VITAMIN D SUPPLEMENTATION REGIMENS FOR HIV-INFECTED PATIENTS: A HISTORICAL CHART REVIEW

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A MASTER OF SCIENCE THESIS

Presented to Graduate Programs in Human Nutrition
and the Oregon Health & Science University School of Medicine
in partial fulfillment of the requirements for the degree of

Master of Science in Clinical Nutrition

June 2015

OREGON HEALTH & SCIENCE UNIVERSITY
SCHOOL OF MEDICINE
GRADUATE PROGRAMS IN HUMAN NUTRITION

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Acknowledgments

First and foremost, I must recognize my parents for their support throughout my entire academic career beginning with the first hug and kiss as I anxiously entered kindergarten, to now, as I near completion of my graduate program. They have not only provided emotional support but gave me the opportunity to understand the best path to pursue. Because of this I am in Portland, Oregon, and following my true passion: dietetics and nutrition.

Beyond the support of my family, I have been blessed with calling the most supportive and unique individuals my friends. They have supported me through stressful moments, which I feared I'd never escape, and they have a sense of humility unlike most. Their gentle reminders kept me on this path even after I lost my vision.

Moving forward to the present, I must acknowledge the support of my thesis advisory committee. Dr. P. Todd Korthuis, Dr. Joyanna Hansen (Joy, preferred), Dr. Melanie Gillingham, and Devon Flynn have all been part of this thoughtful and rewarding journey. Additionally, without Dr. Kathleen Holton's persistence with helping me to develop my thesis topic and connecting with my current mentor, Dr. P. Todd Korthuis, I would not have had the same level of passion compiling my thesis. Additionally, both Mike Lasarev and Hongli Yang were instrumental in completing my data cleaning, variable creation, and resultant statistical analyses.

Finally, I must share my appreciation to Jeri Finn, the Dietetic Internship Director for forwarding my request to expand my Dietetic Internship to include Master of Science degree. If it were not for her efforts, this thesis would not exist.

On one final note, along this academic journey I've acknowledged my weaknesses and

realized my strengths. Though my growth has been complimented by both successes and failures, I've learned to embrace the danger of failure instead of avoiding it completely. Even if the result was not desirable, it has always allowed me to further discover myself.

Thank you to everyone that has made this possible for me.

Abstract

Vitamin D deficiency may be common among HIV infected individuals. Similar to individuals not affected by HIV, HIV-infected patients are affected by limited UV-B exposure and vitamin D deficient diet. However, HIV-infected patients may be at increased risk for insufficient vitamin D due to antiretroviral medication regimens that affect vitamin D metabolism and fat malabsorption due to HIV-related inflammation.

In this historical chart review, we examined the prevalence of vitamin D insufficiency and assessed the comparative effectiveness of varying vitamin D repletion regimens in an HIV-infected cohort. The study population comprised patients from an outpatient HIV clinic who had vitamin D measured and who were prescribed a vitamin D repletion regimen. Demographic and clinical data were extracted from medical records, including 25-(OH)D concentrations drawn at baseline and follow-up appointments. Specific factors were analyzed in association with the main outcome of change in serum 25-(OH)D, including season of blood draw and body mass index. Differences in the change in 25-(OH)D between regimens were calculated by one-way ANOVA.

A total of 228 subjects were studied. There was a high prevalence of 25-(OH)D insufficiency (<30 ng/mL; 71.4%, 160/224). Vitamin D supplementation reduced this prevalence by 10.2% after an average of 14.0 ± 6.3 months (range: 0.7 – 49.0 months). A significant difference in change in 25-(OH)D concentration among vitamin D regimens was noted between the repletion/maintenance regimen (50,000 IU D_2 weekly/400-5,000 D_2 or D_3 IU daily) and the single vitamin D_3 (chole-) based regimens (adjusted p-value = 0.047). Interestingly, when grouped by dosage strength no significant differences in change in serum 25-(OH)D were noted (adjusted p-value = 0.489). When patients were

grouped into respective BMI categories of underweight/normal weight (BMI: 16-24 kg/m^2), overweight (BMI: 25-29 kg/m^2), and obese (BMI: 30-45 kg/m^2), no overall difference was noted among patients' change in serum 25-(OH)D (p-value = 0.10).

Though vitamin D deficiency and insufficiency were both prevalent at baseline and follow-up appointments, there was a significant difference between vitamin D regimens in patients' change of serum 25-(OH)D (adjusted p-value: 0.04). However, no significant differences were noted when average change in serum 25-(OH)D was grouped by dosage strengths (adjusted p-value: 0.489). This research highlights the complexity of increasing vitamin D serum status in an HIV-infected population. Since patient adherence to vitamin D supplementation was not measured in this historical chart review, we were unable to witness any alteration to vitamin D metabolism from an interaction of ARV medications. ARV medication can mimic low adherence rates to vitamin D supplementation as both result in reduced 25-(OH)D change.

Given what is currently known about vitamin D and immune health, further interventional studies assessing the effects of 25-(OH)D supplementation in HIV-infected patients are needed. Future research should investigate the effects not only of different vitamin D doses but also of vitamin D types in raising serum 25-(OH)D status.

Introduction

More than 40% of ambulatory Human Immunodeficiency Virus sero-positive (HIV-infected) patients are vitamin D deficient (VDD).[1-3] HIV-infected patients are considered VDD if serum 25-hydroxyvitamin D or 25-(OH)D levels are below or equal to 20 ng/mL (50 nmol/L).[4] Vitamin D supplementation is essential for those deficient according to their serum 25-(OH)D level.[1-3] Serum 25-(OH)D levels below 20 ng/mL (50 nmol/L) have been associated with cardiovascular disease, multiple sclerosis, and type 1 diabetes in the general population.[5-7] Additionally, in HIV-infected patients, serum 25-(OH)D levels below 20 ng/mL (50 nmol/L) are independently associated with coronary stenosis[8], elevated inflammatory markers (i.e. c-reactive protein) [9], and increased risk of hepatitis C infection[10]. More recently, vitamin D has been suggested to optimize immune function and may have important clinical implications for HIV-infected patients.[11]

Developing research suggests vitamin D inhibits proliferation of Kaposi's sarcoma, a tumor caused by human herpesvirus-8; down-regulates production of pro-inflammatory cytokines; and promotes an anti-microbial environment inhibiting HIV replication.[12-14] Consequentially, VDD in HIV-infected patients may contribute to chronic inflammation and exacerbate infections and malignancies common in this population.[15] Since HIV suppresses the immune system[16] and vitamin D optimizes immune function[17], vitamin D is essential for immune health within an HIV-infected population. However, there is a gap in the current literature regarding the most effective regimen to increase vitamin D status in HIV-infected patients.

Vitamin D insufficiency is defined as a serum 25-(OH)D level equal to 21-29 ng/mL (52.5-72.5 nmol/L), whereas, vitamin D deficiency is defined as 20 ng/mL (50

nmol/L) or less.[4] The goal of supplementation is to normalize serum 25-(OH)D levels to >20 ng/mL (50 nmol/L) for an extended period of time, preferably lifelong for patients at high risk of vitamin D deficiency, such as HIV-infected patients.

HIV-infected patients have several vitamin D deficiency risk factors. First, HIV-infected patients may have difficulties with malabsorption of fat-soluble vitamins in the gastrointestinal (GI) tract [18], which may prevent optimal absorption of vitamin D when supplemented. Other factors that influence vitamin D concentrations include latitude, use of sunscreen, ethnicity, age, gender, and obesity.[19-24] Interestingly, obesity rates among the HIV-infected population have been increasing ever since the introduction of anti-retrovirals.[25] Obesity, along with absorption difficulties, can further undermine vitamin D concentration as excess adipose tissue sequesters vitamin D, thus decreasing serum 25-(OH)D.[25] Due to the risk factors described above and the high prevalence of vitamin D deficiency in this population, research analyzing the most effective vitamin D supplementation regimen in HIV-infected populations will help healthcare providers provide optimal care for HIV-infected individuals.

Within Oregon Health and Science University's clinical setting, the most common vitamin D supplementation is a combined repletion and maintenance regimen. This regimen includes a weekly 50,000 IU capsule of ergocalciferol (vitamin D_2) for 12-16 weeks, the repletion phase, followed by a daily dose of 3,000-4,000 IU, or a daily over-the-counter 2,000-3,000 IU capsule of cholecalciferol (vitamin D_3), also known as the maintenance phase. The exact supplementation regimen is dependent on the healthcare provider's preference.

Specific Aims

The objective of this research project is to determine which regimen of vitamin D supplementation is the most effective for normalizing serum 25-(OH)D levels among HIV-infected patients. The timeframe between baseline and follow-up appointments ranged from 10-17 months in this study population. This historical chart review will utilize Oregon Health and Science University's (OHSU) HIV/AIDS clinic's electronic medical record (EMR) as part of IRB# 464*: Prospective OHSU HIV Database Core Protocol*, which collects various clinical data, modified to include serum 25-(OH)D levels among patients receiving care in the OHSU HIV/AIDS clinic.

Aim 1: To describe the OHSU HIV/AIDS clinic's vitamin D supplementation regimens

Aim 2: To use historical data to compare the mean change of serum 25-(OH)D levels between vitamin D supplementation regimens among patient in this HIV/AIDS clinic after an average range of 10-17 months of supplementation.

Hypothesis 1: Patients prescribed a high dose of vitamin D will have a significantly higher serum 25-(OH)D levels after an average range of 10-17 months of supplementation when compared to patients who were prescribed any other vitamin D supplementation regimen.

Aim 3: To determine if obesity within an HIV-infected population significantly affects serum 25OHD levels after vitamin D supplementation (all regimens).

Hypothesis 2: HIV-infected patients with a baseline BMI > 30 kg/m^2 will have a significantly lower mean change of serum 25-(OH)D than those patients with a BMI <29 kg/m^2.

Background

Prevalence of VDD in HIV-Infected Patients

About 40% of ambulatory Human Immunodeficiency Virus sero-positive (HIV-infected) patients in the United States have low plasma vitamin D levels.[1-3] This prevalence ranges as high as 70% dependent on specific location and severity of illness. [3, 26, 27] Population estimates of vitamin D deficiency range from 42% to 83% of adults living in the United States.[28-30] Though the estimated prevalence of vitamin D deficiency among HIV-infected patients is similar to that of adults living in the United States, HIV-infected patients have additional risk factors for vitamin D deficiency than their non HIV-infected peers.

HIV Specific Vitamin D Deficiency Risk Factors

Due to the high prevalence of VDD within the HIV-infected population, researchers have examined HIV-specific risk factors for vitamin D deficiency. HIV-infected patients are subject to interactions from antiretroviral treatment and malabsorption secondary to inflammation caused by HIV infection and other opportunistic pathogens, which all prevent adequate vitamin D metabolism.[2, 31] Additionally, HIV-infected patients are subject to the same factors that affect vitamin D status as the general population such as obesity, lack of UVB exposure, and nutritionally deficient diets.[1-3, 32, 33] Low dietary vitamin D intake strongly affects HIV-infected individuals due to the additional risk factors within this population such as fat malabsorption. Obesity, defined as a body mass index greater than or equal to 30 kg/m^2, is known to increase the prevalence of VDD among the general population.[23] One aim of

this study will analyze the proportion of obese HIV-infected individuals, compared to non-obese HIV-infected individuals to determine if there is a higher proportion of VDD in this group. Obesity has become more of a chronic health concern for HIV-infected individuals than wasting syndrome due to the advancement of HIV-antiretroviral regimens, making this research question highly relevant.[25]

The increase of obesity prevalence amongst HIV-infected patients is often explained by patients' anti-retroviral (ARV) treatment.[25] Additionally, ARV treatment interacts with vitamin D metabolism.[1] In a cross-sectional study that divided HIV-infected patients into tertiles based on CD4+ T-lymphocyte counts, the highest CD4+ tertile ($\geq$500 cells/mm^3) and the lowest CD4+ tertile (0-199 cells/mm^3) had 73.9% and 64.2% of the patients respectively who consumed deficient amounts of vitamin D (<67% RDI).[34] Even HIV antiretroviral (ARV) based regimens have the potential to lower vitamin D activity through reduced 1,25-dihydroxyvitamin D levels, as compared to those who are not on an ARV-regimen, regardless of the type or regimen of ARV.[35] HIV-protease inhibitors and reverse transcriptase inhibitors, most commonly, have been associated with the lowest serum 1,25-dihydroxyvitamin D levels. HIV protease inhibitors (PIs) indinavir, ritonavir, and nelfinavir and reverse transcriptase inhibitor efavirenz interfere with the metabolic pathways of vitamin D through inhibition of hepatic cytochrome p45027B1 and p45027B2 (CYP27B1 and CYP27B2, respectively). CYP45027B1 and CYP45027B2 are critical for the first hydroxylation of vitamin D and resultant serum 25(OH) D concentration.[35, 36] Beyond medications, co-infections such as Mycobacterium avium complex (MAC) are associated with a lower serum 1,25-dihydroxyvitamin D compared to those without the co-infection in AIDS-diagnosed

patients. Furthermore, a MAC co-infection may activate the TNF system that has been positively correlated with the degree of 1,25-dihydroxyvitamin D deficiency. MAC is also associated with increased morbidity in AIDS diagnosed patients.[33]

HIV potentially causes intestinal fat malabsorption due to the virus infecting the distal small intestinal mucosa; however, this has only been studied in Rhesus monkeys infected with simian immunodeficiency virus (SIV), HIV's precursor. [37] Vitamin D has been shown to reduce overall inflammation in HIV-infected patients and could possibly reduce malabsorption if stores are adequate.[15, 35]

HIV-Associated Dyslipidemia, Statins, and Endogenous Vitamin D Production

Individuals infected with HIV have an increased prevalence rate of dyslipidemia when compared to control groups without HIV. An individual's chance of being diagnosed with dyslipidemia is elevated after sero-conversion and further increased with antiretroviral medications.[38] Often, the HIV-associated dyslipidemia is treated with a statin that inhibits HMG-CoA reductase. Inhibition of HMG-CoA reductase can potentially limit the production of epidermal 7-dehydrocholesterol (7-DHC). Limiting this 7-DHC supply may reduce endogenous vitamin D production. However, a randomized, controlled trial showed no significant effect on serum 25-(OH)D levels after one year of treatment with simvastatin. Additionally, further analysis within this trial discovered that higher vitamin D intake is associated with lower plasma triglycerides, demonstrating a potential positive effect of vitamin D for cardiovascular health.[39]

Vitamin D Sources and Metabolism

Vitamin D is a fat-soluble seco-steroid that is connected with the intestinal absorption of calcium[40, 41], magnesium[42], phosphate[43], and zinc[44]. The two D vitamers available from the diet or supplements include D_2 (ergocalciferol) and D_3 (cholecalciferol).[45] Vitamin D_3 can be synthesized from 7-dehydrocholesterol located in the epidermis via a UVB light dependent reaction. Once the 7-dehydrocholesterol is irradiated, the previtamin D3 undergoes an [1,7] antarafacial sigmatropic rearrangement, thus is isomerized to vitamin D_3. Vitamin D_2 is sourced from diet or supplement only. In order to activate vitamin D and initiate a downstream response, both vitamin D_3 and D_2 are hydroxylated twice, which produces the active steroid calcitriol, or 1,25-dihydroxyvitamin D. Initially, vitamin D_3 converts to calcidiol, 25-(OH)D_3, while vitamin D_2 is hydroxylated to 25-(OH)D_2. Finally, only a portion of the calcidiol supply is converted to calcitriol, 1,25(OH) $_2$D, the biologically active hormone of vitamin D.[46-48] Various tissues express the two cytochrome P450 hydroxylase enzymes that convert calcidiol to calcitriol. The initial vitamin D 25-hydroxylase (P450C25) is primarily expressed in the liver, but is also expressed in other organs including skin, kidney, and intestine. The 25-(OH)D 1-α-hydroxylase (P450C1) is responsible for producing calcitriol from calcidiol and is expressed in the kidney, skin, and intestine as well as in macrophages and bone tissue.[49]

Vitamin D_2 versus Vitamin D_3

Vitamin D_3 varies from D_2 solely due to a structural difference in side chains. Vitamin D_2 contains a methyl group on carbon 24 and a double bond between carbon 22

and 23.[48] In addition to the molecular differences, vitamin D_2 also has a decreased affinity for vitamin D-receptors (VDRs) when compared to vitamin D_3.[50] Despite the side chain difference and decreased VDR affinity, research in rats suggests there is comparable benefit from vitamin D_2 when dosage is about four times greater than vitamin D_3.[51] However, when comparing toxicities in rhesus monkeys, mega-doses of vitamin D_2 resulted in significantly less poly-organ mineral deposition than vitamin D_3.[52] In a recent review of various vitamin D supplementation regimens, Tripkovic et al. (2012) concluded cholecalciferol (D_3) supplementation resulted in a greater increase in serum 25-(OH)D concentrations compared to ergocalciferol supplementation (D_2); however, the metabolic evidence to support this finding is limited in the current literature.[53] Since HIV can lead to multiple malabsorptive effects, potency of the vitamin D supplement is vital. Based on this evidence, vitamin D_3, cholecalciferol, should be more potent and effective for 25OHD repletion within an HIV-infected population than vitamin D_2, ergocalciferol.

Indicators of Serum Vitamin D Supply

25-(OH)D is the best measure of vitamin D concentration to quantify the collective serum vitamin D amount acquired from supplementation, diet, and cutaneous synthesis,.[54, 55] However, this marker, 25-(OH)D, is useful for analyzing intake and absorption, but not vitamin D's overall effect on gene transcription as it needs to be hydroxylated again to 1, 25-dihydroxyvitamin D to be considered fully activated. 1,25-dihydroxyvitamin D (calcitriol), the active hormonal form of vitamin D, is a poor indicator for vitamin D nutriture due to its short half-life (about 4 hours). During VDD, 1,25-dihydroxyvitamin D may be adequate, if not elevated, due to up-regulation of the

renal 1α-hydroxylase enzymes.[56]

The 25-(OH)D critical value to define deficiency or insufficiency is controversial. Mainly, the Institutes of Medicine (IOM) and The Endocrine Society agree that more evidence is needed to further confirm critical values for serum diagnosed vitamin D deficiencies/insufficiencies.[4, 57] In 2011, the IOM recommended defining a VDD as a serum 25-(OH)D level ≤ 12 ng/mL (30 nmol/L) and an insufficient vitamin D concentration as 12-20 ng/mL (30–50 nmol/L) for a generally healthy population.[57]

In order to define the serum 25-(OH)D critical value for insufficiency, researchers have analyzed parathyroid hormone's (PTH) response to varying 25-(OH)D levels. One effect of PTH is increasing serum calcium through up-regulating renal 25-(OH)D 1-α-hydroxylase, thus increasing the 1,25-dihydroxyvitamin D level. 1,25-dihydroxyvitamin D acts as a transcription factor, thus enhancing calcium absorption.[58] When serum 25-(OH)D decreases to about 12 ng/mL (30 nmol/L) or less, PTH increases above baseline. PTH reaches the upper limit of normal, 55 pg/mL, when 25-(OH)D reaches about 4.5 ng/mL (11.25 nmol/L).[59, 60] Elevated PTH has detrimental effects on bone health. As serum PTH increases, skeletal calcium stores decrease.[61] This can prove detrimental for HIV-infected individuals who are already at risk for developing osteopenia, a below normal bone mineral density. According to a longitudinal cohort study, 47.5% HIV-infected individuals were osteopenic regardless of their specific lifestyles and diets.[62]

HIV-Specific Lab Values: Viral load and CD4 Count

In order to understand the degree of health of HIV-infected patients and the progression of disease, clinicians analyze HIV-specific lab values in addition to physical

examinations to note any signs of AIDS. The serum viral load has the strongest association with progression to AIDS and death over CD4+ T-lymphocyte counts. Viral load also predicts the rate of decrease of CD4+ T-lymphocytes. It has been suggested that measurement of plasma HIV-1 RNA viral load *and* CD4+ T-lymphocytes should be considered to fully understand the disease state.[63] Typically, a viral load greater than 100,000 copies/mL is associated with a greater likelihood of developing AIDS and mandates initiating treatment, while a viral load count less than 10,000 copies/mL is considered low-positive and associated with a decreased risk of developing AIDS.[64]

Acquired Immunodeficiency Syndrome (AIDS)

The United States Centers for Disease Control and Prevention (CDC) of the defines AIDS in individuals who are HIV-infected as a CD4+ T-lymphocyte count of 200 per μL of serum or less and/or a CD4+ T-lymphocyte percentage of total lymphocytes (CD4%) less than 14%.[65] As the number of CD4 cells and/or CD4% decreases, the risks and intensity of opportunistic infections increases.[66] The CD4+ T-lymphocyte count and/or percentage has been demonstrated as a marker for HIV related immunosuppression.[67] The CDC expanded this definition to include diagnosing AIDS as an HIV infection plus a diagnosis with one or more opportunistic infections regardless of CD4 count and/or CD4%. Such opportunistic infections include invasive cervical cancer, disseminated histoplasmosis, recurrent pneumonia, encephalitis, and wasting syndrome among many others.[65]

Once HIV-infected patients are diagnosed with AIDS, the diagnosis continues for life even if the patient's health improves. Additionally, the lowest CD4 count and/or

CD4 percentage in an individual's lifetime or the nadir CD4 count percentage determines the extent of overall medical care needed for that individual patient.[65]

It is important to note that HIV-infected patients can live without being diagnosed with AIDS in their lifetime. In the United States, 32,052 HIV-infected patients were diagnosed with AIDS in 2011 out of 1.1 million total HIV-infected patients at that time.[68] Based on this, the risk of AIDS diagnoses in an HIV-infected patient population is about 2.9%. Even without ARV treatment, an HIV-infected individual can live up to 10 years without ever receiving an AIDS diagnosis.[69]

Vitamin D Associated Health Outcomes

Vitamin D has a strong effect on immune function in HIV-infected patients and is important for maintaining bone mineral density in HIV-infected patients, who are at increased risk for developing bone disorders.[70-72] Plasma vitamin D, measured by serum 25-(OH)D, has been positively correlated with calcium absorption.[22] Importantly, bone mineral density has also been positively correlated with calcium absorption while inversely correlated with the length of time infected with HIV.[70, 71] In the general population, VDD can lead to decreases in plasma calcium and eventually secondary hyperparathyroidism.[4, 70] VDD can prove particularly detrimental for an HIV-infected patient due to decreases in bone mineral density in addition to possible negative immune health effects.

Vitamin D's Role in Immune Health for the HIV-Infected

Serum 25-(OH)D levels are not dependent on a patient's HIV viral load or CD4+ T-lymphocyte count.[73] However, micronutrient deficiencies, such as a VDD, has been associated with increased rates of progression to AIDS.[17, 74, 75] Additionally, vitamin D supplementation has been shown to increase CD4 counts and/or percentage and decrease overall viral load.[75] Vitamin D's connection to immune health is still vague; however, in 1980 researchers discovered the nuclear vitamin D receptors (VDR) in T- and B-lymphocytes, monocytes, and dendritic cells.[76, 77] The purpose of vitamin D receptors in immune cells is unclear as this research only highlights preliminary mechanistic evidence to VDD and its relation to disease.[12, 78, 79] Some researchers claim this evidence is "paradoxical" and any response from the VDR depends on other specific stimuli. While vitamin D may down regulate the pro-inflammatory Th1-type reactions, it may also up-regulate the Th2-type pro-inflammatory reactions.[80] Though it is uncertain if vitamin D is positively associated with immunological outcomes related to HIV disease, there is a definite high rate of VDD among HIV-infected patients.[1-3, 59]

Recent in vitro and observational studies, which supplement cholecalciferol, has shown a potential connection to vitamin D's ability to suppress proliferation of Kaposi's sarcoma, down regulate production of pro-inflammatory cytokines, and promote an anti-microbial environment prohibiting HIV replication.[12, 14, 78] More research is needed to fully understand the relationship between vitamin D and its immunomodulation within an HIV-infected population.

Vitamin D Toxicity in HIV-Infected Patients

Currently, there are two notable case studies reporting evidence of vitamin D toxicities and hypercalcemia in HIV-infected patients diagnosed with AIDS. Both case studies were attributed to elevated serum 1,25-dihydroxyvitamin D levels due to an increased activity of dysfunctional macrophages. Researchers hypothesized this toxicity is caused by increased 1,25-dihydroxyvitamin D production from a granuloma or possibly secretion of parathyroid hormone-related protein by a T-cell lymphoma.[81, 82]

It is important to note there are *no* case studies currently available detailing vitamin D toxicities within an HIV-infected population from vitamin D supplementation. Vitamin D toxicity is determined by the same serum marker for deficiency, 25-(OH)D. Vitamin D toxicity is defined as >150 ng/mL (>375 nmol/L) because the risk for potential adverse effects, such as hypercalcemia, increases.[57]

Recommended Daily Intake of Vitamin D for HIV-Infected Patients

For healthy adults aged 19 to 70 years, the recommended daily intake of vitamin D according to the Institutes of Medicine is 600 IU with a tolerable upper limit of 4,000 IU.[57] The Endocrine Society guidelines recommend a daily maintenance dose of 1,500-2,000 IU and an upper limit of 10,000 IU for any adult considered at risk for deficiency, such as an HIV-infected patient who is prescribed ARV treatment or is vitamin D insufficient (21-29 ng/mL (52.5-72.5 nmol/L)). For any adult who is deficient (≤ 20 ng/mL (≤50 nmol/L)) the suggested treatment is 50,000 IU of vitamin D_2 or D_3 weekly for 8 weeks or an equivalent of 6,000 IU daily to achieve a serum 25-(OH)D ≥ 30 ng/mL (≥ 75 nmol/L) followed by a daily maintenance dose of 1,500-2,000 IU daily according to

the Endocrine Society Guidelines.[4]

Current Research in Vitamin D Supplementation Regimens in HIV-Infected Populations

In 2013, J. Schafer and colleagues noted a lack of current knowledge for the most appropriate regimen to replenish vitamin D in deficient HIV-infected patients.[72] Additionally, the British Journal of Clinical Pharmacology recently published in 2013 the first ever population pharmokinetics analysis of 25-(OH)D in HIV-infected adult patients. The study concluded that a vitamin D_3 supplementation regimen is necessary to reach adequate 25-(OH)D levels in HIV-infected adult populations no matter the dietary intake from food or UV-B exposure. A monthly dose of 100,000 IU vitamin D_3 is considered superior due to the population's low adherence rates to the daily and weekly supplementation regimens.[83]

In 2014, K. Falasca and colleagues conducted a prospective cohort study analyzing HIV-infected adults' 25-(OH)D levels after 3 months of different vitamin D_3 supplementation regimens. This study discovered that no matter the vitamin D_3 regimen, every group's 25-(OH)D levels increased, though a few were still insufficient. There were no significant differences between various regimens in the number of HIV-infected patients that increased their serum 25-(OH)D levels above 20 ng/mL (50 nmol/L). However, oral supplementation did demonstrate a higher 25-(OH)D increase compared to the intramuscular regimen throughout the observation phase.[84]

OHSU's General Vitamin D Supplementation Regimen

Due to the limited knowledge regarding the most effective vitamin D supplementation regimen in HIV-infected populations, OHSU does not has a defined protocol for vitamin D supplementation within its HIV/AIDS clinic. Currently, the most common vitamin D supplementation regimens include either a weekly 50,000 IU capsule of ergocalciferol (vitamin D_2) for 6 to 8 weeks followed by a daily maintenance dose of 3,000-4,000 IU cholecalciferol or a daily over-the-counter 2,000-3,000 IU capsule of cholecalciferol. Though these are the most common regimens, other concentrations and frequencies of vitamin D_3 and D_2 are ordered based on the healthcare provider's preference.

Methods

Study Design

This historical chart review was conducted with data acquired from Oregon Health and Science University (OHSU) HIV/AIDS clinic case registry located in Portland, Oregon (latitude: 45.49°). Within this data set, patient names were assigned a unique identifier by an external third party and fully de-identified. Patient data was collected from January 2011 to January 2014 in order to allow for a robust dataset. Importantly, not every patient had 25-(OH)D screening or was prescribed a vitamin D supplementation regimen in this dataset; individuals without two 25-(OH)D screenings or not prescribed a vitamin D supplementation regimen were omitted from the statistical analysis. This study was approved by OHSU's Institutional Review Board as part of Dr. P. Todd Korthuis' *Prospective OHSU HIV Database Core Protocol* (Institutional Review Board protocol #464). Individual IRB modifications regarding specific research analyses were submitted separately under this protocol and approved on September 5th, 2014 (ID# CR00021877).

Patient Population

For the *Prospective OHSU Database Core Protocol*, clinical and administrative data from all adult patients (>18 years of age) receiving outpatient care through OHSU's HIV/AIDS clinic was collected. This protocol included a patient waiver of consent informing the patients of potential research opportunities and specific de-identification procedures. Patient medical record numbers were used to query the OHSU electronic medical record in order to extract data entered during each clinical visit.

Inclusion and Exclusion Criteria

Patients who had a serum 25-(OH)D screening recorded at least twice between January 2011 to January 2014 were included. Inclusion criteria for this sub-analysis was (a) greater than two 25-(OH)D screenings, (b) a history of HIV infection, (c) greater than or equal to 18 years of age, (d) prescribed an ARV treatment without therapeutic changes for greater than 12 months, and (e) prescribed a vitamin D supplementation regimen. All sexes and race/ethnicity groups were included. Patients with (a) less than one month of a single vitamin D supplement, (b) with an unknown vitamin D dose or type, and/or (c) history of chronic kidney disease (all cause) were excluded from this study (Table 1).

Table 1: Inclusion/Exclusion Criteria

Inclusion	Exclusion
A. Greater than 2, 25-(OH)D screenings	A. <1 months of a single vitamin D supplementation regimen
B. History of HIV Infection	B. Vitamin D dose or type not listed in electronic medical record
C. ≥18 years old	C. History of chronic kidney disease (all cause)
D. ARV-treatment without therapeutic changes for >12 months	
E. Prescribed vitamin D supplement	

Variables Requested and Method/Equipment Utilized

The previous database utilized OHSU's medical equipment to record the variables (Table 2). Weight was measured with a Health Scale RGT.B-200-RT with indoor clothes and shoes on and height was recorded with a Charder HM200PW Wall Mount Height Rod. Weight was typically measured at each clinic visit while height was measured once at the initial intake appointment.

The body mass index (BMI) for the third specific aim was calculated as weight in kilograms (kg) and divided by the square of height in meters from measurements recorded during the same appointment as when the vitamin D supplementation regimen was prescribed (baseline BMI). If height was not measured at the appointment when vitamin D supplementation regimen was prescribed, the most recent height was used instead.

OHSU Central Lab collected a venous blood sample for the measurement of serum 25-(OH)D concentration regardless of the fed or fasted status of the patient. Antecubital venous blood samples were ordered by the HIV/AIDS clinic 2 to 3 weeks before the patient's appointment to allow time for the samples' analyses. At minimum, 3 mL of venous blood were collected in a red top tube and analyzed in a DiaSorin LIAISON® 25-OH Vitamin D Total Assay. The DiaSorin LIAISON® 25-OH Vitamin D Total Assay uses a chemiluminescent immunoassay (CLIA) technology for the quantitative determination of 25-(OH)D.[85, 86] The HIV-1 RNA Viral Load count required 5-10 ml of blood collected in a lavender top with EDTA as the anticoagulant. The specimen was centrifuged, separated, and frozen within 6 hours of collection. The sample was analyzed by FDA-approved COBAS® Ampliprep/COBAS® TaqMan® HIV-1 Test.[87] In order to quantify the CD4+/CD8+ T-lymphocyte count, 5 mL blood was collected in a lavender top tube and kept at room temperature. The specific counts were then calculated through flow cytometry.[88] The average processing time from blood draw to laboratory result was between 3-5 days and the month of the result represented the month of the blood draw. Month of the blood draw was used to define season of blood collection and season was controlled for in the statistical analyses.[89]

Since vitamin D metabolism is includes both endogenous and dietary sources, multiple confounder are included in my analyses. The same factors that equally affect the general population and HIV-infected individuals like latitude, the month of blood the blood draw, ethnicity, and age will be controlled for within my analyses.[19, 21-23] In order to adjust for seasonality and diminish the varying effects of endogenous production of vitamin D amongst HIV-infected patients, every patient was grouped according to both baseline and follow-up 25-(OH)D seasons. Two primary seasons were included within the analysis, summer and winter. Summer months, when sunlight and UV-B exposure is greatest, were defined as blood draws taken from March to August. Endogenous production of 25-(OH)D_3 is highest during these months.[19] Conversely, the winter months were defined as blood draws taken from September to February when UV-B exposure is limited, thus endogenous production of 25-(OH)D_3 is lowest.[19]

Table 2: Variables and Corresponding Recording Methods/Equipment

Variables	Method/Equipment
1. Age	1. Patient verbalized response
2. Sex	2. Physician-based observation
3. Weight	3. Health Scale RGT.B-200-RT
4. Height	4. Charder HM200PW Wall Mount Height Rod
5. BMI	5. EPIC Calculated
6. Specific Vitamin D Regimen	6. HIV specialist Prescription
7. Date of Vitamin D Supplement Initiation	7. HIV specialist Prescription
8. Serum 25-OH D Value at Initiation	8. LIAISON® 25 OH Vitamin D TOTAL Assay
9. Serum 25-OH D Value post Vitamin D supplementation Initiation	9. LIAISON® 25 OH Vitamin D TOTAL Assay
10. HIV Viral Load and CD4 count	10. "Amplicor" method and flow cytometry
11. Co-Morbidities	11. PVR and/or MD diagnosis
12. Date of Blood Draw	12. Medical Record
13. ARV Medication Regimen Type & Length	13. Prescription History
14. Date of Dyslipidemia diagnosis, if	14. Medical Diagnosis

applicable	
15. AIDS diagnosis, if applicable	15. Medical Diagnosis
16. Anabolic Agent Use/Prescription	16. Prescription History
17. History of CKD	17. Medical Diagnosis

Statistical Analysis

All statistical analyses were completed with 13th Edition STATA.[90] For Aim 1, the data was analyzed for patterns of vitamin D prescriptions. Patient data based on the frequency, dose, and form of vitamin D supplementation were recorded and grouped. Patterns common among multiple patients were considered a vitamin D regimen. Patients were then organized into their specific vitamin D supplementation regimens and surveyed for an average range of 10-17 months. Patient adherence rates to vitamin D supplementation regimens were not recorded in the electronic medical record and were considered a limitation to this study. Using descriptive statistics, the mean, standard deviations, and confidence intervals of the pre-treatment and subsequent vitamin D concentrations for each type of repletion prescription were calculated. The data was evaluated for normality.

For Aim 2, change between baseline assessment and post repletion regimen were calculated. Baseline for this study was considered the appointment when vitamin D was prescribed and follow-up appointments were on average 14.0 ± 6.3 months from baseline. In order to understand which factors affected the mean change of serum 25-(OH)D levels between vitamin D supplementation regimens in this HIV/AIDS clinic, a linear regression model calculated with residual sum of squares was conducted that included change in serum 25-(OH)D from follow-up to baseline as the main outcome, with vitamin D regimen as the main predictor adjusted by seasons of both baseline and follow-up

blood draws, age, sex, ethnicity (Caucasian or not Caucasian), months between blood draws, and patient specific BMI category. The change in serum 25-(OH)D was also compared among supplementation regimens using an adjusted ANOVA to determine if a significant difference existed amongst regimens with p-values less than 0.05 considered significant. Covariates were included in the model based on common practice in current literature.

For Aim 3, a one-way ANOVA was used to determine if obese patients (BMI >30 kg/m^2) at baseline had significantly different average change in serum 25-(OH)D in ng/mL when compared to overweight (BMI: 25-29 kg/m^2), normal (BMI: 19-24 kg/m^2), and underweight (BMI: <18 kg/m^2) patients. Change in serum 25-(OH)D again was calculated by subtracting follow-up 25-(OH)D serum values from baseline 25-(OH)D serum values.

Results

Specific Aim 1: Description of OHSU's HIV/AIDS Clinic's Vitamin D Supplementation Regimens

Description of the OHSU HIV/AIDS Clinic's vitamin D supplementation regimens was derived from 228 patients that met both inclusion and exclusion criteria. Out of 275 patients that met the inclusion criteria and were prescribed vitamin D between 2011-2014, 12 patients were diagnosed with chronic kidney disease, and 35 had incomplete electronic medical records (i.e. height was not recorded thus BMI could not be calculated). 228 patients remained out of the original 275 patients (Diagram 1).

Three collective vitamin D supplementation regimens were noted. First, a single over the counter supplement with no change in prescription of supplemental vitamin D_3 (average dose = 2687 +/- 29.0 IU daily, 95% CI: 2291.0, 3083.2 IU daily) was noted for 69 patients. Second, a single therapy high-dose supplement with no change in prescription of supplemental vitamin D_2 (average dose = 6521.52 ± 1706.18 IU daily; 95% CI: 6231.1, 6812.0 IU daily) was prescribed for 135 patients. Third, a repletion/maintenance regimen was noted in which patients were supplemented weekly with a high dose 50,000 IU vitamin D_2 and then after an average 6-14 months were changed to a lower, daily dose of either vitamin D_2 or vitamin D_3. For this regimen, 24 patients averaged a daily dose of 5058.3 +/- 406.3 IU, any type (95% CI: 940.7, 9176.0 IU daily).

Diagram 1: Data Cleaning Sample Counts

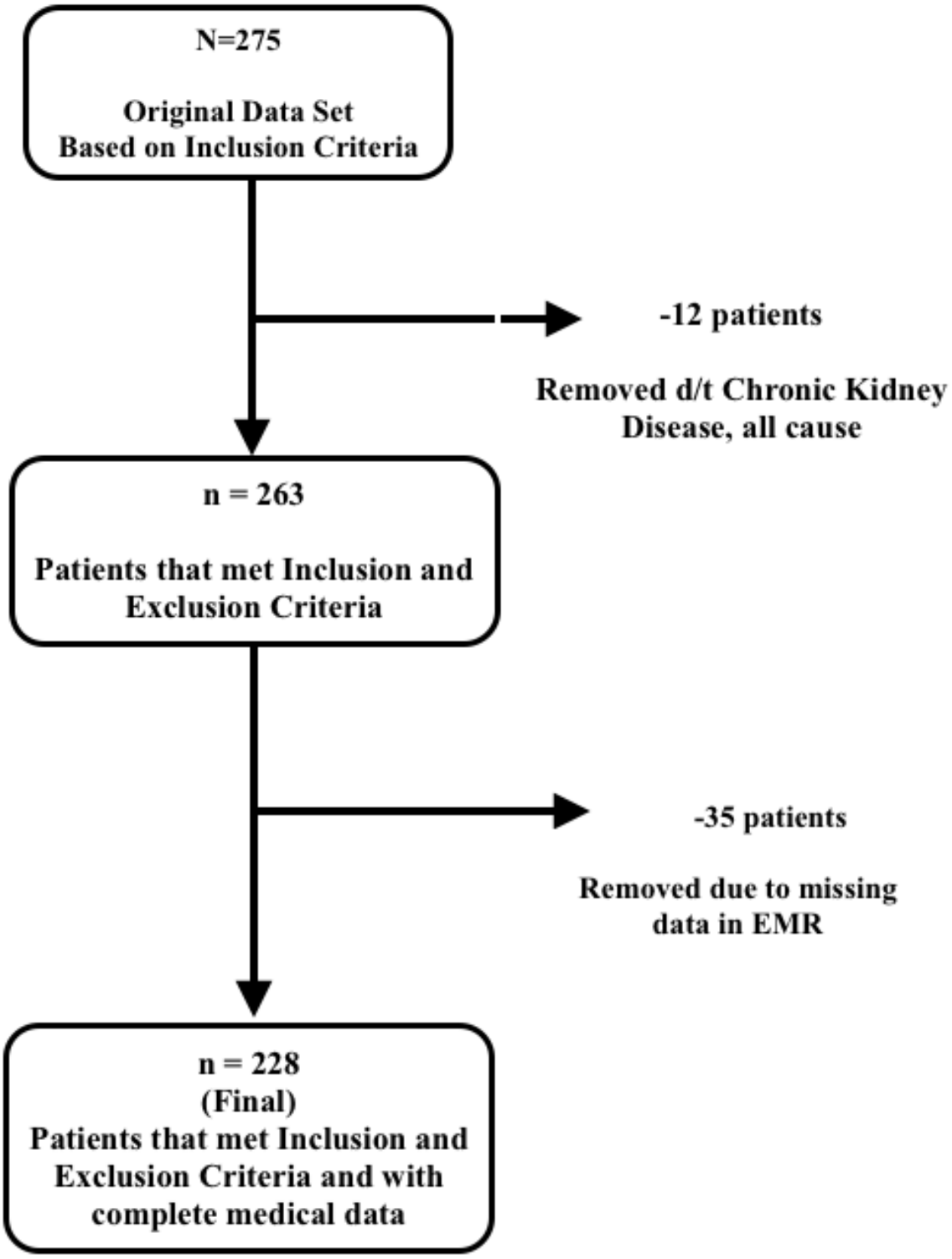

Diagram 1: From the original 275 patients that met the inclusion criteria, 12 patients were diagnosed with chronic kidney disease (all cause) and 35 patients had incomplete electronic medical records, thus were excluded from final analysis. Final sample size included 228 HIV-infected patients.

The majority of vitamin D supplementation regimens were of single therapy vitamin D_2 or D_3, representing 89.5% or 204/228 of the total sample. Only 24 of 228 (10.5%) had an alteration to their vitamin D prescriptions following a repletion/maintenance regimen, which again was a change from a weekly 50,000 IU vitamin D_2 to a lower dose daily vitamin D supplement (either D_2 or D_3). However, the duration from initiation of the weekly 50,000 IU vitamin D_2 supplement to the daily, reduced vitamin D supplement ranged from 6 to 14 months. Importantly, the duration between baseline appointment and follow-up appointments was not significantly different amongst regimens (p-value = 0.251) and was comparable between groups.

Due to the lack of distinction between 25-(OH)D_2 and 25-(OH)D_3 by the LIAISON® 25 OH Vitamin D TOTAL Assay used for vitamin D screenings within this clinic, serum 25-(OH)D was used in the analysis regardless of type. Patients defined as 'single therapy' relates to the type of vitamin D (D_3 or D_2). Vitamin D dosages were not constant for every patient from baseline to follow-up; therefore, the average daily dose was calculated from all vitamin D prescriptions. Change in vitamin D dose was considered a potential confounding variable for all regimens; however, statistical analysis concluded no significant effect (p-value= 0.838) of prescription dose change on serum 25-(OH)D change. Additional characteristics of the patient sample utilized within this study are given in Table 4.

Table 3: Patient Characteristics based on Regimen and Vitamin D Type
(n=228)

	Single Therapy: Vitamin D_3*	Single Therapy: Vitamin D_2*	Repletion/Maintenance (Weekly 50,000 Vitamin D_2 to Daily D_3/D_2)
Sample Size	69	135	24
Initial 25-(OH)D (in ng/mL)			
Mean (±SD)	29.3 (±11.4)	20.9 (±9.8)	23.8 (±0.2)
95% CI	26.5-32.0	19.2-22.60	19.5-28.2
Follow Up 25-(OH)D (in ng/mL)			
Mean (±SD)	31.0 (±12.4)	27.1 (±12.5)	31.2 (±11.9)
95% CI	28.0-34.0	25.0-29.30	26.2-36.3
Mean Difference			
Mean (±SD)	1.7 (±11.8)	6.2 (±12.9)	7.4 (±15.5)
95% CI	-1.2-4.5	4.0- 8.4	0.8-14.0

*Denotes significant difference in serum 25-(OH)D ($\alpha = 0.05$)

Figure 1: 25-(OH)D Change by Regimen

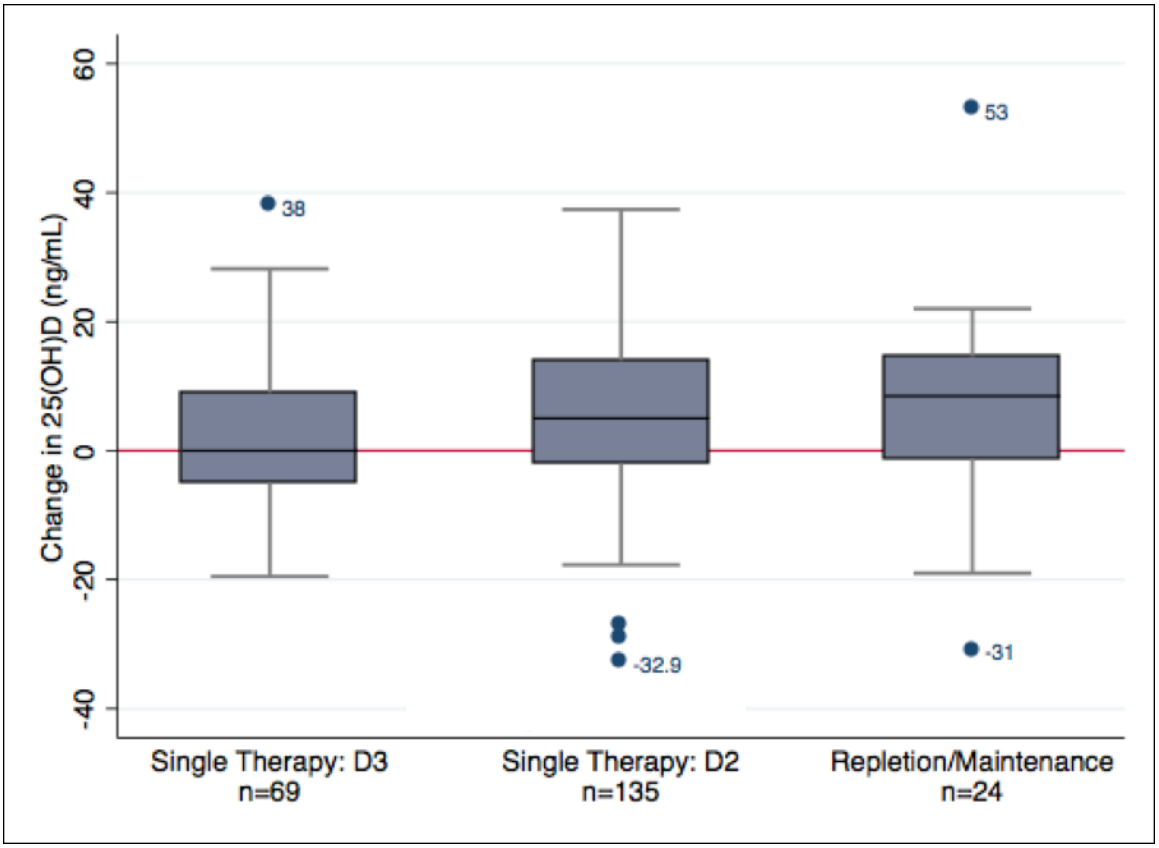

Figure 1: The average change in serum 25(OH)D from baseline to follow-up appointments categorized by vitamin D regimen for all 228 patients (regimen sample sizes are designated below each regimen). Distributions include entire ranges with 75th%-ile and 25th%-ile as the upper and lower borders of each box, respectively. Median (50th%-ile) change is designated by the center, horizontal line.

A significant change was noted amongst all regimens with a one-way ANOVA (crude p-value = 0.04). Pairwise comparisons, adjusted with the Tukey's method, noted significant 4.55 ng/mL increase of patients' serum 25(OH)D levels when prescribed single therapy (no change) of vitamin D_2 versus single therapy (no change) vitamin D_3 (p-value = 0.048, 95% CI: 0.034-9.071). No significant differences noted between any single therapy (D_2 or D_3) and the repletion and maintenance regimen (p-values = 0.92 and 0.15, respectively).

Regardless of which vitamin D supplement patients were prescribed, the prevalence of vitamin D deficiency (serum 25-(OH)D <20 ng/mL) decreased by 16.7% and patients with vitamin D insufficiency ((serum 25-(OH)D 20-30 ng/mL) increased by 5.0% at the follow-up appointments. The proportion of patients with adequate/normal vitamin D status increased by 11.8% of the total population after initiating vitamin D supplementation. Supplementation in this patient population did increase serum 25-(OH)D after an average of 14.0 ± 6.3 months by 4.97 ng/mL +/- 13.07 (2-sided p-value = <0.0001; 95% CI: 3.26, 6.67). At baseline before vitamin D supplementation, 93 (40.8%) patients were vitamin D deficient, 81 (35.5%) were vitamin D insufficient, and 54 (23.7%) had adequate 25-(OH)D concentrations. Follow-up 25-(OH)D screenings included 55 (24.1%) patients with vitamin D deficiency, 92 (40.4%) vitamin D insufficiency, and 81 (35.5%) with adequate vitamin D concentrations (Figure 2).

Table 4: Characteristics of the 228 HIV-Infected Patients by Vitamin D Regimen

Vitamin D Regimen	**Single D_3**	**Single D_2**	**Repletion/ Maintenance** Repletion**: 50,000 (weekly) Maintenance: 400-5000
Daily Dose Range (IU)	400-5000	400-7142.9	
Average Daily Dose Mean (± SD)	2686.9 (± 1649.4)	6521.5 (± 1706.1)	3272.6 (± 2033.8)
Sample Size			
n	69	135	24
Sex			
Male	62	116	21
Female	7	19	3
Ethnic Origin			
White	65	108	18
Other	4	27	6
Age (yrs.)			
Mean (± SD)	48 (±15)	43 (±11)	47 (±13)
BMI (kg/m^2)*			
Median (±IQR)	25 (±6)	24 (±7)	25 (±5)
Season of Baseline Blood Draw			
Summer	43	67	12
Winter	26	68	12
Season of Follow-Up Blood Draw			
Summer	38	70	11
Winter	31	65	13
% Undetectable VL (<50 copies/mL)	46.4%	47.4%	54.2%
Baseline: CD4 (cells/cu mm)*			
Median (±IQR)	451 (±481)	468 (±421)	614 (±551.5)

ARV Drug (Single or Combo)			
NRTI	69	119	20
NonNRTI	27	52	7
PI	35	55	10
EI	3	1	1
INSTI	18	33	7
No Treatment	0	14	3
Baseline: 25-(OH)D (ng/ml)			
Mean (±SD)	29.3 (±11.4)	20.9 (±9.9)	23.8 (±10.2)
% <20 ng/mL	20.3%	50.4%	45.8%
Follow-Up: 25-(OH)D (ng/ml)			
Mean (±SD)	31.0 (±12.4)	27.1 (±12.5)	31.2 (±12.0)
Percentage <20 ng/mL	15.9%	30.4%	12.5%
Change Vitamin D Deficiency	-4.3%	-20.0%	-33.3%
Months Between Draws*			
Median (±IQR)	13.5 (±6.7)	13.2 (±6.7)	14.4 (±6.2)

*Note median (±IQR) utilized for any variable not normally distributed

**50,000 IU weekly is equivalent to 7142.9 daily IU

Figure 2: Vitamin D Deficiency and Insufficiency Before/After Supplementation

n=228

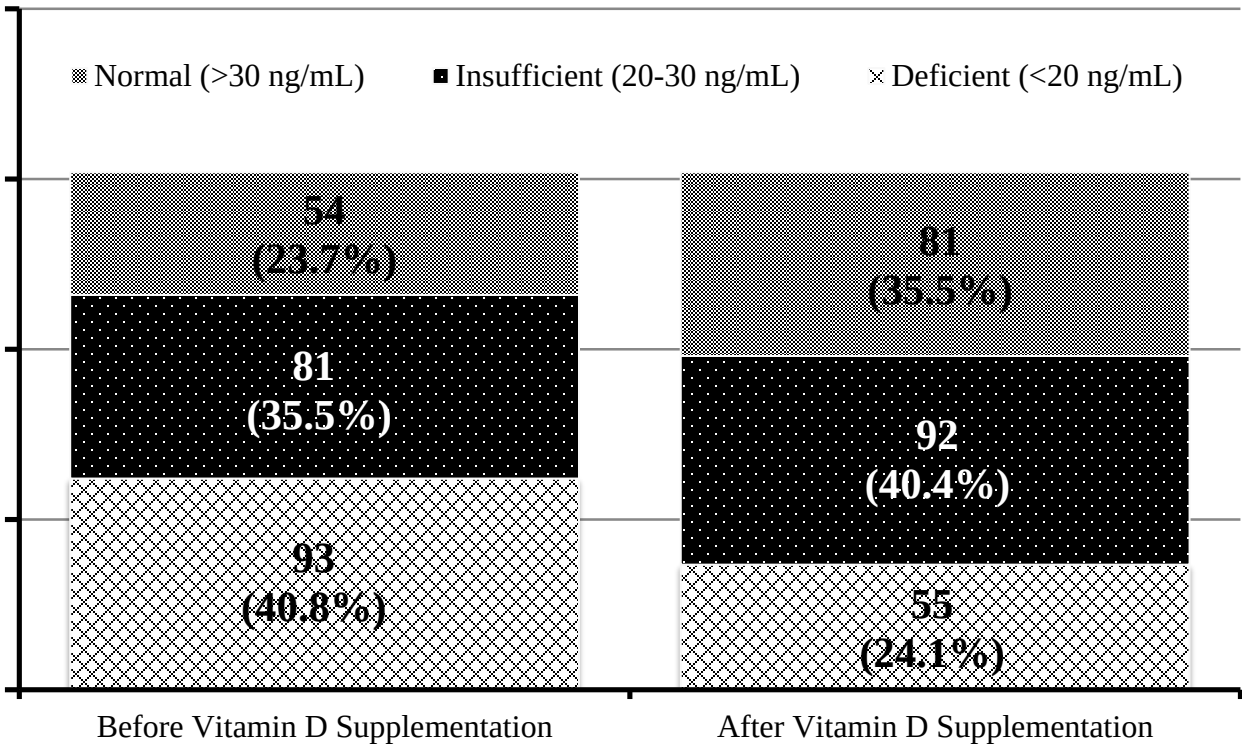

Figure 2: The sub-sample sizes of 228 patients at both baseline and follow-up with a 25(OH)D categorized as adequate (>30 ng/mL), inadequate (20-30 ng/mL), and deficient (<20 ng/mL) 25(OH)D concentrations.

At baseline, 93 (40.8%) deficient, 81 (35.5%) inadequate, and 54 (23.7%) with adequate serum 25(OH)D levels were recorded. At follow-up, 55 (24.1%) deficient, 92 (40.4%) inadequate, and 81 (35.5%) adequate serum 25(OH)D levels were noted.

Specific Aim 2: Change of Serum 25-(OH)D Among Vitamin D Regimens

Given that the dependent variable, change in 25-(OH)D, was continuous and normally distributed, linear regression was considered as an appropriate statistical analysis. Diagnostic analysis of the association model was performed using residual plots, Q-Q plots and Shapiro-Wilks tests, to assess the goodness of fit of the model.[90] The tests were consistent with normal distribution of the residuals ($p > 0.05$ for Shapiro-Wilks test), and there was no concerning pattern to the spread of the residual plots. There was no evidence of co-linearity, based on the results of a VIF (Variance Inflation Factor) analysis (mean VIF = 1.23). Assessment of outliers and influential points was conducted using leverages, Cook's distance, DFITS, and DFBETA analysis. Approximately 15 observations were identified as possible outliers via the leverages assessment; however none of these points were identified using the Cook's distance analysis. None of the measurements seemed implausible, so none of the data were deleted from the model.

Vitamin D regimen was significantly associated with overall change in serum 25-(OH)D concentration (p-value= 0.039) (Figure 1). A significant difference was noted between the repletion/maintenance regimen and the single vitamin D_3 regimen (adjusted p-value = 0.047; 95% CI: 0.09, 11.72); however, no significance differences were noted between the repletion/maintenance and D_2, or D_3 and D_2 single therapies (adjusted p-values >0.05). Since vitamin D status is affected by many factors other than supplementation, the model was adjusted for age, sex, ethnicity, duration between blood draws, season of blood draw, and BMI category. For example, a patient with blood drawn in summer may have elevated serum 25-(OH)D due more to UV-B exposure/endogenous production than their specific vitamin D supplementation.

Adjusting for season in this specific example clarifies the effect of vitamin D supplementation while reducing the variability in change in 25-(OH)D status associated with UV-B exposure/endogenous production.

Vitamin D daily dosage did not seem to be a factor with change in serum 25-(OH)D among all regimens. When patients were compared by average daily dose regardless of vitamin D type, categorized: Low/Medium: 400-2000 IU and High: 5000-7142.9 IU, no significant differences were noted within the average change of serum 25-(OH)D between baseline and follow-up appointments (adjusted p-value = 0.489). Therefore, the specific regimen that altered both vitamin D dose and type had a more significant effect on patients' serum 25-(OH)D change than simply the dose of vitamin D.

Patient's age, duration between blood draws, and season of blood draw were significant confounders of the association between change in serum 25-(OH)D concentrations (p-values <0.05) and vitamin D regimen. Dose strength, type, sex, ethnicity, and BMI (continuous or categorical) were not significant confounders (Table 5). To avoid over-complicating the association model, ARV medications were not included. Additionally, no significant interaction was observed between change in serum 25-(OH)D and ARV drug treatment, which included nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INSTI), or entry inhibitors (EIs).

Table 5: Effect of Confounders on Follow-Up Serum 25-(OH)D by Dose Strength
(n=228) $R^2 = 0.188$

	Standardized Coefficients (± Std. Error)	95% CI	p-value
Regimen			
Single Vitamin D_2	7.02 (±4.18)	-1.22, 15.27	0.095
Single Vitamin D_3	Reference		
Repletion/Maintenance	5.90 (±2.95)	0.09, 11.72	0.047*
Dose Strength			
High (5000-7142.9 IU)	-1.44 (±2.08)	-5.55, 2.66	0.489
Low/Med (400-2000 IU)	Reference		
Dose Type			
Vitamin D_2 (ergo-)	-2.29 (±4.15)	-10.46, 5.89	0.582
Vitamin D_3 (chole-)	Reference		
Age	0.153 (±0.068)	0.019, 0.287	0.026*
Sex			
Male	-3.13 (±2.48)	-8.03, 1.76	0.209
Female	Reference		
Ethnicity			
Caucasian	-1.46 (±2.31)	-6.01, 3.10	0.529
Not Caucasian	Reference		
Months Between Draws	-0.678 (±0.13)	-0.93, -0.42	<0.0001*
Season of Draws (Baseline → Follow Up)			
Summer→Summer	5.71 (±2.31)	1.16, 10.26	0.014*
Winter→Winter	Reference		
Summer→Winter	5.66 (±2.38)	0.97, 10.36	0.018*
Winter→Summer	3.77 (±2.4)	-0.98, 8.53	0.120
BMI, continuous	-0.01 (±0.36)	-0.70, 0.72	0.975
Underweight/Normal	Reference		
Overweight	-3.18 (±2.64)	-8.30, 2.03	0.231
Obese	-5.26 (±4.91)	-14.93, 4.41	0.285
β_0 (constant)	5.58 (± 9.10)	-12.36, 23.51	0.541

*Denotes statistical significance

Full Model: $E(\text{Change in 25-(OH)D}) = \beta_0 + \beta_{1,2}*I\_Regimen + \beta_3*I\_DoseStrength + \beta_4*I\_Type + \beta_5*Age + \beta_6*I\_Sex + \beta_7*I\_Ethnicity + \beta_8*MonthsBetweenBloodDraw + \beta_{9,10,11}*I\_Season + \beta_{12}*BMI + \beta_{13,14}*I\_BMIcategory + \varepsilon$

Specific Aim 3: Effect of BMI on Change in Serum 25-(OH)D in HIV-Infected Patients

When patients were categorized by baseline BMI category, no significant difference was noted (crude p-value = 0.121) (Figure 3). Pairwise comparisons adjusted by Tukey's method also did not find any statistical significance of serum 25-(OH)D change amongst BMI categories (p-values >0.05). After adjusting for all covariates in Table 5, no additional statistical significance was noted (adjusted p-value = 0.975). Though not significant, for every kg/m^2 BMI increased, the change in 25-(OH)D was 0.01 (±0.36) ng/mL less (95% CI: 0.70 less to 0.72 ng/mL more).

Additionally, when underweight and normal weight patients were grouped to balance out sample sizes, an overall difference was not noted (crude p-value = 0.10). Pairwise comparisons adjusted with the Tukey's method mirrored this result (p-values >0.05). A diminished change in serum 25-(OH)D amongst obese HIV-infected patients compared to the under-/normal weight reference was visually noted amongst the data (Figure 4).

At baseline obese patients accounted for only 19.4% of the vitamin D deficient subgroup, while this percentage increased to 23.6% at follow-up, indicating that a larger percentage of obese individuals compared to normal/overweight individuals remained vitamin D deficient despite supplementation. Additionally, when change in serum 25-(OH)D was compared between obese (BMI $\geq$30 kg/m^2) and non-obese (BMI <29 kg/m^2) patients, no significant difference was noted amongst changes in serum 25-(OH)D (2-sided, adjusted, p-value = 0.217). Therefore, a significant difference exists between obese and underweight/normal weight patients, but not obese (BMI: >30 kg/m^2)

compared to non-obese (BMI: ≤ 30 kg/m^2) patients when analyzing change in serum 25-(OH)D.

Figure 3: Change in 25-(OH)D Categorized by BMI

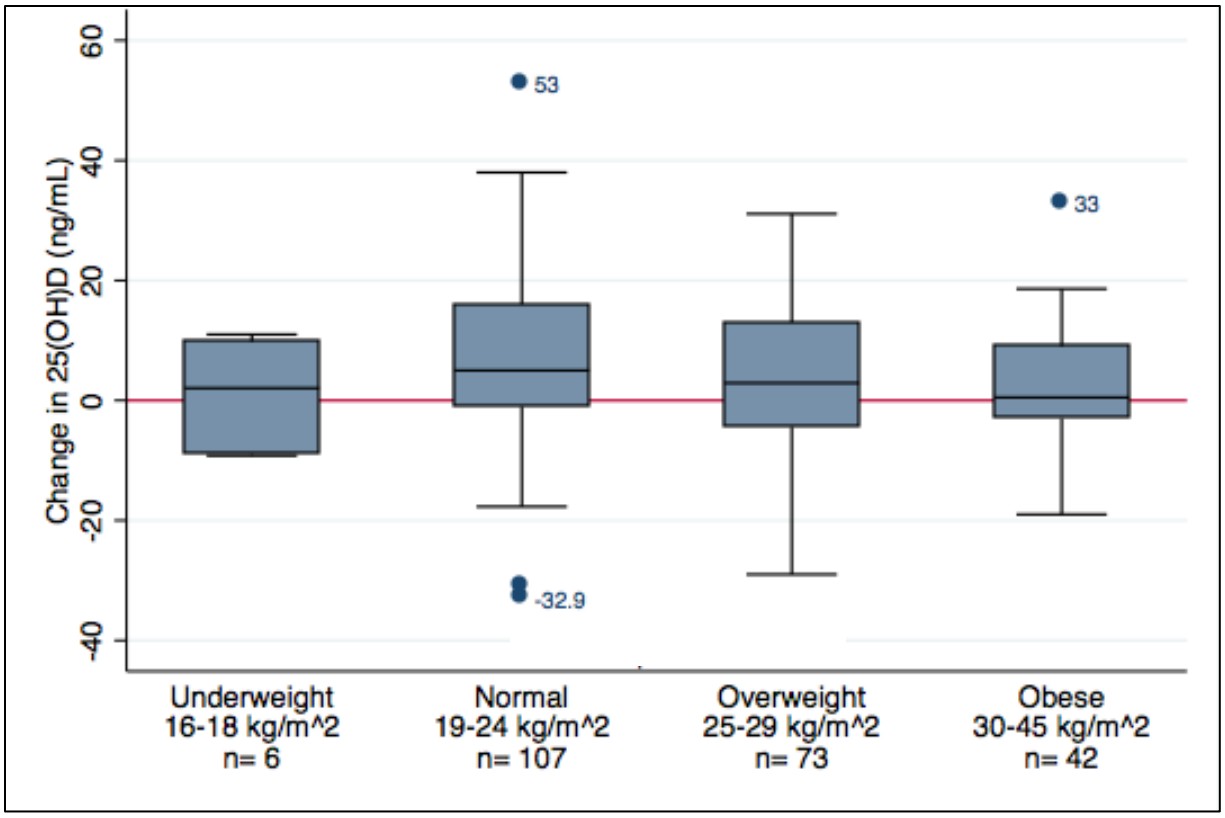

Figure 3: The average change in serum 25(OH)D from baseline to follow-up appointments categorized by baseline BMI (kg/m^2). Categories were defined as underweight (16-18 kg/m^2), normal (19-24 kg/m^2), overweight (25-29 kg/m^2), and obese (30-45 kg/m^2). Respective sample sizes are listed below each.

One-way ANVOA lacked significance amongst BMI categories (p-value = 0.121). Pairwise comparisons adjusted by Tukey's method also did not find any statistical (p-values >0.05).

Figure 4: Change in 25-(OH)D Categorized by BMI, Underweight and Normal Weight Combined

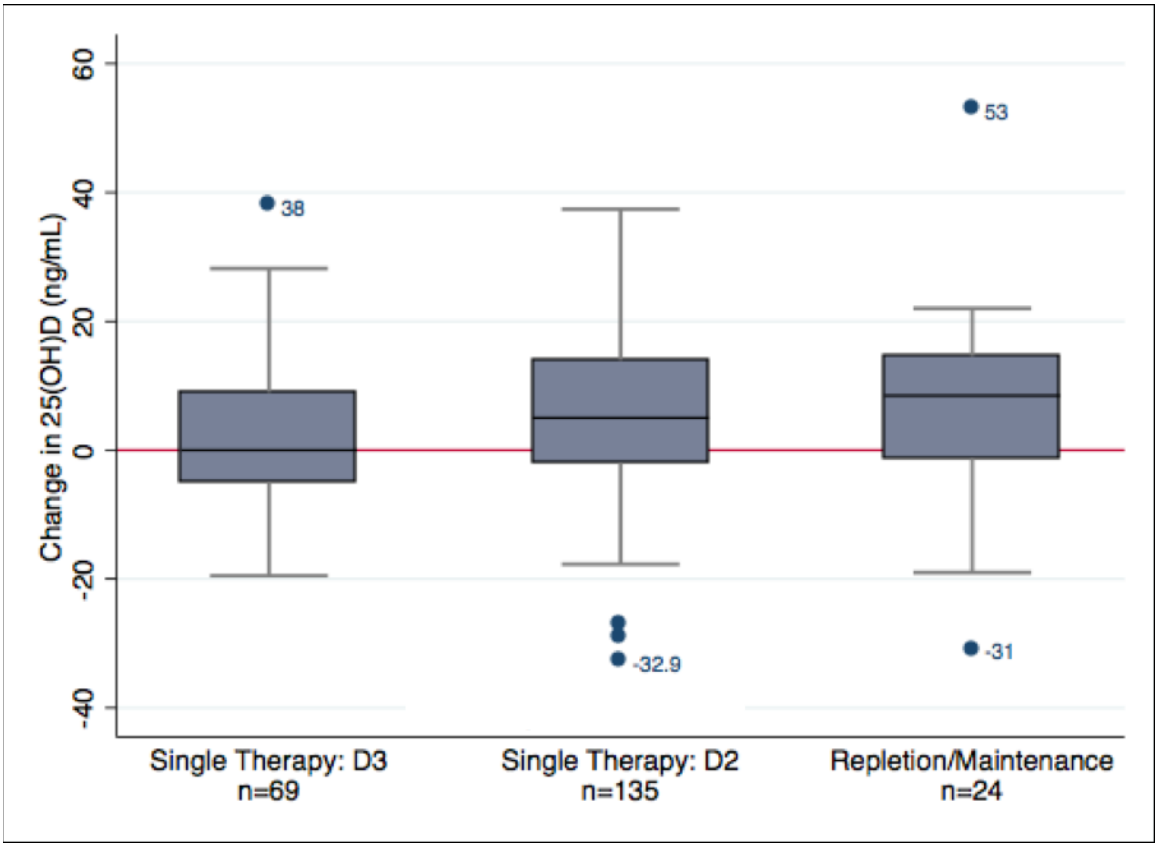

Figure 3: The average change in serum 25(OH)D from baseline to follow-up appointments categorized by baseline BMI (kg/m^2). Categories were defined as underweight/normal (16-24 kg/m^2), overweight (25-29 kg/m^2), and obese (30-45 kg/m^2). Respective sample sizes are listed below each.

One-way ANVOA did not note significant group difference (p-value = 0.10). Pairwise comparisons adjusted by Tukey's method also did not find any statistical (p-values >0.05).

Discussion

Prevalence of Vitamin D Deficiency Among HIV-Infected Patients

Previous population-wide vitamin D research has estimated the prevalence of vitamin D deficiency (25-(OH)D <20 ng/mL) among HIV-Infected patients to be about 40%.[1-3, 35] Within this patient sample 93 out 228 (40.3%) had a serum 25-(OH)D concentration less than 20 ng/mL before any vitamin D supplementation and 55 out of 228 (24.1%) remained vitamin D deficient at follow up. The prevalence of vitamin D deficiency in our historical cohort is consistent with previously reported high prevalence of vitamin D deficiency among HIV-infected patients.

HIV-Specific Risk Barriers to Vitamin D Metabolism

HIV-infected patients have a high rate of vitamin D deficiency but the reason for this is not completely understood. In the general population vitamin D status is decreased by obesity, limited UVB exposure, ethnicity, and a nutritionally deficient diet. In addition to these factors, an HIV-infected population is further burdened by potential interactions from antiretroviral medications and malabsorption secondary to inflammation caused by HIV infection and other opportunistic pathogens.[1-3, 32, 33] Since all patients lived in the same latitude, location was not considered a factor for this study. Patient's age, duration between blood draws, and season of blood draw were significant confounders for the response to vitamin D supplementation in this group of patients. Dose strength, vitamin D type, sex, ethnicity, and BMI (continuous or categorical) were not significant confounders in our data (Table 5). Even though vitamin D dose strength[27, 53], ethnicity[21], and ARV regimen[1] are widely accepted as factors affecting serum vitamin D

concentrations, this was not observed in our cohort. This could potentially be due to lack of patient adhearance to vitamin D supplementation regimen, thus altering overall change in serum 25-(OH)D among these factors. Patient adherence to prescribed vitamin D regimens was not included in patients' electronic medical records. In addition, small sample size and limited variability in dose strength, ethnicity, and ARV regimen may explain the observed lack of association of these factors with change in vitamin D.

Effect of Vitamin D Dosage

Contradictory to current literature[27, 53], we failed to highlight a significant effect of taking an average higher dose of vitamin D daily with increasing serum 25-(OH)D at follow-up compared to a lower average daily dose even after adjusting for patient age, sex, time between appointments (in months), seasons of both baseline and follow up appointments, BMI, and ethnicity (Caucasian/Not Caucasian). However, a trend was noted for increased 25-(OH)D levels for patients supplementing greater than 5,000 IU daily of vitamin D. Additionally, both the single therapy vitamin D_2 and the combined repletion/maintenance regimen trended towards a greater increase in serum 25-(OH)D than the single therapy lower dose vitamin D_3 supplement. Similar to Foissac et al's (2013) investigation, there was no significant difference among change in serum 25-(OH)D at follow-up between varying vitamin D_3 supplementation doses.[83] Within this study vitamin D supplementation increased serum 25-(OH)D in 141 (61.8%) HIV-infected patients and decreased prevalence of vitamin D deficiency by 38 patients (16.7%) at the follow-up appointments. This finding strongly agrees with both Lake et al (2011) and Pinzone et al (2013)'s research, which suggest vitamin D supplementation is

vital to increasing 25-(OH)D stores in an HIV-infected population regardless of dietary intake.[15, 35]

Effect of Regimens: Importance of Varying Vitamin D Dose and Type

Our research analyzes not only varying vitamin D doses but also varying vitamin D type. Previous vitamin D supplementation investigations for HIV-infected patients have focused solely on vitamin D_3 (cholecalciferol) and not on supplementing vitamin D_2 (ergocalciferol) for HIV-Infected patients. [83, 84] However, a meta-analysis of randomized, controlled vitamin D supplementation trials for the non-HIV-infected persons noted that despite vitamin D_3 being more effective at increasing serum 25-(OH)D concentrations at lower doses, vitamin D_2 still had a significant effect when supplementation was dosed significantly greater than the vitamin D_3 dose.[53] However, some studies suggested equal efficacy of vitamin D_3 to vitamin D_2 on raising serum 25-(OH)D concentrations. [53] The exact metabolism of vitamin D_2 is weakly understood in current literature and a definite area of future research.

The repletion/maintenance regimen had a significantly greater effect on raising serum 25-(OH)D concentrations than any single vitamin D type regimen (p-value = 0.047). Before adjustments by age, sex, ethnicity, duration between blood draws, season of blood draw, and the patient's specific BMI category, only the vitamin D_2 (ergo-) had a significant change of serum 25-(OH)D concentrations (p-value= 0.048). However, after adjustments, single therapy vitamin D_2 did not significantly increase serum 25-(OH)D concentrations (adjusted p-value = 0.095). Importantly, the average daily dose for the regimen and the repletion/maintenance regimen (50,000 D_2 weekly → lowered dose of

either D_2 or D_3) was a higher daily dose than the single vitamin D_3 regimen and lower than the single therapy D_2 therapy (repletion/maintenance: 3272.6 (± 2033.8) IU daily, single D_3: 2686.9 (± 1649.4) IU daily, single vitamin D_2: 6521.5 (± 1706.1) IU daily). Importantly, patients in the repletion/maintainence regimen did not have a higher daily dose of vitamin D than the other regimens. Also, the single therapy vitamin D_2 regimen, which had an average daily dose of 6521.5 (± 1706.1) IU did not significantly increase serum 25-(OH)D concentrations greater than all other regimens. Therefore, the vitamin D regimen that included both alterations in vitamin D dose and changed from vitamin D_2 to vitamin D_3 was more effective at changing serum 25-(OH)D concentration in HIV-infected patients.

Obesity's Effect on Serum 25-(OH)D Concentration

When HIV-infected patients categorized as obese (BMI: >30 kg/m^2) were compared to the non-obese patients, no significant difference was noted even after adjusting for dose strength, age, sex, ethnicity, months between draws, and season of both baseline and follow-up draws, which contradicts Wortsman et al (2000)'s research that highlighted a strongly significant effect of obesity due to adipocyte sequestration of serum 25-(OH)D. [23] Despite combining both underweight (BMI: 16-18 kg/m^2) and normal weight (BMI: 19-24 kg/m^2) HIV-infected patients to balance out categories, no significant differences were noted among BMI categories (crude p-value 0.10). Similarly no significance was noted when BMI was included in the association model after adjusting for patient's age, sex, ethnicity, duration between blood draws, and season of blood draw (adjusted p-value= 0.975). It is possible this lack of association is related to

power and few numbers of patients in the obese category.

ARV Medication's Interaction with Vitamin D Metabolism

Current literature suggests that specific ARV medication, such as efavirenz (NonNRTI), reduces serum 25-(OH)D levels. [31] This was not noted, however, in a separate analysis among any ARV medication including NonNRTIs within this cohort (p-values >0.05). This finding can be explained by the unknown patient adherence rates to vitamin D supplementation. Patients not supplementing vitamin D as prescribed would have decreased follow-up serum 25-(OH)D concentrations, mirroring ARV medications interacting with vitamin D metabolism.

Study Limitations

Despite efforts to control for seasonality by limiting serum 25-(OH)D concentrations to similar seasons at both baseline and follow-up appointments, the majority of patients had follow-up appointments with varying seasons from baseline. Thus, seasons of blood draw were limited to summer months (March to August) or to the winter months (September to February). Condensing twelve months to two seasons limited this study's generalizability as to which specific month altered 25(OH) D serum concentrations the greatest.

Also, the lack of differentiation between 25-(OH)D_2 and 25-(OH)D_3 due to OHSU's serum 25-(OH)D assay limited interpretability of which vitamin D type is most effective at increasing vitamin D nutriture, especially since vitamin D_2 supplementation in both the single D type and repletion/maintenance regimen increased serum 25-(OH)D

concentrations greater than vitamin D_3 supplementation.

Each patient's specific regimens were categorized based on the healthcare provider's manual input. As a result, accuracy of a patient's vitamin D supplementation regimen depends on the degree of human error. As this study is historical, there is a potential for inaccurate data entry related to vitamin D regimen categories.

Patient compliance and adherence to the prescribed vitamin D supplementation regimen was not recorded for this study, which is a significant study limitation. If patients failed to regularly take their vitamin D supplements, the consecutive serum 25-(OH)D concentrations will not increase as expected and follow-up serum 25-(OH)D concentrations will be decreased.

Future Research Directions

The average 25-(OH)D change per vitamin D regimen was 1.7 (±11.8) ng/mL, 6.2 (±12.9) ng/mL, and 7.4 (±15.5) ng/mL in single vitamin D_3, single vitamin D_2, and the repletion/maintenance regimens, respectively. Between vitamin D regimens, there were 4.55 ng/mL, 5.69 ng/mL, and 1.14ng/mL differences, thus this study was adequately powered (>80%). Due to limited clinical benefit from a serum 25-(OH)D increase of 5 ng/mL, a future study should have enough power to detect at least a 10 ng/mL as this would increase the chances of a vitamin D deficient patient to become either insufficient (20-30 ng/mL) or adequate (>30 ng/mL). In order to detect a single vitamin D regimen that increases serum 25-(OH)D concentrations more than 10 ng/mL greater than any other regimen with 80% power at 95% confidence, a desirable sample size would be 20 patients per regimen (60 total) understanding the overall variance is equal amongst

regimens.

There were no adverse reports noted in the electronic medical records from the vitamin D supplementation and no evidence of toxicity among any vitamin D regimens. Future investigations should consider supplementing only high-dose vitamin D within an HIV-Infected population in order to increase serum 25-(OH)D concentrations significantly.

Conclusion

Vitamin D deficiency is prevalent in HIV-infected patients, including among HIV-infected patients treated at OHSU. Supplementation, therefore, is vital to increasing 25-(OH)D stores within this complex population. Vitamin D status is generally decreased by lack of sunlight (UV-B exposure), obesity, and ethnicity.[4, 27] HIV-infected patients' metabolism of vitamin D is further challenged by ARV medications, HIV-related inflammation, and potential fat-malabsorptive issues.[27] A repletion/maintenance regimen of high-dose vitamin D_2 at 50,000 IU weekly and then a lower daily dose of either vitamin D_2 or D_3 increased serum 25-(OH)D concentrations more than any other single D type regimen. Future studies should continue to adjust for known confounders when analyzing change in vitamin D status but also adjust for signs/symptoms of fat-malabsorption (i.e. steatorrhea) and indicators of patient adherence. Although this study provides guidance on optimal vitamin D supplementation regimens for HIV-infected patients, further research is needed, including studies specifically investigating HIV-specific vitamin D deficiency.

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Appendix I: Evidence Table of Influential Research

Study Identification	Design	Participants/ Specimens	Outcomes	Duration	Selection Criteria
1. *High frequency of vitamin D deficiency in HIV-infected patients: effects of HIV-related factors and antiretroviral drugs*	Cross-Sectional (Data from 5 cohorts)	2994 eligible participants (Average 45 y/o & male)	Vitamin D deficiency/ insufficiency in 86.7% (31.1% w/ deficiency)	Screening every 3-6 months	Exclusion: No Vitamin D screen, 3 months ARV Tx, Hx of osteoporosis & severe renal impairment
2. *High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients*	Retrospective assessment of vit D by season and initiation of cART	211 HIV Infected patients' stored plasma samples, mainly male	Deficiency (< 30nmol/l) was 42% in spring and 14% in fall and cART was not a covariate	Plasma collected every 6 months	Exclusion: HIV >50 copies/mL 6 months prior
3. *High frequency of vitamin D deficiency in ambulatory HIV-Infected patients*	Cross-sectional (limited to the winter/spring)	57 Ambulatory, 77% male and average 46 y/o	36.8% Vit D deficient	4 months (collected plasma throughout)	Exclusion: Not seeking HIV medical care at MGH HIV Clinic (Boston, MA)
4. *Vitamin D deficiency and risk of cardiovascular disease*	Longitudinal	No prev. CV disease, Mean: 59 y/o, 55% female, all white	Graded CV risk increase: hazard ratios of 1.53 for 10-15 ng/mL and 1.80 for 10 ng/mL	7.6 years of follow-up (Mean 5.4 yrs.)	Exclusion: Mainly, no previous Hx of chronic disease (esp. CV)

5. *Vitamin D intake and incidence of multiple sclerosis*	Prospective cohort	Female, I: 121,700 aged 30 -55 years; II: 116,671 aged 25- 42 years	Total vitamin D intake at baseline was inversely associated with risk of MS (Food source did not alter risk)	10 yrs. (1991-2001)	Exclusion: Incomplete food frequency or500-3,500 kcal/day and occurrence of neurologic symptoms before baseline
6. *Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study*	Birth-cohort	10,334 infants (51% boy/49% girl)	Dietary vitamin D supplementation is associated with reduced risk of type 1 diabetes	31 years (1966-1997)	Pregnant Finnish women (post 24th week of gestation w/estimated delivery falling on 1966)
7. *Vitamin D concentration of HIV-infected women and its association with HIV disease progression, anemia, and mortality.*	Clinical Trial (large)	HIV Infected Females of Tanzania (inc. pregnant)	Vitamin D has protective association with HIV disease progression, all-cause mortality, and development of anemia	~69.5 months	HIV Infected, female, and in Tanzania (inc. pregnant)
9. *Meinken C. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response*	In-Vitro	Monocyte/Macrophages (TLR2/1)	Supplementation of the African American serum with 25-(OH)D3 to a physiologic range restored TLR repletion of cathelicidin mRNA (+antimicrobial)		
10. *The antimicrobial peptide LL-37 inhibits HIV-1*	In-Vitro	Antimicrobial peptide LL-37	HIV-1 inhibitory effect independent of FPRL-1 signaling		

replication					
11. *Vitamin D in HIV-infected patients*	Observational	HIV Infected patients (<18 yrs. old)	Very high rates of low 25-(OH)D (vitamin D) levels in both general and HIV-infected populations. In HIV-Infected: low 25-(OH)D levels are likely a combination of both traditional risk factors and HIV- and antiretroviral therapy-specific contributors and may respond to standard repletion regimens differently	varies	HIV Infected and adult (>18 years)
12. *Dietary Reference Intakes for Calcium and Vitamin D*	Systematic evidence-based reviews	American & Canadian citizens	Vitamin D deficiency is a serum 25-(OH)D level equal to 20 ng/ml (50 nmol/liter) or less	varies	American & Canadian citizens (w/ involvement in Vit. D study)
13. *Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin*	Cross-sectional	Boston & Edmonton residents	Latitudinal increase in the length of the "vitamin D winter" during which dietary supplementation of the vitamin may be advisable	Seasonal	Must reside in specific cities

14. *Chronic sunscreen use decreases circulating concentrations of 25-(OH)D: a preliminary study*	Placebo-Controlled	20 long-term users of p-aminobenzoic acid (PABA) and 20 in controls matched by age and exposure to sunlight	Mean (±SEM) serum concentration of 25-OH-D level was lower in the sunscreen users than in their matched controls	One-time lab visit	Must live within the same latitude
15. *Demographic Differences and Trends of Vitamin D Insufficiency in the US Population, 1988-2004*	Observational (survey)	18,883 participants in NHANES III and 13,369 participants in NHANES 2001-2004	Decrease in serum 25-(OH)D levels from the 1988-1994 to the 2001-2004 NHANES data collections and racial/ethnic differences persist	~9 years	United States Citizen
16. *Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients: effect of age and dietary calcium*	Placebo-Controlled	94 normal subjects (77 women and 17 men) 30-90 y/o	Insufficient metabolism of 25-OH-D to 1,25(OH)2D contributes significantly to decreased calcium absorption and adaptation in both osteoporotic and elderly vs. nonelderly	3 months	Ambulatory, good health, not on supplements, w/o hip fractures or back pain

17. *Decreased bioavailability of vitamin D in obesity*	Placebo-Controlled	Healthy, white, obese [body mass index (BMI; in kg/m2) ≥ 30] and matched lean control subjects (BMI ≤ 25)	Obese subjects had significantly lower basal 25-(OH)D concentrations and higher parathyroid hormone concentrations	48 hours (24 hr. to screen)	Exclusion: Hx of hepatic/renal disorders and taking vitamin D supplements, anticonvulsant medications, or corticosteroids
18. *Obesity among patients with HIV: the latest epidemic*	Retrospective	661 HIV-infected patients	Patients with HIV in the HAART era are commonly overweight and/or obese with rates similar to the general population	1 yr. (June 2004-2005)	HIV Infected and ART-initiated
19. *Different strategies of 25OH vitamin D supplementation in HIV+ subjects*	Prospective Cohort	191 HIV-infected patients	Analyzed w/ Pearson's correlation coefficient and determined	March-May 2011	25OH vitamin D levels <30 ng/mL, Caucasian ethnicity, HIV infection, and current cART treatment w/o changes for >12 mo.
20. *Bone Health and Human Immunodeficiency Virus Infection*	Review	Multiple studies (Notable: SUN, HOPS, ASSERT, PREPARE, iPrEx, and EuroSIDA)	LMD is common among persons w/ HIV and lack of knowledge for most appropriate vitamin D supplementation	Variable time frame of study (Review pub. 2013)	HIV Infected Patients