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## Associations of Vitamin D Intake with 25-Hydroxyvitamin D in Overweight and Racially/Ethnically Diverse US Children

## Lauren E. Au, PhD, RD [researcher],

Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA.

## Gail T. Rogers, MA [senior statistical programmer],

Jean Mayer USDA Human Nutrition Research Center on Aging, Boston, MA.

## Susan S. Harris, DSc [associate professor],

Friedman School of Nutrition Science and Policy, Tufts University, and a scientist I, Jean Mayer USDA Human Nutrition Research Center on Aging, Boston, MA.

## Johanna T. Dwyer, DSc, RD [professor],

Friedman School of Nutrition Science and Policy, Tufts University, and a senior scientist, Jean Mayer USDA Human Nutrition Research Center on Aging, Boston, MA.

## Paul F. Jacques, DSc [professor], and

Friedman School of Nutrition Science and Policy, Tufts University, and a senior scientist, Jean Mayer USDA Human Nutrition Research Center on Aging, Boston, MA.

## Jennifer M. Sacheck, PhD [associate professor]

Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA.

## Abstract

Overweight children and minorities are at risk of vitamin D deficiency. Little information exists on whether overweight children and minorities who do not meet dietary vitamin D recommendations are at risk for low 25-hydroxyvitamin D (25OHD) status. Vitamin D intake from foods and dietary supplements was estimated in 3,310 children/adolescents who were examined as part of the 2005-2006 National Health and Nutrition Examination Survey. Weight status was dichotomized into healthy weight or overweight/obese. Parent-reported race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Mexican American, or other. Adjusted logistic regression was used to determine whether children who did not achieve the Estimated Average Requirement (EAR) were at increased risk for inadequate 25OHD. Nearly 75% of children failed to meet the EAR. Overall, not meeting the EAR was associated with inadequate 25OHD (odds ratio=2.5; 95% CI 1.4 to 4.5). However, this association differed by weight status (P=0.02) and race/ethnicity (P=0.02). Overweight/obese children who failed to meet the EAR were five times more likely to be at risk for inadequate 25OHD than overweight/obese children who met it (95% CI 2.0 to 12.7; P<0.001). Non-Hispanic blacks with intakes below the

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Address correspondence to: Lauren Au, PhD, RD, Tufts University Friedman School of Nutrition Science and Policy, 150 Harrison Ave, Boston, MA 02111. lauren.au@alumni.tufts.edu.

STATEMENT OF POTENTIAL CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

EAR were nearly four times more likely to be at risk for inadequate 25OHD than those who met the EAR (95% CI 1.5 to 9.7; P<0.01). The majority of US children failed to meet current vitamin D recommendations. Overweight/obese and non-Hispanic black children were especially likely to be at risk for inadequate 25OHD when not consuming the EAR.

#### Keywords

Vitamin D intake; 25-Hydroxyvitamin D; Obesity; Children; Ethnically diverse

MOST CIRCULATING SERUM 25-HYDROXY-vitamin D (250HD) comes from exposure of the skin to ultraviolet rays in natural sunlight. However, seasonal changes in sunlight exposure, living at high latitudes, dark skin pigmentation, and other factors can impair the exposure,<sup>1</sup> making it important to meet dietary vitamin D recommendations to maintain adequate serum 250HD concentrations. In 2011, the Institute of Medicine (IOM) revised its recommendations for dietary reference intakes for vitamin D, increasing it from 200 IU (5  $\mu$ g) (the previous Adequate Intake) to an Estimated Average Requirement (EAR) of 400 IU (10  $\mu$ g), to meet the needs of half of children aged 1 to 18 years and set 600 IU (15  $\mu$ g) daily as the Recommended Dietary Allowance to meet the needs of 97.5% of individuals.<sup>2</sup> However, many children failed to meet even the previous Adequate Intake recommendations,<sup>3</sup> and intakes were also found to vary by weight status<sup>4</sup> and race/ ethnicity.<sup>5,6</sup>

In addition, various studies have found that attaining adequate 25OHD concentrations ( 20 ng/mL [ 50 nmol/L]) is more challenging for overweight/obese individuals and racial/ethnic minorities.<sup>4,7,8</sup> Serum 25OHD has been found to be low in obese adults,<sup>4,8</sup> but the association of 25OHD with overweight and obesity has not been well characterized in children. In addition, 20% of children aged 6 to 11 years in recent cycles of National Health and Nutrition Examination Survey (NHANES) conducted in 2003-2004 and 2005-2006, were reported to have serum 25OHD concentrations <20 ng/mL<sup>9</sup> (<50 nmol/L), and non-Hispanic blacks had higher rates of inadequate 25OHD status than non-Hispanic whites (51% vs 9%).

To the best of our knowledge, since the release of the IOM report, the risk of vitamin D inadequacy relative to the 2011 Dietary Reference Intakes has not been shown in US children by weight status or race/ethnicity. The presence of lower 25OHD concentrations in overweight/obese and racially/ethnically diverse populations needs to be confirmed and the possible reasons for differences in these concentrations explored to better manage inadequate vitamin D status in children.<sup>8</sup> The objective of this study was to examine the association between not meeting the EAR and inadequate 25OHD status, and how this relationship differed by weight status and race/ethnicity in a nationally representative population-based sample of US children and adolescents aged 1 to 18 years who were surveyed in the NHANES 2005-2006.

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## METHODS

#### **Overview of NHANES**

NHANES is a nationally representative, cross-sectional population-based survey that samples noninstitutionalized, civilian US residents using a complex, stratified, multistage probability cluster sampling design.<sup>10</sup> NHANES oversamples certain subgroups of people including black, Mexican-American, and low-income individuals. Written informed consent was obtained from all participants or proxies and the survey protocol was approved by the Research Ethics Review Board at the National Center for Health Statistics. Each person was interviewed to collect data on demographics, socioeconomic status, dietary habits including dietary supplement use, and health-related topics. Examinations were conducted on each participant to obtain medical and laboratory measures. Information on latitude of residence is not available in the public-use dataset to protect respondent confidentiality. Latitude data were accessed through the Research Data Center at the National Center for Health Statistics.

#### **Study Sample**

For this study, data on children and adolescents aged 1 to 18 years from NHANES 2005-2006 were included. Children with missing data for 24-hour dietary recalls, serum 25OHD, height or weight, and those pregnant or lactating were excluded (n=722). After applying the exclusion criteria, 3,310 total eligible subjects were available for analyses.

#### **Questionnaire Data**

Age was categorized into 1- to 8-years-old and 9- to 18-years-old groups. Race/ethnicity was self-reported as non-Hispanic white, non-Hispanic black, Mexican American, and other (ie, individuals of several races and those whose race/ethnicity was other than non-Hispanic white, non-Hispanic black, or Mexican).<sup>11</sup> Poverty-to-income ratio was defined as the ratio of a family's income relative to the US poverty threshold and was categorized into three groups: <130%, 130% to <350%, and 350%.<sup>12</sup> Sedentary time was calculated based on the number of hours per day spent watching television and videos and using a computer. Data in the northern United States were collected in summer (May 1 through October 31) and data in the south were collected in winter (November 1 through April 30). Latitude was dichotomized to north (35°N) and south (<35°N) based on estimated levels of ultraviolet light exposure.<sup>13,14</sup>

#### Anthropometrics

Height, weight, and waist circumference were collected based on NHANES anthropometry and physical activity manual standards.<sup>15</sup> Weight status was classified into three groups: underweight (body mass index [BMI] <5th percentile for age), healthy weight (BMI 5th and <85th percentile for age), and overweight/obese children (BMI 85th percentile for age) based on the Centers for Disease Control and Prevention's classifications.<sup>16</sup> The underweight category was removed for regression analyses based on the small sample size (n=75).

#### **Dietary Intake**

Dietary intake was calculated from two 24-hour recalls using the US Department of Agriculture's Automated Multiple-Pass method.<sup>17</sup> For both 24-hour recalls, proxy respondents reported for children 5 years old and younger and proxy-assisted interviews were conducted with children 6 to 11 years of age.<sup>18</sup> Data on dietary intakes of vitamin D were available in the continuous NHANES 2005-2006 and were released in 2009.<sup>19</sup> The National Cancer Institute (NCI) method<sup>20</sup> was used to estimate usual dietary vitamin D intake utilizing the amount-only part of the NCI method following similar methods of Bailey and colleagues.<sup>3</sup> The complete details of the NCI method and the SAS macros necessary to fit this model can be found at the NCI website.<sup>21</sup> The 24-hour recalls were also used to derive average total calories and total fat in grams.<sup>22</sup>

Information on dietary supplement use is collected during the household interview as part of the Dietary Supplement Questionnaire. Information includes the participant's use of vitamins, minerals, herbs, and other dietary supplements during the past 30 days. The average daily intake of vitamin D from dietary supplements was calculated for individuals based on methods by Bailey and colleagues<sup>3</sup> using the number of days of supplement use reported, the reported amount taken per day, and the serving size unit from the product label. The total contribution of vitamin D from foods and supplemental sources was then dichotomized as intake above or below the EAR (ie, 400 IU/day), the level appropriate for assessing intakes of population groups.<sup>23</sup>

#### **Biochemical Measurements**

During the NHANES health examination, blood samples were obtained by venipuncture and immediately centrifuged, ali-quoted, and frozen to  $-20^{\circ}$ C. The frozen serum and plasma samples were then shipped on dry ice to central laboratories and stored at  $-70^{\circ}$ C until analysis. Detailed descriptions of laboratory methodology can be found on the NHANES website.<sup>19</sup> During the 2005-2006 survey, serum 25OHD levels were measured using the two-step procedure with the Dia-Sorin, Inc 25OHD assay. The assay was designed to detect serum 25OHD values from 5 to 100 ng/mL (12.5 to 250 nmol/L). Values of <5.0 ng/mL (<12.5 nmol/L) and >70 ng/mL (>175 nmol/L) were verified by means of re-assay, including re-extraction. Any sample batches with coefficients of variation >10% were also re-assayed. Vitamin D status was classified as inadequate (<20 ng/mL [<50 nmol/L]) according to IOM criteria<sup>2</sup> for the main analyses. This level was chosen based on previous NHANES studies<sup>9,24</sup> that have used serum 25OHD <20 ng/mL (<50 nmol/L) as the cut point for inadequacy. For descriptive analyses, vitamin D status was classified into the following three categories: inadequate (<20 ng/mL [<50 nmol/L]), adequate (20 to <30 ng/mL [50 to <75 nmol/L]), and optimal ( 30 ng/mL [ 75 nmol/L]).

#### Statistical Analysis

All statistical analyses were performed using SAS software (version 9.2, SAS Institute Inc) and SUDAAN software (version 10.0, Research Triangle Institute) and weighted to the US population to provide nationally representative estimates. As per NHANES guidelines,<sup>18</sup> dietary day-1 sample weights were used to produce statistically reliable estimates taking the

complex survey design into account. Detailed descriptions on sample weights and variance estimation methods are provided in the NHANES analytic guidelines.<sup>25</sup>

For the purpose of descriptive and regression analyses, serum 25OHD concentrations were stratified into three categories (inadequate <20 ng/mL [<50 nmol/L], adequate 20 to <30ng/mL [50 to <75 nmol/L], and optimal 30 ng/mL [75 nmol/L]) for analyses. Concentrations in nmol/L are obtained from multiplying by 2.496. Potential confounding variables considered in the data analysis were age, sex, poverty income ratio, dietary energy intake, season of examination, sedentary time, and latitude. Descriptive categorical variables were compared by vitamin D status groups by using the Rao-Scott  $\chi^2$  test. Continuous variables were compared by vitamin D status groups using LSMEANS in SUDAAN. For variables (waist circumference, dietary vitamin D, dietary energy, and dietary fat) that were positively skewed, natural log transformations were applied before analyses and geometric means were presented. The association between not meeting the EAR and inadequacy as determined by 25OHD level was analyzed with multivariate logistic regression analysis after adjusting for age, sex, race/ethnicity, weight status, dietary energy, sedentary time, povertyto-income ratio, season, and latitude. Because of missing variables and excluding the underweight category, there were 2,465 participants included in the adjusted analyses. Multivariate-unadjusted and adjusted odds ratios (ORs) and 95% CIs were calculated for inadequate serum 25HOHD status. Interactions were determined between vitamin D intake and weight status and race/ethnicity by including cross-product terms in regression analyses. Significant interactions between vitamin D intake and weight status and race/ethnicity were further explored by stratified analyses. A P value <0.05 was considered significant in all analyses.

## **RESULTS AND DISCUSSION**

Selected characteristics of the 3,310 children are shown by serum 25OHD status in Table 1. The study population was 48% female. More than one third of children (33%) were overweight or obese. The sample was racially/ethnically diverse, with 39% characterized as non-Hispanic black, Mexican American, or other. Overall, 74% of children did not meet the EAR for vitamin D intake.

Table 2 displays mean serum 25OHD, anthropometrics, dietary intake, and sedentary activity time by vitamin D status of the cohort. Mean 25OHD was 26.0 ng/mL (65 nmol/L) (95% CI 25.5 to 26.5). Children with inadequate serum 25OHD were older, taller, weighed more, and had higher BMI percentiles and larger waist circumferences than those with adequate and optimal serum 25OHD. Total dietary energy and total fat did not vary by serum 25OHD status. Sedentary activity time differed by vitamin D status groups, with children with inadequate 25OHD reporting the greatest time spent in sedentary activity. The IOM committee assumed minimal sun exposure when establishing the Dietary Reference Intakes for vitamin D, but most children acquire at least some vitamin D from sun exposure. It is not surprising that average total vitamin D intake for the optimal 25OHD status group was below the EAR.

The use of dietary supplements containing vitamin D was low (28%), and was greater in the adequate (14%) and optimal (11%) vitamin D status groups compared with the inadequate (3%) status group (P<0.001). Of children who reported taking dietary supplements with vitamin D, 70% were non-Hispanic white children with only 7% of non-Hispanic blacks, 11% of Mexican Americans, and 12% of other/multiracial groups reporting use (P<0.001). Use also differed by weight status: 74% healthy weight compared with 26% overweight/ obese children (P=0.005).

Overall, total dietary vitamin D intake was positively associated with serum 25OHD status, which is consistent with a previous but smaller study including one of 145 9- to 15-year-old racially/ethnically diverse children in the northeast.<sup>26</sup> Not meeting the EAR was significantly associated with inadequate 25OHD status in unadjusted (OR=3.7; 95% CI 2.6 to 5.2) and multivariate-adjusted (OR=2.5; 95% CI 1.4 to 4.5) logistic regression analyses. This association was modified by weight status (*P* for interaction=0.02) and race/ethnicity (*P* for interaction=0.02).

Table 3 shows the odds=ratios for inadequate 25OHD for those not meeting the EAR compared with those that did, stratified by weight and race/ethnicity categories. Overall, children that were taller, heavier, and had larger waist circumferences were more likely to have inadequate vitamin D levels. Because vitamin D is fat soluble and is readily stored in adipose tissue, it is possible that vitamin D could be taken up by a larger body pool of fat in larger individuals.<sup>8</sup> Therefore, it might take more dietary vitamin D to achieve recommended serum 25OHD levels in overweight/obese children and, for this reason, they have lower amounts of circulating serum 25OHD than do healthy weight children. In the present study, overweight/obese children were five times more likely to be at risk for inadequate 25OHD if they failed to meet the EAR compared with overweight/obese children. This suggests that overweight/obese children were especially likely to be at risk for inadequate serum 25OHD when their intakes were below the EAR.

There were significantly fewer non-Hispanic black, Mexican-American, and other/ multiracial children who met the EAR than non-Hispanic whites. For those who failed to meet the EAR, non-Hispanic black and other/multiracial children were almost four times more likely to be at risk for inadequate 25OHD compared with non-Hispanic black or other/ multiracial children who met the EAR. Similar to overweight/obese children, this suggests that non-Hispanic black and other/multiracial children were especially likely to be at risk for inadequate 25OHD status when not consuming the EAR. In addition, fewer minorities achieved adequate or optimal serum 25OHD levels compared with non-Hispanic white children, which is consistent with previous work in Canadians.<sup>6</sup> In addition, <6% of non-Hispanic black children had serum 25OHD levels above the threshold ( 30 ng/mL [ 75 nmol/L]) that some researchers believe is optimal for bone health and, emerging evidence suggests, possibly for other chronic diseases as well.<sup>1,27,28</sup> More research is needed to determine whether current dietary vitamin D recommendations are adequate for these populations to meet recommended serum 25OHD levels that are thought to be adequate for bone health.<sup>6</sup>

The study's strengths include its nationally representative, population-based sample of US children and adolescents and its examination of current dietary vitamin D recommendations using established usual total nutrient intake methods.<sup>3</sup> A limitation of our study was that because of geographic preference for sampling in NHANES, serum 25OHD was collected in northern states in the summer and southern states in the winter, which might have increased overall 25OHD levels and explained the null effect of season and latitude on serum 25OHD. In addition, although specific foods and beverages were not examined in this study, other data document that the main sources of dietary vitamin D in US children's diets are milk and dairy products,<sup>29</sup> although children's milk intakes have been declining in recent years.<sup>30</sup>

## CONCLUSIONS

These findings suggest that children who did not consume current dietary vitamin D recommendations were at increased risk for inadequate 25OHD status. Overweight/obese and non-Hispanic black children were especially likely to be at risk when they do not meet the EAR. Future research is needed to confirm these findings. In the meantime, efforts should be made to ensure that all children achieve recommended intakes of vitamin D. In addition, registered dietitians and others should be aware that low dietary vitamin D intakes can be especially detrimental among overweight children and non-Hispanic black children.

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#### Table 1

Select characteristics by serum 25OHD<sup>a</sup> status in children ages 1 to 18 years in the National Health and Nutrition Examination Survey 2005-2006 (N=3,310)

			250HD			
	Inadequate (<20 ng/mL <sup>b</sup> ) (n=1,204)	Adequate (20 to <30 ng/mL <sup>b</sup> ) (n=1,463)	Optimal ( 30 ng/mL <sup>b</sup> ) (n=643)	P value <sup>c</sup>		
	<i>~</i>	——————————————————————————————————————				
Age				< 0.001		
1-8 у	9.4 (0.8)	48.4 (2.7)	42.2 (2.7)			
9-18 y	30.2 (1.9)	46.5 (1.9)	23.2 (1.5)			
Sex				0.03		
Male	18.4 (1.4)	48.2 (2.2)	33.4 (2.6)			
Female	26.0 (1.6)	46.3 (2.6)	27.7 (2.0)			
Race/ethnicity				< 0.001		
Non-Hispanic white	10.1 (1.6)	48.8 (2.2)	41.1 (1.6)			
Non-Hispanic black	58.4 (1.8)	35.6 (2.1)	5.9 (1.0)			
Mexican American	31.6 (1.7)	51.0 (2.1)	17.9 (1.7)			
Other	30.0 (3.2)	49.2 (4.6)	20.8 (2.9)			
Weight category (n=2,789)				< 0.001		
Underweight (<5th percentile)	25.2 (8.1) <sup>e</sup>	29.8 (7.8)	45.0 (11.2)			
Healthy (5th to 84.9th percentile)	20.1 (1.1)	47.3 (1.5)	32.5 (1.5)			
Overweight (85th to 95th percentile)	26.0 (2.6)	47.7 (3.0)	26.3 (3.6)			
Obese (>95th percentile)	36.8 (4.0)	44.6 (3.7)	18.6 (3.7)			
Poverty-to-income ratio				< 0.001		
<130%	30.9 (1.5)	45.8 (2.3)	23.3 (2.0)			
130% to <350%	20.6 (1.3)	49.5 (2.3)	30.0 (2.1)			
350%	15.3 (1.8)	46.1 (2.5)	38.7 (2.5)			
Season <sup><math>f</math></sup> (n=3,167)				0.33		
Winter	21.7 (1.6)	49.3 (2.1)	29.0 (1.0)			
Summer	22.3 (1.4)	45.8 (1.8)	31.9 (2.1)			
Latitude <sup>g</sup>				0.17		
South	20.1 (1.9)	50.8 (2.1)	29.1 (1.3)			
North	22.9 (1.3)	45.7 (1.9)	31.4 (1.7)			

<sup>a</sup>250HD=25-hydroxyvitamin D.

 $^{b}$  To convert ng/mL 25OHD to nmol/L, multiply ng/mL by 2.496. To convert nmol/L 25OHD to ng/mL, multiply nmol/L by 0.4. 25OHD of 20 ng/mL=49.92 nmol/L.

<sup>*c*</sup>Analyzed with Rao-Scott  $\chi 2$  test.

 $^{d}$ SE=standard error.

 $^{e}$ The relative SE is >30%, this estimate is unreliable.

 $f_{\mbox{Data}}$  collected during May 1 through October 31 (summer) and November 1 through April 30 (winter).

 $^{g}$ South is <35° and North is 35°.

#### Table 2

Serum 25OHD<sup>*a*</sup>, anthropometrics, dietary intake, and sedentary time by vitamin D status in children ages 1 to 18 years old in the National Health and Nutrition Examination Survey 2005-2006 (N=3,310)

	25-OHD						
	Inadequate (<20 ng/mL <sup>b</sup> ) (n=1,204)		Adequate (20 to <30 ng/mL <sup>b</sup> ) (n=1,463)		Optimal ( 30 ng/mL <sup>b</sup> ) (n=643)		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Overall P value <sup>c</sup>
25OHD (ng/mL <sup><math>b</math></sup> )	14.6	14.3-14.9	25.0	24.7-25.2 <sup>d</sup>	35.8	35.1-36.4 <sup>d</sup>	< 0.001
Height (cm) (n=3,108)	153	151-155	143	140-145 <sup>d</sup>	136	132-140 <sup>d</sup>	< 0.001
Weight (kg) (n=3,300)	57.1	52.6-61.5	43.2	40.6-45.8 <sup>d</sup>	35.6	32.1-39.0 <sup>d</sup>	< 0.001
BMI <sup>e</sup> percentile (n=2789)	70.1	66.7-73.4	64.5	61.7-67.4 <sup>f</sup>	57.9	62.4-54.1 <sup>d</sup>	< 0.001
Waist circumference (cm) (n=3,082) <sup>g</sup>	76.1	73.4-78.9	68.0	66.5-69.5 <sup>d</sup>	63.1	60.9-65.6 <sup>d</sup>	<0.001
Total dietary vitamin D (IU/d) <sup>g</sup>	213	199-228	298	284-314 <sup>d</sup>	312	288-338 <sup>d</sup>	< 0.001
Total dietary energy (kcal/d) <sup>g</sup>	1,837	1,773-1,904	1,893	1,824-1,963	1,834	1,770-1,900	0.25
Total fat (g/d) <sup>g</sup>	67.7	65.1-70.4	68.2	64.9-71.7	66.9	64.2-69.8	0.85
Sedentary time (h/d)	1.7	1.5-1.9	1.4	1.3-1.5 <sup>d</sup>	1.0	0.9-1.2 <sup>d</sup>	< 0.001

<sup>a</sup>250HD=25-hydroxyvitamin D.

b To convert ng/mL 25OHD to nmol/L, multiply ng/mL by 2.496. To convert nmol/L 25OHD to ng/mL, multiply nmol/L by 0.4. 25OHD of 20 ng/mL=49.92 nmol/L.

<sup>c</sup>All mean values presented are calculated with LSMEANS.

 $^{d}$ Mean is significantly different from inadequate 25OHD, P<0.001.

<sup>e</sup>BMI=body mass index.

fMean is significantly different from inadequate 25OHD, *P*<0.05.

<sup>g</sup>Values presented are geometric mean and 95% CI for waist circumference, dietary vitamin D, dietary energy, and dietary fat because of skewed distribution.

#### Table 3

Odds ratio for being at risk for inadequate 25OHD<sup>*a*</sup> in US children not meeting the Estimated Average Requirement (relative to meeting the Estimated Average Requirement) stratified by weight status and race/ ethnicity  $(N=2,465)^b$ 

Modifier	Odds ratio	95% CI	P value
Weight status			
Healthy weight	1.86	1.04-3.33	0.04
Overweight/obese	5.05	2.02-12.65	< 0.001
Race/ethnicity			
Non-Hispanic white	2.28	0.85-6.13	0.10
Non-Hispanic black	3.80	1.48-9.70	< 0.01
Mexican American	1.49	0.88-2.53	0.14
Other	4.05	1.01-16.28	< 0.05

<sup>a</sup>250HD=25-hydroxyvitamin D.

 $^b{}_{\rm Adjusted}$  for age, sex, poverty-to-income ratio, dietary calories, sedentary time, season, and latitude.