Public Health Nutrition: 15(2), 324–330 doi:10.1017/S1368980011000188

High prevalence of vitamin D deficiency in school-age children in Tehran, 2008: a red alert

Tirang R Neyestani[1,*], Majid Hajifaraji[2], Nasrin Omidvar[3], Mohammad R Eshraghian[4], Nastaran Shariatzadeh[1], Ali Kalayi[1], A'azam Gharavi[1], Niloufar Khalaji[1], Homa Haidari[5], Telma Zowghi[5] and Bahareh Nikooyeh[1]

[1]Laboratory of Nutrition Research, National Research Institute, Faculty of Nutritional Science and Food Technology, Shahid Beheshti University of Medical Sciences, PO Box 19395-4741, Tehran, Iran: [2]Department of Nutritional Policy-making Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran: [3]Department of Community Nutrition, Faculty of Nutritional Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran: [4]Department of Epidemiology and Biostatistics, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran: [5]Department of Nutrition Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Submitted 23 June 2010: Accepted 3 December 2010: First published online 28 February 2011

Abstract

Objective: To assess the vitamin D status of 9–12-year-old primary-school children in Tehran during autumn and winter 2007–2008.

Design: A descriptive cross-sectional study.

Setting: Primary schools of Tehran city, Iran.

Subjects: A total of 1111 children aged 9–12 years (573 boys and 538 girls) from sixty primary schools were enrolled in the study. Weight, height, BMI and serum levels of Ca, P, Mg, 25-hydroxyvitamin D (25(OH)D), intact parathyroid hormone (iPTH), osteocalcin and bone-specific alkaline phosphatase of all the participants were assessed. Dietary Ca intake was also evaluated using a quantitative FFQ for a subsample of the study population (n 503). Vitamin D sufficiency was defined on the basis of serum levels of 25(OH)D as either ≥37 nmol/l (criterion 1) or ≥50 nmol/l (criterion 2).

Results: Daily intake of Ca did not differ significantly between boys and girls (929·6 (SD 436·7) mg and 909·5 (SD 465·5) mg, respectively). However, on the basis of the first criterion, approximately 86 % of the children had vitamin D deficiency, with 38·3 % being severely deficient (25(OH)D < 12·5 nmol/l). According to the second criterion, prevalence of vitamin D deficiency rose to 91·7 %. Prevalence of vitamin D deficiency was higher in girls than in boys by either criterion. Serum levels of 25(OH)D inversely correlated with iPTH ($r = -0{\cdot}154$, $P < 0{\cdot}001$) and BMI ($r = -0{\cdot}092$, $P = 0{\cdot}002$) but directly correlated with duration of sun exposure ($r = 0{\cdot}115$, $P < 0{\cdot}001$).

Conclusions: The high prevalence of vitamin D deficiency among schoolchildren (especially among girls) warrants immediate interventions for proper nutritional support.

Keywords
Vitamin D
School-age children
Prevalence

Vitamin D insufficiency is a major health problem in developed as well as developing countries[1], including Iran[2,3]. Vitamin D is formed in the skin from 7-dehydrocholesterol under the influence of UV radiation. To be fully activated, however, vitamin D needs to undergo two hydroxylation reactions in the liver and kidney to form 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), respectively[4]. Apart from calcaemic actions, vitamin D has many non-calcaemic functions, among which are immune function[5], cellular differentiation[6] and anti-cancer actions[7–11]. Vitamin D insufficiency in the long term may not only lead to metabolic bone disorders such as osteomalacia and osteoporosis but may also have a role in the development of many chronic diseases such as auto-immune disorders[12] (e.g. type 1 diabetes mellitus (T1DM)[13], lupus[14] and multiple sclerosis (MS)[15]) and malignancies[16]. Wherever sun exposure is insufficient for any reason, dietary intake of vitamin D becomes a necessity[17]. For this reason, food fortification with vitamin D has been implemented as a prophylactic measure in many countries for several years[18,19]. However, foods are not commonly fortified in Iran at the moment in spite of scattered reports of a

*Corresponding author: Email t.neyestani@nnftri.ac.ir, neytr@yahoo.com

remarkably high prevalence of various degrees of vitamin D deficiency among those 20–69 years of age in the Tehran population (79·6 %)[2] and among 14–18-year-old adolescents in Isfahan (46·2 %)[20].

Children in accelerated growth spurts have increased requirements for many nutrients, including vitamin D. Although a well-balanced diet may provide almost all the required nutrients, vitamin D is an exception since it has no appreciable food source, especially in the Iranian diet[21]. The present study was therefore undertaken to assess vitamin D status of 9–12-year-old primary-school children in Tehran during autumn and winter 2007–2008.

Materials and methods

To estimate the prevalence of vitamin D insufficiency, a two-stage sampling was performed. In the first stage, sixty primary schools were selected through systematic random sampling from all nineteen districts of the Ministry of Education in Tehran. In the second phase, from each school sixteen to twenty children from grades 4 and 5 were enrolled in the study. The parents of all the children were invited to visit the school. The objectives of the study were fully explained to the parents in an open session, after which an informed written consent was signed by the parents who wished their child to participate in the study. After making arrangements with the schools, 3 ml of non-fasting venous blood was taken from the children on pre-determined days. Those children who were unwilling to donate blood for any reason were excluded. Finally, 1111 children (573 boys and 538 girls) of 1120 selected (99·2 % response) were enrolled in the study. The present study was scientifically and ethically approved by the Research Council and the Ethical Committee of the National Nutrition and Food Technology Research Institute, respectively.

Assessment of Ca intake

Ca intake was assessed in a subsample of the study population (n 503) using a sixty-item quantitative FFQ (qFFQ) specifically designed for dietary sources of Ca. The validity and reproducibility of the questionnaire were evaluated before the study[22]. The questionnaires were completed by face-to-face interviews with the children at the schools. A food album and measuring cups were used to ensure accuracy of the reported servings.

Anthropometric assessments

Weight was measured using a digital scale to the nearest of 0·1 kg (model 840; Seca, Hamburg, Germany) with the child wearing light clothes and no shoes. Height was measured by a measuring tape to the nearest 0·1 cm. BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2).

Laboratory investigations

All blood samples were stored in the dark until serum separation. After 2 h at room temperature (RT), blood samples were centrifuged at 2500**g** at RT for 20 min. Sera thus recovered were transferred to fresh clean microtubes in aliquots and kept at −80°C until the day of analysis.

Serum 25(OH)D was determined using a competitive protein-binding assay (Immunodiagnostik, Bensheim, Germany). In the present study, two sets of criteria were used to categorize various degrees of vitamin D deficiency, both based on serum levels of 25(OH)D. The first set was: sufficient ≥37·0 nmol/l; 25·0 nmol/l ≤ mild deficiency < 37·0 nmol/l; 12·5 nmol/l ≤ moderate deficiency < 25·0 nmol/l; and severe deficiency <12·5 nmol/l. This set of criteria was used by the Iranian Ministry of Health in a national survey on micronutrient status in both 15–23-month-old children and pregnant women[23] and also in a pilot study on Iranian adults[24]. The second set of criteria, which is more recent[25], was: sufficient ≥50·0 nmol/l; 27·5 nmol/l ≤ insufficiency < 50·0 nmol/l; and deficiency <27·5 nmol/l.

Serum levels of osteocalcin (OST; Biosource Europe SA, Nivellles, Belgium), intact parathyroid hormone (iPTH; DRG Instruments GmbH, Marburg, Germany) and bone-specific alkaline phosphatase (BAP; Immunodiagnostic Systems Ltd, Boldon, UK) were all determined using enzyme immunoassay. Concentrations were calculated from the absorbances using an automatic system (StatFax 3200 microplate ELISA reader; Awareness Technology, Inc., Palm City, FL, USA).

Statistical analyses

Data were expressed as mean and SD. Normality of distribution was evaluated using the Kolmogrov–Smirnov test. Comparison of data between two groups was carried out using Student's t test (for normal distribution) or the Mann–Whitney U test (for non-normal distribution). Correlations were evaluated using Pearson's (for normal distribution) or Spearman's (for non-normal distribution) coefficients. The χ^2 test was used to compare qualitative data between groups. Differences were considered statistically significant at $\alpha < 0{\cdot}05$. All statistical analyses were performed using the Statistical Package for the Social Sciences statistical software package version 16·0 (SPSS Inc., Chicago, IL, USA).

Results

Data on daily Ca intake, serum levels of 25(OH)D, OST, iPTH and duration of sun exposure did not show normal distribution and they were not normalized even after data transformation (including logarithm). The data on other factors had a normal distribution.

Anthropometrics

Although girls were significantly taller than boys ($P = 0{\cdot}010$), no significant difference was observed between the BMI of boys and girls (Table 1).

https://doi.org/10.1017/S1368980011000188 Published online by Cambridge University Press

Table 1 Comparison of variables by sex: 9–12-year-old primary-school children, Tehran, autumn and winter 2007–2008

	Boys		Girls				Total	
Variable	Mean	SD	Mean	SD	*P* value	95 % CI	Mean	SD
Weight (kg)	37·9	9·6	39·0	10·6	0·065	−0·14, 2·20	38·4	10·1
Height (cm)	140·5	6·8	141·6	7·3	0·010	0·26, 1·92	141·0	7·1
BMI (kg/m^2)	19·0	3·8	19·2	4·0	0·332	−0·22, 0·69	19·1	3·9
Ca (mg/dl)	9·8	0·6	9·7	0·6	0·510	−0·09, 0·04	9·7	0·6
P (mg/dl)	4·6	0·6	4·5	0·6	0·311	−0·11, 0·03	4·6	0·6
Mg (mg/dl)	2·0	0·2	2·2	0·2	0·005	0·01, 0·05	2·2	0·2
OST (ng/ml)	41·5	17·4	44·4	19·1	0·016†	27·4‡	42·9	18·4
	40·4*		43·4				41·6	
iPTH (μg/l)	38·3	18·3	56·5	38·1	<0·001†	27·8‡	49·3	40·6
	35·3*		45·9				40·1	
BAP (μg/l)	128·4	34·5	142·4	40·4	<0·001†	9·43, 18·55	135·2	38·1
25(OH)D (nmol/l)	26·6	20·6	18·8	15·7	<0·001†	22·3‡	23·6	22·2
	21·7*		13·3				17·4	
Sun exposure (min/d)	43·0	35·1	34·5	22·5	<0·001†	25·0‡	38·8	29·9
	30·0*		30·0				30·0	

OST, osteocalcin; iPTH, intact parathyroid hormone; BAP, bone-specific alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D.
*Data distribution was not normal: values given are medians.
†Mann–Whitney *U* test.
‡Values given are interquartile range.

Ca intake

Daily intakes of Ca in boys and girls were not significantly different (929·6 (SD 436·7) mg and 909·5 (SD 465·5) mg, respectively). Dietary sources of Ca in the study population are shown in Table 2.

Serum levels of Ca, P and Mg

Concentrations of Ca and P did not differ significantly between boys and girls; however, those of Mg were slightly, but significantly, higher in girls than in boys ($P = 0·005$). Boys had significantly lower serum levels of OST ($P = 0·016$), iPTH and BAP ($P < 0·001$ for both) but higher 25(OH)D ($P < 0·001$). Duration of sun exposure was reported longer in boys than in girls ($P < 0·001$; Table 1).

Table 3 shows the prevalence of various degrees of vitamin D deficiency, according to the first set of criteria, among the whole study population and in both sexes. Approximately 86 % of the children were vitamin D deficient, of whom 38·3 % were severely deficient. Although the percentage of children with moderate and mild deficiency did not differ significantly between the two sexes, severe deficiency was more prevalent in girls than in boys (χ^2 test; $P < 0·001$).

However, when the second set of criteria was used to define vitamin D status, the prevalence of various degrees of vitamin D deficiency rose to 91·7 %. Again in this situation the frequency of boys with adequate vitamin D status was significantly higher than that of girls (χ^2 test; $P < 0·001$; Table 4).

Serum levels of 25(OH)D inversely correlated with iPTH ($r = -0·16$, 95 % CI −0·17, −0·06; $P < 0·001$), BAP ($r = -0·150$, 95 % CI −0·22, −0·11; $P < 0·001$) and Mg ($r = -0·129$, 95 % CI −0·17, −0·07; $P < 0·001$) and also with weight ($r = -0·091$, 95 % CI −0·13, −0·04; $P = 0·003$), height ($r = -0·084$, 95 % CI −0·13, −0·034; $P = 0·005$) and BMI ($r = -0·074$, 95 % CI −0·13, −0·01; $P = 0·014$).

Table 2 Dietary sources of Ca in 9–12-year-old primary-school children, Tehran, autumn and winter 2007–2008

	Ca intake (g/d)					
	Girls (*n* 244)		Boys (*n* 257)		Total (*n* 501)	
Food group	Mean	SD	Mean	SD	Mean	SD
Milk and dairies	653·7	379·0	666·2	375·2	690·3	395·3
Bread and cereals	90·1	52·2	100·8	52·8	96·7	52·8
Fruit	62·5	45·1	73·4	53·9	71·9	55·6
Vegetables	41·6	38·2	38·8	29·8	40·9	34·4
Meat						
Meat and eggs	13·3	8·8	16·1	10·1	15·4	10·0
Legumes	10·9	18·0	12·6	20·9	11·8	19·6
Nuts	29·9	54·6	26·3	45·5	32·1	58·5

Table 3 Frequency of different degrees of vitamin D deficiency according to criteria 1* in 9–12-year-old primary-school children, Tehran, autumn and winter 2007–2008

	Frequency						
	Boys		Girls			Total	
Vitamin D status	*n*	%	*n*	%	*P* value	*n*	%
Severe deficiency	167	29·1	260	48·3	<0·001	427	38·3
Moderate deficiency	149	26·0	132	24·5	0·311	281	25·3
Mild deficiency	124	21·6	101	18·8	0·133	225	20·3
Adequate	133	23·2	45	8·4	<0·001	178	16·1
Total	573	100·0	538	100·0		1111	100·0

Frequencies were compared using the χ^2 test.
*Severe deficiency, 25-hydroxyvitamin D (25(OH)D) < 12·5 nmol/l; moderate deficiency, 12·5 ≤ 25(OH)D < 25·0 nmol/l; mild deficiency, 25·0 ≤ 25(OH)D < 37·0 nmol/l; adequate, 25(OH)D ≥ 37·0 nmol/l.

Interestingly, OST showed a positive correlation with height ($r = 0·130$, 95 % CI 0·08, 0·16; $P < 0·001$) but a negative correlation with BMI ($r = -0·072$, 95 % CI −0·131, −0·01; $P = 0·018$). For those variables without normal distribution, Spearman's correlations were also evaluated.

https://doi.org/10.1017/S1368980011000188 Published online by Cambridge University Press

Table 4 Frequency of different degrees of vitamin D deficiency according to criteria 2* in 9–12-year-old primary-school children, Tehran, autumn and winter 2007–2008

	Frequency						
	Boys		Girls			Total	
Vitamin D status	*n*	%	*n*	%	*P* value	*n*	%
Deficiency	357	62·3	411	76·4	<0·001	768	69·1
Insufficiency	152	26·5	99	18·4	0·001	251	22·6
Adequate	64	11·2	28	5·2	<0·001	92	8·3
Total	573	100·0	538	100·0		1111	100·0

Frequencies were compared using the χ^2 test.
*Deficiency, 25-hydroxyvitamin D (25(OH)D) < 27·5 nmol/l; insufficiency, 27·5 ≤ 25(OH)D < 50·0 nmol/l; adequate, 25(OH)D ≥ 50·0 nmol/l.

In this case, serum levels of 25(OH)D inversely correlated with iPTH ($r_s = -0{\cdot}154$, 95% CI −0·22, −0·10; $P < 0{\cdot}001$), BAP ($r_s = -0{\cdot}151$, 95% CI −0·22, −0·10; $P < 0{\cdot}001$), Mg ($r_s = -0{\cdot}142$, 95% CI −0·22, −0·11; $P < 0{\cdot}001$), weight ($r_s = -0{\cdot}116$, 95% CI −0·17, −0·06; $P < 0{\cdot}001$) and BMI ($r_s = -0{\cdot}092$, 95% CI −0·14, −0·04; $P = 0{\cdot}002$) but directly correlated with duration of sun exposure ($r_s = 0{\cdot}115$, 95% CI 0·11, 0·22; $P < 0{\cdot}001$).

Discussion

A remarkably high prevalence of poor vitamin D status among 9–12-year-old schoolchildren during cold seasons in Tehran convincingly proved that vitamin D deficiency is a major health problem in this age group living in Tehran. Severity of this deficiency was higher in girls than in boys. Our data are comparable to those reported from Isfahan, central Iran (95·2% in girls *v.* 49·0% in boys)[20]. However, there are some differences between these two studies as in the Isfahan study: (i) participants were between 14 and 18 years of age; (ii) sampling was performed during all four seasons; (iii) adequacy and insufficiency of vitamin D were defined as serum levels of 25(OH)D above 80 nmol/l and below 50 nmol/l, respectively; and (iv) RIA was used to measure serum 25(OH)D[20].

One of the factors affecting vitamin D status is body weight, notably BMI. The negative influence of fat mass (FM) on vitamin D status has already been reported[26]. It has been suggested that FM may act as a 'metabolic well' to reduce the bioavailability of vitamin D and its transformation to 25(OH)D[27]. This theory is well supported not only by our finding of an inverse correlation between serum 25(OH)D levels and BMI, but also by the epidemiological observation that vitamin D insufficiency is more prevalent among overweight and obese people[28].

Duration of direct sun exposure was clearly not sufficient to protect the children, and especially girls, from vitamin D insufficiency. Higher prevalence of vitamin D deficiency among girls has been repeatedly reported by others[20,29,30]. Considering the very limited food sources of vitamin D in the Iranian diet[21], one can relate the higher vitamin D deficiency in girls to their lower outdoor activities as well as to their dress code.

According to Islamic regulations, girls after pubescence must be veiled. It is noteworthy that even long sun exposure in this situation may not be effective to produce adequate vitamin D in the body. The amount of UV energy for dermal vitamin D synthesis is about 18–20 mJ/cm[2,31]. Exposure of limited surfaces of the body, mainly face and hands, does not seem sufficient for vitamin D adequacy[32]. The dermal response of individuals to UV may be different. In a study on ninety-three adults (thirty men and sixty-three women) with a mean age of 24·0 (SD 0·7) years and mean BMI of 23·6 (SD 0·4) kg/m^2, even after 28·9 (SD 1·5) h/week of sun exposure 51% of the participants had some degree of vitamin D insufficiency. In that study, serum levels of 25(OH)D below 75 nmol/l were considered as insufficient[33].

Tehran is located at a latitude of 35·34°, where dermal synthesis of vitamin D in cold seasons is almost negligible[34]. During this time, the body inevitably relies on vitamin D body stores. The extremely high prevalence of vitamin D deficiency in our study participants reflected well their inefficient vitamin D storage during warm seasons. In accordance with this finding, in a study on 168 girls aged 4–8 years, race and season were the strongest predictors of vitamin D status[35]. Prevalence of vitamin D insufficiency in Lebanese children was reported as 52% in 1999, with a peak in winter (65%) and a fall in summer (40%). In those children, socio-economic status and BMI were the most important predictors of serum vitamin D[36].

Human diets usually fail to provide sufficient amounts of vitamin D[37]. Even in North America, with the higher intakes of dairy products[38] compared with Iran[21] and fortification of milk with vitamin D[39], prevalence of vitamin D insufficiency is more than expected[40,41]. This could be, at least in part, because of insufficient intakes of dairy products. It has recently been reported that: first, dairy intake in the 4–18-year-old population of the USA is insufficient; and second, most children consume high-fat dairy products, which, compared with low-fat products, have a lower amount of Ca in a certain volume[38]. Ca intake may decrease even more during transition from childhood to adolescence[42]. The importance of Ca intake, with regard to vitamin D status, lies in the fact that sufficient Ca intake can improve serum levels of 25(OH)D by decreasing PTH secretion, thus decreasing hydroxylation of 25(OH)D to 1,25(OH)$_2$D[43]. The Ca intake in our study participants, however, was not enough to protect them from vitamin D insufficiency. This observation is quite explainable as there is no fortification and/or supplementation programme currently ongoing in Iran.

Significantly higher iPTH in girls than in boys is in accordance with girls' lower vitamin D status. Regarding the lower age of puberty in girls, there is a compensatory increase in PTH secretion to produce enough 1,25(OH)$_2$D

https://doi.org/10.1017/S1368980011000188 Published online by Cambridge University Press

needed for the accelerated growth. Higher levels of OST and BAP, together with iPTH, in girls compared with boys clearly indicate their higher bone turnover[44].

The high prevalence of vitamin D insufficiency and deficiency during cold seasons in primary-school children in Tehran should be regarded as a public health emergency by all stakeholders. Vitamin D deficiency-induced immune dysfunction can bring about such auto-immune disorders as T1DM in children and MS in adults[45]. There are some reports of a higher occurrence of T1DM[46] and MS[47] during the cold seasons and it was related to the lower vitamin D status[48–50]. Viral infections, which are usually more common during cold seasons, have been proposed as possible aetiologies of T1DM[51–53]. Vitamin D may exert its protective effects by inducing the expression of an antimicrobial peptide, cathelicidin[54,55]. Therefore, improvement of vitamin D intake in schoolchildren can help their bone health and growth and act, at least to some extent, as a 'vaccination' against auto-immune disorders and some other prevalent diseases such as CVD later in life[56].

It is noteworthy that the present study was conducted during cold seasons and does not necessarily show the vitamin D status of the children for the entire year. In a recent study conducted in 7–18-year-old children in Tehran, the prevalence of vitamin D insufficiency was reported as 53·6 % in girls and as 11·3 % in boys[57]. In a separate study on 313 children aged 8–18 years in Tehran, 26 % had vitamin D insufficiency[58]. These two studies were conducted in four seasons and both used RIA to measure serum 25(OH)D. Therefore, the time[59] and the method of assessment[60] can both influence our judgement on the prevalence.

We achieved a very high response rate in all schools and there was no obvious difference between children with and without blood samples.

In conclusion, our data showed that the problem of vitamin D deficiency in children still persists 35 years after the earliest reports of clinical rickets in Iran[61]. Vitamin D deficiency was more prevalent in girls than in boys. These findings warrant immediate appropriate interventions for nutritional support of 9–12-year-old (and most likely all) children.

Acknowledgement

The present study was funded by the National Nutrition and Food Technology Research Institute (NNFTRI). The authors do not have any duality of conflict of interest. The whole project was designed and conducted by T.R.N.; M.H., as a co-supervisor, actively participated in designing and conducting the project; dietary assessment, including the validation study, was carried out under the supervision of N.O.; sampling methodology and statistical analyses were performed under directions of M.R.E.; all laboratory analyses and fieldwork were conducted by T.R.N., A.G., A.K., N.S., N.K., H.H., T.Z. and B.N. The authors acknowledge the Deputy of Health of the Iranian Ministry of Education who allowed them to perform the present research in the primary schools of Tehran. They also appreciate all the school directors, principals and health instructors for their sincere cooperation. They especially thank all the children who participated in the present study and the parents who permitted their children to do so. All the laboratory bench works were carried out at the Laboratory of Nutrition Research at NNFTRI.

References

1. Calvo MS, Whiting SJ & Barton CN (2005) Vitamin D intake: a global perspective of current status. *J Nutr* **135**, 310–316.
2. 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.
3. Salek M, Hashemipour M, Aminorroaya A *et al.* (2008) Vitamin D deficiency among pregnant women and their newborns in Isfahan, Iran. *Exp Clin Endocrinol Diabetes* **116**, 352–356.
4. Heaney RP (2008) Vitamin D in health and disease. *Clin J Am Soc Nephrol* **3**, 1535–1541.
5. Maruotti N & Cantatore FP (2010) Vitamin D and the immune system. *J Rheumatol* **37**, 491–495.
6. Piek E, Sleumer LS, van Someren EP *et al.* (2010) Osteo-transcriptomics of human mesenchymal stem cells: accelerated gene expression and osteoblast differentiation induced by vitamin D reveals c-MYC as an enhancer of BMP2-induced osteogenesis. *Bone* **46**, 613–627.
7. Krishnan AV, Trump DL, Johnson CS *et al.* (2010) The role of vitamin D in cancer prevention and treatment. *Endocrinol Metab Clin North Am* **39**, 401–418.
8. Anderson LN, Cotterchio M, Vieth R *et al.* (2010) Vitamin D and calcium intakes and breast cancer risk in pre- and postmenopausal women. *Am J Clin Nutr* **91**, 1699–1707.
9. Bao Y, Ng K, Wolpin BM *et al.* (2010) Predicted vitamin D status and pancreatic cancer risk in two prospective cohort studies. *Br J Cancer* **102**, 1422–1427.
10. Barysch MJ, Hofbauer GF & Dummer R (2010) Vitamin D, ultraviolet exposure, and skin cancer in the elderly. *Gerontology* **56**, 410–413.
11. Khan QJ, Kimler BF & Fabian CJ (2010) The relationship between vitamin D and breast cancer incidence and natural history. *Curr Oncol Rep* **12**, 136–142.
12. Arnson Y, Amital H & Shoenfeld Y (2007) Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis* **66**, 1137–1142.
13. Zipitis CS & Akobeng AK (2008) Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child* **93**, 512–517.
14. Amital H, Szekanecz Z, Szucs G *et al.* (2010) Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D? *Ann Rheum Dis* **69**, 1155–1157.
15. Ascherio A, Munger KL & Simon KC (2010) Vitamin D and multiple sclerosis. *Lancet Neurol* **9**, 599–612.
16. Trump DL, Deeb KK & Johnson CS (2010) Vitamin D: considerations in the continued development as an agent for cancer prevention and therapy. *Cancer* **16**, 1–9.

17. Bischoff-Ferrari HA, Giovannucci E, Willett WC *et al.* (2006) Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* **84**, 18–28.
18. Keane EM, Rochfort A, Cox J *et al.* (1992) Vitamin-D-fortified liquid milk – a highly effective method of vitamin D administration for house-bound and institutionalised elderly. *Gerontology* **38**, 280–284.
19. Keane EM, Healy M, O'Moore R *et al.* (1998) Vitamin D-fortified liquid milk: benefits for the elderly community-based population. *Calcif Tissue Int* **62**, 300–302.
20. Moussavi M, Heidarpour R, Aminorroaya A *et al.* (2005) Prevalence of vitamin D deficiency in Isfahani high school students in 2004. *Horm Res* **64**, 144–148.
21. 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.
22. Omidvar N, Zaini-Nezhad A & Eshraghian MR (2009) *Validity and Reproducibility of a Quantitative Food Frequency Questionnaire to Assess Calcium Intake in 9–13-Year-Old Students in Tehran* (*Research Report*). Tehran: NNFTRI.
23. Ministry of Health & National Nutrition and Food Technology Research Institute (2005) *National Assessment of Iron, Zinc, Vitamin A and Vitamin D, 2001 Final Report of a National Survey*. Tehran: Ministry of Health.
24. Neyestani TR, Gharavi A & Kalayi A (2008) Iranian diabetics may not be vitamin D deficient more than healthy subjects. *Acta Med Iran* **46**, 337–341.
25. 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.
26. 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.
27. Wortsman J, Matsuoka LY, Chen TC *et al.* (2000) Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* **72**, 690–693.
28. Hypponen E & Power C (2006) Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity. *Diabetes Care* **29**, 2244–2246.
29. Bener A, Al-Ali M & Hoffmann GF (2009) High prevalence of vitamin D deficiency in young children in a highly sunny humid country: a global health problem. *Minerva Pediatr* **61**, 15–22.
30. Sahu M, Bhatia V, Aggarwal A *et al.* (2009) Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India. *Clin Endocrinol (Oxf)* **70**, 680–684.
31. Matsuoka LY, Wortsman J, Haddad JG *et al.* (1989) In vivo threshold for cutaneous synthesis of vitamin D3. *J Lab Clin Med* **114**, 301–305.
32. Matsuoka LY, Wortsman J & Hollis BW (1990) Use of topical sunscreen for the evaluation of regional synthesis of vitamin D3. *J Am Acad Dermatol* **22**, 772–775.
33. Binkley N, Novotny R, Krueger D *et al.* (2007) Low vitamin D status despite abundant sun exposure. *J Clin Endocrinol Metab* **92**, 2130–2135.
34. Holick MF, Chen TC, Lu Z *et al.* (2007) Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res* **22**, Suppl. 2, S28–S33.
35. Stein EM, Laing EM, Hall DB *et al.* (2006) Serum 25-hydroxyvitamin D concentrations in girls aged 4–8 y living in the southeastern United States. *Am J Clin Nutr* **83**, 75–81.
36. El-Hajj Fuleihan G, Nabulsi M, Choucair M *et al.* (2001) Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107**, 53–60.
37. Vieth R, Bischoff-Ferrari H, Boucher BJ *et al.* (2007) The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* **85**, 649–650.
38. Kranz S, Lin PJ & Wagstaff DA (2007) Children's dairy intake in the United States: too little, too fat? *J Pediatr* **151**, 642–646.
39. Calvo MS, Whiting SJ & Barton CN (2004) Vitamin D fortification in the United States and Canada: current status and data needs. *Am J Clin Nutr* **80**, Suppl. 6, S1710–S1716.
40. Hanley DA & Davison KS (2005) Vitamin D insufficiency in North America. *J Nutr* **135**, 332–337.
41. Calvo MS & Whiting SJ (2003) Prevalence of vitamin D insufficiency in Canada and the United States: importance to health status and efficacy of current food fortification and dietary supplement use. *Nutr Rev* **61**, 107–113.
42. Larson NI, Neumark-Sztainer D, Harnack L *et al.* (2009) Calcium and dairy intake: longitudinal trends during the transition to young adulthood and correlates of calcium intake. *J Nutr Educ Behav* **41**, 254–260.
43. Wilson HD, Horst RL & Schedl HP (1982) Calcium intake regulates 1,25-dihydroxy-vitamin D formation in the diabetic rat. *Diabetes* **31**, 401–405.
44. Levine MA (2003) Biochemical markers of bone metabolism: application to understanding bone remodeling in children and adolescents. *J Pediatr Endocrinol Metab* **16**, Suppl. 3, S661–S672.
45. Mark BL & Carson JA (2006) Vitamin D and autoimmune disease – implications for practice from the multiple sclerosis literature. *J Am Diet Assoc* **106**, 418–424.
46. Moltchanova EV, Schreier N, Lammi N *et al.* (2009) Seasonal variation of diagnosis of type 1 diabetes mellitus in children worldwide. *Diabet Med* **26**, 673–678.
47. McDowell TY, Amr S, Langenberg P *et al.* (2010) Time of birth, residential solar radiation and age at onset of multiple sclerosis. *Neuroepidemiology* **34**, 238–244.
48. Holick MF (2005) Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease, type 1 diabetes, autoimmune diseases, and some cancers. *South Med J* **98**, 1024–1027.
49. Eikelenboom MJ, Killestein J, Kragt JJ *et al.* (2009) Gender differences in multiple sclerosis: cytokines and vitamin D. *J Neurol Sci* **286**, 40–42.
50. Orton SM, Morris AP, Herrera BM *et al.* (2008) Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. *Am J Clin Nutr* **88**, 441–447.
51. Richer MJ & Horwitz MS (2009) Coxsackievirus infection as an environmental factor in the etiology of type 1 diabetes. *Autoimmun Rev* **8**, 611–615.
52. Goldberg E & Krause I (2009) Infection and type 1 diabetes mellitus – a two edged sword? *Autoimmun Rev* **8**, 682–686.
53. Aarnisalo J, Veijola R, Vainionpää R *et al.* (2008) Cytomegalovirus infection in early infancy: risk of induction and progression of autoimmunity associated with type 1 diabetes. *Diabetologia* **51**, 769–772.
54. White JH (2010) Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: past, present and future. *J Steroid Biochem Mol Biol* **121**, 234–238.
55. Misawa Y, Baba A, Ito S *et al.* (2009) Vitamin D(3) induces expression of human cathelicidin antimicrobial peptide 18 in newborns. *Int J Hematol* **90**, 561–570.
56. Holick MF (2004) Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* **79**, 362–371.
57. Rabbani A, Alavian SM, Motlagh ME *et al.* (2009) Vitamin D insufficiency among children and adolescents living in Tehran, Iran. *J Trop Pediatr* **55**, 189–191.

58. 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.
59. Bolland MJ, Chiu WW, Davidson JS *et al.* (2008) The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency. *N Z Med J* **121**, 63–74.
60. Neyestani TR, Gharavi A, Kalayi A *et al.* (2007) Determination of serum 25-hydroxy cholecalciferol using high-performance liquid chromatography: a reliable tool for assessment of vitamin D status. *Int J Vitam Nutr Res* **77**, 341–346.
61. Salimpour R (1975) Rickets in Tehran. Study of 200 cases. *Arch Dis Child* **50**, 63–66.

https://doi.org/10.1017/S1368980011000188 Published online by Cambridge University Press