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Investigation of the 3-epi-25(OH)D$_3$ of 25-hydroxyvitamin D3 in urban schoolchildren

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Abstract

Background: The physiological relevance C-3 epimer of 25-hydroxyvitamin D [3-epi-25(OH)D] is not well understood among youth.

Objective: To assess whether demographic/physiologic characteristics were associated with 3-epi-25(OH)D\textsubscript{3} concentrations in youth.

Methods: Associations between 3-epi-25(OH)D\textsubscript{3} and demographics, 3-epi-25(OH)D\textsubscript{3}, total 25-hydroxyvitamin (25(OH)D) (25(OH)D\textsubscript{2} + 25(OH)D\textsubscript{3}), total cholesterol, HDL, LDL and triglycerides were examined in racially/ethnically diverse schoolchildren (n=682, 8-15yrs) at Boston-area urban schools.

Results: Approximately 50% of participants had detectable 3-epi-25(OH)D\textsubscript{3} (range 0.95-3.95 ng/mL). The percentage of 3-epi-25(OH)D\textsubscript{3} of total 25(OH)D ranged from 2.5 to 17.0% (median 5.5%). Males were 38% more likely than females to have detectable 3-epi-25(OH)D\textsubscript{3} concentrations. Both Asian and black race/ethnicity were associated with lower odds of having detectable 3-epi-25(OH)D\textsubscript{3} compared to non-Hispanic white children ((Asian vs. white, OR 0.28, 95%CI 0.14-0.53; black vs. white, OR 0.38, 95%CI 0.23-0.63, p<0.001). Having an adequate (20-29 ng/ml) or optimal (>30 ng/ml) 25(OH)D concentration was associated with higher odds of having detectable 3-epi-25(OH)D\textsubscript{3} than having an inadequate (<20 ng/ml) concentration (OR 4.78, 95%CI 3.23-6.94 or OR 14.10, 95%CI 7.10-28.0, respectively). There was no association between 3-epi-25(OH)D\textsubscript{3} and blood lipids. However, when considering 3-epi-25(OH)D\textsubscript{3} as a percentage of total 25(OH)D, total cholesterol was lower in children with percent 3-epi-25(OH)D\textsubscript{3} above the median (mean difference -7.1 mg/dL, p=0.01).

Conclusions: Among schoolchildren, sex, race/ethnicity, and total serum 25(OH)D concentration is differentially associated with 3-epi-25(OH)D. The physiological relevance of 3-epi-25(OH)D\textsubscript{3} may be related to the 3-epi-25(OH)D\textsubscript{3} as a percentage of total 25(OH)D and should be considered in future investigations.

Trial registration number: ClinicalTrials.gov, NCT01537809

Keywords: 25-Hydroxyvitamin D3, C-3 epimer, Vitamin D, Daily D Health Study
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**Introduction**

Low concentrations of 25-hydroxyvitamin D [25(OH)D], the standard biomarker of vitamin D status, have been associated with increased cardiovascular disease risk in adolescents and adults. (Giovannucci 2008, Reis et al. 2009) According to the National Health and Nutrition Examination Survey (NHANES) 2001-2006, approximately 6 million children in the United States were vitamin D deficient, defined as a serum 25(OH)D concentration less than 20 ng/mL. (Looker et al. 2011) Given the high prevalence of vitamin D deficiency in children and that children with cardiovascular risk factors are at higher risk for cardiovascular disease in adulthood, it is critical to understand the relationships that exist between serum 25(OH)D and cardiovascular risk factors among youth when early prevention is possible. (Bao et al. 1997)

Vitamin D status is characterized by serum concentrations of 25(OH)D₃ because it has a long half-life, is not tightly regulated compared to 1,25-dihydroxyvitamin D [1,25(OH)₂D] (the active form), and is considered an accurate representation of vitamin D intake from diet, supplements, and synthesis in the skin from exposure to UVB radiation. (Ross et al. n.d., van den Ouweland et al. 2013) It is now known that 25(OH)D undergoes epimerization during metabolism, and the 3-epi-25(OH)D₃ of 25(OH)D₃ is most prevalent, forming 3-epi-25-hydroxyvitamin D₃ (3-epi-25(OH)D). (Singh et al. 2006) Significant blood concentrations of the 3-epi-25(OH)D₃ have been found in infants, (Singh et al. 2006, Stepman et al. 2011, Granado-Lorencio et al. 2012) and, more recently, in adults. (Lensmeyer et al. 2012, Engelman et al. 2014, Cashman et al. 2014, Lutsey et al. 2015) However, the 3-epi-25(OH)D₃ is not routinely measured, and without chromatographic resolution, it could be mistakenly reported as 25(OH)D₃ due to their identical molecular weights. (Lensmeyer et al. 2006)

Laboratory studies have found potential physiological relevance of 3-epi-25(OH)D₃ with some binding capacity to the vitamin D receptor, (Kamao et al. 2005) but limited data exist on its relationship with anthropometrics and blood lipids. Studies in adults found that being female (Cashman et al. 2014, Lutsey et al. 2015) and having a higher waist circumference (Engelman et al. 2014, Cashman et al. 2014) were negative determinants of 3-epi-25(OH)D₃. (Cashman et al. 2014) One study examining 3-epi-
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25(OH)D, anthropometrics and blood lipids, separately among black and white adults, found higher concentrations of 3-epi-25(OH)D were associated with a better cardiovascular risk profile. (Lutsey et al. 2015) Furthermore, the relationship among 3-epi-25(OH)D, anthropometrics, and blood lipids in children is especially unclear. Research is needed to understand the potential physiological relevance of the 3-epi-25(OH)D to determine if it is necessary to consider the 3-epi-25(OH)D when characterizing vitamin D status. To understand the significance of the 3-epi-25(OH)D in children, we examined the 3-epi-25(OH)D cross-sectionally among a diverse sample of schoolchildren in the Boston area. Our goals were to: 1) determine if demographic characteristics were associated with 3-epi-25(OH)D concentrations; 2) examine associations between 3-epi-25(OH)D concentration and blood lipids; and 3) identify the impact of considering 3-epi-25(OH)D on vitamin D status classification.

Methods

Participants

This cross-sectional study investigated 3-epi-25(OH)D using data drawn from 682 children enrolled in the Daily D Health Study. A detailed description of the recruitment and design of the Daily D Health Study has been published elsewhere. (Sacheck et al. 2015) Briefly, the Daily D Health Study was a randomized, double-blind trial assessing the impact of 6-month vitamin D supplementation on serum 25(OH)D and cardiometabolic risk factors in Boston-area schoolchildren (8-15 years). The Daily D study protocol was approved by Tufts University’s Institutional Review Board.

Demographics, Anthropometrics and Vitamin D Intake

Birth date, race/ethnicity, and eligibility for free or reduced-price school meals, as a proxy measure of socioeconomic status, were collected from parents during informed consent procedures. The mean age was 11.2 ± 1.4 years, and age was categorized into a binary variable (≥12 years old vs. <12 years old) for analysis. Racial/ethnic categories were grouped as non-Hispanic white, Hispanic/Latino, black/African American, Asian/Asian American, and multiracial/other. Height and weight were measured and body mass index (BMI) calculated as previously reported. (BMI Percentile Calculator for Child and Teen: English Version 2013, Sacheck et al. 2015) Weight status was categorized into obese (≥95th
Investigation of the 3-epimer in children percentile) and non-obese (<95\textsuperscript{th} percentile) categories. Pubertal status was assessed using a brief pubertal questionnaire designed and validated for this age group as previously described.(Carskadon and Acebo 1993, Sacheck et al. 2015) Dietary intake of vitamin D was assessed using the 2004 Block Food Frequency Questionnaire (FFQ) for Children, which inquired about intake over the past week (NutritionQuest, Berkeley, CA).

25(OH)D, 3-epi-25(OH)D\textsubscript{3} Measurements and Blood Lipids

Blood was drawn from the antecubital vein following an overnight fast. Total serum 25(OH)D was measured by validated liquid chromatography-mass spectrometry (LC-MS/MS) method including fractionation of 25(OH)D\textsubscript{2}, 25(OH)D\textsubscript{3}, and the 3-epi-25(OH)D\textsubscript{3} of 25(OH)D\textsubscript{3}.(Holick et al. 2005) Serum samples of 25(OH)D were prepared and analyzed through a turbulent flow LC system (Cohesive Technologies, Franklin, MA) followed by traditional laminar flow chromatography and analyzed relative to the National Institute of Standards and Technology (NIST) vitamin D standard for detection and quantification of 25(OH)D\textsubscript{3}, 25(OH)D\textsubscript{2}, and 3-epi-25(OH)D\textsubscript{3}. The analysis was performed using a Triple Stage Quadrupole (TSQ) Quantum Ultra triple mass-spectrometer (Thermo Finnigan Corp., San Jose, CA). The intra-assay coefficient of variation is 6.0%. Concentrations of 25(OH)D <20 ng/mL were classified as inadequate, 20-29 ng/mL as adequate, and ≥30 as optimal. (“Dietary Reference Intakes for Calcium and Vitamin D” n.d., Dawson-Hughes et al. 2005) Concentrations of 3-epi-25(OH)D\textsubscript{3} were considered detectable at ≥0.95 ng/mL based on assay sensitivity. Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides and were measured as previously described.(Van Rompay et al. 2015)

Statistical Analysis

Pearson’s \(\chi^2\) test was used to compare the distribution of participant characteristics across groups by 3-epi-25(OH)D\textsubscript{3} status (detectable vs. non-detectable). Statistically significant differences between groups in continuous variables were determined with Student’s independent t-test. For variables with skewed distributions, the Kruskal-Wallis test was used. To calculate 3-epi-25(OH)D\textsubscript{3} as a percent of total 25(OH)D, we added concentrations of 25OHD\textsubscript{2} and 25OHD\textsubscript{3} and the 3-epi-25(OH)D\textsubscript{3} of 25OHD\textsubscript{3}.
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134 divided 3-epi-25(OH)D₃ concentration by the summed total 25(OH)D, and multiplied the result by 100.

135 Logistic regression was used to examine the relationship between participant characteristics and the odds
136 of having detectable 3-epi-25(OH)D₃, as well as the odds of being above or below the median of percent
137 3-epi-25(OH)D₃ (5.5%). Multiple linear regression was used to examine linear relationships between 3-
138 epi-25(OH)D₃ concentration and blood lipids, adjusting for participant characteristics (age, sex, race, weight
139 status, puberty status, & free/reduced lunch eligibility). The distribution of 3-epi-25(OH)D₃ and triglycerides
140 were right-skewed and logarithmically transformed for this analysis.

141 Generalized linear models were used to examine the mean difference in blood lipids between
142 children with and without detectable 3-epi-25(OH)D₃ and children above or at or below median of percent
143 3-epi-25(OH)D₃ and each was adjusted for participant characteristics and vitamin D status. Least-square
144 means and standard errors were reported for total, LDL and HDL cholesterol. P values from multiple
145 comparisons were adjusted with Tukey’s HSD test. Because triglyceride data were skewed, results are
146 reported as geometric mean and standard error, as well as ratio of geometric means and 95% confidence
147 interval.

148 To assess the impact of including 3-epi-25(OH)D₃ in calculating total 25(OH)D concentration on
149 vitamin D status classification, we compared the proportions of children with inadequate, adequate and
150 optimal 25(OH)D concentrations with and without consideration of 3-epi-25(OH)D₃. Agreement between
151 the two methods was evaluated with weighted kappa statistics. We also examined the relationship
152 between 3-epi-25(OH)D₃ and 25(OH)D as a percent of total 25(OH)D. Spearman’s rank correlation was
153 used to determine the correlation between 3-epi-25(OH)D₃ and percent 3-epi-25(OH)D₃ and 25(OH)D₃
154 concentrations. A P value of <0.05 was considered statistically significant for all analyses. Data were
155 analyzed using SAS version 9.3 (SAS Institute, Cary, NC).

156 Results

157 Participant Characteristics

158 Overall, 55% of children were <12 years old; 49% were male; 41% were non-Hispanic white,
159 15% black/African American, 22% Hispanic/Latino, 8% Asian/Asian American, and 14%
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multiracial/other; 64% had reached puberty; 67% were eligible for free or reduced/price lunch; 25%
were classified as obese. Mean 25(OH)D concentration was 21.9 ± 6.8 ng/ml. Of 682 children in this
study, 42% had inadequate, 47% had adequate, and 11% had optimal 25(OH)D concentrations. Mean
total cholesterol was 156.5 ± 26.8 mg/dL, mean HDL cholesterol was 50.5 ± 12.2 mg/dL, mean LDL
cholesterol was 88.0 ± 23.8 mg/dL and median triglycerides were 65.0 ± 40.0 mg/dL.

**Detectable vs. Non-detectable 3-epi-25(OH)D$_3$**

Half of the children (337 of 682) had detectable 3-epi-25(OH)D$_3$ concentrations. Of those with
detectable 3-epi-25(OH)D$_3$, the median 3-epi-25(OH)D$_3$ value was 1.4 ng/mL (range 0.95-3.95 ng/mL;
Figure 1A). Participant characteristics by detectable 3-epi-25(OH)D$_3$ status are summarized in Table 1.
The proportion of children classified within each level of vitamin D status as significantly different by
detectable 3-epi-25(OH)D$_3$ status (p<0.001). Among children with inadequate vitamin D concentration,
75% did not have detectable 3-epi-25(OH)D$_3$. Among children with adequate and optimal vitamin D
concentrations, 62% and 83% had detectable 3-epi-25(OH)D$_3$, respectively. Dietary vitamin D was
greater in children with detectable 3-epi-25(OH)D$_3$ (median 120 IU vs. 103 IU, p<0.005). Among
children with detectable 3-epi-25(OH)D$_3$, there was a significantly higher proportion of males (p=0.02),
pre-pubertal children (p=0.008), and non-Hispanic whites (p<0.0001), and fewer black/African American
and Asian/Asian American children than whites. There were no significant differences in age, weight
status, or free or reduced-price lunch eligibility between children with and without detectable 3-epi-
25(OH)D$_3$ concentrations.

In the multivariate analysis only being male (OR 1.38, 95%CI 1.01-1.89) was associated with a
significantly higher odds of having detectable 3-epi-25(OH)D$_3$ concentrations (Table 1). Being either
Asian or black was associated with lower odds of having detectable 3-epi-25(OH)D$_3$ (Asian/Asian
American vs. white, OR 0.28, 95%CI 0.14-0.53; black/African American vs. white, OR 0.38, 95%CI
0.23-0.63). After adjustment for vitamin D status, the associations between detectable 3-epi-25(OH)D$_3$
and sex as well as race were attenuated. Only vitamin D status was significantly associated with higher
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odds of having detectable 3-epi-25(OH)D_3 (adequate vs. inadequate: OR 4.78, 95%CI 3.23-6.94; optimal vs. inadequate: OR 14.10, 95%CI 7.10-28.0).

Among those with detectable 3-epi-25(OH)D_3, continuous 3-epi-25(OH)D_3 was not associated with age, sex, weight status or puberty status. Median 3-epi-25(OH)D_3 was significantly higher in children without free or reduced price lunch eligibility compared to children with free or reduced price lunch eligibility (median [IQR]: 1.50 [0.61] ng/mL vs. 1.35 [0.63] ng/mL, p=0.049). 3-epi-25(OH)D_3 was also associated with race/ethnicity, in that white/Caucasian children had higher median 3-epi-25(OH)D_3 compared to all other race/ethnicity groups (1.60 [0.61] ng/mL vs. 1.17-1.36 [0.29-0.69] ng/mL, p=0.001).

**Median Percent 3-epi-25(OH)D_3 of 25(OH)D**

Among children with detectable 3-epi-25(OH)D_3, the median percent 3-epi-25(OH)D_3 of 25(OH)D was 5.5%, ranging from 2.5-17.0% (Figure 1B). There were differences in percent 3-epi-25(OH)D_3 by vitamin D status. Specifically, among children with inadequate 25(OH)D, 79% were above the median percent 3-epi-25(OH)D_3, while among those with optimal 25(OH)D, only 31% were above the median. The proportion of females above the median was significantly higher than males (54% vs. 38%, respectively, p=0.002), but no other differences with participant characteristics were found.

Multivariate logistic regression results were similar, with males being 50% less likely to have percent 3-epi-25(OH)D_3 above the median compared with females (OR 0.50, 95%CI 0.32-0.78), and those with adequate or optimal 25(OH)D less likely to be above the median (adequate vs. inadequate: OR 0.22, 95%CI 0.11-0.42; optimal vs. inadequate: OR 0.10, 95%CI 0.05-0.24). With additional adjustment for vitamin D status, sex retained significance. Similarly to the median analysis, continuous percent 3-epi-25(OH)D_3 was associated with sex. Females had significantly higher percent 3-epi-25(OH)D_3 than males (mean [SD]: 6.16 [2.0] % vs. 5.77 [1.9] %, p=0.033).
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3-epi-25(OH)D<sub>3</sub>, Percent 3-epi-25(OH)D<sub>3</sub> and Blood Lipids

We did not find a linear relationship between 3-epi-25(OH)D<sub>3</sub> and blood lipids (data not shown). In comparing blood lipid concentrations between children with detectable 3-epi-25(OH)D<sub>3</sub> and non-detectable, we found no statistically significant differences in mean total cholesterol, LDL, HDL, or triglycerides. However, the adjusted mean difference in total cholesterol between children above the median percent 3-epi-25(OH)D<sub>3</sub> was 7 mg/dL lower than those at or below the median (149.6 ± 2.8 mg/dL vs. 156.7 ± 2.6; mean difference: -7.1, 95%CI -12.7-1.5 mg/dL; p = 0.01).

Relationship between 3-epi-25(OH)D<sub>3</sub> and total 25(OH)D

The relationship between 3-epi-25(OH)D<sub>3</sub> and total 25(OH)D did not appear linear, with large variation in 3-epi-25(OH)D<sub>3</sub>, particularly at higher concentrations of total 25(OH)D; nonetheless, 3-epi-25(OH)D<sub>3</sub> concentration was positively correlated with 25(OH)D<sub>3</sub> (Spearman’s correlation coefficient, r=0.40; p<0.05; Figure 2a). The relationship between percent 3-epi-25(OH)D<sub>3</sub> and total 25(OH)D was also non-linear, with stable variability in percent 3-epi-25(OH)D<sub>3</sub> with increasing concentrations of total 25(OH)D (Figure 2b). Percent 3-epi-25(OH)D<sub>3</sub> of total 25(OH)D was negatively correlated with total 25OHD (Spearman’s correlation coefficient, r = -0.40; p<0.05).

The 3-epi-25(OH)D<sub>3</sub> and Vitamin D Status

Table 2 illustrates reclassification of children when considering the 3-epi-25(OH)D<sub>3</sub> in total 25(OH)D. Good agreement was found between the two vitamin D status classification methods (κ = 0.78, 95%CI 0.72-0.84). However, among the 70 children with inadequate vitamin D without 3-epi-25(OH)D<sub>3</sub> in the total, 33% were reclassified from inadequate to adequate after addition of 3-epi-25(OH)D<sub>3</sub> to total 25(OH)D. Similarly, among the 203 children with adequate vitamin D levels without addition of 3-epi-25(OH)D<sub>3</sub>, 14% were reclassified from adequate to optimal. Overall, 15% of children with detectable 3-epi-25(OH)D<sub>3</sub> concentrations were reclassified to a higher vitamin D status when 3-epi-25(OH)D<sub>3</sub> was added to total 25(OH)D.
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Discussion

Evidence is limited on the physiological relevance of the 3-epi-25(OH)D$_3$ in children; thus a clearer understanding of the 3-epi-25(OH)D$_3$ can help determine the necessity of considering the 3-epi-25(OH)D$_3$ clinically and in studies examining vitamin D. With half of the children in the Daily D Health Study having detectable 3-epi-25(OH)D$_3$, we were able to determine that male, pre-pubertal, and non-Hispanic white children were more likely to have detectable 3-epi-25(OH)D$_3$ concentrations, which suggests that having detectable 3-epi-25(OH)D$_3$ is not a random phenomenon.

To our knowledge, there is no literature to compare these findings to other studies in children, but several of our findings coincide with existing literature on the 3-epi-25(OH)D$_3$ in adult population studies. The range of 3-epi-25(OH)D$_3$ concentration in the Daily D Health Study was similar to adults in the Survey of the Health of Wisconsin (SHOW), a cross-sectional population-based study in non-Hispanic white adults (0-4.9 ng/mL)(Engelman et al. 2014) and in the Atherosclerosis Risk in Communities Study (ARIC), which provided analyses of 3-epi-25(OH)D$_3$ separately by white and black adults (2.12 ng/mL and 2.16 ng/mL, respectively).(Lutsey et al. 2015) The concentration of 3-epi-25(OH)D$_3$ in infants has been shown to be much higher, with concentrations as high as 188 ng/mL,(Singh et al. 2006) suggesting that 3-epi-25(OH)D$_3$ concentrations drop from infancy to childhood, but do not drop from childhood to adulthood.

The correlation between 3-epi-25(OH)D$_3$ and 25(OH)D$_3$ was lower in our study than in two adult studies (r=0.40 vs. r=0.66)(Engelman et al. 2014), r=0.78(Cashman et al. 2014), respectively) but similar to the ARIC study (r=0.54 in whites and r=0.36 in blacks). This is surprising given that the SHOW and the National Adult Nutrition Survey (NANS) studies were observational, some participants were using supplements, and there was variation in season of data collection. Whereas, in the Daily D Health Study, our analysis was cross-sectional at the beginning of the trial prior to vitamin D supplementation and during the same season. Our lower correlation could be explained, however, by racial/ethnic differences, in that the SHOW and NANS studies were primarily non-Hispanic white and the Daily D Health Study had a diverse population.
We found that males were more likely to have detectable 3-epi-25(OH)D, but females were more likely to be above the median percent 3-epi-25(OH)D of 5.5%. The ARIC Study and a national survey in Thailand found that males had higher 3-epi-25(OH)D concentrations, (Lutsey et al. 2015, Chailurkit et al. 2015) thus sex seems to impact having detectable 3-epi-25(OH)D concentrations and the magnitude of concentrations, but the relationship requires further investigation. Our findings also indicate that non-Hispanic white children were more likely to have detectable 3-epi-25(OH)D, which is similar to the findings from ARIC, in which 33% of whites and 15% of blacks had detectable 3-epi-25(OH)D concentrations (Lutsey et al. 2015) and NHANES, in which 91% of non-Hispanic whites and 64% of non-Hispanic blacks had detectable 3-epi-25(OH)D. (Schleicher et al. 2011, 2016) In these studies, higher vitamin D intake was also associated with higher 3-epi-25(OH)D concentrations. Among our findings, dietary vitamin D was significantly higher in children with detectable 3-epi-25(OH)D, but dietary vitamin D intake was not associated with higher 3-epi-25(OH)D concentrations or higher percent 3-epi-25(OH)D. The NHANES and SHOW studies found lower waist circumference to be a predictor of higher 3-epi-25(OH)D concentrations, (Engelman et al. 2014, Cashman et al. 2014) which also does not coincide with our findings of no relationship between 3-epi-25(OH)D and weight status. Nonetheless, a study of healthy term infants aged newborn to 1 year also found that the 3-epi-25(OH)D was not predictive of lean mass, in contrast with the normal 25OHD variant. (Hazell et al. 2014)

When evaluating the relationship between 3-epi-25(OH)D and blood lipids, 3-epi-25(OH)D was associated with blood lipids only when examining 3-epi-25(OH)D as a percentage of total 25(OH)D. Total cholesterol was 7 ng/mL lower in children with percent 3-epi-25(OH)D above the median (5.5%). This suggests that there may be a physiological relevance of the 3-epi-25(OH)D, but not independent of 25(OH)D; rather the ratio of the two values could be important. Scant evidence exists on associations between the 3-epi-25(OH)D and blood lipids. In the ARIC Study, among both whites and blacks, HDL concentrations were higher with increasing tertiles of 3-epi-25(OH)D concentration, but no associations were found with LDL or triglycerides, and associations with total cholesterol were not reported. (Lutsey et al. 2015) In general, higher concentrations of 3-epi-25(OH)D were associated with a better
Investigation of the 3-epimer in children cardiovascular risk profile, including lower BMI, lower prevalence of diabetes, and lower CRP concentration, but this study did not examine these relationships using percent of 3-epi-25(OH)D₃ of total 25(OH)D. (Lutsey et al. 2015) Additional studies investigating potential physiologic relevance of the 3-epi-25(OH)D₃ are needed, particularly in children.

Currently, an individual’s vitamin D status is determined by 25(OH)D₃ concentrations, and whether 3-epi-25(OH)D₃ is included as part of 25(OH)D₃ is dependent on the assay used. The consideration of 3-epi-25(OH)D₃ in total 25(OH)D concentrations increased the proportion of children classified with a higher vitamin D status. There is potential practical relevance in 15% (51 out of 337 children with detectable 3-epi-25(OH)D₃) of children moving from inadequate to adequate status or adequate to optimal. Other studies have reported smaller percentages of individuals’ being reclassified to adequacy status with inclusion of the 3-epi-25(OH)D₃ in total 25OHD, such as 2-3% of adults in ARIC, (Lutsey et al. 2015) and 7% (APrON), (Aghajafari et al. 2016) to 9% of newborns. (Strathmann et al. 2012) This suggests that, particularly in younger age groups, regardless of the small serum concentrations, 3-epi-25(OH)D₃ could impact the identification of an individual being considered deficient or not, and contribute to determining supplementation needs. This may also be particularly important in individuals with lower 25(OH)D where a change in classification might be clinically meaningful, (Bailey et al. 2013) such as in individuals living in northern latitudes during the winter, those with darker skin, and those who are overweight or obese. Our data support the general consensus that the 3-epi-25(OH)D₃ should be quantified in infant/pediatric populations to avoid complicating interpretation of total 25(OH)D concentrations.

This was the first study to our knowledge to examine the 3-epi-25(OH)D₃ in a diverse group of school-aged children which provided a distribution of 3-epi-25(OH)D₃ detectability to make a useful comparison between children with and without detectable 3-epi-25(OH)D₃. Due to known differences in vitamin D by race/ethnicity, it may be appropriate to examine the association between 3-epi-25(OH)D₃ and cardiometabolic risk factors separately by racial/ethnic group, but small sample sizes in the present study precluded these analyses.
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In conclusion, we have shown that there are demographic characteristics that can impact 3-epi-25(OH)D$_3$ concentration, including sex and race/ethnicity. We have also demonstrated certain characteristics that are associated with having detectable 3-epi-25(OH)D$_3$. Even small concentrations of 3-epi-25(OH)D$_3$ can impact how individuals are classified based on vitamin D status. Our findings show that considering the 3-epi-25(OH)D$_3$ as a percent of 25(OH)D provides alternative but important insight for potential physiological relevance. Finally, further research is needed to clearly elucidate the physiological relevance of 3-epi-25(OH)D$_3$, particularly its relationship to cardiovascular risk factors.

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References


Hazell, T.J., Gallo, S., Berzina, L., Vanstone, C.A., Rodd, C., and Weiler, H.A. 2014. Plasma 25-hydroxyvitamin D, more so than its epimer, has a linear relationship to leaner body
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Table 1. Subject characteristics by detectable 3-epimer status and logistic regression for characteristics associated with odds of having detectable 3-epimer (n=682)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Detectable 3-epimer (n=337)</th>
<th>Non-detectable 3-epimer (n=345)</th>
<th>P value</th>
<th>Odds of having detectable 3-epimer (OR 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D Status (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate (&lt;20)</td>
<td>70 (25)</td>
<td>209 (75)</td>
<td>&lt;0.001</td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>Adequate (20-29)</td>
<td>203 (62)</td>
<td>123 (38)</td>
<td></td>
<td>4.78 (3.23, 6.94)</td>
</tr>
<tr>
<td>Optimal (≥30)</td>
<td>64 (83)</td>
<td>13 (17)</td>
<td></td>
<td>14.10 (7.10, 28.0)</td>
</tr>
<tr>
<td>Vitamin D Intake*** (IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥12</td>
<td>139 (46)</td>
<td>166 (54)</td>
<td>0.07</td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>&lt;12</td>
<td>198 (53)</td>
<td>179 (47)</td>
<td></td>
<td>1.15 (0.81, 1.64)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.02</td>
<td>1.38 (1.01, 1.89)</td>
</tr>
<tr>
<td>Female</td>
<td>156 (45)</td>
<td>192 (55)</td>
<td></td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>Male</td>
<td>181 (54)</td>
<td>153 (46)</td>
<td></td>
<td>1.38 (1.01, 1.89)</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>White/Caucasian</td>
<td>162 (57)</td>
<td>120 (43)</td>
<td></td>
<td>0.38 (0.23, 0.63)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>33 (34)</td>
<td>65 (66)</td>
<td></td>
<td>0.72 (0.41, 1.27)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>74 (49)</td>
<td>77 (51)</td>
<td></td>
<td>1.05 (0.66, 1.67)</td>
</tr>
<tr>
<td>Asian/Asian American</td>
<td>15 (27)</td>
<td>41 (73)</td>
<td></td>
<td>0.45 (0.22, 0.91)</td>
</tr>
<tr>
<td>Multiracial/Other</td>
<td>53 (56)</td>
<td>42 (44)</td>
<td></td>
<td>1.38 (0.81, 2.35)</td>
</tr>
<tr>
<td>Puberty Status</td>
<td></td>
<td></td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Pre-pubertal</td>
<td>233 (53)</td>
<td>205 (47)</td>
<td></td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>Pubertal</td>
<td>104 (43)</td>
<td>140 (57)</td>
<td></td>
<td>1.31 (0.91, 1.89)</td>
</tr>
<tr>
<td>Weight Status</td>
<td></td>
<td></td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Non-obese</td>
<td>243 (48)</td>
<td>262 (52)</td>
<td></td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>Obese</td>
<td>94 (53)</td>
<td>83 (47)</td>
<td></td>
<td>1.09 (0.76, 1.55)</td>
</tr>
<tr>
<td>Free or Reduced-price Lunch Eligibility</td>
<td></td>
<td></td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Not eligible</td>
<td>113 (51)</td>
<td>107 (49)</td>
<td></td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>Eligible</td>
<td>224 (49)</td>
<td>238 (51)</td>
<td></td>
<td>1.17 (0.82, 1.67)</td>
</tr>
</tbody>
</table>

*Krusall-Wallis test used for statistical significance between group medians
**Bold p values and odds ratios are statistically significant (p<0.05)
***Median (IQR) is reported for Vitamin D Intake.

a Model 1: Adjusted for age, sex, race, weight status, puberty status, & free/reduced lunch eligibility
b Model 2: Model 1 + vitamin D status
Table 2. Total number of children with detectable 3-epimer classified as inadequate, adequate, or optimal for serum 25(OH)D status with and without inclusion of the 3-epimer of 25(OH)D.

<table>
<thead>
<tr>
<th>Total 25(OH)D without 3-epimer</th>
<th>Inclusion of 3-epimer in total 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inadequate</td>
</tr>
<tr>
<td>Inadequate N=70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47 (67%)</td>
</tr>
<tr>
<td>Adequate N=203</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Optimal N=64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Inadequate <20 ng/mL, adequate 20-29 ng/mL, optimal ≥ 30 ng/mL.
Bold indicates change in status with consideration of 3-epimer.
Percentages are indicative of row percent.
*Weighted Kappa = 0.78
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Figure Captions

Figure 1. A) Distribution of 3-epimer of 25(OH)D among racially/ethnically diverse schoolchildren. B) Distribution of % 3-epi(25(OH)D$_3$). *

*One child not included in figure had a 3-epimer concentration of 12.3 ng/mL and % 3-epi-25(OH)D of 37.6.

Figure 2. A) Scatter plot of 3-epimer of 25(OH)D$_3$ and 25(OH)D$_3$. B) Scatterplot of percent 3-epimer and 25OHD$_3$.

*Individual with 3-epimer of 12.3 ng/mL not included in graph