



# Vitamin D status of 6- to 7-year-old children living in Isfahan, Iran

Stężenie witaminy D u 6–7-letnich dzieci zamieszkałych w Isfahanie, Iran

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## Abstract

**Introduction:** Vitamin D is essential for the maintenance of good health, and vitamin D deficiency has been reported from many countries, including those with a lot of sunshine. This study was conducted to evaluate the vitamin D status in healthy 6- to 7-year-old children in Isfahan, Iran.

**Material and methods:** Five hundred and thirteen healthy children were enrolled. Serum PTH and 25-hydroxyvitamin D (25-OHD) were measured. Dietary vitamin D intake, duration of daily sunlight exposure, and percentage of exposed body surface area were determined. 25-OHD levels < 20 ng/mL and < 10 ng/mL were defined as mild and severe vitamin D deficiency, respectively. The ROC curve was utilized to obtain a local cut-off point of vitamin D deficiency.

**Results:** 25-OHD was < 20 ng/mL in 3% and < 33 ng/mL (local cut-off point of vitamin D deficiency) in 26% of subjects. Duration of sunlight exposure and daily intake of vitamin D had significant effects on serum level of vitamin D.

**Conclusions:** A high prevalence of vitamin D deficiency in Isfahan children was observed in this study. Improvements in duration of sunlight exposure and daily intake of vitamin D can prevent vitamin D deficiency in these children.

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**Key words:** vitamin D status, vitamin D deficiency, Iran, diet, sunlight

## Streszczenie

**Wstęp:** Witamina D ma podstawowe znaczenie dla utrzymania dobrego zdrowia. O niedoborach witaminy D donoszą autorzy z wielu krajów, również tych o dużym stopniu nasłonecznienia. Niniejsze badanie przeprowadzono w celu dokonania oceny stężenia witaminy D u zdrowych dzieci w wieku 6–7 lat, zamieszkałych w Isfahanie, Iran.

**Materiał i metody:** Do badania włączono 513 zdrowych dzieci. Zmierzone u nich stężenie PTH i 25-hydroksywitaminy D (25-OHD) w surowicy. Określono spożycie witaminy D w diecie, czas ekspozycji na słońce w ciągu doby i odsetek powierzchni ciała wystawionej na działanie słońca. Przyjęto, że stężenia 25-OHD wynoszące < 20 ng/ml i 10 ng/ml odpowiadają odpowiednio łagodnemu i ciężkiemu niedoborowi witaminy D. Do ustalenia lokalnych punktów odcięcia dla niedoborów witaminy D użyto krzywych ROC.

**Wyniki:** U 3% dzieci stężenie witaminy 25-OHD wynosiło < 20 ng/ml, a u 26% wynosiło < 33 ng/ml (lokalny punkt odcięcia dla niedoboru witaminy D). Czas ekspozycji na słońce i dzienne spożycie witaminy D w diecie miały istotny wpływ na stężenie witaminy D w surowicy.

**Wnioski:** W omawianym badaniu wykazano częste występowanie niedoboru witaminy D u dzieci zamieszkałych w Isfahanie. Wydłużenie czasu ekspozycji na słońce i zwiększenie dziennego spożycia witaminy D w diecie może zapobiec niedoborom witaminy D u tych dzieci.

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**Słowa kluczowe:** stężenie witaminy D, niedobór witaminy D, Iran, dieta, światło słoneczne

## Introduction

Vitamin D deficiency is an unrecognized epidemic and a common health problem worldwide [1]. Vitamin D status is very different among various countries, which is due to the difference in many factors including sun-

light exposure (e.g. season, kind of clothes, and effect of latitude), dietary intake of vitamin D, and air pollution [2]. Vitamin D deficiency causes poor mineralization of the collagen matrix in young children's bones leading to growth retardation and bone deformities known as rickets [3]. Recent evidence suggests that



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vitamin D deficiency is associated with an increased risk of type I diabetes, multiple sclerosis, rheumatoid arthritis, hypertension, cardiovascular heart disease, and many common deadly cancers [1, 4–7]. The main source of this vitamin in humans is synthesis from the ultraviolet B light fraction of sunlight in skin and a small part from the vitamin D which exists in our diet. Although vitamin D deficiency is unexpected in sunny regions, it has been reported to be highly prevalent in such countries [8–12]. The purpose of this study was to assess vitamin D status in 6- to 7-year-old children living in Isfahan, the third largest city in Iran.

## Material and methods

This cross-sectional study was conducted on 513 children aged 6–7 years, living in Isfahan city (32° 39' N), Iran. In Iran all children should give a complete physical examination at special sites before being registered in primary school. In the summer of 2006, we selected children by multistage cluster random sampling from the five administrative parts of primary schools. Children who took vitamin D supplements or had any diseases based on their medical history were excluded from the study. Informed written consent was obtained from the parents of the children.

Each parent filled a 7-day food frequency questionnaire and a questionnaire which was used to determine the children's daily sunlight exposure (minutes/day). The percentage of body surface area exposed to sunlight was estimated by asking about the children's most commonly worn daytime cloths. Face and neck 3%, plantar surface of hands 2.5%, half of the arm besides forearm 4%, feet 2.5%, and calves 6% were estimated and the average body surface of children was estimated at 7000 cm<sup>2</sup>. By multiplying the measured percentage of body surface exposed by 7000, the exposed body surface area was measured in cm<sup>2</sup>. The face validity of the questionnaires was approved by a pilot study among 20 individuals.

In the chosen clinics, 5 ml venous blood samples were drawn from the children. The blood samples were centrifuged and sera were stored at –20°C until analysis. Serum 25-hydroxyvitamin D (25-OHD) and parathyroid hormone (PTH) were measured in the laboratory of the Isfahan Endocrine and Metabolism Research Centre which is under the quality control of the National Reference Laboratory (WHO-Collaborating centre) in Tehran.

Serum 25-OHD and PTH levels were assayed by radioimmunoassay (INCSTAR corp., MN, and USA). Intra- and inter-assay coefficients of variation (CV) for 25-OHD were 3.3% and 5.2%, respectively. Intra- and inter-assay CV for PTH were 9.7% and 5%, respectively.

Mild and severe vitamin D deficiency were defined as 25-OHD levels below 20 ng/ml and 10 ng/ml, respectively [13].

Normality of data distribution was assessed with the Kolmogorov-Smirnov test. Descriptive data were expressed as mean  $\pm$  SD. Correlations were tested by computing Pearson's correlation coefficients. Differences were considered statistically significant at  $p < 0.05$ . Statistical analysis was conducted using SPSS version 13 (SPSS Corp, Chicago, IL, USA). There is no universal definition for vitamin D deficiency and it seems that it varies in different populations [14]. Most scientists have defined vitamin D deficiency as levels of 25-OHD  $< 20$  ng/mL, while studies that have examined PTH levels have suggested that the inflection point is closer to 30 ng/mL [11, 14]. Therefore, in addition to the international definitions, we determined a cut-off point for vitamin D deficiency in our population using a receiver operating characteristic (ROC) curve.

## Results

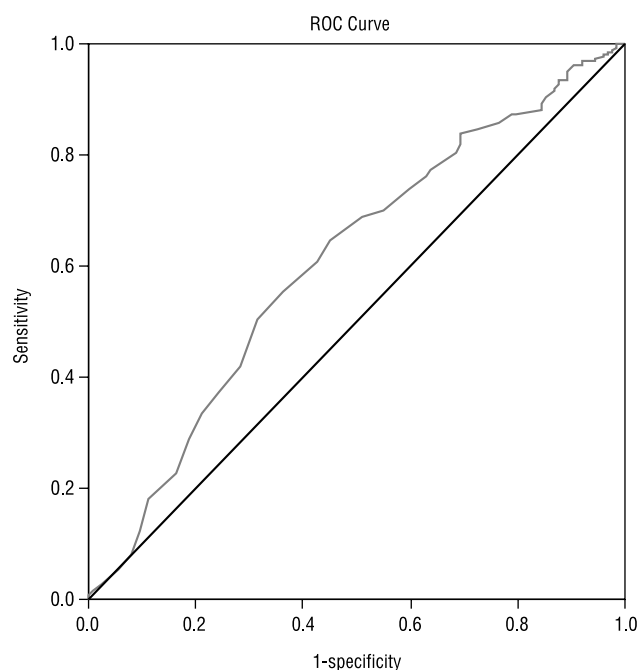
Five hundred and thirteen healthy children (271 boys and 242 girls) aged 6 to 7 years old were enrolled. The mean serum 25-OHD and PTH were  $46.01 \pm 17$  ng/mL and  $23.3 \pm 12$  pg/mL, respectively.

Three per cent of subjects had mean serum 25-OHD  $< 20$  ng/mL (mild vitamin D deficiency) and none had mean serum 25-OHD  $< 10$  ng/mL (severe vitamin D deficiency).

There was a reverse correlation between serum 25-OHD and PTH level ( $p < 0.001$ ,  $r = -0.157$ ). Using the ROC curve we determined 25-OHD level = 33 ng/ml as the vitamin D cut-off point with the surface under the curve at 60.5% (CI 95% = 54–66%,  $p = 0.001$ ) in our population (Fig. 1). Having considered this local cut-off point, 26% of subjects were classified as vitamin D deficient.

The mean duration of sunlight exposure (min/day), the mean body surface area exposed (cm<sup>2</sup>), the mean vitamin D intake (IU/day), and serum levels of PTH and 25-OHD in the studied population are shown in Table I. In all subjects, the mean daily intake of vitamin D was less than 400 units per day (the recommended intake of vitamin D) and in just 2.5% ( $n = 13$ ) of subjects it was more than 200 units per day, which is the adequate intake of vitamin D for 4- to 8-year-old children who do not receive adequate exposure to sunlight [15]. The mean daily sunlight exposure was  $44 \pm 29$  min/day (range: 4.29–180 min/day).

Using a regression test, a significant association between exposure to sunlight (min. cm<sup>2</sup>/day) ( $\beta = 0.28$ ,  $p < 0.001$ ) and daily intake of vitamin D (IU/L) ( $\beta = 0.13$ ,  $p = 0.002$ ) with serum 25-OHD level was found.



**Figure 1.** Receiver operating characteristic curve showing 25-OHD level = 33 ng/mL as the local cut-off point of vitamin D deficiency (the surface under the curve of 60.5% [CI 95% = 54–66%,  $p = 0.001$ ])

**Rycina 1.** Krzywe ROC przedstawiające lokalne stężenie 25-OH D wynoszące 33 ng/ml jako lokalny punkt odcięcia dla niedoboru witaminy D (pole pod krzywą 60,5% [95-procentowy przedział ufności = 54–66%,  $p = 0,001$ ])

## Discussion

The present study was the first to evaluate vitamin D status in young children living in Isfahan. In our study 26% of children had a serum 25-OHD level less than 33 ng/mL, which is the local cut-off point of vitamin D in

our population. In 5% of subjects 25-OHD was less than 20 ng/mL. We found that vitamin D deficiency was common among healthy 6- to 7-year-old children in Isfahan, which is in keeping with the results of other studies in sun-rich countries and the Middle East [8, 10–12, 16].

Although both daily intake of vitamin D and sun exposure were both significant predictors of vitamin D status, the latter was more significant, which is in agreement with other studies [17, 18] and the fact that the main source of vitamin D is exposure to sunlight. The mean duration of sun exposure in our subjects was more than that of the 11- to 15-year-old girls of Tehran [19] but lower than that of high school students of Isfahan [11]. It seems that these differences are partly because of the method of study, using different questionnaires, and the difference in age groups. Although in our study we found less exposure to sunlight than the study in Isfahan high schools, the better status of vitamin D in our subjects was predictable because our preschool participants did not use the dress style of school children in our region.

All our subjects reported a daily intake of vitamin D less than the recommended amount (400 IU/day) and just 2.5% received the adequate intake of vitamin D for the age group [15]. A survey of vitamin D intake in the United States revealed that neither children nor adults were receiving the recommended adequate intake of vitamin D [20]. These results highlight the need to provide children with supplements and a diet rich in vitamin D.

Although we did not have any literature about the vitamin D status of this age group in other cities and in extreme latitudes of Iran, a comparison of our results with studies at different latitudes is in agreement with existing evidence of latitudinal differences in the 25-OHD level of children [21, 22]. Mean serum 25-OHD

**Table I.** The daily sunlight exposure, body surface area exposed to sunlight, daily intake of vitamin D, and serum levels of 25-OHD and PTH in Isfahan 6-year-old children

**Tabela I.** Dobowa ekspozycja na słońce, powierzchnia ciała wystawiona na działanie słońca, dobowe spożycie witaminy D oraz stężenie witaminy 25-OH D i PTH w surowicy u 6-letnich dzieci z Isfahanu

	Girls	Boys	P value	Total
Number	242	271	–	513
25-OHD [ng/mL]	46.4 ± 17.9	46.7 ± 18.7	0.86	46.01 ± 17
25-OHD < 20 ng/mL n (%)	5 (2.1)	10 (3.7)	0.34	15 (3)
25-OHD < 33 ng/mL n (%)	62 (26.5)	65 (25)	0.65	127 (26)
Daily sunlight exposure [min/day]	42 ± 27	46 ± 30	0.06	44 ± 29
Body surface area exposed [cm <sup>2</sup> ]	1037 ± 376	1046 ± 356	0.1	1041 ± 365
Vitamin D intake [IU/day]	82 ± 49	88 ± 47	0.68	85 ± 48
PTH [pg/mL]	23.4 ± 12	23.3 ± 12	0.45	23.3 ± 12

values for these participants who lived at 32° 39' N were higher than those of children who lived at higher latitudes in Spain (43°N) [23], Tasmania (42°S) [21], and the south-eastern United States (34°N)[22].

Despite the effect of latitude, it was highlighted that living at low latitudes does not entirely prevent vitamin D deficiency [24]. Data from the National Health and Nutrition Examination Survey (NHANES) III showed that 47% of a sample of 12- to 19-year-old girls from sunlight-rich locations with a mean latitude of 32°N had vitamin D level < 25 ng/mL in winter [25].

Compared with former studies in Iran [26, 27], we found a higher vitamin D status in our study, which is partly due to the time this study was performed (summer) and the young age group of our subjects. According to many studies evaluating seasonal variations in vitamin D levels, it is expected that one would observe a lower vitamin D status in winter than in summer. In a study on US girls, the mean serum level of 25-OHD was 32% lower in winter than in summer [22]. Studies have also found a lower vitamin D status in older ages [25], and an age-related decline in cutaneous synthesis of vitamin D may be responsible for this [28].

We found a negative correlation between 25-OHD and PTH, which is in agreement with other studies [2, 25].

Age, sex, pubertal status, latitude, season, race, and ethnicity influence serum concentrations of 25-OHD [2, 3]. Therefore, there is no consensus on the ideal 25-OHD concentration, and there are many suggested values to set the lower limit of normality. However, it is now recognized that maintenance of a serum 25-OHD level of  $\geq 32$  ng/mL improves muscle strength and bone mineral density in adults [29, 30]. Researchers suggest that the concentration at which vitamin D suppresses PTH can be used as a functional index of sufficient vitamin D status [31]. In the present study, using an ROC curve we determined a serum level of 33 ng/mL as the local cut off point, which is similar to the cut off point determined in another study in Isfahan high school children [11].

One of the main limitations of our study is that we just evaluated the vitamin D status of subjects in summer, and therefore we cannot discuss the seasonal variation of vitamin D status. Also we did not ask subjects about using sun block. Further studies are needed to evaluate vitamin D status in older children and also during winter.

## Conclusions

We found that vitamin D deficiency is common in young children living in Isfahan. Since there is not any governmental regulation mandating vitamin D supplementation of food products in Iran, the main source of vitamin D is exposure to sunlight. Sensible sun expo-

sure and the use of vitamin D supplements can provide children with good vitamin D status and therefore good health.

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