

7-2-2013

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## Repository Citation

Nwosu, Benjamin U.; Maranda, Louise; Cullen, Karen; Ciccarelli, Carol A.; and Lee, Mary M., "Vitamin D status is associated with early markers of cardiovascular disease in prepubertal children" (2013). *Endocrinology/Diabetes*. 42.

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# Vitamin D status is associated with early markers of cardiovascular disease in prepubertal children

## Abstract

**Background:** The associations of 25-hydroxyvitamin D [25(OH)D], non-high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL), and related markers of early cardiovascular disease (CVD) are unclear in prepubertal children.

**Objective:** To investigate the association of 25(OH)D with markers of CVD. The hypothesis was that 25(OH)D would vary inversely with non-HDL-C.

**Subjects and methods:** A prospective cross-sectional study of children (n=45; 26 males, 19 females) of mean age 8.3±2.5 years to investigate the relationships between 25(OH)D and glucose, insulin, high-sensitivity C-reactive protein, and lipids. Vitamin D deficiency was defined as 25(OH)D <20 ng/mL; overweight as body mass index (BMI) ≥85th but <95th percentile; and obesity as BMI >95th percentile.

**Results:** Twenty subjects (44.4%) had BMI <85%, and 25 had BMI of ≥85%. Eleven participants (24.4%) had 25(OH)D of <20 ng/mL, and 10 (22.2%) had 25(OH)D of >30 ng/mL. Patients with 25(OH)D of <20 ng/mL had significantly elevated non-HDL-C (136.08±44.66 vs. 109.88±28.25, p=0.025), total cholesterol (TC)/HDL ratio (3.89±1.20 vs. 3.21±0.83, p=0.042), and triglycerides (TG) (117.09±71.27 vs. 73.39±46.53, p=0.024), while those with 25(OH)D of >30 ng/mL had significantly lower non-HDL-C, TC/HDL, TG, and LDL (82.40±18.03 vs. 105.15±28.38, p=0.006). Multivariate analysis showed significant inverse correlations between 25(OH)D and non-HDL cholesterol ( $\beta=-0.337$ , p=0.043), and TC/HDL ratio ( $\beta=-0.339$ , p=0.028), and LDL ( $\beta=-0.359$ , p=0.016), after adjusting for age, race, sex, BMI, and seasonality.

**Conclusions:** Vitamin D varied inversely with non-HDL, TC/HDL, and LDL. A 25(OH)D level of 30 ng/mL is associated with optimal cardioprotection in children.

**Keywords:** cardiovascular disease; prepubertal children; vitamin D status.

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## Introduction

Several studies have reported a link between vitamin D deficiency and cardiometabolic risk factors such as inflammation, insulin resistance, abnormal lipid profile, and high blood pressure in adults (1–4). Few studies, however, have investigated these relationships in prepubertal children (5, 6). Most of the pediatric studies were either conducted in older children and adolescents exclusively or in a mixed group of children and adolescents (7–9). For example, data from the 2001–2004 National Health and Nutrition Examination Surveys (NHANES) analysis reported that 25-hydroxyvitamin D [25(OH)D] concentrations were inversely associated with systolic blood pressure (BP) and plasma glucose concentrations in 3577 youth of 12–19 years (10), while a second report on the same data in 6257 children and adolescents of ages 1–21 years showed that 25(OH)D level of <15 ng/mL was associated with increased BP and lower high-density lipoprotein (HDL) cholesterol (8). A third report on 5867 adolescents of ages 12–19 years using three cycles of NHANES data from 2001 through 2006 found that serum 25(OH)D level was related to homeostasis model assessment-insulin resistance index (HOMA-IR), systolic blood pressure (SBP), and HDL cholesterol, but not to high-sensitivity C-reactive protein (hsCRP) (9). A study of 701 adolescents of ages 14–18 years living in the sunny parts of the US showed significant inverse correlations between serum 25(OH)D concentrations and leptin, fibrinogen, glucose, HOMA-IR, SBP, and diastolic blood pressure (DBP), and significant positive correlations between adiponectin and HDL cholesterol (6). Another study (11) evaluated the relationship between vitamin D status, total and abdominal adiposity, and lipids in 237 preadolescent and adolescent black and white subjects of ages 8–18 years and found that lower levels of 25(OH)D were associated with higher adiposity measures and lower HDL cholesterol levels. A recent

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study (5) from the UK examined the differential associations of each of the 25(OH)D metabolites, namely, 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, with cardiovascular risk factors in 4274 prepubertal children of mean age of 9.9 years and, surprisingly, found that lower 25(OH)D<sub>2</sub> status was associated with lower levels of chronic inflammatory markers – CRP and interleukin-6 – and higher levels of apolipoprotein A1. In contrast, higher circulating 25(OH)D<sub>3</sub> was associated with elevated levels of HDL cholesterol, apolipoprotein A1, and adiponectin. There was no evidence for associations of either 25(OH)D<sub>2</sub> or 25(OH)D<sub>3</sub> with DBP or low-density lipoprotein (LDL) cholesterol. The mixed results from this study made it rather difficult to accurately characterize the relationships of vitamin D and cardiovascular risk factors in prepubertal children.

Although the above studies have provided useful information to advance the field, there are some vital areas that need to be addressed to effectively determine the exact relationship between vitamin D and cardiometabolic risks in prepubertal children. Given the mixed findings from the only exclusively preadolescent study above (5), the relationship between vitamin D status and markers of cardiovascular disease in prepubertal children remains unclear. Furthermore, because the rest of the studies were either performed exclusively in adolescents (6, 9, 10) or in a mixed group of children and adolescents (8, 11), the role of pubertal hormonal fluctuations and body fat changes on the findings of these studies is unclear. In order to exclude the influence of such confounders and determine the exact nature of the relationship between vitamin D and cardiometabolic risk factors, it is necessary to conduct such a study in a cohort of healthy prepubertal children.

Another important reason to conduct this study in an exclusive cohort of prepubertal children was to evaluate the recent report from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents which recommends that all prepubertal children be screened for risk factors of cardiovascular disease (CVD) (12) using non-HDL cholesterol as a core screening tool for the identification of dyslipidemia in childhood (12). Non-HDL cholesterol is defined as the difference between TC and HDL cholesterol and, thus, represents cholesterol carried on all of the potentially proatherogenic apoB-containing particles [primarily very low density lipoprotein, intermediate-density lipoprotein, and LDL, as well as chylomicron remnants and lipoprotein(a)] (13). It is a significant predictor of atherosclerosis and future cardiovascular events compared to TC, LDL-cholesterol, or HDL-cholesterol levels alone (12, 14). However, the relationship between vitamin D and non-HDL cholesterol in prepubertal children has not

been adequately studied. Our primary aim was to determine the association of serum 25(OH)D concentrations with non-HDL cholesterol and related cardiometabolic risk factors in healthy prepubertal children. Our primary hypothesis was that non-HDL cholesterol would vary inversely with the serum concentrations of total body vitamin D as measured by 25(OH)D level.

## Subjects and methods

The study protocol was approved by the University of Massachusetts Institutional Review Board. The study's clinical trial identification number is NCT00756899. Written informed consent was obtained from each subject's parent or legal guardian, and assent was obtained from each subject prior to participating in the study.

### Subjects

All participants were healthy children who were recruited by means of advertisement using paper flyers distributed to the primary care physician offices in Central New England, USA. Fifty subjects signed consent for the study. Four patients failed to return for the study. One patient developed needle phobia after initial assessment and refused phlebotomy. Forty-five subjects (26 males and 19 females) between 3 and 12 years of age were studied. The mean age of the cohort was 8.3±2.5 years, while the mean age of the females was 7.28±2.4 years, and the mean age of the males was 9.0±2.4 years. All participants were of prepubertal status: males, with testicular volume of ≤3 mL, and females, with Tanner 1 breasts as determined by the principal investigator. Subjects were excluded if they had any known metabolic or genetic diseases resulting in obesity such as severe hypothyroidism, pseudohypoparathyroidism, or Cushing's disease. We also excluded patients with known familial dyslipidemias and secondary causes of dyslipidemia such as diabetes mellitus, nephrotic syndrome, chronic renal disease, history of Kawasaki disease, and chronic inflammatory disorders. Subjects were also excluded if they were receiving lipid-lowering medications or taking medications known to impact body weight or calcium homeostasis. Subjects with a history of significant weight loss or gain (change of >10% body weight in 6 months) were excluded from the study. Methods used for exclusion included history, physical examination, and screening laboratory tests for fasting blood glucose, cortisol, urinalysis, comprehensive metabolic panel, serum creatinine, and thyroid function tests. Because vitamin D status could vary with sunlight exposure and the seasons, we categorized each subject's visit according to the seasons as follows: fall (September 22–December 21), winter (December 22–March 21), spring (March 22–June 21), and summer (June 22–September 21) (15).

### Study methods

All participants in this cross-sectional study were evaluated at the UMass Memorial Children's Medical Center between 0800 and 0900 h following an overnight fast.

## Anthropometry

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Ltd., Crymych, Dyfed, UK). Weight was measured to the nearest 0.1 kg using an upright scale. Body mass index (BMI) was derived using the formula weight in kilograms divided by the square of the height in meters ( $\text{kg}/\text{m}^2$ ) and expressed as standard deviation score (SDS) for age and gender based on National Center for Health Statistics standards (16).

## Biochemical studies

Fasting blood sample was collected for the following analytes: glucose, insulin, 25(OH)D, lipid profile, and hsCRP. All samples were centrifuged and stored at  $-70^\circ\text{C}$  until assay.

## Assay

Serum levels of 25(OH)D were analyzed using 25-hydroxy chemiluminescent immunoassay (DiaSorin Liaison; Stillwater, MN, USA), which has a 100% cross-reactivity with both metabolites of 25(OH)D, namely, 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, and thus measures total serum 25(OH)D content. Its functional sensitivity is 4 ng/mL, and its intra- and inter-assay coefficients of variation are 5% and 8.2%, respectively. Vitamin D status was classified according to American Academy of Pediatrics and the Institutes of Medicine criteria as deficient [25(OH)D <20 ng/mL] or sufficient [25(OH)D >20 ng/mL] (17).

Lipid levels were measured enzymatically on a Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN, USA) using reagents and calibrators from Roche Diagnostics in a laboratory certified by the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute's Lipid Standardization Program (17). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol (14).

Serum hsCRP was measured by a latex particle-enhanced immune-turbidimetric immunoassay (Immulite 1000 High-Sensitivity CRP, Diagnostic Products, Los Angeles, CA, USA). The minimal detected CRP level was 0.01 mg/L, with intra-assay and interassay CV of 4.2%–6.4% and 4.9%–10.0%, respectively (17).

Insulin was measured by microparticle-enhanced immuno-metric assay (Abbott, Wiesbaden, Germany), and glucose on a Vitros analyzer (Ortho Clinical Diagnostics, Neckargemünd, Germany). Insulin resistance was estimated from fasting plasma measurements using the homeostasis model of insulin resistance (HOMA-IR) [insulin (mU/L) × glucose (mmol/L)/22.5] (9).

## Statistical analyses

Statistical analyses were performed using SPSS v. 20 (IBM Corporation, Somers, NY, USA). Means, standard deviations, and percentages were calculated for descriptive summary statistics. The values for 25(OH)D, non-HDL cholesterol, cholesterol/HDL ratio, and HOMA-IR were logarithmically transformed to approximate normal distributions. Univariate comparisons between groups were made using the Student's t-test. Data were expressed as mean ± standard deviation.

Proportions were compared using Fisher's exact test. Simple and multiple linear regression models were specified to describe the relationships between the parameters of interest. For the analysis of seasonality, data on the seasons associated with high 25(OH)D levels (summer and fall) were combined and contrasted with the seasons with lower 25(OH)D levels (winter and spring) based on an earlier report by Ostergard et al. (18), which demonstrated a significant seasonal difference in 25(OH)D, with higher levels during summer-autumn compared with winter-spring ( $p=0.021$ ) after controlling for BMI.

## Results

Subjects were first categorized into vitamin D-deficient [25(OH)D level of <20 ng/mL] and vitamin D-sufficient [25(OH)D of >20 ng/mL] groups (Table 1) (19). Table 1 shows the mean ( $\pm$ SD) values for age, gender, seasonality, anthropometric, and biochemical parameters. Subjects with vitamin D deficiency had significantly elevated values for non-HDL cholesterol, TC/HDL ratio, and triglycerides (TG). There were no significant differences between the groups for systolic or diastolic blood pressure, LDL cholesterol, and hsCRP. Further analysis showed a significant positive correlation between 25(OH)D and HDL cholesterol, and significant inverse correlations between 25(OH)D and HOMA-IR, non-HDL cholesterol, LDL, and TC/HDL ratio. However, only the relationships between 25(OH)D and LDL ( $\beta=-0.359$ ,  $p=0.016$ ), non-HDL cholesterol (Figure 1), and TC/HDL ratio (Figure 2) remained significant after adjusting for age, sex, race, BMI, and seasonality.

Further analysis showed that 22.2% of the subjects had 25(OH)D levels  $\geq 30$  ng/mL. Participants with 25(OH)D level of  $\geq 30$  ng/mL had a significant reduction in LDL cholesterol ( $82.40 \pm 18.03$  vs.  $105.15 \pm 28.38$ ,  $p=0.006$ ) and in all the lipid parameters that were significantly lower at a 25(OH)D cut-off value of 20 ng/mL, viz, triglycerides ( $61.1 \pm 27.41$  vs.  $89.84 \pm 61.29$ ,  $p=0.044$ ), TC/HDL ratio ( $2.72 \pm 0.52$  vs.  $3.56 \pm 0.99$ ,  $p=0.001$ ), and non-HDL cholesterol ( $94.60 \pm 16.55$  vs.  $123.12 \pm 36.84$ ,  $p=0.002$ ).

In subsequent analysis, the subjects were stratified into normal-weight vs. overweight and obese groups to determine the influence of obesity on the CVD parameters (Table 2). Overweight was defined as body mass index (BMI) of  $\geq 85$ th but <95th percentile, while obesity was defined as a BMI of  $\geq 95$ th percentile for age and sex. The overweight/obese prepubertal children had significantly lower levels of 25(OH)D and HDL cholesterol, but significantly elevated values for hsCRP, total cholesterol (TC)/HDL ratio, triglycerides, non-HDL, systolic blood pressure, and diastolic blood pressure. There was no difference in the level of LDL cholesterol between the normal-weight and overweight/obese groups.

**Table 1** Physical and biochemical characteristics of subjects stratified by vitamin D status.

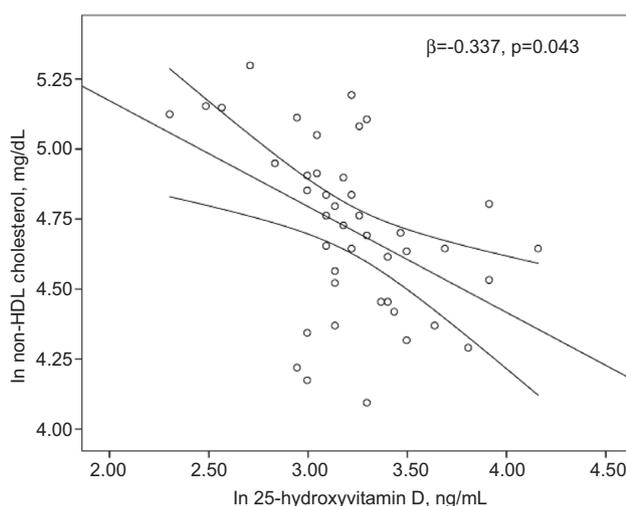
Parameters	25(OH)D $\leq$ 20 ng/dL	25(OH)D >20 ng/dL	p-Value <sup>a</sup>
n	11	34	
Age, years	8.72 $\pm$ 2.48	8.16 $\pm$ 2.58	0.534
Sex, males	3	13	0.720
Race, white	9 (81.8%)	30 (88.2%)	0.841
Weight, kg	47.78 $\pm$ 25.43	36.48 $\pm$ 20.61	0.143
Height, cm	131.47 $\pm$ 15.0	127.51 $\pm$ 17.59	0.505
BMI SDS	1.67 $\pm$ 1.01	0.90 $\pm$ 1.55	0.133
SBP, mm Hg	105.55 $\pm$ 9.47	103.88 $\pm$ 14.37	0.722
DBP, mm Hg	65.27 $\pm$ 8.34	61.18 $\pm$ 9.55	0.228
25(OH)D, ng/mL	16.82 $\pm$ 3.71	29.88 $\pm$ 9.92	<0.001
hsCRP, mg/mL	2.28 $\pm$ 1.58	1.55 $\pm$ 1.29	0.134
Total cholesterol (TC), mg/dL	185.18 $\pm$ 43.86	163.91 $\pm$ 27.36	0.064
Triglycerides, mg/dL	117.09 $\pm$ 71.27	73.39 $\pm$ 46.53	0.024
HDL-cholesterol, mg/dL	49.45 $\pm$ 7.27	53.12 $\pm$ 11.38	0.324
LDL-cholesterol, mg/dL	112.27 $\pm$ 37.39	96.12 $\pm$ 22.85	0.093
TC/DL ratio	3.89 $\pm$ 1.20	3.21 $\pm$ 0.83	0.042
Non-HDL cholesterol, mg/dL	136.08 $\pm$ 44.66	109.88 $\pm$ 28.25	0.025
Fasting blood glucose, mg/dL	88.64 $\pm$ 7.61	85.39 $\pm$ 6.56	0.236
Insulin, $\mu$ U/mL	10.70 $\pm$ 8.27	7.48 $\pm$ 8.04	0.302
HOMA-IR	2.27 $\pm$ 2.12	1.62 $\pm$ 1.81	0.423
Season (summer-fall)	3	11	1.0

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; 25(OH)D, 25-hydroxyvitamin D; hsCRP, high sensitivity C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance. <sup>a</sup>Results for two tailed t-tests. Significant t-tests are italicized.

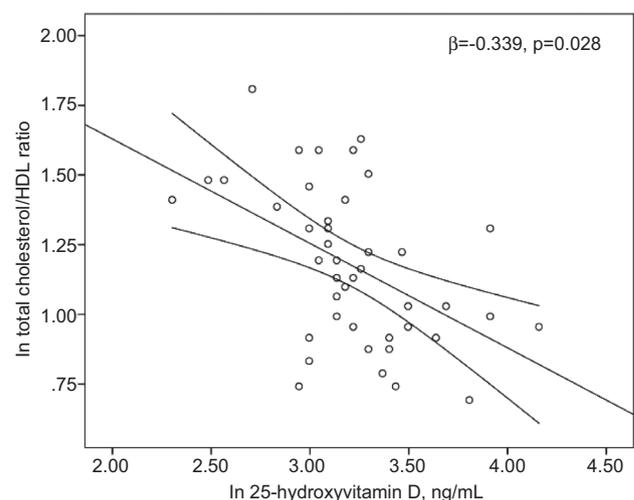
## Discussion

In summary, these data showed significantly elevated levels of non-HDL cholesterol, triglycerides, and TC/HDL ratio in prepubertal children with serum 25(OH)D level

of <20 ng/mL. There were significant inverse correlations between 25(OH)D and non-HDL cholesterol, LDL, and TC/HDL ratio in all patients when adjusted for age, sex, race, BMI, and seasonality. When compared to normal-weight children, overweight/obese prepubertal children had



**Figure 1** The relationship between 25-hydroxyvitamin D and non-HDL cholesterol in prepubertal children. This inverse correlation remained significant after adjusting for body mass index, age, sex, race, and seasonality.



**Figure 2** The relationship between 25-hydroxyvitamin D and total cholesterol/HDL ratio in prepubertal children. This inverse correlation remained significant after adjusting for body mass index, age, sex, race, and seasonality.

**Table 2** Physical and biochemical characteristics of subjects stratified by body mass index.

Parameters	Normal weight (BMI <85%)	Overweight/obese (BMI ≥85%)	p-Value <sup>a</sup>
n	20	25	
Age, years	7.45±2.36	8.98±2.51	0.042
Sex, males	11	15	0.770
Race, white	18 (90%)	21 (84%)	0.710
Weight, kg	22.00±5.17	53.04±20.75	<0.001
Height, cm	117.73±10.35	137.07±16.32	<0.001
BMI, SDS	-0.27±1.03	2.18±0.56	<0.001
SBP, mm Hg	96.73±6.85	110.04±14.09	<0.001
DBP, mm Hg	57.80±6.36	65.56±9.95	0.003
25OHD, ng/mL	30.90±13.22	23.04±5.51	0.020
CRP, ng/mL (median)	1.06±0.17	2.30±1.68	0.001
Total cholesterol, mg/dL	160.70±26.70	176.33±36.54	0.109
Triglycerides, mg/dL	60.70±28.44	104.00±65.94	0.007
HDL, mg/dL	56.35±10.19	48.75±9.73	0.016
LDL, mg/dL	92.20±23.85	106.79±29.27	0.076
TC/DL ratio	2.95±0.72	3.74±1.00	0.004
Non-HDL cholesterol, mg/dL	104.35±28.07	127.58±37.20	0.027
Fasting blood glucose, mg/dL	83.72±6.62	88.13±6.63	0.040
Insulin, μU/mL	2.75±1.48	12.17±8.61	<0.001
HOMA-IR	0.58±0.35	2.71±2.03	<0.001
Season (summer-fall)	5	9	0.525

<sup>a</sup>Results for two-tailed t-tests. Significant t-tests are italicized.

significantly lower HDL cholesterol and 25(OH)D levels, but significantly elevated levels for hsCRP, TC/HDL ratio, non-HDL cholesterol, triglycerides, HOMA-IR, and blood pressure. Children with 25(OH)D level of  $\geq 30$  ng/mL had significantly reduced LDL cholesterol level in addition to the other lipid parameters. These findings are detailed below.

## Cholesterol

Although several studies have established that high BMI in children and adolescents is associated with adverse levels of lipids, insulin, and blood pressure (20–22), the independent associations of vitamin D status with these markers of cardiovascular disease in a cohort of prepubertal children have not been adequately studied (17, 23). Such studies are necessary to evaluate the recent summary report from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents which recommends that all prepubertal children be screened for risk factors of cardiovascular disease (CVD) (12). The central focus of this recommendation, which has drawn sharp criticisms (24) from some clinicians and praise from others (25), is the early initiation of primordial prevention of CVD starting

in the prepubertal years. *Primordial* prevention is a term that describes the avoidance of risk-factor development, in contrast to *primary* prevention which is the prevention of future CVD by effective management of identified risk factors (12). The strong emphasis on the prepubertal period stemmed from studies showing that the pathologic process of atherosclerotic CVD (2) begins in childhood with the deposition of fatty streaks within the arterial walls and subsequent progression into fibrous plaques throughout adolescence and early adulthood (25). While the centerpiece of primordial prevention is lifestyle modification (26), adherence is poor (27). Hence, the necessity to identify modifiable risk factors that could augment the primordial prevention of cardiometabolic risk factors in prepubertal children. The expert panel based its recommendation that children be screened for risk factors for CVD before the onset of puberty on the finding that TC and LDL-cholesterol levels decreased by as much as 10%–20% or more during puberty (12). They recommended that 10 years of age (range: 9–11 years) is a stable time for lipid assessment in children based on the above-stated normal pattern of change in lipid and lipoprotein levels with growth and maturation (12). The panel recommended the use of non-HDL cholesterol as a key screening tool for the identification of dyslipidemia in childhood because non-HDL cholesterol is a strong predictor of the

presence of atherosclerosis in children and adolescents (12). A major advantage of non-HDL cholesterol is that it can be accurately calculated in a non-fasting state and is therefore practical to obtain in routine, regular evaluation of patients (12). While the data showed that there was neither a significant difference in LDL cholesterol between the normal-weight and overweight/obese groups, nor between the vitamin D-deficient and the vitamin D-sufficient groups using a cut-off value of <20 ng/mL to define vitamin D deficiency, there was a significant difference in LDL-cholesterol level when a cut-off value of >30 ng/mL was used. Further analysis showed that prepubertal children with either a 25(OH)D level of <20 ng/mL or a BMI of  $\geq$ 85th percentile had a significantly higher level of non-HDL cholesterol level compared to those with either a 25(OH)D level of >20 ng/mL or a BMI of <85th percentile. Taken together, our data suggest that the beneficial effects of vitamin D on TG, TC/HDL ratio, and non-HDL cholesterol are apparent at a 25(OH)D level of 20 ng/mL, while the beneficial effects of vitamin D on LDL cholesterol are apparent at a much higher level of 30 ng/mL.

The postulated mechanism for the significant reduction in non-HDL cholesterol and TG levels in vitamin D-sufficient subjects is based on the actions of vitamin D to improve insulin sensitivity (28) and reduce free fatty acid (FFA) generation (29). In general, insulin resistance or defects in FFA incorporation into adipocytes lead to increased mobilization of FFA to the liver, leading to increased TG formation, decreased LDL proteolysis, and enhanced very low density lipoprotein production and secretion (30). These processes result in elevated serum concentrations of non-HDL cholesterol and TG. Vitamin D improves insulin sensitivity and limits FFA generation, thus leading to reduced serum concentrations of both non-HDL cholesterol and TG. The significant reduction in 25(OH)D level in obese subjects is believed to be primarily due to the sequestration of vitamin D in fat depots (31). Therefore, in clinical practice, adequate vitamin D supplementation may be necessary to maintain normal serum concentrations of non-HDL cholesterol in obese patients.

### High-sensitivity C-reactive protein

Recent reports indicate that elevated levels of baseline hsCRP, a marker of inflammatory state, are independently associated with carotid artery intima-media thickness progression, which is a measure of preclinical atherosclerosis (32). CRP is produced in the liver and its concentration could be increased by malignancy and infections that show no relationship to atherosclerosis (32). The proposed

mechanisms by which hsCRP may play a causal role in the atherosclerotic process and CVD include recruitment of monocytes to the atherosclerotic lesion (33), intimal growth (34), and endothelial dysfunction (35, 36). Our data showed a significantly elevated hsCRP level in obese prepubertal children compared to their non-obese peers. There was no significant difference in hsCRP level between the vitamin D-deficient and -sufficient children. Our finding is similar to other reports (5, 6) but differed from that of Sackeck et al. (17) who found an association between 25(OH)D and hsCRP in a mixed cohort of pubertal and postpubertal children and adolescents. A recent study (23) reported a high prevalence of vitamin D insufficiency in severely obese children with elevated levels of markers of oxidative/nitrosative stress, inflammation, and endothelial activation, such as soluble vascular cell adhesion molecule 1, plasma malondialdehyde, myeloperoxidase activity, and interleukin-6, but low levels of HDL cholesterol and apolipoprotein A1. These findings suggest a link between vitamin D deficiency and the pathogenic factors involved in the initiation and propagation of atherosclerosis. Thus, even though the traditional cardiovascular risk factors for atherosclerosis are well known (37, 38), increasing evidence implicates inflammation in the pathogenesis of atherosclerosis and CVD (32). The coexistence of vitamin D deficiency and elevated hsCRP in obese children in our study calls for interventional trials to examine the role of vitamin D supplementation on hsCRP levels in obese children.

### Blood pressure

There was no significant difference in blood pressure between vitamin D-deficient and vitamin D-sufficient prepubertal children. However, the obese prepubertal children had significantly elevated SBP and DBP when compared to their normal-weight peers. These results are similar to the findings by Williams et al. (5) who reported no association between 25(OH)D and SBP or DBP. However, other studies (8–10) showed a negative correlation between 25(OH)D and BP in children and adolescents. Although the association between low levels of 25(OH)D and hypertension is well documented in cross-sectional studies, the evidence is not so strong in prospective studies (39, 40). Vitamin D is believed to affect blood pressure (BP) through several mechanisms such as the up-regulation of the renin-angiotensin system in vitamin D-deficient states (41, 42), the suppression of parathyroid hormone activity (40), and direct modulatory effects on vascular smooth muscle cells (43) as many cells that are

important for cardiovascular health express the vitamin D receptor and respond to calcitriol (44).

## Insulin resistance

There was a significant inverse correlation between 25(OH)D and HOMA-IR, a marker of insulin resistance, in our cohort of prepubertal children prior to adjustment for covariates. This is consistent with published reports (6, 9) except that the associations persisted after adjusting for covariates. Although insulin resistance is not part of the diagnostic criteria for metabolic syndrome, it is a major cardiometabolic risk factor associated with the metabolic syndrome (45). The mechanisms for the association between 25(OH)D and insulin resistance are not fully known, but one hypothesis is that 25(OH)D promotes insulin sensitivity (28) through insulin-receptor expression and the regulation of intracellular calcium (46). It is also believed that parathyroid hormone could mediate the effects of vitamin D on insulin sensitivity and glucose tolerance (47).

## Strengths and weaknesses

This study has some limitations. First, the cross-sectional study design excluded causal inference about the effects of low vitamin status on cardiovascular risk factors in prepubertal children. This study also had a small sample size which could have precluded the detection of subtle differences between the groups. The participants were primarily made up of non-Hispanic white children. Therefore, our findings may not be generalized to other racial groups. We did not measure serum concentrations of parathyroid hormone which is believed to mediate some of the effects of vitamin D on insulin sensitivity and glucose tolerance (47). We did not measure the effect of seasons on the degree of physical activity of the children either as this could have impacted the results of our findings. It is known that sun exposure is associated with increased outdoor physical activity which could improve insulin sensitivity independent of serum 25(OH)D concentrations (46).

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The unique strength of this study is that it was conducted exclusively in healthy prepubertal children. When compared with studies in adults, associations in children are less prone to confounding lifestyle factors, such as smoking and alcohol consumption, and are also less likely to be biased by the effects of existing medical conditions and their treatments on both 25(OH)D status and cardiovascular risk (5). Thus, studies in children permit the assessment of potential associations of vitamin D with cardiovascular risk than studies in adults. Additionally, studies in prepubertal children exclude the effects of pubertal sex steroids on 25(OH)D status and cardiovascular risks. Furthermore, the blood samples were collected throughout all seasons of the year, and all outcomes of interest were adjusted for seasons.

## Conclusions

This study showed that prepubertal children with 25(OH)D level of >20 ng/mL but <30 ng/mL had significantly lower levels of non-HDL cholesterol, TC/HDL ratio, and TG only, while those with 25(OH)D of  $\geq 30$  ng/mL had significantly reduced levels of non-HDL, TC/HDL, TG, and LDL cholesterol. Therefore, a 25(OH)D level of 30 ng/mL, not 20 ng/mL, is associated with optimal cardioprotection in healthy prepubertal children. Non-HDL cholesterol, LDL, and TC/HDL ratio varied inversely with 25(OH)D in all subjects after adjusting for covariates. This study provides preliminary association data for considering larger prospective trials on the possible role of vitamin D supplementation in the primordial prevention of CVD.

**Acknowledgments:** This study was supported in part by a grant to Benjamin U. Nwosu from the Department of Pediatrics and the Faculty Diversity Scholars Program, University of Massachusetts Medical School, Worcester, MA, USA. BUN and MML are members of the UMass Diabetes and Endocrine Research Center (DK32520).

Received March 7, 2013; accepted June 3, 2013; previously published online July 2, 2013

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