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Effects of cholecalciferol supplementation on serum and urinary vitamin D metabolites and binding protein in HIV-infected youth

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ABSTRACT

Vitamin D insufficiency is widespread in HIV-infected patients. HIV and/or antiretroviral therapy (ART), particularly efavirenz (EFV), may interfere with vitamin D metabolism. However, few data from randomized, controlled trials exist. Here, we investigate changes in vitamin D metabolites and binding protein (VDBP) after 6 months of supplementation in a randomized, active-control, double-blind trial investigating 2 different monthly cholecalciferol (vitamin D_3) doses [60,000 (medium) or 120,000 (high) IU/month] vs. a control arm of 18,000 IU/month in 8–25 year old HIV-infected youth on ART with HIV-1 RNA <1000 copies/mL and baseline 25-hydroxycholecalciferol ($25(OH)D_3$) ≤30 ng/mL. A matched healthy uninfected group was enrolled in a similar parallel study for comparison. Changes after 6 months were analyzed as intent-to-treat within/between groups [control group (low dose) vs. combined supplementation doses (medium + high)]. At 6 months, 55% vs. 82% of subjects in control and supplementation groups, respectively, reached $25(OH)D_3$ ≥30 ng/mL ($P = 0.01$) with no difference between medium and high doses (both 82% ≥30 ng/mL). There were few differences for those on EFV vs. no-EFV, except serum VDBP decreased in EFV-treated subjects (both within- and between-groups $P \leq 0.01$). There were no significant differences between the HIV-infected vs. healthy uninfected groups. The major finding of the present study is that cholecalciferol supplementation (60,000 or 120,000 IU/month) effectively raises serum $25(OH)D_3$ in the majority of HIV-infected subjects, regardless of EFV use. Notably, response to supplementation was similar to that of uninfected subjects.

1. Introduction

While viral suppression with combination antiretroviral therapy (cART) dramatically restores health, HIV-1-infected individuals have more age-related co-morbidities, such as osteoporosis and an increased risk of fractures, cardiovascular disease, neurocognitive impairment, renal disease and non-AIDS-defining malignancies and occur at an earlier age than individuals in the general population [1–3]. Similar to their adult counterparts, data show that HIV-1-infected youth are also at an increased risk of development of these HIV-related co-morbidities later in life, despite few clinical manifestations at their younger age [4–9]. Given that they will live for many decades with exposure to this state of chronic infection and immune dysfunction, the implications could be profound as the population ages. Developing complementary strategies to

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $24,25(OH)_2D_3$, 24,25-dihydroxycholecalciferol; $24,25(OH)_2D$, 24,25-dihydroxyvitamin D; $25(OH)D_3$, 25-hydroxycholecalciferol; 25(OH)D, 25-hydroxyvitamin D; vitamin D_3, cholecalciferol; VDBP, vitamin D binding protein; PTH, parathyroid hormone; cART, combination antiretroviral therapy (cART); EFV, efavirenz; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; RCT, randomized-controlled trial; CV, coefficients of variance.

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cART aimed at decreasing the risk of HIV-associated co-morbidities before clinical manifestations develop may greatly improve quality and length of length in this vulnerable population.

Vitamin D supplementation is arguably an under-utilized potential adjuvant to cART based on the currently available data in both the HIV and general populations. The naturally-synthesized vitamin D hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), calcitriol, plays a substantial role in many of the complications related to chronic HIV infection [10–14]. In addition, vitamin D endocrine system has long been known to play a key role in the immune system, making it even more important to be studied in the HIV-infected population. A number of observational and cross-sectional studies have shown that vitamin D deficiency, as measured by the concentration of circulating 25-hydroxyvitamin D (25(OH)D), an established marker of overall vitamin D status [15], hastens HIV disease progression and mortality. Higher 25(OH)D concentrations contribute to a more favorable immune restoration after initiating cART and are associated with higher CD4+ T-cell counts and lower HIV-1 RNA levels [16–24]. Likewise, we have previously shown a significant association between vitamin D status and common carotid artery intima-media thickness, a surrogate marker of subclinical atherosclerosis, in a group of HIV-infected subjects on stable cART [18]. In the few randomized-controlled trials (RCTs) conducted in the HIV-infected population, limited data suggest that vitamin D supplementation may improve immune function, decrease immune activation, and increase bone mineral density [25–30].

Importantly, the prevalence of vitamin D insufficiency is very high and widespread in both HIV-infected children and adults. Some studies have demonstrated >90% of subjects with HIV have 25(OH)D concentrations <30 ng/mL [18,22,31–34], a blood concentration suggested by the Endocrine Society to represent a suboptimal vitamin D status [35]. In addition, data suggest that HIV infection and cART, especially the non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV), may interfere with vitamin D metabolism [32,33,36–43]; however, data from RCTs are limited and none of these studies showed a negative effect of EFV on raising 25(OH)D concentrations [44–46]. In addition, the precise mechanisms whereby HIV and ART may alter vitamin D pathways are poorly-defined. In fact, to date, no vitamin D supplementation study has investigated the mechanisms by which EFV may interfere with vitamin D metabolism. Likewise, there are relatively few RCTs in HIV that have evaluated dosing regimens needed to raise 25(OH)D to optimal blood concentrations [26,45,47–50].

Thus, the primary objectives of this study were to 1) comprehensively investigate the changes in serum and urine vitamin D metabolites and vitamin D binding protein (VDBP) with three different monthly dosing regimens in cART-stable, virally-suppressed HIV-infected subjects with vitamin D insufficiency within a RCT of cholecalciferol supplementation, 2) evaluate the efficacy of raising blood concentrations of $25(OH)D_3$ to >30 ng/mL with these different dosing regimens in this specific HIV population, and 3) determine if the changes in serum and urine vitamin D metabolites and VDBP proteins differ among HIV-infected subjects on EFV compared to those not on EFV. Secondary objectives included to: 1) determine if the results differed between HIV-infected subjects and healthy uninfected controls; and 2) examine factors associated with changes in vitamin D metabolites and VDBP within the HIV-infected subjects. Our focus on HIV-1-infected youth represents an innovative approach to potentially identify efficacious strategies to prevent the development of HIV-related co-morbidities before the onset of established disease.

2. Materials and methods

2.1. Study Design/Population

This is a randomized, active-control, double-blinded trial designed to measure the effect of cholecalciferol supplementation in HIV-infected youth. HIV-infected subjects were recruited from the HIV clinics of University Hospitals Case Medical Center, Cleveland, OH and Grady Health System, Atlanta, GA via electronic medical record system queries and case manager/provider referrals. HIV-infected subjects were eligible if they were between 8 and 25 years of age with documented HIV-1 infection on a stable cART regimen for ≥12 weeks, with ≥6 months cumulative cART duration, HIV-1 RNA level <1000 copies/mL, with no intent to change cART regimen.

Controls were 8–25 years of age and healthy and were selected so that the group matched the HIV-infected subjects in regards to sex, race, and age. Controls were recruited in multiple ways, including a) friends or family members of the HIV-infected subjects, b) physician referrals from local pediatric and adult clinics, c) extensive outreach to various local organizations, churches, and schools, and d) recruitment flyers in targeted locations throughout the two cities. Documentation of absence of HIV infection was obtained in controls ≥13 years of age prior to study inclusion with OraQuick Advance Rapid HIV Test (OraSure Technologies, Inc., Bethlehem, PA, USA) for subjects at the Emory University site, given the high prevalence of HIV in this age group in Atlanta, Georgia.

Additional inclusion criteria for both HIV-infected subjects and healthy controls included baseline serum 25(OH)D concentration ≤30 ng/mL with no intent to change diet, sun exposure or exercise routine during study period. Exclusion criteria included regular daily use of vitamin D supplementation of >400 IU/day, pregnancy or lactation, acute illness or inflammatory condition, malignancy, parathyroid or calcium disorder, diabetes, creatinine clearance <50 mL/min, liver enzymes ≥2.5 times the upper limit of normal, hemoglobin ≤9.0 g/dL, medication use (*e.g.*, chemotherapy agents, systemic steroids) which could affect results, or unwillingness/inability to comply with study procedures. Enrollment into the study occurred ≤30 days after screening.

Intervention consisted of 2 different monthly cholecalciferol doses [60,000 (medium) or 120,000 (high) IU/month] vs. a control arm of 18,000 IU/month (Tischon Corp., Salisbury, MD). A monthly dosing strategy was chosen to minimize additional pill burden, given the risk of poor adherence to medication among HIV-infected adolescents and young adults. These particular monthly doses were designed to represent doses of 600 IU/day (control arm: Institute of Medicine's current recommended daily allowance for those ages 1–70 years), 4000 IU/day (high dose: Institute of Medicine's recommended upper daily limit), and 2000 IU/day (medium dose) [51].

The randomization scheme was computer-generated, stratified by EFV use at entry and administered by an investigational pharmacist. Regardless of randomized group, subjects took two capsules of cholecalciferol orally at baseline and then monthly after being prompted by a reminder phone call from study staff; capsules looked identical regardless of dose. Representative capsules were sent to an independent laboratory (Analytical Research Laboratories, Oklahoma City, OK) at regular intervals during the study period to ensure continued potency of each dose.

The study was reviewed and approved by the Institutional Review Boards of University Hospital Case Medical Center, Emory University and Grady Health System. All parents or legal guardians and participants ≥18 years of age gave written informed consent to participate in the study. Participants aged 17 years of age signed a written consent along with their parent or legal guardian.