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Associations of Low Vitamin D and Elevated Parathyroid Hormone Concentrations with Bone Mineral Density in Perinatally HIV-Infected Children

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Abstract

Background—Perinatally HIV-infected (PHIV) children have, on average, lower bone mineral density (BMD) than perinatally HIV-exposed uninfected (PHEU) and healthy children. Low 25-hydroxy vitamin D [25(OH)D] and elevated parathyroid hormone (PTH) concentrations may lead to suboptimal bone accrual.

Methods—PHIV and PHEU children in the Pediatric HIV/AIDS Cohort Study had total body (TB) and lumbar spine (LS) BMD and bone mineral content (BMC) measured by dual-energy x-ray absorptiometry; BMD z-scores (BMDz) were calculated for age-sex. Low 25(OH)D was defined as ≤20 ng/mL and high PTH as >65 pg/mL. We fit linear regression models to estimate average adjusted differences in BMD/BMC by 25(OH)D and PTH status, and log-binomial models to determine adjusted prevalence ratios (aPR) of low 25(OH)D and high PTH in PHIV relative to PHEU children.

Results—PHIV children (N=412) were older (13.0 vs.10.8 yr) and more often black (76% vs. 64%) than PHEU (N=207). Among PHIV, children with low 25(OH)D had lower TB-BMDz (−0.38 SD; 95%CI: −0.60 to −0.16) and TB-BMC (−59.1 g; 95%CI: −108.3 to −9.8); high PTH accompanied by low 25(OH)D was associated with lower TB-BMDz. Among PHEU, children with low 25(OH)D had lower TB-BMDz (−0.34 SD; 95%CI: −0.64 to −0.03). Prevalence of low 25(OH)D was similar by HIV status (aPR1.00; 95%CI: 0.81 to 1.24). High PTH was 3.17 (95%CI: 1.25 to 8.06) times more likely in PHIV children.

Conclusion—PHIV and PHEU children with low 25(OH)D may have lower BMD. Vitamin D supplementation trials during critical periods of bone accrual are needed.

Keywords

25-hydroxy-vitamin D; parathyroid hormone; HIV infection; children; bone mineral density

Introduction

Adequate attainment of peak bone mass by young adulthood decreases the risk of osteoporosis and fragility fractures later in life[1] and can be optimized with adequate intakes of calcium, phosphorus and vitamin D; sunlight exposure; and regular physical activity.[1] Bone mineralization may be adversely affected by delays in growth and puberty, nutrient malabsorption, and chronic infections and inflammation, including human immunodeficiency virus infection (HIV). Children with perinatally acquired HIV (PHIV) have lower bone mineral density (BMD) compared to healthy children[2] and perinatally HIV-exposed uninfected (PHEU) children.[3] There remains a paucity of information regarding factors underlying the observed low BMD in PHIV children.

It is likely that low vitamin D status, as reflected by low serum levels of 25-hydroxy vitamin D [25(OH)D], and suggested by elevated parathyroid hormone (PTH) levels, contribute to

suboptimal bone accumulation in PHIV children. Studies in HIV-infected adults report low 25(OH)D in 13–77% of participants[4,5] and associations of low 25(OH)D with bone loss.[6] Among the few studies conducted in HIV-infected children and adolescents, the prevalence of low 25(OH)D ranged from 21% to 39% in PHIV adolescents[7–11] and ≥50% in adolescents behaviorally-infected by HIV.[12,13] In PHIV adolescents, lumbar spine BMD was lower in those with both low 25(OH)D and high PTH.[11] In HIV-infected adults, 25(OH)D levels were lower among those initiating antiretroviral therapy (ART) with efavirenz (EFV) compared to without EFV[14] and baseline PTH levels were higher in HIV-infected adolescents receiving ART with tenofovir (TDF) compared to without.[12]

Few studies have evaluated the relationship of low 25(OH)D and high PTH on bone outcomes in large cohorts of PHIV and PHEU children.[11] The primary objectives of this cross-sectional study were to: a) evaluate associations of low 25(OH)D and high PTH concentrations with total body and spine BMD and bone mineral content (BMC); and b) assess distributions of 25(OH)D and PTH by HIV status and EFV and TDF exposure.

Methods

The Adolescent Master Protocol (AMP) of the NIH-supported Pediatric HIV/AIDS Cohort Study (PHACS) evaluates outcomes of HIV infection and ART among PHIV compared to PHEU pre-adolescents and adolescents across 15 clinical sites. The protocol was approved by the Institutional Review Boards (IRBs) at each site and the Harvard T.H. Chan School of Public Health. Informed consent from the parent(s) or guardian(s), and assent from participants were obtained per local IRB guidelines.

Data Collection

Socio-demographic and clinical history—Clinical and laboratory data, including sociodemographics and Tanner staging, were obtained as previously described.[15] Tanner stage was ascertained by inspection of breasts and pubic hair for females and of genitalia and pubic hair for males at semi-annual visits until Tanner stage 5 was reached. For boys and girls, the more advanced stage of the two respective pubertal components was used for classification if there was discordance between the examined body sites (e.g., breast vs. pubic hair in females). Weight, height and BMI were expressed as z-scores.[16] Season at the time of blood draw was categorized as winter, spring, summer, or fall based on solstice dates. Latitude was considered both as a categorical [sites classified as northern (≥39°) or southern (< 39°)] and a continuous variable.

Continental ancestry—Since circulating levels of both 25(OH)D and BMD vary by race, we included genetic ancestry informative markers to adjust for residual confounding in addition to self-reported race and ethnicity. A panel of 41 single nucleotide polymorphisms (SNP) ancestry informative markers was used to determine continental ancestry which was estimated by comparing each child's genotype to allele frequencies in a reference set of 3517 individuals.[17] Reference populations were originally grouped into the seven world-regions: Europe, Africa, America, Central/South Asia, South/West Asia, East Asia, and Oceania. We determined the percentage of each region present within an individual which totals 100%.[18,19] Due to the small number of Asians in the study, we combined Europe,

Central/South Asia, and South/West Asia (Europe/CSW Asia) by adding the regional ancestry percentage.

Bone outcomes—Total body (TB)-BMD including head, and lumbar spine BMD (LS-BMD), TB bone mineral content including head (TB-BMC) and less head (TBLH-BMC), lumbar spine BMC (LS-BMC), percent body fat (%), and extremity lean mass (g) were measured on a Lunar or Hologic dual energy x-ray absorptiometry (DXA) scanner (General Electric Healthcare, UK or Hologic Inc., Bedford, MA) as previously described.[3] A phantom was circulated to each clinical site to standardize results. Scans were sent to the Body Composition Analysis Center at Tufts University School of Medicine for central analysis and standardization. Hologic scans were analyzed using Hologic QDR version 12.3 and APEX version 3.3. Lunar scans were analyzed using Prodigy Advance enCORE 2005 version 9 and enCORE 2011 version 13.6. All scans were analyzed by a single technician blinded to the participants' HIV status. Bone age was assessed using x-rays of the left hand and wrist by a radiologist at each clinical site blinded to HIV status, and TB and LSBMD z-scores for age and sex (BMDz) were calculated using normative BMD data.[3,20] For children between Tanner stages 1–4 with a chronologic age (CA) that differed by more than 1 SD from the bone age (BA), the BA was used instead of the CA in the TB-BMD and LS-BMD calculations.[3] For children at Tanner stage 5, CA was used. Children between Tanner stages1–4 were excluded from analyses of BMDz if their bone age was missing.

Laboratory measures—For this cross-sectional analysis, we selected a repository blood specimen (see Table, Supplemental Digital Content 5) drawn within 1 year of a DXA scan (generally the first DXA scan). If no specimen was available within 1 year of the DXA or no DXA was completed, we selected the earliest available repository specimen. Measurements of laboratory variables were performed in batch at the USDA-ARS WHNRC, (Davis, California).25(OH)D was measured by enzyme-linked immunosorbent assay (ELISA, Immunodiagnostic System, (ids) Inc. Gaithersburg, MD). Serum intact PTH was measured using a solid phase, two-site chemiluminescent enzyme-labeled immunometric assay (Intact-PTH, Siemens Medical Solutions Diagnostics, Tarrytown, NY). Calcium, phosphate, and creatinine were determined using a clinical chemistry analyzer (Cobas Integra 400 Plus [04469658]: Roche Diagnostics Corp., Indianapolis, IN). Samples were assayed in duplicate and the mean of each pair was calculated. Low 25(OH)D status was defined as ≤20.0 ng/mL and deficient 25(OH)D as <12 ng/mL.[21] High PTH was defined as >65pg/mL. (http://labmed.ucsf.edu/labmanual/db/resource/Immulite_2000_Intact_PTH.pdf.)

Database records from children with high PTH and /or high phosphate levels (>5.4 mg/dL) were reviewed for evidence of concurrent clinical diseases (pancreatitis, chronic kidney disease, inflammatory bowel disease, neonatal renal failure, and nephrolithiasis).

Dietary recall and physical activity—The Block Dietary Questionnaire and Physical Activity Screener for Children and Adolescents were administered to assess dietary intake including supplement use and physical activity, respectively, over the past week (Block Dietary Data Systems, Nutriquest, Berkeley, CA). The recommended daily dietary allowance (RDA) for calcium was ≥1100 mg for ages 4–8 years and ≥1300 mg for ages 9–18 years, and vitamin D was ≥600 IU/day for ages 1–18 years of age.[22] Minutes of vigorous

physical activity were categorized into >75th percentile (> 25.2 minutes/day) or ≤75th percentile (≤25.2 minutes/day) based on our data distribution.

Statistical Methods—The distribution of socioeconomic and clinical covariates by HIV, 25(OH)D, and PTH status was compared using Wilcoxon test/Kruskal Wallis for continuous and Chi-square for categorical variables. A variable was constructed for combinations of low 25(OH)D and high PTH.

Linear regression models were fit with the robust variance to evaluate differences in bone outcomes by 25(OH)D and PTH status in PHEU and PHIV separately. Effect modification of 25(OH)D by HIV status was tested in models that included both PHIV and PHEU children. Potential confounders were chosen *a priori* based on literature review and expert knowledge. Adjusted models of bone outcomes included age at vitamin D measure, black race, continental ancestry (African, Europe/CSW Asia, and other), height z-score, extremity lean mass, and vigorous activity >75th percentile. We additionally adjusted for percent body fat when 25(OH)D status was the exposure and for Tanner stage and sex when the outcome was TB-BMC, TBLH-BMC, or LS-BMC. We did not include season and latitude as potential confounders because we did not hypothesize an effect on bone other than through 25(OH)D. Missing indicators were included for those missing information about race and vigorous activity.

Log-binomial regression models were fit to obtain the unadjusted (uPR) and adjusted prevalence ratio (aPR) of low 25(OH)D and high PTH in PHIV compared to PHEU, and for current TDF and EFV use compared to PHEU children. When the log-binomial model did not converge, a Poisson model was fit.[23] To estimate average differences in 25(OH)D and PTH levels by the above groups, we fit linear regression models with a robust variance estimator. All models were adjusted for black race and continental origin. 25(OH)D models were additionally adjusted for season and latitude. PTH levels may be higher in TDF users possibly due to phosphate wasting. Thus, as a secondary aim, we fit linear regression models to determine the relationship of PTH with calcium levels by HIV and TDF status. PHIV children without ARV information or not using ARVs at the 25(OH)D assessment were excluded from analyses of EFV or TDF. Analyses were performed in SAS 9.4 (SAS Institute, Cary, NC).

Results

Characteristics

Of the 448 PHIV and 226 PHEU children with an entry visit in AMP, 25(OH)D was measured in 426 and 219, respectively. Continental ancestry was available as well as 25(OH)D on 412 PHIV and 207 PHEU. In the final dataset, 394 of 412 PHIV and 199 of 207 PHEU had a DXA scan within one year of the 25(OH)D measure, with a median (interquartile range, IQR) of 0 days (1, 39).

The distribution and unadjusted comparison of socioeconomics, lifestyle characteristics, anthropometrics, and laboratory values between PHIV and PHEU children are shown in Table 1. The prevalence of low 25(OH)D was 40% overall and higher in PHIV compared to

PHEU (42% vs. 34%). Average PTH concentrations were higher in PHIV and 9% of PHIV and 2% of PHEU had high PTH. Among PHIV with high PTH and without low 25(OH)D, the range of 25(OH)D levels was 21.1to 25.5 ng/mL in 10children, 29.4 to 32.6 in 4children, and was52.4 ng/mL in one child.

Diagnoses among children with high PTH and/or phosphate levels

Among the 50 children with high PTH and/or high phosphate, only one child had a relevant clinical diagnosis other than HIV, i.e., chronic kidney disease. That child was included in all analyses. None of the PHEU children had a relevant diagnosis.

Association of 25(OH)D and PTH with bone

Among PHIV children (Table 2), those with low 25(OH)D compared to those without had adjusted TB-BMD z-scores that were on average 0.38 SD lower (95% confidence interval (95%CI): −0.60 to −0.16) and TB-BMC levels that were59.1 g lower (95%CI: −108.3 to −9.8). In an unadjusted analysis, this represents a 5.9% lower TB-BMC. Similar results were observed for TBLH-BMC. Among PHEU children, adjusted TB-BMD z-scores were on average 0.34 SD lower (95%CI−0.64, −0.03)in children with low 25(OH)D, but there were no differences for TB-BMC and TBLH-BMC. In models including PHIV and PHEU children, there was no strong indication of effect modification of HIV status by low 25(OH) for any of the bone outcomes (P>0.29), but power may be limited.

The average adjusted difference in LS-BMD z-scores between those with versus without low 25(OH)D was −0.21 SD (95%CI −0.42, 0.0) for PHIV and 0.10 SD (95%CI −0.30, 0.51) for PHEU. There were no differences in LS-BMC by 25(OH)D status in PHIV or PHEU children.

There was no apparent difference in any bone outcome by PTH status among PHIV children (Table 2). The average difference in TB-BMD z-scores was −0.02 (95%CI: −0.37, 0.42) between those with high versus normal PTH. However, TB-BMD z-scores were on average 0.48 SD lower (95%CI: −0.92 to −0.03) in children with low 25(OH)D and high PTH and 0.28 SD units lower (95%CI: −0.51 to−0.05) in those with low 25(OH)D and normal PTH, compared to the reference group (25(OH)D >20 ng/mL and PTH ≤65 pg/mL) (See Table, Supplemental Digital Content 1). LS-BMC was on average 3.1 g lower (95%CI: −6.2 to 0.05) in PHIV with low 25(OH)D and high PTH compared to the reference.

Differences in characteristics by 25(OH)D and PTH status in PHIV and PHEU children

Characteristics associated with low 25(OH)D for PHIV and PHEU children combined included older age, female sex, black race, African ancestry, blood drawn in winter or spring, northern latitude, Tanner stage >1, higher percent body fat, lower calcium and phosphate, and higher PTH and creatinine (Table 3) (See Table, Supplemental Digital Content 2). Among the PHIV, children with low 25(OH)D had lower CD4 counts and were more likely to receive EFV.

Factors associated with high PTH were older age, African ancestry, Tanner stage >1, lower 25(OH)D and calcium, and higher creatinine. Among PHIV children, those with high PTH

had a lower frequency of EFV use, a higher frequency of TDF use, and higher HIV viral load. (Supplemental Digital Content 3). (48 of 401 received both TDF and EFV).

Prevalence of low 25(OH)D in PHIV compared to PHEU children

The adjusted prevalence ratio (95%CI) of low 25(OH)D was 1.00 (0.81 to 1.24) in PHIV relative to PHEU (Table 4, Model 1B), 1.30 (0.98 to 1.74) in PHIV receiving EFV compared to PHEU (Model 2B), and 0.95 (0.76 to 1.18) in PHIV not receiving EFV compared to PHEU children (Table 4, Model 2B). The opposite trend was observed for PHIV receiving TDF compared to PHEU (aPR= 0.77 (0.56 to 1.06, Model 3B). Differences in mean 25(OH)D levels by HIV, and by EFV and TDF use, suggest similar results (Table 4, Models 7B–9B).

Prevalence of high PTH in PHIV compared to PHEU children

PHIV had a 3.17 (1.25 to 8.06) times greater adjusted prevalence of high PTH than PHEU children (Table 4 Model 4B). The average difference between groups was 5.26pg/mL (−2.35 to 8.17). PHIV who were not receiving EFV (Table 4, Model 5B) had a 3.67 times higher prevalence of high PTH than PHEU (1.44 to 9.36). The prevalence of high PTH (Table 4, Model 6B) was 5.50 (1.95 to 15.49) times higher in PHIV receiving TDF compared to PHEU, and 2.64 times higher in PHIV not receiving TDF (1.01 to 6.94) than in PHEU children. Compared to PHEU, PTH concentrations were on average 5.54 pg/mL higher in PHIV not receiving EFV (Table 4, Model 11B) and 11.65 pg/mL higher in those receiving TDF (Table 4, Model 12B), compared to PHEU.

Relationship between calcium and PTH by HIV status and TDF use

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).

Discussion

In this multi-center cohort of PHIV and PHEU children in the US, 40% had vitamin D deficiency overall and the prevalence did not differ by HIV status after careful adjustment for confounding. In both PHIV and PHEU children, low 25(OH)D was associated with lower TB-BMD, but not with LS-BMD in either cohort. PTH levels were highest in PHIV children receiving TDF. While PTH levels were, on average, higher in PHIV children with low 25(OH)D, high PTH was only associated with lower TB-BMD when accompanied by low 25(OH)D. The relationship between calcium and PTH was weak in PHIV children receiving TDF.

Several randomized clinical trials (RCTs) have been done examining effects of vitamin D supplementation in healthy children and adolescents.[24] A meta-analysis of six such RCTs found that children with normal baseline 25(OH)D concentrations (>18 ng/mL) did not have benefits in BMD accrual from supplementation, but that there was a significant positive effect of supplementation on total body and a borderline significant effect on spine BMD among those with low levels of 25(OH)D at baseline.[25] Four cross-sectional studies in

normal children of the relationship of 25(OH)D as a continuous variable with BMD all found positive associations with whole body BMD[26–29], two with lumbar spine[26,29], one with total hip[29], and one with the forearm.[28] No associations were found in five other cross-sectional studies.[30–34] We previously found that TB-BMD was greater in PHIV children using vitamin supplements.[35] In a study of vitamin D and calcium supplementation over 2 years in PHIV children, 25(OH)D levels increased, but this did not significantly increase total body or spine BMC or BMD measures over time.[7] In the aforementioned study, those who advanced through puberty during the study period had a greater increase in TB-BMC and LS-BMC in the supplemented group, but this result did not achieve significance. Thus, our finding that low 25(OH)D was associated with decreased total body BMD and BMC is supported by some, but not all studies. Since the effect sizes on BMD from vitamin D supplementation appear to be modest, and many prior studies have used low doses of vitamin D supplementation (as little as 133 IU/day) and included children who at baseline were vitamin D-replete, further adequately powered studies examining effects of vitamin D supplementation to target adequate 25(OH)D serum concentrations across puberty, particularly in children with PHIV with baseline vitamin D deficiency or insufficiency, are needed.

It is unclear why we found no associations with LS-BMD/BMC in either group or with TB-BMC in PHEU children. In contrast to these studies, we evaluated the effect of categorically low 25(OH)D compared to 25(OH)D as a continuous variable. This is based on clinical evidence that the relationship between 25(OH)D and BMD may not be linear and that those at the lowest levels of 25(OH)D may benefit most from supplementation.[21] The lack of association between high PTH and bone outcomes in our study is possibly a result of competing reasons for higher PTH values, including low 25(OH)D, additional requirements for calcium and phosphate during rapid bone accrual, and effects of TDF on PTH.[12] This is supported by our finding of lower TB-BMD when high PTH was accompanied by low 25(OH)D.

Vitamin D deficiency is common in the US.[36–38] The 40% prevalence of low 25(OH)D in our cohort was similar to those of cohorts of healthy children in the northeastern US[37] and PHIV children in New York City.[7] Our prevalence of vitamin D deficiency was higher than the 24% observed in 6–18 year-old children in the NHANES representative US sample of this age group,[38] but lower than the ≥50% reported in behaviorally HIV-infected adolescents.[12,13] Differences across studies are likely attributable to age, race, prevalence of obesity, latitude, sun exposure, season of sampling, and dietary intake.

While low 25(OH)D was equally prevalent in our PHIV and PHEU children overall after adjustment for known confounders[37,39] low 25(OH)D concentrations may be more common in PHIV receiving EFV than in PHEU, although our power to detect a significant difference between groups may be too low. This is consistent with adult studies,[14] and may be explained by *in vitro* evidence of EFV induction of the P450 enzyme CYP24,[40] which converts 25(OH)D to its inactive form, calcitroic acid. PHIV children had higher vitamin D intake than did PHEU, but HIV providers may check 25(OH)D levels in their PHIV patients and recommend supplementation.

Calcium is important to optimize bones and tooth mineralization, and for catalytic and mechanical functions throughout the body. The body tightly regulates ionized calcium levels. When the calcium supply is insufficient, PTH concentrations increase. PTH increases serum calcium through a variety of mechanisms. PTH mobilizes calcium adsorbed to the bone surface by breaking down bone mineral and matrix and it stimulates conversion of 25(OH)D to 1,25-dihydroxyvitamin D (1,25(OH)(2)D) which increases gut calcium absorption and renal calcium reabsorption. During the rapid linear growth and bone accrual of puberty, there is increased demand for calcium and phosphate, and PTH levels increase.[4142] PTH measurements also provide an assessment of the level of "stress" on the system as a result of low 25(OH)D concentrations, although the degree of serum PTH suppression may not determine optimal vitamin D status in children.[43]

TDF use has been previously associated with increases in serum concentrations of PTH,[12,44] with multiple mechanisms implicated. TDF appears to have effects on both bone and hormones [such as fibroblast growth factor-23 and 1,25(OH)2D] that may indirectly increase PTH.[45–47] TDF use can also result in renal tubular dysfunction stimulating production of PTH and in renal phosphate-wasting[44] which may, in part, be PTH-mediated.[12] In HIV-infected adolescents, baseline PTH levels were higher in TDF users regardless of 25(OH)D status. With vitamin D supplementation, PTH levels decreased in the TDF group, but did not change in those not receiving TDF.[12] In our cohort, PTH levels were higher in those with low 25(OH)D; highest at Tanner stages 3–4, the time of most rapid bone accrual; and higher among TDF users. PTH concentrations were not strongly associated with serum calcium among TDF users, suggesting other mechanisms for elevated PTH.

Our study has several limitations. While 25(OH)D is a stable vitamin D metabolite with a biological half-life of 2–3 weeks, levels vary by season; thus, one measurement may not represent the average yearly level.[48] However, children with 25(OH)D deficiency at one time of the year tend to be deficient at other times during the year[49] which favors using the ≤20 ng/mL cut-off recommended by the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets.[21] While imperfect, we adjusted for season when evaluating prevalence or differences in 25(OH)D by subgroups. We recognize that the 20 ng/mL cutoff is based upon what is needed to prevent rickets and osteomalacia,[21] but concentrations required for optimal bone and immunological health are likely higher.[50] Using this lower threshold, we could examine characteristics of those most likely to have clinically significant deficiency. Since this was a cross-sectional study where 25(OH)D, PTH, and DXA parameters were measured within one year of each other, we could not establish a temporal relationship between low 25(OH)D or high PTH, and subsequent bone accrual. While this is an important limitation, BMD ranking tracks well over time in healthy children such that those who ranked among the lowest earlier in childhood also ranked among the lowest later in adolescence, and those who ranked highest generally remained high.[51] When evaluating prevalence of high PTH, confidence intervals were wide due to few children having high PTH. Finally, our findings might not be generalizable to populations other than the US where there is a difference in the distribution of continental ancestry and nutritional status.

Children gain more than half of their peak bone mass during adolescence with the greatest increase following the pubertal growth spurt, highlighting the need to identify modifiable factors during this critical period that could improve bone accrual. This study afforded a unique opportunity to further quantify risk factors for poor bone health, specifically low 25(OH)D. This may lead to novel vitamin D supplementation trials that could ameliorate deficits and improve bone health at critical developmental stages.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Sociodemographic, Clinical and Laboratory Characteristics at the Time of Blood Draw by HIV Status

Characteristic[1]		Cohort PHIV (N=412)	Cohort PHEU (N=207)	P Value
		Median (Q1, Q3) or N(%)		
Age (yr)		13.0 (10.6, 14.7)	10.8 (9.0, 12.8)	< 0.001
Sex-male		195 (47%)	106 (51%)	0.36
Hispanic (self-reported)		100 (24%)	70 (34%)	0.01
Black race (self-reported)		298 (76%)	129 (64%)	0.002
Continental ancestry (%)[2]				
Africa		69.8 (23.5, 81.4)	62.0 (8.7, 77.1)	0.01
Europe/CSK Asia		17.5 (6.69, 50.4)	23.5 (9.5, 58.4)	0.02
Americas		1.9 (1.3, 3.8)	2.1 (1.4, 4.7)	0.06
East Asia		3.1 (2.1, 4.9)	3.4 (2.1, 5.2)	0.15
Oceania		3.4 (2.0, 5.9)	3.4 (2.1, 6.2)	0.57
Season of blood draw	Spring	110 (27%)	54 (26%)	< 0.001
	Summer	124 (30%)	58 (28%)	
	Fall	95 (23%)	26 (13%)	
	Winter	83 (20%)	69 (33%)	
Northern latitude[3]	Northern	250 (61%)	103 (50%)	0.01
Vigorous activity (min/day)		8.4 (0.0, 30.0)	9.6 (0.0, 34.2)	0.09
Vitamin D intake (IU/day)		160 (93, 320)	135 (79, 248)	0.03
Vitamin D intake < 600 IU/day		363 (94%)	193 (97%)	0.12
Calcium intake (mg/day)		713 (480, 1,029)	700 (480, 1,004)	0.65
Calcium intake < 1300 mg/day[4]		326 (84%)	173 (87%)	0.42
Phosphorus intake (mg/day)		1,016 (726, 1,452)	9670 (701, 1,387)	0.37
Weight z-score		0.15 (−0.76, 1.01)	0.84 (−0.20, 1.83)	< 0.001
Height z-score		−0.30 (−1.13, 0.38)	0.27 (−0.43, 0.97)	< 0.001
Percentage body fat (%)[5]		22.3 (15.1, 30.4)	26.1 (18.6, 36.9)	< 0.001
Tanner stage[6]	1	86 (21%)	65 (32%)	< 0.001
	2	77 (19%)	58 (28%)	
	3	77 (19%)	28 (14%)	
	4	79 (19%)	32 (16%)	
	5	91 (22%)	22 (11%)	
Laboratory				
25(OH)D (ng/mL)		22.0 (16.3, 27.7)	22.6 (18.4, 27.0)	0.17
25(OH)D ≤ 20 ng/ml		175 (42%)	71 (34%)	0.05
25(OH)D (ng/ml)	< 12.0	34 (8%)	10 (5%)	0.11
	12.1–20.0	141 (34%)	61 (29%)	
	>20.0 to 29.9	161 (39%)	100 (48%)	

Characteristic[1]		Cohort PHIV (N=412)	PHEU (N=207)	P Value
	≥30	76 (18%)	36 (17%)	
PTH (pg/mL)		31.1 (22.3, 45.9)	26.2 (20.1, 37.4)	< 0.001
PTH >65 pg/ml	> 65 pg/mL	38 (9%)	5 (2%)	0.002
25(OH)D and PTH	25D ≤20 PTH >65	23 (6%)	2 (1%)	0.006
	25D ≤20 PTH ≤65	152 (37%)	69 (33%)	
	25D>20 PTH >65	15 (4%)	3 (1%)	
	25D >20 PTH ≤65	222 (54%)	132 (64%)	
Calcium (mg/dl)		9.6 (9.3, 9.9)	9.7 (9.5, 10.0)	< 0.001
Phosphorus (mg/dL)		4.5 (4.1, 5.0)	4.9 (4.5, 5.2)	< 0.001
Creatinine (mg/dL)		0.56 (0.47, 0.65)	0.54 (0.48, 0.62)	0.14
Among PHIV				
Type of ART regimen				
	PI	40 (9.7)		
	NNRTI	65 (15.8)		
	PI	263 (63.8)		
	Other ART	40 (9.7)		
	No ART	4 (0.10)		
Lifetime duration of ARV		10.7 (8.6, 12.7)		
Efavirenz – current use[7]		73 (18%)		
Lifetime duration (yr)		3.1 (1.5, 6.1)		
Tenofovir – current use[7]		91 (23%)		
Lifetime duration (yr)		1.5 (0.5, 2.8)		
CD4 T cell count (cells/mm^3)		699 (508, 919)		
CD4 percent before ART initiation (%)		30.0 (21.0, 38.0)		
HIV RNA (log10 copies/mL)		2.2 (1.7, 3.1)		
HIV RNA < 400 copies/mL		277 (67%)		

Abbreviations: PHIV-perinatally HIV-infected; PHEU-perinatally HIV-exposed uninfected; 25(OH)D -25 hydroxy-vitamin D; ART-antiretroviral.

[1]Missing data for PHIV and PHEU : Hispanic (N=1, N=3); Black race (N=21, N=6); Birthplace (N=2.N=0); vigorous activity (N=79, N=14);); dietary intake (N=26, N=8); Tanner stage (N=2, N=2); PTH (N=0, N= 1); calcium (N=5, N=0), phosphate/creatinine (N=1, N=0); CD4 count (N=2); HIV viral load (N=0).

[2]Continental 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: ≥ 39° degree latitude.

[4]The RDA for calcium is 1100 for children 4–8 years old and 1,300 for children 9–18 years old.

[5]No percent body fat measured on N=21 PHIV, N=8 PHEU) because there was no DXA scan within 365 days of the 25(OH)D.

[6]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.

[7]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 for2 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.

Table 2

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

		PHIV[1]		PHEU[1]	
Outcome	**Exposure**	**Adjusted difference[3] (95%CI)**	**P value**	**Adjusted difference[3] (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	-	-

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; BMD z-Bone mineral density z score; BMC-bone mineral content.

[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 count. When 25(OH)D status was the exposure, we additionally adjusted for percent body fat. When the outcome was Total body-BMC, Total body less head-BMC or Lumbar spine-BMC, we additionally adjusted for Tanner stage and sex.

Table 3

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

Characteristic[1]		25(OH)D Level ≤ 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
Black race		198 (83%)	229 (65%)	< 0.001
Continental ancestry (%)				
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
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 latitude[2]		175 (71%)	178 (48%)	<0.001
Vitamin D intake (IU/day)		138 (78, 267)	160 (95, 306)	0.10
Calcium intake (mg/day)		713 (476, 1,009)	710 (483, 1,027)	0.84
Percentage of body fat (%)[3]		25.3 (17.9, 33.2)	22.3 (15.1, 33.1)	0.06
Vitamin 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 use[4]		38 (23%)	33 (15%)	0.047
TDF use – current use[4]		34 (21%)	54 (25%)	0.35
CD4 T cell count (cells/mm^3)		661 (462, 852)	728 (540, 974)	0.008
HIV RNA (log_{10} 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, 25(OH)D – 25 hydroxy-vitamin D.

[1] Missing data for 25(OH)D ≤20 ng/mL and >20 ng/mL: Black race (N=7, N=20); dietary intake (N=8, N=26); percent body fat (N=14, N=15), 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: ≥ 39° degrees latitude

[3] No percent body fat measure because there was no total body DXA scan performed within 365 days of 25(OH)D (N=14, N=15).

[4]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.

Table 4

Prevalence of Low 25(OH)D and High PTH and Differences in Levels by HIV status, and by Use of Efavirenz and Tenofovir

	Prevalence of Low 25(OH)D or High PTH		Difference in 25(OH)D or PTH Levels	
	Prevalence Ratio (95%CI) P value		Difference (95%CI) P value	
Exposure	**Unadjusted**	**Adjusted[1,2]**	**Unadjusted**	**Adjusted[1]**
	25(OH)D ≤ 20 ng/mL		**25(OH)D (ng/mL)**	
	Model 1A	**Model 1B[2]**	**Model 7A**	**Model 7B**
PHIV vs. PHEU	1.24 (0.99,1.54)	1.00 (0.81,1.24)	−0.77 (−2.05,0.51)	0.48 (−0.69,1.65)
	0.06	0.98	0.24	0.42
	Model 2A	**Model 2B**	**Model 8A**	**Model 8B**
PHIV EFV+ vs. PHEU[3]	1.52 (1.14,2.03)	1.30 (0.98,1.74)	−2.83 (−4.93, −0.72)	−1.94 (−3.88,0.01)
	0.01	0.07	0.008	0.05
PHIV EFV− vs. PHEU[3]	1.17 (0.93,1.48)	0.95 (0.76,1.18)	−0.31 (−1.64,1.01)	0.99 (−0.23,2.21)
	0.17	0.63	0.643	0.11
	Model 3A	**Model 3B**	**Model 9A**	**Model 9B**
PHIV TDF+ vs. PHEU[3]	1.09 (0.79,1.51)	0.77 (0.56,1.06)	−1.20 (−3.16,0.75)	1.02 (−0.87,2.92)
	0.61	0.10	0.23	0.29
PHIV TDF− vs. PHEU[3]	1.28 (1.02,1.6)	1.06 (0.86,1.31)	−0.65 (−1.99,0.70)	0.37 (−0.85,1.59)
	0.03	0.58	0.35	0.55
	PTH > 65 pg/mL		**PTH (pg/mL)**	
	Model 4A	**Model 4B**	**Model 10A**	**Model 10B**
PHIV vs. PHEU	3.80 (1.52,9.51)	3.17 (1.25,8.06)	7.10 (4.17,10.02)	5.26 (2.35,8.17)
	0.004	0.01	<0.001	<0.001
	Model 5A	**Model 5B**	**Model 11A**	**Model 11B**
PHIV EFV+ vs. PHEU[3]	1.13 (0.22,5.69)	0.85 (0.17,4.56)	5.44 (0.61,10.28)	3.37 (−1.85,8.60)
	0.88	0.89	0.03	0.21
PHIV EFV− vs. HEU[3]	4.40 (1.75,11.04)	3.67 (1.44,9.36)	7.27 (4.11,10.44)	5.54 (2.47,8.61)
	0.002	0.006	<0.001	<0.001
	Model 6A	**Model 6B**	**Model 12A**	**Model 12B**
PHIV TDF+ vs. PHEU[3]	6.79 (2.54,18.12)	5.50 (1.95,15.49)	14.22 (9.11,19.32)	11.65 (6.47,16.83)
	<0.001	0.001	<0.001	<0.001
PHIV TDF− vs. PHEU[3]	2.92 (1.13,7.6)	2.64 (1.01,6.94)	4.80 (1.69,7.91)	3.71 (0.61,6.82)
	0.03	0.05	0.002	0.02

Abbreviations: PHIV-Perinatally HIV-infected; PHEU-perinatally HIV-exposed uninfected; TDF-tenofovir; EFV-efavirenz, 25(OH)D – 25 hydroxy-vitamin D.

[1]For the outcome 25(OH)D, the models were adjusted for age, black race, ancestry markers, latitude as a continuous variable, and season. For the outcome PTH, models were adjusted for age, black race, and ancestry markers.

[2]Model 1B, 2B, and 3B were fit using the Poisson link and the robust standard error.

[3]In sensitivity analyses including children with ARV data just prior to the 25(OH)D date, and not on the 25(OH)D date, the results did not change for the TDF or EFV analyses.