# Vitamin D Status and Bone Outcomes in Perinatally HIV-Infected Children

*Jacobson DL[1], Geffner M[2], Stephensen C[3], Hazra R[4], Patel K[5], Miller TL[6], Van Dyke RB[7], Ellis A[8] and DiMeglio LA[9] for the Pediatric HIV/AIDS Cohort Study (PHACS)*

[1]Center for Biostatistics in AIDS Research, Harvard T. H. Chan School of Public Health, Boston, MA; [2]The Saban Research Institute, Children's Hospital Los Angeles, Keck School of Medicine of USC, Los Angeles, CA; [3]United States Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Davis, CA; [4]Maternal and Pediatric Infectious Disease (MPID) Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; [5]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA; [6]Division of Pediatric Clinical Research, Department of Pediatrics, Miller School of Medicine at the University of Miami, Miami, FL; [7]Tulane University School of Medicine, New Orleans, LA; [8]Frontier Science and Technology Research Foundation, Amherst, NY; [9]Indiana University School of Medicine, Indianapolis, IN

## Abstract #931

**Denise L Jacobson**
**Center for Biostatistics in AIDS Research**
**Harvard T.H. Chan School of Public Health**
**617-432-3266 (phone)**
**617-432-2843 (fax)**
**jacobson@sdac.harvard.edu**

## ABSTRACT*

**Background:** HIV-infected children (HIV+) are at risk for poor bone mineral accrual, but the predisposing etiological factors are ill-defined (Jacobson 2010). We previously reported lower than recommended intakes of vitamin D in over half of HIV+ children enrolled in the US-based Pediatric HIV/AIDS Cohort Study Adolescent Master Protocol (AMP) (DiMeglio 2013). In this study, we hypothesized that serum 25-hydroxy vitamin D (25D) levels would be lower in HIV+ children than HIV-exposed uninfected (HEU) children and would be associated with lower bone mineral density (BMD) in HIV+ children.
**Methods:** We measured serum levels of 25D, parathyroid hormone (PTH), calcium, and phosphate in perinatally HIV+ (N=426) and HEU (N=219) children. Dual-energy X ray absorptiometry (DXA) was performed in HIV+ to assess total body and spine BMD and percent body fat. Z-scores were calculated for bone measures based on reference data. Among HIV+ children, 396 had a DXA scan within 365 days of the 25D assay. Low serum 25D was defined as < 20 ng/mL. The prevalence ratio (PR) and 95% confidence interval (95%CI) of low 25D in HIV+ relative to HEU children was determined as were associations of 25D levels with BMD outcomes in HIV+ children, unadjusted and adjusted for confounding.
**Results:** The median age of the HIV+ and HEU children was 13.0 and 10.7 years, respectively. The majority of children were Non-Hispanic Black (62%) or Hispanic (28%). Prevalence of low 25D was 43% in HIV+ versus 34% in HEU children (P=0.027), with a prevalence ratio of 1.27 (95%CI 1.02, 1.58) and 1.03 (95%CI 0.86 1.24) unadjusted and adjusted, respectively. On univariable analysis, low 25D was associated with older age, Non-Hispanic Black race/ethnicity, female sex, born on mainland US versus Puerto Rico, winter or spring season, and higher total body fat % by DXA. In children with low 25D, mean level of PTH was higher and calcium and phosphate levels were lower. Among HIV+ children, for each 1 ng/mL decrease in 25D level there was a 0.013 SD decrease in total body BMD z-score (P=0.079), adjusted for confounders (age, race/ethnicity and height). No association was observed between 25D and spine BMD z-score (adjusted estimate 0.0042, P=0.56).
**Conclusions:** Low 25D is common in both HIV+ and HEU children. There was a trend for an association between vitamin D deficiency and lower total body BMD in HIV+ children. HIV+ children should be monitored routinely for 25D deficiency due to possible adverse effects on BMD.

## BACKGROUND

- HIV-infected children are at risk for poor bone mineral accrual, but the predisposing etiological factors are ill-defined (Jacobson 2010). We previously reported lower than recommended intakes of vitamin D in over half of HIV+ children enrolled PHACS-AMP (DiMeglio 2013). Here we hypothesized that serum 25-hydroxy vitamin D (25D) levels would be lower in perinatally HIV-infected (HIV+) children than HIV-exposed uninfected (HEU) children and would be associated with lower bone mineral density (BMD) in HIV+ children.

## OBJECTIVES

- To evaluate adjusted differences in the distribution of 25D in perinatally HIV+ compared to HIV-exposed uninfected children.
- To evaluate associations of 25D with total body and spine bone mineral density among HIV+ children.

## METHODS

### Study Population and Assessments

**Study population:** Perinatally HIV+ and perinatally HEU children from 7 to <15 years of age were enrolled into the Adolescent Master Protocol (AMP) of the Pediatric HIV/AIDS Cohort Study between 2007 and 2009 from 15 clinical sites across the mainland US and Puerto Rico.

**Annual visits:** Blood draw, anthropometric measurements, clinical and medication history abstracted from clinical charts.

**Dual energy X-ray absorptiometry (DXA) scan:** At entry visit in HIV+ Total body (TBBMD) and lumbar spine L1-L4 (LSBMD) bone mineral density. Percent body fat (%).

**Bone age**: At time of DXA, children at < Tanner 5 pubertal status had a left hand and wrist radiograph performed to determine bone age (Greulich 1959).

**BMD z-scores** (age and sex-adjusted standard deviation scores) were calculated using Baylor University Reference Data for children. Age adjustments were done as follows: If chronological age was within 1 SD of the bone age, then z-score was based on chronological age. However, if the chronological age was > 1 SD from the bone age, then z-score was based on the bone age.

**Serum assays:**

**25-OH vitamin D (25D):** EIA (Immunodiagnostic System, LTD). Categorized as: < 20 ng/mL (low), > 20 ng/mL (normal)

**Parathyroid hormone:** Intact PTH (Siemens Medical Solutions Diagnostics)

**Calcium, phosphate and creatinine.**

### Statistical Methods

**Differences between groups**: Wilcoxon/Kruskal Wallis for continuous and Chi-square/Fisher's exact for categorical variables.

**Models:** Regression models were fit for a binary outcome using the log-binomial to obtain a prevalence ratio and for a continuous outcome using linear regression, unadjusted and adjusted for potential confounders.

## RESULTS

**Table 1: Characteristics of HIV+ and HEU Children at Time of 25D (by column percent)**

| Characteristic | | HIV+ (N=426) | HEU (N=219) | P-Value |
|---|---|---|---|---|
| Age (yr) | Median (Q1, Q3) | 13.0 (10.5, 14.7) | 10.7 (9.0, 12.7) | <0.001 |
| Male Gender | | 198 (46%) | 114 (52%) | 0.180 |
| Race/ethnicity | White Non-Hispanic | 26 (6%) | 16 (7%) | 0.015 |
| | Black Non-Hispanic | 281 (66%) | 117 (53%) | |
| | Hispanic | 104 (24%) | 78 (36%) | |
| | Multi/Other/Unknown | 15 (4%) | 8 (4%) | |
| Birthplace | Africa | 9 (2%) | 0 (0%) | <0.001 |
| | Puerto Rico | 26 (6%) | 28 (13%) | |
| | USA Mainland | 371 (88%) | 191 (87%) | |
| | Other | 17 (4%) | 0 (0%) | |
| Season | Spring | 115 (27%) | 58 (26%) | <0.001 |
| | Summer | 128 (30%) | 61 (28%) | |
| | Fall | 97 (23%) | 28 (13%) | |
| | Winter | 86 (20%) | 72 (33%) | |

**Table 2: Characteristics of Children by 25D Category - HIV+ and HEU Combined (by row percent)**

| Characteristic | | < 20 ng/mL | ≥ 20 ng/mL | P-Value |
|---|---|---|---|---|
| Cohort | HIV+ | 183 (43%) | 243 (57%) | 0.024 |
| | HEU | 74 (34%) | 145 (66%) | |
| Age (yr) | Median (Q1, Q3) | 13.0 (10.7, 14.8) | 11.7 (9.3, 13.7) | <0.001 |
| Male Gender | | 102 (33%) | 210 (67%) | <0.001 |
| Race/ethnicity | White Non-Hisp. | 12 (29%) | 30 (71%) | <0.001 |
| | Black Non-Hisp. | 194 (49%) | 204 (51%) | |
| | Hispanic | 44 (24%) | 138 (76%) | |
| | Multi/Other/Unk. | 7 (30%) | 16 (70%) | |
| Birthplace | Africa | 4 (44%) | 5 (56%) | <0.001 |
| | Puerto Rico | 2 (4%) | 52 (96%) | |
| | USA Mainland | 245 (44%) | 317 (56%) | |
| | Other | 5 (29%) | 12 (71%) | |
| Season | Spring | 80 (46%) | 93 (54%) | <0.001 |
| | Summer | 54 (29%) | 135 (71%) | |
| | Fall | 48 (38%) | 77 (62%) | |
| | Winter | 75 (47%) | 83 (53%) | |
| Latitude | Northern | 171 (53%) | 154 (47%) | <0.001 |
| | Southern | 86 (27%) | 234 (73%) | |
| BMI Z-score | Median (Q1, Q3) | 0.56 (-0.26, 1.4) | 0.40 (-0.38, 1.3) | 0.34 |
| Percent body fat | Median (Q1, Q3) | 25.4 (18.0, 33.2) | 22.4 (15.3, 33.1) | 0.062 |
| Laboratory Assays | | | | |
| Vitamin D (ng/mL) | Median (Q1, Q3) | 15.6 (13.3, 17.6) | 26.3 (23.2, 30.8) | <0.001 |
| PTH mean (pg/mL) | Median (Q1, Q3) | 34.0 (23.8, 49.4) | 27.1 (20.1, 38.1) | <0.001 |
| Calcium (mg/dL) | Median (Q1, Q3) | 9.5 (9.3, 9.8) | 9.7 (9.4, 9.9) | <0.001 |
| Phosphate (mg/dL) | Median (Q1, Q3) | 4.6 (4.1, 5.1) | 4.7 (4.3, 5.1) | 0.023 |
| Creatinine (mg/dL) | Median (Q1, Q3) | 0.56 (0.49, 0.65) | 0.53 (0.46, 0.63) | 0.013 |

**Table 3: Prevalence of Low 25D in HIV+ Compared to HEU Children**

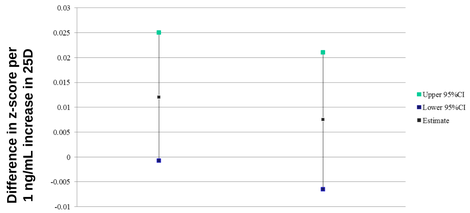
| | | Low 25D (<20 ng/mL) | Prevalence Ratio (PR) of Low 25D - HIV vs. HEU | | | |
|---|---|---|---|---|---|---|
| | | | Unadjusted | | Adjusted* | |
| | Median (range) | Percent | PR (95%CI) | P value | PR (95%CI) | P value |
| HIV+ | 21.9 (7.4, 53) | 43.0 | 1.27 (1.02, 1.58) | 0.029 | 1.03 (0.86, 1.24) | 0.74 |
| HEU | 22.6 (8.9, 53) | 33.8 | Ref. | | Ref. | |

* Adjusted for age, race/ethnicity, site region, season, place of birth

**Table 4: Univariable Models of 25D and Potential Confounders on Total body BMD and Lumbar Spine BMD Z-Scores in HIV+ children**

| Characteristic for each Model | Total Body BMD z-score (TBBMD) | | Spine BMD z-score LSBMD) | |
|---|---|---|---|---|
| | Univariable Estimate | 95%CI | Univariable Estimate | 95%CI |
| 25D (ng/mL) | -0.0012 | -0.49, 0.26 | -0.00081 | -0.016, 0.014 |
| Age (yr) | 0.061 | 0.013, 0.11 | 0.012 | -0.034, 0.058 |
| Race/ethnicity | | | | |
| White Non-Hispanic | -0.75 | -1.31,-0.19 | -0.30 | -0.85, 0.25 |
| Black Non-Hispanic | 0.33 | 0.03, 0.62 | 0.26 | -0.027, 0.54 |
| Multiple race/Other | -0.62 | -1.32, 0.10 | -0.086 | -0.78, 0.60 |
| Hispanic | Ref. | | Ref. | |
| Height z | 0.45 | 0.35, 0.55 | 0.44 | 0.35, 0.53 |

**Figure 1: Difference in Total Body and Spine BMD Z-Score per 1 ng/mL Increase in 25 D in HIV+ children, Adjusted for Race/ethnicity, Age and Height Z-Score**




## SUMMARY

**Table 1: Characteristics of HIV+ and HEU**

- HIV+ were older than HEU and more likely to be Non-Hispanic black.

**Table 2: Characteristics of low 25D**

- Low 25D is common in both HIV+ and HEU children (43% vs. 34%).
- Low 25D was associated with older age, Black Non-Hispanics, female sex, born on mainland US versus Puerto Rico, winter or spring season, and higher percent body fat .
- Children with low 25D had higher PTH and lower calcium and phosphate.

**Table 3: Adjusted prevalence of low 25D in HIV+ vs. HEU**

- Prevalence of low 25D was similar in HIV+ and HEU, adjusted for potential confounders.

**Table 4: Univariable models of total body and lumbar spine z in HIV+**

- Total body BMD z was increased by 0.06 SD for each one year increase in age, by 0.33 SD in Black Non-Hispanic vs. Hispanic, by 0.45 SD with each 1 SD increase in height z. No significant associations with 25D.
- Lumbar spine BMD was increased by 0.44 SD for each 1 SD increase in height z. No significant associations with 25D.

**Figure 1: Adjusted models of 25 D with total body and lumbar spine z in HIV+**

- **Total Body BMD:** For each 1 ng/mL increase in 25D there was a 0.013 increase in total body BMD z-score (P = 0.079).
- **Spine BMD:** For each 1 ng/mL increase in 25D there was a 0.0075 increase in spine BMD z-score (P= 0.29).

## CONCLUSIONS

- Given the high prevalence of 25-D deficiency in HIV+ children and the observed associations of low 25D with higher PTH and lower BMD, consideration should be given to monitoring children with HIV for 25D deficiency and to supplementing when needed to maintain 25D adequacy.

## REFERENCES

- Jacobson DL, Lindsey JC, Gordon CM, Moye J, Hardin D, Mulligan K, Aldrovandi GM. for the Pediatric AIDS Clinical Trials Group P1045. Total body and spinal bone mineral density across Tanner stage in perinatally HIV-infected and uninfected children and youth in PACTG 1045. AIDS. 2010 Mar 13;24(5):687-96.
- DiMeglio LA, Wang JJ, Siberry GK, Miller TL, Geffner ME, Hazra R, Borkowsky W, Chen JS, Dooley L, Patel K, Van Dyke RB, Fielding RA, Gurmu Y, Jacobson DL for the Pediatric HIV/AIDS Cohort Study. Bone mineral density in children and adolescents with perinatal HIV infection. AIDS. 2013 Jan 14;27(2):211-20.
- Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Stanford, CA: Stanford University Press; 1959.

## ACKNOWLEDGMENTS

National Institutes of Health
*Turning Discovery Into Health*

Funded by the National Institutes of Health, under cooperative agreements HD052104 (PHACS Coordinating Center, Tulane University School of Medicine) and HD052102 (PHACS Data and Operations Center, Harvard School of Public Health). We thank the study participants, clinical sites, PHACS CAB, Frontier Science & Technology Research Foundation, and Westat.