

## CLINICAL STUDY

# Low 25(OH)D<sub>3</sub> levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents

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## Abstract

**Objectives:** Evidence of the association between vitamin D and cardiovascular risk factors in the young is limited. We therefore assessed the relationships between circulating 25-hydroxyvitamin D<sub>3</sub> (25(OH)D<sub>3</sub>) and metabolic syndrome (MetS), its components, and early atherosclerotic changes in 452 (304 overweight/obese and 148 healthy, normal weight) Caucasian children.

**Methods:** We determined serum 25(OH)D<sub>3</sub> concentrations in relation to MetS, its components (central obesity, hypertension, low high-density lipoprotein (HDL)-cholesterol, hypertriglyceridemia, glucose impairment, and/or insulin resistance (IR)), and impairment of flow-mediated vasodilatation (FMD) and increased carotid intima-media thickness (cIMT) – two markers of subclinical atherosclerosis.

**Results:** Higher 25(OH)D<sub>3</sub> was significantly associated with a reduced presence of MetS. Obesity, central obesity, hypertension, hypertriglyceridemia, low HDL-cholesterol, IR, and MetS were all associated with increased odds of having low 25(OH)D<sub>3</sub> levels, after adjustment for age, sex, and Tanner stage. After additional adjustment for SDS-body mass index, elevated blood pressure (BP) and MetS remained significantly associated with low vitamin D status. The adjusted odds ratio (95% confidence interval) for those in the lowest (<17 ng/ml) compared with the highest tertile (>27 ng/ml) of 25(OH)D<sub>3</sub> for hypertension was 1.72 (1.02–2.92), and for MetS, it was 2.30 (1.20–4.40). A similar pattern of association between 25(OH)D<sub>3</sub>, high BP, and MetS was observed when models were adjusted for waist circumference. No correlation was found between 25(OH)D<sub>3</sub> concentrations and either FMD or cIMT.

**Conclusions:** Low 25(OH)D<sub>3</sub> levels in Caucasian children are inversely related to total adiposity, MetS, and hypertension.

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## Introduction

Several cross-sectional and prospective studies have shown an association between low vitamin D status, as indicated by concentrations of serum 25-hydroxyvitamin D (25(OH)D), and increased prevalence of the metabolic syndrome (MetS) and individual cardiovascular disease (CVD) risk factors (1). A recent systematic review and meta-analysis, using data from eight cross-sectional studies, showed that the prevalence of the MetS was reduced by ~50% (odds ratio (OR), 0.49; 95% confidence intervals (CIs), 0.38–0.64) for those with high 25(OH)D concentration (2). Of substantial concern is that subjects with excess weight – a component of the MetS – are at risk of having 25(OH)D deficiency (3). On the other hand, epidemiologic studies suggest that intake of calcium or dairy products is associated with reduced fat mass and/or weight (4, 5). Further, dietary calcium and dairy products have been

shown to enhance weight loss or fat mass loss in several intervention studies (6, 7).

An association between vitamin D and fat or lipid metabolism has been suggested. In a randomized, placebo-controlled, calcium- or vitamin D-fortified dairy product intervention study, Teegarden *et al.* (8) provided evidence that overweight women given supplementary dairy products had increased fat oxidation. Also, in a cross-sectional study of obese men and women, Botella-Carretero *et al.* (9) reported that vitamin D deficiency was associated with, so-called, atherogenic dyslipidemia, consisting of both high triglycerides and low high-density lipoprotein (HDL) cholesterol levels.

Studies showing an inverse association of serum concentration of 25(OH)D with insulin resistance (IR) and  $\beta$ -cell dysfunction provided a further possible explanation for the inverse association between serum concentrations of vitamin D and the prevalence of the MetS (10–13). Impaired glucose-mediated insulin

production and/or IR are key components of the MetS. Previous studies have shown that IR is associated with impairment of flow-mediated vasodilatation (FMD) and increased carotid intima-media thickening (cIMT) (14, 15), early surrogate markers of CV risk. Thus, it is possible to argue that hypovitaminosis D may trigger IR as well as functional and morphological vascular changes, all of which play a role in the development of atherosclerosis. Finally, observational studies suggest that low 25(OH)D levels are associated with a higher risk of hypertension (16).

With the increasing prevalence of obesity and a greater emphasis on the continuum that represents metabolic dysfunction and IR, the research focus has recently shifted from studies on specific metabolic components to investigations of the interlinked metabolic irregularities, which constitute the MetS (1).

Although CVD events occur most frequently during or after the fifth decade of life, atherosclerosis often begins in childhood or young adulthood (17), and therefore, studies on the link between vitamin D and CVD risk factors in the young are important. A significant association between 25(OH)D and CVD risk factors in children and adolescents would suggest that successful vitamin D supplementation has the potential to improve CVD risk markers and to lower the risk of CVD in adulthood. However, compared with adults, the associations between vitamin D and MetS as well as its individual CVD risk factors have not been fully explored in children (18–27). We therefore conducted a cross-sectional study to examine the association of serum vitamin D levels with the MetS, its individual components, and early atherosclerotic abnormalities in a large sample of Italian overweight/obese and normal-weight children and adolescents.

## Methods

### Study subjects

To avoid seasonal variations, all study children were considered for inclusion during the winter months (November to March) of 2008–2009 and 2009–2010. A total of 452 Caucasian children and adolescents were included in the study. Among them, 304 were overweight/obese children who were consecutively enrolled at the outpatient clinics of the Department of Pediatrics, Sapienza University of Rome, Italy. None of the participants had a recent history of traveling to warmer and sunnier areas prior to enrollment. Exclusion criteria were primary hyperparathyroidism or other skeletal disease; malabsorptive disorders; the presence of renal disease; type 1 or 2 diabetes; any condition known to influence body composition, insulin action, or insulin secretion (e.g. glucocorticoid therapy, hypothyroidism, and Cushing's disease); a history of pre-existing heart disease; familial or secondary dyslipidemia other than that due to

the state of obesity; and history of alcohol consumption and smoking (where appropriate). The study also included the enrollment of 148 healthy normal-weight children. They were recruited during the study periods from two elementary and two middle schools in the Rome area in a pilot program to prevent CVD in childhood. No child in the control group had a history of alcohol consumption and smoking (where appropriate).

All study participants underwent physical examination including measurements of weight and standing height (from which body mass index (BMI) was calculated), waist circumference (WC), determination of the Tanner stage, systolic blood pressure (BP), and diastolic BP, as previously reported in detail (28). The degree of obesity was quantified by Cole's least mean square method, which normalizes the skewed distribution of BMI and expresses BMI as an SDS (29). The study was approved by the hospital ethics committee, and informed consent was obtained from subjects' parents before assessment.

### Dietary survey

Dietary intakes of calcium and vitamin D were assessed via 3-day (including two weekdays and one weekend day) recall interview during the study visit. Depending on the age of the child, the parent or guardian and the child were interviewed with the use of food models, portion booklets, or serving containers to assist in estimating serving size. Nutrient analyses were obtained from the Food Composition Database for Epidemiological Studies in Italy (Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia – BDA). For the subjects reporting the use of dietary supplements, the calcium and vitamin D contents from supplement sources were recorded during the study interview and, when necessary, confirmed by telephone after the study visit. Adequate intake of vitamin D was defined as 200 IU/day as recommended by the Italian Recommended Dietary Allowances.

### Laboratory methods

Blood samples were taken from each study participant, after an overnight fast, to estimate serum concentrations of 25(OH)D<sub>3</sub>, glucose, insulin, total cholesterol, HDL cholesterol, and triglycerides. Serum 25(OH)D<sub>3</sub> concentrations were measured by the electrochemiluminescence immunoassay ECLIA using an automated clinical chemistry analyzer (Elecsys 2010, Roche Diagnostics). According to the supplier's specifications, within-run and total analytical coefficient of variations were 4.0–5.7 and 6.6–9.9%, respectively, while functional sensitivity was 4 ng/ml. Insulin concentrations were determined by an electrochemiluminescent method using a COBAS 6000 immunometric analyzer (Roche Diagnostics). The remaining analytes were measured on a COBAS INTEGRA 800 analyzer (Roche Diagnostics). Total cholesterol, HDL-

cholesterol, and triglyceride concentrations were assessed with the cassettes COBAS INTEGRA total cholesterol version 2; HDL-cholesterol was assessed with version 3; triglyceride was assessed according to International Federation of Clinical Chemistry (IFCC) by enzymatic colorimetric methods; and glucose concentration was assessed with the cassette version 3 by a hexokinase method (Roche Diagnostics).

### Definitions

According to the American Heart Association (AHA), MetS is diagnosed by the presence of any three of the following five constituent conditions: central obesity as determined by WC, hypertension, low HDL-cholesterol values, elevated triglyceride values, and glucose impairment (30). We used the pediatric AHA definition, which is based on the AHA adult definition but uses pediatric reference standards for BP, WC, triglycerides, and HDL-cholesterol (31). Thus, in our study, central obesity was defined as WC  $\geq$  90th percentile for age and gender (32); hypertriglyceridemia as triglycerides  $\geq$  90th percentile for age and gender (33); low HDL-cholesterol as concentrations  $\leq$  10th percentile for age and gender (33); elevated BP as systolic or diastolic BP  $\geq$  90th percentile for age, gender, and height (34); and impaired fasting glucose as glucose  $\geq$  5.6 mmol/l. IR was determined by a homeostasis model assessment of IR (HOMA-IR). HOMA-IR was calculated using the formula: fasting insulin ( $\mu$ U/ml)  $\times$  fasting glucose (mmol/l)/22.5 (35).

### Subclinical atherosclerosis

Recent improvements in imaging technology have identified early vascular changes that can be assessed by the use of ultrasonography. These early changes include impairment of FMD and increased cIMT – two reliable markers of subclinical atherosclerosis. The measurement of FMD and cIMT by high-resolution ultrasound is increasingly used for CV risk evaluation in young individuals with obesity, MetS or its components, and pre-diabetes.

Longitudinal ultrasonographic scans of the carotid artery were obtained on the same day as the studies of the brachial artery reactivity and included evaluation of the right and left common carotid arteries near the bifurcation during end diastole. We measured four values on each side, and the maximum and mean cIMT were calculated. The coefficient of variation was  $< 3\%$  (28).

Assessment of FMD was performed as previously reported (36). Briefly, brachial artery diameters were measured before and then 45 and 70 s after 5 min of reduced blood flow (induced by inflation of a standard sphygmomanometer cuff to at least 50 mmHg above resting systolic BP). FMD was assessed as the percentage change from baseline to maximal diameter of the brachial artery with reactive hyperemia. The average of three measurements at each time point was used to derive the maximum FMD.

### Statistical analysis

Statistical analyses were performed using the SPSS package (Chicago, IL, USA). Data are expressed either as frequencies or as median and interquartile range (IQR). Differences between groups in quantitative variables were evaluated by Mann–Whitney *U* test (two tailed) or Kruskal–Wallis test, as appropriate. Proportions were compared by the  $\chi^2$  test. Partial correlation and linear regression coefficients were used to examine the relationship between variables, both in the whole population and separately in lean and obese children. Multiple logistic regression models of the tertiles of 25(OH)D<sub>3</sub> were used to estimate ORs and 95% CIs for MetS and its components. Models were adjusted for age, gender, and Tanner stage. Subsequent risk factor models were adjusted additionally for SDS-BMI (or WC).

### Results

#### Baseline characteristics of the entire study population according to serum 25(OH)D<sub>3</sub> tertiles

Baseline characteristics of all participants according to serum 25(OH)D<sub>3</sub> tertiles are summarized in Table 1. The median total daily vitamin D intake, calculated as the sum of the dietary intake and supplemental intake of vitamin D as well as total calcium intake were not significantly different among the three groups. Significant trends for a lower BMI, SDS-BMI, and WC were observed with the increasing tertile of 25(OH)D<sub>3</sub> (lowest tertile to highest). With increasing serum 25(OH)D<sub>3</sub> tertiles, there was also a significant decrease in median systolic and diastolic BP values, fasting glucose and insulin concentrations, and HOMA-IR values and a significant increase in HDL-cholesterol level. Serum 25(OH)D<sub>3</sub> concentrations were not significantly related to age, gender, Tanner stage, total cholesterol, or triglyceride concentrations, as well as to functional or structural vascular changes as assessed by measurements of FMD and cIMT respectively.

#### 25(OH)D<sub>3</sub> concentrations in relation to clinical, anthropometric, and metabolic variables

Within the entire study population, after adjustment for age, gender, and Tanner stage, the 25(OH)D<sub>3</sub> concentrations were negatively correlated with SDS-BMI, WC, systolic BP, fasting glucose, insulin and triglyceride concentrations, and HOMA-IR values but positively with HDL-cholesterol levels (Table 2). When the association was restricted to the group of obese subjects, 25(OH)D<sub>3</sub> concentrations were significantly associated with SDS-BMI, WC, systolic BP, fasting glucose and insulin concentrations, and HOMA-IR values, after adjustment for age, gender, and Tanner stage. In the control group,

**Table 1** Baseline characteristics of the participants by serum 25(OH)D<sub>3</sub> tertile categories. Data are expressed as *n* (%) or median (interquartile range).

	Serum 25(OH)D <sub>3</sub> tertile categories <sup>a</sup>			P for trend
	I	II	III	
Participants, <i>n</i> (%)	156 (34.5)	149 (33.0)	147 (32.5)	
25(OH)D <sub>3</sub> (ng/ml)	12.0 (11.0)	22.0 (5.0)	36 (17)	<0.0001
Age (years)	11.5 (4.2)	11.2 (4.3)	11.0 (4.0)	0.10
Male gender, <i>n</i> (%)	77 (49.4)	67 (45)	67 (45.6)	0.45
Tanner stage, <i>n</i> (%)				0.85
I	35 (22.4)	41 (27.5)	34 (23.1)	
II–III	66 (42.3)	66 (44.3)	77 (52.4)	
IV–V	55 (35.3)	42 (28.2)	36 (24.5)	
BMI (kg/m <sup>2</sup> )	24 (7)	22 (7)	21 (5)	<0.0001
SDS-BMI	1.64 (1.30)	1.47 (1.33)	1.21 (1.27)	0.002
Waist circumference (cm)	80 (20)	76 (21)	72 (15)	<0.0001
Systolic blood pressure (mmHg)	110 (20)	105 (10)	100 (15)	<0.0001
Diastolic blood pressure (mmHg)	70 (10)	65 (10)	62 (10)	0.012
Total cholesterol (mg/dl)	170 (51)	166 (54)	172 (47)	0.09
HDL-cholesterol (mg/dl)	50 (17)	50 (18)	54 (8)	0.008
Triglycerides (mg/dl)	80 (58)	73 (49)	68 (41)	0.12
Fasting glucose (mmol/l)	4.8 (0.6)	4.6 (0.6)	4.6 (0.5)	0.005
Insulin (mU/l)	13 (10)	10 (9)	8.5 (10)	<0.0001
HOMA-IR values	2.78 (2.22)	2.15 (1.84)	1.73 (2.12)	<0.0001
Carotid IMT (mm)	0.51 (0.13)	0.50 (0.10)	0.50 (0.11)	0.60
FMD (%)	14 (13)	13 (13)	13 (13)	0.22
Total daily vitamin D intake (IU/day)	210 (50)	221 (52)	217 (49)	0.20
Adequate intake of vitamin D (%)	19.2	18.9	19.7	0.10
Total calcium intake (mmol/day)	35 (7)	40 (10)	39 (11)	0.33

<sup>a</sup>Tertile I, 25(OH)D<sub>3</sub> <17.0 ng/ml; tertile II, 25(OH)D<sub>3</sub>: 17.0–27.0 ng/ml; tertile III, 25(OH)D<sub>3</sub> >27.0 ng/ml.

25(OH)D<sub>3</sub> concentrations were significantly correlated only with systolic BP, insulin concentrations, and HOMA-IR values. No correlation was found between 25(OH)D<sub>3</sub> concentrations and either FMD or cIMT in the entire population or in obese or control children separately.

### 25(OH)D<sub>3</sub> concentrations in relation to MetS and its components

Serum 25(OH)D<sub>3</sub> concentrations were significantly lower in patients with MetS compared with those without (median 19.0 (IQR, 17.0) versus 23.2 (15.8) ng/ml; *P*<0.001), and they decreased as the number, 1, 2, 3, ≥4, of MetS components increased (23.0 (12.0) versus 20.3 (18.2) versus 19.0 (15.9) versus 17.0 (21.0) ng/ml; *P*<0.05 for trend).

As shown in Table 3, higher 25(OH)D<sub>3</sub> was significantly associated with a reduced presence of MetS. Among the components, significant inverse associations were present for tertiles of concentration of 25(OH)D<sub>3</sub> and obesity, central obesity, elevated BP, hypertriglyceridemia, low HDL-cholesterol, and IR.

The multivariable-adjusted associations between 25(OH)D<sub>3</sub> and obesity, central obesity, elevated BP, hypertriglyceridemia, low HDL-cholesterol, impaired fasting glucose, and IR are also shown in Table 3. Obesity, central obesity, elevated BP, hypertriglyceridemia, low HDL-cholesterol, IR, and MetS were all associated with an increased odds of having low 25(OH)D<sub>3</sub> levels, after adjustment for age, gender, and

Tanner stage. After additional adjustment for SDS-BMI, elevated BP as well as MetS remained significantly associated with low 25(OH)D<sub>3</sub> levels. The adjusted OR (95% CI) for those in the lowest (<17 ng/ml) compared with the highest tertile (>27 ng/ml) of 25(OH)D<sub>3</sub> for hypertension was 1.72 (1.02–2.92), and for MetS, it was 2.30 (1.20–4.40). A similar pattern of association between 25(OH)D<sub>3</sub>, high BP, and MetS was observed when models were adjusted for central adiposity, as reflected by WC as opposed to SDS-BMI.

### Discussion

This study demonstrated that 25(OH)D<sub>3</sub> was significantly associated with obesity status, high BP, and MetS. The association between low 25(OH)D<sub>3</sub> levels and hypertension as well as MetS was not attenuated after adjustment for total adiposity.

Findings from a number of epidemiologic studies indicate a protective role of 25(OH)D<sub>3</sub> against various metabolic disorders and MetS. The biological mechanisms by which vitamin D may influence the MetS have not been completely elucidated. Several studies reported that 25(OH)D<sub>3</sub> was inversely related to percentage body fat (37, 38), elevated BP (39–41), elevated glucose or IR (11, 42–44), and triglycerides (45) and positively associated with HDL-cholesterol (42). In particular, using data from the Third National Health Examination and Nutrition Survey (NHANES)

**Table 2** Age-, gender-, and Tanner stage-adjusted correlation coefficients between 25(OH)D<sub>3</sub> concentrations and variables measured in the study participants.

	All cases	Overweight/obese	Normal weight
SDS-BMI	-0.234 <sup>§</sup>	-0.176 <sup>†</sup>	-0.073
Waist circumference (cm)	-0.274 <sup>§</sup>	-0.189 <sup>†</sup>	-0.080
Systolic blood pressure (mmHg)	-0.183 <sup>§</sup>	-0.185 <sup>†</sup>	-0.168 <sup>†</sup>
Diastolic blood pressure (mmHg)	-0.102	0.080	-0.085
Total cholesterol (mg/dl)	-0.022	-0.059	-0.039
HDL-cholesterol (mg/dl)	0.142 <sup>†</sup>	0.127	0.050
Triglycerides (mg/dl)	-0.124 <sup>*</sup>	-0.053	-0.119
Fasting glucose (mmol/l)	-0.171 <sup>†</sup>	-0.134 <sup>*</sup>	-0.125
Insulin (mU/l)	-0.285 <sup>§</sup>	-0.221 <sup>†</sup>	-0.227 <sup>*</sup>
HOMA-IR values	-0.275 <sup>§</sup>	-0.220 <sup>†</sup>	-0.228 <sup>*</sup>
Carotid IMT (mm)	-0.072	-0.081	-0.045
FMD (%)	0.041	0.110	0.095

\**P*<0.05; <sup>†</sup>*P*<0.01; <sup>§</sup>*P*<0.0001.

conducted between 1988 and 1994, Martins *et al.* (44) found that among US adults the age-, gender-, and race-adjusted prevalence of hypertension (OR, 1.30), diabetes mellitus (OR, 1.98), obesity (OR, 2.29), and high serum triglyceride levels (OR, 1.47) was significantly higher in the bottom than in the top quartile of 25(OH)D<sub>3</sub>. Using the same NHANES data, Ford *et al.* (13) found that after multiple adjustments (age, gender, race or ethnicity, and smoking status), the odds of having the MetS decreased progressively across increasing quintiles of concentrations of 25(OH)D<sub>3</sub>. An inverse association between concentrations of vitamin D and IR provided a possible explanation for their findings of an inverse association between serum concentrations of vitamin D and the prevalence of the MetS (13). A study, using more recent data from NHANES 2003–2004, also found among US adults an inverse association of 25(OH)D<sub>3</sub> with MetS (according to the recently revised Third Report of the National Cholesterol Education Program, Adult treatment Panel III), which was independent of potential confounding factors (age, gender, race or ethnicity, smoking status, and alcohol use), calcium intake, and parathyroid hormone (PTH) (46). However, the explanation of the inverse associations of metabolic derangements with deficiency of vitamin D is very complex.

If one looks at the NHANES III data set, there is a significant stepwise quartile drop in the serum 25(OH)D level with the reverse quartile trend upward in both percentage body weight and BMI (47). Metabolically, obesity is associated with IR/hyperinsulinemia, low HDL-cholesterol, high triglycerides, increases in serum total cholesterol, and small, dense, low-density lipoprotein cholesterol particles (48). Although there are direct causal relationships of some of the metabolic derangements with obesity, in some situations, the metabolic change (including vitamin D deficiency) can be a consequence of obesity *per se* (48).

Overall, our current results indicate that the risk of low 25(OH)D<sub>3</sub> levels in a Caucasian pediatric population was driven by the degree of (gender-age specific) total adiposity, being more closely associated with SDS-BMI

than with central body fat distribution. BMI in adulthood and SDS-BMI in childhood are not a measure of fatness but an index of total adiposity. Even in individuals who are not obese, body size and adiposity are inversely associated with blood 25(OH)D concentrations (37, 49). It has been shown that concentrations of 25(OH)D<sub>3</sub> in biopsies of subcutaneous fat tissue from obese men and women were inversely related to the degree of overweight and positively to the concentrations of circulating 25(OH)D<sub>3</sub> (50). The positive association of serum and subcutaneous fat tissue vitamin D<sub>3</sub> concentrations is compatible with the long-standing concept that adipose tissue is a storage site for vitamin D (51).

There is evidence of an association between IR and insufficiency of 25(OH)D<sub>3</sub> in some pediatric studies (18, 23) but not in other studies (52, 53). Vitamin D status has been associated with dyslipidemia in some instances, but the literature is conflicting and the evidence base is still gathered (1). Vitamin D is thought to be essential for maintaining adequate levels of apolipoprotein A-1 (the main component of HDL). A possible mechanism by which vitamin D may be associated with triglyceride levels is through increased activity of lipoprotein lipase, which has been shown to be regulated by vitamin D in adipocytes (54). In this study, the association between 25(OH)D<sub>3</sub> and some components of the MetS including IR and hypertriglyceridemia became nonsignificant after adjustment for SDS-BMI, suggesting that the most important determinant of low 25(OH)D<sub>3</sub> levels is the increased body size, as previous studies have asserted (55). Thus, cardiometabolic risk factors in overweight and obese children may be just a reflection of IR due to a function of overall adiposity, independent of 25(OH)D<sub>3</sub>. The study findings also suggest that in overweight and obese children, the relation between low 25(OH)D<sub>3</sub> levels and metabolic traits cannot be fully explained by its association with the visceral compartment. It may be of interest that in a cross-sectional study involving 58 obese adolescents whose visceral fat and body fat mass were measured by computed tomography and dual-energy X-ray absorptiometry, respectively, higher fat mass was associated with higher

**Table 3** Prevalence, adjusted odds ratios, and 95% confidence intervals (CIs) of metabolic syndrome and its components according to serum 25-hydroxyvitamin D<sub>3</sub> (25(OH)D<sub>3</sub>) tertile categories among 452 children and adolescents.

	Serum 25(OH)D <sub>3</sub> tertile categories			P trend
	I (156)	II (149)	III (147)	
<b>Obesity (SDS-BMI ≥ 2)</b>				
Prevalence, % (95% CI)	60.0 (53.0–66.0)	33.0 (27.0–36.0)	21.0 (17.0–24.0)	<0.0001
Adjusted OR (95% CI) <sup>a</sup>	4.39 (2.19–8.83) <sup>§</sup>	1.24 (0.87–1.78)	1.00 (referent)	<0.0001
Adjusted OR (95% CI) <sup>b</sup>	2.59 (1.10–6.30)*	1.14 (0.71–1.83)	1.00 (referent)	0.046
<b>Central obesity</b>				
Prevalence, % (95% CI)	67.3 (59.8–74.8)	61.1 (54.7–67.5)	56.5 (48.3–64.7)	0.008
Adjusted OR (95% CI) <sup>a</sup>	2.33 (1.33–4.08) <sup>†</sup>	1.15 (0.89–1.49)	1.00 (referent)	0.005
Adjusted OR (95% CI) <sup>c</sup>	1.98 (0.83–4.73)	1.20 (0.75–1.91)	1.00 (referent)	0.21
<b>Elevated blood pressure</b>				
Prevalence, % (95% CI)	42.3 (34.4–50.2)	37.6 (29.7–45.5)	28.6 (21.2–36.0)	0.003
Adjusted OR (95% CI) <sup>a</sup>	2.12 (1.28–3.51) <sup>†</sup>	1.60 (0.96–2.63)	1.00 (referent)	0.003
Adjusted OR (95% CI) <sup>b</sup>	1.70 (1.01–2.90)*	1.60 (0.94–2.73)	1.00 (referent)	0.049
Adjusted OR (95% CI) <sup>c</sup>	1.72 (1.02–2.92)*	1.18 (0.90–1.55)	1.00 (referent)	0.038
<b>Hypertriglyceridemia</b>				
Prevalence, % (95% CI)	34.6 (27.0–42.2)	24.8 (17.8–31.8)	22.4 (15.5–29.3)	0.008
Adjusted OR (95% CI) <sup>a</sup>	2.0 (1.18–3.42) <sup>†</sup>	1.14 (0.66–1.96)	1.00 (referent)	0.011
Adjusted OR (95% CI) <sup>b</sup>	1.37 (0.75–2.51)	1.07 (0.74–1.34)	1.00 (referent)	0.26
Adjusted OR (95% CI) <sup>c</sup>	1.45 (0.82–2.6)	1.13 (0.64–2.01)	1.00 (referent)	0.26
<b>Low HDL-cholesterol</b>				
Prevalence, % (95% CI)	19.9 (13.5–26.3)	17.4 (11.2–23.6)	10.2 (5.21–15.2)	0.014
Adjusted OR (95% CI) <sup>a</sup>	2.14 (1.10–4.16)*	1.92 (0.96–3.8)	1.00 (referent)	0.20
Adjusted OR (95% CI) <sup>b</sup>	1.57 (0.76–3.22)	1.29 (0.89–1.86)	1.00 (referent)	0.16
Adjusted OR (95% CI) <sup>c</sup>	1.95 (0.97–3.9)	1.57 (0.78–3.15)	1.00 (referent)	0.19
<b>Glucose ≥ 5.6 mmol/l</b>				
Prevalence, % (95% CI)	2.6 (0.06–5.14)	2.0 (0–4.0)	1.4 (0–2.6)	0.42
Adjusted OR (95% CI) <sup>a</sup>	1.99 (0.35–11.2)	1.57 (0.26–9.6)	1.00 (referent)	0.52
Adjusted OR (95% CI) <sup>b</sup>	1.30 (0.21–8.7)	1.36 (0.54–3.42)	1.00 (referent)	0.57
Adjusted OR (95% CI) <sup>c</sup>	1.50 (0.25–9.0)	1.61 (0.26–9.8)	1.00 (referent)	0.45
<b>Insulin resistance</b>				
Prevalence, % (95% CI)	40.4 (32.6–48.2)	29.5 (22.0–37.0)	26.5 (19.2–33.8)	0.003
Adjusted OR (95% CI) <sup>a</sup>	2.22 (1.33–3.7) <sup>†</sup>	1.17 (0.69–1.98)	1.00 (referent)	0.008
Adjusted OR (95% CI) <sup>b</sup>	1.64 (0.92–2.91)	1.03 (0.77–1.36)	1.00 (referent)	0.084
Adjusted OR (95% CI) <sup>c</sup>	1.61 (0.91–2.86)	1.14 (0.65–1.97)	1.00 (referent)	0.72
<b>Metabolic syndrome</b>				
Prevalence, % (95% CI)	30.1 (22.8–37.4)	20.1 (13.5–26.6)	12.9 (7.4–18.4)	<0.0001
Adjusted OR (95% CI) <sup>a</sup>	3.10 (1.69–5.68) <sup>§</sup>	1.67 (0.89–3.13)	1.00 (referent)	<0.0001
Adjusted OR (95% CI) <sup>b</sup>	2.21 (1.10–4.22)*	1.20 (0.84–1.72)	1.00 (referent)	0.028
Adjusted OR (95% CI) <sup>c</sup>	2.30 (1.20–4.40)*	1.80 (0.90–3.50)	1.00 (referent)	0.026

\* $P < 0.05$ ; <sup>†</sup> $P < 0.01$ ; <sup>§</sup> $P < 0.0001$  versus referent.<sup>a</sup>Data are adjusted for age, gender, and Tanner stage.<sup>b</sup>Data are adjusted for age, gender, Tanner stage, and waist circumference.<sup>c</sup>Data are adjusted for age, gender, Tanner stage, and SDS-BMI.

risk of vitamin D deficiency, whereas higher visceral fat was associated with lower PTH, after adjustment for potential confounding variables (53).

On the other hand, the association between vitamin D and MetS was independent of overall obesity, suggesting that this association cannot be completely accounted for by the low vitamin D levels of those with excess general adiposity. However, there is biologic plausibility to this argument. There is emerging evidence that levels of circulating 25(OH)D decrease significantly during the acute-phase response (56, 57) (as do other circulating vitamins (57, 58)), providing another mechanism whereby lowered levels of 25(OH)D may be a response to disease rather than an antecedent (59).

We also found that the inverse association of 25(OH)D<sub>3</sub> with elevated BP was independent of general adiposity. Over the last decade, accumulating evidence

has indicated that blood 25(OH)D is inversely associated with BP (16). Some clinical studies favor the hypothesis that vitamin D has a protective role in CVD risk (60). The increased CV risk associated with vitamin D deficiency among the Framingham Offspring Study participants who were free of CVD at baseline was magnified in the subgroup of patients with hypertension (61). Some studies, using data from the NHANES III, have shown an inverse association between 25(OH)D and hypertension or prehypertension (44, 62). It has been suggested that the antihypertensive properties of vitamin D include renoprotective effects, suppression of the renin–angiotensin–aldosterone system, direct effects on vascular cells, and effects on calcium metabolism (63). Interestingly, the high prevalence of vitamin D deficiency in African Americans beginning at an early age (26) and persisting throughout life may play a

major role in the genesis and maintenance of hypertension (64). Although it is our contention that this group is at increased risk because of their high prevalence of vitamin D deficiency, there are no data to suggest that Caucasian adults and children with vitamin D deficiency would be at lesser risk (64). However, additional evidence is required before recommending widespread vitamin D supplementation in the primary prevention of hypertension (16), in particular in the young. In fact, the lack of evidence from randomized, controlled trials measuring vitamin D intake and incident disease, rather than serum levels of 25(OH)D, make recommendations regarding vitamin D supplementation in patients with hypertension and below-target values of 25(OH)D somewhat tenuous (65). Because vitamin D receptor (VDR) polymorphisms have been described that can influence the relationship of blood levels of 25(OH)D<sub>3</sub>, with target organ damage in hypertensive patients (66), a further question needed to be answered is whether such polymorphisms may affect VDR activation, producing variable responses to vitamin D supplementation to prevent hypertension.

We did not find a statistically significant relationship between 25(OH)D<sub>3</sub> and markers of subclinical atherosclerosis such as FMD and cIMT. If the relationship between low 25(OH)D<sub>3</sub> and CVD risk is causal, other potential mechanisms might include incident hypertension (40), incident diabetes (67), left ventricular hypertrophy (68), and/or regulation of the renin/angiotensin pathway (69), which may not be reflected in the arterial phenotypes measured. The effects of vitamin D on vascular smooth muscle cells are complex and are modulated by other hormones, such as PTH and estrogenic compounds, which upregulate 1 $\alpha$ -hydroxylase in these cells (63, 70, 71). Our findings in children are in line with the very recent findings by Richart *et al.* (72), who showed that in a general population cIMT increased with higher PTH/25(OH)D<sub>3</sub> ratio and higher PTH but was not significantly associated with 25(OH)D<sub>3</sub>. Since both vitamin D and PTH operate within a tightly controlled feedback system to maintain extracellular calcium concentrations (73), elevations in PTH may be a reflection of true vitamin D deficiency (in contrast to normal PTH and low vitamin D seen in obese subjects that may be a reflection of overall adiposity status). Hence, it is possible that true vitamin D deficiency may be associated with increased CVD risk. Therefore, a simultaneous PTH measurement with 25(OH)D<sub>3</sub> might have been a better test to assess subclinical atherosclerosis.

Limitations of the study are that the sample was conducted at a single site and included one ethnic group. Another limitation is the lack of a direct measurement of IR, as well as the lack in obese and in normal-weight children, of a direct measure of total body adiposity, such as dual-energy X-ray absorptiometry, and abdominal adiposity, such as computed tomography or magnetic resonance imaging. This study suffers from the limitation of the non-measurement of the PTH concentration. It is

therefore impossible to determine the relationship between low vitamin D levels and PTH or to determine whether an increased ratio of PTH/25(OH)D<sub>3</sub> is associated with an increased abdominal adiposity, an increased prevalence of the MetS, and its components and an increased risk of atherosclerotic abnormalities. Notwithstanding the above limitations, this study has several strengths. Our study sample was large and diverse, consisting of Caucasian overweight, obese, and healthy, normal-weight children and adolescents. In addition, blood samples were obtained during the same season. This study also shows that there are extraskeletal associations between vitamin D deficiency and metabolic outcomes beyond the relationships between adiposity and biochemical vitamin D deficiency. Although we did not find a statistically significant relationship between 25(OH)D<sub>3</sub> and markers of subclinical atherosclerosis, our data suggest that Caucasian individuals with vitamin D deficiency would not be at lesser risk for hypertension than African American subjects.

In conclusion, we found that low 25(OH)D<sub>3</sub> levels in Caucasian children were inversely associated with the degree of total adiposity, MetS, and hypertension. Additional research is necessary to determine whether low vitamin D levels may have an impact on the subsequent development of CVD during adulthood.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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