

## Association of Vitamin D Status and Metabolic Syndrome Components in Iranian Children

### Abstract

**Background:** Recently, it has been found that Vitamin D can affect cardiometabolic risk factors. However, these findings have not been confirmed in younger population. We aimed to assess the associations of serum 25-hydroxyvitamin D (25(OH)D) and metabolic syndrome (MetS) components in Iranian children. **Methods:** This cross-sectional study was conducted on 240 children aged 6–9 years old. Anthropometric indices (weight, height, waist circumference, and body fat), biochemical parameters (low-density lipoprotein, high-density lipoprotein, triglyceride [TG], fasting blood sugar, and serum 25(OH)D), systolic blood pressure (SBP) and diastolic blood pressure (DBP) blood pressure, and dietary intake and physical activity were measured. Multivariate linear regression analysis was used to assess the association of MetS components and serum 25(OH)D. **Results:** Mean age of children was  $7.8 \pm 1.06$  year. Mean serum 25(OH)D concentration was  $14.6 \pm 10.64$  ng/ml, and the prevalence of Vitamin D deficiency (serum 25(OH)D lower than 10 ng/ml) was 41.66%. Dietary intake of Vitamin D was  $1.91 \pm 1.8$  mcg/day. Serum 25(OH)D was inversely associated with TG ( $\beta = -0.16$ ; CI:  $-0.27, -0.04$ ) after adjusting by age, gender, body mass index, physical activity, and some dietary components. Serum 25(OH)D was negatively associated with SBP ( $\beta = -0.02$ ; CI:  $-0.05, -0.004$ ), and DBP ( $\beta = -0.02$ ; CI:  $-0.05, -0.003$ ); however, it was not significant anymore after adjustment for sodium, potassium, and fiber. **Conclusions:** Vitamin D deficiency is alarming among Iranian children. Among the components of MetS, lower serum Vitamin D concentration was only associated with TG that could contribute in onset and progression of cardiometabolic disorders later in life.

**Keywords:** Children, metabolic syndrome, triglyceride, Vitamin D

### Introduction

Majority of chronic diseases and their risk factors start early in life. Thus, the prevention of them at primary level should be considered from childhood.<sup>[1]</sup> Long-term effects of childhood obesity could be the main risk factor for cardiovascular disease and metabolic disorders in adult years. However, chronic diseases are not restricted to adults, children, and adolescents are also prone to such disorders.<sup>[2]</sup> Besides, increasing prevalence of chronic diseases due to unhealthy food habits, physical inactivity, and obesity involved both developing and developed countries.<sup>[2,3]</sup> Metabolic syndrome (MetS) composed of central obesity, hyperglycemia, dyslipidemia, and hypertension which are the strongest predictors of cardiovascular diseases and/or diabetes.<sup>[4-6]</sup> It is estimated that a range of 27.46%–33.7% of Iranian adults have MetS.<sup>[7]</sup>

Physical inactivity, genetic predisposition, and dietary factors, including fatty acid composition, intake of calorie dense and high-fat diets, are risk factors for the development of MetS. Abdominal obesity, insulin resistance, and chronic inflammation were also contributed to this syndrome.<sup>[8-10]</sup> Besides, MetS may be exacerbated by other factors such as Vitamin D deficiency. Epidemiologic studies reported an inverse relation between serum 25-hydroxyvitamin (25(OH)D) and the risk of MetS.<sup>[3]</sup> Moreover, studies have indicated that other than muscle-skeletal diseases, a widespread range of disorders, including hypertension, diabetes, obesity, and coronary heart disease, are related to Vitamin D deficiency<sup>[11,12]</sup> even at a young age.<sup>[2]</sup> It is also possible that obesity has a negative effect on Vitamin D status. Vitamin D is a fat-soluble vitamin that may be trapped and saved in adipose tissue.<sup>[13]</sup> Experimental and human studies reported more storage of Vitamin D in adipose tissue

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and lower bioavailability of endogenous Vitamin D in blood circulation of obese persons.<sup>[13]</sup>

Despite the importance of studying the association of Vitamin D with cardiometabolic risk factors in early life,<sup>[14]</sup> this issue has been less investigated in children and/or adolescence. Thus, we aimed to evaluate the association of serum 25(OH)D and MetS components in school-aged children in Shiraz-Iran.

## Methods

### Study participants

This study was carried out in autumn, 2015 on 240 children aged 6–9 years selected by stratified sampling in the elementary schools of Shiraz, Iran. There are four educational districts in Shiraz. At first, we selected two schools in each districts. Then, we chose 30 children from first to third grades in each school. All stages were done randomly. We excluded children who used special diets and/or drugs which have any effects on metabolic status, and children with chronic diseases such as diabetes, cardiac disorders, and thyroid malfunction. Written consents were taken from parents or child caregivers.

### Anthropometric parameters and dietary intake

Anthropometric measurements, including weight, height and waist circumference (WC), were done for all participants. Weight and body fat were measured in light clothing by OMRON body composition monitor BF511 (made in China). Using a nonstretchable tape measure, height was measured barefoot to the nearest 0.1 cm. WC was measured at the narrowest part of the body between the lowest rib and the iliac crest while subject standing, to the nearest 0.1 cm.<sup>[15]</sup> Nutritional intake was evaluated by three 24 h food records, including 2 weekdays and one weekend. Then, nutrients composition was estimated using Nutritionist IV software (Nutritionist IV Diet Analysis, First Data Bank Division, Hearst Corp., San Bruno, CA).

### Biochemical assay

Following an overnight fasting, a sample of venous blood was obtained to measure fasting blood sugar (FBS), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and serum 25(OH)D. FBS concentration and lipid profile were measured using enzymatic colorimetric method by a BT1500 auto-analyzer. Serum 25(OH)D was measured by ELISA technique. Serum 25(OH)D concentrations were categorized into five levels; severe deficiency (<5 ng/ml), deficiency (5–10 ng/ml), insufficiency (10–20 ng/ml), marginal status (20–30 ng/ml), and sufficiency (>30 ng/ml).<sup>[16]</sup> Using a mercury sphygmomanometer (ALPK2, Japan) with suitable cuff size for children, blood pressure was measured twice after a 5-min rest and the mean of two measurements was recorded. Blood pressure measurement was done by a trained staff for all participants.

## Statistical analyses

Statistical analyses were performed using SPSS software (ver. 16 for Windows; SPSS Inc., Chicago, USA). Data were expressed as mean  $\pm$  standard deviation or number and percentage. Kolmogorov–Smirnov test was used to check the normal distribution of data. Multivariate linear regressions were applied to find the association of MetS components and serum 25(OH)D concentrations with adjustment for age, gender, body mass index (BMI), physical activity, and dietary components including polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), cholesterol, fiber, sodium, potassium, and energy intake.  $P < 0.05$  was considered statistically significant.

## Results

A total of 240 children consisted of 127 (52.9%) boys, and 113 (47.1%) girls were studied. Baseline characteristics of the participants are presented in Table 1. Mean age of participants was  $7.8 \pm 1.06$  years. Almost three quarter of children lived in less crowded families with lower than 4 members. In total, 52.3% of participants were in medium socioeconomic status. Nearly 41.66% of children were Vitamin D deficient and 8.75% of them were in sufficient group. Serum 25(OH)D of boys ( $14.86 \pm 12.98$  ng/ml) and girls ( $14.51 \pm 9.09$  ng/ml) were not significantly different. About 10.83 and 8.3% of participants were overweight and obese, respectively.

Table 2 demonstrates the association of MetS components and serum 25(OH)D concentration. An inverse association between serum 25(OH)D and systolic blood pressure (SBP) ( $\beta = -0.02$ ; CI:  $-0.05, -0.004$ ), diastolic blood pressure (DBP) ( $\beta = -0.02$ ; CI:  $-0.05, -0.003$ ), and TG ( $\beta = -0.22$ ; CI:  $-0.30, -0.14$ ) were found in multivariate linear regression. After adjusting for age, gender, BMI, physical activity, PUFA, MUFA, SFA, cholesterol, and fiber, TG was still significantly associated with serum 25(OH)D. However, the association of serum 25(OH)D with SBP and DBP did not remain significant after adjusting by sodium, potassium, and fiber. Furthermore, the association of serum 25(OH)D and total cholesterol, LDL, HDL, FBS, WC, waist-to-hip ratio, and body fat were not statistically significant.

## Discussion

This study revealed that 41.66% of children aged 6–9-year-old were Vitamin D deficient. We also found an inverse association between serum 25(OH)D and TG, as a component of MetS. Vitamin D deficient children comprised nearly half of participants. Recent literatures also reported the prevalence of Vitamin D deficiency is considerably high among Iranian children and adolescents.<sup>[17-20]</sup>

We found a significant inverse association between serum 25(OH)D concentration and TG, which remained significant

**Table 1: Baseline characteristics of 6-9-year-old children (n=240)**

Variables	Boys (n=127)	Girls (n=113)	Total
Age	7.69±1.11	8±0.97	7.8±1.06
Birth order n (%)			
1	83 (65.4)	60 (53.1)	143 (59.6)
2	29 (22.8)	42 (37.2)	71 (29.6)
3	11 (8.7)	9 (8)	20 (8.3)
>3	4 (3.1)	2 (1.8)	6 (2.5)
Family size n (%)			
≤4	96 (76.2)	86 (76.1)	182 (76.2)
≥5	30 (23.8)	27 (23.9)	57 (23.8)
Child live with n (%)			
Both parents	119 (93.7)	107 (94.7)	226 (94.2)
Father	1 (0.8)	2 (1.8)	3 (1.3)
Mother	6 (4.7)	4 (3.5)	10 (4.2)
Other	1 (0.8)	0 (0)	1 (0.4)
Mothers education n (%)			
Elementary	33 (25.6)	32 (27.7)	65 (26.6)
Secondary	68 (54.4)	64 (57.1)	132 (55.7)
Academic	26 (20)	17 (15.2)	43 (17.7)
Fathers education n (%)			
Elementary	38 (29.8)	35 (30.6)	73 (30.2)
Secondary	56 (44.6)	48 (43.2)	104 (44)
Academic	33 (25.6)	30 (26.1)	63 (25.9)
Socioeconomic status n (%)			
Low	38 (30.2)	33 (29.2)	71 (29.7)
Medium	66 (52.4)	59 (52.2)	125 (52.3)
High	23 (9.2)	21 (18.6)	44 (18)
25(OH)D (ng/mL)	14.67±11.90	14.51±9.08	14.6±10.64
<5 (ng/mL)	23 (18.11)	5 (4.42)	28 (11.66)
5-10 (ng/mL)	30 (23.67)	42 (37.16)	72 (30)
10-20 (ng/mL)	48 (37.79)	40 (35.39)	88 (36.66)
20-30 (ng/mL)	12 (9.44)	19 (16.81)	31 (12.91)
>30 (ng/mL)	14 (11.02)	7 (6.19)	21 (8.75)
BMI (kg/m <sup>2</sup> )	16.06±2.55	16.04±2.95	16.05±2.74
Underweight	20 (15.74)	11 (9.73)	31 (12.91)
Normal	82 (64.56)	83 (73.45)	163 (67.91)
Overweight	14 (11.02)	10 (8.84)	26 (10.83)
Obesity	11 (8.66)	9 (7.96)	20 (8.3)
Physical activity index (MET-h/week)	34.13 (5.03)	31.05 (2.2)	32.6 (4.24)
WC (cm)	57.08±6.63	56.42±7.19	56.77±6.89
WHR (cm)	0.88±0.06	0.83±0.03	0.86±0.06
Body fat (%)	20.86±8.75	20.77±8.58	20.83±8.67
SBP (mm Hg)	107.64±11.44	104.93±10.91	106.41±11.26
DBP (mm Hg)	69.72±8.49	67.07±8.2	68.52±5.45
FBG (mg/dl)	82.94±9.47	88.75±12.34	85.66±11.27
TG (mg/dl)	108.67±57.81	104.26±52.31	106.6±55.52
TC (mg/dl)	154±23.06	156.61±30.24	155.22±26.66
LDL-C (mg/dl)	91.85±19.28	99.13±23.28	95.28±21.53
HDL-C (mg/dl)	44.07±10.52	46.26±12.06	45.1±11.30

Data are presented as mean±SD, The italic data are presented as n (%), BMI=Body mass index, WC=Waist circumference, WHR=Waist-to-hip ratio, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, FBG=Fasting blood glucose, TG=Triglyceride, TC=Total Cholesterol, low-density lipoprotein, LDL-C=Low-density lipoprotein cholesterol, HDL-C=High-density lipoprotein cholesterol, MET=Metabolic equivalent of task

even after adjusting by age, gender, BMI, physical activity, and dietary intakes of PUFA, MUFA, SFA, cholesterol, and fiber. In accordance with our results, Kwon *et al.* found

inverse association between serum Vitamin D and TG.<sup>[21]</sup> Vitamin D may decrease TG in several ways. It could enhance the intestinal absorption of calcium, and reduce

**Table 2: Associations of metabolic syndrome components and serum 25(OH)D concentration among 6-9-year-old children**

	Unadjusted		Model 1		Model 2		Model 3	
	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI
WC (cm)	-0.007	-0.02-0.01	-0.002	-0.01-0.008	-0.002	-0.01-0.008	-0.004	-0.01-0.009 <sup>d</sup>
WHR (cm)	0.004	-0.007-0.01	0.006	-0.003-0.16	0.008	-0.003-0.01	0.008	-0.007-0.02 <sup>d</sup>
Body fat (%)	0.24	-1.56-2.05	0.47	-0.63-1.58	0.44	-0.67-1.56	0.32	-1.35-1.99 <sup>d</sup>
SBP (mm Hg)	-0.02	-0.05-22120.004*	-0.02	-0.04--0.001*	-0.02	-0.04--0.002*	-0.004	-0.04-0.03 <sup>c</sup>
DBP (mm Hg)	-0.02	-0.05--0.003*	-0.02	-0.04--0.001*	-0.02	-0.04--0.001*	-0.02	-0.05-0.007 <sup>c</sup>
FBG (mg/dl)	1.16	-0.74-3.07	0.59	-1.24-2.43	0.63	-1.2-2.47	1.68	-0.82-4.2 <sup>b</sup>
TG (mg/dl)	-0.22	-0.30--0.14**	-0.21	-0.29--0.13**	-0.21	-0.29--0.13**	-0.16	-0.27--0.04** <sup>a</sup>
TC (mg/dl)	-0.32	-5.04-4.39	-0.70	-5.50-4.09	-0.84	-5.63-3.95	-1.35	-8-5.37 <sup>a</sup>
LDL-C (mg/dl)	1.86	-1.8-5.5	1.35	-2.33-5.04	1.2	-2.46-4.9	0.95	-3.76-5.68 <sup>a</sup>
HDL-C (mg/dl)	-0.004	-0.04-0.03	-0.004	-0.04-0.03	-0.006	-0.04-0.03	-0.02	-0.09-0.03 <sup>a</sup>

\*The differences were significant at  $P < 0.05$ , \*\*The differences were significant at  $P < 0.01$ . Model 1: Data are adjusted for age, gender, and BMI. Model 2: Data are adjusted for age, gender, BMI, and physical activity. Model 3: Data are adjusted for age, gender, BMI, physical activity and (<sup>a</sup>Ploy unsaturated fatty acids + monounsaturated fatty acids + saturated fatty acids + cholesterol + fiber), (<sup>b</sup>Fiber), (<sup>c</sup>Sodium + potassium + fiber), (<sup>d</sup>Fiber + energy intake). WC=Waist circumference, WHR=Waist to hip ratio, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, FBG=Fasting blood glucose, TG=Triglyceride, TC=Total cholesterol, BMI=Body mass index, LDL-C=Low-density lipoprotein cholesterol, HDL-C=High-density lipoprotein cholesterol, CI: Confidence interval

hepatic formation and secretion of TG through calcium action. Vitamin D also suppress parathyroid hormone, which inhibits the lipoprotein lipase activity, thereby decreasing plasma TG level.<sup>[22]</sup> Moreover, an experimental study displayed that Vitamin D may increase the VLDL receptor mRNA in HL-60 cells,<sup>[23]</sup> thereby could decrease serum TG through inducing VLDL receptor expression, *in vivo*. However, inconsistent results were reported by a study conducted on 3577 adolescents participated in 2001–2004 National Health and Nutrition Examination Survey.<sup>[24]</sup> In addition, a meta-analysis of randomized controlled trials demonstrated nonsignificant effect of Vitamin D supplementation on serum TG level.<sup>[25]</sup>

Our study did not find any association between serum 25(OH)D and TC, LDL, and HDL. The same results were confirmed by Kwon *et al.*<sup>[21]</sup> However, some other studies conducted on older children and adolescents reported serum Vitamin D was related to all blood lipids.<sup>[17,26]</sup> This controversy may be due to age differences of studies' population. In addition, high percentage of children in our study was categorized in a small range of serum 25(OH)D (5–20 ng/ml), therefore, we could not find the association of lipid profile, other than TG, with level of Vitamin D. On the other hand, some other factors such as dietary diversity, lifestyle, and/or genetic factors may affect serum cholesterol variation.<sup>[27]</sup> These factors could resulted in differences between studies and make the interpretation of results complicated.

In this study, serum 25(OH)D was not significantly associated with BMI, WC, and body fat. Similarly, Mark *et al.* did not find a positive relation between serum Vitamin D and fat mass, measured by DEXA in 8–11-year-old children.<sup>[28]</sup> Moreover, Rodríguez-Rodríguez *et al.* conducted a study on Spanish schoolchildren and

reported no association between percentage of body fat, estimated by measurement of subcutaneous fat, and serum level of Vitamin D.<sup>[29]</sup> However, some recent studies reported Vitamin D status was related to obesity, either in children or in adults<sup>[30-32]</sup> Although it has been proposed that Vitamin D synthesis does not differ between obese and nonobese persons, due to its trapping in adipose tissue, release of Vitamin D to the bloodstream is lower in persons with obesity.<sup>[33]</sup> Moreover, sedentary lifestyle and low UV exposure in obese people may be the causes of lower Vitamin D levels in this population.<sup>[34]</sup> Results of some clinical trials showed that Vitamin D supplementation increased its serum concentration with no effects on BMI.<sup>[2,35]</sup> On the other hand, a cohort study on 479 children reported that Vitamin D deficiency resulted in obesity in childhood.<sup>[36]</sup> It can be deduced that Vitamin D is more powerful in the prevention of weight gain than promoting weight loss.

Our study results showed that serum Vitamin D was inversely associated with systolic and DBP, although this association was confounded by dietary intake of sodium, potassium, and fiber. Therefore, it seems that dietary factors including sodium, potassium, and/or fiber may modulate blood pressure stronger than Vitamin D. In addition, other studies reported that the association of blood pressure and Vitamin D status was not statistically significant after adjusting for some confounding factors including BMI, WC, and serum calcium and/or phosphate.<sup>[31,37,38]</sup> Nevertheless, a proposed mechanism is that Vitamin D can decrease blood pressure by suppressing the renin-angiotensin system, facilitate calcium entering to muscle cell, and a direct effect on endothelium.<sup>[14,39]</sup> Furthermore, opposite to our findings a cross-sectional study on children and adolescents showed that blood pressure was inversely associated with

serum 25(OH)D.<sup>[14,39]</sup> However, this inverse association was not adjusted for dietary intakes.

Moreover, we found no significant association between serum Vitamin D concentration and FBS. The same results were also reported by some other studies,<sup>[21,31]</sup> for instance Kelishadi *et al.* reported no significant association between serum Vitamin D and FBS among Iranian children and adolescents.<sup>[17]</sup> However, some previous literatures declared the Vitamin D function in glucose hemostasis and insulin resistance and found an inverse relation between serum Vitamin D concentration and FBS among children and adolescents.<sup>[19,33,40]</sup> Levy-Marchal *et al.*<sup>[41]</sup> presented that FBS or serum insulin could not appropriately determined insulin resistance in children. In addition, Erdönmez *et al.* did not find any relation between serum Vitamin D and FBS and/or insulin resistance indicators.<sup>[42]</sup> Thus, it could be concluded that the role of Vitamin D on glucose hemostasis has not been clearly manifested in childhood yet.

One of the limitations of our study was its probable not enough large sample size that affect the significant level of some results. Moreover, we studied children aged 6–9 years, although comparison a wide range of age may show the age differences more obviously.

## Conclusions

This study showed that the prevalence of Vitamin D deficiency was in an alarming level in Iranian children. In conclusion, we found serum 25(OH)D was only inversely associated with TG among the MetS components. We assume that the effect of Vitamin D on MetS components could be affected by other factors such as dietary intakes. Furthermore, as the most participants in our study were Vitamin D deficient we could not find its beneficial roles. Hence, this issue needs to be more investigated in the future studies.

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## Conflicts of interest

There are no conflicts of interest.

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