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Association of serum 25-hydroxyvitamin D with race/ethnicity and constitutive skin color in urban schoolchildren

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Abstract

The objective of this study was to determine the extent to which constitutive skin color explains racial/ethnic differences in serum 25-hydroxyvitamin D (25OHD) concentrations in urban schoolchildren. Analysis of covariance (ANCOVA) was used to determine associations of 25OHD with parent-reported race/ethnicity and constitutive skin color as measured by reflectance colorimeter [individual typology angle (ITA°; higher value corresponds to lighter skin)] in 307 Greater Boston schoolchildren aged 9–15 during October–December 2011. Nearly 60% of all children were inadequate in 25OHD (<20 ng/mL). Prevalence of inadequate 25OHD differed by race/ethnicity ($p < 0.001$): white (46.6%), black (74.5%), Hispanic (64.7%), Asian (88.9%), and multi-racial/other (52.7%). Serum 25OHD increased 0.6 ng/mL per 10° increase in ITA° value ($p < 0.001$). The prediction of 25OHD by race/ethnicity was slightly stronger than the prediction by skin color in separate models ($R^2 = 0.19$, $R^2 = 0.16$, respectively). Most of the variability in 25OHD in race/ethnicity was due to constitutive skin color in this group of racially diverse US children.

Keywords

children; race; serum-25-hydroxyvitamin D; skin color; vitamin D

Introduction

Vitamin D deficiency may be associated with a variety of diseases that can affect children, including upper respiratory infections, autoimmune diseases, certain cancers, and

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cardiometabolic diseases (1). Some vitamin D is obtained from dietary sources, however, the majority of vitamin D in many populations is synthesized in the skin through exposure to UVB light (1). Solar UVB radiation (wavelength 290 to 315 nm) penetrates the skin and converts 7-dehydrocholesterol to provitamin D₃, which is then converted to vitamin D₃ (1, 2)

The efficiency of provitamin D₃ synthesis in the skin is dependent on the number of UVB photons that penetrate the skin. The skin pigment, melanin, absorbs UVB and determines the number of photons that reach the lower malpighian cellular layers of the skin, where vitamin D₃ synthesis takes place (3). Darker-skinned racial/ethnic groups have been shown to have lower serum 25-hydroxyvitamin D (25OHD) concentrations than lighter skinned groups living in the same geographic area (4–6). However, race/ethnicity is only a proxy measure of skin color. There is a large gradation of skin color within groups and considerable overlap between groups (5).

This is the first study to examine the relationship between race/ethnicity, skin color, and serum 25OHD level using quantitative measures of skin color in racially/ethnically diverse groups of US children from northern latitudes. While it remains unknown how much of the variation in 25OHD levels may be explained by differences in skin color across populations, it is well understood that 25OHD levels are influenced by a large variety of factors and that racial/ethnic differences may not be due only to skin color differences. Future identifications of other factors that influence 25OHD status may provide new targets for clinical and public health interventions. The hypothesis was that constitutive skin color would explain most or all of the racial/ethnic differences in serum 25OHD.

Materials and methods

Study subjects

Subjects were enrolled in the Daily D Health Study, a randomized, double-blind trial, assessing the impact of a 6-month vitamin D supplementation on serum 25OHD and cardiometabolic risk factors in 4th–8th grade schoolchildren. Schoolchildren in the 4th–8th grades (9–15 years) were recruited from three urban school districts (Everett, Malden, and Somerville, MA, USA) north of Boston (42° N) during October–December of 2011. This age range was chosen because previous research in children of similar age from the Boston area showed high rates of low 25OHD status (7, 8). Participants were recruited from five elementary/middle schools through classroom presentations, school newsletters, and fliers sent home with the children. Children who were currently taking vitamin D or multi-vitamin supplements were required to have a minimum of a 2-week wash-out period prior to the start of the study due to the relatively short half-life of serum 25OHD of 15 days in the circulation (9). Children were also excluded if they were taking oral glucocorticoids, or had rickets, cystic fibrosis, kidney disease, sarcoidosis, irritable bowel syndrome, epilepsy, or HIV/AIDS. Each student was given a gift card to a large local retailer for participating. Consent forms and study information materials were available in English, Spanish, Portuguese, Haitian-Creole, and Chinese, the major languages spoken in the communities. Both parental informed consent and the child's assent were obtained. The study protocol was approved by Tufts University's Institutional Review Board (#1103016).

Subjects arrived between 6:45 and 8:00 am, which was before the start of the school day. In addition to blood collection, study measurements included height and weight, skin color measurements, dietary and physical activity questionnaires, and a self-administered questionnaire to assess pubertal status.

Sociodemographics

Birth date, race/ethnicity, and maternal education were reported by parents during the informed consent procedure. Age in months was calculated as examination date minus birth date and converted to age in years. Parents were asked to choose one of the following to describe their children: white/Caucasian, black/African American, Mexican/Mexican-American, other Hispanic/Latino, Asian/Asian-American/Asian-Indian, Native American/American Indian, multi-racial/ethnic, or other (10). Parents were asked to provide the highest level of education received by the mother: no formal schooling, <8th grade, 8th grade but less than high school, high school graduate, post-high school trade/technical school, 1–3 years of college, college graduate, or graduate work/higher degree.

Anthropometric and pubertal status measures

Height and weight were measured in triplicate with light clothing and without shoes. Height was measured using a portable stadiometer (Model 214, Seca Weighing and Measuring Systems, Hanover, MD, USA) with the head in the Frankfurt plane made with a right angle height procedure (11) and recorded to the closest 1/8th inch. Weight was measured on a portable balance beam scale (Healthometer, Boca Raton, FL, USA) and recorded to the closest 0.25 pound. Body mass index (BMI) was calculated and then expressed as a z-score (BMIz) using the US Centers for Disease Control sex-specific growth charts (12). Pubertal status was assessed by asking the female subjects if they had reached menarche (yes/no) and male subjects if their voice had changed (not yet started/barely started/definitely underway/seems complete) (13, 14). Answering “yes” for menarche for girls or answering “definitely underway” or “seems complete” for voice change for boys was considered a marker for puberty.

Dietary intake and physical activity

Dietary intakes were assessed using the Block Kid 2004 Food Frequency Questionnaire (15, 16) (NutritionQuest, Berkeley, CA, USA), which was used in a previous study in a similar population of children (8). The 8-page questionnaire asked about frequency and quantity of 78 foods eaten, as well as multivitamin intake over the past week and took approximately 20–30 min to complete. The dietary data were quantified and reported as daily intakes of energy and nutrients. Dietary vitamin D was adjusted for total energy intake for analyses. The Block Kids Physical Activity Screener (NutritionQuest, Berkeley, CA, USA) was used to estimate total energy expenditure in calories expended. The one-page form asked subjects to report frequency and duration for leisure activities, school activities, chores, part-time jobs, and hours per day watching television, playing video games, and using the internet.

Skin color

Skin color was measured at an anatomical site by reflectance colorimetry using the ChromaMeter 400 (CR-400; Konica Minolta, Osaka, Japan) by trained research assistants following an established protocol (17–19). Research assistants were instructed to remove and subsequently reposition the ChromaMeter head following each reading at each site. Measurements on the upper inner arm, midway between the axilla and the medial epicondyle, were taken to estimate constitutive skin color. Measurements on the posterior forearm, midway between the olecranon and the ulnar epicondyle, were taken to estimate facultative skin color, which consists of constitutive skin color plus tanning. Measurements were taken around dense freckling or moles present. If a reading was compromised because the subject moved, the research assistant lost grip on the ChromaMeter handle, or other errors, that reading was immediately replaced with another (fifth) reading. Two variables were reported (L^* and b^*) based on the mean of four measurements, representing the degrees along the lightness-darkness (L^*) and yellow-blue axes (b^*), respectively. These two values were used to calculate the individual typology angle (ITA°), indicative of skin color: $ITA^\circ = [(\text{ArcTangent}(L-50/b)) \times 180/\pi]$ (20, 21).

Serum 25OHD

Total serum 25OHD was measured by the validated (22) liquid chromatography-mass spectrometry (LC-MS/MS) method including fractionation of 25OHD₃ and 25OHD₂ in serum (23); 25OHD₃ and 25OHD₂ calibration control solutions were generated from National Institute of Standards and Technology (NIST) reference standards (24) provided by Calbiochem (San Diego, CA, USA). 25OHD samples from study subjects were prepared and analyzed through a turbulent flow LC system (Cohesive Technologies, Franklin, MA, USA) followed by traditional laminar flow chromatography. The study samples were analyzed relative to the control solutions (NIST vitamin D standard references) for detection and quantification of the 25OHD₃ and 25OHD₂ component of each sample. The analysis was performed using a TSQ Quantum Ultra triple mass-spectrometer (Thermo Finnigan Corp., San Jose, CA, USA). The intra-assay coefficient of variation was 6.0%. NIST internal standards were used to confirm consistency of the assay over time. Serum 25OHD status was classified as inadequate (<20 ng/mL) according to the Institute of Medicine criteria (22, 25) and 30 ng/mL based on research suggesting that this may be the optimal level of 25OHD for both children and adults (26, 27). Concentrations in nmol/L were obtained from multiplying by 2.496.

Statistical analyses

The study originally included 310 subjects. Data on three participants were excluded from analyses because they were missing parent-reported race/ethnicity ($n=1$), skin color measurement ($n=1$), or had parent-reported hyperpigmentation, leaving 307 subjects. Intra-class correlation coefficients to test the reliability of the ChromaMeter skin color readings were calculated on four measurements of skin color on a sub-sample of children ($n=34$), following similar methods of previous researchers (19, 28). Racial/ethnic categories were aggregated to white, Hispanic, black, Asian, and multi-racial/other. Constitutive and facultative skin color was used as a continuous variable (ITA°) and categorized into three

groups: light ($ITA^{\circ} > 41^{\circ}$), intermediate ($41^{\circ} > ITA^{\circ} > 28^{\circ}$), and brown ($28^{\circ} > ITA^{\circ}$). Dietary vitamin D and total energy were used as continuous variables (IU/day and kcals/day, respectively). Physical activity was used as a continuous variable as total calories expended/day. Self-reported pubertal status was coded as a binary variable as puberty (yes/no). Weight status was dichotomized to healthy weight (BMI 5th and <85th percentile-for-age) and overweight/obese (BMI ≥85th percentile-for-age) for descriptive analyses. Six subjects were classified as underweight and their data were excluded from descriptive analyses. Maternal education was coded as a binary variable as post-secondary education (yes/no). Chi-square tests were used to compare the distributions of categorical variables between racial/ethnic groups. Analysis of variance with Bonferroni adjustments were used to compare continuous variables between the five racial/ethnic groups. Analysis of covariance (ANCOVA) was used to determine the association of constitutive skin color with total serum 25OHD before and after including race/ethnicity. An F-test for comparison of R^2 was completed to determine if the addition of race/ethnicity to the model that included skin color was significantly different from the model without race/ethnicity. Covariates that were controlled for included age, sex, BMIz, energy-adjusted dietary vitamin D, total energy expenditure, reported maternal education, and pubertal status. The potential interaction between race/ethnicity and constitutive skin color as predictors of serum 25OHD was investigated by including a cross-product term in ANCOVA analyses. A p-value of <0.05 was considered statistically significant. Data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Subject characteristics

The study population consisted of 52% females. Nearly half (46%) of the children were overweight/obese. Approximately 60% had inadequate concentrations of 25OHD (<20 ng/mL) and only 5% had levels ≥30 ng/mL. Most (61%) of the children were from racial/ethnic minorities. Hispanics were predominantly from El Salvador and Puerto Rico. Blacks included African-Americans and Haitians. Most Asians were Chinese, Indian, or Nepalese.

Selected characteristics of the 307 study subjects are shown by race/ethnicity in Table 1. Seventy-percent of racial/ethnic minorities compared to 47% of white children were inadequate in serum 25OHD. Race/ethnicity was also associated with BMIz, weight status, serum 25OHD, inadequate 25OHD status, energy-adjusted total vitamin D intake, total energy expenditure, maternal education, and constitutive and facultative skin color. Age, sex, total energy intake, or reported puberty did not differ by race/ethnicity.

Association of skin color, race/ethnicity, and serum 25OHD

All intra-class correlations of L^* and b^* constitutive skin color measurements fell within a range of 0.90 to 0.99 ($p < 0.001$). Figure 1 shows the relationship between constitutive ITA° skin color and serum 25OHD. As ITA° values increase, indicating lighter skin, serum 25OHD increases (model $R^2 = 0.05$, $p < 0.001$). White children had the lightest skin and highest 25OHD concentrations. Black children had the darkest skin color with the widest range in constitutive skin color ITA° values and lowest 25OHD concentrations. Specifically,

serum 25OHD increased 0.6 ng/mL for each 10° increase in ITA° value ($p<0.001$). The majority of black children fell in the lowest ITA° values.

Table 2 shows associations of serum 25OHD with race/ethnicity and skin color separately and together. When race/ethnicity and constitutive skin color were considered separately, race/ethnicity explained 3% more of the variability in 25OHD than skin color. However, when both variables were included in the same model, the results suggest that skin color is completely captured by race/ethnicity ($F=2.67$, $p=0.03$). The interaction between race/ethnicity and constitutive skin color and serum 25OHD was not significant.

Discussion

The results suggest that having a lighter skin color in winter is associated with higher serum 25OHD concentrations. This is consistent with previous research showing that the level of skin pigmentation is inversely associated with serum 25OHD concentrations (18, 29). While most studies have shown that individuals with dark skin tend to have lower serum 25OHD concentrations than those with lighter skin (25, 30), this study is, to our knowledge, the first to examine the association between quantitative measures of skin color, race/ethnicity, and serum 25OHD in a diverse group of US children. These data indicate that both parentally defined race/ethnicity and constitutive skin color predicted 25OHD in this population, however, most of the variability in 25OHD in race/ethnicity was due to constitutive skin color. Furthermore, black and Asian children had lower serum 25OHD concentrations than white children, a finding that is consistent with other studies that included darker-skinned subjects (5, 18).

Overall, race/ethnicity was a modestly stronger predictor of serum 25OHD concentrations than an objective measure of skin color in this population. Despite the darker skin color and lower 25OHD concentrations observed among racial/ethnic minorities than among whites, constitutive skin color did not improve prediction of 25OHD concentrations when included in the model with race/ethnicity. These findings are similar to Nessvi et al. (18) who reported that the association between constitutive skin color and serum 25OHD in adults disappeared after including ethnicity. This could be due to race/ethnicity capturing genetic differences and/or additional lifestyle determinants of 25OHD other than skin color that are associated with race/ethnicity.

Among this study's strengths was the use of a reliable and reproducible quantitative skin color tool (31–33). In addition, the study population comprised a racially and ethnically diverse sample of schoolchildren with a large gradation in skin colors compared to previous studies (17, 18, 34, 35). This study was limited by its cross-sectional nature. The modest sample size of some of the racial/ethnic groups may have been insufficient to detect the association between skin color, race/ethnicity, and serum 25OHD. For the few children who reported taking supplements containing vitamin D, the wash-out period of 2 weeks may not have been sufficient as the half-life of serum 25OHD is 15 days. Another limitation was that there was no measure of exposure to UV radiation, including outdoor behavior, such as the clothing worn, or use of sunbeds. However, in Boston, latitude 42 degrees north, little vitamin D synthesis occurs in October and virtually none occurs from November through

February (36). Thus, this study was well-timed with respect to minimizing seasonal differences between students in the effects of sun exposure on 25OHD. Further, while adjustments were made for a wide range of factors that influence 25OHD, such as age, sex, diet, socioeconomic, and pubertal status, a large amount (~80%) of variability in 25OHD remains unexplained. Future research examining the factors that account for this large variability in 25OHD, such as genetic differences, is warranted.

In summary, both race/ethnicity and constitutive skin color were associated with serum 25OHD; however, most of the variability in 25OHD in race/ethnicity was due to constitutive skin color in this group of racially diverse US children. Future research identifying the other factors that affect 25OHD status may provide a new focus for public health interventions.

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Figure 1. Constitutive skin color and serum 25-hydroxyvitamin D by race/ethnicity of urban schoolchildren (n=307) during October to December 2011.

Table 1

Selected characteristics by race/ethnicity in urban schoolchildren (n=307).

	White (n=118)	Black (n=47)	Hispanic/ Latino (n=51)	Asian (n=36)	Multi-racial/ other (n=55)	Overall p-Value
Age ^a	11.3 (1.6)	11.4 (1.5)	11.3 (1.4)	11.6 (1.8)	11.4 (1.3)	0.78
Female, % ^c	45.8%	57.5	60.8	50.0	54.6	0.38
BMIz ^d	0.8 (1.0)	1.0 (0.9)	1.1 (1.0)	0.0 (1.3) ^b	0.7 (1.1)	<0.001
Overweight/obese, % (n=301) ^e	47.0%	48.9	60.8	25.0	40.7	0.03
Serum 25OHD, ng/mL ^f	20.7 (6.6)	16.1 (6.5) ^b	17.2 (6.0) ^b	14.4 (5.4) ^b	19.3 (7.1)	<0.001
Inadequate 25OHD (<20 ng/mL), %	46.6%	74.5	64.7	88.9	52.7	<0.001
25OHD, 30 ng/mL, %	7.6%	4.3	2.0	0	5.5	0.31
Total energy intake (kcal/day) ^g , (n=306)	1435 (1101)	1859 (2094)	1491 (1174)	1192 (408)	1822 (1347)	0.07
Dietary vitamin D intake (IU/day) ^{h,i} , (n=306)	147 (82)	104 (83) ^b	111 (71) ^b	133 (65)	106 (68) ^b	0.001
Total energy expenditure (kcal/day), (n=306)	470 (440)	577 (513)	400 (418)	323 (315)	614 (538)	0.01
Maternal education, (% post-secondary), (n=300)	65.2%	43.5	29.4	45.7	52.8	<0.001
Self-reported pubertal status (% yes), (n=290)	37.8%	54.4	37.0	48.6	42.3	0.32
Constitutive skin color ^j						
ITA° mean	50.1° (10.1)	−10.7° (25.2) ^b	34.1° (10.7) ^b	28.7° (14.6) ^b	31.3° (14.9) ^b	<0.001
ITA° category						<0.001
Light, %	83.9	6.4	25.5	22.2	29.1	
Intermediate, %	13.6	0	47.1	27.8	38.2	
Brown, %	2.5	93.6	27.5	50.0	32.7	
Facultative skin color ^j						
ITA° mean	28.6° (10.9)	−28.0° (22.3) ^b	12.0° (12.5) ^b	9.1° (14.7) ^b	11.8° (14.1) ^b	<0.001
ITA° category						<0.001
Light, %	11.0	0	3.9	0	0	
Intermediate, %	39.8	2.1	5.9	13.9	9.1	
Brown, %	49.2	97.9	90.2	86.1	90.9	

^a ANOVA tests with Bonferonni adjustments.^b (p<0.05) compared to white children.^c Chi-square tests.^d BMI, body mass index z-score.^e Overweight/obese weight status is classified as BMI ≥85th percentile for age; n=6 were underweight and removed from analyses.^f To convert ng/mL to nmol/L, multiply by 2.5.^g To convert kcal to kJ, multiply by 4.184.^h To convert IU to µg, divide by 40.ⁱ Adjusted by total energy intake.

^j Skin color: light: individual typology angle (ITA°) >41; intermediate: 41>ITA°>28; brown: 28>ITA°; 25OHD, 25-hydroxyvitamin D.

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Table 2Associations of serum 25-hydroxyvitamin D with constitutive skin color and race/ethnicity.^a

	Model 1 Constitutive skin color ^c	p-Value	Model 2 Race ^c	p-Value	Model 3 Race+ constitutive skin color ^c	p-Value
Race/ethnicity (mean±SE)				<0.001		0.04
White			20.0 (0.6)		19.5 (0.8)	
Black			16.3 (0.9) ^b		17.4 (1.4)	
Hispanic			18.1 (0.9)		18.1 (0.9)	
Asian			15.3 (1.1) ^b		15.4 (1.1) ^b	
Other			18.8 (0.9)		18.8 (0.9)	
Constitutive skin color, β (SE) ^d	0.5 (0.2)	<0.001			0.3 (0.3)	0.30
Model R ²		0.16		0.19		0.19

^aLeast square means.^b(p<0.05) compared to white children.^cAdjusted for age, gender, body mass index z-score, energy-adjusted dietary vitamin D, total energy expenditure, maternal education, and pubertal status.^dConstitutive skin color (individual typology angle)×10. SE, standard error.