

Havens, PL et al. Vitamin D3 Supplementation Increases Spine Bone Mineral Density in Adolescents and Young Adults with HIV Infection Being Treated with Tenofovir Disoproxil Fumarate: A Randomized, Placebo-Controlled Trial

Supplementary material

## **Laboratory methods**

Serum intact PTH was measured using an electrochemiluminescence immunoassay (Intact-PTH, Cobas e411; Roche Diagnostics Corp., Indianapolis, IN). Serum fibroblast growth factor 23 (FGF23) was measured by ELISA (Kainos Laboratories, Inc., Tokyo, Japan). Serum VDBP was measured by ELISA (Quantikine VDBP ELISA kit; R&D Systems, Inc., Minneapolis, MN).

Serum C-terminal telopeptides (CTX) were measured by ELISA (Serum CrossLaps ELISA, Immunodiagnostic Systems (IDS) Inc., Gaithersburg, MD). Serum intact osteocalcin and bone-specific alkaline phosphatase were determined by enzyme immunoassay (EIA) (MicroVue; Quidel Corporation, San Diego, CA).

Calcium, phosphate, creatinine, and albumin from serum, and glucose from plasma (collected with a vacutainer containing potassium oxalate and sodium fluoride) were determined using a clinical chemistry analyzer (Cobas Integra 400 Plus; Roche Diagnostics Corp., Indianapolis, IN).

Serum 25-OHD was measured by EIA and 1,25-OH(2)D was measured by radioimmunoassay (Immunodiagnostic Systems (IDS) Inc., Gaithersburg, MD).

Urinary retinol binding protein (URBP) was measured by ELISA (DetectX urinary RBP EIA kit; Arbor Assays, Ann Arbor, MI). URBP values were normalized for urine creatinine concentrations. Urinary beta-2 microglobulin was determined using a solid phase electrochemiluminescence assay (Meso Scale Discovery, LLC, Rockville, MD).

The concentrations of calcium, phosphate, protein, glucose, and creatinine in urine were determined using a clinical chemistry analyzer (Cobas Integra 400 Plus; Roche Diagnostics Corp., Indianapolis, IN).

**Supplemental Table 1. Calcium and Vitamin D Intake During 48 Weeks of the Study**

Characteristic	Overall <sup>1</sup>	Randomized Group		P value <sup>6</sup>
		Vitamin D3	Placebo	
Multivitamin adherence by pill count				
N with data	146	75	71	
Adherence (%) <sup>2</sup>				
Median	49%	49%	49%	0.68
Q1, Q3	27%, 69%	27%, 68%	27%, 70%	
Study drug adherence				
N with data	188	99	89	
Administered within visit window (%) <sup>3</sup>				
Median	100%	100	100	0.21
Q1, Q3	83%, 100%	83%, 100%	92%, 100%	
Total doses administered (%)				
Median	100%	100%	100%	0.49
Q1, Q3	100%, 100%	92%, 100%	100%, 100%	
Calcium intake (mg/day)				
Dietary <sup>4</sup>				
Median	845	862	828	0.45
Q1, Q3	526, 1290	584, 1280	472, 1295	
Multivitamin <sup>5</sup>				
Median	79	80	79	0.68
Q1, Q3	44, 112	44, 110	43, 113	
Total calcium intake				
Median	888	906	853	0.47
Q1, Q3	609, 1321	637, 1317	559, 1349	
Inadequate <sup>7</sup> total daily calcium intake for age N (%)	108 (58%)	55 (56%)	53 (60%)	0.66
Vitamin D intake (IU/day)				
Dietary <sup>4</sup>				
Median	124	124	123	0.55
Q1, Q3	68, 199	73, 206	67, 191	
Multivitamin <sup>5</sup>				
Median	195	196	195	0.68
Q1, Q3	108, 277	110, 271	107, 279	
Study drug				
Median		1780	0	---
Q1, Q3		1637, 1786	0, 0	
Total vitamin D intake				
Median		2020	284	<0.001
Q1, Q3		1914, 2168	179, 394	

1. Evaluable participants only. The data represent cumulative experience over the duration of the study.
2. Median (Q1, Q3) unless otherwise specified. Calculated as total number of pills taken divided by the number of days on study.
3. Visit window was one week before or after the every 28-day target visit for directly observed therapy. A minimum of 21 days was required between DOT visits for study drug administration. Denominator was the total number of DOT visits (=12).
4. Calculated from the food frequency questionnaire at week 48
5. For the multivitamin and for study drug, intake = Received dose = prescribed dose multiplied by adherence (ref 17). Inadvertently, the initially distributed preparation contained calcium 45 mg, taken by 48 participants for a median of 2.8 (range 0.9 to 2.5) months, at which time the error was identified and the originally planned preparation, with calcium 162 mg, was distributed to study sites. The calculated received dose for these individuals accounts for this error.
6. P-value by Wilcoxon Rank Sum test for continuous variables and Fisher's Exact test for categorical variables.
7. Total calcium intake was defined as inadequate if a subject was  $\leq 18$  years old and had total calcium intake  $< 1300$  mg or if  $\geq 19$  years old and had total calcium intake  $< 1000$  mg. All other subjects not meeting criteria for being inadequate were classified as adequate.

**Supplemental Table 2. Endocrine, Renal, and Bone Mineral Density Measurements at Baseline, Weeks 12, 24, and 48, and Change from Baseline to Weeks 12, 24, and 48 by Randomized Study Group**

Variable <sup>1</sup>	Rando mized Group	N <sup>2</sup>		Study Week – Median (Q1, Q3)				P value <sup>3</sup>
				Baseline	12	24	48	
<b>Vitamin D-related Variables</b>								
Serum 25-OHD (ng/mL)	VITD	100	value change	15.7 (12.1, 24.9)	35.1 (29.8, 39.8) 16.3 (9.3, 21.2) <sup>†</sup>	37.0 (30.4, 44.1) 18.6 (10.5, 25.6) <sup>†</sup>	36.9 (30.5, 42.4) 17.8 (11.8, 24.0) <sup>†</sup>	<0.001
	PL	91	value change	16.8 (10.2, 22.7)	24.0 (18.4, 30.2) 6.9 (2.3, 11.6) <sup>†</sup>	23.3 (17.9, 28.5) 6.8 (1.4, 22.2) <sup>†</sup>	20.6 (14.4, 25.8) 2.6 (-1.7, 7.5) <sup>†</sup>	
Serum 1,25- OH(2)D (pmol/L)	VITD	99	value change	72.5 (58.3, 89.5)	87.7 (70.5, 112.1) 15.6 (-0.3, 35.2) <sup>†</sup>	90.7 (72.2, 112.9) 12.9 (-7.6, 36.6) <sup>†</sup>	83.6 (70.1, 112.5) 10.5 (-2.1, 31.5) <sup>†</sup>	0.014
	PL	91	value change	68.9 (57.1, 96.1)	81.5 (60.2, 106.1) 6.1 (-7.6, 25.5)*	80.4 (66.7, 106.0) 8.6 (-9.0, 27.0) <sup>^</sup>	78.3 (61.4, 95.7) 2.7 (-14.7, 24.5)	
Serum free 1,25- OH(2)D (fmol/L)	VITD	99	value change	418 (315, 638)	561 (361, 792) 107 (-13, 222) <sup>†</sup>	582 (383, 768) 99 (-30, 243) <sup>†</sup>	572 (364, 757) 64 (-19, 203) <sup>†</sup>	0.021
	PL	91	value change	445 (312, 638)	496 (355, 697) 53 (-29, 149) <sup>†</sup>	496 (354, 689) 59 (-42, 162) <sup>†</sup>	452 (333, 646) 26 (-101, 118)	
Serum vitamin D binding protein (mcg/mL)	VITD	100	value change	153 (1058, 251)	161 (109, 259) -2 (-11, 9)	156 (104, 259) -1 (-17, 7)	151 (99, 259) -4 (-16, 5)*	0.11
	PL	91	value change	159 (104, 230)	147 (103, 233) -1 (-10, 9)	151 (104, 231) -2 (-8, 8)	144 (105, 228) 1 (-12, 13)	
<b>Bone Mineral Density and Anthropometric Variables</b>								
Lumbar spine (L1-L4) BMD (g/cm <sup>2</sup> )	VITD	99	value % change	1.06 (0.99, 1.19)	NA	1.09 (1.00, 1.19) 0.94 (-0.72, 2.16) <sup>†</sup>	1.08 (1.01, 1.16) 1.15 (-0.75, 2.74) <sup>†</sup>	0.12
	PL	89	value % change	1.08 (0.98, 1.20)	NA	1.09 (0.97, 1.21) 0.42 (-1.02, 1.91)*	1.08 (0.97, 1.18) 0.09 (-1.49, 2.61)	
Lumbar spine (L1-L4) BMD Z- score	VITD	99	value change	-0.65 (-1.40, 0.00)	NA	-0.50 (-1.30, 0.10) 0.00 (-0.10, 0.20)	-0.70 (-1.40, 0.00) 0.00 (-0.10, 0.10)	0.14
	PL	89	value change	-0.70 (-1.60, 0.20)	NA	-0.70 (-1.60, 0.10) 0.00 (-0.10, 0.10)	-0.90 (-1.70, -0.10) -0.10 (-0.30, 0.20)*	

Total hip BMD (gm/cm <sup>2</sup> )	VITD	99	value % change	1.06 (0.95, 1.16)	NA	1.06 (0.95, 1.15) -0.27 (-1.08, 1.53)	1.07 (0.94, 1.15) -0.17 (-2.12, 1.73)	0.40
	PL	89	value % change	1.05 (0.95, 1.17)	NA	1.04 (0.96, 1.17) 0.00 (-1.14, 1.46)	1.03 (0.94, 1.14) -0.42 (-1.66, 0.71)	
Total hip BMD Z-score	VITD	97	value change	-0.40 (-1.10, 0.10)	NA	-0.40 (-1.00, 0.10) 0.00 (-0.10, 0.10)	-0.40 (-0.90, 0.10) 0.00 (-0.10, 0.10)	0.12
	PL	89	value change	-0.65 (-1.30, 0.00)	NA	-0.70 (-1.35, 0.00) 0.00 (-0.10, 0.10)	-0.80 (-1.40, -0.10) 0.00 (-0.10, 0.10)	
Total body BMD (g/cm <sup>2</sup> )	VITD	99	value % change	1.18 (1.10, 1.25)	NA	1.18 (1.11, 1.25) -0.07 (-1.20, 1.01)	1.18 (1.11, 1.24) 0.00 (-1.78, 1.36)	0.91
	PL	89	value % change	1.17 (1.07, 1.26)	NA	1.17 (1.8, 1.25) 0.18 (-1.15, 1.30)	1.16 (1.09, 1.25) -0.27 (-1.39, 1.18)	
Total body BMD Z-score	VITD	99	value change	-0.60 (-1.35, 0.10)	NA	-0.70 (-1.30, 0.10) 0.00 (-0.10, 0.10)	-0.60 (-1.20, 0.00) 0.00 (-0.30, 0.10)	0.56
	PL	89	value change	-0.80 (-1.65, 0.10)	NA	-0.70 (-1.60, 0.20) 0.00 (-0.10, 0.10)	-0.70 (-1.80, 0.10) 0.00 (-0.20, 0.10)	
Total body bone mineral content (g)	VITD	99	value % change	2636 (2368, 2939)	NA	2670 (2395, 2949) 0.25 (-1.23, 1.44)	2694 (2380, 2941) 0.08 (-1.47, 1.61)	0.84
	PL	89	value % change	2596 (2316, 2936)	NA	2612 (2284, 2913) 0.07 (-0.82, 1.09)	2633 (2304, 2943) 0.07 (-1.42, 0.98)	
Body weight (kg)	VITD	100	value change	75.7 (64.9, 87.2)	NA	75.3 (66.7, 87.5) 0.0 (-1.8, 2.0)	77.5 (66.6, 88.7) 0.6 (-2.0, 3.4)	0.80
	PL	91	value change	70.8 (62.6, 85.6)	NA	71.9 (62.3, 89.1) 0.1 (-1.6, 1.6)	71.2 (62.6, 90.2) 0.6 (-0.9, 2.7) <sup>^</sup>	
Height (cm)	VITD	100	value change	173 (168, 178)	NA	173 (168, 179) 0.0 (-0.2, 0.0)	173 (169, 179) 0.0 (0.0, 0.3)	0.77
	PL	91	value change	175 (168, 180)	NA	174 (168, 180) 0.0 (-0.2, 0.0)	174 (168, 180) 0.0 (0.0, 0.2)	
<b>Endocrine and Bone Turnover Variables</b>								
Serum parathyroid hormone (pg/mL)	VITD	98	value change	38.7 (28.8, 48.5)	35.8 (26.5, 47.3) -2.2 (-10.3, 4.0)*	36.1 (26.5, 44.0) -0.3 (-10.4, 6.7)	33.7 (26.8, 42.0) -2.2 (-10.1, 4.3)*	0.47
	PL	89	value change	37.0 (28.7, 46.6)	34.2 (28.5, 42.8) -0.8 (-7.8, 4.0)	34.9 (26.1, 45.4) -1.7 (-7.8, 5.7)	35.8 (28.6, 43.5) -1.0 (-9.3, 4.9)	
Serum fibroblast growth factor 23 (pg/mL)	VITD	100	value change	33.2 (26.7, 42.7)	37.6 (28.5, 44.7) 3.3 (-3.0, 9.2) <sup>†</sup>	36.3 (30.2, 44.3) 2.9 (-4.1, 8.4)*	37.7 (30.6, 43.9) 4.8 (-2.8, 10.3) <sup>^</sup>	0.71
	PL	91	value change	34.5 (27.0, 42.1)	37.4 (31.3, 43.2) 3.9 (-2.3, 9.2) <sup>†</sup>	38.2 (32.2, 45.0) 3.7 (-4.5, 10.5) <sup>^</sup>	38.3 (30.5, 46.5) 3.2 (-3.8, 10.2) <sup>^</sup>	

Serum bone specific alkaline phosphatase (U/L)	VITD	100	value change	31.5 (25.6, 36.7)	30.0 (24.3, 36.4) -1.1 (-4.3, 1.9) <sup>^</sup>	27.9 (23.7, 34.8) -2.5 (-5.3, 0.1) <sup>†</sup>	27.9 (22.8, 33.9) -2.7 (-6.4, -0.5) <sup>†</sup>	0.20
	PL	91	value change	31.8 (25.4, 37.9)	29.3 (24.2, 34.3) -1.7 (-3.8, 0.8) <sup>†</sup>	28.5 (24.0, 36.5) -2.0 (-4.5, 0.4) <sup>†</sup>	27.9 (22.5, 34.2) -2.2 (-5.6, 1.1) <sup>†</sup>	
Serum osteocalcin (mcg/L)	VITD	100	value change	9.60 (6.47, 11.63)	9.38 (7.19, 11.73) 0.15 (-0.78, 1.67)	9.17 (7.22, 11.78) 0.09 (-1.06, 1.46)	8.79 (6.56, 11.15) -0.93 (-2.10, 0.62) <sup>†</sup>	0.78
	PL	90	value change	8.94 (5.97, 11.82)	9.01 (6.34, 12.66) -0.10 (-1.15, 1.66)	8.92 (5.70, 10.97) -0.22 (-1.43, 1.12)	8.71 (6.20, 11.02) -0.48 (-1.95, 0.41) <sup>†</sup>	
Serum C terminal telopeptides (mcg/L)	VITD	99	value change	0.76 (0.56, 1.10)	0.72 (0.59, 0.99) -0.03 (-0.21, 0.13)	0.73 (0.54, 1.00) -0.03 (-0.24, 0.18)	0.68 (0.51, 0.90) -0.08 (-0.25, 0.10) <sup>*</sup>	0.37
	PL	91	value change	0.78 (0.59, 1.08)	0.77 (0.56, 1.00) -0.02 (-0.19, 0.15)	0.74 (0.60, 1.03) -0.02 (-0.16, 0.15)	0.69 (0.54, 0.93) -0.09 (-0.27, 0.02) <sup>†</sup>	
Serum calcium (mg/dL)	VITD	100	value change	9.4 (9.2, 9.6)	9.4 (9.2, 9.6) -0.0 (-0.3, 0.2)	9.4 (9.2, 9.6) -0.0 (-0.3, 0.2)	9.4 (9.2, 9.7) 0.0 (-0.2, 0.3)	0.27
	PL	91	value change	9.3 (9.1, 9.6)	9.4 (9.2, 9.6) 0.1 (-0.1, 0.3)	9.5 (9.2, 9.7) 0.1 (-0.2, 0.3) <sup>*</sup>	9.4 (9.2, 9.7) 0.0 (-0.1, 0.3)	
Serum phosphate (mmol/L)	VITD	100	value change	3.6 (3.3, 3.9)	3.5 (3.2, 3.7) 0.0 (-0.3, .03)	3.5 (3.2, 3.8) -0.1 (-0.4, 0.3)	3.5 (3.2, 4.0) -0.0 (-0.3, 0.4)	0.48
	PL	91	value change	3.5 (3.2, 3.9)	3.6 (3.2, 3.9) 0.0 (-0.3, 0.4)	3.6 (3.3, 3.9) 0.0 (-0.3, 0.3)	3.5 (3.2, 3.8) -0.1 (-0.5, 0.3)	
<b>Renal Related Variables</b>								
Serum creatinine (mg/dL)	VITD	100	value change	0.89 (0.76, 0.99)	0.90 (0.79, 1.04) 0.03 (-0.03, 0.08) <sup>†</sup>	0.92 (0.81, 1.01) 0.02 (-0.03, 0.08) <sup>†</sup>	0.91 (0.80, 1.03) 0.03 (-0.02, 0.08) <sup>†</sup>	0.51
	PL	91	value change	0.86 (0.76, 0.96)	0.88 (0.76, 1.01) 0.02 (-0.03, 0.07) <sup>*</sup>	0.88 (0.76, 0.99) 0.02 (-0.04, 0.06)	0.89 (0.75, 1.00) 0.01 (-0.03, 0.07) <sup>*</sup>	
Estimated glomerular filtration rate (mL/min)	VITD	100	value change	125 (114, 140)	123 (109, 138) 0 (-12, 6.3)	122 (107, 134) 0 (-15, 2.4) <sup>^</sup>	123 (109, 137) -2 (-16, 3.7) <sup>*</sup>	0.43
	PL	91	value change	126 (113, 142)	125 (110, 140) 0 (-7, 7)	123 (117, 140) 0 (-10, 8)	122 (108, 142) 0 (-11, 6)	
Urine glucose (mg/dL)	VITD	100	value change	7.2 (5.1, 9.4)	7.4 (4.6, 10.0) -0.4 (-3.3, 3.1)	6.8 (4.9, 9.5) -0.1 (-3.4, 3.1)	6.9 (4.7, 8.7) -0.4 (-3.3, 3.2)	0.92
	PL	91	value change	7.4 (5.5, 11.3)	7.5 (5.7, 10.1) -0.2 (-3.6, 2.4)	7.5 (4.5, 9.8) -0.4 (-3.5, 2.5)	7.4 (5.2, 10.0) -0.4 (-3.2, 2.6)	
Urine retinol binding protein to creatinine ratio (mcg/g)	VITD	98	value change	101 (70, 142)	93 (71, 120) -4 (-39, 26)	95 (67, 134) -6 (-41, 26)	107 (67, 137) -1 (-38, 33)	0.40
	PL	88	value change	104 (76, 163)	99 (67, 155) 0 (-26, 25)	95 (60, 136) -4 (-36, 23)	90 (70, 156) -5 (-34, 18)	

Urine beta-2 microglobulin (mcg/L)	VITD	98	value change	111 (53, 389)	137 (53, 368) 17 (-103, 128)	143 (74, 340) 8 (-96, 149)	139 (62, 418) 5 (-95, 112)	0.58
	PL	89	value change	134 (52, 306)	156 (65, 339) -9 (-91, 104)	143 (63, 290) 8 (-129, 95)	163 (68, 312) 11 (-91, 157)	
Urine protein to creatinine ratio (mg/mg)	VITD	98	value change	0.07 (0.05, 0.08)	0.06 (0.05, 0.08) -0.00 (-0.02, 0.01)	0.06 (0.05, 0.08) -0.00 (-0.02, 0.01)*	0.06 (0.05, 0.07) -0.00 (-0.02, 0.01)*	0.16
	PL	90	value change	0.07 (0.05, 0.09)	0.06 (0.05, 0.09) -0.00 (-0.02, 0.01)	0.06 (0.05, 0.08) -0.01 (-0.02, 0.01)	0.07 (0.05, 0.08) -0.00 (-0.01, 0.01)	
Tubular reabsorption of phosphate (%)	VITD	98	value change	91.7 (89.2, 93.8)	91.2 (88.4, 93.8) -0.7 (-3.5, 2.0)	90.0 (87.2, 93.5) -0.6 (-4.4, 1.8)	90.1 (86.5, 93.8) -1.6 (-4.7, 1.8)*	0.08
	PL	87	value change	91.5 (88.8, 94.2)	91.8 (88.7, 94.7) 0.0 (-2.8, 3.4)	91.9 (87.8, 94.2) -0.9 (-3.0, 2.3)	91.4 (88.1, 93.4) 0.6 (-2.9, 3.1)	
Urine calcium to creatinine ratio (mg/mg)	VITD	98	value change	0.04 (0.02, 0.07)	0.05 (0.02, 0.08) 0.00 (-0.02, 0.04)	0.05 (0.03, 0.10) 0.01 (-0.01, 0.05)*	0.05 (0.03, 0.09) 0.00 (-0.01, 0.03)	0.97
	PL	87	value change	0.04 (0.02, 0.07)	0.05 (0.02, 0.09) 0.00 (-0.01, 0.04)*	0.05 (0.03, 0.08) 0.01 (-0.01, 0.02)*	0.05 (0.02, 0.08) 0.01 (-0.01, 0.04)^	

Abbreviations: PL, placebo randomized group; VITD, vitamin D3 randomized group.

Calculated variables: Serum free 1,25-OH(2)D was calculated from serum albumin, vitamin D binding protein (VDBP), and 1,25-OH(2)D[28]. Estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI equation[36] for participants age  $\geq 18$  years and by the bedside Schwartz formula[37] for those age  $< 18$  years. Urine calcium/creatinine (UCa/UCr; normal  $< 0.21$  mg/mg) ratio was used to estimate urinary calcium excretion. Tubular reabsorption of phosphate (TRP) was calculated as  $(1 - [(UPhos \times SCr)/(SPhos \times UCr)]) \times 100$ .

1. All data presented as median (Q1, Q3).
2. N = number with data available at both baseline and week 48.
3. P value: Wilcoxon rank sum test for change between baseline and week 48 for VITD3 versus placebo group
4. Value is the value of each variable at a given time point.
5. Change is the difference in the value between baseline and each time point. For BMD, change is presented as percent change. For all other variables, change is the absolute change.
6. \*  $0.01 < P \text{ value} < 0.05$ ; ^  $0.001 < P \text{ value} \leq 0.01$ ; †,  $P \leq 0.001$  for Wilcoxon signed rank test for difference from baseline to a given time point.