both arms. These data did not affirm the hypothesis that vitamin D plays a crucial role in lipid metabolism or insulin sensitivity. While vitamin D deficiency has previously been associated with impaired pancreatic beta cell function and insulin resistance, as well as the metabolic syndrome in the general population and among persons with HIV [17, 18, 35], we did not identify these associations in a randomized clinical trial. It may be that

Table 2. Changes in Metabolic Parameters at Weeks 24 and 48 by Treatment Arm

Parameter	Vitamin D/Calcium	Placebo	P^a	Vitamin D/Calcium	Placebo	P^a
	Median (Q1, Q3) Change From Baseline to Week 24			Median (Q1, Q3) Change From Baseline to Week 48		
25-OH vitamin D, ng/mL	27.5* (15.0, 38.0)	–0.8 (–5.9, 4.9)	<.001	24.2* (14.3, 35.8)	0.6 (–6.1, 4.3)	<.001
Insulin, uIU/mL	0.4 (–1.5, 3.8)	1.4* (–1.1, 4.2)	.24	0.5 (–1.5, 4.2)	0.9 (–1.8, 3.2)	.78
Glucose, mg/dL	5* (–4, 12)	5* (–3, 12)	.91	4* (1, 8)	6* (–3, 12)	.73
HOMA-IR	0.17* (–0.21, 0.91)	0.39* (–0.11, 1.15)	.21	0.13 (–0.26, 1.11)	0.26 (–1.09, 2.73)	.87
Total chol, mg/dL	11* (–4, 29)	18* (1, 31)	.22	13* (–6, 28)	14* (–1, 37)	.55
HDL-c, mg/dL	8* (0, 15)	8* (2, 15)	.72	8* (1, 14)	8* (1, 14)	.44
LDL-c, mg/dL	0 (–10, 17)	8* (–11, 20)	.26	2 (–9, 14)	4 (–14, 27)	.93
Triglycerides, mg/dL	3 (–17, 45)	4 (–18, 28)	.90	3 (–14, 31)	4 (–18, 39)	.87
BMI, kg/m^2	0.0 (–0.5, 1.1)	0.1 (–0.6, 0.9)	.67	0.1 (–0.8, 1.1)	0.1 (–0.7, 1.0)	.98
Waist circ, cm	0.0 (–2.5, 3.0)	0.7* (–1.0, 3.7)	.17	0.3 (–2.5, 3.3)	0.9* (–2.0, 4.8)	.38
	Number of Participants (%) at Week 24		P^b	Number of Participants (%) at Week 48		P^b
Presence of metabolic syndrome	7/71 (10%)	11/78 (14%)	.55	9/66 (14%)	11/75 (15%)	.97
Central obesity	19%	16%	-	21%	18%	-
Elevated triglycerides	22%	21%	-	24%	21%	-
Low HDL-c	27%	28%	-	24%	28%	-
Elevated BP	39%	42%	-	37%	41%	-
Elevated glucose	6%	5%	-	6%	7%	-

Statistical comparisons for the individual components of the metabolic syndrome were not performed.

Abbreviations: BMI, body mass index; chol, cholesterol; circ, circumference; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, insulin resistance as assessed by homeostatic model assessment; LDL-c, low-density lipoprotein cholesterol.

*Wilcoxon signed rank *P* value < .05 for within–treatment arm change from baseline.

[a]Stratified Wilcoxon rank sum *P* value evaluating the the difference in changes from baseline between the 2 treatment arms, stratified by screening 25-OH vitamin D levels.

[b]Stratified normal score *P* value evaluating the difference between the 2 treatment arms, stratified by screening 25-OH vitamin D levels.

metabolic changes related to ART initiation far outweigh the effects of vitamin D supplementation. Alternatively, vitamin D supplementation may only be useful in persons who develop these metabolic derangements after some duration of exposure to the ART medications or that the level of 25(OH)D required to cause metabolic derangements is lower than that needed to maintain bone health. Alternatively, it is possible that the previous trials were confounded by some unmeasured variables.

The current analysis has several limitations. The duration of follow-up was only 1 year, which may limit the ability to determine the long-term effect of vitamin D supplementation. Because we only studied supplementation with 1 ART regimen (EFV/FTC/TDF); we cannot assert whether vitamin D would have a different effect with another ART regimen. The study population was young and metabolically healthy. The effect of vitamin D supplementation may have beneficial effects on metabolic parameters for persons with diabetes or dyslipidemia but limited impact in persons with normal metabolic parameters. The low prevalence of the comorbidities of interest (ie, diabetes, metabolic syndrome, dyslipidemia) in the cohort may have limited the effect of the intervention. We only measured 25(OH)D levels, while previous work has suggested that the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) is more appropriate in assessing the link between vitamin D and lipids [36]. Also, baseline data on physical activity, alcohol intake, and smoking status were not collected, which limited our ability to assess their effect on lipids and glucose metabolism in our sample. Previous studies have shown beneficial effects of vitamin D supplementation in women; however, the low number of women in our sample limits the generalizability of our findings.

In summary, vitamin D and calcium supplementation in HIV-infected persons initiating ART increased vitamin D levels and improved bone health but did not alter metabolic parameters of relevance for cardiometabolic disease risk. Whether vitamin D supplementation would have different effects with other ART regimens or in patients with a higher prevalence of baseline metabolic abnormalities should be examined.

Acknowledgments

We wish to gratefully acknowledge all of the study sites and study participants who have devoted their time and effort to this research endeavor.

Financial support. Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701 and by the National Institute of Mental Health, National Institute of Dental and Craniofacial Research. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Potential conflicts of interest. Ms. Melbourne works for Gilead Sciences, which provided study medications and funding to complete some of the assays performed as part of the trial.

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