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Effects of Vitamin D Supplementation on Carotid Intima-Media Thickness in HIV+ Youth

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CASE WESTERN RESERVE UNIVERSITY EST. 1826 think beyond the possible

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ABSTRACT

Background: HIV+ youth are at an increased risk of cardiovascular disease (CVD). Vitamin D deficiency is associated with CVD risk in HIV, but it is not known whether supplementation could affect this risk. **Methods:** This is a 24-month randomized, active-control, double-blind trial comparing 2 different monthly vitamin D_3 doses [60,000 (medium) or 120,000 (high) IU/month] vs. control of 18,000 IU/month in 8-26 year old HIV+ youth on ART with baseline 25-hydroxyvitamin D (25(OH)D) ≤30 ng/mL & HIV-1 RNA <1000 copies/mL. Carotid IMT was measured at baseline & 24 months. Comparisons of changes in IMT were made between the HIV+ control arm vs. combined supplementation arms (medium+high) & within these groups using appropriate two-sample tests. A matched healthy uninfected group was enrolled in a similarly-designed parallel study for comparison. **Results:** We enrolled 102 HIV+ subjects: 64% men, 89% black, median age of 20 years. HIV & ART duration were 8 & 3 years, respectively with a CD4 count of 652 cells/mm^3. Baseline 25(OH)D was similar between groups (controls: 17 (11, 21) vs. supplementation group: 18 (14, 22) ng/mL; P=0.49) and increased to 32 (25, 38) and 41 (31, 46) ng/mL in the control and supplementation (medium+high) dose groups at 24 months, respectively (within & between groups P<0.001). Baseline bulb (0.65 vs. 0.63 mm, P=0.13) and common carotid artery (CCA) IMT (0.69 vs. 0.56 mm, P=0.81) were similar between groups. Over 24 months, bulb & CCA IMT decreased only in the control arm (Figure 1), with changes in bulb IMT being significantly different than supplementation arm at 24 months (P=0.02). Overall, changes in bulb IMT were significantly correlated with changes in 25(OH)D (R=0.43, P=0.001). In multivariable regression models, larger increases in 25(OH)D were associated with greater IMT increases. In contrast to the findings in HIV+ subjects, among the healthy uninfected group (N=88), there were no differences in changes in IMT between the control vs. supplementation arms and no significant correlations between changes in 25(OH)D and changes in IMT. **Conclusions:** A modest vitamin D_3 dose of 18,000 IU/month given over 24 months resulted in significant decreases in carotid IMT compared to high monthly doses of 60,000 or 120,000 IU. These results suggest a potential for a detrimental effect of high-dose vitamin D supplementation in this population, an effect that was not seen in the parallel HIV-uninfected study. On-going analyses are underway to better understand these surprising findings.

BACKGROUND

- HIV-infected patients are at an increased risk of cardiovascular disease (CVD)
- HIV-infected youth also appear to be at an increased CVD risk as assessed by carotid intima-media thickness (IMT), a measure of subclinical atherosclerosis
- Vitamin D insufficiency is associated with CVD risk in HIV
- No studies to date have assessed changes in carotid IMT with vitamin D repletion in HIV

OBJECTIVES

- The primary objective of this study was to determine the effects of vitamin D on carotid IMT in HIV-infected youth after 24 months of supplementation
- Secondary objective included comparing the results to an uninfected healthy control group

METHODS

- **STUDY DESIGN**
  - Randomized, active-controlled, double-blind trial investigating two doses of oral vitamin D_3: 60,000 IU (medium dose) and 120,000 IU (high dose) vs. a standard dose of 18,000 IU (control dose) given monthly for 24 months
  - 18,000, 60,000, 120,000 IU/monthly equivalent to 600, 2,000, 4,000 IU/daily, respectively
  - Randomization stratified by current EFV use
- **SELECTION OF SUBJECTS**
  - **Inclusion criteria:** HIV infection, age between 8-25 years, cumulative ARV duration ≥6 months, current ARV regimen consistently for ≥3 months prior to enrollment, HIV-1 RNA level ≤1,000 copies/mL
  - **Exclusion criteria:** Acute illness ≤30 days prior to enrollment, inflammatory condition, malignancy, medication use which could affected results, pregnancy/breastfeeding
- **STUDY ASSESSMENTS**
  - **Clinical and laboratory evaluation:** Questionnaires, extensive chart review, weight, height, HIV-1 RNA, CD4 count, fasting lipids, glucose, insulin
  - **Vitamin D evaluation:** plasma 25-hydroxyvitamin D (25(OH)D)
  - **Carotid ultrasound:** 3 measurements of the IMT were obtained at both the near and far walls of the right and left common carotid artery (CCA); all measurements were averaged and reported as a single measurement; process repeated for the internal carotid artery (ICA) and bulb region
- **STATISTICAL ANALYSIS**
  - Subjects in medium and high dose groups were considered together (supplementation dose) and compared to subjects in control dose
  - All analyses were conducted as intent-to-treat
  - **Within-group and between-group comparisons:** Appropriate one-sample and two-sample tests, respectively
  - **Correlations:** Spearman correlation coefficients between changes in carotid IMT and variables of interest
  - **Multivariable regression analysis**: Used to determine variables independently associated with changes in carotid IMT.

RESULTS

Table 1. Baseline Characteristics

Median (Q1, Q3) or no. (%)	HIV-infected Subjects: Combined (N=102)	Supplementation Dose (N=66)	Control Dose (N=36)	P[1]	Healthy Uninfected Subjects: Combined (N=88)	Supplementation Dose (N=58)	Control Dose (N=30)	P[2]	P[3]
Demographics & Clinical Variables									
Age, years	20.3 (16.6, 22.8)	20.9 (17.0, 23.6)	19.9 (15.9, 21.6)	0.27	19.3 (14.4, 23.2)	19.6 (14.5, 23.7)	18.4 (14.4, 22.2)	0.54	0.34
Male sex	65 (64%)	44 (67%)	21 (58%)	0.07	53 (60%)	38 (66%)	15 (50%)	0.16	0.66
Black race	91 (89%)	57 (86%)	34 (94%)	0.52	76 (86%)	47 (81%)	29 (97%)	0.05	0.66
BMI, kg/m^2	22.5 (19.5, 25.3)	23.2 (19.6, 25.8)	21.1 (18.9, 21.6)	0.32	23.6 (19.8, 28.1)	23.6 (19.7, 28.1)	23.3 (20.2, 28.3)	0.62	0.17
Tanner stage									
<5	25 (25%)	15 (15%)	10 (28%)	0.96	32 (36%)	18 (31%)	14 (47%)	0.31	0.14
5	77 (75%)	51 (77%)	26 (72%)		56 (64%)	40 (69%)	16 (53%)		
Systolic BP, mmHg	120 (112, 128)	122 (113, 129)	118 (105, 125)	0.09	117 (107, 124)	105 (88, 118)	114 (92, 114)	0.99	0.06
Current smoking	27 (27%)	22 (33%)	5 (14%)	**0.03**	13 (15%)	11 (19%)	2 (7%)	0.20	0.05
Current alcohol use	45 (45%)	3 (46%)	15 (42%)	0.66	30 (34%)	21 (36%)	9 (30%)	0.56	0.14
Waist-to-hip ratio	0.85 (0.80, 0.88)	0.85 (0.81, 0.90)	0.84 (0.79, 0.86)	0.19	0.83 (0.78, 0.89)	0.82 (0.74, 0.88)	0.83 (0.78, 0.91)	0.59	0.40
LDL cholesterol, mg/dL	91 (71, 112)	87 (72, 106)	99 (71, 116)	0.23	91 (71, 113)	94 (76, 112)	81 (66, 117)	0.23	0.99
HDL cholesterol, mg/dL	46 (38, 54)	44 (38, 52)	48 (41, 56)	0.36	53 (43, 63)	53 (43, 63)	53 (42, 60)	0.43	**0.0005**
Triglycerides, mg/dL	80 (61, 116)	83 (66, 133)	70 (58, 95)	0.08	63 (48, 87)	63 (49, 81)	65 (44, 101)	0.92	**0.0004**
HOMA-IR	2.1 (1.4, 4.0)	2.2 (1.4, 4.0)	2.0 (1.4, 3.7)	0.61	2.1 (1.5, 3.4)	2.0 (1.4, 3.1)	2.3 (1.5, 4.4)	0.39	0.61
25(OH)D, ng/mL	17 (13, 22)	18.0 (14.0, 22.0)	17.3 (11.03, 20.9)	0.49	16 (12, 21)	16 (12, 20)	17 (13, 22)	0.62	0.30
Carotid IMT									
CCA IMT, mm	0.56 (0.55, 0.59) (N=100)	0.56 (0.55, 0.60) (N=65)	0.56 (0.54, 0.59) (N=35)	0.81	0.55 (0.51, 0.58) (N=87)	0.55 (0.48, 0.58) (N=58)	0.56 (0.52, 0.58) (N=29)	0.82	**0.009**
Bulb IMT, mm	0.63 (0.55, 0.69) (N=82)	0.63 (0.54, 0.68) (N=54)	0.65 (0.60, 0.71) (N=28)	0.13	0.57 (0.49, 0.62) (N=71)	0.57 (0.50, 0.64) (N=49)	0.55 (0.44, 0.61) (N=22)	0.47	**0.0002**
ICA IMT, mm	0.56 (0.50, 0.61) (N=62)	0.55 (0.47, 0.61) (N=41)	0.56 (0.53, 0.59) (N=21)	0.63	0.49 (0.38, 0.54) (N=61)	0.49 (0.39, 0.54) (N=42)	0.52 (0.36, 0.57) (N=19)	0.86	**0.001**
HIV Variables									
Current CD4, cells/mm^3	652 (449, 872)	652 (451, 879)	608 (417, 792)	0.58					
Nadir CD4, cells/mm^3	293 (174, 424)	317 (190, 472)	246 (109, 363)	0.06					
HIV RNA <80 copies/mL	91 (89%)	59 (89%)	32 (89%)	1.00					
HIV RNA, copies/mL (N=11)	190 (127, 630)	143 (90, 590)	654 (382, 805)	0.10					
Perinatal transmission	50 (49%)	35 (47%)	19 (53%)	0.68					
Previous AIDS diagnosis	41 (41%)	24 (38%)	17 (47%)	0.34					
HIV duration, years	8.1 (3.4, 15.6)	8.2 (2.1, 15.8)	8.0 (2.7, 15.6)	0.86					
ARV duration, years	3.2 (1.3, 10.0)	3.2 (1.3, 10.0)	2.7 (1.3, 10.1)	1.00					
NRTI duration, years	3.1 (1.3, 9.5)	3.2 (1.3, 9.7)	2.6 (1.3, 7.7)	0.84					
PI duration, years	2.3 (0.6, 7.3)	2.8 (0.5, 8.3)	1.8 (0.8; 6.5)	0.87					
EFV duration, months	2 (0, 16)	6 (0, 16)	0 (0, 15)	0.34					
TDF duration, months	18 (7, 32)	18 (7, 33)	18 (8, 30)	0.95					
Current PI use	57 (56%)	38 (58%)	19 (53%)	0.64					
Current EFV use	27 (25%)	18 (27%)	9 (25%)	1.00					
Current TDF use	82 (80%)	55 (83%)	27 (75%)	0.31					

[1]P value between supplementation and control dosing arms; [2]P value between combined HIV+ and combined healthy uninfected subjects

Table 2A. Changes over 24 Months in HIV-infected Subjects

Median (Q1, Q3)	All Subjects (N=68)	P[1]	Suppl. Dose (N=42)	P[1]	Control Dose (N=26)	P[1]	P[2]
Clinical and Laboratory Variables							
BMI, kg/m^2	+1.1 (+0.1, +2.3)	**<0.0001**	+1.0 (-0.2, +2.9)	**0.0001**	+1.4 (+0.6, +1.9)	**<0.0001**	0.82
Waist-to-hip ratio	+0.01 (-0.04, +0.04)	0.80	+0.01 (-0.03, +0.05)	0.49	-0.00 (-0.06, +0.03)	0.53	0.43
CD4 count, cells/mm^3	+42 (-85, +159)	0.07	+57 (-77, +186)	0.07	+24 (-85, +158)	0.29	0.49
Systolic BP, mmHg	+4 (-5, +9)	0.12	+4 (-5, +10)	**0.02**	+10 (+9, +18)	0.39	0.60
LDL cholesterol, mg/dL	-6 (-19, +3)	**0.003**	-8 (-19, -1)	**0.01**	-5 (-18, +3)	0.12	0.57
HDL cholesterol, mg/dL	+2 (-4, +6)	0.35	0 (-4, +5)	1.00	+4 (-1, +7)	0.16	0.17
Triglycerides, mg/dL	+2.5 (-20, +19)	0.95	+8 (-26, +21)	0.79	-1 (-16, +17)	0.74	0.63
HOMA-IR	-0.01 (-0.89, +0.96)	0.64	+0.13 (-0.75, +1.56)	0.43	-0.06 (-1.27, +0.73)	0.80	0.42
25(OH)D, ng/mL	+18 (+9, +25)	**<0.0001**	+23 (+13, +33)	**<0.0001**	+3 (-4.5, +5.0)	**<0.0001**	**0.004**
Carotid IMT							
CCA IMT, mm	-0.01 (-0.06, +0.02) (N=67)	0.06	-0.01 (-0.05, +0.02) (N=42)	0.44	-0.02 (-0.08, +0.02) (N=25)	**0.06**	0.26
Bulb IMT, mm	-0.06 (-0.21, +0.02) (N=53)	**0.0006**	-0.03 (-0.13, +0.04) (N=35)	0.11	-0.19 (-0.27, -0.03) (N=18)	**0.0008**	**0.01**
ICA IMT, mm	-0.04 (-0.17, +0.04) (N=40)	0.10	-0.03 (-0.06, +0.03) (N=26)	0.30	-0.09 (-0.19, +0.04) (N=14)	0.15	0.34

[1]P value within group; [2]P value between the two dosing groups

Table 2B. Changes over 24 Months in Healthy Uninfected Subjects

Median (Q1, Q3)	All Subjects (N=54)	P[1]	Suppl. Dose (N=33)	P[1]	Control Dose (N=21)	P[1]	P[2]	P[3]
Clinical and Laboratory Variables								
BMI, kg/m^2	+1.4 (+0.0, +2.78)	**<0.0001**	+1.4 (+0.0, +2.8)	**0.001**	+1.5 (+0.0, +2.8)	**0.01**	0.79	0.95
Waist-to-hip ratio	+0.00 (-0.01, +0.03)	0.44	+0.00 (-0.01, +0.03)	0.70	+0.00 (-0.02, +0.02)	0.48	0.83	0.96
Systolic BP, mmHg	+6 (+2, +15)	**<0.0001**	+7 (+2, +14)	**<0.0001**	+6 (+1, +15)	**0.005**	0.70	**0.01**
LDL cholesterol, mg/dL	-2 (-19, +6)	0.15	-7 (-20, +1)	0.06	+0 (-12, +14)	0.90	0.13	0.43
HDL cholesterol, mg/dL	-2 (-5, +6)	0.44	-2 (-4, +6)	0.96	+0 (-7, +3)	0.35	0.51	0.23
Triglycerides, mg/dL	+0 (-12, +22)	0.38	+8 (-7, +27)	0.09	-4 (-15, +9)	0.46	0.06	0.61
HOMA-IR	+0.12 (-0.88, +1.27)	0.71	+0.33 (-0.97, +1.36)	0.73	+0.11 (-0.50, +0.52)	0.79	0.78	0.96
25(OH)D, ng/mL	+14 (+4, +23)	**<0.0001**	+21 (+12, +32)	**<0.0001**	+4 (+0, +12)	**0.0003**	**<0.0001**	0.17
Carotid IMT								
CCA IMT, mm	-0.01 (-0.07, +0.02) (N=52)	0.07	-0.01 (-0.07, +0.02) (N=32)	0.22	-0.02 (-0.06, +0.02) (N=20)	0.14	0.69	0.82
Bulb IMT, mm	-0.03 (-0.13, +0.04) (N=44)	0.06	-0.03 (-0.12, +0.06) (N=28)	0.33	-0.03 (-0.16, +0.04) (N=16)	0.08	0.71	0.23
ICA IMT, mm	-0.03 (-0.15, +0.07) (N=33)	0.07	-0.05 (-0.15, +0.07) (N=20)	0.22	-0.02 (-0.22, +0.07) (N=13)	0.27	0.88	0.97

[1]P value within group; [2]P value between the two dosing groups; [3]P value between combined HIV+ and combined healthy uninfected subjects

Table 3A. Variables at 24 Months in HIV-infected Subjects

Median (Q1, Q3)	All Subjects (N=68)	Suppl. Dose (N=42)	Control Dose (N=26)	P[1]
25(OH)D, ng/mL	37 (28, 44)	41 (31, 46)	32 (25, 38)	**0.006**
CCA IMT, mm	0.55 (0.51, 0.58)	0.56 (0.51, 0.58)	0.54 (0.49, 0.57)	0.39
Bulb IMT, mm	0.54 (0.47, 0.58)	0.55 (0.50, 0.60)	0.48 (0.43, 0.56)	**0.02**
ICA IMT, mm	0.50 (0.39, 0.57)	0.51 (0.39, 0.57)	0.50 (0.40, 0.57)	0.98

[1]P value within group; [2]P value between the two dosing groups

Table 3B. Variables at 24 Months in Healthy Uninfected Subjects

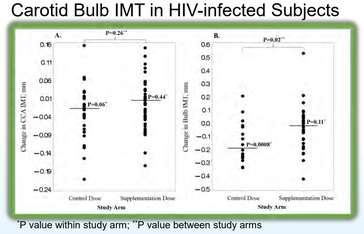
Median (Q1, Q3)	All Subjects (N=54)	Suppl. Dose (N=33)	Control Dose (N=21)	P[1]	P[2]
25(OH)D, ng/mL	30 (23, 39)	38 (29, 51)	24 (20, 30)	**0.0005**	**0.046**
CCA IMT, mm	0.54 (0.51, 0.57)	0.55 (0.48, 0.58)	0.53 (0.51, 0.55)	0.36	0.17
Bulb IMT, mm	0.54 (0.42, 0.58)	0.39 (0.20, 0.54)	0.53 (0.43, 0.56)	0.49	0.52
ICA IMT, mm	0.49 (0.32, 0.54)	0.44 (0.32, 0.54)	0.50 (0.28, 0.54)	0.91	0.14

[1]P value within group; [2]P value between the two dosing groups; [3]P value between combined HIV+ and combined healthy uninfected subjects

N.B. Other variables tested but did not have significant associations include changes in CCA IMT; changes in ICA IMT; total use of EFV; nadir CD4; HIV duration; PI y/n at entry, previous AIDS diagnosis, current use of EFV, changes in SBP, changes in BMI, changes in WHR, changes in HDL, changes in LDL, changes in TG, changes in HOMA, changes in CD4; BL age, BL SBP, BL BMI, BL WHR, BL HDL, BL LDL, BL TG, BL HOMA, sex, current smoking, race, current alcohol use, tanner stage 5 vs. <5.

Correlations between change in 25(OH)D and change in IMT not significant for healthy uninfected controls.

Figure 1. Changes in (A). CCA IMT and (B). Carotid Bulb IMT in HIV-infected Subjects



*P value within study arm; **P value between study arms

Figure 2. Correlations for (A). all HIV+ Subjects Combined and (B). by Dosing Arm

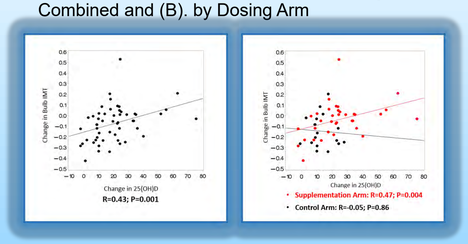


Table 4. Significant Correlations Between Changes in 25(OH)D and Variables of Interest in HIV-infected Subjects

Current CD4 count	R=0.34; P=0.01
ARV duration	R=0.29; P=0.045
PI duration	R=0.32; P=0.045
Changes in bulb IMT	R=0.43; P=0.001

R = Spearman Correlation Coefficient

Table 5. Multivariable Regression Models to Predict Changes in Bulb IMT in HIV+ Subjects

Variable	β	B (SE)	P
Study arm*	-0.13267	0.05642	**0.03**
CD4 count	-0.00003	0.00008	0.73
ARV duration	0.00036	0.00072	0.62
PI duration	0.00038	0.00077	0.63
Sex	0.00606	0.06606	0.93
Race	0.03328	0.10437	0.75
Smoking	-0.25887	0.07391	**0.04**
	R^2 = 0.32618		

*Control dose associated with greater decreases in IMT

Variable	β	B (SE)	P
Change in 25(OH)D	0.00288	0.00157	0.08
CD4 count	-0.00003	0.00008	0.70
ARV duration	0.00052	0.00073	0.48
PI duration	0.00040	0.00080	0.62
Sex	0.03486	0.06557	0.60
Race	-0.02692	0.10497	0.80
Smoking	-0.07995	0.07355	0.29
	R^2 = 0.28493		

SUMMARY OF RESULTS

- Among the HIV-infected subjects, plasma 25(OH)D concentrations increased significantly after 24 months of monthly oral vitamin D_3 in both study arms: those receiving a medium or high (supplementation) dose and those receiving a standard (control) dose; however, 25(OH)D increased significantly more in the supplementation arm.
- Carotid IMT did not change significantly within the supplementation arm, but decreased significantly in the bulb region and approached significance in the CCA within the control arm.
- Differences in bulb IMT changes were statistically significant between the supplementation and control arms, and bulb IMT was statistically lower at 24 months in the control arm.
- In bivariate analyses, increases in bulb IMT were significantly correlated with increases in plasma 25(OH)D, with this association driven by subjects in the supplementation arm.
- Being in the supplementation arm was independently associated with increases in bulb IMT.
- Increases in plasma 25(OH)D concentrations trended toward an independent relationship with increases in bulb IMT.
- HIV-infected subjects had increased CCA, ICA, and bulb IMT compared to healthy uninfected controls at baseline.
- Over 24 months, healthy uninfected controls had similar increases in plasma 25(OH)D concentrations compared to the HIV-infected subjects, but only a trend toward a significant decrease in bulb IMT within the control group (without any significant between group differences between arms for IMT).
- The relationship between changes in IMT and 25(OH)D observed in the HIV-infected group were not seen among the healthy uninfected controls.

CONCLUSIONS

A modest vitamin D_3 dose of 18,000 IU/month given over 24 months resulted in significant decreases in carotid bulb IMT compared to high monthly doses of 60,000 or 120,000 IU. These results suggest a potential for a detrimental effect of high-dose vitamin D supplementation in this population, an effect that was not seen in the parallel HIV-uninfected study. On-going analyses are underway to better understand these surprising findings.

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