**Supplemental Digital Content 1.**

**Adjusted Difference in Bone Outcomes by 25(OH)D and PTH Status in PHIV and PHEU children**

|  |  | **PHIV1** | **PHEU**1 |
| --- | --- | --- | --- |
| **Outcome** | **Exposure** | **Adjusted** **difference****95%CI** | **P****value** | **Adjusted difference****(95%CI)** | **P****value** |
|  | **25(OH)D (ng/mL)** |  |  |  |  |
| **BMD z** |  |  |  |  |  |
|  Total body | < 20 vs. >20 | -0.38 (-0.60,-0.16) | <0.001 | -0.34 (-0.64,-0.03) | 0.03 |
|  Lumbar spine  | < 20 vs. >20 | -0.21 (-0.42,0.00) | 0.05 | 0.10 (-0.30,0.51) | 0.62 |
|  |  |  |  |  |  |
| **BMC (g)** |  |  |  |  |  |
|  Total body | < 20 vs. >20 | -59.1 (-108.3,-9.8) | 0.02 | -0.01 (-73.5,73.5) | 1.00 |
|  Total body less head | < 20 vs. >20 | -52.3 (-96.7,-7.8) | 0.02 | -0.30 (-69.3,68.7) | 0.99 |
|  Lumbar spine | < 20 vs. >20 | -0.89 (-2.2,0.47) | 0.20 | 0.57 (-1.2,2.4) | 0.54 |
|  |  |  |  |  |  |
|  | **PTH (pg/mL)**2 |  |  |  |  |
| **BMD z** |  |  |  |  |  |
|  Total body | >65 vs. <65 | -0.02 (-0.37,0.42) | 0.91 | - | - |
|  Lumbar spine  | >65 vs. <65 | -0.06 (-0.49,0.36) | 0.77 | - | - |
|  |  |  |  |  |  |
| **BMC (g)** |  |  |  |  |  |
|  Total body | >65 vs. <65 | 14.6 (-79.7,108.9) | 0.76 | - | - |
|  Total body less head | >65 vs. <65 | 16.9 (-62.9,96.7) | 0.68 | - | - |
|  Lumbar spine | >65 vs. <65 | -0.54 (-3.1,2.0) | 0.68 | - | - |
|  |  |  |  |  |  |
|  | **25(OH)D ng/mL****and PTH (pg/mL)** |  |  |  |  |
| **BMD** z |  |  |  |  |  |
|  Total body | 25D <20 and PTH >65 | -0.48 (-0.92,-0.03) | 0.03 | - | - |
|  | 25D <20 and PTH <65 | -0.28 (-0.51,-0.05) | 0.02 | - | - |
|  | 25D>20 and PTH >65 | 0.50 (-0.15,1.14) | 0.13 | - | - |
|  | 25D >20 and PTH <65 | Ref. |  | - | - |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  Lumbar spine | 25D <20 and PTH >65 | -0.42 (-0.96,0.12) | 0.12 | - | - |
|  | 25D <20 and PTH <65 | -0.12 (-0.33,0.10) | 0.29 | - | - |
|  | 25D >20 and PTH >65 | 0.38 (-0.27,1.02) | 0.25 | - | - |
|  | 25D >20 and PTH <65 | Ref. | . | - | - |
| **BMC (g)** |  |  |  |  |  |
|  Total body | 25D <20 and PTH >65 | -89.6 (-208.9,29.7) | 0.14 | - | - |
|  | 25D <20 and PTH <65 | -33.8 (-86.1,18.4) | 0.20 | - | - |
|  | 25D >20 and PTH >65 | 141.8 (17.2,266.4) | 0.03 | - | - |
|  | 25D >20 and PTH <65 | Ref. | . | - | - |
|  |  |  |  |  |  |
|  Total body less head | 25D <20 and PTH >65 | -69.3 (-169.6,31.0) | 0.18 | - | - |
|  | 25D <20 and PTH <65 | -32.4 (-79.8,15.0) | 0.18 | - | - |
|  | 25D >20 and PTH >65 | 116.5 (6.4,226.6) | 0.04 | - | - |
|  | 25D >20 and PTH <65 | Ref. | . | - | - |
|  |  |  |  |  |  |
|  Lumbar spine | 25D <20 and PTH >65 | -3.1 (-6.2,0.05) | 0.05 | - | - |
|  | 25D <20 and PTH <65 | -0.34 (-1.7,1.1) | 0.64 | - | - |
|  | 25D >20 and PTH >65 | 3.2 (-0.51,6.8) | 0.09 | - | - |
|  | 25D >20 and PTH <65 | Ref. |  | - | - |

**Abbreviations:** PHIV-Perinatally HIV-infected; PHEU- Perinatally HIV-exposed uninfected; 95%CI-95% confidence interval; 25(OH)D – 25 hydroxy-vitamin D; PTH-parathyroid hormone; Ref-reference group.

1 The number of children included in the model for PHIV and PHEU was as follows: total body z-scores N=388, N=198; spine z-scores N=382, N=196; total body BMC N=387, N= 197; spine BMC N=381, N=395.

2 No models were fit for high PTH among HEU children because only 5 had high PTH.

3 Adjusted models of bone outcomes included age at vitamin D measurement, black race, ancestral markers (African, Europe/CSW Asia, other), height z, extremity lean mass, vigorous activity > 75th percentile and CD4 cell count. When 25(OH)D status was the exposure, we additionally adjusted for percent body fat. When the outcome was TB-BMC, TBLH-BMC or SP-BMC, we additionally adjusted for Tanner stage and sex.

**Supplemental Digital Content 2.**

**Differences in Sociodemographic, Clinical and Laboratory Characteristics by 25(OH)D Status at Time of Blood Draw - PHIV and PHEU Children Combined**

|  | **25(OH)D level** |  |
| --- | --- | --- |
| **Characteristic**1 |  | **< 20 ng/mL(N=246)** | > **20 ng/mL(N=373)** | **P-Value** |
|  |  | **Median (Q1, Q3) or N(%)** |  |
| Age (yr) at 25(OH)D assessment |  | 13.1 (10.7, 14.8) | 11.9 (9.3, 13.8) | < 0.001 |
| Sex-M |  | 98 (40%) | 203 (54%) | < 0.001 |
| Hispanic |  | 40 (17%) | 130 (35%) | <0.001 |
| Black race |  | 198 (83%) | 229 (65%) | < 0.001 |
| Continental origin (%) |  |  |  |  |
|  Africa |  | 72.0 (52.4, 81.9) | 55.5 (8.0, 78.4) | < 0.001 |
|  Europe/CSK Asia |  | 13.9 (6.3, 30.8) | 27.2 (9.0, 62.9) | < 0.001 |
|  Americas |  | 1.9 (1.3, 3.4) | 2.1 (1.3, 4.7) | 0.09 |
|  East Asia |  | 3.1 (2.1, 4.7) | 3.2 (2.1, 5.1) | 0.52 |
|  Oceania |  | 3.3 (2.2, 5.6) | 3.5 (2.0, 6.1) | 0.97 |
| Birthplace of child | USA Mainland | 235 (96%) | 306 (82%) | < 0.001 |
|  | Puerto Rico | 2 (1%) | 49 (13%) |  |
|  | Other | 5 (2%) | 11 (3%) |  |
|  | Africa | 4 (2%) | 5 (1%) |  |
| Season at time of blood draw | Spring | 75 (30%) | 89 (24%) | 0.004 |
|  | Summer | 53 (22%) | 129 (35%) |  |
|  | Fall | 48 (20%) | 73 (20%) |  |
|  | Winter | 70 (28%) | 82 (22%) |  |
| Northern vs southern latitude2 | Northern | 175 (71%) | 178 (48%) | <0.001 |
| Latitude (degrees) |  | 40.2 (35.1, 41.9) | 35.1 (25.8, 40.7) | < 0.001 |
| Vigorous activity (min/day) |  | 7.5 (0.0, 30.0) | 8.4 (0.0, 32.1) | 0.15 |
| Vigorous activity >75th %ile3 |  | 46 (22%) | 86 (27%) | 0.22 |
| Vitamin D intake (IU/day) |  | 137.9 (78.5, 266.6) | 160.1 (95.4, 306.3) | 0.10 |
| Vitamin D intake < 600 IU/day |  | 230 (97%) | 326 (94%) | 0.14 |
| Calcium intake (mg/day) |  | 713.4 (476.3, 1,009.4) | 710.0 (482.9, 1,027.4) | 0.84 |
| Calcium intake < 1300 mg/day4 |  | 208 (87%) | 291 (84%) | 0.24 |
| Phosphorus intake (mg/day) |  | 1,008.2 (696.4, 1,427.7) | 983.6 (730.7, 1,421.5) | 0.91 |
| Weight z-score |  | 0.48 (-0.47, 1.36) | 0.25 (-0.65, 1.27) | 0.22 |
| Height z-score |  | -0.01 (-0.87, 0.82) | -0.17 (-1.03, 0.47) | 0.09 |
| BMI z-score |  | 0.56 (-0.28, 1.46) | 0.40 (-0.39, 1.33) | 0.42 |
| Percentage of body fat (%)5 |  | 25.3 (17.9, 33.2) | 22.3 (15.1, 33.1) | 0.06 |
| Tanner stage | 1 | 43 (18%) | 108 (29%) | < 0.001 |
|  | 2 | 43 (18%) | 92 (25%) |  |
|  | 3 | 49 (20%) | 56 (15%) |  |
|  | 4 | 54 (22%) | 57 (15%) |  |
|  | 5 | 54 (22%) | 59 (16%) |  |
| 25(OH)D (ng/mL) |  | 15.5 (13.1, 17.7) | 26.3 (23.2, 30.8) | < 0.001 |
| PTH (pg/mL) |  | 33.7 (23.8, 48.6) | 27.2 (20.3, 38.1) | < 0.001 |
| PTH >65 pg/ml |  | 25 (10%) | 18 (5%) | 0.01 |
| Calcium (mg/dl) |  | 9.5 (9.3, 9.8) | 9.7 (9.4, 9.9) | < 0.001 |
| Phosphate (mg/dl) |  | 4.6 (4.1, 5.1) | 4.7 (4.3, 5.1) | 0.02 |
| Creatinine (mg/dL) |  | 0.56 (0.49, 0.65) | 0.54 (0.46, 0.64) | 0.05 |
| **Among PHIV** |  |  |  |  |
| Efavirenz – current use6 |  | 38 (23%) | 33 (15%) | 0.047 |
| TDF use – current use6 |  | 34 (21%) | 54 (25%) | 0.35 |
| CD4 T cell count (cells/mm3) |  | 661 (462, 852) | 728 (540, 974) | 0.008 |
| HIV RNA (log10 copies/mL) |  | 2.60 (1.70, 3.28) | 1.98 (1.70, 2.99) | 0.11 |

Abbreviations:PHIV-perinatally HIV-infected; PHEU-perinatally HIV-exposed uninfected. PTH-parathyroid hormone; TDF-tenofovir; EFV-efavirenz.

1 Missing data for 25(OH)D <20 ng/mL and >20 ng/mL: Hispanic (N=4, N=0); Black race (N=7, N=20); Birthplace (N=0,N=2); vigorous activity (N=39, N=54); ); dietary intake (N=8, N=26); percent body fat (N=14, N=15), Tanner stage (N=3, N=1); PTH (N=0, N= 1); calcium (N=4, N=1), phosphate/creatinine (N=1, N=0); EFV/TDF (N=4, N=6); CD4 count (N=0, N=2).

2 Northern latitude: > 39o degrees latitude

3 >75th %ile: Greater than the 75th percentile of the distribution of minutes of physical activity among the children in this study.

4 The RDA for calcium is 1100 mg/day for children 4-8 years old and 1,300 mg/day for children 9-18 years old.

5 No percent body fat measure becausethere was no total body DXA scan performed within 365 days of 25(OH)D (N=14, N=15).

6 Twelve PHIV children are not included in these numbers. For 8 children the last information on ARV use was just prior to the 25(OH)D date, for 2 children ARV was started for the first time after the 25(OH)D date, and for 2 children ARVs were never used. Of the 8 children with previous ARV use just prior to the 25(OH)D date, 1 had been on TDF, 2 on EFV, 1 on TDF and EFV, and 4 on neither TDF or EFV.

**Supplemental Digital Content 3.**

**Differences in Sociodemographic, Clinical and Laboratory Characteristics by PTH Status - PHIV and PHEU Children Combined**

|  | **PTH level** |  |
| --- | --- | --- |
| **Characteristic**1 |  | **< 65 pg/mL** **(N=575)** | **> 65 pg/mL** **(N=43)** | **P-Value** |
|  |  | **Median (Q1, Q3) or N (%)** |  |
| Age (yr) |  | 12.2 (9.7, 14.2) | 13.4 (12.1, 14.4) | 0.01 |
| Sex-M |  | 284 (49%) | 16 (37%) | 0.12 |
| Hispanic |  | 160 (28%) | 10 (23%) | 0.50 |
| Black race |  | 391 (71%) | 35 (83%) | 0.09 |
| Continental Origin (%)2 |  |  |  |  |
|  Africa |  | 68.0 (12.3, 80.0) | 73.8 (39.5, 84.7) | 0.05 |
|  Europe/CSK Asia |  | 19.1 (7.9, 55.6) | 14.6 (6.2, 39.1) | 0.13 |
|  Americas |  | 2.0 (1.3, 4.1) | 1.8 (1.1, 3.7) | 0.20 |
|  East Asia |  | 3.2 (2.1, 5.1) | 2.8 (2.2, 4.6) | 0.36 |
|  Oceania |  | 3.4 (2.1, 5.9) | 3.4 (1.8, 7.3) | 0.75 |
| Birthplace of child | USA Mainland | 504 (88%) | 36 (84%) | 0.81 |
|  | Puerto Rico | 46 (8%) | 5 (12%) |  |
|  | Other | 15 (3%) | 1 (2%) |  |
|  | Africa | 8 (1%) | 1 (2%) |  |
| Season of blood draw | Spring | 151 (26%) | 13 (30%) | 0.80 |
|  | Summer | 168 (29%) | 13 (30%) |  |
|  | Fall | 115 (20%) | 6 (14%) |  |
|  | Winter | 141 (25%) | 11 (26%) |  |
| Northern latitude3 | Northern | 322 (56%) | 30 (70%) | 0.08 |
| Latitude (degrees) |  | 39.7 (29.7, 40.8) | 40.2 (35.1, 41.9) | 0.14 |
| Vigorous activity (min/day) |  | 8.4 (0.0, 31.8) | 4.2 (0.0, 30.0) | 0.29 |
| Vigorous activity >75th pctle4 |  | 124 (25%) | 7 (21%) | 0.54 |
| Vitamin D (IU/day) |  | 151 (90, 297) | 145 (67, 296) | 0.67 |
| Vitamin D intake < 600 IU/day |  | 515 (95%) | 40 (98%) | 0.44 |
| Calcium (mg/day) |  | 710 (485, 1,015) | 737 (419, 1,098) | 0.57 |
| Calcium intake < 1300 mg/day5 |  | 463 (85%) | 35 (85%) | 0.99 |
| Dietary phosphorus (mg) |  | 989 (722, 1,412) | 948 (590, 1,475) | 0.81 |
| Weight z-score |  | 0.33 (-0.62, 1.30) | 0.46 (-0.82, 1.12) | 0.71 |
| Height z-score |  | -0.10 (-0.97, 0.58) | -0.02 (-1.03, 0.45) | 1.00 |
| BMI z-score |  | 0.45 (-0.36, 1.39) | 0.28 (-0.33, 1.34) | 0.58 |
| Percentage of body fat (%)6 |  | 23.7 (16.3, 33.1) | 23.0 (15.5, 32.1) | 0.87 |
| Tanner stage7 | 1 | 147 (26%) | 3 (7%) | 0.02 |
|  | 2 | 126 (22%) | 9 (21%) |  |
|  | 3 | 93 (16%) | 12 (28%) |  |
|  | 4 | 99 (17%) | 12 (28%) |  |
|  | 5 | 106 (19%) | 7 (16%) |  |
| **Laboratory** |  |  |  |  |
| Vitamin D (ng/mL) |  | 22.6 (17.1, 27.9) | 17.8 (12.4, 22.7) | < 0.001 |
| Vitamin D < 20 ng/ml |  | 221 (38%) | 25 (58%) | 0.01 |
| PTH (pg/mL) |  | 27.9 (21.0, 39.3) | 74.6 (68.4, 90.8) | < 0.001 |
| Calcium (mg/dl) |  | 9.7 (9.4, 9.9) | 9.3 (9.0, 9.7) | < 0.001 |
| Phosphorus (mg/dl) |  | 4.7 (4.2, 5.1) | 4.7 (4.3, 5.1) | 0.82 |
| Creatinine (mg/dL) |  | 0.55 (0.47, 0.64) | 0.59 (0.49, 0.69) | 0.03 |
| **Among PHIV** |  |  |  |  |
| Efavirenz – current use8 |  | 69 (20%) | 2 (5%) | 0.03 |
| Tenofovir – current use8 |  | 73 (21%) | 15 (41%) | 0.007 |
| CD4 T cell count (cells/mm3) |  | 699 (50, 910) | 672 (523, 985) | 0.79 |
| HIV RNA (log10 copies/mL) |  | 2.1 (1.7, 3.0) | 2.6 (1.7, 3.4) | 0.06 |

**Abbreviations:** PHIV-perinatally HIV-infected; PHEU-perinatally HIV-exposed uninfected.

1 Missing data for PTH <65 pg/mL and PTH > 65 pg/mL: Hispanic (N=4, N=0); Black race (N=26, N=1); Birthplace (N=2.N=0); vigorous activity (N=84, N=9); ); dietary intake (N=32, N=2); stage (N=4, N=0); calcium (N=4, N=1), phosphate/creatinine (N=1, N=0); CD4 count (N=2); HIV viral load (N=0).

2Continental origin (%) - The percent for each region is the percent present within an individual for that region. When looking by HIV status, it is the median of those individual percents for that region.

**3** Northern: > 39o degree latitude

4 >75th %ile: Greater than the 75th percentile of the distribution of minutes of physical activity among the children in this study.

5 The RDA for calcium is 1100 for children 4-8 years old and 1,300 for children 9-18 years old.

6 No percent body fat measured on N=28 PHIV, N=1) becausethere was no DXA scan within 365 days of the 25(OH)D.

7 If Tanner stage was missing at the time of 25(OH)D assessment, we carried forward the Tanner stage assessment from the previous annual or semi-annual visit. Except for 4 children on which there was no previous Tanner stage assessment in AMP.

8 There were 7 PHIV children on whom the last information of ARV use was just prior to the 25(OH)D date, 2 who started ARV after the 25(OH)D date and 2 had never used ARVs. Of the 7 with previous information, 4 had been using EFV and 5 had been using TDF.

**Supplemental Digital Content 4.**

**Figure 1. Relationships between calcium and PTH levels by HIV status and TDF use.** This figure shows the relationships between calcium and PTH levels in PHIV children receiving TDF (PHIV TDF+), PHIV not receiving TDF (PHIV TDF-) and in PHEU children (HEU). Serum calcium and PTH were negatively associated in PHIV not receiving TDF (slope -10.5, P=0.001) and PHEU (slope -7.6, P=0.002) but not associated among PHIV receiving TDF (slope -3.8, P=0.56) (See Figure, Supplemental Digital Content 4).

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**Supplemental Digital Content 5.**

**Repository Protocol for Blood Specimens**

The blood was spun 400xg for 10 mins. The plasma was removed and the plasma was re-spun at 800xg for 10 minutes. The sample was processed into 0.5mL aliquots and stored locally at –70°C.  On regular intervals, the specimens were then shipped to a central repository on dry ice and stored there at –70°C until they were requested for testing.  The specimens were shipped on dry ice to the testing lab.