
Vijay Ganji, Xu Zhang, Nida Shaikh, and Vin Tangpricha

ABSTRACT

Background: The effect of assay drifts over time on serum 25-hydroxyvitamin D [25(OH)D] concentrations were not accounted for in previous national survey studies. Thus, previously reported associations between 25(OH)D with cardiometabolic risk factors using data from NHANES were likely over- or underestimated. Moreover, associations between serum 25(OH)D and metabolic syndrome (MetSyn) in children have been inconsistent, due to the rise in children. Vitamin D is required not only for bone health but also has been reported to play a role in a range of ailments such as autoimmune disease, cardiovascular disease, type 2 diabetes (T2D), hypertension, depression, and certain types of cancer.

Metabolic syndrome (MetSyn) is characterized by abdominal adiposity, dyslipidemia, and elevated glucose and blood pressure (BP). Although insulin resistance (IR) is not part of diagnostic criteria for MetSyn, it is a major cardiometabolic abnormality associated with MetSyn. IR contributes to the pathogenesis of T2D and MetSyn. Although, there is a lack of consistent criteria in diagnosing MetSyn in children, the National Cholesterol Education Program—Adult Treatment Panel III (NCEP-ATP III) criteria are the most widely used. In US children, the prevalence of MetSyn has risen from 6.4% in 1999–2000 to 8.6% in 2001–2006.

Low circulating 25(OH)D has been linked to MetSyn and other cardiometabolic risk factors. It has been reported that vitamin D reduces the risk of T2D by preserving insulin secretion and reducing IR. However, there are inconsistent data that relate vitamin D status with glucose homeostasis. In addition, vitamin D insufficiency has been linked to elevated inflammation. C-reactive protein (CRP) is a predictor of elevated inflammation, which has been linked to increased risk of CVD, obesity, and MetSyn. Due to the presence of vitamin D receptors (VDRs) on pancreatic β cells, inflammatory cells, there is a potential role for vitamin D in IR and inflammation. No epidemiologic studies based on nationally representative data have investigated the relation between serum 25(OH)D and IR and inflammation in children.

INTRODUCTION

Serum 25-hydroxyvitamin D [25(OH)D] is a commonly used marker of vitamin D nutritional status. Circulating 25(OH)D represents endogenous vitamin D synthesis from the skin and dietary intake of limited foods containing vitamin D. It has been reported that suboptimal vitamin D status is widespread in the United States. Health concerns associated with low vitamin D status are on the rise in children. Vitamin D is required not only for bone health but also has been reported to play a role in a range of ailments such as autoimmune disease, cardiovascular disease, type 2 diabetes (T2D), hypertension, depression, and certain types of cancer.

Recently, the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) updated serum 25(OH)D data files, replacing previous data files for public use in November 2010. The revised adjusted data account for drifts in the serum 25(OH)D assay performance over time. In the data advisory, the NCHS specifically recommended that the adjusted data rather than previously available unadjusted data be used for all analyses of serum 25(OH)D concentrations for NHANES 2000–2006 (33). Thus, previously reported associations in children using the unadjusted serum 25(OH)D data from NHANES 2001–2002, 2003–2004, and 2005–2006 cycles were likely either over- or underestimated inadvertently (3, 15, 16). Therefore, in this study, we report the association between serum 25(OH)D and the prevalence of MetSyn, individual components of MetSyn, CRP, and homeostatic model assessment–insulin resistance index (HOMA-IR) in children with the use of the recently updated serum 25(OH)D data from nationally representative sample surveys of the US population.

METHODS

Overview of survey description

The NCHS conducts large, nationally representative sample surveys known as NHANES on the civilian, noninstitutionalized US population by using a stratified, multistage, probability sample survey design. NHANES were conducted as annual surveys beginning in 1999. Data are released in 2-year cycles for public use. Data on demographic characteristics, diet, and health are collected from personal interviews. Physical examinations and collection of blood and urine samples are conducted in the Mobile Examination Center (MEC). Low-income persons, adolescents, persons aged ≥60 y, non-Hispanic blacks, and Hispanics/Mexicans were oversampled to yield reliable estimates for these groups.

The detailed description of the survey methodologies and analytic guidelines were reported elsewhere (34). Briefly, NHANES 2001–2002 was conducted between January 2001 and December 2002 in 11,039 individuals (10,477 were examined in MECs), NHANES 2003–2004 was conducted between January 2003 and December 2004 in 12,761 individuals (9643 were examined in MECs), and NHANES 2005–2006 was conducted between January 2005 and December 2006 in 12,862 individuals (9950 were examined in MECs). The NCHS Ethics Review Board approved all NHANES protocols before data collection.

Periodically, NHANES data files are updated by the NCHS, which replaces previous data files. One such update occurred in November 2010 for serum 25(OH)D data because of changes in serum 25(OH)D assay over time. At that time, NCHS issued a data advisory for vitamin D recommending that investigators use the assay-adjusted data rather than previously available unadjusted data. A detailed description of this data advisory for serum 25(OH)D is found elsewhere (33).

Description of metabolic syndrome

MetSyn in children and adolescents was defined according to the modified NCEP-ATP III criteria (13). According to these criteria, the MetSyn phenotype requires the presence of ≥3 of the following: 1) waist circumference (WC) ≥90th percentile for age and sex; 2) triglycerides ≥110 mg/dL (1.24 mmol/L); 3) HDL cholesterol <40 mg/dL (1.03 mmol/L); 4) either systolic BP (SBP) or diastolic BP (DBP) ≥90th percentile for age and sex, use of BP medication, or previous diagnosis of BP; and 5) fasting glucose ≥100 mg/dL (5.55 mmol/L), current diabetes status, or current insulin or hypoglycemic medication use. These criteria (≥3 out of 5) were applied to generate the cases of MetSyn phenotypes.

Description of the study sample

For this study, data on children aged 12–19 y from NHANES 2001–2002, 2003–2004, and 2005–2006 were concatenated into one master database: NHANES 2001–2006 (n = 6873). Of 6873 total eligible participants, 662 subjects were excluded due to lack of data for serum 25(OH)D. Of the remaining 6211 persons, 281, 59, and 4 individuals were excluded due to missing data for MetSyn phenotype, body mass index (BMI), and supplement use, respectively. After applying the above exclusion criteria, the sample consisted of 5867 children and adolescents (weighted sample: 27,812,647). This sample was used for the data analysis of measurement of association between prevalence of MetSyn and serum 25(OH)D concentrations.

Sample sizes for the data analysis of measurement of association between various individual cardiometabolic risk factors and serum 25(OH)D concentrations varied. Out of 6873 total eligible persons, after applying the above-mentioned exclusion criteria sequentially (serum 25(OH)D, cardiometabolic risk factors, BMI, and supplement use), 5377 subjects had data for WC, 2599 subjects had data for fasting blood glucose, 5398 subjects had data for serum HDL cholesterol, 2581 had data for serum triglycerides, 5281 had data for SBP, 5313 had data for DBP, and 5470 subjects had data for serum CRP. The sample size for IR measurement was 2573. These subjects had data for both fasting glucose and serum insulin concentrations.

Description of study variables

Potential confounding variables considered in the data analysis were age, sex, race-ethnicity, BMI, poverty-income ratio (PIR), season of survey, use of supplements, smoking, and alcohol intake. Subjects were categorized into 12–15-y and 16–19-y age groups. Race-ethnicity was self-reported as non-Hispanic white, non-Hispanic black, Hispanic/Mexican American, and other (persons of multirace and persons with race-ethnicity other than non-Hispanic white, non-Hispanic black, or Mexican/Hispanic). PIR is the ratio of income to the family’s appropriate poverty threshold (35). PIR was categorized as below poverty (<1.0), middle income (1.0–2.5), and higher income (>2.5). Individuals with no data for PIR were placed into the fourth category, i.e., “not reported.” To avoid damage to the MECs, data in the northern United States were collected in summer (1 May–31 October) and data in the south were collected in winter (1 November–30 April).

Data for WC, BMI, and BP were obtained from the examination component of NHANES. Age- and sex-specific 90th percentiles of WC were derived. Measurement of WC has been described elsewhere in detail (36). BMI was categorized as normal weight (<85th percentile) and overweight and obese (≥85th percentile) for age and sex. BP was measured with a mercury sphygmomanometer (36). Age- and sex-specific 90th percentiles of SBP and DBP measurements were derived. The participants who answered “yes” to the question “Did you take supplements in the past 30 d?”...
were regarded as supplement users. Smoking status and alcohol intake variables were also considered in the data analysis. However, these variables were not significantly related to either vitamin D or MetSyn in this study sample; therefore, smoking and alcohol variables were dropped from the analysis.

Biochemical measurements

Blood samples were collected by venipuncture in MECs according to standard protocols (37–39). Serum 25(OH)D concentrations were determined by using the Diasorin RIA kit assay (Stillwater, MN) at the National Center for Environmental Health, CDC, Atlanta, GA. Serum HDL cholesterol was analyzed by using the heparin manganese precipitation method in NHANES 2001–2002 and a direct HDL-cholesterol immunnoassay method in NHANES 2005–2006. Triglyceride was measured enzymatically at Johns Hopkins Hospital, Baltimore, MD. Glucose concentration was determined enzymatically. Because the assay for glucose measurement has changed between surveys and in order for the glucose data to be valid when NHANES 2005–2006 and NHANES 2003–2004 files were merged, the following regression equation was used as per the NCHS guidelines (37, 38):

\[
\text{Fasting blood glucose}_{\text{NHANES 2003–2004}} = (0.9815 \times \text{fasting blood glucose}_{\text{NHANES 2005–2006}}) + 3.5707
\]

Serum insulin was measured with a radioimmunoassay, a 2-site immunoenzymometric assay, and an ELISA immunoassay in NHANES 2001–2002, NHANES 2003–2004, NHANES 2005–2006, respectively. The mean serum insulin concentration in the NHANES 2003–2004 was \(\approx\)11% lower than the mean in the NHANES 2001–2002. To account for this change in laboratory measurement procedure between these 2 surveys, the NCHS recommended the following linear regression to adjust the NHANES 2001–2002 serum insulin values when comparing them with NHANES 2003–2004 values (37–39):

\[
\text{Serum insulin}_{\text{NHANES 2003–2004}} = (1.0027 \times \text{serum insulin}_{\text{NHANES 2001–2002}}) - 2.2934
\]

After applying the above regression model, we found one serum insulin observation with negative value, and this was excluded from the analysis. In order for the merged insulin data from NHANES 2005–2006 and NHANES 2003–2004 to be valid, the following regression equation was used as per the NCHS guidelines (37–39):

\[
\text{Serum insulin}_{\text{NHANES 2003–2004}} = (1.0526 \times \text{serum insulin}_{\text{NHANES 2005–2006}}) - 1.5674
\]

Both fasting glucose and serum insulin were measured at the Diabetes Diagnostic Laboratory, University of Missouri, Columbia, MO, for NHANES 2001–2004 and at the Fairview Medical Center Laboratory, University of Minnesota, Minneapolis, MN, for NHANES 2005–2006. IR was assessed by using HOMA-IR [fasting insulin (\(\mu\)U/mL) \(\times\) fasting glucose (mg/dL)/405] (40, 41). Serum CRP was measured with the latex-enhanced nephelometry method at University of Washington, Seattle, WA.

Statistical analysis

SAS-callable SUDAAN (version 10.0.1; Research Triangle Institute, Research Triangle Park, NC) and SAS (version 9.2; SAS Institute Inc, Cary, NC) statistical software packages were used in the data analysis. As per NCHS guidelines, 6-y sample weights were used to produce statistically reliable estimates taking the complex survey design into account. The Taylor linearization method was used to compute variance estimates. Detailed descriptions on sample weights and variance estimation methods are provided in the NHANES analytic guidelines (34).

Combining the data from 3 NHANES cycles (2001–2002, 2003–2004, and 2005–2006) yielded more stable estimates of means, percentages, and prevalences for serum 25(OH)D. For the purpose of regression analysis, serum 25(OH)D concentrations were stratified into tertiles. The tertile ranges for serum 25(OH)D were <48.1, 48.1 to <66.2, and \(\geq\)66.2 nmol/L. Concentrations in ng/mL are obtained by dividing by 2.496. The proportion of subjects with MetSyn in the first-tertile serum 25(OH)D group was compared with that of the third-tertile 25(OH)D group by using the Rao-Scott chi-square test. The association between prevalence of MetSyn and serum 25(OH)D concentration was analyzed with multivariate logistic regression analysis after taking sex, age, race-ethnicity, supplement use, season of survey, BMI, and PIR variables into consideration. Nonsignificant variables such as PIR, season of survey, and use of supplements were subsequently dropped from the logistic regression model. Multivariate-adjusted odds ratios (ORs) and 95% CIs were calculated for the presence of MetSyn phenotype for each serum 25(OH)D tertile category after the analysis was adjusted for age, sex, race-ethnicity, and BMI. ORs for tertiles 1 and 2 in relation to tertile 3 (referent category) were compared.

The multivariate-adjusted means for WC, fasting glucose, triglycerides, BP, HDL cholesterol, HOMA-IR, and CRP across tertiles of serum 25(OH)D concentrations were generated by using regression analysis after the data were adjusted for sex, age, race-ethnicity, supplement use, season of survey, BMI, and PIR. Individual multivariate linear regression models were developed to ascertain the association between serum 25(OH)D and WC, fasting blood glucose, HDL cholesterol, triglyceride, SBP, DBP, CRP, and HOMA-IR. Because the distribution of fasting glucose, triglycerides, CRP, and HOMA-IR were skewed, these variables were log-transformed in the analysis. Multiple comparisons with Bonferroni correction were performed to determine significant differences between the adjusted means for serum 25(OH)D tertiles for each indicator of MetSyn \((P < 0.0167)\). We also determined interactions between serum 25(OH)D and confounding variables. The interaction terms were included in the model. A \(P \leq 0.05\) was considered significant in all analyses.

RESULTS

The study population consisted of 50.6% boys and 49.4% girls. The mean age of the sample was 15.4 y. Of the 5867 subjects, 62.7% were non-Hispanic white, 14.8% were non-Hispanic black, and 17% were Hispanics/Mexicans. The percentage of subjects in 12–15-y and 16–19-y age groups was similar (51.6% and 48.4%, respectively). Approximately 25% of the study population reported having taken a supplement within 1 mo before the survey. Approximately 58% of the subjects were examined in the summer. The
prevalence of MetSyn in this study sample was 5.4%. The prevalence of MetSyn was significantly higher in boys than in girls (6.9% compared with 3.9%; \( P < 0.001 \)) and in persons with a BMI \( \geq 85\text{th} \) percentile than in those with a BMI <85th percentile (29.6% compared with 19%; \( P < 0.001 \)). The geometric mean serum 25(OH)D concentration of the study sample was 55.5 nmol/L. Concentrations of serum 25(OH)D <50, 50 to <75 nmol/L, and \( \geq 75 \) nmol/L were considered vitamin D deficient, insufficient, and sufficient, respectively (42). The prevalence of MetSyn was significantly higher in those with serum 25(OH)D <50 nmol/L compared with those with serum 25(OH)D \( \geq 75 \) nmol/L (7.5% compared with 3.5%; \( P < 0.001 \)). The prevalence of MetSyn was significantly different across 3 serum 25(OH)D tertiles (\( P < 0.001 \)) (Figure 1). The cardiometabolic characteristics of the study population are given in Table 1.

The proportion of subjects in the lowest 25(OH)D tertile category (<48.1 nmol/L) was significantly higher for females than for males (\( P < 0.001 \)), for non-Hispanic blacks than for non-Hispanic whites (\( P < 0.001 \)), for the PIR <1.0 group than for the PIR \( \geq 2.5 \) group (\( P < 0.001 \)), for those examined in the winter than for those examined in the summer (\( P < 0.001 \)), for non–supplement users than for supplement users (\( P < 0.001 \)), and for persons with a BMI \( \geq 85\text{th} \) percentile than for those with a BMI <85th percentile (\( P < 0.001 \)) (Table 2).

The association between serum 25(OH)D concentrations and prevalence of MetSyn is presented in Table 3. The proportion of subjects with MetSyn in the first-tertile serum 25(OH)D group was significantly higher than those in the third-tertile serum 25(OH)D group (\( P < 0.001 \) for chi-squared statistic). Serum 25(OH)D was significantly associated with prevalence of MetSyn in unadjusted (\( P < 0.005 \) for trend) and multivariate-adjusted (\( P < 0.04 \) for trend) logistic regression analysis. In the multivariate-adjusted analysis, the likelihood of children having MetSyn in the lowest serum 25(OH)D tertile category was 1.71 (95% CI: 1.11, 2.65; \( P < 0.01 \)) compared with subjects in the highest serum 25(OH)D tertile category.

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25(OH)D (nmol/L) (( n = 5867 ))</td>
<td>55.5 ± 1.0</td>
</tr>
<tr>
<td>BMI (kg/m(^2)) (( n = 5867 ))</td>
<td>23.1 ± 0.1</td>
</tr>
<tr>
<td>Waist circumferenc (cm) (( n = 5813 ))</td>
<td>80.5 ± 0.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg) (( n = 5735 ))</td>
<td>108.8 ± 0.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg) (( n = 5732 ))</td>
<td>60.4 ± 0.3</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL) (( n = 5848 ))</td>
<td>50.3 ± 0.2</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dL) (( n = 2934 ))</td>
<td>81.4 ± 1.3</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL) (( n = 2955 ))</td>
<td>92.7 ± 0.3</td>
</tr>
<tr>
<td>Serum insulin (( \mu \text{U/mL} )) (( n = 2933 ))</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>C-reactive protein (( \mu \text{g/dL} )) (( n = 5867 ))</td>
<td>52.0 ± 1.2</td>
</tr>
<tr>
<td>HOMA-IR(^2) (( n = 2929 ))</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

\(^2\) NHANES 2001–2002, 2003–2004, and 2005–2006 were combined into one master database: NHANES 2001–2006. To convert nmol/L to ng/mL, divide by 2.496. Values are arithmetic means ± SEs for BMI, waist circumference, blood pressure, and HDL cholesterol because the distribution of these variables was normal. 25(OH)D, 25-hydroxyvitamin D; HOMA-IR, homeostatic model assessment–insulin resistance index. Values for BMI, waist circumference, and blood pressure are arithmetic means ± SEs because the distribution of these variables was normal. All other variables are geometric means ± SEs because of skewed distribution.

\(^1\) Fasting insulin (\( \mu \text{U/mL} \)) \times \) fasting glucose (mg/dL)/405.
The association between serum 25(OH)D concentrations and various cardiometabolic risk factors is presented in Table 4. An inverse association between serum 25(OH)D and WC (P for linear trend <0.001), SBP (P for linear trend = 0.01), and HOMA-IR score (P for linear trend = 0.002) and a positive association between serum 25(OH)D and HDL cholesterol (P for linear trend <0.001) were found in the multivariate-adjusted regression analysis. WC, SBP, and HOMA-IR were significantly higher and HDL cholesterol was significantly lower in the lowest serum 25(OH)D tertile group than in the highest serum 25(OH)D tertile group (<0.0167). There was no association between serum 25(OH)D and fasting plasma glucose (P = 0.81), serum triglycerides (P = 0.94), DBP (P = 0.51), and CRP (P = 0.18). Additional analysis showed no association between serum 25(OH)D and CRP in children with a BMI ≥85th percentile (P = 0.18; data not shown).

**TABLE 2**


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tertile 1: &lt;48.1 nmol/L (n = 2880)</th>
<th>Tertile 2: 48.1 to &lt;66.2 nmol/L (n = 1689)</th>
<th>Tertile 3: ≥66.2 nmol/L (n = 1298)</th>
<th>P value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Boys (n = 2935)</td>
<td>1305 (28.1)</td>
<td>906 (33.5)</td>
<td>724 (38.4)</td>
<td></td>
</tr>
<tr>
<td>Girls (n = 2932)</td>
<td>1575 (34.3)</td>
<td>783 (31.6)</td>
<td>574 (34.0)</td>
<td></td>
</tr>
<tr>
<td>Race-ethnicity [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-Hispanic white (n = 1603)</td>
<td>239 (15.0)</td>
<td>564 (35.0)</td>
<td>800 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic black (n = 1962)</td>
<td>1517 (77.8)</td>
<td>326 (16.3)</td>
<td>119 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Mexican (n = 2067)</td>
<td>996 (43.2)</td>
<td>734 (39.4)</td>
<td>337 (17.4)</td>
<td></td>
</tr>
<tr>
<td>Other (n = 235)</td>
<td>128 (53.7)</td>
<td>65 (27.0)</td>
<td>42 (19.3)</td>
<td></td>
</tr>
<tr>
<td>Age [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>12–15 y (n = 2913)</td>
<td>1342 (29.8)</td>
<td>940 (35.3)</td>
<td>631 (34.9)</td>
<td></td>
</tr>
<tr>
<td>16–19 y (n = 2954)</td>
<td>1538 (32.7)</td>
<td>749 (29.7)</td>
<td>667 (37.6)</td>
<td></td>
</tr>
<tr>
<td>Poverty-income ratio [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;1.0 (n = 1723)</td>
<td>1024 (45.5)</td>
<td>464 (32.1)</td>
<td>235 (22.4)</td>
<td></td>
</tr>
<tr>
<td>1.0–2.5 (n = 1910)</td>
<td>982 (34.1)</td>
<td>534 (32.7)</td>
<td>394 (33.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;2.5 (n = 1937)</td>
<td>728 (23.1)</td>
<td>608 (33.4)</td>
<td>601 (43.5)</td>
<td></td>
</tr>
<tr>
<td>Not reported (n = 297)</td>
<td>146 (46.8)</td>
<td>83 (25.5)</td>
<td>68 (24.7)</td>
<td></td>
</tr>
<tr>
<td>Season of survey [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Summer (n = 2745)</td>
<td>1073 (22.5)</td>
<td>836 (32.8)</td>
<td>836 (44.6)</td>
<td></td>
</tr>
<tr>
<td>Winter (n = 3122)</td>
<td>1807 (43.0)</td>
<td>853 (21.2)</td>
<td>462 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Use of supplements [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes (n = 1198)</td>
<td>420 (21.2)</td>
<td>411 (36.4)</td>
<td>367 (42.4)</td>
<td></td>
</tr>
<tr>
<td>No (n = 4669)</td>
<td>2460 (35.6)</td>
<td>1278 (31.3)</td>
<td>931 (34.1)</td>
<td></td>
</tr>
<tr>
<td>BMI [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;85th percentile (n = 5011)</td>
<td>2321 (28.6)</td>
<td>1474 (32.6)</td>
<td>1216 (38.9)</td>
<td></td>
</tr>
<tr>
<td>≥85th percentile (n = 856)</td>
<td>559 (49.1)</td>
<td>215 (25.6)</td>
<td>82 (18.3)</td>
<td></td>
</tr>
</tbody>
</table>

1 n = 5867, weighted n = 27,812.647. NHANES 2001–2002, 2003–2004, and 2005–2006 were combined into one master database: NHANES 2001–2006. Analysis was based on recently released assay-adjusted serum 25(OH)D concentrations. Percentages are shown in parentheses and are based on the weighted sample. To convert nmol/L to ng/mL, divide by 2.496.

2 Significance determined by Rao-Scott chi-square test.

3 Ratio of income to the family’s appropriate poverty threshold, provided by the US Census Bureau. A ratio of <1.0 is considered to be below-poverty threshold.

4 Data collected during 1 May–31 October (summer) and 1 November–30 April (winter).

5 Participants who took supplements 1 mo before the survey was conducted.

**DISCUSSION**

In this study, we report the first cross-sectional association between serum 25(OH)D and cardiometabolic risk factors in children based on recently updated serum 25(OH)D data that were released by the NCHS in November 2010. The NCHS released updated data because previous publically available serum 25(OH)D data did not account for assay drift over time (method bias and imprecision). On the basis of the quality-control studies by the CDC, it was estimated that the mean serum 25(OH)D in NHANES 2003–2004 and NHANES 2005–2006 were either over- or underestimated by ≤10% (33, 43, 44). The adjusted serum 25(OH)D for NHANES 2003–2004 was lower and for NHANES 2005–2006 was higher compared with the respective unadjusted serum 25(OH)D concentrations. This variation in serum 25(OH)D between surveys was more likely due to assay reformulation by the manufacturer and lot-to-lot variation in calibration. Hence, the NCHS issued an advisory note that the adjusted data rather than unadjusted data should be used for all analyses involving serum 25(OH)D (33). The previously reported cross-sectional associations between serum 25(OH)D and cardiometabolic risk factors in children based on unadjusted 25(OH)D data from NHANES 2001–2006 were either under- or overestimated (3, 15, 16). To date, this study is the most comprehensive investigation of the relation between serum 25(OH)D and various cardiometabolic...
Multivariate-adjusted odds ratios (ORs) and 95% CIs for metabolic syndrome according to serum 25-hydroxyvitamin D [25(OH)D] concentration in 12–19-y-old children and adolescents in NHANES 2001–2006

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile 1: &lt;48.1 nmol/L</th>
<th>Tertile 2: 48.1 to &lt;66.2 nmol/L</th>
<th>Tertile 3: ≥66.2 nmol/L</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for metabolic syndrome (n = 2880)</td>
<td>181</td>
<td>78</td>
<td>40</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Unadjusted OR (95% CI)</td>
<td>2.22 (1.38, 3.57)</td>
<td>1.60 (1.03, 2.48)</td>
<td>1.0*</td>
<td>0.005*</td>
</tr>
<tr>
<td>Multivariate-adjusted OR (95% CI)</td>
<td>1.71 (1.11, 2.65)</td>
<td>1.16 (0.71, 1.91)</td>
<td>1.0*</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

*Significance determined by Rao-Scott chi-square test.
†Significantly different from the referent category, tertile 3.
‡Referent category.
§Significant for the effect of metabolic syndrome prevalence in the unadjusted logistic regression analysis.
‖Significant for the effect of metabolic syndrome prevalence in the multivariate-adjusted logistic regression analysis. The analysis was adjusted for sex, age, race-ethnicity, and BMI. Interaction between race-ethnicity and BMI was significant (P = 0.023). Age, poverty-income ratio, season of survey, and use of supplements were not significant in this model.

Multivariate-adjusted ORs and 95% CIs for metabolic syndrome according to serum 25-hydroxyvitamin D [25(OH)D] concentration. Analysis was based on recently released assay-adjusted serum 25(OH)D concentrations. To convert nmol/L to ng/mL, divide by 2.496. Metabolic syndrome phenotype is defined according to the modified National Cholesterol Education Program–Adult Treatment Panel III criteria (13). The presence of ≥3 of the following criteria are required: waist circumference >90th percentile for age and sex; triglycerides >110 mg/dL (1.24 mmol/L); HDL cholesterol <40 mg/dL (1.03 mmol/L); either systolic or diastolic blood pressure >90th percentile for age, sex, and height or use of blood pressure medication; and fasting glucose >100 mg/dL (5.55 mmol/L), current diagnosis of diabetes, or current use of insulin or oral hypoglycemic drugs.

TABLE 3

Serum 25(OH)D concentration

Risk factors in children in a nationally representative sample survey using the assay-adjusted serum 25(OH)D data. In this study, we report that serum 25(OH)D was inversely related to prevalence of MetS phenotype, WC, SBP, and HOMA-IR. Serum 25(OH)D was also directly related to HDL cholesterol regardless of obesity. No relation was found between serum 25(OH)D and CRP.

To our knowledge, this is the first study to investigate the association between serum 25(OH)D and IR as assessed by HOMA in a nationally representative sample of children. In this large population-based study, we found that children in the lowest serum 25(OH)D tertile had significantly higher HOMA-IR scores than did those in the highest 25(OH)D tertile. A few cross-sectional studies in adults (12, 19, 21) and a small cohort study on children (45) have reported an association between serum 25(OH)D and IR. In contrast, vitamin D intervention studies have yielded inconsistent results on lowering IR (46). This is likely due to differences in dosage and duration of vitamin D. Similar to our findings, a small cross-sectional study in 85 children recently found that serum 25(OH)D was inversely associated with HOMA-IR (45). Previous studies in adults have suggested an inverse association between serum 25(OH)D and IR and a direct association between 25(OH)D and insulin sensitivity (12, 19, 21). In contrast, others (47) found no association between serum 25(OH)D and IR in obese children. The lack of association may be due to a small sample size or a limited range of serum 25(OH)D with more than one-quarter of the subjects with severe vitamin D deficiency [25(OH)D <30 nmol/L] (47).

The mechanism by which vitamin D reduces IR includes its effects on enhancing insulin sensitivity by up-regulating the insulin receptors and activating peroxisome proliferator-activated receptor-γ expression, a transcription factor important for fatty acid metabolism in adipose and muscle tissues (48). Also, in response to 1,25-dihydroxyvitamin D [1,25(OH)2D], calcitriol is synthesized, which alters insulin action on the adipocyte and affects the secretion of insulin from pancreatic β cells (12, 18). The association between vitamin D and IR is further supported by the presence of polymorphisms in the VDR gene. These polymorphisms (Apa I, Bsm I, Tag I, and Fok I) have been found to modify insulin secretion leading to IR and dysregulation of glucose homeostasis (49, 50). Although the evidence for vitamin D supplementation is building, randomized controlled studies are needed before vitamin D supplementation can be considered as a potential intervention strategy for the management of IR and T2D.

With the use of previously available unadjusted data from NHANES 2001–2004, Reis et al (16) reported an association of serum 25(OH)D with MetSyn. Our study extends these findings with the adjusted values for serum 25(OH)D and provides additional information regarding the relation between vitamin D status and IR. With the use of unadjusted NHANES 2001–2004 data, Kumar et al (3) found that vitamin D deficiency (defined as <37.44 nmol/L) was inversely associated with elevated BP and directly associated with HDL cholesterol in persons aged 1–21 y. With the use of unadjusted data from NHANES III, Ford et al (17) reported an inverse association between serum 25(OH)D and abdominal obesity, hyperglycemia, and hypertriglyceridemia in adults. Reis et al (51) found an inverse association between serum 25(OH)D and MetSyn independent of BMI, parathyroid hormone, and calcium intake in adults (aged ≥20 y) in unadjusted NHANES 2003–2004. With the use of the assay-adjusted serum 25(OH)D, we confirm some previously reported associations of serum 25(OH)D with cardiometabolic risk factors in children.

We found that serum 25(OH)D was inversely associated with WC, which correlates with a previously reported inverse association between serum 25(OH)D and obesity (51). Although the exact reasons for low serum 25(OH)D in obese individuals are not clear, it has been shown that there was no difference in endogenous synthesis of vitamin D in the dermis between obese and nonobese persons when exposed to sunlight; however, the release of vitamin D from the dermis into the circulation is reduced in obese persons, leading to depressed circulating 25(OH)D (52). Sequestering of vitamin D within the adipose tissue might also explain low circulating 25(OH)D in obesity (52). In addition, overweight and obese children generally spend less time outdoors due to sedentary
VITAMIN D AND CARDIOMETABOLIC RISK FACTORS


<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum 25(OH)D concentration</th>
<th>P for trend²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1: &lt;48.1 nmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tertile 2: 48.1 to &lt;66.2 nmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tertile 3: ≥66.2 nmol/L</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)† (n = 5377)</td>
<td>85.3 (0.7)a</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mm Hg)† (n = 5281)</td>
<td>109.8 (0.5)a</td>
<td></td>
</tr>
<tr>
<td>Diastolic (mm Hg)† (n = 5313)</td>
<td>59.9 (0.4)</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL) (n = 5398)</td>
<td>48.4 (1.0)a</td>
<td></td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dL) (n = 2581)</td>
<td>79.8 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL) (n = 2599)</td>
<td>92.8 (1.0)</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (µg/dL) (n = 5470)</td>
<td>48.3 (1.1)</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR10 (n = 2573)</td>
<td>2.56 (1.0)a</td>
<td></td>
</tr>
</tbody>
</table>

¹ All values are multivariate-adjusted means (SEs in parentheses). NHANES 2001–2002, 2003–2004, and 2005–2006 were combined into one master database: NHANES 2001–2006. HOMA-IR, homeostatic model assessment–insulin resistance index. Analysis was based on recently released assay-adjusted serum 25(OH)D concentrations. To convert nmol/L to ng/mL, divide by 2.496. Multiple comparisons were made with Bonferroni correction for the cardiometabolic variables by using a family-wise significance level of P < 0.05 for Wald F. Values with different superscript letters are significantly different from each other within the row if the contrast between 2 values is P < 0.0167. The lack of superscripts indicate nonsignificance of that cardiometabolic risk variable in relation to serum 25(OH)D in the regression model.

² Significance for the association of serum 25(OH)D with cardiometabolic risk factor variables in the multivariate-adjusted regression analyses.

³ Adjusted for sex, age, race-ethnicity, season of survey, and use of supplements. Poverty-income ratio and BMI were not significant. Analysis was performed by using natural log-transformed values. Significant for the interaction between sex and race-ethnicity (P < 0.001).

4 Adjusted for age, sex, race-ethnicity, and BMI. Significant for the interaction between sex and race-ethnicity (P < 0.001). Supplement use, poverty-income ratio, and season of survey were not significant in the model.

5 Adjusted for age and sex. Race-ethnicity, BMI, supplement use, poverty-income ratio, and season of survey were not significant in the model.

6 Adjusted for age, sex, race-ethnicity, poverty-income ratio, and BMI. Significant for the interaction between sex and race-ethnicity (P < 0.0001). Season of survey and supplement use were not significant. Analysis was performed by using natural log-transformed values.

7 Adjusted for race-ethnicity, BMI, and season of survey. Age, sex, supplement use, and poverty-income ratio were not significant in the model. Analysis was performed by using natural log-transformed values. Significant for the interaction between race-ethnicity and BMI (P < 0.001).

8 Adjusted for age, sex, race-ethnicity, season of survey, and BMI. Poverty-income ratio and supplement use were not significant. Analysis was performed by using natural log-transformed values.

9 Adjusted for age, race-ethnicity, BMI, and poverty-income ratio. Sex, supplement use, and season of survey were not significant in the model. Analysis was performed by using natural log-transformed values.

10 Fasting insulin (µU/mL) × fasting glucose (mg/dL)/405. Adjusted for race-ethnicity, BMI, and use of supplements. Sex, age, poverty-income ratio, and season of survey were not significant in the model. Analysis was performed by using natural log-transformed values.

Lifestyles, which lead to less exposure to ultraviolet B radiation (53). These are possible explanations for depressed endogenous vitamin D synthesis and circulating 25(OH)D in obesity.

Similar to our findings using unadjusted vitamin D data from NHANES III, Judd et al (8) found that serum 25(OH)D (<50 nmol/L) was inversely associated with SBP in adults. Also, in the prospective cohort of the first Nurses’ Health Study, it was shown that plasma 25(OH)D was inversely related to the incidence of hypertension (54). Vitamin D is known to play a role in the regulation of BP (55). The renin-angiotensin-aldosterone system (RAS) regulates BP, electrolyte, and blood volume homeostasis (56). In obese persons with hypovitaminosis D, the perturbation in RAS may be the cause of elevated BP (57). The RAS is up-regulated in obesity, and excessive RAS stimulation leads to elevated BP (58). A possible explanation for the association between vitamin D and BP in obesity may be because vitamin D is stored in adipose tissue where all components of the RAS are also synthesized (59). The role of vitamin D in regulating RAS is further supported by the fact that the VDR knockout mice experienced elevated renin and BP (60). On the basis of the evidence, vitamin D supplementation for lowering BP has been proposed (8).

Because VDRs are located on immune cells that synthesize and secrete 1,25(OH)₂D, it is believed that vitamin D plays a role in inflammation (61). The underlying mechanism behind the inverse relation between vitamin D and the inflammatory marker CRP is not clear. It has been found that the expression of cytokines is down-regulated by 1,25(OH)₂D via VDR signaling through the regulation of nuclear transcription factor kB (32). However, in the Framingham study, Shea et al (62) found no association of 25(OH) D with inflammation, which was similar to our findings. The lack of a relation with CRP is likely due to fewer overweight and obese children in this study.

The use of national surveys with a large sample size of children allowed us to examine the relation of serum 25(OH)D with various cardiometabolic risk factors after taking several confounding variables into account. The confirmation of a cause-and-effect relation is not possible due to the cross-sectional design of the study. In conclusion, the assay-adjusted NHANES 2001–2006 vitamin D data show that children with poor vitamin D status are at increased risk of several cardiometabolic risk factors regardless of obesity. The relation between low vitamin D status and MetSyn and IR is a concern because children with MetSyn are at high risk of future CVD and T2D. Because of negative health outcomes associated with MetSyn and poor vitamin D status, an examination of vitamin D supplementation in reversing components of MetSyn ultimately in the prevention of CVD appears to be warranted.

The authors’ responsibilities were as follows—VG, XZ, and VT: contributed to the study design and interpretation of results; VG: wrote the manuscript; XZ...
and NS; contributed to data acquisition, data management, and data analysis; and VG, XZ, NS, and VT: contributed to review, editing, and revision of the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES
2. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Preva-

cence of vitamin D deficiency among healthy adolescents. Arch Pediatr

cence and Associations of 25-Hydroxyvitamin D Deficiency in US
4. Holick MF. Sunlight and vitamin D for bone health and prevention of
autoimmune diseases, cancers, and cardiovascular disease. Am J Clin
Nutr 2004;80:1678S–88S.
5. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake
of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet
2001;358:1500–3.
independently associated with high-density lipoprotein cholesterol and
7. Baz-Hecht M, Goldfine AB. The impact of vitamin D deficiency on
diabetes and cardiovascular risk. Curr Opin Endocrinol Diabetes Obes
vitamin D status attenuates the age-associated increase in systolic
blood pressure in white Americans: results from the third National
Health and Nutrition Examination Survey. Am J Clin Nutr 2008;87:
136–41.
D concentrations are related to depression in young adult US pop-
ulation: the Third National Health and Nutrition Examination Survey.
10. Spina CS, Tangpricha V, Uskokovic M, Adorinic L, Maehr H, Holick
11. Potenza MV, Mechaniak JL. The metabolic syndrome: definition, global
12. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin
Education Program (NCEP) Expert Panel on Detection, Evaluation, and
Treatment of High Blood Cholesterol in Adults (Adult Treatment
14. Duncan GE, Li SM, Zhou XH. Prevalence and trends of a metabolic
Care 2004;27:4384–9.2
15. Johnson WD, Krono JJ, Greenway FL, Bouchard C, Ryan D, Karmazyn
PT. Prevalence of risk factors for metabolic syndrome in adolescents:
National Health and Nutrition Examination Survey (NHANES),
D. Status and cardiometabolic risk factors in the United States ado-
17. Ford ES, Ajani UA, Mcguire LC, Liu S. Concentrations of serum
vitamin D and the metabolic syndrome among U.S. adults. Diabetes
18. Teegarden D, Donkien Ss. Vitamin D: emerging new roles in insulin
19. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated
with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004;
79:560–8.
20. Huh SY, Gordon CM. Vitamin D deficiency in children and adoles-
cents: epidemiology, impact and treatment. Rev Endocr Metab Disord
21. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline
serum 25-hydroxy vitamin D is predictive of future glycemic status and
insulin resistance: the Medical Research Council Ely Prospective Study
22. Aleemzadeh R, Kichler J, Babar G, Calhoun M. Hypovitaminosis D in
obese children and adolescents: relationship with adiposity, insulin
23. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W. Vitamin D
status and parathyroid hormone in obese children before and after weight
24. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-
Hydroxyvitamin D deficiency is associated with inflammation-linked
vascular endothelial dysfunction in middle-aged and older adults.
25. Oliveira AC, Oliveira AM, Adan LF, Oliveira NF, Silva AM, Ladeia
AM. C-reactive protein and metabolic syndrome in youth: a strong
26. Giannoudis RF, Association C. C-reactive protein and indices of
body fat distribution and overweight in Mexican American children.
27. de Ferranti SD, Rifai N. C-reactive protein: a nontraditional serum
28. Szmitko PE, Verma S. C-reactive protein and the metabolic syndrome:
useful addition to the cardiovascular risk profile? J Cardiometab Syndr
29. Skinner AC, Steiner MJ, Henderson FW, Perrin EM. Multiple markers
of inflammation and weight status: cross-sectional analyses throughout
30. Ford ES, Ajani UA, Mokdad AH. The metabolic syndrome and con-
centrations of C-reactive protein among U.S. youth. Diabetes Care
31. Reinehr T, Gauvreau K, Ludwig DS, Newburger JW, Rifai N. Inflamma-
tion and changes in metabolic syndrome abnormalities in US adoles-
32. Sun J, Kong J, Duan Y, et al. Increased NF-kappa B activity in fibro-
basts lacking the vitamin D receptor. Am J Physiol Endocrinol Metab
33. National Center for Health Statistics. Revised analytic note for NHANES
nhanes3/VitaminD_analyticnote.pdf (cited 3 December 2010).
34. National Center for Health Statistics. National Health and Nutrition
jenue_04.pdf (cited 1 December 2010).
35. National Center for Health Statistics. National Health and Nutrition
December 2010).
www.cdc.gov/nchs/nhanes/nhanes2001-2002/current_nhanes_01_02_
htm (cited 2 December 2010).
37. National Center for Health Statistics. National Health and Nutrition
www.cdc.gov/nchs/nhanes/nhanes2005-2006/lab05_06.htm (cited 1
December 2010).
38. National Center for Health Statistics. National Health and Nutrition
Examination Survey laboratory protocol. 2004. Available from http:
www.cdc.gov/nchs/nhanes/nhanes2003-2004/lab03_04.htm (cited 3
December 2010).
www.cdc.gov/nchs/nhanes/nhanes2001-2002/lab01_02.htm (cited 1
December 2010).
40. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF,
Turner RC. Homeostasis model assessment: insulin resistance and
beta-cell function from fasting plasma glucose and insulin concen-
41. Le Stunff C, Dechartres A, Miraglia Del Giudice E, Froguel P,
Bougres P. A single-nucleotide polymorphism in the p110beta gene
promoter is associated with partial protection from insulin resistance
42. Mansbach JM, Ginde AA, Camargo CA Jr. Serum 25-hydroxyvitamin
D levels among US children aged 1-11 years: do children need more