

RESEARCH ARTICLE

Sex differences in the association of vitamin D and metabolic risk factors with carotid intima-media thickness in obese adolescents

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Abstract

Background

It has been shown that vitamin D is associated with obesity and the development of atherosclerosis. Less is known about this association among adolescents with obesity.

Objectives

To determine the association of vitamin D level and metabolic risk factors with carotid intima-media thickness (CIMT) among obese adolescents.

Methods

We conducted a cross-sectional study among obese children aged 15 to 17 years in Yogyakarta, Indonesia. The association of vitamin D and other metabolic risk factors (triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), and insulin resistance using homeostasis model assessment of insulin resistance (HOMA-IR)) with CIMT was explored by multivariable linear regression models.

Results

Out of 156 obese adolescents, 55.8% were boys. Compared to girls, boys had higher BMI z-score, waist circumference, and HDL-cholesterol. After adjustment for age, sex and second-hand smoke exposure, high HOMA-IR, total cholesterol, LDL-cholesterol and triglyceride levels were associated with higher odds of elevated CIMT. In analyses stratified by sex, a similar trend was observed in boys, while none of the risk factors were associated with CIMT in girls. We observed no association between vitamin D and CIMT.

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Conclusions

Hyperinsulinemia, higher total cholesterol and LDL cholesterol were associated with greater odds of elevated CIMT among obese adolescent boys.

Introduction

Atherosclerosis is one of the important causes of cardiovascular and cerebrovascular diseases which lead to significant morbidity and mortality [1]. Subclinical atherosclerosis is an early indicator of atherosclerotic burden [2], and carotid intima-media thickness (CIMT) is regarded as a reliable marker of subclinical atherosclerosis since increased CIMT can reflect an increase in arterial wall thickness [3] including in children and adolescents [4]. Increased CIMT has been associated with obesity in children, familial hypercholesterolemia, type 1 diabetes, hypertension [5] and vitamin D deficiency [4].

Vitamin D is a steroid hormone with both endocrine and autocrine functions [6]. The endocrine function of vitamin D is mainly on the maintenance of calcium homeostasis and bone metabolism [7]. One of the autocrine functions of vitamin D is the modulation of inflammatory pathways which plays a role in cardiovascular diseases [8]. Vitamin D deficiency has been associated with the development of atherosclerotic and abnormality of arterial wall thickness mostly in adults [3, 9]. Vitamin D may play an important role in the endothelial or smooth muscle vascular cells and can also be involved in immune or inflammatory modulation [10, 11]. Therefore, vitamin D deficiency may contribute to an imbalance of vascular homeostasis, decreased arterial compliance, and the development of atherosclerosis [11]. Sufficient level of vitamin D in adults leads to a reduction in circulating inflammatory and endothelial function biomarkers; therefore, vitamin D might have a potential role as an anti-inflammatory therapy for the prevention and the treatment of cardiovascular disease [9]. Deficiency of vitamin D might be common in obese populations because of reduced sun exposure due to increased sedentary activities and sequestration of vitamin D in lipocytes [12]. There were few studies reporting the association between vitamin D deficiency with increased CIMT in children and adolescents with conflicting results [4, 13–15].

Studies evaluating the relationship of vitamin D deficiency and increased CIMT in obese adolescents are few and most studies were conducted in high-income countries among Caucasian populations [13–17]. Given limited data from low- and middle-income and tropical countries, studies are needed to better identify the association of vitamin D level and cardiovascular risk on vascular thickness among obese adolescents. This study aimed to determine the relationship of vitamin D status and other metabolic risk factors with subclinical atherosclerosis among obese adolescents in Indonesia (a low-to-middle income country with naturally abundant sun exposure). We were particularly interested in looking at CIMT as predicted by vitamin D status independent of other predictors of CIMT.

Methods

Study design and population

We conducted a cross-sectional study among adolescents in Yogyakarta, Indonesia. The recruitment process was done by screening for adolescents with obesity in seven public and three private high schools in Yogyakarta, a city in the Southern part of Java, Indonesia. The participants were recruited at their schools and we screened 4,268 students from 10 schools in

Yogyakarta using three body mass index (BMI) reference cut-off points: World Health Organization (WHO) [18], Centre for Disease Control and Prevention (CDC) [19], and International Obesity Task Force (IOTF) [20]. BMI was converted into BMI z-scores based on WHO Growth Reference 2007 using WHO AnthroPlus (<https://www.who.int/growthref/tools/en/>). The screening was done from January to February 2016. Obesity was considered when fulfilling all of the three obesity criteria [18–20]. Obese adolescents who agreed to participate in the next step of the study, i.e. assessment of their metabolic risk, were included in this study. The blood collection was taken at their high schools by a trained laboratory technician, while the assessment of carotid intima media thickness was performed at the Dr Sardjito Hospital, Yogyakarta. These data collections were done from March to October 2016.

Inclusion criteria were obese children aged 15 to less than 18 years who agreed to participate in this study. We excluded children with diabetes mellitus, renal disease, cardiovascular disease, any history of any systemic disease or history of current steroid use. Approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (KE/FK/333/EC/2016). Written informed consent was obtained from all parents/guardians included in the study.

Data collection

All eligible children underwent history taking, physical examination, and blood collection. We collected demographic information as well as information on smoking exposures and family history of hypertension. We measured body weight, height, and waist circumference. The children's weight was measured using a portable weighing scale (CAMRY, EB9003) while they were in light clothing without shoes or slippers. The weight was recorded in kilograms (kg) to the nearest 0.1 kg. We measured height using a portable stadiometer or microtoise (GEA), and height was recorded in centimeters (cm) to the nearest 0.1 cm.

Waist circumference was measured using standardised procedures by placing a tape midway of the hipbone and the bottom of ribs and wrapping it around the child's waist. Abdominal obesity was defined as waist to height ratio ≥ 0.5 [21].

Blood pressure was reported as the average of three measurements collected after a 10-minute rest. Elevated blood pressure, including hypertension, was defined according to the Clinical Practice Guidelines for Screening and Management of High Blood Pressure in Children and Adolescents proposed by The American Academy of Pediatrics 2017. For adolescents aged ≥ 13 years, elevated blood pressure was systolic blood pressure of ≥ 120 mmHg, irrespective of diastolic blood pressure [22].

A total of 10 ml blood was collected to measure serum levels of triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose, insulin, and glycated hemoglobin (HbA1c). Fasting plasma lipid profile was measured using enzymatic assays. Increased risk of diabetes and insulin resistance were assessed using HbA1c, fasting plasma glucose, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR). Fasting plasma insulin was measured using immunoassay, while the fasting plasma glucose was measured using the hexokinase method. HOMA-IR was calculated from fasting plasma glucose and insulin using the specified formula: (fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5) [23].

For impaired glucose metabolism, the criteria defined by the American Diabetes Association for increased risk of diabetes was fasting glucose ≥ 100 mg/dL and HbA1c $\geq 5.7\%$ [24]. Insulin resistance was defined when HOMA-IR was >2.5 [24]. High insulin level was defined as insulin level above 15.7 microU/mL [25].

To define dyslipidemia, we used pediatric-specific cut-off values identified by the National Cholesterol Education Program (NCEP) Expert Panel on Cholesterol Levels in Children for: high triglyceride ≥ 130 mg/dL, high total cholesterol ≥ 200 mg/dL, LDL cholesterol ≥ 130 mg/dL, HDL cholesterol < 40 mg/dL [26].

Level of vitamin D was measured using enzyme-linked immunosorbent assay or ELISA (DRG® 25-Hydroxyvitamin D Total ELISA, EIA 5396), which had been validated in pediatric population [27]. Vitamin D status was defined as deficiency of serum vitamin D level at < 20 ng/mL, insufficiency of serum vitamin D level 20–30 ng/mL, and sufficient for serum vitamin D level at > 30 ng/mL [4, 8].

Carotid intima-media thickness (CIMT) was measured using common carotid artery B-mode ultrasound [2]. The carotid arteries were imaged using a standard echocardiography machine of Phillips HD 15 with vascular probe L12.3. CIMT was measured on the posterior (far) wall of the left carotid artery at end diastolic phase. At least three measurements were taken at approximately 10 mm proximal to the bifurcation to derive mean CIMT. A trained technician who measured the carotid arteries was blinded to the subject's clinical information. Abnormal CIMT was determined if the value was ≥ 95 th percentile for age, sex, and height. Elevated CIMT was defined when CIMT measurement ≥ 0.047 mm in girls and ≥ 0.049 mm in boys [28].

Statistical analysis

Data analyses were performed using STATA version 12.1, StataCorp LP, Texas. Continuous data are presented as mean and standard deviation (SD) or median and quartiles 1 (Q1) and Q3, for normally distributed and skewed data, respectively. Categorical variables are presented as counts and percentages. Normality of variables was checked using Kolmogorov-Smirnov tests.

Normally distributed data are compared using independent sample t-test, while skewed data are compared using independent-samples Mann-Whitney U test. Categorical data are presented as percentages and compared using chi-square tests. The strength of correlation between vitamin D and metabolic risk factors with carotid intima-media thickness between boy and girl obese adolescents were analyzed using Pearson correlation.

The dependent variable was CIMT as a marker of early vascular impairment. The independent variables were vitamin D and other metabolic risk factors including waist-to-hip ratio (marker of abdominal obesity), blood glucose, HbA1c (impaired glucose metabolism), fasting insulin, HOMA-IR (insulin resistance), LDL and HDL cholesterol, triglyceride (impaired lipid metabolism), blood pressure, smoking and family history of hypertension.

The association of vitamin D and other metabolic risk factors with CIMT was explored using logistic regression and presented as odds ratio (OR) with 95% confidence interval (CI). Each factor was tested in a separate regression model. Models that were significantly associated with elevated CIMT, were re-tested with adjustment for age, sex and secondary smoke exposures. All analyses were performed on a sample as a whole and also stratified by sex based on biological differences in body fat accumulation and potentially vitamin D levels between boys and girls.

Results

We screened 4,268 students from seven public and three private high schools in Yogyakarta, Indonesia and identified 298 (7%) adolescents classified as obese based on all three obesity criteria of World Health Organization (WHO) [18], Centre for Disease Control and Prevention (CDC) [19], and International Obesity Task Force (IOTF) [20]. Of those, 229 (76.8%) obese

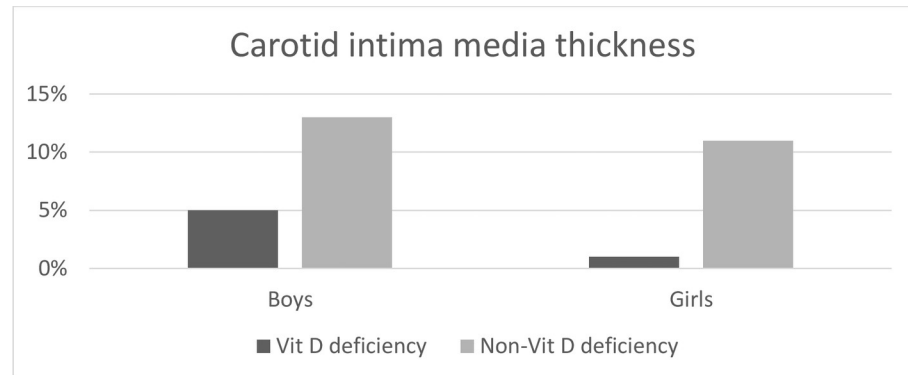


Fig 1. Prevalence of increased carotid intima media thickness across vitamin D status among obese adolescents.

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adolescents agreed to participate in the next step of the study, i.e. assessment of their metabolic risk. The results of this assessment have been reported elsewhere [29].

This study included 156 (out of 229) obese adolescents who had data on CIMT. There were no differences in metabolic risk parameters between adolescents who had their CIMT measured versus those who had not (S1 Appendix). The exceptions were triglyceride levels that were on average higher among adolescents with data missing on CIMT. We considered that the sample of this study could be considered representative of a larger population.

Vitamin D deficiency (vitamin D at < 20 ng/mL) was observed in 30.1% of children, while 13.6% had vitamin D insufficiency (vitamin D level 20–30 ng/mL); and the rest of children (56.4%) had sufficient vitamin D level (vitamin D level at >30 ng/mL) (Fig 1).

Compared to girls, boys had significantly higher BMI z-score, waist circumference, and HDL cholesterol (Table 1). We found no correlation between vitamin D and CIMT, but LDL and total cholesterol were positively correlated with carotid intima media thickness among obese adolescent boys (Table 2). Boys with elevated CIMT showed increased values of LDL cholesterol (Table 3). Table 4 demonstrated that, overall, elevated HOMA-IR, total cholesterol, LDL-cholesterol and triglyceride levels were associated with greater odds of elevated CIMT. The associations persisted after adjustment for age, sex and second-hand smoke exposure. However, after the data were analyzed within each sex, similar results were only observed in boys. None of the risk factors were associated with CIMT in girls.

Discussion

This study explored the relationship of vitamin D and other metabolic risk factors with CIMT in 156 obese adolescents in Indonesia. The results of the study indicate a positive association of hyperinsulinemia, hypercholesterolemia, and LDL hypercholesterolemia with CIMT among boys. No association was observed between vitamin D and CIMT among obese adolescents.

In our study, only about half of the Indonesian obese adolescents had normal vitamin D level, and vitamin D deficiency was observed in 30.1% of the adolescents. This is lower than the prevalence of vitamin D deficiency noted for adolescents aged 10–18 years from other countries (49% [30], 75% [31]); however, there are other studies that reported a lower prevalence of vitamin D deficiency than that observed in our study (4.5% [14], 20% [15]). We also observed the higher prevalence of vitamin D deficiency in boys compared to girls. In another study from Indonesia where researchers recruited 120 children between ages 7 and 12 years, there were 15% of adolescents with deficient vitamin D levels [32]. They observed that 75.8% of adolescents were with insufficient vitamin D levels, which was higher than that observed

Table 1. Characteristics of between obese adolescent boys and girls from Yogyakarta, Indonesia.

Variables	All subjects	Boys	Girls	p value
	n = 156 (%)	n = 87 (%)	n = 69 (%)	
Age, years	16.4 (0.7)	16.3(0.7)	16.5(0.6)	0.030
Weight, kg	85.2 (78.0;95.7)	91.7 (84.3;101.0)	78.1 (73.0;84.6)	<0.001
Height, cm	162.7 (9.2)	168.6(7.0)	155.3(5.5)	<0.001
BMI z-scores	2.54 (2.30;2.90)	2.62 (2.34;3.02)	2.48 (2.26;2.74)	0.004
Waist circumference, cm	93.3 (86.3;98.6)	96.6 (92.3;104.5)	86.1 (83.2;92.3)	<0.001
Waist-to-Height Ratio	0.57 (0.54;0.60)	0.58 (0.54;0.62)	0.56 (0.53;0.59)	0.007
Systolic blood pressure, mmHg	115.0 (106.0;124.0)	119.0 (110.0;127.0)	111.0 (102.0;120.0)	<0.001
Diastolic blood pressure, mmHg	74.0 (69.0;81.0)	73.0 (68.0;81.0)	75.0 (70.0;82.0)	0.143
High waist-to-hip ratio, $\geq 0.5^a$	151 (96.8)	87 (100)	64 (92.8)	0.002*
High waist-to-hip ratio, $\geq 0.6^a$	39 (25.0)	28 (32.2)	11(15.9)	0.020
Vitamin D ≤ 30 ng/mL ^b	68 (43.6)	43 (49.4)	25 (36.2)	0.099
Vitamin D ≤ 20 ng/mL ^b	47 (30.1)	31(35.8)	16 (23.2)	0.092
Elevated fasting insulin, ≥ 15.7 mg/dL	134 (86.5)	78 (89.7)	56 (82.4)	0.187
Elevated fasting blood glucose, ≥ 100 mg/dL	6 (3.8)	4 (4.6)	2 (2.9)	0.584*
HOMA-IR, >2.5	146 (96.2)	83 (95.4)	63 (92.6)	0.467*
High HbA1C, $\geq 5.7\%$	6 (3.8)	3 (3.4)	3 (4.3)	0.772*
High total cholesterol, ≥ 200 mg/dL	32 (20.5)	14 (16.1)	18 (26.1)	0.125
High LDL cholesterol, ≥ 130 mg/dL	50 (32.1)	28 (32.2)	22 (31.9)	0.968
Low HDL cholesterol, < 40 mg/dL	41 (26.3)	29 (33.3)	12 (17.4)	0.025
High triglyceride, ≥ 130 mg/dL	58 (37.2)	38 (43.7)	20 (29.0)	0.062
Active smoking	10 (6.4)	10 (11.5)	0 (0.0)	0.002*
Second hand smoke exposure	105 (67.3)	62 (71.3)	43 (62.3)	0.237
Family history of hypertension	47 (30.1)	28 (32.2)	19 (27.5)	0.530
Elevated CIMT (≥ 0.47 mm in girls, ≥ 0.49 mm in boys)	23 (14.7)	15 (17.2)	8 (11.6)	0.323

Normally distributed and skewed continuous data are presented as mean (standard deviation or SD) and median (Quartile 1 or Q1;Q3), respectively. Normally distributed data are compared using independent sample t-test, while skewed data are compared using independent-samples Mann-Whitney U test. Categorical data are presented as n (%) and compared using chi-square tests unless otherwise indicated. P values report the significance comparing boys and girls.

* Fischer Exact Test.

^a Different cut off was used: high waist-to-hip ratio ≥ 0.5 or ≥ 0.6 .

^b Different cut off was used: Vitamin D level ≤ 30 ng/mL or ≤ 20 ng/mL.

BMI = body mass index; HOMA-IR = homeostatic model assessment of insulin resistance; HbA1C = glycated hemoglobin, LDL = low density lipoprotein; HDL = high density lipoprotein; CIMT = carotid intima media thickness.

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among our study participants (13.6%). They also observed the higher prevalence of vitamin D insufficiency in girls compared to boys, which is in contrast to the findings of our study [32]. This discrepancy in findings may be due to differences in study sample, whereby most of recruited subjects were girls (62.5%) in the other Indonesian study, while in our study the proportion of girls was 44.2%. We also only recruited obese adolescents, and mean BMI z-scores in boys (2.7) were higher compared to girls (2.5). Vitamin D has been considered to be lower in people with obesity because it is sequestered in their lipocytes [12].

We observed no association between vitamin D and CIMT in obese adolescents. This is consistent with the results of previously published studies in adolescents from Italy [14], and the United States [15, 16]. However, our results are in contrast to the studies from Turkey [4] and the United States [13], which noted a positive association between vitamin D deficiency and CIMT. We extended the results of previous studies by reporting a positive association of

Table 2. Correlations between of vitamin D levels and other metabolic risk factors to predict carotid intima-media thickness in obese adolescent boys and girls.

	Boys (n = 87)		Girls (n = 69)	
	ρ	P	ρ	P
Age, years	-0.027	0.801	0.044	0.717
Waist-to-hip ratio	0.119	0.273	-0.144	0.237
Systolic blood pressure, mmHg	0.078	0.472	0.094	0.444
Diastolic blood pressure, mmHg	-0.032	0.767	0.104	0.396
Vitamin D, ng/mL	0.077	0.481	0.163	0.182
Fasting insulin, mg/dL	0.104	0.339	-0.127	0.303
Fasting blood glucose, mg/dL	-0.139	0.198	0.082	0.501
HOMA-IR	0.088	0.420	-0.125	0.309
HbA1C, g/dL	-0.015	0.889	0.114	0.349
Total cholesterol, mg/dL	0.316	0.003	-0.029	0.814
LDL cholesterol, mg/dL	0.337	0.001	-0.012	0.920
HDL cholesterol, mg/dL	-0.085	0.433	-0.132	0.279
Triglyceride, mg/dL	0.152	0.160	0.105	0.392

LDL = low density lipoprotein; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; HbA1C = glycated hemoglobin.

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LDL hypercholesterolemia, total hypercholesterolemia, hypertriglyceridemia and hyperinsulinemia with CIMT among obese adolescent boys. It is important to note that atherosclerosis can start in childhood showing lipid accumulation in the arterial intima [33]. Initially, children with atherosclerosis have at least some degrees of aortic fatty streaks [34, 35] and atherosclerotic plaques can be found in the coronary arteries during adolescence [35].

We observed no association between vitamin D and subclinical atherosclerosis in obese adolescents. It is possible that the lack of observing the association is due to vitamin D only serving as a marker for sunlight exposure [17, 36]. It is also likely that vascular changes may occur only after a significant period of vitamin D deficiency exposure, and therefore, the vascular effects may manifest later in children with chronic vitamin D deficiency [15]. Since we only measured vitamin D serum once, this may not reflect a long-term period of vitamin D deficiency. Therefore, our study population may have lacked time to develop atherosclerosis from vitamin D deficiency. In addition, a simultaneous measurement of inflammatory markers, parathyroid hormone, and estrogen with vitamin D level may strengthen the studies on the relationship of vitamin D and subclinical atherosclerosis [11, 37].

One study reported associations of vitamin D deficiency with adverse cardiovascular risk factors in children and adolescents since receptors of vitamin D can be found in vascular smooth muscles, endothelium, and cardiomyocytes [35]. Vitamin D deficiency contributes to the development of cardiovascular disease by promoting vascular stiffness and calcification, which lead to atherosclerosis [38]. Vitamin D deficiency is also associated with hypertension because of the renin-angiotensin-aldosterone system activation and endothelial system dysfunction [11, 39]. These can lead to the development of plaque as a degenerative vascular process that might result in myocardial infarction or stroke; and these processes start at a younger age [40].

The role of vitamin D deficiency in the development of atherosclerosis is also mediated by systemic and vascular inflammation [8, 11]. These include increased levels of inflammatory cytokines, such as C-reactive protein (CRP), tumor necrosis factor- α (TNF- α), and interleukin-6, and low levels of interleukin-10 [11]. The role of vitamin D on vascular smooth muscle

Table 3. Differences of vitamin D levels and other metabolic risk factors in obese adolescent boys and girls with elevated carotid intima-media thickness and not elevated carotid intima-media thickness.

Variables	Boys (n = 87)			Girls (n = 69)		
	Elevated CIMT n = 15	Not Elevated	p value	Elevated CIMT n = 8	Not Elevated	p value
		n = 72			n = 61	
Age, years	16.15 (0.81)	16.32 (0.67)	0.394	16.56 (0.47)	16.52 (0.61)	0.849
Systolic blood pressure, mmHg*	116.0 (107.0;124.0)	119.5 (108.5;126.0)	0.749	115.5 (107.8;119.0)	110.0 (102.5;119.0)	0.343
Diastolic blood pressure, mmHg*	71.0 (69.0;80.0)	72.0 (67.3;80.0)	0.951	79.5 (72.0;84.5)	74.0 (70.0;79.0)	0.212
BMI, kg/m ²	34.08 (4.51)	32.67 (3.70)	0.199	33.53 (3.86)	32.64 (2.91)	0.435
BMI z-scores	2.86 (0.49)	2.67 (0.43)	0.136	2.63 (0.46)	2.53 (0.35)	0.474
Waist circumference, cm	101.7 (10.6)	97.9 (9.4)	0.163	87.0 (7.4)	86.7 (7.1)	0.908
Waist-to-Height Ratio*	0.59 (0.55;0.64)	0.57 (0.54;0.61)	0.096	0.55 (0.52;0.60)	0.55 (0.52;0.58)	0.779
Vitamin D, ng/mL*	21.9 (16.0;51.3)	30.9 (17.3;49.1)	0.835	45.4 (32.4;57.0)	36.9 (21.1;53.6)	0.358
Insulin, μ IU/mL*	35.6 (13.9;64.2)	35.9 (24.0;55.2)	0.991	24.7 (16.0;48.2)	30.1 (19.5; 44.4)	0.775
Fasting Plasma Glucose, mg/dL	84.80 (6.93)	86.85 (6.63)	0.283	89.38 (11.90)	86.38 (15.74)	0.606
HOMA-IR*	7.0 (2.8–11.4)	6.6 (4.4;10.1)	0.937	4.9 (3.0; 9.4)	5.5 (3.9;7.9)	0.805
HbA1C, %	5.26 (0.26)	5.20 (0.27)	0.448	5.25 (0.39)	5.16 (0.98)	0.801
Cholesterol, mg/dL	187.9 (24.9)	172.6 (30.3)	0.071	191.6 (47.0)	174.8 (31.9)	0.190
LDL cholesterol, mg/dL	131.9 (21.3)	116.7 (27.9)	0.050	133.0 (42.0)	116.5 (29.5)	0.160
HDL cholesterol, mg/dL	43.13 (7.43)	42.76 (8.12)	0.871	41.75 (11.94)	48.43 (8.86)	0.059
Triglyceride, mg/dL *	141.0 (95.0;173.0)	118.5 (94.0;168.0)	0.609	133.0 (83.5; 203.7)	95.0 (73.5;133.5)	0.097

Normally distributed and skewed continuous data are presented as mean (standard deviation or SD) and median (Quartile 1 or Q1;Q3), respectively. Normally distributed continuous data are compared using independent sample t-test, while skewed data are compared using independent-samples Mann-Whitney U test. Categorical data are presented as n (%) and compared using chi-square tests unless otherwise indicated. BMI = body mass index; HOMA-IR = homeostatic model assessment of insulin resistance; HbA1C = glycated hemoglobin, LDL = low density lipoprotein; HDL = high density lipoprotein; CIMT = carotid intima media thickness.

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cells is also modulated by parathyroid hormones and estrogen [37]. Vitamin D and estrogen have already proven to help prevent Metabolic Syndrome and cardiovascular diseases in post-menopausal women [37].

We demonstrated the association of LDL hypercholesterolemia and hyperinsulinemia with CIMT in obese adolescent boys. This message could inform policy makers to formulate an effective prevention strategy for the development of cardiovascular diseases in obese adolescents. Vitamin D deficiency, obesity and its comorbidities and particularly high levels of insulin, HOMA-IR, LDL cholesterol, and total cholesterol should be prevented among adolescents, and boys especially, to avoid the development of cardiovascular disease.

The strength of our study is that it is among the first to evaluate the association of vitamin D and metabolic disease risk factors with vascular thickness in obese adolescents living in a country where sun exposure is abundant. However, the study is limited by its cross-sectional design and small sample size, and as a result, we may have not had enough power to detect the association between vitamin D and CIMT. In addition, since this study was only performed in the city of Yogyakarta, the results could not be generalized to other obese adolescents in Indonesia, or other low- and middle-income country settings with abundant sun exposure. More robust studies with larger sample sizes and using a longitudinal design are needed to further explore the association between vitamin D and sub-clinical atherosclerosis among obese adolescents living in low- and middle-income countries with abundant sun exposure.

Table 4. The association between vitamin D levels and other metabolic risk factors to predict carotid intima-media thickness among obese adolescent boys and girls.

	All subjects (n = 156)		Boys (n = 87)		Girls (n = 69)	
	OR (95%CI)	P	OR (95%CI)	p	OR (95%CI)	p
Age, years	0.76 (0.39–1.50)	0.430	0.69 (0.30–1.60)	0.390	1.13 (.32–3.99)	0.846
High waist-to-hip ratio, ≥ 0.5 ^a	na	Na	na	na	na	na
High waist-to-hip ratio, ≥ 0.6 ^a	2.21 (0.87–5.60)	0.096	2.13 (0.68–6.61)	0.193	1.93 (0.34–11.08)	0.463
Elevated blood pressure	1.03 (0.42–2.55)	0.948	1.42 (0.46–4.40)	0.545	0.70 (0.15–3.23)	0.645
Vitamin D ≤ 30 ng/mL	1.01 (0.41–2.46)	0.991	0.83 (0.27–2.52)	0.740	1.82 (0.34–9.76)	0.487
Vitamin D ≤ 20 ng/mL	1.66 (0.58–4.78)	0.346	1.65 (0.48–5.70)	0.429	2.28 (0.26–20.09)	0.457
Elevated fasting insulin, ≥ 15.7 mg/dL	2.75 (0.94–8.06)	0.065	4.87 (1.13–21.01)	0.034 ^d	1.67 (0.29–9.48)	0.565
Elevated fasting blood glucose, ≥ 100 mg/dL	0.86 (0.10–7.71)	0.859	na	na	0.12 (0.007–2.08)	0.144
HOMA-IR, > 2.5	5.35 (1.32–21.69)	0.019 ^c	17.75 (1.70–185.1)	0.016 ^d	2.00 (0.20–20.51)	0.559
High HbA1C, $\geq 5.7\%$	3.07 (0.53–17.83)	0.211	2.50 (0.21–29.50)	0.467	4.21 (0.34–52.65)	0.264
High total cholesterol, ≥ 200 mg/dL	3.88 (1.51–9.96)	0.005 ^c	5.33 (1.50–18.94)	0.010 ^d	3.36 (0.74–15.18)	0.116
High LDL cholesterol, ≥ 130 mg/dL	3.37 (1.36–8.36)	0.009 ^c	4.48 (1.31–13.33)	0.015 ^d	2.39 (0.54–10.61)	0.252
Low HDL cholesterol, < 40 mg/dL	1.62 (0.63–4.15)	0.319	1.00 (0.72–6.92)	1.00	3.47 (0.70–17.11)	0.127
High triglyceride, ≥ 130 mg/dL	2.54 (1.03–6.25)	0.042 ^c	2.22 (0.72–6.92)	0.168	2.81 (0.63–12.59)	0.176
Active smoking	na	Na	na	na	na	na
Second hand smoke exposure	1.45 (0.53–3.93)	0.466	1.76 (0.45–6.86)	0.416	1.01 (0.22–4.62)	0.991
Family history of hypertension	1.61 (0.64–4.03)	0.311	1.52 (0.48–4.78)	0.478	1.69 (0.36–7.88)	0.506

Abnormal conditions were given code = 1, normal = 0; LDL = low density lipoprotein; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; HbA1C = glycated hemoglobin; na = not applicable.

^a Different cut off was used: high waist-to-hip ratio ≥ 0.5 or ≥ 0.6 .

^b Different cut off was used: Vitamin D level ≤ 30 ng/mL or ≤ 20 ng/mL, ≥ 0.47 mm in girls, ≥ 0.49 mm in boys.

^c The association remained significant after controlling for age, gender and second hand smoke exposure.

^d The association remained significant after controlling for age and second hand smoke exposure.

The dependent variable was CIMT as a marker of early vascular impairment.

The independent variables were vitamin D and other metabolic risk factors including waist-to-hip ratio (marker of abdominal obesity), blood glucose, HbA1c (impaired glucose metabolism), fasting insulin, HOMA-IR (insulin resistance), LDL and HDL cholesterol, triglyceride (impaired lipid metabolism), blood pressure, smoking and family history of hypertension.

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Conclusions

This study explored the relationship of vitamin D and metabolic disease risk factors with CIMT in obese adolescents in Indonesia. The results indicate a positive association of insulin resistance and dyslipidemia with CIMT among obese adolescent boys, while no association was observed between vitamin D and subclinical atherosclerosis in obese adolescents.

Supporting information

S1 Appendix. Characteristics of obese adolescents who had and had not CIMT measured. (DOCX)

S1 Fig. Prevalence of increased carotid intima media thickness across vitamin D status among obese adolescents. (TIFF)

S1 Data. Dataset of vitamin D and CIMT. (XLSX)

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References

1. World Health Organization. Cardiovascular diseases (CVDs) Fact sheet, 2017. <http://www.who.int/mediacentre/factsheets/fs317/en/>. Accessed by 8 Dec 2019.
2. Holewijn S, den Heijer M, Stalenhoef AF, de Graaf J. Non-invasive measurements of atherosclerosis (NIMA): current evidence and future perspectives. *Neth J Med* 2010; 68: 388–99. PMID: [21209464](https://pubmed.ncbi.nlm.nih.gov/21209464/)
3. Chen FH, Liu T, Xu L, Zhang L, Zhou XB. Association of Serum Vitamin D Level and Carotid Atherosclerosis: A Systematic Review and Meta-analysis. *J Ultrasound Med* 2018; 37:1293–303 <https://doi.org/10.1002/jum.14494> PMID: [29171066](https://pubmed.ncbi.nlm.nih.gov/29171066/)
4. Atabek ME, Ekliloglu BS, Akyürek N, Alp H. Association between vitamin D level and cardiovascular risk in obese children and adolescents. *J Pediatr Endocr Met.* 2014; 27:661–6. <https://doi.org/10.1515/jpem-2013-0379> PMID: [24756047](https://pubmed.ncbi.nlm.nih.gov/24756047/)
5. Gilardini L, Pasqualinotto L, Di Matteo S, Caffetto K, Croci M, et al. Factors associated with early atherosclerosis and arterial calcifications in young subjects with a benign phenotype of obesity. *Obesity* 2011; 19:1684–89. <https://doi.org/10.1038/oby.2010.342> PMID: [21273999](https://pubmed.ncbi.nlm.nih.gov/21273999/)
6. Morris HA, Anderson PH. Autocrine and paracrine action of vitamin D. *Clin Biochem Rev* 2010; 31:129. PMID: [21170259](https://pubmed.ncbi.nlm.nih.gov/21170259/)
7. Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. *Osteoporosis Int* 2002; 13:187–94. <https://doi.org/10.1007/s001980200012> PMID: [11991436](https://pubmed.ncbi.nlm.nih.gov/11991436/)
8. Cohen-Lahav M, Douvdevani A, Chaimovitz C, Shany S. The anti-inflammatory activity of 1,25-dihydroxyvitamin D3 in macrophages. *J Steroid Biochem* 2007; 103:558–62 <https://doi.org/10.1016/j.jsbmb.2006.12.093> PMID: [17267205](https://pubmed.ncbi.nlm.nih.gov/17267205/)
9. Agbalalah T, Hughes SF, Freeborn EJ, Mushtaq S. Impact of vitamin D supplementation on endothelial and inflammatory markers in adults: A systematic review. *J Steroid Biochem* 2017. <https://doi.org/10.1016/j.jsbmb.2017.01.015> PMID: [28126565](https://pubmed.ncbi.nlm.nih.gov/28126565/)

10. Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab* 2009; 94:4023–30. <https://doi.org/10.1210/jc.2008-1212> PMID: 19584181
11. Ferder M, Inserra F, Manucha W, Ferder L. The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system. *Am J Physiol Cell Physiol* 2013; 304: C1027–39. <https://doi.org/10.1152/ajpcell.00403.2011> PMID: 23364265
12. Vu D, Ong JM, Clemens TL, Kern PA. 1,25-Dihydroxyvitamin D induces lipoprotein lipase expression in 3T3-L1 cells in association with adipocyte differentiation. *Endocrinology* 1996; 137:1540–4. <https://doi.org/10.1210/endo.137.5.8612483> PMID: 8612483
13. Bacha F, Arslanian SA. Race or vitamin D: A determinant of intima media thickness in obese adolescents? *Pediatric diabetes* 2017; 18:619–21. <https://doi.org/10.1111/pedi.12472> PMID: 27860112
14. Pacifico L, Anania C, Osborn JF, et al. Low 25(OH)D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. *Eur J Endocrinol* 2011; 165:603–11. <https://doi.org/10.1530/EJE-11-0545> PMID: 21753070
15. Cheraghi N, Dai H, Raghuvver G. Vitamin D deficiency is associated with atherosclerosis-promoting risk factor clustering but not vascular damage in children. *Med Sci Monit* 2012; 18:CR687–92. <https://doi.org/10.12659/msm.883593> PMID: 23197228
16. Singh J, Merrill ED, Sandesara PB, Schoeneberg L, Dai H, Raghuvver G. Vitamin D, low-grade inflammation and cardiovascular risk in young children: a pilot study. *Pediatr Cardiol* 2015; 36:1338–43 <https://doi.org/10.1007/s00246-015-1162-0> PMID: 25832850
17. Murni IK, Sulistyoningrum DC, Oktaria V. Association of vitamin D deficiency with cardiovascular disease risk in children: implications for the Asia Pacific Region. *Asia Pac J Clin Nutr* 2016; 25(Suppl 1): S8–19. <https://doi.org/10.6133/apjcn.122016.s1> PMID: 28027627
18. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 2007; 85:660–7. <https://doi.org/10.2471/blt.07.043497> PMID: 18026621
19. Centers for Disease Control and Prevention. 2000 CDC Growth charts for the United States: Methods and Development. *Vital and Health Statistics* 2002; series 11(246). PMID: 12043359
20. Cole TJ, Lobstein T. Extended international (IOFT) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; 7:284–94. <https://doi.org/10.1111/j.2047-6310.2012.00064.x> PMID: 22715120
21. IDF. The IDF consensus definition of the Metabolic Syndrome in children and adolescents. 2007
22. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics* 2017; 140:e20171904. <https://doi.org/10.1542/peds.2017-1904> PMID: 28827377
23. Kimm H, Lee SW, Lee HS, Shim KW, Cho CY, Yun JE et al. Associations between lipid measures and Metabolic Syndrome, insulin resistance and adiponectin: usefulness of lipid ratios in Korean men and women. *Circ J* 2010; 74: 931–7. <https://doi.org/10.1253/circj.cj-09-0571> PMID: 20215701
24. Keskin M, Kurtoglu S, Kendirci M, Atabek E, Yazici C. Homeostasis model assessment s more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics* 2005; 115:e500–3. <https://doi.org/10.1542/peds.2004-1921> PMID: 15741351
25. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 2003; 139:802–9. <https://doi.org/10.7326/0003-4819-139-10-200311180-00007> PMID: 14623617
26. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report. *Pediatrics* 2011; 128 (Suppl 5).
27. Elhoseiny SM, Morgan DS, Rabie AM, Bishay ST. Vitamin D Receptor (VDR) gene polymorphisms (FokI, BsmI) and their relation to vitamin D status in pediatric beta Thalassemia major. *Indian J Hematol Blood Transfus* 2016; 32:228–38.
28. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr*. 2008; 21:93–111. <https://doi.org/10.1016/j.echo.2007.11.011> PMID: 18261694
29. Murni IK, Sulistyoningrum DC, Susilowati R, Julia M. Risk of metabolic syndrome and early vascular markers for atherosclerosis in obese Indonesian adolescents. *Paediatr Int Child Health* 2019. <https://doi.org/10.1080/20469047.2019.1697568> PMID: 31775576

30. Ganji V, Zhang X, Shaikh N, Tangpricha V. Serum 25-hydroxyvitamin D concentrations are associated with prevalence of metabolic syndrome and various cardiometabolic risk factors in US children and adolescents based on assay-adjusted serum 25-hydroxyvitamin D data from NHANES 2001–2006. *Am J Clin Nutr* 2011; 94:225–33. <https://doi.org/10.3945/ajcn.111.013516> PMID: 21613551
31. Lee DY, Kwon AR, Ahn JM, et al. Relationship between serum 25-hydroxyvitamin D concentration and risks of metabolic syndrome in children and adolescents from Korean National Health and Nutrition Examination survey 2008–2010. *Ann Pediatr Endocrinol Metab* 2015; 20:46–52. <https://doi.org/10.6065/apem.2015.20.1.46> PMID: 25883927
32. Soesanti F, Pulungan A, Tridjaja B, Batubara J. Vitamin D profile in healthy children aged 7–12 years old in Indonesia. *Int J Pediatr Endocrinol* 2013:P167.
33. McGill HC Jr. The pathogenesis of atherosclerosis. *Clin Chem* 1988; 34(8B):B33–9 PMID: 3042197
34. Napoli C, Pignatola O, de Nigris F, Sica V. Childhood infection and endothelial dysfunction: a potential link in atherosclerosis? *Circulation* 2005; 111:1568–70. <https://doi.org/10.1161/01.CIR.0000161816.52136.66> PMID: 15811865
35. Strong JP, Mc GH Jr. The natural history of coronary atherosclerosis. *Am J Pathol* 1962; 40:37–49. PMID: 13917861
36. Mao PJ, Zhang C, Tang L, et al. Effect of calcium or vitamin D supplementation on vascular outcomes: a meta-analysis of randomized controlled trials. *Int J Cardiol* 2013; 169:106–11. <https://doi.org/10.1016/j.ijcard.2013.08.055> PMID: 24035175
37. Somjen D, Weisman Y, Kohen F, et al. 25-hydroxyvitamin D3-1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation* 2005; 111:1666–71. <https://doi.org/10.1161/01.CIR.0000160353.27927.70> PMID: 15795327
38. Susilowati R, Sulistyoningrum DC, Witari NP, Huriyati E, Luglio HF, Julia M. Sexual dimorphism in interleukin 17A and adipocytokines and their association with insulin resistance among obese adolescents in Yogyakarta, Indonesia. *Asia Pac J Clin Nutr* 2016; 25(Suppl 1):S93–101. <https://doi.org/10.6133/apjcn.122016.s13> PMID: 28027637
39. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002; 110:229–38. <https://doi.org/10.1172/JCI15219> PMID: 12122115
40. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med* 1998; 338:1650–6. <https://doi.org/10.1056/NEJM199806043382302> PMID: 9614255