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High-Dose Vitamin D and Calcium Attenuates Bone Loss with Antiretroviral Therapy Initiation:

A Prospective, Randomized Placebo-Controlled Trial for Bone Health in HIV-Infected Individuals

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Abstract

Background—Antiretroviral therapy (ART) initiation for HIV-1 infection is associated with 2-6% loss in bone mineral density (BMD).

Objective—To evaluate vitamin D3 (4000 IU daily) plus calcium (1000 mg calcium carbonate daily) supplementation on bone loss associated with ART initiation.

Design—48-week prospective, randomized, double-blind, placebo-controlled study.

Setting—Thirty nine AIDS Clinical Trials Network research units.

Participants—ART-naïve HIV-infected adults.

Measurements—BMD by dual-energy X-ray absorptiometry (DXA); 25-hydroxy vitamin D (25(OH)D) levels, parathyroid hormone (PTH), phosphate metabolism, markers of bone turnover and systemic inflammation.

Results—165 eligible subjects were randomized (79 Vitamin D/calcium (VitD/Cal); 86 placebo); 142 subjects with evaluable DXA data were included in the primary analysis. The study

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arms were well-balanced at baseline: median age 33 years; 90% male; 33% non-Hispanic black; median CD4 count 341 cells/mm^3; and median 25(OH)D 23 ng/mL (57 nmol/L). At 48 weeks, subjects receiving placebo had greater decline in total hip BMD than VitD/Cal: −3.19% median change (1^{st}-3^{rd} quartile (Q1, Q3) −5.12%, −1.02%) vs. (−1.46% −3.16%,−0.40%). respectively (p=0.001). Lumbar spine BMD loss for the two groups was similar: −2.91% (−4.84%, −1.06%) vs. −1.41% (−3.78%, 0.00%), (p=0.085). At week 48, 90% of participants achieved HIV-1 RNA <50 copies/mL. Levels of $25(OH)D_3$ increased in the VitD/Cal but not the placebo group: median change of 24.5 (14.6, 37.8) vs. 0.7 (−5.3, 4.3) ng/mL, respectively (p<0.001). Additionally, increases in markers of bone turnover were blunted in the VitD/Cal group.

Limitations—No international sites were included; only 48 weeks of follow up

Conclusion—Vitamin D/calcium supplementation mitigates the loss of BMD seen with initiation of efavirenz/emtricitabine/tenofovir, particularly at the total hip, which is the site of greatest concern for fragility fracture.

Primary Funding Source—National Institute of Allergy and Infectious Diseases, Bristol-Meyers Squibb, Gilead Sciences.

Introduction

Antiretroviral therapy (ART) has transformed HIV infection from a terminal disease to a manageable chronic illness. While incidence of AIDS-defining conditions has declined, other comorbidities have increased (1), including osteoporosis and fragility fractures (2-7). Both viral and host factors likely contribute to bone loss and fracture risk: HIV infection mediated by certain viral proteins, HIV-associated inflammation, lifestyle and behavioral factors, underlying genetic predisposition, comorbidities, and ART (8-14).

ART initiation studies have confirmed that 2-6% loss of hip and spine BMD occurs over the first 24-48 weeks after ART initiation with subsequent stabilization (15-18). The magnitude of bone loss is similar to that observed with glucocorticoids or during the first year of menopausal transition (19,20). This initial bone loss is marked by an increase in serum bone resorption markers followed by a delayed compensatory increase in bone formation markers [21]; therefore, this catabolic window, a high bone turnover state with excess bone resorption, may be a central mechanism of bone loss with ART initiation.

Tenofovir (TDF), a nucleotide analogue reverse transcriptase inhibitor (NRTI), has been associated with greater bone loss than other NRTIs (15,16). TDF use is associated with increased PTH, elevated vitamin D binding protein and reduced free 1,25-dihydroxy vitamin D ($1,25(OH)_2D_3$) levels (22,23), suggesting that functional vitamin D deficiency with TDF use potentially contributes to excess bone loss. Initiation of efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor, is associated with a 2.5-5 ng/ml (6.2-12.5nmol/L) decrease in 25(OH)D levels (24,25). Efavirenz induces cytochrome P450 enzymes involved in vitamin D metabolism and may accelerate the catabolism of 25(OH)D and $1,25(OH)_2D_3$, the latter being the active vitamin D metabolite (26). These two ART agents are combined with emtricitabine (FTC), another NRTI, into a fixed dose combination (FDC) once-a-day pill (EFV/FTC/TDF) that is highly effective for treating HIV infection (27).

Beyond bone metabolism effects, vitamin D has immunomodulatory effects mediated through the vitamin D receptor present on cells in both the innate and the adaptive immune system (28,29). Vitamin D increases monocyte expression of CD14 and cathelicidin, molecules involved in innate immune responses (30,31), and down-regulates cytokine expression in activated T cells and suppresses T cell proliferation and the production of IFN-gamma and IL-2, thus reducing net state of inflammation(32,33). These pathways are particularly relevant in HIV infection where excess monocyte activation and T cell activation are important drivers of morbidity and mortality (34-37).

We hypothesized that bone loss associated with ART initiation with EFV/FTC/TDF would be attenuated with high dose vitamin D and calcium supplementation. Additionally, we evaluated immunomodulatory effects of vitamin D in the setting of treatment of HIV infection. Herein, we report the results of ACTG A5280: a multi-center, randomized, double-blind, placebo-controlled study assessing the effect of daily oral 4000 IU vitamin D_3 and 1000 mg calcium carbonate in HIV-infected adults initiating their first ART regimen with EFV/FTC/TDF.

Methods

HIV-infected subjects, naïve to ART without evidence of resistance to the antiretrovirals in the regimen and with HIV-1 RNA>1000 copies/ml, were eligible if they met the following criteria: screening 25(OH)D level ≥ 10 and <75 ng/mL (≥25 and <188 nmol/L), CrCl ≥60 ml/min by Cockcroft-Gault, and serum calcium <10.5 mg/dl. We excluded subjects with daily calcium supplementation >500mg, daily vitamin D supplementation >800 IU, any bisphosphonate use, recent steroid or chemotherapy treatments, clinically active thyroid disease, active substance or alcohol abuse, history of fragility fracture, documented osteoporosis, nephrolithiasis, or weight>300 lbs (limit of DXA scanner). Pregnant and breastfeeding women were excluded. We performed three-day dietary recalls at entry to estimate vitamin D and calcium intake. Participants were randomized to 4000 IU cholecalciferol (vitamin D3) daily plus 500mg calcium carbonate twice daily or identically matching placebos (Tishcon Corporation, Westbury, NY), and counseled to take with food to facilitate absorption. Some experts consider the current upper U.S. Dietary Reference Intake of 2,000 IU below actual physiologic requirements; therefore, we tested the highest supplementation without risk of toxicity (39-41). Vitamin D_3 (4000 IU/day) is the highest daily dose considered safe for adults by the Institute of Medicine (38) and has previously been evaluated in HIV-infected persons with excellent tolerability and safety data (42). We randomized subjects to treatment arms in a 1:1 ratio using permuted blocks stratified by screening serum 25(OH)D (≤ 20 and >20 ng/mL (50nmol/L)). The Institutional Review Boards of all participating sites approved the study; all subjects provided written informed consent. (clinicalTrials.gov Identifier NCT01403051)

Primary endpoint was percentage change in total hip BMD from baseline to 48 weeks. Secondary endpoints included percentage change in lumbar spine BMD at 48 weeks, change in plasma 25(OH)D, PTH, markers of bone turnover, soluble inflammatory biomarkers, and CD4 cell counts at 24 and 48 weeks. Incidence of hypercalcemia and nephrolithiasis were

monitored. All DXA scans were read in a blinded fashion at the Body Composition Analysis Center at Tufts Medical Center (Boston, MA) using a standardized protocol.

Biomarker Assays

Screening laboratories were performed at local CLIA certified laboratories. Serum samples were stored at −70°C until batched analysis at the Irving Institute Biomarkers Core at Columbia University Medical Center (New York, NY). We measured 25(OH) D_2 and D_3 by liquid chromatography tandem mass spectrometry; intact PTH (radioimmunoassay; Scantibodies, Santee, CA); N-terminal propeptide of procollagen type 1 (P1NP; RIA; IDS, Scottsdale, AZ); C-telopeptide (CTX; ELISA; IDS Scottsdale, AZ); IL-6 (ELISA; R&D Systems, Minneapolis, MN); soluble receptors of TNFα (sTNFr-I and –II; ELISA; R&D Systems, Minneapolis, MN), and soluble CD14 (sCD14, ELISA; R&D Systems, Minneapolis, MN). Inflammatory biomarkers were chosen based upon association with relevant endpoints (43-46). Except for 25(OH)D, biomarkers were measured in duplicate and values averaged for analysis.

Statistical Analysis

To provide an intent-to-treat interpretation, analyses were performed regardless of status regardless of status on randomized treatment. Two subjects who did not have the correct Vitamin D test performed at baseline were excluded from the efficacy analyses. The primary analysis utilized a multiple imputation approach to impute missing data with a Markov chain Monte Carlo (MCMC) method. These analyses used 5 imputations based upon screening 25(OH)D stratum, age, sex, and race for the primary outcomes of change in hip and spine BMD from baseline to 48 weeks. A pre-specified complete case approach was utilized for analyses by stratum and to assess interactions. Stratified Wilcoxon rank sum tests were used to test for distribution shifts between the treatment groups, stratified by screening vitamin D levels. Fisher's exact tests and Wilcoxon rank sum tests were used to evaluate for differences between groups for categorical and continuous secondary outcomes, respectively. Wilcoxon signed rank test was used to evaluate within treatment group change. The 95% confidence intervals for median changes within treatment group were estimated using distribution-free method via percentiles. For the BMD outcomes modification of the treatment effect by screening vitamin D stratum was evaluated via linear regression. All statistical tests were two-sided and interpreted at the 5% nominal level of significance without adjustment for multiple comparisons. Analyses were performed using the following procedures in SAS version 9.2 and Cytel Proc StatXact package version 9: FREQ, STRATIFY, PAIRED, UNIVARIATE, REG, MI, and MIANALYZE).

Role of the Funding Source

The National Institute of Allergy and Infectious Diseases funded the study. Industry sponsors provided antiretrovirals, vitamin D, calcium and matching placebos and additional funding was provided for completion of DXA scans and laboratory assays. Representatives from Bristol Meyers Squibb and Gilead Sciences served as members of the study team. NIAID had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or decision

to submit the manuscript for publication. The manuscript was reviewed by industry sponsors prior to submission.

Results

We enrolled 167 subjects between September 2011 and February 2012, (81 in VitD/Cal group and 86 in placebo group) from 39 sites in the US and Puerto Rico. Two subjects, both from VitD/Cal group, were found to have eligibility violations due to incorrect screening test being performed ($1,25(OH)_2D$ rather than 25(OH)D). At the time of discovery and blinded to treatment group assignment and all on-study data, the protocol team decided that these subjects could continue to receive study treatment, but their data would not be included in efficacy analyses. Baseline demographic, immunologic, virologic, and other parameters of the 165 eligible study subjects are summarized in table 1. Overall, the cohort was 90% male, 33% non-Hispanic black, 37% non-Hispanic white, and 25% Hispanic; median CD4 cell count of 341 cells/mm^3 (230, 490 cells/mm^3); and no subjects reported HBV or HCV co-infection. Overall, 148 subjects (90%) completed study follow-up with 9 discontinuations in VitD/Cal and 8 in placebo group. Loss to follow-up (including relocation and incarceration) and noncompliance were the most commonly cited reasons; additionally 3 subject (1 in VitD/Cal, 2 in placebo group) completed follow-up off study treatment. Twenty-five subjects discontinued EFV/FTC/TDF prematurely (13 in VitD/Cal and 12 in placebo group). While week 48 outcomes were unavailable for a greater number of subjects in the VitD/Cal group compared to the placebo, reasons for missing outcomes did not appear related to any of the outcomes of interest (figure 1).

Vitamin D Changes

Median screening 25(OH)D level was 23 ng/mL (Q1,Q3: 18, 31 ng/mL) (57nmol/L; 45, 77 nmol/L). The median (Q1,Q3) estimated daily dietary calcium and vitamin D intake at entry were 813 mg (435, 1239 mg) and 131 IU (59, 260 IU), respectively. Of the entire cohort, 18% and 22% reported calcium and vitamin D supplementation, respectively, within 30 days of baseline. As expected, from entry to weeks 24 and 48, median $25(OH)D_3$ levels did not change in the placebo group: −0.6 (−5.9, 5.0) and 0.7 (−5.3, 4.3) ng/mL, respectively. Levels of $25(OH)D_3$ increased in the VitD/Cal group from entry to weeks 24 and 48: 28.6 (15.0, 38.0) and 24.5 (14.6, 37.8) ng/mL, respectively ($p<0.001$ at both time points). Group $25(OH)D_3$ distributions differed at both weeks 24 and 48 ($p<0.001$ for both, figure 2).

BMD Changes

At baseline, median BMD (Q1,Q3) for the entire cohort at the total hip and lumbar spine were 1.05 g/cm^2 (0.96, 1.17 g/cm^2) and 1.11 g/cm^2 (1.02, 1.21 g/cm^2), respectively, with similar distributions between groups (table 1). Median Z-scores (Q1,Q3) for the entire cohort at hip and lumbar spine were 0.00 (−0.80, 0.50) and −0.30 (−1.20, 0.50), respectively, with similar distributions between groups. At 48 weeks, both groups demonstrated significant decreases in BMD at total hip from baseline ($p< 0.001$, for both). Using the multiple imputations approach, percentage decline in BMD at total hip was smaller in the VitD/Cal group compared to the placebo group: median (Q1, Q3) −1.36% (−3.43, 0.50%) and −3.22% (−5.56, −0.88%), respectively (P=0.004). Between-group differences at the

lumbar spine were similar in magnitude to differences at the total hip (−1.23%, (−3.73, 0.20%) and −2.94% (−4.87, −0.94%), respectively) (p=0.033).

Our pre-specified complete case analysis approach yielded similar results; median (Q1, Q3) at the hip −1.46% (−3.16, −0.40%) versus −3.19% (−5.12, −1.02%) (p= 0.001) and (−1.41%; −3.78, 0.00% versus −2.91% (−4.84, −1.06%) (p=0.085) (figure 3). To evaluate whether changes in BMD were related to 25(OH)D levels, we assessed the changes in total hip BMD by screening 25(OH)D strata: 25(OH)D 10-20 ng/ml (25-50 nmol/L), or 20-75ng/mL (50-188 nmol/L). For the VitD/Cal group, the median decline (Q1,Q3) at 48 weeks was −1.33% (−3.24, −0.00%) in the low stratum (n=20) and −1.48% (−3.13, −0.66%) in the high stratum (n=45). For the placebo group, the median decline was −2.87% (−5.20, −1.34%) in the low stratum (n=24) and −3.19 (−5.12, −1.02%) in the high stratum (n=53). Modification of the treatment effect by screening 25(OH)D stratum was not apparent (interaction p=0.15).

Bone Turnover Marker and PTH Changes

At baseline, bone turnover marker distributions were similar in the VitD/Cal and placebo groups for both P1NP, a bone formation marker (52 ng/mL (41, 67ng/mL) versus 45 ng/mL (36, 65 ng/mL), respectively) and CTX, a bone resorption marker (0.41 ng/mL (0.30, 0.53 ng/mL) versus 0.34 ng/mL (0.25, 0.51 ng/mL), respectively). Both groups experienced increases in these markers at 24 and 48 weeks after ART initiation but increases were attenuated in the VitD/Cal group compared to the placebo group at week 24 (p=0.002 and 0.005 for P1NP and CTX, respectively) (figure 4, table 2). Also, PTH increased 15% and 22% from baseline to week 24 and week 48 ($p \leq 0.001$ for both) in the placebo group, while a change from baseline was not apparent in the VitD/Ca group (1.4%, p=0.18 and 4.3%, p=0.17), (between group comparison: $P<0.011$, figure 4). Changes in urinary fractional excretion of phosphate from baseline to week 24 or 48 were not apparent in either group (Table 2).

Inflammatory biomarker changes

At baseline, the distributions of IL-6, sTNFr-I, sTNFr-II, and sCD14, were similar between the two groups (Table 2). There were no significant changes in IL-6 at week 24 or 48 in either treatment group ($p> 0.07$ for all) or between groups at either time point ($p \geq 0.70$ for both). A within-group decline in sTNFr-I at week 24 and week 48 was seen in the placebo group (p =0.016, and 0.041, respectively) although changes were modest (median decrease: 64 and 62 pg/mL at week 24 and 48, respectively). For the VitD/Cal group, there was a similar modest decline at week 48 (median decrease: 52 and 94 pg/mL at week 24 and 48 (p=0.071 and 0.005, respectively). However, differences in magnitude of changes between groups were not apparent ($p> 0.65$ for both time points). Decreases in sTNFr-II were seen at weeks 24 and 48 for both treatment groups ($p<0.001$ for all), but there were no differences between arms ($p> 0.73$ for both). While a 17% increase in sCD14 from baseline to week 48 was observed in the VitD/Cal group (p=0.008), a change was not apparent in the placebo group (5% median increase, p=0.18). Differences in the change in sCD14 between groups at week 24 and 48 were not apparent ($p>0.16$ for both).

HIV disease parameters

Total CD4 T-cell count increased at weeks 24 and 48 in both groups ($p<0.001$ for all) with no differences between groups ($p\geq0.28$ for both). At 48 weeks, the median gain (Q1,Q3) was 192 (113, 305) and 201 (108, 292) cells/μL in the VitD/Cal and placebo groups, respectively (Table 2). Overall, 90% of subjects achieved virologic suppression (plasma HIV RNA < 50cp/mL) at 48 weeks; four subjects in the VitD/Cal group and 10 subjects in the placebo group experienced virologic failure (defined as >200cp/mL confirmed with a second evaluation on or after week 24).

Safety

Overall, 103 subjects (62%) reported at least one adverse event during the study: 50 subjects in VitD/Cal group (33 Grade 1-2, 15 Grade 3, 2 Grade 4) and 53 subjects in placebo group (33 Grade 1-2, 15 Grade 3, 5 Grade 4). No clear pattern emerged with regards to AEs (Table 3). There were no cases of hypercalcemia, and 1 case of nephrolithiasis in the placebo group. One death occurred in the VitD/Cal group due to renal failure in the context of rapid HIV disease progression.

Discussion

Supplementation with high dose vitamin D3 (4000IU) and calcium carbonate (1000mg) with ART initiation increased 25(OH)D levels, attenuated bone turnover marker increases and bone loss at the hip and lumbar spine by approximately 50% at 48 weeks. These results mark the first successful intervention to attenuate bone loss with ART initiation and demonstrate the benefit of vitamin D and calcium supplementation to promote bone health in HIV-infected persons. The bone loss associated with ART initiation leaves HIV-infected persons with lower bone mass, potentially contributing to increased fracture risk (2-7). With the lifespan of HIV-infected persons now approaching that of seronegative individuals(47), the incidence of fragility fractures will undoubtedly increase (3,6). Few guidelines have addressed preventive measures in HIV-infected individuals (48).

Tenofovir (TDF) is associated with BMD decline (16) not only among HIV-infected persons starting ART but also in seronegative individuals using TDF as a component of Pre-Exposure Prophylaxis (PrEP) and in seronegative newborns exposed to TDF in utero (49,50). Recent studies have explored the association between TDF bone toxicity, proximal tubule toxicity and increased PTH levels (50). In our study, ART initiation with a TDF-based regimen increased PTH and vitamin D and calcium supplementation prevented increases in PTH levels, without altering urinary phosphate excretion, suggesting that TDF does not cause increase PTH through proximal renal tubule toxicity. Similar results were reported by Havens and colleagues (22,23) in a study of vitamin D supplementation, reporting that PTH levels decreased with vitamin D supplementation without change in urinary phosphate excretion, and the greatest effect on PTH was seen in persons receiving TDF-containing ART. Given that PTH secretion is regulated by serum calcium, it is plausible that increased intestinal calcium absorption mediated by vitamin D is the key mechanism blocking the negative bone effects of TDF.

Efavirenz is a potent inducer of cytochrome P450 enzymes. Several of these enzymes are involved in vitamin D metabolism and efavirenz may have the detrimental off-target effect of reducing available vitamin D substrate and active metabolites (25,51). In contrast to prior observations, we saw only a modest non-significant decline in the 25(OH)D levels at 24 weeks in the placebo group (24,25,52). In the group that received VitD/Cal, efavirenz did not prevent increases in 25(OH)D. We did not measure $1,25(OH)_2D_3$, the active metabolite, and thus cannot comment how supplementation affected ongoing vitamin D metabolism.

Given recent data on the immunomodulatory effects of vitamin D (29,53), we explored whether the diminished BMD loss in the VitD/Cal group was immune-mediated. Immune reconstitution with ART-initiation may result in a surge in pro-resorptive cytokines, and potentially explain the relatively consistent magnitude of BMD decline within the first 24-48 weeks after initiation of ART across different regimens, and the more modest effect when ART is used as PrEP. In our study, sCD14 increased in the VitD/Cal group, likely related to vitamin D effects on monocyte CD14 expression (30), while TNFr levels decreased similarly from baseline to 48 weeks in both arms, likely related to virologic suppression, as previously reported (54). It is possible that the anti-inflammatory effects of ART initiation obscured any potential immunomodulatory effects of vitamin D. Additional research is needed to define the mechanism behind the salutary effect of vitamin D and calcium supplementation.

Our study has some limitations. We had an imbalance in the amount of missing data between groups, but a review of reasons for missing data (relocation, loss to follow up, death and missing or uninterpretable DXA data) did not reveal a systemic bias, but rather a chance imbalance in discontinuations. Furthermore, results for our primary outcome were similar when analyzed using a multiple imputation approach for missing data or a complete case approach that assumes that missing data was random. We followed subjects for only 48 weeks and did not evaluate longer-term effects of vitamin D supplementation on fracture prevention. Data on smoking status, alcohol intake, falls, and physical activity were not collected. We cannot determine if the beneficial effect was due to vitamin D, calcium or their combination. Our study was performed in the US and Puerto Rico and comprised of 90% male subjects, thus generalizability to other areas and females may be limited. We evaluated only one ART regimen (EFV/FTC/TDF); therefore, our conclusions may not be applicable to other antiretroviral regimens.

In summary, daily high dose vitamin D and calcium supplementation over 48 weeks attenuates BMD decline associated with ART initiation by approximately 50%. This beneficial effect corresponded with reductions in bone turnover markers and limited excursion of PTH levels. We did not observe a specific anti-inflammatory effect of vitamin D supplementation. Vitamin D and calcium is a low cost, well-tolerated intervention to prevent ART-related bone loss. Future studies will examine alternative vitamin D doses, effects when utilized with other ART regimen and in international settings, and longer-term efficacy.

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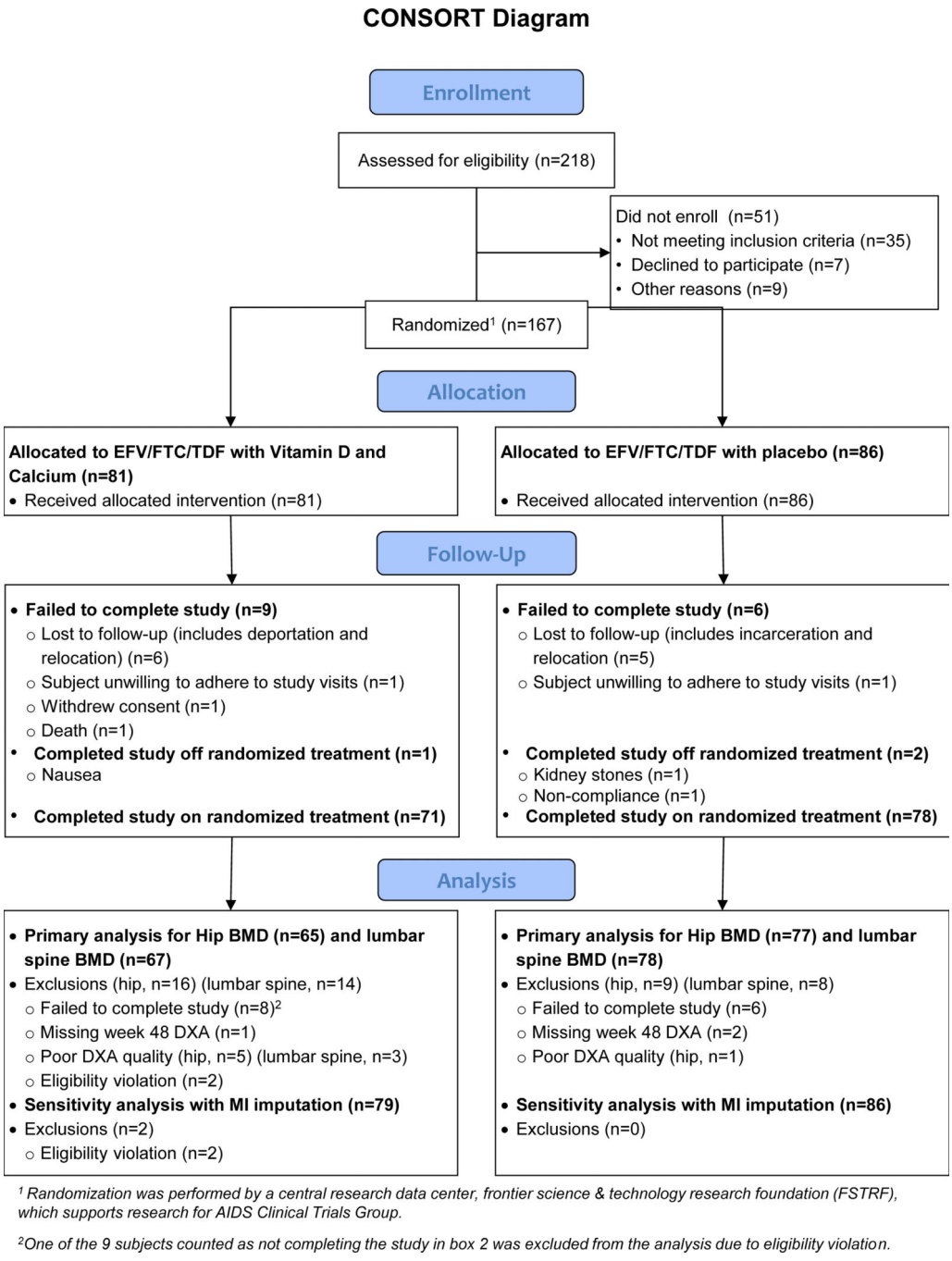


Figure 1.

Details and Disposition of Study Participants.

No footnotes.

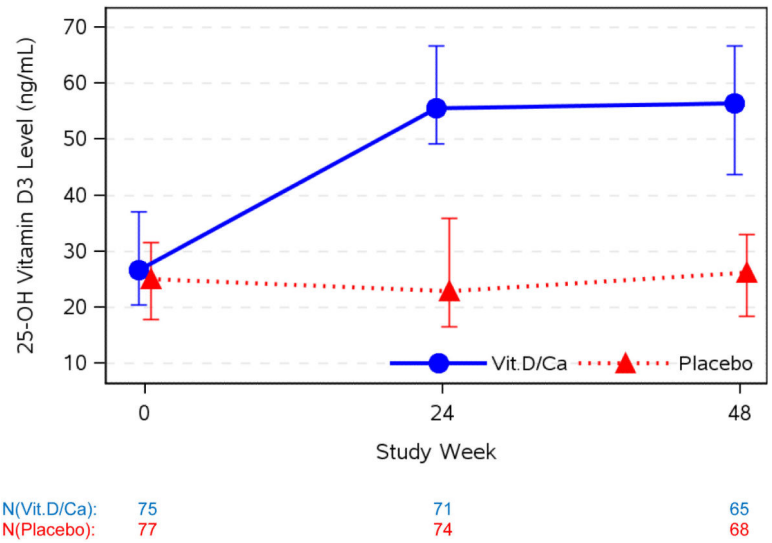
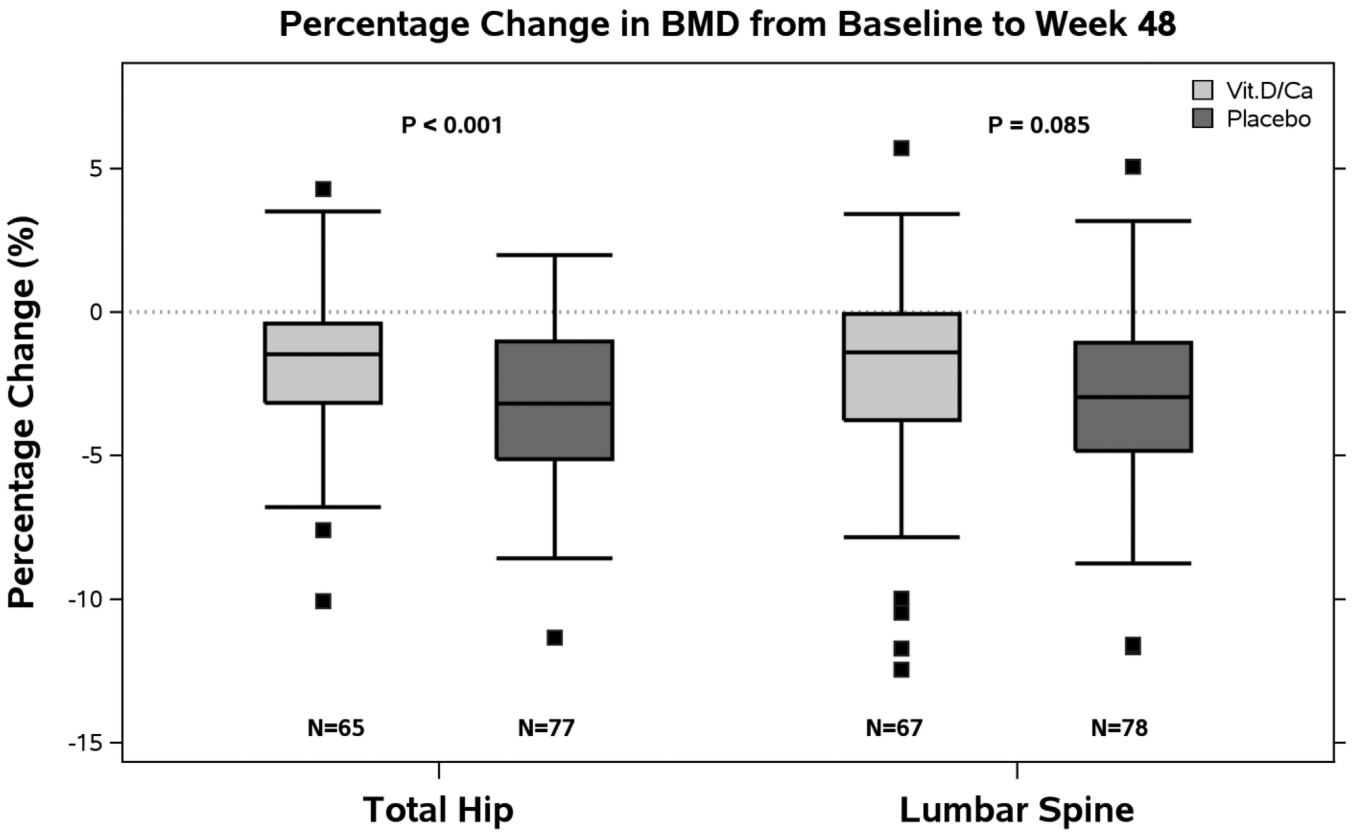


Figure 2. 25(OH) Vitamin D3 Levels at Baseline, 24 and 48 Weeks

25(OH) vitamin D3 levels at baseline, weeks 24 and 48 are presented in by study group. Data is presented as median value in ng/mL with error bars representing first and third quartiles (25^{th} and 75^{th} percentiles). From entry to weeks 24 and 48, $25(OH)D_3$ levels did not significantly change in the placebo group (median change (Q1,Q3): −0.6 (−5.9, 5.0) and 0.7 (−5.3, 4.3) ng/mL, respectively) but levels increased significantly in the VitD/Cal group (median change: 28.6 (15.0, 38.0) and 24.5 (14.6, 37.8) ng/mL, respectively; $p<0.001$ at both time points). The consequence of these changes between group differences in the $25(OH)D_3$ distributions was significant at both weeks 24 and 48 ($p<0.001$ for both).



The line inside the box indicates the median value;
The lower/upper edges of the box indicate the first/third quartiles (the 25th/75th percentiles);
The lower/upper whiskers are the first/third quantiles -/+ 1.5 times interquartile range (IQR);
Observations outside the whiskers are identified as outliers (with square symbols).

Figure 3. Percent Change in Bone Mineral Density from Baseline to week 48

Percent changes in bone mineral density from baseline to week 48 are presented in this figure: total hip on the left and lumbar spine on the right. For both groups there was a significant decline in median percent BMD change (p<0.001 for both sites and both groups). The percentage decline in BMD at total hip was significantly smaller in the VitD/Cal group (−1.46%; −3.16, −0.40%) compared to the placebo group (−3.19%; −5.12, −1.02%) (p= 0.001). The difference in BMD at lumbar spine between arms was of similar magnitude as the total hip, it did not achieve statistical significance: VitD/Cal group (−1.41%; −3.78, 0.00%) compared to the placebo group (−2.91%; −4.84, −1.06%) (p=0.085).

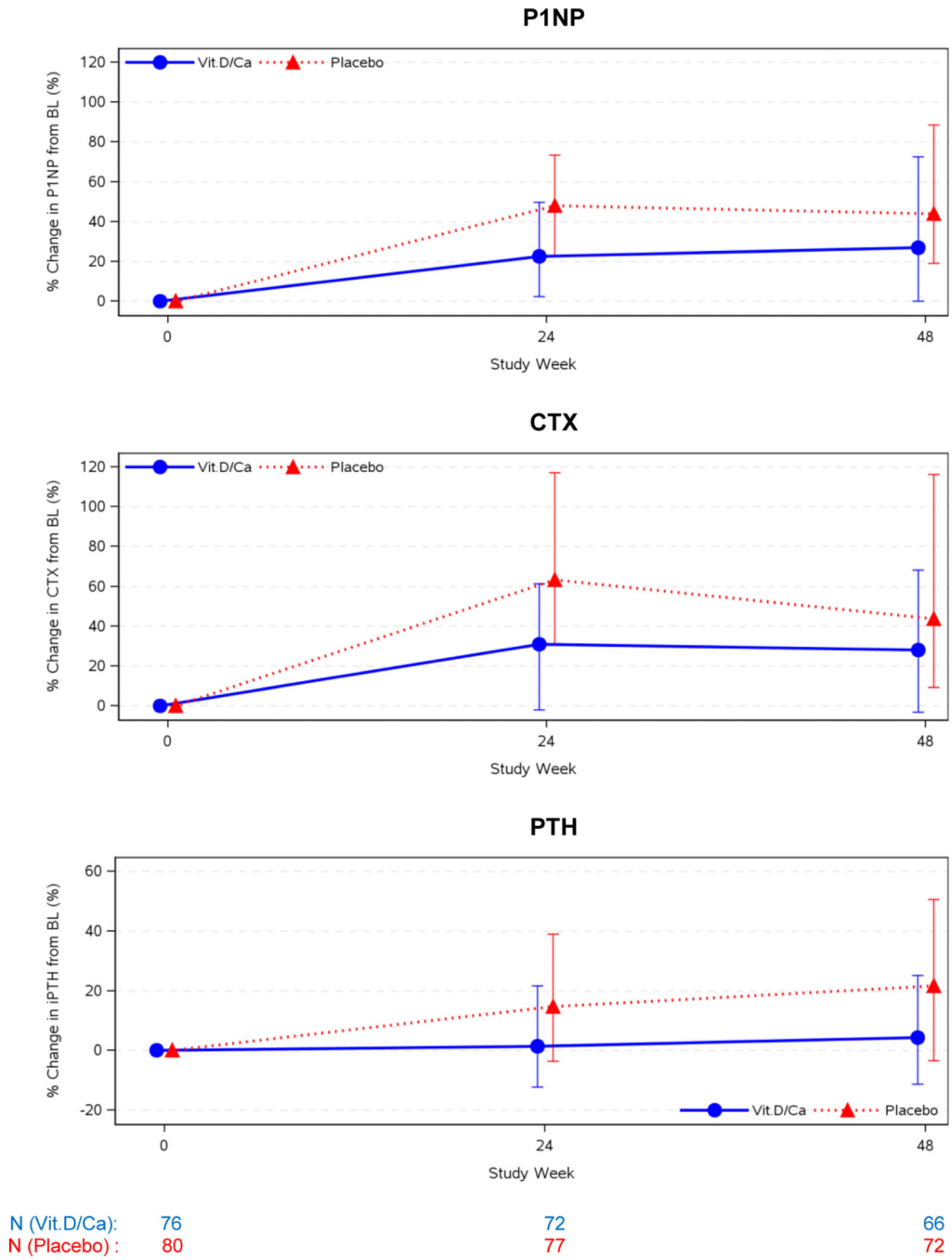


Figure 4. Percent Change in Bone Turnover Markers and PTH at 24 and 48 Weeks

Percent changes in biomarkers related to bone turnover from baseline to week 24 and 48 are presented in this figure: N-terminal propeptides of procollagen type 1 (P1NP), C-telopeptide (CTX), and parathyroid hormone (PTH) are presented in the top, middle, and bottom panels, respectively. Data is presented as median percent increase from baseline with error bars representing first and third quartiles (25^{th} and 75^{th} percentiles. In the upper and middle panels, P1NP and CTX increased significantly from baseline to week 24 and 48 in both groups ($p< 0.001$, for all). At week 24, the between group differences were also statistically

significant (p= 0.002 for P1NP, p=0.005 for CTX) but the differences were not statistically significant at week 48 (p> 0.088 for both). The lower panel displays the percent change in PTH. In the VitD/Cal group, there was no significant change in PTH at week 24 or 48 (p>0.31 for both), while the placebo group experienced a significant increase at both 24 and 48 weeks (p< 0.001, for both). The between group differences were statistically significant for both time points (p=0.011 at 24 weeks and p=0.004 at 48 weeks)

Table 1

Baseline Demographics

Parameter	Vitamin D / Calcium (n=79)	Placebo (n=86)
Age in years *	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)
Plasma HIV RNA (log$_{10}$cp/mL) *	4.5 (4.1, 5.1)	4.5 (4.0, 5.1)
> 100,000 cp/mL	21 (27%)	22 (26%)
CD4 cell count (cells/μl) *	339 (230, 500)	342 (232, 454)
< 200 cells/ μl	17 (22%)	15 (17%)
BMD at hip (g/cm^2) *	1.08 (0.96, 1.19)	1.02 (0.94, 1.11)
Z score for BMD at hip *	0.10 (−0.70, 0.70)	−0.20 (−0.80, 0.40)
< −2.0	4 (5%)	5 (6%)
BMD at lumbar spine (g/cm^2) *	1.15 (1.03, 1.26)	1.07 (0.99, 1.21)
Z score for BMD at lumbar spine *	0.00 (−0.90, 0.70)	−0.50 (−1.30, 0.40)
< −2.0	7 (9%)	9 (10%)
Estimated daily Ca intake (mg) *#	813 (492, 1303)	811 (365, 1227)
Estimated daily VitD intake (IU) *#	120 (62, 215)	137 (59, 279)

Note: Two ineligible subjects are not included in the table. These 2 subjects are both non-Hispanic males, ages 30 and 41,and were randomized to the Vitamin D/Calcium group.

Abbreviations: BMI: body mass index

*Denotes median value with 25th and 75th percentile value in parentheses.

#We performed three-day dietary recalls at entry to estimate daily vitamin D and calcium intake.

Table 2

Summary of BMD, Vitamin D, Bone Turnover Markers, and Soluble Inflammatory Biomarkers at Baseline, 24 weeks, and 48 weeks

Parameter	Group	Baseline	Week 24	P value*	Week 48	P value*
BMD at hip (g/cm^2)	VitD/Ca	1.08 (0.96, 1.19)	Not Assessed	N/A	1.05 (0.98, 1.17)‡	**<0.001**
	Placebo	1.02 (0.94, 1.11)			0.98 (0.90, 1.07)‡	
BMD at lumbar spine (g/cm^2)	VitD/Ca	1.15 (1.04, 1.26)	Not Assessed	N/A	1.12 (1.03, 1.24)‡	0.085
	Placebo	1.07 (0.99, 1.21)			1.05 (0.94, 1.18)‡	
Total 25(OH) D_3 (ng/mL)	VitD/Ca	26.7 (20.4, 37.1)	55.6 (49.1, 66.7)‡	**<0.001**	56.4 (43.7, 66.6)‡	**<0.001**
	Placebo	25.1 (17.8, 31.5)	22.9 (16.6, 35.9)		26.2 (18.4, 32.9)	
P1NP (ng/mL)	VitD/Ca	52 (41, 67)	72 (81, 86)‡	**0.002**	68 (48, 80)‡	0.088
	Placebo	45 (36, 65)	75 (55, 88)‡		73 (51, 91)‡	
CTX (ng/mL)	VitD/Ca	0.41 (0.30, 0.53)	0.53 (0.37, 0.74)‡	**0.005**	0.51 (0.37, 0.75)‡	0.12
	Placebo	0.34 (0.25, 0.51)	0.62 (0.37, 0.85)‡		0.56 (0.36, 0.74)‡	
PTH (pg/mL)	VitD/Ca	28.3 (24.5, 34.5)	29.2 (24.7, 35.5)	**0.011**	30.0 (25.2, 37.2)	**0.004**
	Placebo	27.6 (22.1, 33.9)	32.9 (25.7, 42.5)‡		33.1 (26.8, 42.1)‡	
Percent Fractional	VitD/Ca	8.4 (5.9, 11.1)	8.4 (6.1, 12.2)	0.77	8.4 (6.2, 12.5)	0.66
Excretion of PO4 (%)	Placebo	7.9 (4.9, 10.6)	8.4 (5.7, 10.7)		9.2 (5.9, 12.4)	
CD4 Count (cells/μl)	VitD/Ca	346 (238, 495)	487 (346, 650)‡	0.73	551 (414, 733)‡	0.90
	Placebo	337 (266, 468)	507 (361, 646)‡		526 (410, 732)‡	
IL-6 (log_{10} pg/mL)	VitD/Ca	0.08 (−0.10, 0.31)	0.02 (−0.19, 0.26)	0.98	0.00 (−0.12, 0.25)	0.70
	Placebo	0.12 (−0.09, 0.29)	0.08 (−0.12, 0.23)		−0.01 (−0.20, 0.32)	
sTNFr-I (log_{10} pg/mL)	VitD/Ca	3.04 (2.97, 3.13)	3.01 (2.95, 3.09)	0.65	3.02 (2.97, 3.07)‡	0.81
	Placebo	3.04 (2.98, 3.10)	3.02 (2.98, 3.08)‡		3.03 (2.95, 3.08)‡	
sTNFr-II (log_{10} pg/mL)	VitD/Ca	3.57 (3.44, 3.68)	3.43 (3.33, 3.51)‡	0.76	3.39 (3.30, 3.49)‡	0.73
	Placebo	3.59 (3.47, 3.68)	3.43 (3.35, 3.54)‡		3.43 (3.31, 3.49)‡	
sCD14 (log_{10} ng/mL)	VitD/Ca	3.14 (3.06, 3.26)	3.19 (3.09, 3.31)	0.76	3.22 (3.14, 3.33)‡	0.16
	Placebo	3.20 (3.10, 3.29)	3.22 (3.13, 3.34)		3.24 (3.12, 3.30)	

All data presented as median values with 25th and 75th percentile value in parentheses.

Abbreviations: P1NP: N-terminal propeptide of procollagen type 1; CTX: C-telopeptide; IL-6: interleukin 6; sTNFr-I and II: soluble receptors of tumor necrosis factor alpha; sCD14: soluble CD14

‡denotes within-arm difference from baseline is statistically significant ($p < 0.05$).

*P values -evaluate the difference in changes from baseline to week 24/48 between the two treatment groups, stratified by screening 25-OH vitamin D levels; statistically significant differences are bolded.

Table 3

Summary of Grade 3 and 4 Adverse Events

Primary event	Grade	VitD/Ca	Placebo
Headache	3	0	1
Seizure	3	1	0
Aseptic Meningitis	3	1	0
Depression	3	1	1
Mallory Weiss Tear	3	1	0
Gastroenteritis	3	0	2
Abdominal pain	3	1	0
Cholecystitis	4	1	0
Alcoholic Hepatitis	3	0	1
Anal AIN 3	3	0	1
Asthma exacerbation	1	0	1
Bacterial pneumonia	3	0	1
Back pain	3	0	1
Shoulder dislocation	3	0	1
Left hand laceration	3	1	0
Right knee effusion	3	0	1
R foot abscess	3	0	1
Weight loss	3	1	0
Coagulopathy	3	0	1
Lyme Disease	3	0	1
Immune Reconstitution Inflammatory syndrome	3	1	0
DRESS Syndrome	3	0	1
LAB ABNORMALITIES			
Absolute Neutrophil Count	3	1	3
Serum Phosphate	3	4	1
ALT	3	1	3
AST	3	1	2
	4	0	1
Alkaline Phosphatase	3	0	1
Total Bilirubin	3	1	0
Triglycerides	3	0	1
Total Cholesterol	3	1	0
Glucose	3	2	3
	4	0	2