Original Article

# Safety and Efficacy of High-Dose Daily Vitamin $D_3$ Supplementation in Children and Young Adults Infected With Human Immunodeficiency Virus

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**Background.** Suboptimal vitamin D (vitD) status is common in children and young adults infected with human immunodeficiency virus (HIV). The vitD supplemental dose needed to normalize vitD status in this population is unknown.

**Methods.** In this double-blind trial, subjects infected with HIV ages 8.3 to 24.9 years were randomized to vitD$_3$ supplementation of 4000 IU/day or 7000 IU/day and evaluated at 6 and 12 week for changes in vitD status and HIV indicators. A dose was considered unsafe if serum calcium was elevated (above age and sex-specific range) associated with elevated serum 25 hydroxyvitamin D (25(OH)D); >160 ng/mL).

**Results.** At baseline, 95% of subjects (n = 44; 43% with perinatally acquired HIV, 57% with behaviorally acquired HIV) had a suboptimal serum 25(OH)D concentration of <32 ng/mL (mean ± standard deviation, 19.3 ± 7.4; range, 4.4–33.6 ng/mL). After 12 weeks (main outcome) of $D_3$ supplementation, both $D_3$ doses were safe and well tolerated, with no evidence of elevation of serum calcium concentrations or deterioration in HIV immunologic or virologic status. Sufficient vitD status, defined as serum 25(OH)D $\geq$32 ng/mL, was achieved in 81% of all subjects, and only the 7000 IU/day group (86%) achieved this a priori efficacy criterion in >80% of subjects. Change in serum 25(OH)D did not differ between HIV acquisition groups.

**Conclusions.** A 7000 IU/day $D_3$ supplementation was safe and effective in children and young adults infected with HIV.

**Key words.** HIV; nutrition; pediatrics; supplementation; vitamin D.

Suboptimal vitamin D (vitD) status is common in individuals infected human immunodeficiency virus (HIV) [1–4], and it is associated with increased risk of HIV disease severity and death [5–7]. Contributing factors may include inadequate sunlight exposure, low vitD intake, skin pigmentation, specific drug therapies, malabsorption, or unknown HIV-associated factors. Mechanisms are poorly understood; however, a role for vitD in immunomodulation of lymphocytes and monocytes is suggested [2, 8]. Studies have demonstrated an association between better vitD status and higher $CD4^+$ cell counts [3, 5, 9] and lower RNA viral load [6, 10] in subjects infected with HIV. The supplemental dose needed to optimize vitD status is unknown.

Safety and efficacy of 2 oral daily doses (4000 vs 7000 IU) of cholecalciferol ($D_3$) over a 12-week period were assessed in children and young adults infected with HIV. Safety was determined by serum calcium and 25 hydroxyvitamin D (25(OH)D) concentrations, and efficacy was determined by attaining 25(OH)D $\geq$32 ng/mL. We

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hypothesized the following: (1) both $D_3$ doses were safe, with <5% incidence of elevated calcium (age- and sex-specific range) associated with elevated 25(OH)D (>160 ng/mL); and (2) daily $D_3$ supplementation for 12 weeks would result in the study-defined target of 25(OH)D ≥32 ng/mL in >80% of subjects in both dose groups.

## SUBJECTS AND METHODS

Subjects with perinatally acquired HIV (PHIV) were recruited from The Children's Hospital of Philadelphia (CHOP) Special Immunology Family Care Clinic, and subjects with behaviorally acquired HIV (BHIV) were recruited from CHOP Adolescent Initiative Program and Jonathan Lax Treatment Center (Philadelphia, PA). Exclusion criteria included participation in another study impacting 25(OH)D, pregnant or lactating females, and other conditions affecting growth, dietary intake, or nutritional status. Subjects taking supplements that contained vitD were not eligible. Those willing to discontinue supplementation with approval of their medical provider were eligible after a 2-month washout period. The first subject enrolled in January 2010 and the last subject was completed on January 2011, with visits at baseline, 6, and 12 weeks.

For this study, vitD status (25(OH)D concentration) was defined based upon the literature at the time of the onset of the study [11, 12]: sufficient, ≥32 ng/mL; insufficient, <32–20 ng/mL; and deficient, <20 ng/mL. Intestinal calcium absorption efficiency has been shown to be positively associated with 25(OH)D until a threshold of 30 ng/mL is achieved [13], and regulation of intestinal calcium absorption is considered optimal at 25(OH)D >32 ng/mL [14]. The results of these published data were used in this study to define the relationships between calcium and 25(OH)D. A 25(OH)D of 200 ng/mL has been considered the lower bound for potential toxicity; however, above 200 ng/mL, the risk for elevated calcium increases [15, 16]. In this study, a vit$D_3$ dose was considered unsafe if it resulted in elevated 25(OH)D >160 ng/mL coupled with an elevated calcium (age- and sex-specific range). Current evidence suggests that, compared to people with 25(OH)D <30 ng/mL, those with higher concentrations have reduced risk for many nonbone-related health outcomes, including immunomodulatory effects, potentially less cancer, and overall mortality [15, 17]. However, the ideal level to minimize the risk of these nonbone-related outcomes is not known. Thus, subjects with optimal and suboptimal vitD status were enrolled in this study.

This protocol was approved by Institutional Review Board at CHOP. Written informed consent was obtained from subjects ages 18.0 to 24.9 years, emancipated minors presenting for care alone, and parents or legal guardians of subjects <18.0 years. Verbal assent was obtained from subjects 6.0 to <18.0 years. Safety was monitored weekly by a study team and quarterly by an Independent Monitoring Committee.

### Randomization and Masking

In this randomized, double-blind study, subjects were stratified to dose by PHIV/BHIV group and season of the year. The design was to enroll 2 cohorts by HIV acquisition group, 1 cohort of 22 subjects with PHIV, and 1 cohort of 22 subjects with BHIV. To accommodate likely seasonal vitD changes, subjects within cohorts were enrolled as follows: (1) January and February (winter); (2) May (spring); (3) June (summer); and (4) September, October, and November (fall). Each cohort was randomized to 4000 or 7000 IU $D_3$/day. Doses were independently verified before and at the conclusion of the study (Tampa Bay Analytical Research, Inc., Largo, FL). The 4000 IU/day group received two 2000 IU $D_3$ capsules (Nutraceutical Science Institute, Lexington, NC), which were over-encapsulated by 1 size-0 gelatin capsule. The 7000 IU/day group received one 2000 IU $D_3$ capsule and one 5000 IU $D_3$ softgel (NOW Foods; NOW Health Group, Bloomingdale, IL), which were over-encapsulated by 1 size-0 gelatin capsule. For both doses, the resulting single capsule was identical in size, shape, and color. Those subjects who were unable to swallow capsules took 0.28 mL or 0.49 mL of 400 IU/day $D_3$ drops (Carlson Ddrops; J.R. Carlson Laboratories, Inc., Arlington Heights, IL) in the 4000 or 7000 IU/day group, respectively.

### Anthropometry, Pubertal Status, Dietary Intake, and Biochemistry

Anthropometric measurements were obtained at each visit. Details of growth, body composition, and pubertal status methods have been described previously [18]. In brief, body mass index (BMI) was calculated from weight (digital scale; Scaletronix, White Plains, NY) and height (stadiometer; Holtain, Crymych, United Kingdom) and compared with reference standards that generated age- and sex-specific Z scores. Triceps, biceps, subscapular, and suprailiac skinfold thickness (skinfold caliper: Holtain, Crymych, United Kingdom) were used to estimate fat stores. Upper arm muscle and fat areas were compared with reference data to generate Z scores. Baseline pubertal status was determined by self-assessment of Tanner stages [19, 20]. Dietary intake was estimated as the average of three 24-hour recalls (1 at each visit) by interview using visual aids for portion size (Nutrition Counseling Enterprises, Framingham, MA) and analyzed (Minnesota Nutrition Data System, Minneapolis, MN) for vitD and calcium expressed as percentage recommended dietary allowance (RDA) [21].

The following were assessed at all visits. The 25(OH)D was determined using liquid chromatography tandem mass spectrometry (Clinical Laboratory, CHOP) with intra- and interassay coefficients of variation (CV) below 8%. Bioavailable 25(OH)D (ng/mL; 25(OH)D not bound to vitD binding protein or albumin) and bioavailable and total 25(OH)D (percentage) were calculated [22]. Serum 1,25 dihydroxyvitamin D (1,25(OH)D) and intact parathyroid hormone (PTH) were assessed by radioimmunoassay with radioiodinated tracer (Heartland Assays, Inc., Ames, IA). Inter- and intra-assay CVs were 12.6% and 9.8% for 1,25(OH)D and 2.7% and 4.3% for PTH, respectively. Serum magnesium was assessed using standard techniques (CHOP). Vitamin D binding protein was assessed by enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN) with inter- and intra-assay CVs below 10%.

Safety assessments at each visit included the following: complete blood count with differential, comprehensive metabolic panel, gamma glutamyl transferase, phosphorus, and ionized calcium. Spot urines were collected for calcium and creatinine (CHOP).

### Immunology and Virology

An HIV-1 RNA plasma quantitative assay for viral load was performed at each visit (CHOP). Human immunodeficiency virus-specific multicolor flow cytometry immunophenotyping was assessed at baseline and 12-weeks (Becton Dickinson LSR II flow cytometer, BD FACSDiva software; Becton Dickinson, San Diego, CA) [23]. T cells, B cells, T helper cells, T cytotoxic cells, natural killer (NK) cells, activated cytotoxic T cells, naïve T cells, and memory T helper cells were determined. Percentages of NK cells expressing each of the natural cytotoxicity receptors (NCRs) NKp46, NKp44, and NKp30 were determined. See results for phenotype definitions.

### Adherence, HIV Status, and Questionnaires

Subjects returned supplement bottles at 12 weeks, and residual tablets or volumes were recorded. Adherence was also assessed by (1) questionnaire at 6 and 12 weeks and (2) phone calls at weeks 1, 3, 5, 8, and 10.

Human immunodeficiency virus disease status was assessed by medical records for inpatient admissions, emergency room visits, and scheduled and unscheduled clinic visits, and HIV-related and nonrelated HIV diagnoses were recorded. The Centers for Disease Control and Prevention (CDC) classification for HIV status in children <13 years used the following clinical categories: N = nonsymptomatic, A = mildly symptomatic, B = moderately symptomatic, and C = severely symptomatic with acquired immune deficiency syndrome (AIDS) defining illness [24]. In adolescents and adults, clinical categories of A, B, and C and Immunological Categories 1 through 3 corresponding to $CD4^+$ cell counts of >500, 200–499, and <200, respectively, were used [25]. The same immunologic categories used for adolescents and adults were used for children ages 6 to 12 years. Subjects were interviewed at each visit, and intensity and frequency of adverse events were documented. Medication and supplement uses were extracted from medical records, and socio-demographic information was obtained by questionnaire.

### Statistical Analysis

All variables were tested for normality, and nonparametric tests were used as appropriate. Statistical analyses were performed using SAS software 9.3 (SAS Institute, Cary, NC). Nonparametric methods were used to test whether median change from baseline within each dose group was significantly different from zero (Wilcoxon signed-rank test) and whether there were differences between dose groups (Mann-Whitney *U* test). Change from baseline within and between dose groups at 6 and 12 weeks was assessed by analysis of covariance. Models controlled for baseline value and dose group was treated as a fixed effect. Results were considered significant at $P < .05$ (unless otherwise indicated) and presented as means±standard deviation (normal distribution) or median (range; skewed distribution).

## RESULTS

Forty-four subjects were enrolled, 19 (8.3 to 24.2 years) with PHIV and 25 (18.9 to 24.7 years) with BHIV (Figure 1). Subject characteristics (Table 1) show adequate growth and nutritional status with positive Z scores for height, weight, BMI, upper arm muscle, and fat areas. Approximately one fifth of subjects had AIDS-defining illness (CDC classification C), half were nonsymptomatic, and the majority were on HIV medications. Median dietary intake of vitD and calcium was low, with half consuming ≤15% and ≤64% RDA, respectively [21].

After 12 weeks of supplementation, both $D_3$ doses were safe (Table 2). No subject had elevated serum calcium and elevated serum 25(OH)D, and there was no evidence of adverse biochemical, hematologic, immunologic, or virologic events. There was no change in total bilirubin, total protein, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, white blood cells, hemoglobin, hematocrit, or platelets (data not shown).

Vitamin D status by dose group at each visit is shown in Table 3. At baseline, 95% had suboptimal vitD status (<32 ng/mL), 50% were deficient (<20 ng/mL), and the mean 25(OH)D was 19.3 ± 7.4 (range, 4.4 to 33.6 ng/mL).

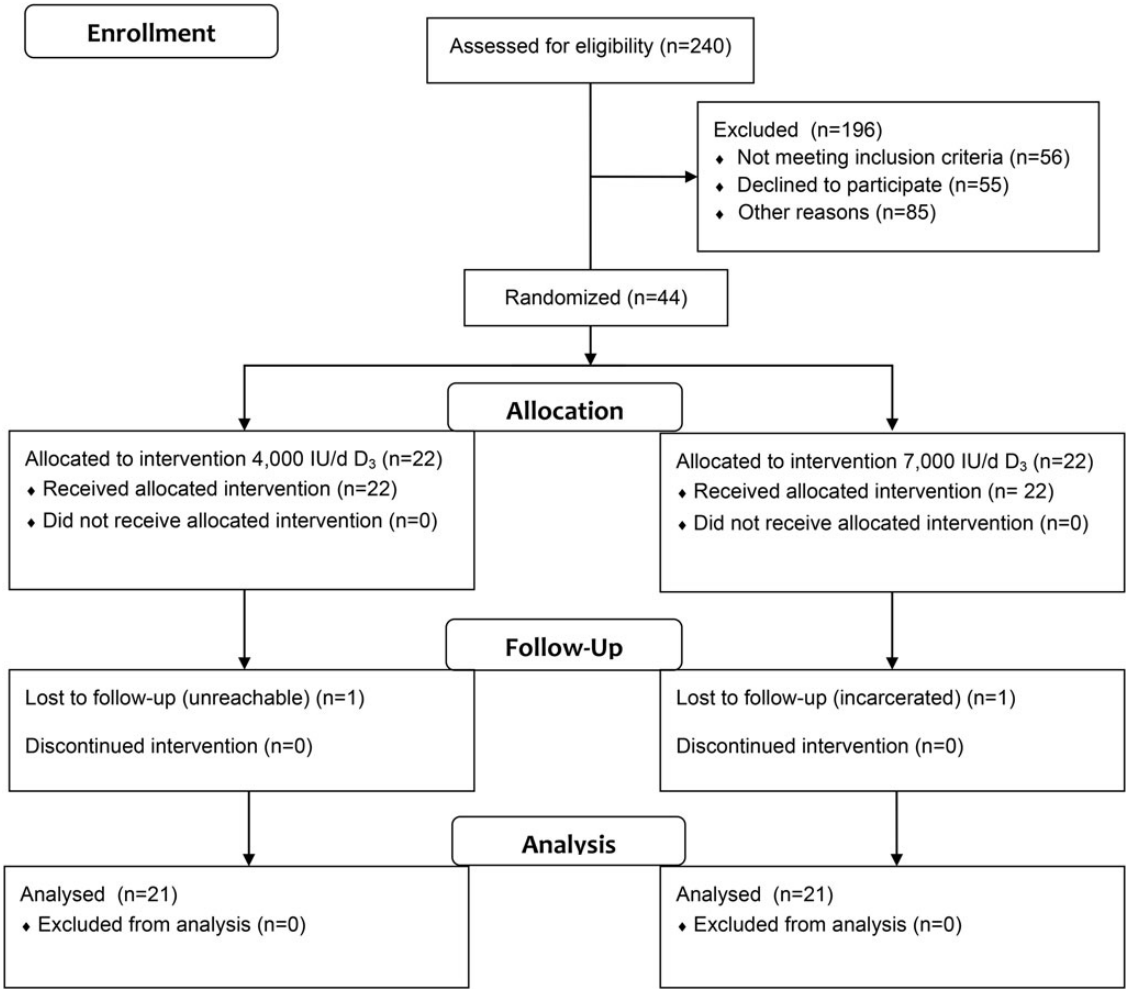


**Figure 1.** Flow diagram of group assignment and follow up.

After 12 weeks of supplementation, although overall 81% achieved sufficient vitD status, only those in the 7000 IU/day group achieved a 25(OH)D ≥32 ng/mL in >80% of subjects. Mean 12-week increase in 25(OH)D was 27.9 ± 21.5 ng/mL (24.8 ± 18.5 and 31.0 ± 24.2 ng/mL in the 4000 and 7000 IU/day groups, respectively). The significant increase in 25(OH)D with supplementation was accompanied by significant (all $P < .02$) increases in bioavailable 25(OH)D (+5.2 ± 4.3 ng/mL), 1,25(OH)D (+20.5 ± 32.0 pg/mL), and decrease in PTH (–4.6 ± 12.2 pg/mL). However, the proportion of the total 25(OH)D that was bioavailable did not change. The change in 25 (OH)D was not different by acquisition group. At baseline, 25(OH)D was significantly ($P = .001$) lower in subjects enrolled during winter (mean, 14 ng/mL) versus other seasons (range, 21 to 23 ng/mL). After 12 week of supplementation, there was no significant difference by season in 25 (OH)D for either dose group. Estimated adherence to supplementation was 94% overall using the biweekly phone calls, 91% from questionnaires, and 88% from pill or volume counts (n = 31) with no differences between dose groups in any measure of adherence.

There were no significant differences at baseline or in the change in virology and immunology measures by dose group, thus Table 4 presents data combining dose groups. For all subjects, by 12 weeks, there were significant reductions in RNA, RNA log, NK%, HLA-DR%, and NKp46%, and a significant increase in CD4% (all $P < .05$). However, there was no significant change in RNA log or CD4% in subjects not on highly active antiretroviral treatment (HAART).

The 12-week change in vitD and selected immunologic and virologic markers for subjects on and off HAART and with detectable and undetectable viral load at baseline is shown in Table 5. The change in vitD and immunology status over 12 weeks did not differ between HAART and no HAART. However, 25(OH)D change was significantly less in subjects whose baseline RNA was detectable versus

**Table 1.** Characteristics of HIV-Infected Subjects at Baseline Presented by Dose Group[a]

| | All | 4000 IU | 7000 IU |
|---|---|---|---|
| N | 44 | 22 | 22 |
| Age (yr) | 18.7 ± 4.7[b] | 18.4 ± 4.5 | 19.1 ± 5.0 |
| Gender (% male) | 68 | 68 | 68 |
| Perinatally acquired (%) | 43 | 41 | 45 |
| Season (%) | | | |
| Summer | 27 | 27 | 27 |
| Fall | 32 | 32 | 32 |
| Winter | 32 | 32 | 32 |
| Spring | 9 | 9 | 9 |
| Height Z score | 0.2 ± 1.1 | 0.4 ± 0.9 | –0.1 ± 1.3 |
| Weight Z score | 0.6 ± 1.3 | 0.8 ± 1.1 | 0.4 ± 1.6 |
| BMI Z score | 0.5 ± 1.2 | 0.6 ± 1.2 | 0.5 ± 1.3 |
| UAMA Z score | 1.1 ± 1.5 | 1.0 ± 1.1 | 1.2 ± 1.8 |
| UAFA Z score | 0.3 ± 1.3 | 0.3 ± 1.0 | 0.2 ± 1.6 |
| Body fat (%) | 19.5 ± 8.4 | 20.7 ± 7.6 | 18.1 ± 9.1 |
| Tanner (no.) | | | |
| 1 | 2 | 1 | 1 |
| 2 | 2 | 0 | 2 |
| 3 | 6 | 5 | 1 |
| 4 | 2 | 1 | 1 |
| 5 | 32 | 15 | 17 |
| Dietary intake | | | |
| Vitamin D (IU/day) | 90 [11, 346][c] | 86 [11, 269] | 101 [24, 346] |
| Vitamin D (%RDA) | 15 [2, 58] | 14 [2, 45] | 17 [4, 58] |
| Calcium (mg/day) | 648 [131, 2034] | 629 [242, 2034] | 668 [131, 1360] |
| Calcium (%RDA) | 63 [13, 203] | 63 [24, 203] | 65 [13, 136] |
| On HIV medication (%) | 82 | 77 | 86 |
| Viral RNA log (copies/mL)[d] | 1.8 [1.6, 4.8] | 2.1 [1.6, 4.6] | 1.6 [1.6, 4.8] |
| Viral RNA undetected (%) | 47 | 41 | 52 |
| Type of medication (%)[e] | | | |
| Tenofovir containing regimens | 69 | 71 | 68 |
| PI | 53 | 41 | 63 |
| NNRTI (77% on efavirenz) | 53 | 65 | 42 |
| CDC HIV Classification (%) | | | |
| A | 14 | 9 | 19 |
| B | 16 | 14 | 19 |
| C | 21 | 27 | 14 |
| N | 49 | 50 | 48 |
| Immunity category: worst (%) | | | |
| CD4 count ≥500 | 33 | 27 | 38 |
| CD4 count 200–499 | 47 | 45 | 47 |
| CD4 count <200 | 21 | 27 | 14 |
| Immunity category: current (%) | | | |
| CD4 count ≥500 | 65 | 64 | 67 |
| CD4 count 200–499 | 30 | 32 | 29 |
| CD4 count <200 | 5 | 5 | 5 |

Abbreviations: BMI, body mass index; CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral treatment; HIV, human immunodeficiency virus; NNRTI, nonnucleoside reverse transcriptase-based HAART; PI, protease inhibitor-based HAART; RDA, recommended daily allowance; UAFA, upper arm fat area; UAMA, upper arm muscle area.

[a]Significant differences were assessed by using a Student's *t* test, Wilcoxon rank-sum test, or $\chi^2$ test. No differences were detected.

[b]Mean ± standard deviation (all such values).

[c]Median: range in brackets (all such values).

[d]Viral load for subjects who are detectable and subjects who are undetectable.

[e]All on HIV medication n = 36; 4000 IU n = 17; 7000 IU n = 19.

undetectable, and their RNA log declined over time (both $P < .05$).

Subjects receiving tenofovir did not differ at baseline or in the change in 25(OH)D, 1,25(OH)D, or PTH over time. However, subjects receiving efavirenz compared with those on other drug regimens had lower 25(OH)D at baseline (16.2 ± 5.5 vs 20.9 ± 7.8 ng/mL; $P = .045$) and a significantly greater increase in 25(OH)D at 12 weeks (41.1 ± 23.5 vs 21.3 ± 17.3 ng/mL; $P = .004$), with no significant differences in 1,25(OH)D or PTH. Adjusted for baseline 25(OH)D and viral load, subjects taking efavirenz had a significantly greater increase in 25(OH)D over time of 1.65 ng/mL per week or 19.8 ng/mL over 12 weeks than those on nonefavirenz regimens.

## DISCUSSION

Suboptimal vitD status was prevalent in children and young adults infected with HIV. At baseline, 95% of

**Table 2.** Laboratory Values for HIV-Infected Subjects by Dose Group At Each Visit[a]

| | Baseline | 12 Weeks |
|---|---|---|
| N | | |
| 4000 IU | 22 | 21 |
| 7000 IU | 22 | 21 |
| Calcium (mg/dL) | | |
| 4000 IU | 9.3 ± 0.2[b] | 9.5 ± 0.4[†] |
| 7000 IU | 9.5 ± 0.4* | 9.5 ± 0.3 |
| Ionized calcium (mM/L) | | |
| 4000 IU | 1.22 ± 0.04 | 1.23 ± 0.05 |
| 7000 IU | 1.22 ± 0.04 | 1.22 ± 0.04 |
| Magnesium (mg/dL) | | |
| 4000 IU | 2.0 ± 0.2 | 2.0 ± 0.2 |
| 7000 IU | 1.9 ± 0.2 | 1.9 ± 0.1 |
| Phosphorous (mg/dL) | | |
| 4000 IU | 4.0 ± 0.7 | 3.9 ± 0.8 |
| 7000 IU | 4.9 ± 0.8 | 4.1 ± 0.7 |
| Glucose (mg/dL) | | |
| 4000 IU | 77.2 ± 11.5 | 78.9 ± 19.7 |
| 7000 IU | 76.2 ± 12.2 | 82.9 ± 19.6 |
| Albumin (g/dL) | | |
| 4000 IU | 4.5 [3.7, 4.9][c] | 4.5 [3.7, 5.0] |
| 7000 IU | 4.4 [3.7, 5.4] | 4.4 [3.7, 5.1] |
| Alkaline phosphatase (U/L) | | |
| 4000 IU | 98 [58, 659] | 99 [62, 726] |
| 7000 IU | 108 [69, 294] | 112 [69, 281] |
| Urine calcium:creatinine ratio (mg/dL) | | |
| 4000 IU | 0.04 [0.01, 0.16] | 0.07 [0.01, 0.23] |
| 7000 IU | 0.06 [0.01, 0.37] | 0.06 [0.01, 0.28] |

Abbreviations: HIV, human immunodeficiency virus.
[a]Nonparametric methods used to test whether median change from baseline within each dose group was significantly different from 0 (Wilcoxon signed-rank test) and whether differences exist between dose groups (Mann-Whitney *U* test). Change from baseline within and between dose groups at week 6 and 12 assessed by analysis of covariance with dose group treated as a fixed effect and controlling for baseline value.
[b]Mean ± standard deviation (all such values).
[c]Median: range in brackets (all such values).
*$P < .05$, 4000 vs 7000 IU at baseline.
[†]$P < .05$, 4000 IU 12 weeks vs baseline.

subjects had suboptimal vitD status (serum 25(OH)D <32 ng/mL) with 50% in deficient range (<20 ng/mL). Daily oral high-dose $D_3$ supplementation of 4000 and 7000 IU was safe and well tolerated. Both doses increased 25(OH)D to ≥32 ng/mL by 12 weeks. However, only those receiving 7000 IU/day achieved the target 25(OH)D in >80% of subjects. Other measures of vitD status improved: 1,25(OH)D increased, PTH declined, and 25(OH)D seasonal differences were eliminated. Taken together, these findings suggest that daily high-dose vitD supplementation for this population was safe, effective, and required to achieve optimal response, as indicated by serum 25(OH)D.

In a few studies evaluating children and young adults with HIV infection, researchers found a high prevalence of suboptimal 25(OH)D, ranging from 71% to 89% <30 ng/mL [3, 26–28] to 33% to 78% <20 ng/mL [1, 4, 29–31], with 1 group reporting 87% <15 ng/mL [2]. Our results support the finding that suboptimal 25(OH)D status is common in unsupplemented youth, with 95% at baseline having a 25(OH)D <32 ng/mL. Low vitD in HIV may be due to a combination of factors, including inadequate sunlight exposure, low vitD intake, skin pigmentation, specific drugs therapies, malabsorption, or unknown HIV-associated factors. These findings highlight the need to determine the vitD supplementation dose that safely results in optimal year-round serum 25(OH)D in children and adults infected with HIV.

The doses of $D_3$ chosen for this study were based on several studies. In healthy adults, 3600 to 4200 IU/day of $D_3$ were required to sustain 25(OH)D >32 ng/mL [14]. A 6-month, randomized, placebo-controlled $D_3$ supplementation study in healthy African American and white adults found a daily dose of 3440 IU was necessary for most to attain 25(OH)D >30 ng/mL [32]. For those with baseline values <22 ng/mL, 5000 IU was required. For the present study, our pilot 25(OH)D data for children and young adults infected with HIV showed an average of 12.6 ng/mL (8.1 ng/mL for BHIV subjects [2] and 17.1 ng/mL for PHIV subjects [3]). Vieth et al [33] showed a $D_3$ dose of 4000 IU/day resulted in a minimum 25(OH)D plateau of 28 ng/mL in adults. Thus, due to HIV status, medications, low dietary intake, and darker skin pigmentation in many patients with HIV, 4000 and 7000 IU/day were chosen for our sample of predominantly African American children and young adults infected with HIV. We hypothesized these doses would assure that nearly all subjects would achieve the a priori 25(OH)D efficacy criteria ≥32 ng/mL. However, this result was achieved only in the 7000 IU/day group.

Studies investigating optimal dosing regimens, safety, and efficacy of vitD for sufficient 25(OH)D status in children and adults infected with HIV are sparse. Arpadi et al [1] randomized 56 subjects, aged 6 to 16 years, to $D_3$ supplementation of 100 000 IU (equivalent to 1667 IU/day) or placebo every 2 months. After 12 months, 6.7% of treated subjects had 25(OH)D <20 ng/mL, and 50% of treated subjects had between 20 and 30 ng/mL, suggesting that this dose approach was insufficient to raise 25(OH)D above 30 ng/mL in most children infected with HIV. In subjects aged 18 to 24 years, Havens et al [31] randomized 203 subjects to monthly $D_3$ supplementation of 50 000 IU (equivalent to 1667 IU/day, as in the Arpadi et al [1] study) or placebo. After 3 months, 95% of subjects receiving vitD had 25(OH)D ≥20 ng/mL, including 76% in 20–50 ng/mL range and 8% exceeding 50 ng/mL. Compared with Arpadi et al [1], this result suggests that a more frequent dosing regimen may be needed to achieve optimal 25(OH)D in this population. Kakalia et al [26] randomized 53 children infected with HIV (mean age, 10 years) to receive 5600 IU/week (800 IU/day), 11 200 IU/week (1600

**Table 3.** Vitamin D Status for HIV-Infected Subjects by Dose Group At Each Visit[a]

| | Baseline | 6 Weeks | 12 Weeks |
|---|---|---|---|
| N | | | |
| 4000 IU | 22 | 22 | 21 |
| 7000 IU | 22 | 21 | 21 |
| Total 25(OH)D (ng/mL) | | | |
| 4000 IU | 18.6 [4.4, 32.5][b] | 39.4 [13.8, 69.3]* | 43.2 [11.8, 76.2]† |
| 7000 IU | 22.5 [11.3, 33.6] | 47.6 [31.0, 82.2]**§ | 51.2 [22.4, 124.0]‡ |
| Total 25(OH)D <20 ng/mL (%) | | | |
| 4000 IU | 59 | 5 | 5 |
| 7000 IU | 41 | 0 | 0 |
| Total 25(OH)D <32-20 ng/mL (%) | | | |
| 4000 IU | 36 | 18 | 19 |
| 7000 IU | 54 | 5 | 14 |
| Total 25(OH)D ≥32 ng/mL (%) | | | |
| 4000 IU | 5 | 77* | 76† |
| 7000 IU | 5 | 95** | 86‡ |
| Bioavailable 25(OH)D (ng/mL) | | | |
| 4000 IU | 2.6 [0.5, 7.0] | 7.0 [2.6, 18.6]* | 7.8 [1.9, 17.2]† |
| 7000 IU | 3.8 [0.9, 8.8] | 10.5 [2.9, 24.7]** | 8.7 [3.7, 18.6]‡ |
| Bioavailable/Total 25(OH)D (%) | | | |
| 4000 IU | 21.1 [7.7, 35.9] | 19.5 [7.1, 37.7] | 19.3 [6.7, 37.1] |
| 7000 IU | 19.2 [6.8, 26.8] | 21.3 [7.9, 33.4] | 18.1 [8.3, 28.8] |
| 1,25(OH)D (pg/mL) | | | |
| 4000 IU | 43.0 [19.1, 103.0] | 63.8 [32.0, 229.1]* | 69.2 [35.0, 143.7]† |
| 7000 IU | 48.2 [21.4, 108.1] | 66.6 [26.8, 142.1]** | 61.1 [25.2, 130.8]‡ |
| VDBP (μmol/L) | | | |
| 4000 IU | 2.6 [1.2, 7.9] | 2.9 [1.1, 8.6] | 2.8 [1.2, 9.3] |
| 7000 IU | 2.6 [1.7, 8.7] | 2.5 [1.4, 7.7] | 2.7 [1.6, 7.9] |
| PTH (pg/mL) | | | |
| 4000 IU | 27.3 [4.9, 58.8] | 19.1 [9.0, 45.9] | 16.9 [8.7, 79.0]† |
| 7000 IU | 23.9 [5.6, 40.5] | 24.5 [10.1, 46.2] | 22.7 [7.0, 40.3] |

Abbreviations: HIV, human immunodeficiency virus; PTH, parathyroid hormone; VDBP, vitamin D binding protein; 25(OH)D, 25-hydroxyvitamin D; 1, 25(OH)D, 1,25-dihydroxyvitamin D.

[a]Nonparametric methods used to test whether median change from baseline within each dose group was significantly different from 0 (Wilcoxon signed-rank test) and whether differences exist between dose groups (Mann-Whitney *U* test). Change from baseline within and between dose groups at week 6 and 12 assessed by analysis of covariance with dose group treated as a fixed effect and controlling for baseline value.

[b]Median: range in brackets (all such values).

*$P < .05$, 4000 IU 6 weeks vs baseline.

**$P < .05$, 7000 IU 6 week vs baseline.

†$P < .05$, 4000 IU 12 weeks vs baseline.

‡$P < .05$, 7000 IU 12 weeks vs baseline.

§$P < .05$, 4000 IU vs 7000 IU at 6 weeks.

IU/day), or no supplementation. After 6 months, only 38% and 67% of subjects in the 5600 IU/week and 11 200 IU/week groups, respectively, achieved a 25(OH)D >30 ng/mL. In aggregate, these studies demonstrated the safety of vitD supplementation in HIV at various doses and frequencies. Our daily dosing study resulted in more participants in the target range, no seasonal variation, and no adverse events. However, in prior studies, it is possible that other factors such as the short half-life of vitD, which resulted in too low of a monthly dose, accounted for some of their results. The high dose used in the present study (7000 IU/day) is equivalent to what many providers use to replete adults (50 000/week), which has been shown to be efficacious. This approach has not been studied in children and young adults infected with HIV. We recommend daily dosing as an approach for achieving and maintaining 25(OH)D status in the HIV care setting.

This regimen of $D_3$ supplementation did not result in any deterioration in immunologic or virologic status. In subjects on HAART, a small but significant increase in percentage of $CD4^+$ T cells, a small decrease in viral load, and a significant decrease in the percentage of activated cytotoxic T cells ($CD8^+/CD38^+/HLA\text{-}DR^+$) were observed, and these results suggest a possible favorable impact on HIV status. This finding supports other studies in which vitD status was associated with higher $CD4^+$ cell counts or reduction in RNA viral load [3, 5, 6, 9, 10]. However, this may reflect improved adherence to HAART regimen because we did not detect an improvement in CD4% or virologic control in the small number of subjects who were not on HAART. A significant decrease in the percentage of NK cells was observed and all values remained within normal range. A significant decrease in percentage of NK cells expressing NKp46 was noted, whereas expression of

**Table 4.** Virology and Immunology for HIV-Infected Subjects (Dose Group Combined for This Analysis) At Baseline and 12 Weeks[a]

| | Baseline | 12 Weeks |
|---|---|---|
| N | 39 | 39 |
| Viral RNA (copies/mL) | 114 [40,54397][b] | 40 [40,43821]* |
| Viral RNA log (copies/mL) | 2.0 [1.6, 4.8] | 1.6 [1.6, 4.6]** |
| Viral RNA undetected (%) | 45 | 56 |
| WBC ($\times 10^3/\mu L$) | 4.8 [3.4, 11.6] | 5.3 [2.7, 10.8] |
| ALC ($\times 10^3/\mu L$) | 2.0 [1, 4.4] | 1.8 [1.0, 3.7] |
| Lymphocytes (% of WBC) | 39 [21.0, 61.0] | 39 [23.1, 74.0] |
| B cells (%) | 10.6 [2.2, 23.5] | 9.8 [1.8, 28.2] |
| T cells (%) | 79.6 [57.3, 96.0] | 79.6 [64.5, 93.7] |
| T helper cells (%) | 29.8 [7.0, 57.7] | 31.6 [7.7, 58.7]** |
| Naive T helper cells (%) | 43.6 [23.1, 73.7] | 43.4 [19.8, 73.9] |
| Memory T helper cells (%) | 48.1 [24.4, 72.2] | 47.5 [25.2, 74.8] |
| Cytotoxic T cells (%) | 45.4 [24.0,84.0] | 44.7 [18.9, 82.9] |
| Activated cytotoxic T cells (%) | 26.0 [5.4, 60.4] | 21.3 [4.0, 52.3]* |
| NK cells (%) | 8.3 [1.2, 26.3] | 5.5 [1.5, 19.5]** |
| $NKp46^+$ NK cells (%) | 56.5 [2.2, 92.5] | 44.7 [1.7, 96.4]** |
| $NKp30^+$ NK cells (%) | 42.2 [1.0, 92.8] | 37.1 [6.6, 96.5] |
| $NKp44^+$ NK cells (%) | 0.9 [0, 4.6] | 0.4 [0, 3.5] |

Abbreviations: ALC, absolute lymphocyte count; HIV, human immunodeficiency virus; NK, natural killer; WBC, white blood cells.

[a]T cells: $CD45^+/CD3^+$; B cells: $CD45^+/CD3^-/CD19^+$; T helper: $CD45^+/CD3^+/CD4^+$; T cytotoxic: $CD45^+/CD3^+/CD8^+$; activated cytotoxic T cells: $CD3^+/CD8^+/CD38^+/HLA\text{-}DR^+$; naive T helper cells: $CD3^+/CD4^+/CD45RA^+/CD62L^+$; memory T helper cells: $CD3^+/CD4^+/CD45RA^-/CD45RO^+$; NK cells: $CD45^+/CD3^-/CD16^+CD56^+/NKp46^+$; $NKp46^+$ NK cells: $CD45^+/CD3^-/CD16^+CD56^+/NKp46^+$; $NKp44^+$ NK cells: $CD45^+/CD3^-/CD16^+CD56^+/NKp44^+$; $NKp30^+$ NK cells: $CD45^+/CD3^-/CD16^+CD56^+/NKp30^+$. Longitudinal mixed effects analyses were used to examine change over time where subject was treated as a random effect and measurement and time as fixed effects.

[b]Median: range in brackets (all such values).

*$P < .05$, 12 weeks vs baseline after adjusting for baseline values (%).

**$P < .01$, 12 weeks vs baseline after adjusting for baseline values (%).

**Table 5.** Change From Baseline to 12-Weeks in Vitamin D and Selected Immunologic and Virologic Markers for HIV-Infected Subjects On and Off HAART and With Detectable and Undetectable Viral Load at Baseline

| | All | On HAART[a] | Off HAART[a] | RNA Undetectable (<40 cpm)[b] | RNA Detectable[b] |
|---|---|---|---|---|---|
| N | 39 | 32 | 7 | 17 | 21 |
| 25(OH)D (ng/mL) | 28.6 ± 22.0 | 30.8 ± 22.3[c] | 18.3 ± 18.7 | 42.0 ± 21.6 | 17.7 ± 16.4* |
| RNA log (copies/mL) | –0.3 ± 0.6 | –0.3 ± 0.7 | –0.0 ± 0.2 | No change | –0.5 ± 0.8* |
| RNA (% undetectable at baseline) | 45 | 52 | 14 | 100 | 0 |
| RNA (% undetectable at 12 wks) | 56 | 66 | 14 | 100 | 23 |
| CD4 (%) | 2.4 ± 5.5 | 2.8 ± 5.8 | 0.8 ± 3.7 | 4.0 ± 5.1[c] | 1.2 ± 5.6 |
| NK (%) | –1.4 ± 3.1 | –1.5 ± 2.9 | –1.0 ± 4.1 | –2.0 ± 3.6 | –1.0 ± 2.7 |
| HLA-DR (%) | –4.2 ± 11.2 | –5.7 ± 10.3 | 2.2 ± 13.4 | –3.8 ± 10.8 | –5.1 ± 11.7 |
| NK46 (%) | –7.4 ± 14.6 | –7.4 ± 15.8 | –7.5 ± 7.7 | –3.9 ± 13.8 | –9.7 ± 15.1 |
| HIV acquisition group (%BHIV) | 54 | 44 | 100 | 47 | 57 |

Abbreviations: BHIV, behaviorally acquired HIV; HAART, highly active antiretroviral treatment; HIV, human immunodeficiency virus; HLA-DR, major histocompatibility complex class II antigen; NK, natural killer; WBC, white blood cells.

[a]Thirty-nine subjects with longitudinal data for Peripheral Immunological Data.

[b]Thirty-eight subjects with longitudinal data for Peripheral Immunological Data & RNAlog data at baseline. All 17 subjects undetectable at baseline remained undetectable at 12 weeks.

[c]Increase in CD4% significant from baseline to 12 weeks, $P < .05$.

Significant differences were assessed by using a Student's $t$ test or $\chi^2$ test.

[c]Mean ± standard deviation (all such values).

*$P < .05$, RNA detectable vs RNA undetectable.

the other NCR studied was not affected by supplementation. It is interesting to note that, in a similar 12-week dose study (4000 and 7000 IU/day vit$D_3$) conducted in 60 children and adults infected with HIV (5 to 50 years) on antiretroviral therapy and living in Botswana, our group reported significant increases in 25(OH)D accompanied by a modest increase in CD4% and decrease in viral load (Steenhoff et al, manuscript in preparation). Additional studies are needed to understand how $D_3$ affects immunologic or virologic status in patients infected with HIV.

Although others have observed differences in vitD status, including 25(OH)D, 1,25(OH)D, and PTH for people receiving tenofovir, results of the present study showed no effect before or after supplementation [34, 35]. Efavirenz has been associated with poorer vitD status [31, 35, 36].

Results from the present study are in agreement, with subjects on efavirenz having a significantly lower 25(OH) D at baseline. In addition, our finding of a better response to supplementation with efavirenz treatment has not been noted in a daily supplementation trial setting. It is unclear why subjects on efavirenz treatment had a better response to supplementation, and this effect warrants further study.

Subjects in this study were supplemented with 3 different brands of vitD to provide the necessary study doses. Comparative gut absorption characteristics were not available for these commercially available products. As a dose-finding protocol, this study did not have a placebo group. Since all subjects were aware they were taking a vitD supplement, this knowledge may have changed the participants' dietary intake or other behaviors known to alter 25(OH)D.

In summary, suboptimal vitD status was prevalent in children and young adults infected with HIV, and daily oral 7000 IU/day $D_3$ supplementation was both safe and effective in optimizing serum 25(OH)D. Taken together, these findings highlight the need for randomized, double-blind, placebo-controlled trials to test the impact of this optimal dose on clinically important, HIV-related immunological status and other clinically meaningful outcomes.

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