

Vitamin D status and its relationship with metabolic syndrome risk factors among adolescent girls in Boukan, Iran

Maryam Rafraf¹, Saadat Karimi Hasanabad^{2,*} and Mohammad Asghari Jafarabadi³

¹Nutrition Research Center, Department of Community Nutrition, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Islamic Republic of Iran: ²Students' Research Committee, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Islamic Republic of Iran: ³Medical Education Research Center, Department of Statistics and Epidemiology, Faculty of Health, Tabriz University of Medical Sciences, Tabriz, Islamic Republic of Iran

Submitted 26 February 2013: Final revision received 15 October 2013: Accepted 17 October 2013: First published online 20 December 2013

Abstract

Objective: To investigate vitamin D status and its association with components of the metabolic syndrome (MetS) in adolescent girls attending high school in Boukan, Iran during winter 2012.

Design: A cross-sectional study.

Setting: High schools of Boukan city, Iran.

Subjects: A sample of 216 girls aged 14–17 years was selected by multistage random sampling from four districts of Boukan. Weight, height, waist circumference, blood pressure, daily energy intake, physical activity and serum 25-hydroxyvitamin D (25(OH)D), fasting blood glucose, TAG and HDL-cholesterol levels of all participants were evaluated. Serum 25(OH)D level <20 ng/ml was defined as vitamin D deficiency.

Results: Mean serum 25(OH)D was 7.26 (sd 2.81) ng/ml and 96% of the participants had vitamin D deficiency. According to age-modified definitions of the National Cholesterol Education Program Adult Treatment Panel III, MetS was diagnosed in 10.6% of the participants. In multivariable regression analysis after adjustment for BMI, energy intake and physical activity level, serum 25(OH)D was inversely associated with fasting blood glucose ($\beta = -0.143$, $P = 0.04$). No significant relationship was found between serum 25(OH)D and other components of MetS.

Conclusions: The high prevalence of vitamin D deficiency warrants national policies and interventions towards improving this major health problem among adolescent girls. Low 25(OH)D level was significantly associated with increased fasting blood glucose. Prospective studies are needed to determine the effects of vitamin D deficiency on the development of MetS and related metabolic diseases in adolescent girls.

Keywords
Vitamin D
Prevalence
Metabolic syndrome
Adolescent girls

Vitamin D deficiency is a major public health problem and an unrecognizable condition of epidemic proportions in both developed and developing countries, with widespread reports of 30–93% prevalence in children and adults^(1–3). Determination of vitamin D status in different age groups in a community and in different climates of a country is necessary and has important implications for general health. Recent studies suggest that vitamin D deficiency is no longer just a problem of the older generations but also is an important health concern among younger generations⁽⁴⁾. Studies in the last two decades have shown a high prevalence of vitamin D deficiency in healthy adolescents in different countries^(5–15). In Iran, Razzaghy-Azar and Shakiba and Moussavi *et al.* reported that 67% of female children and adolescents in Tehran and 72.1% of teenage girls in Isfahan had vitamin D deficiency, with higher rates of deficiency in girls than boys^(16,17). Vitamin D is known as the 'sunshine vitamin' because the

mean exposure to sunlight should be sufficient for most people to produce their own vitamin D using UV light and cholesterol in the skin⁽¹⁸⁾. Whenever sun exposure is insufficient, dietary intake of this vitamin becomes a necessity. However, there are few dietary sources of vitamin D available to provide the daily recommended requirements⁽¹⁹⁾. Serum 25-hydroxyvitamin D (25(OH)D) is a commonly used marker of vitamin D nutritional status^(20–24). Vitamin D is the most important determinant of growth and body development during childhood and adolescence⁽²⁵⁾. Apart from its necessity for bone metabolism, vitamin D has multiple extraskelatal beneficial effects^(12,26). Vitamin D plays a role in glucose homeostasis, insulin secretion and action, and regulation of lipolysis^(18,21,23,27,28). Low levels of serum 25(OH)D have been linked to some chronic diseases, including the metabolic syndrome (MetS), diabetes mellitus, CVD, myocardial infarction, peripheral arterial disease, hypertension, multiple

*Corresponding author: Email saadatkarimi63@gmail.com

sclerosis and cancers^(29–37). Even though the underlying mechanism has not been well understood, 25(OH)D appears to exert effects through direct modulation of gene expression via vitamin D receptors⁽²¹⁾. A possible role for vitamin D deficiency in the pathogenesis of MetS has been recently proposed⁽³⁸⁾. MetS, a constellation of cardiometabolic disease risk factors, has become a global epidemic⁽²¹⁾. MetS is characterized by abdominal adiposity, dyslipidaemia and elevated glucose and blood pressure (BP)⁽²⁴⁾. The prevalence and risk factors of MetS have been extensively studied in adults, while data from children and adolescents are more limited. In a national study conducted by Esmailzadeh *et al.* on 3036 Iranian adolescents aged 10–19 years, the prevalence of MetS, according to the National Cholesterol Education Program Adult Treatment Panel III definition, was 10.1%⁽³⁹⁾. A relationship between 25(OH)D status and MetS was suggested in several epidemiological studies in Western populations^(40,41). Nevertheless, evidence from Asian populations, especially in adolescents, is limited^(21,26,38,42). Because of ethnic differences in 25(OH)D metabolism and its nutritional status among different populations, it is not clear whether the findings from Western populations could be extrapolated directly to Asian individuals⁽²¹⁾. Considering that there is little information about vitamin D status in Iranian adolescents and that its probable role in MetS has not been investigated, we therefore initiated the present study to evaluate vitamin D status and its relationship with MetS components in Iranian adolescent girls attending high school in Boukan, a city of Western Azerbaijan, in north-west Iran.

Methods

Study population

In the current cross-sectional study, a sample of 216 girls (14–17 years old) was selected from high schools in Boukan city during winter (in February) 2012 by a multi-stage stratified random sampling technique. Sample size was determined using as primary end point the relationship between fasting blood glucose (FBG) and vitamin D. Considering a 95% confidence level and a power of 80%, in two-tailed tests using G-Power 3.1.2 software the sample size was calculated to be at least 210 subjects. In the first stage, eight high schools were selected through systematic random sampling from all four districts (two schools per each district) of Boukan. In the second phase, total determined samples (n 240) were allocated to schools and then classes, the number of students was determined proportionately based on the schools' populations and also by student grade, then classes in each school (two to four classes in schools for each grade) were randomly selected by using even and odd numbers of each grade. Finally, in each class the participants were selected based on a simple random sampling scheme. The final sample

included 216 adolescent girls. The ethical committee of Tabriz University of Medical Sciences approved the study protocol. Girls with a history of disease (including diabetes, heart disease, hepatic, kidney and gastrointestinal diseases, and cancer) or with any use of medication or nutritional supplements (such as vitamin D, calcium, etc.) were excluded from the study. Written informed consent was obtained from the students and their parents prior to the study. Information about general characteristics was obtained by interviewing the participants.

Anthropometric assessments

Body weight was measured using a calibrated beam scale and was approximately recorded to 0.5 kg. The participants were measured barefoot wearing light clothing. Height was measured using a mounted tape with the participants' arms hanging freely at their sides and was approximately recorded to 0.5 cm. BMI was calculated as the weight in kilograms divided by the square of height in metres. Underweight, healthy weight, overweight and obesity were defined according to the international BMI cut-off points for adolescents. At risk or overweight was defined as ≥ 85 th to < 95 th percentile of BMI for age and sex, and obese was defined as ≥ 95 th percentile of BMI for age and sex^(43,44). Waist circumference (WC) was approximately recorded to 0.1 cm by a measuring tape. WC was determined at the midpoint between the lowest rib and the iliac crest while the participant was standing and after expiration⁽³⁹⁾.

Blood pressure measurements

Systolic BP (SBP) and diastolic BP (DBP) were measured in the morning by a mercury sphygmomanometer with an adult cuff. BP was measured on the upper right arm, with the arm horizontally on a table, while the participant in the sitting position and after 5 min rest. SBP was measured as the first detectable sound (phase 1) and DBP was measured as the disappearance of Korotkoff sounds (phase 5). Two readings were recorded at an interval of 1–2 min, and the cuff was completely deflated between readings. The mean of the two readings was calculated for analysis. Sex-, age- and height-specific percentile levels were defined using US normative BP tables for children and adolescents. Hypertension was defined as SBP and/or DBP ≥ 95 th percentile⁽⁴⁵⁾.

Energy intake

Information about daily energy intake was obtained by the 24 h recall method for 3 d, including two weekdays and one weekend day, and analysed by Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, CA, USA).

Physical activity

The validated short form of the Iranian version of the International Physical Activity Questionnaire was used to estimate levels of physical activity⁽⁴⁶⁾. For each activity

level, the metabolic equivalent of task (MET) value was multiplied by the time spent at that particular level. The MET-time at each level was added to obtain a total over 24 h MET-time, representing the physical activity level on an average weekday. In the current study, we categorized the physical activity level as sedentary (<3 MET), moderate (3–6 MET) or vigorous (≥ 6 MET)⁽⁴⁷⁾.

Laboratory methods

Venous blood samples (5 ml) were obtained for all participants after a 12 h overnight fast. Serum was separated by centrifugation and stored frozen at -70°C until assay. Serum 25(OH)D concentration was measured by an ELISA kit (EQ 6411-9601, lot E 120203 BL; EUROIMMUN AG, Lübeck, Germany) using an ELISA plate reader (model Stat Fax[®] 2100; Awareness Technology Inc., Palm City, FL, USA). The intra- and inter-assay CV were 2.4–4.4% and 5.9–8.2%, respectively. The following definitions were used for stratification of vitamin D status: deficiency, 25(OH)D level <20 ng/ml; insufficiency, 25(OH)D level <30 ng/ml; and sufficiency, 25(OH)D level ≥ 30 ng/ml⁽⁷⁾. FBG was measured by an enzymatic colorimetric method (Alcyon 300 automated biochemistry analyser; Abbott Laboratories, Abbott Park, IL, USA) using a glucose oxidase kit (Pars Azmoon Inc., Tehran, Iran); inter- and intra-assay CV were both less than 2.2%. For lipids, measurement kits (Pars Azmoon Inc.) and an biochemistry analyser (Alcyon 300) were used. TAG were assayed using an enzymatic colorimetric assay with glycerol phosphate oxidase. HDL-cholesterol (HDL-C) was measured after precipitation of the apoB-containing lipoproteins with phosphotungstic acid. All samples were analysed when internal quality control met the acceptable criteria. Inter- and intra-assay CV were 2.0% and 0.5% for HDL-C and 1.6% and 0.6% for TAG, respectively.

Description of the metabolic syndrome

MetS was defined according to the modified criteria of the National Cholesterol Education Program Adult Treatment Panel III. Participants with three or more characteristic of the following components were categorized as having MetS: (i) abdominal obesity (WC > age- and sex-specific 75th percentile for this population); (ii) elevated BP (SBP/DBP > age, sex- and height-specific 90th percentile); (iii) low HDL-C level (<50 mg/dl); (iv) elevated serum TAG (≥ 100 mg/dl); (v) elevated FBG (≥ 110 mg/dl)^(48–50).

Statistical analyses

Data were expressed as mean and standard deviation for quantitative variables and as number and percentage for qualitative variables. Correlations between two sets of data were evaluated using Pearson's correlation. A multiple linear regression analysis was conducted to examine the relationship between serum 25(OH)D as independent variable and MetS components as dependent variables adjusting for energy, BMI and physical activity level. Data

were analysed using the data entry and statistical software package IBM SPSS Statistics 17.0. A *P* value less than 0.05 was considered statistically significant.

Results

The general and clinical characteristics of the participants are shown in Table 1. Mean daily vitamin D intake of the participants was lower than the recommended dietary reference intake (15 μg). MetS was present in 10.6% of the girls. Table 2 shows the vitamin D status and

Table 1 General and clinical characteristics of adolescent girls aged 14–17 years, Boukan, Iran, winter 2012 (*n* 216)

	Summary statistics	
	Mean or <i>n</i>	SD or %
Age (years)	15.9	1.0
Weight (kg)	54.7	9.9
Height (cm)	160.7	6.0
BMI (kg/m ²)	21.1	3.5
BMI < 5th percentile	35	16.2
5th percentile \leq BMI < 85th percentile	138	63.9
85th percentile \leq BMI < 95th percentile	40	18.5
BMI \geq 95th percentile	3	1.4
WC (cm)	69.3	7.6
WC > 75th percentile	57	26.4
SBP (mmHg)	107.2	10.1
DBP (mmHg)	69.2	8.0
BP > 90th percentile	10	4.6
Energy intake (kJ/d)	8331.4	2035.9
Vitamin D intake ($\mu\text{g}/\text{d}$)		
Median		0.0
25th–75th percentile		0.0–5.0
Carbohydrate intake (g/d)	282.1	73.8
Fat intake (g/d)	71.7	21.9
Protein intake (g/d)	60.5	19.4
Physical activity†		
Low	6	2.8
Moderate	51	23.8
High	159	73.4
MetSt	23	10.6

WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; MetS, metabolic syndrome. †Data are expressed as *n* and %.

Table 2 Serum 25(OH)D and biochemical components of MetS in adolescent girls aged 14–17 years, Boukan, Iran, winter 2012 (*n* 216)

	Summary statistics	
	Mean or <i>n</i>	SD or %
25(OH)D (ng/ml)	7.26	2.81
25(OH)D < 20 ng/ml†	207	95.8
20 ng/ml < 25(OH)D < 30 ng/ml†	9	4.2
FBG (mg/dl)	90.0	9.7
FBG ≥ 110 (mg/dl)†	30	13.9
TAG (mg/dl)	84.4	37.1
TAG ≥ 100 (mg/dl)†	57	26.4
HDL-C (mg/dl)	50.0	10.9
HDL-C < 50 (mg/dl)†	116	53.7

25(OH)D, serum 25-hydroxyvitamin D; MetS, metabolic syndrome; FBG, fasting blood glucose; HDL-C, HDL-cholesterol. †Data are expressed as *n* and %.

Table 3 Comparison of general, clinical and metabolic characteristics of adolescent girls aged 14–17 years without and with MetS, Boukan, Iran, winter 2012 (*n* 216)

	Without MetS (<i>n</i> 193)		With MetS (<i>n</i> 23)		<i>P</i>
	Mean	SD	Mean	SD	
Age (years)	15.8	1.0	16.17	1.1	0.226
Weight (kg)	53.4	9.2	65.2	10.2	<0.001*
Height (cm)	160.7	6.1	160.7	4.3	0.997
BMI (kg/m ²)	20.6	3.1	25.3	3.8	<0.001*
WC (cm)	68.2	6.8	78.7	8.2	<0.001*
SBP (mmHg)	106.9	9.9	109.8	11.3	0.185
DBP (mmHg)	68.9	7.9	72.0	8.8	0.088
Vitamin D intake (μg/d)	0.5	1.3	0.3	0.4	0.491
Energy intake (kJ/d)	8281.6	2027.1	8749.2	2107.2	0.291
Physical activity†					0.119
Low	6	2.8	0	0	
Moderate	51	23.6	0	0	
High	136	62.9	23	10.6	
Serum 25(OH)D (ng/ml)	7.1	2.6	9.2	5.4	0.035*
FBG (mg/dl)	88.9	9.1	98.8	10.4	<0.001*
TAG (mg/dl)	79.4	33.0	125.6	44.8	<0.001*
HDL-C (mg/dl)	50.9	11.0	42.4	5.8	<0.001*

MetS, metabolic syndrome; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; 25(OH)D, serum 25-hydroxyvitamin D; FBG, fasting blood glucose; HDL-C, HDL-cholesterol.

**P*<0.05 is significant.

†Data are expressed as *n* and %.

Table 4 Relationship between serum 25(OH)D and components of the MetS in adolescent girls aged 14–17 years, Boukan, Iran, winter 2012 (*n* 216)

	25(OH)D					
	Adjusted†			Unadjusted‡		
	<i>B</i> (unstandardized)	SE	β (standardized)	<i>P</i>	<i>r</i>	<i>P</i>
WC (cm)	0.031	0.025	0.091	0.206	0.065	0.173
SBP (mmHg)	−0.048	0.029	−0.184	0.107	−0.030	0.335
DBP (mmHg)	0.056	0.037	0.173	0.131	0.033	0.316
FBG (mg/dl)	−0.039	0.019	−0.143	0.040*	−0.126	0.034
TAG (mg/dl)	0.001	0.005	0.017	0.813	−0.006	0.466
HDL-C (mg/dl)	0.026	0.017	0.110	0.120	0.087	0.104

25(OH)D, serum 25-hydroxyvitamin D; MetS, metabolic syndrome; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, HDL-cholesterol.

**P*<0.05 is significant.

†Values were adjusted for energy intake, physical activity and BMI.

‡Pearson correlation.

biochemical components of MetS in the studied population. Mean serum 25(OH)D was 7.26 (SD 2.81) ng/ml, ranging between 1.7 and 22.8 ng/ml. Prevalence of vitamin D deficiency and insufficiency was 95.8% (95% CI 93.2%, 98.5%) and 4.2%, respectively. No one had vitamin D sufficiency. Characteristics of participants without and with MetS are presented in Table 3. Mean weight, BMI, WC and serum FBG and TAG were significantly higher, while mean serum HDL-C levels were significantly lower, in girls with MetS in comparison to girls without MetS (*P*<0.001 for all except serum 25(OH)D, where *P*=0.035). Table 4 shows the association between serum 25(OH)D and components of MetS. In the multiple-adjusted linear regression analysis, serum 25(OH)D was inversely associated with FBG (β = −0.143, *P* = 0.04). No significant

associations were found between serum 25(OH)D and other risk factors of MetS. Age was entered into the model; however, there was no significant effect of age and therefore age was removed from the model. There was also no significant relationship (*r* = 0.066, *P* = 0.331) between serum 25(OH)D and daily vitamin D intake (data not shown).

Discussion

The present study is the first one to investigate vitamin D status among adolescent high-school girls in Boukan, Iran. Our data demonstrated that an unexpected proportion of healthy adolescents (96%) had vitamin D

deficiency (serum 25(OH)D \leq 20 ng/ml). The prevalence of vitamin D deficiency in our study population is higher than that in other provinces of Iran, including 72.1% for adolescent girls aged 14–18 years in Isfahan and 67% for girls aged 8–18 years in Tehran^(16,17). It is even higher than that reported for adolescent girls of other countries, such as Turkey (64.8%), the USA (54%) and Brazil (36.3%)^(3,12,25). Similar to our results, Zhu *et al.* reported that 100% of the adolescents (male and females) aged 12–16 years in Hangzhou, China had low serum 25(OH)D levels in winter (<30 ng/ml)⁽⁵¹⁾. In a study conducted by Shin *et al.* on Korean adolescents aged 12–13 years, vitamin D insufficiency or deficiency was found in 100% of girls⁽⁴⁾. Our finding clearly indicates that vitamin D deficiency is an important health problem in the studied adolescent girls.

Poor 25(OH)D status in these adolescent girls likely results from low dietary intake, inadequate exposure to sunshine and skin covering. Unless fortified, most foods are a poor source of vitamin D, with the exception of fish oil, egg yolk, and certain types of fish and seafood⁽⁵²⁾. These food sources are limited in the Iranian diet⁽⁵³⁾ and our findings confirmed low vitamin D intake in our study participants. There is also no vitamin D fortification programme ongoing in Iran now. Moreover, according to our religious regulations, females should wear long-sleeved clothes, so that only the face and hands (from wrist to fingers) are allowed to be exposed. This might also have contributed to the high occurrence of poor vitamin D status in our participants despite them having a relatively high physical activity level which included outdoor activities, too. Boukan is located at latitude of 37°N, where dermal synthesis of vitamin D in cold seasons is nearly minimal. During this time, the body inevitably relies on vitamin D body stores. The very high prevalence of vitamin D deficiency in our study participants reflected well their inefficient vitamin D storage during warm seasons.

According to our results, the frequency of MetS was found to be 10.6% (n 23). Esmailzadeh *et al.* demonstrated a 9.9% prevalence of MetS in girls aged 10–19 years in Tehran, Iran⁽³⁹⁾. In a study in Mashhad, another province of Iran, MetS was recorded in 6.5% of girls aged 15–17 years⁽⁵⁴⁾. Results of a study by Kelishadi *et al.* in a national sample of youths in Iran indicated that MetS was present in 11.7% of girls aged 14–18 years⁽⁵⁵⁾. No other published data are available about MetS prevalence in Iranian adolescents. In studies conducted in other countries, presence of MetS was reported in 8.6%, 2.5% and 6.5% of US adolescent girls and Korean and Mexican adolescents (girls and boys), respectively^(56–58). Behavioural factors such as poor dietary habits (including high intakes of carbohydrate, fat and processed foods) and other environmental factors, as well as ethnic predisposition to MetS, may implicate in developing this health problem in different populations⁽⁵⁹⁾.

The link between 25(OH)D status and MetS has been, and still is, under debate^(21,38). Serum 25(OH)D levels in our study were shown to be inversely correlated with FBG. In the present study, participants with MetS had significantly lower 25(OH)D levels than participants without MetS. The inverse relationship between serum 25(OH)D and FBG was reported in other studies, too^(20,21,26,28,60). However, in a study by Ashraf *et al.* on obese African-American adolescent females, no relationship was found between serum 25(OH)D and FBG⁽²³⁾. Although the exact mechanisms linking 25(OH)D insufficiency with hyperglycaemia and subsequent diabetes risk are not completely understood, there is accumulating evidence that 25(OH)D may directly influence pancreatic β -cell secretory function through nuclear 25(OH)D receptors and may influence insulin sensitivity through insulin receptors' expression regulation of intracellular calcium⁽²⁸⁾. The finding of no significant associations between serum 25(OH)D and other components of MetS, including WC, serum TAG, serum HDL-C and BP, in our study is similar to the results of studies conducted on women in Iran and on adults (men and women) in Jordan^(27,38). Another study performed among adolescents of French origin in Canada failed to reveal an association between serum 25(OH)D and the existence of at least two components of MetS⁽⁶⁰⁾. Erdönmez *et al.* found no association of serum 25(OH)D with components of MetS in Turkish adolescent girls⁽⁶¹⁾. On the other hand, the results of the 2001–2004 National Nutrition and Health Survey in the USA showed a strong association between low 25(OH)D level and MetS⁽²⁸⁾. The discrepancies seen in the results of various studies may be due to the different study populations, polymorphism of the vitamin D receptor, ethnic differences in vitamin D metabolism, the season of the studies and the methods used for determination of 25(OH)D^(12,28,38). It should be noted that we found a remarkably high prevalence of vitamin D deficiency among the 14–17 years age group included in the present sample. It is possible that the narrow range of serum 25(OH)D might contribute to mask any significant relationships between vitamin D and most of the components of MetS in our sample.

In present study, there was no significant relationship between serum 25(OH)D and BMI. In the literature, some reports showed no interaction of BMI and 25(OH)D, but particularly studies on adults and a few on children and adolescents demonstrated a relationship between obesity status and serum 25(OH)D^(15,26,62). In the study by Çizmeciöglü *et al.* in obese and overweight adolescents with hypovitaminosis D, serum 25(OH)D level decreased as BMI increased⁽⁶⁾. It is believed that extra body fat, by sequestration of 25(OH)D, may reduce its bioavailability⁽⁶³⁾. As mentioned above in connection with MetS components, the narrow range of serum 25(OH)D in our participants might also have limited the possible relationship of serum 25(OH)D with BMI, too.

Our study had some limitations. Blood samples were drawn during the cold season, when dermal synthesis of vitamin D is negligible, so the vitamin D status of the study participants did not necessarily reflect their status for the whole year. Other limitations include the relatively small sample size and the lack of information about sun exposure and use of sunscreen. The cross-sectional nature of our data also impedes the ability to infer causation between vitamin D and MetS risk factors.

Conclusion

The results of the present study indicated the problem of vitamin D deficiency in adolescent Iranian girls. Low serum 25(OH)D was associated inversely with FBG. Future studies with a long-term longitudinal design are warranted to determine whether low vitamin D status during adolescence may impact MetS and related disorders such as diabetes and CVD during adulthood. At the present time, development and implementation of national policies and interventions towards improving vitamin D status among adolescent girls are needed.

Acknowledgements

Sources of funding: The present study was funded by the Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. The Nutrition Research Center had no role in the design, analysis or writing of this article. *Conflicts of interest:* The authors declare no conflicts of interest, both financial and non-financial, for this study. *Ethical approval:* The study was approved by the ethical committee of Tabriz University of Medical Sciences, Tabriz, Iran. *Authors' contributions:* The whole project was designed and conducted by M.R., as a supervisor; and S.K.-H. contributed to data collection and on-site study management. Sampling methodology and statistical analysis were performed under the direction of M.A.-J. All laboratory work was carried out at the Drug Applied Research Center, Tabriz, Iran. All authors contributed to manuscript preparation. *Acknowledgements:* The authors thank all the school directors and students who participated in the study, and the parents who permitted their children to do so.

References

1. Hashemipour S, Larijani B, Adibi H *et al.* (2004) Vitamin D deficiency and causative factors in the population of Tehran. *BMC Public Health* **4**, 38.
2. Mansour MM & Alhadidi KM (2012) Vitamin D deficiency in children living in Jeddah, Saudi Arabia. *Indian J Endocrinol Metab* **6**, 263–269.
3. Andiran N, Çelik N, Akça H *et al.* (2012) Vitamin D deficiency in children and adolescents. *J Clin Res Pediatr Endocrinol* **4**, 25–29.
4. Shin YH, Kim KE, Lee C *et al.* (2012) High prevalence of vitamin D insufficiency or deficiency in young adolescents in Korea. *Eur J Pediatr* **171**, 1475–1480.
5. Melamed ML & Kumar J (2010) Low levels of 25-hydroxyvitamin D in the pediatric populations: prevalence and clinical outcomes. *Ped Health* **4**, 89–97.
6. Çizmecioglu FM, Etiler N, Görmüş U *et al.* (2008) Hypovitaminosis D in obese and overweight schoolchildren. *J Clin Res Pediatr Endocrinol* **1**, 89–96.
7. Scharla SH (1998) Prevalence of subclinical vitamin D deficiency in different European countries. *Osteoporos Int* **8**, 7–12.
8. Guillemant J, Taupin P, Le HT *et al.* (1999) Vitamin D status during puberty in French healthy male adolescents. *Osteoporos Int* **10**, 222–225.
9. Lehtonen-Veromaa M, Möttönen T, Irjala K *et al.* (1999) Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. *Eur J Clin Nutr* **53**, 746–751.
10. El-Hajj Fuleihan G, Nabulsi M, Choucair M *et al.* (2001) Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107**, 53.
11. Du X, Greenfield H, Fraser DR *et al.* (2001) Vitamin D deficiency and associated factors in adolescent girls in Beijing. *Am J Clin Nutr* **74**, 494–500.
12. Saintonge S, Bang H & Gerber LM (2009) Implications of a new definition of vitamin D deficiency in a multiracial US adolescent population: the National Health and Nutrition Examination Survey III. *Pediatrics* **123**, 797–803.
13. Lapatsanis D, Moulas A, Cholevas V *et al.* (2005) Vitamin D: a necessity for children and adolescents in Greece. *Calcif Tissue Int* **77**, 348–355.
14. Ginty F, Cavadini C, Michaud PA *et al.* (2004) Effects of usual nutrient intake and vitamin D status on markers of bone turnover in Swiss adolescents. *Eur J Clin Nutr* **58**, 1257–1265.
15. Neyestani TR, Hajifaraji M, Omidvar N *et al.* (2012) High prevalence of vitamin D deficiency in school-age children in Tehran, 2008: a red alert. *Public Health Nutr* **15**, 324–330.
16. Razzaghy-Azar M & Shakiba M (2010) Assessment of vitamin D status in healthy children and adolescents living in Tehran and its relation to iPTH, gender, weight and height. *Ann Hum Biol* **37**, 692–701.
17. Moussavi M, Heidarpoor R, Aminorroaya A *et al.* (2005) Prevalence of vitamin D deficiency in Isfahani high school students in 2004. *Horm Res* **64**, 144–148.
18. Cashman KD (2007) Vitamin D in childhood and adolescence. *Postgrad Med J* **83**, 230–235.
19. Khadilkar AV (2010) Vitamin D deficiency in Indian adolescents. *Indian Pediatr* **47**, 755–756.
20. Alemzadeh R, Kichler J, Babar G *et al.* (2008) Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism* **57**, 183–191.
21. Lu L, Yu Z, Pan A *et al.* (2009) Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. *Diabetes Care* **32**, 1278–1283.
22. Gordon CM, DePeter KC, Feldman HA *et al.* (2004) Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* **158**, 531–537.
23. Ashraf A, Alvarez J, Saenz K *et al.* (2009) Threshold for effects of vitamin D deficiency on glucose metabolism in obese female African-American adolescents. *J Clin Endocrinol Metab* **94**, 3200–3206.
24. Ganji V, Zhang X, Shaikh N *et al.* (2011) 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* **94**, 225–233.
25. Santos BR, Mascarenhas LP, Satler F *et al.* (2012) Vitamin D deficiency in girls from South Brazil: a cross-sectional study on prevalence and association with vitamin D receptor gene variants. *BMC Pediatr* **12**, 62.
 26. Gannagé-Yared MH, Chedid R, Khalife S *et al.* (2009) Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol* **160**, 965–971.
 27. Khader YS, Batiha A, Jaddou H *et al.* (2011) Relationship between 25-hydroxyvitamin D and metabolic syndrome among Jordanian adults. *Nutr Res Pract* **5**, 132–139.
 28. Reis JP, von Mühlen D, Miller ER *et al.* (2009) Vitamin D status and cardiometabolic risk factors in the United States adolescent population. *Pediatrics* **124**, 371–379.
 29. Ford ES, Zhao G, Li C *et al.* (2001) Serum concentrations of vitamin D and parathyroid hormone and prevalent metabolic syndrome among adults in the United States. *J Diabetes* **1**, 296–303.
 30. Scragg R, Sowers M, Bell C *et al.* (2004) Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* **27**, 2813–2818.
 31. Pittas AG, Lau J, Hu FB *et al.* (2007) The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* **92**, 2017–2029.
 32. Wang TJ, Pencina MJ, Booth SL *et al.* (2008) Vitamin D deficiency and risk of cardiovascular disease. *Circulation* **117**, 503–511.
 33. Giovannucci E, Liu Y, Hollis BW *et al.* (2008) 25-Hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* **168**, 1174–1180.
 34. Forman JP, Giovannucci E, Holmes MD *et al.* (2007) Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* **49**, 1063–1069.
 35. Munger KL, Levin LI, Hollis BW *et al.* (2006) Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* **296**, 2832–2838.
 36. Feskanich D, Ma J, Fuchs CS *et al.* (2004) Plasma vitamin D metabolites and risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev* **13**, 1502–1508.
 37. Ahonen MH, Tenkanen L, Teppo L *et al.* (2000) Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* **11**, 847–852.
 38. Salekzamani S, Neyestani TR, Alavi-Majd H *et al.* (2011) Is vitamin D status a determining factor for metabolic syndrome? A case-control study. *Diabetes Metab Syndr Obes* **4**, 205–212.
 39. Esmailzadeh A, Mirmiran P, Azadbakht L *et al.* (2006) High prevalence of the metabolic syndrome in Iranian adolescents. *Obesity (Silver Spring)* **14**, 377–382.
 40. Botella-Carretero JL, Alvarez-Blasco F, Villafruela JJ *et al.* (2007) Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. *Clin Nutr* **26**, 573–580.
 41. Hjelmsaeth J, Hofso D, Aasheim ET *et al.* (2009) Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* **8**, 7.
 42. Moy FM & Bulgiba A (2011) High prevalence of vitamin D insufficiency and its association with obesity and metabolic syndrome among Malay adults in Kuala Lumpur, Malaysia. *BMC Public Health* **11**, 735.
 43. Cole TJ, Flegal KM, Nicholls D *et al.* (2007) Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ* **335**, 194.
 44. Cole TJ, Bellizzi MC, Flegal KM *et al.* (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* **320**, 1240–1243.
 45. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004) The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* **114**, 555–576.
 46. Kelishadi R, Rabiee K, Khosravi A *et al.* (2004) Assessment of physical activity in adolescents of Isfahan. *Shabrekord Univ Med Sci* **3**, 55–65.
 47. Kelishadi R, Ardalan G, Gheiratmand R *et al.* (2007) Association of physical activity and dietary behaviours in relation to the body mass index in a national sample of Iranian children and adolescents: CASPIAN Study. *Bull World Health Organ* **85**, 19–26.
 48. Kelishadi R (2010) *Prevention and Control of Childhood Obesity: Today Better Than Tomorrow*, 1st ed. Isfahan: Isfahan University of Medical Sciences.
 49. Kelishadi R, Gouya MM, Ardalan G *et al.* (2007) First reference curves of waist and hip circumferences in an Asian population of youths: CASPIAN study. *J Trop Pediatr* **53**, 158–164.
 50. de Ferranti SD, Gauvreau K, Ludwig DS *et al.* (2004) Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. *Circulation* **110**, 2494–2497.
 51. Zhu Z, Zhan J, Shao J *et al.* (2012) High prevalence of vitamin D deficiency among children aged 1 month to 16 years in Hangzhou, China. *BMC Public Health* **12**, 126.
 52. Prentice A (2008) Vitamin D deficiency: a global perspective. *Nutr Rev* **66**, 153–164.
 53. Kalantari N & Ghafarpour M (2005) *National Comprehensive Study on Household Food Consumption Pattern and Nutritional Status, IR Iran, 2001–2003 (National Report)*. Tehran: Shaheed Beheshti Medical University, NNFTRI.
 54. Mirhosseini NZ, Yusoff NA, Shahar S *et al.* (2009) Prevalence of the metabolic syndrome and its influencing factors among adolescent girls in Mashhad, Iran. *Asia Pac J Clin Nutr* **18**, 131–136.
 55. Kelishadi R, Gouya MM, Adeli K *et al.* (2008) Factors associated with the metabolic syndrome in a national sample of youths: CASPIAN Study. *Nutr Metab Cardiovasc Dis* **18**, 461–470.
 56. Johnson WD, Kroon JJ, Greenway FL *et al.* (2009) Prevalence of risk factors for metabolic syndrome in adolescents: National Health and Nutrition Examination Survey (NHANES), 2001–2006. *Arch Pediatr Adolesc Med* **163**, 371–377.
 57. Kim HM, Park J, Kim HS *et al.* (2006) Obesity and cardiovascular risk factors in Korean children and adolescents aged 10–18 years from the Korean National Health and Nutrition Examination Survey, 1998 and 2001. *Am J Epidemiol* **164**, 787–793.
 58. Rodríguez-Morán M, Salazar-Vázquez B, Violante R *et al.* (2004) Metabolic syndrome among children and adolescents aged 10–18 years. *Diabetes Care* **27**, 2516–2517.
 59. Park J, Hilmers DC, Mendoza JA *et al.* (2010) Prevalence of metabolic syndrome and obesity in adolescents aged 12 to 19 years: comparison between the United States and Korea. *J Korean Med Sci* **25**, 75–82.
 60. Delvin EE, Lambert M, Levy E *et al.* (2010) Vitamin D status is modestly associated with glycemia and indicators of lipid metabolism in French-Canadian children and adolescents. *J Nutr* **140**, 987–991.
 61. Erdönmez D, Hatun S, Çizmeçioğlu FM *et al.* (2011) No relationship between vitamin D status and insulin resistance in a group of high school students. *J Clin Res Pediatr Endocrinol* **3**, 198–201.
 62. Smotkin-Tangorra M, Purushothaman R, Gupta A *et al.* (2007) Prevalence of vitamin D insufficiency in obese children and adolescents. *J Pediatr Endocrinol Metab* **20**, 817–823.
 63. Wortsman J, Matsuoka LY, Chen TC *et al.* (2000) Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* **72**, 690–693.