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Investigation of the C-3-epi-25(OH)D_3 of 25-hydroxyvitamin D_3 in urban schoolchildren

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Abstract

The physiological relevance C-3 epimer of 25-hydroxyvitamin D (3-epi-25(OH)D) is not well understood among youth. The objective of this study was to assess whether demographic/physiologic characteristics were associated with 3-epi-25(OH)D_3 concentrations in youth. Associations between 3-epi-25(OH)D_3 and demographics and between 3-epi-25(OH)D_3, total 25-hydroxyvitamin (25(OH)D) (25(OH)D_2 + 25(OH)D_3), total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides were examined in racially/ethnically diverse schoolchildren (n = 682; age, 8–15 years) at Boston-area urban schools. Approximately 50% of participants had detectable 3-epi-25(OH)D_3 (range 0.95−3.95 ng/mL). The percentage of 3-epi-25(OH)D_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_3 concentrations. Both Asian and black race/ethnicity were associated with lower odds of having detectable 3-epi-25(OH)D_3 compared with non-Hispanic white children (Asian vs. white, odds ratio (OR) 0.28, 95% confidence interval (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_3 than having an inadequate (<20 ng/mL) concentration (OR

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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_3 and blood lipids. However, when considering 3-epi-25(OH)D_3 as a percentage of total 25(OH)D, total cholesterol was lower in children with percent 3-epi-25(OH)D_3 above the median (mean difference −7.1 mg/dL, $p = 0.01$). In conclusion, 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_3 may be related to the 3-epi-25(OH)D_3 as a percentage of total 25(OH)D and should be considered in future investigations.

Résumé:

La pertinence physiologique de l'épimère C-3 de la 25-hydroxyvitamine D (« 3-epi-25(OH)D ») chez les jeunes n'est pas bien établie. Cette étude se propose de vérifier si des caractéristiques démographiques/physiologiques sont associéesà la concentration de 3-epi-25(OH)D chez les jeunes. On examine, chez des enfants d'âge scolaire de diverses races/ethnies ($n = 682$, âge : 8–15 ans) dans la région urbaine de Boston, la relation entre 3-epi-25(OH)D_3 et des variables démographiques, entre 3-epi-25(OH)D_3 et 25-hydroxyvitamine (« 25(OH)D ») (25(OH)D_2 + 25(OH)D_3) totale, cholestérol total, lipoprotéines de haute densité, lipoprotéine de faible densité et les triglycérides. Environ 50 % des enfants présentent une concentration détectable de 3-epi-25(OH)D_3 (écart : 0,95−3,95 ng/mL). Le pourcentage de 3-epi-25(OH)D_3 parrapportà25(OH)Dtotalevariede2,5à17,0%(médiane:5,5%). Lesgarçonssont38%plusà mêmequelesfillesdeprésenterune concentration détectable de 3-epi-25(OH)D_3. Les enfants de race/ethnie noire et asiatique ont une plus faible probabilité de présenter une concentration détectable de 3-epi-25(OH)D_3 comparativement aux enfants de race blanche non hispaniques (Asiatiques vs Blancs, rapport des cotes (RC) 0,28, intervalle de confiance (IC) 95 % 0,14–0,53; Noirs vs Blancs, RC 0,38, IC 95 % 0,23−0,63, $p < 0,001$). Un taux adéquat (20–29 ng/mL) ou optimal (>30 ng/mL) de 25(OH)D est associéà une probabilité plus élevée de présenter une concentration détectable de 3-epi-25(OH)D_3 comparativementà un taux inadéquat (<20 ng/mL) : RC 4,78, IC 95 % 3,23–6,94 ou RC 14,10, IC 95 % 7,10–28,0, respectivement. Il n'y a pas de relation entre 3-epi-25(OH)D_3 et les lipides sanguins. Toutefois, en considérant 3-epi-25(OH)D_3 en pourcentage de 25(OH)D totale, les enfants présentant un taux de 3-epi-25(OH)D_3 supérieurà la médiane (différence moyenne : −7,1 mg/dL, $p = 0,01$) ont un plus faible taux de cholestérol total. *Conclusion*. Chez les enfants d'âge scolaire, le sexe, la race/ethnie et la concentration sérique de 25(OH)D totale sont différemment associés au taux de 3-epi-25(OH)D. La pertinence physiologique de 3-epi-25(OH)D_3 pourrait être envisagée en pourcentage de 25(OH)D totale et devrait être prise en considération dans les études ultérieures. [Traduit par la Rédaction]

Keywords

25-hydroxyvitamin D3; C-3 epimer; vitamin D; Daily D Health Study

Mots-clés

25-hydroxyvitamine D_3; épimère C-3; vitamine D; étude de la vitamine D journalière sur la santé

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 $25(OH)D_3$ because it has a long half-life, is not tightly regulated compared with 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 ultraviolet B radiation (Institute of Medicine (IOM) 2011; van den Ouweland et al. 2013). It is now known that 25(OH)D undergoes epimerization during metabolism, and the 3-epi-$25(OH)D_3$ of $25(OH)D_3$ is most prevalent, forming 3-epi-25-hydroxyvitamin D_3 (3-epi-25(OH)D) (Singh et al. 2006). Significant blood concentrations of the 3-epi-$25(OH)D_3$ 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-$25(OH)D_3$ is not routinely measured, and without chromatographic resolution, it could be mistakenly reported as $25(OH)D_3$ because of their identical molecular weights (Lensmeyer et al. 2006).

Laboratory studies have found potential physiological relevance of 3-epi-$25(OH)D_3$ 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-$25(OH)D_3$ (Cashman et al. 2014). One study examining 3-epi-25(OH)D, anthropometrics, and blood lipids, separately among black and white adults, found higher concentrations of 3-epi-$25(OH)D_3$ were associated with a better cardiovascular risk profile (Lutsey et al. 2015). Furthermore, the relationship among 3-epi-$25(OH)D_3$, anthropometrics, and blood lipids in children is especially unclear. Research is needed to understand the potential physiological relevance of the 3-epi-$25(OH)D_3$ to determine if it is necessary to consider the 3-epi-$25(OH)D_3$ when characterizing vitamin D status. To understand the significance of the 3-epi-$25(OH)D_3$ in children, we examined the 3-epi-$25(OH)D_3$ cross-sectionally among a diverse sample of schoolchildren in the Boston area. Our goals were to (*i*) determine if demographic characteristics were associated with 3-epi-$25(OH)D_3$ concentrations; (*ii*) examine associations between 3-epi-$25(OH)D_3$ concentration and blood lipids; and (*iii*) identify the impact of considering 3-epi-$25(OH)D_3$ on vitamin D status classification.

Materials and methods

Participants

This cross-sectional study investigated 3-epi-25(OH)D_3 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. The ClinicalTrials.gov registration number is NCT01537809.

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 (Centers for Disease Control and Prevention (CDC) 2013, Sacheck et al. 2015). Weight status was categorized into obese (≥95th percentile) and nonobese (<95th 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 for Children, which inquired about intake over the past week (NutritionQuest, Berkeley, Calif., USA).

25(OH)D and 3-epi-25(OH)D_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_2, 25(OH)D_3, and the 3-epi-25(OH)D_3 of 25(OH)D_3 (Holick et al. 2005). Serum samples of 25(OH)D were prepared and analyzed through a turbulent flow LC system (Cohesive Technologies, Franklin, Mass., USA) followed by traditional laminar flow chromatography and analyzed relative to the National Institute of Standards and Technology vitamin D standard for detection and quantification of 25(OH)D_3, 25(OH)D_2, and 3-epi-25(OH)D_3. The analysis was performed using a Triple Stage Quadrupole Quantum Ultra triple mass-spectrometer (Thermo Finnigan Corp., San Jose, Calif., USA). 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 (Dawson-Hughes et al. 2005; IOM 2011). Concentrations of 3-epi-25(OH)D_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 χ^2 test was used to compare the distribution of participant characteristics across groups by 3-epi-25(OH)D_3 status (detectable vs. nondetectable). 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_3 as a percent of total 25(OH)D, we added concentrations of 25(OH)D_2 and 25(OH)D_3 and the 3-epi-25(OH)D_3 of 25OHD_3, divided 3-epi-25(OH)D_3 concentration by the summed total 25(OH)D, and multiplied the result by 100. Logistic regression was used to examine the relationship between participant characteristics and the odds of having detectable 3-epi-25(OH)D_3, as well as the odds of being above or below the median of percent 3-epi-25(OH)D_3 (5.5%). Multiple linear regression was used to examine linear relationships between 3-epi-25(OH)D_3 concentration and blood lipids, adjusting for participant characteristics (age, sex, race, weight status, puberty status, and free/reduced lunch eligibility). The distribution of 3-epi-25(OH)D_3 and triglycerides were right-skewed and logarithmically transformed for this analysis.

Generalized linear models were used to examine the mean difference in blood lipids between children with and without detectable 3-epi-25(OH)D_3 and children above or at or below median of percent 3-epi-25(OH)D_3 and each was adjusted for participant characteristics and vitamin D status. Least-square means and standard errors were reported for total, LDL, and HDL cholesterol. *P* values from multiple comparisons were adjusted with Tukey's Honestly Significant Difference test. Because triglyceride data were skewed, results are reported as geometric mean and standard error, as well as ratio of geometric means and 95% confidence interval.

To assess the impact of including 3-epi-25(OH)D_3 in calculating total 25(OH)D concentration on vitamin D status classification, we compared the proportions of children with inadequate, adequate, and optimal 25(OH)D concentrations with and without consideration of 3-epi-25(OH)D_3. Agreement between the 2 methods was evaluated with weighted kappa statistics. We also examined the relationship between 3-epi-25(OH)D_3 and 25(OH)D as a percent of total 25(OH)D. Spearman's rank correlation was used to determine the correlation between 3-epi-25(OH)D_3 and percent 3-epi-25(OH)D_3 and 25(OH)D_3 concentrations. A *P* value of <0.05 was considered statistically significant for all analyses. Data were analyzed using SAS version 9.3 (SAS Institute, Cary, N.C., USA).

Results

Participant characteristics

Overall, 55% of children were <12 years old; 49% were male; 41% were non-Hispanic white, 15% black/African American, 22% Hispanic/Latino, 8% Asian/Asian American, and 14% multiracial/other; 64% had reached puberty; 67% were eligible for free or reduced/priced 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 versus nondetectable 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; Fig. 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 was significantly different between those with and without detectable 3-epi-25(OH)D_3 ($p < 0.001$). 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 ($p = 0.02$), prepubertal 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, male participants (odds ratio (OR) 1.38, 95% confidence interval (CI) 1.01−1.89) had significantly higher odds of having detectable 3-epi-25(OH)D_3 concentrations than female participants (Table 1). Asian or black participants had 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 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 with children with free or reduced-price lunch eligibility (median [interquartile range]: 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 with 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% (Fig. 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) %, $p = 0.033$).

3-epi-25(OH)D_3, percent 3-epi-25(OH)D_3 and blood lipids

We did not find a linear relationship between 3-epi-25(OH)D_3 and blood lipids (data not shown). In comparing blood lipid concentrations between children with detectable 3-epi-25(OH)D_3 and nondetectable 3-epi-25(OH)D_3, 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_3 was 7 mg/dL lower thanthoseatorbelowthemedian(149.6±2.8mg/dLvs. 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_3 and total 25(OH)D

The relationship between 3-epi-25(OH)D_3 and total 25(OH)D did not appear linear, with large variation in 3-epi-25(OH)D_3, particularly at higher concentrations of total 25(OH)D; nonetheless, 3-epi-25(OH)D_3 concentration was positively correlated with 25(OH)D_3 (Spearman's correlation coefficient, $r = 0.40$; $p < 0.05$; Fig. 2A). The relationship between percent 3-epi-25(OH)D_3 and total 25(OH)D was also nonlinear, with stable variability in percent 3-epi-25(OH)D_3 across increasing concentrations of total 25(OH)D (Fig. 2B). Percent 3-epi-25(OH)D_3 of total 25(OH)D was negatively correlated with total 25(OH)D (Spearman's correlation coefficient, $r = -0.40$; $p < 0.05$).

3-epi-25(OH)D_3 and vitamin D status

Table 2 illustrates reclassification of children when considering the 3-epi-25(OH)D_3 in total 25(OH)D. Good agreement was found between the 2 vitamin D status classification methods ([H9260] = 0.78, 95%CI 0.72–0.84). However, among the 70 children with inadequate vitamin D without 3-epi-25(OH)D_3 in total 25(OH)D, 33% were reclassified from inadequate to adequate after addition of 3-epi-25(OH)D_3 to total 25(OH)D. Similarly, among the 203 children with adequate vitamin D levels without addition of 3-epi-25(OH)D_3, 14% were reclassified from adequate to optimal. Overall, 15% of children with detectable 3-epi-25(OH)D_3 concentrations were reclassified to a higher vitamin D status when 3-epi-25(OH)D_3wasaddedto total 25(OH)D.

Discussion

Evidence is limited on the physiological relevance of 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 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, prepubertal, 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 with 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 (ARIC) study, 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 2 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_3, but females were more likely to be above the median percent 3-epi-25(OH)D_3 of 5.5%. The ARIC study and a national survey in Thailand found that males had higher 3-epi-25(OH)D_3 concentrations (Chailurkit et al. 2015; Lutsey et al. 2015), thus sex seems to impact having detectable 3-epi-25(OH)D_3 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_3, which is similar to the findings from the ARIC study, in which 33% of whites and 15% of blacks had detectable 3-epi-25(OH)D_3 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_3 (Schleicher et al. 2011, 2016). In these studies, higher vitamin D intake was also associated with higher 3-epi-25(OH)D_3 concentrations. Among our findings, dietary vitamin D was significantly higher in children with detectable 3-epi-25(OH)D_3, but dietary vitamin D intake was not associated with higher 3-epi-25(OH)D_3 concentrations or higher percent 3-epi-25(OH)D_3. The NHANES and SHOW studies found lower waist circumference to be a predictor of higher 3-epi-25(OH)D_3 concentrations (Cashmanetal.2014;Engelmanetal.2014), which also does not coincide withourfindingsofnorelationshipbetween3-epi-25(OH)D_3 and weight

status. Nonetheless, a study of healthy-term infants aged newborn to 1 year also found that the 3-epi-25(OH)D_3 was not predictive of lean mass, in contrast with the normal 25(OH)D_3 variant (Hazell et al. 2014).

When evaluating the relationship between 3-epi-25(OH)D_3 and blood lipids, 3-epi-25(OH)D_3 was associated with blood lipids only when examining 3-epi-25(OH)D_3 as a percentage of total 25(OH)D. Total cholesterol was 7 ng/mL lower in children with percent 3-epi-25(OH)D_3 above the median (5.5%). This suggests that there may be a physiological relevance of the 3-epi-25(OH)D_3, but not independent of 25(OH)D; rather the ratio of the 2 values could be important. Scant evidence exists on associations between the 3-epi-25(OH)D_3 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_3 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_3 were associated with a better cardiovascular risk profile, including lower BMI, lower prevalence of diabetes, and lower C-reactive protein concentration, but this study did not examine these relationships using percent of 3-epi-25(OH)D_3 for total 25(OH)D (Lutsey et al. 2015). Additional studies investigating potential physiologic relevance of the 3-epi-25(OH)D_3 are needed, particularly in children.

Currently, an individual's vitamin D status is determined by 25(OH)D_3 concentrations, and whether 3-epi-25(OH)D_3 is included as part of 25(OH)D_3 is dependent on the assay used. The consideration of 3-epi-25(OH)D_3 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)D_3) of children moving from inadequate to adequate status or adequate to optimal status. Other studies have reported smaller percentages of individuals being reclassified to adequacy status with inclusion of the 3-epi-25(OH)D_3 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)D_3 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)D_3 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)D_3 in a diverse group of school-aged children, which provided a distribution of 3-epi-25(OH)D_3 detectability to make a useful comparison between children with and without detectable 3-epi-25(OH)D_3. Due to known differences in vitamin D by race/ethnicity, it may be appropriate to examine the association between 3-epi-25(OH)D_3 and cardiometabolic risk factors separately by racial/ethnic group, but small sample sizes in the present study precluded these analyses.

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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Conflict of interest statement and funding disclosure

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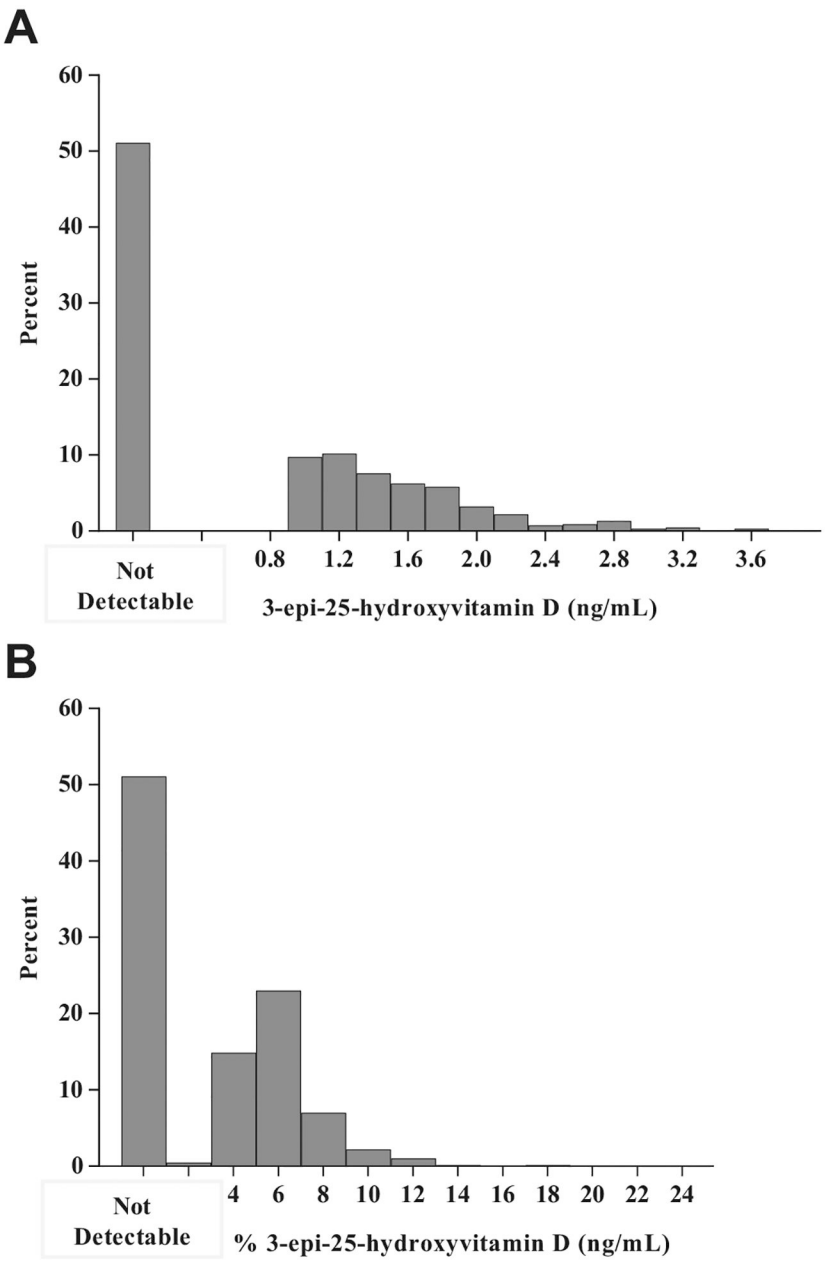

Fig. 1.

(A) Distribution of 3-epimer of 25-hydroxyvitamin D (3-epi-25(OH)D_3) among racially/ethnically diverse schoolchildren. (B) Distribution of percentage of 3-epi(25(OH)D_3. One child not included in the figure had a 3-epimer concentration of 12.3 ng/mL and a percentage of 3-epi-25(OH)D of 37.6%.

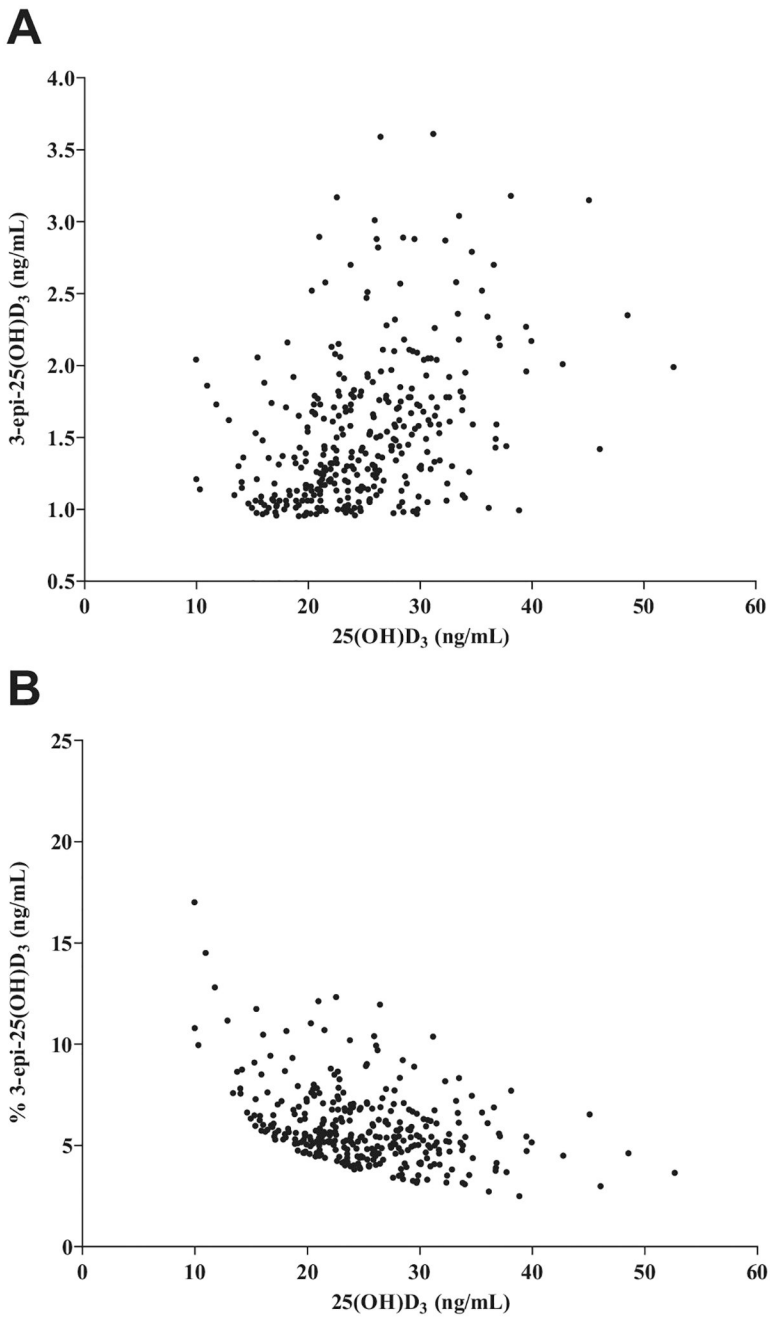

Fig. 2.

(A) Scatter plot of 3-epimer of 25-hydroxyvitamin D (3-epi-25(OH)D_3) and 25-hydroxyvitamin D (25(OH)D_3). (B) Scatterplot of percentage of 3-epi-25(OH)D_3 and 25(OH)D_3. Individual with 3-epimer of 12.3 ng/mL was not included in graph.

Table 1.

Subject characteristics by detectable epimer status and logistic regression for characteristics associated with odds of having detectable 3-epimer (n = 682).

	Detectable versus nondetectable			Odds of having detectable 3-epimer	
Subject characteristics	**Detectable 3-epimer, N = 337; n (row %)**	**Nondetectable 3-epimer, N = 345; n (row %)**	***P***	**Model 1[a]; OR (95% CI)**	**Model 2[b]; OR (95% CI)**
Vitamin D status (ng/mL)			**<0.001**		
Inadequate (<20)	70 (25)	209 (75)		1.00	1.00
Adequate (20–29)	203 (62)	123 (38)		—	**4.78 (3.23, 6.94)**
Optimal (≥30)	64(83)	13 (17)		—	**14.10 (7.10, 28.0)**
Vitamin D intake (IU)*	120 (70,199)	103 (56, 163)	**0.005**†	—	—
Age (y)			0.07		
≥12	139 (46)	166 (54)		1.00	1.00
<12	198 (53)	179 (47)		1.15 (0.81, 1.64)	1.08 (0.74, 1.58)
Sex			**0.02**		
Female	156 (45)	192 (55)		1.00	1.00
Male	181 (54)	153 (46)		**1.38 (1.01, 1.89)**	1.18 (0.79, 1.57)
Race/ethnicity			**<0.001**		
White/Caucasian	162 (57)	120 (43)		1.00	1.00
Black/African American	33 (34)	65 (66)		**0.38 (0.23, 0.63)**	0.72 (0.41, 1.27)
Hispanic/Latino	74 (49)	77 (51)		0.73 (0.53, 0.99)	1.05 (0.66, 1.67)
Asian/Asian American	15 (27)	41 (73)		**0.28 (0.14, 0.53)**	**0.45 (0.22, 0.91)**
Multiracial/other	53 (56)	42 (44)		0.96 (0.60, 1.56)	1.38 (0.81, 2.35)
Puberty status			**0.008**		
Prepubertal	233 (53)	205 (47)		1.00	1.00
Pubertal	104 (43)	140 (57)		1.31(0.91,1.89)	1.25 (0.83, 1.86)
Weight status			0.25		
Nonobese	243 (48)	262 (52)		1.00	1.00
Obese	94 (53)	83 (47)		1.09 (0.76,1.55)	1.38 (0.93, 2.03)
Free or reduced-price lunch eligibility			0.48		
Not eligible	113 (51)	107 (49)		1.00	1.00
Eligible	224 (49)	238 (51)		1.17 (0.82, 1.67)	1.26 (0.86, 1.86)

Note: Bold p values and ORs are statistically significant ($p < 0.05$). CI, confidence interval; OR, odds ratio.

[a] Model 1: Adjusted for age, sex, race, weight status, puberty status, and free/reduced lunch eligibility.

[b] Model 2: Model 1 + vitamin D status.

[*] Median (interquartile range) is reported for vitamin D intake.

[†] Kruskal–Wallis test used for statistical significance between group medians.

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 ($N = 337$).*

Total 25(OH)D without 3-epimer	Inclusion of 3-epimer in total 25(OH)D		
	Inadequate	Adequate	Optimal
Inadequate, $n = 70$	47 (67%)	**23 (33%)**	0 (0%)
Adequate, $n = 203$	0(0%)	175 (86%)	**28 (14%)**
Optimal, $n = 64$	0(0%)	0 (0%)	64 (100%)

Note: 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. 25(OH)D, 25-hydroxyvitamin D.

*Weighted Kappa = 0.78.