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

Tirang R Neyestani1,*, Majid Hajifaraji2, Nasrin Omidvar3, Mohammad R Eshraghian4, Nastaran Shariatzadeh1, Ali Kalayi1, A’azam Gharavi1, Niloufar Khalaji1, Homa Haidari5, Telma Zowghi5 and Bahareh Nikooyeh1
1Laboratory 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: 2Department of Nutritional Policy-making Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran; 3Department of Community Nutrition, Faculty of Nutritional Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran: 4Department of Epidemiology and Biostatistics, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 5Department of Nutrition Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Abstract


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 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 = −0.154, P < 0.001) and BMI (r = −0.092, P = 0.002) but directly correlated with duration of sun exposure (r = 0.115, P < 0.001).

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

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 anticancer 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 autoimmune disorders(12) (e.g. type 1 diabetes mellitus (T1DM)(13), lupus(14) and multiple sclerosis (MS)(15)) and malignancies(16). Whereas 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...
remarkably high prevalence of various degrees of vitamin D deficiency among those 20–69 years of age in the Tehran population (79-6%) 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 used by the Iranian Ministry of Health in a national survey on micronutrient status in both 15–23-month-old children and pregnant women and also in a pilot study on Iranian adults (22). The second set of criteria, which is more recent (25), was: sufficient \( \geq 37.0 \text{ nmol/l} \); mild deficiency \( 25.0 \text{ nmol/l} \leq \text{insufficiency} < 37.0 \text{ nmol/l} \); and severe deficiency \( < 25.0 \text{ nmol/l} \). This set of criteria was used to categorize various degrees of vitamin D deficiency, both based on serum levels of 25(OH)D. The first set was: sufficient \( \geq 37.0 \text{ nmol/l} \); mild deficiency \( 25.0 \text{ nmol/l} \leq \text{insufficiency} < 37.0 \text{ nmol/l} \); and severe deficiency \( < 25.0 \text{ nmol/l} \). Serum levels of osteocalcin (OST; Biosource Europe SA, Nivelles, Belgium), intact parathyroid hormone (iPTH; DRG Instruments GmbH, Marburg, Germany) and bone-specific alkaline phosphatase (BAP; Immunodiagnostics 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).

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

Height 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/m²).

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 \(-80^\circ\text{C}\) 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 \( \geq 37.0 \text{ nmol/l} \); mild deficiency \( 25.0 \text{ nmol/l} \leq \text{insufficiency} < 37.0 \text{ nmol/l} \); moderate deficiency \( < 25.0 \text{ nmol/l} \); and severe deficiency \( < 25.0 \text{ nmol/l} \). The second set of criteria was used to categorize various degrees of vitamin D deficiency, both based on serum levels of 25(OH)D. The first set was: sufficient \( \geq 50.0 \text{ nmol/l} \); mild deficiency \( 27.5 \text{ nmol/l} \leq \text{insufficiency} < 50.0 \text{ nmol/l} \); and deficiency \( < 27.5 \text{ nmol/l} \).

Serum levels of osteocalcin (OST; Biosource Europe SA, Nivelles, Belgium), intact parathyroid hormone (iPTH; DRG Instruments GmbH, Marburg, Germany) and bone-specific alkaline phosphatase (BAP; Immunodiagnostics 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 Kolmogorov–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 \( \chi^2 \) test was used to compare qualitative data between groups. Differences were considered statistically significant at \( \alpha < 0.05 \). 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 (\( P = 0.010 \)), no significant difference was observed between the BMI of boys and girls (Table 1).
Table 1 Comparison of variables by sex: 9–12-year-old primary-school children, Tehran, autumn and winter 2007–2008

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boys</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>P value</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>37.9</td>
<td>9.6</td>
<td>39.0</td>
<td>10.6</td>
<td>0.065</td>
<td>−0.14, 2.20</td>
<td>38.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>140.5</td>
<td>6.8</td>
<td>141.6</td>
<td>7.3</td>
<td>0.010</td>
<td>0.26, 1.92</td>
<td>141.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.0</td>
<td>3.8</td>
<td>19.2</td>
<td>4.0</td>
<td>0.332</td>
<td>−0.22, 0.69</td>
<td>19.1</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>9.8</td>
<td>0.6</td>
<td>9.7</td>
<td>0.6</td>
<td>0.510</td>
<td>−0.08, 0.04</td>
<td>9.7</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>4.6</td>
<td>0.6</td>
<td>4.5</td>
<td>0.6</td>
<td>0.311</td>
<td>−0.11, 0.03</td>
<td>4.6</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>2.0</td>
<td>0.2</td>
<td>2.2</td>
<td>0.2</td>
<td>0.005</td>
<td>0.01, 0.05</td>
<td>2.2</td>
</tr>
<tr>
<td>OST (ng/ml)</td>
<td>41.5</td>
<td>17.4</td>
<td>44.4</td>
<td>19.1</td>
<td>0.016†</td>
<td>27.4‡</td>
<td>42.9</td>
</tr>
<tr>
<td>iPTH (µg/l)</td>
<td>40.4†</td>
<td></td>
<td>43.4</td>
<td></td>
<td></td>
<td></td>
<td>41.6</td>
</tr>
<tr>
<td>Cement exposure (min/d)</td>
<td>43.0</td>
<td>35.1</td>
<td>34.5</td>
<td>22.5</td>
<td>&lt;0.01†</td>
<td>25.0‡</td>
<td>38.8</td>
</tr>
</tbody>
</table>

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 are given in 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 (χ² 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 (χ² 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 = −0.15, 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 height (r = −0.091, 95% CI −0.13, −0.04; P = 0.003), weight (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

<table>
<thead>
<tr>
<th>Food group</th>
<th>Girls (n 244)</th>
<th>Boys (n 257)</th>
<th>Total (n 501)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Milk and dairies</td>
<td>653.7</td>
<td>379.0</td>
<td>376.2</td>
</tr>
<tr>
<td>Bread and cereals</td>
<td>50.1</td>
<td>26.5</td>
<td>50.3</td>
</tr>
<tr>
<td>Fruit</td>
<td>62.6</td>
<td>48.9</td>
<td>63.3</td>
</tr>
<tr>
<td>Vegetables</td>
<td>41.6</td>
<td>36.8</td>
<td>41.9</td>
</tr>
<tr>
<td>Meat</td>
<td>21.7</td>
<td>13.3</td>
<td>21.9</td>
</tr>
<tr>
<td>Meat and eggs</td>
<td>13.3</td>
<td>9.8</td>
<td>14.1</td>
</tr>
<tr>
<td>Legumes</td>
<td>10.9</td>
<td>6.0</td>
<td>11.4</td>
</tr>
<tr>
<td>Nuts</td>
<td>26.7</td>
<td>11.7</td>
<td>28.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Boys</th>
<th>Girls</th>
<th>Total</th>
<th>P value</th>
<th>n</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe deficiency</td>
<td>167</td>
<td>29.1</td>
<td>48.3</td>
<td>&lt;0.001</td>
<td>427</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>Moderate deficiency</td>
<td>149</td>
<td>26.0</td>
<td>13.2</td>
<td>0.311</td>
<td>281</td>
<td>25.3</td>
<td></td>
</tr>
<tr>
<td>Mild deficiency</td>
<td>124</td>
<td>21.6</td>
<td>101.0</td>
<td>0.133</td>
<td>225</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>Adequate</td>
<td>133</td>
<td>23.2</td>
<td>45.8</td>
<td>&lt;0.001</td>
<td>178</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>573</td>
<td>100.0</td>
<td>538.0</td>
<td>100.0</td>
<td>1111</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Frequencies were compared using the χ² test.

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.
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

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Boys</th>
<th>Girls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Deficiency</td>
<td>357</td>
<td>62.3</td>
<td>411</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>152</td>
<td>26.5</td>
<td>99</td>
</tr>
<tr>
<td>Adequate</td>
<td>64</td>
<td>11.2</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>573</td>
<td>100</td>
<td>538</td>
</tr>
</tbody>
</table>

Frequencies were compared using the χ² test.

In this case, serum levels of 25(OH)D inversely correlated with iPTH (r = −0.015, 95% CI −0.22, 0.10; P < 0.001), BAP (r = −0.115, 95% CI −0.22, 0.10; P < 0.001), Mg (r = −0.142, 95% CI −0.22, 0.11; P < 0.001), weight (r = −0.116, 95% CI −0.22, 0.17; P < 0.001) and BMI (r = −0.092, 95% CI −0.14, 0.04; P = 0.002) but directly correlated with duration of sun exposure (r = 0.115, 95% CI 0.11, 0.22; P < 0.001).

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 vs. 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 (20). 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 (20).

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/cm² (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 ± 7) years and mean BMI of 23 ± 6 (SD ± 4) kg/m², 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)₂D (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)₂D.
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**

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**References**


Vitamin D status of schoolchildren


