

# ASSESSMENT OF VITAMIN D STATUS IN CZECH CHILDREN

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## SUMMARY

**Objective:** Vitamin D deficiency is a global health problem. The aim of this study was to determine the proportion of Czech children with vitamin D deficiency and examine related factors.

**Methods:** The study subjects were 419 healthy children aged 5 and 9 years. Severe vitamin D deficiency was defined as a serum 25(OH)D level of < 25 nmol/L (3% of children), deficiency as that of 25–50 nmol/L (24% of children), insufficiency as that of 50–75 nmol/L (40% of children) and sufficiency as that of > 75 nmol/L (34% of children).

**Results:** Serum 25(OH)D levels varied mainly with season. The highest levels of 25(OH)D were reached in autumn (median: 79.0 nmol/L), followed by summer (median: 67.8 nmol/L) and winter (median: 56.0 nmol/L). The lowest median value (49.8 nmol/L) was detected in spring.

**Conclusion:** Children with sufficiency 25(OH)D levels were more frequently exposed to the sun and exposed a larger part of their body to the sun while spending time outdoors. Levels of 25(OH)D were also associated with using vitamin D supplements within six weeks before sampling.

**Key words:** vitamin D, 25(OH)D levels, human biomonitoring, children, Czech Republic

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## INTRODUCTION

Human biomonitoring is a useful tool for assessing the levels of toxic substances ingested into the body from the environment as well as for evaluating the levels of several substances essential to health. In the Czech Republic, there has existed since 1991 a human biomonitoring network that functions as part of the Environmental Health Monitoring System that was established by the Czech Government. Over the years, monitoring has undergone major developmental stages which have improved its functioning (1). In 2016, it was decided to include vitamin D as a new biomarker into the ongoing Czech Human Biomonitoring System. Until now, there have been conducted only a few studies on vitamin D serum levels in the Czech population (2). Vitamin D plays an important role in calcium homeostasis maintenance and bone health. It also has been reported as a relevant factor in decreasing the risk of many chronic conditions, such as cardiovascular diseases, autoimmune diseases, metabolic disorders and others (3–5). The main objective of the present study was to assess vitamin D saturation in the population of Czech children and possible effects of certain factors (season, personal behaviour, etc.) on serum vitamin D concentration.

## MATERIALS AND METHODS

### Sample Collection and Preparation

A total of 419 blood samples were collected from healthy children aged 5 and 9 years to obtain serum for vitamin D analysis. The study was conducted in five Czech cities – Prague, Ostrava, Liberec, Žďár nad Sázavou, and Kutná Hora from March through December in 2016 and in January and February of 2017. The monitoring took place during regular medical check-ups of children aged 5 and 9 in surgeries of collaborating paediatricians. The children were enrolled in the study consecutively as they presented for check-ups according to the month of birth. Their parents signed an appropriate informed consent form and filled out a questionnaire about the child's socio-demographic characteristics and general lifestyle factors, including the length of sun exposure expressed in hours per day.

Blood samples were collected by trained nurses either during the preventive paediatric check-ups or at a designated department of medical laboratories. Serum was separated by centrifugation, frozen, transported to the analytical laboratory and stored at –20 °C until analysis. The vitamin D (25OHD total – 25OHD<sub>2</sub>+25OHD<sub>3</sub>) level was measured by the electrochemilu-

minescence immunoassay (ECLIA) method (Cobas 6000, Roche, Mannheim, Germany, IVD-CE). Analyses were performed in an accredited laboratory in compliance with the standard operating procedures and quality assurance and quality control (QA/QC) protocols. The study protocol was approved by the Ethical Committee of the National Institute of Public Health.

## Requirements for Vitamin D Threshold

As reported by Pludowski, the majority of studies include 25(OH)D levels in the range of 75–125 nmol/L as the necessary minimal concentration for human well-being (6). However, in children, it is important to note that there are sparse outcome data to help define a healthy or optimal level of 25(OH)D (7). Vitamin D status was categorized as deficient (<25 nmol/L), insufficient (<50 nmol/L), sufficient (<75 nmol/L), and optimal ( $\geq 75$  nmol/L) (8).

## Statistical Analyses

The time when samples were collected was reported only as to the season of the year, namely spring, summer, autumn or winter, defined as March, April and May for spring, June, July and August for summer, September, October and November for autumn, and December, January and February for winter.

Differences between the 25(OH)D levels were tested using the Kruskal-Wallis test or Mann-Whitney U test. Statistical significance was set as  $\alpha = 0.05$ . Data were processed using SPSS ver. 23 (SPSS Inc. Chicago, IL).

## RESULTS

Table 1 shows the baseline characteristics of the studied children. Table 2 indicates that the level of 25(OH)D was measured in 419 serum samples and that the levels detected ranged between 12.1 nmol/L and 146.5 nmol/L, with a median value of 63.0 nmol/L. The highest level of 25(OH)D was reached in the autumn (median: 79.0 nmol/L) and was followed by those in the summer (median: 67.8 nmol/L) and winter (median: 56.0 nmol/L). The lowest median value (49.8 nmol/L) was detected in the spring (Fig. 1). On average, only 34% of children in our study group reached the optimal serum concentrations of 25(OH)D. Significant

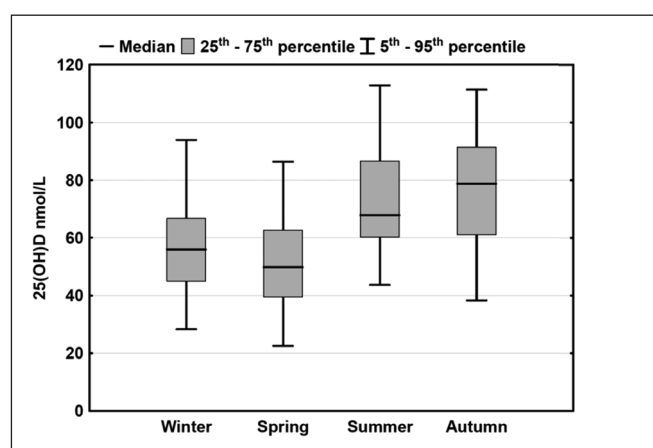


Fig. 1. 25(OH)D concentrations by season.

Table 1. Descriptive statistics of the participants enrolled in the study (N = 419)

Demographic characteristic	n (%)
Age (years)	
5	161 (38.4)
9	256 (61.1)
Not specified	2 (0.5)
Gender	
Boys	227 (54.2)
Girls	185 (44.2)
Not specified	7 (1.6)
Locality	
Kutná Hora	34 (8.1)
Žďár nad Sázavou	99 (23.6)
Ostrava	105 (25.1)
Liberec	82 (19.6)
Prague	99 (23.6)
Maternal education	
Primary (ISCED 0–2)	89 (22.2)
Secondary (ISCED 3–4)	185 (44.2)
Tertiary (ISCED 5–6)	128 (30.5)
Not specified	17 (4.1)
Season of sample collection	
Spring	124 (29.6)
Summer	85 (20.3)
Autumn	144 (34.4)
Winter	66 (15.8)
Vitamin D supplementation	
Yes	58 (13.8)
No	240 (57.3)
Not specified	121 (28.9)

ISCED – International Standard Classification of Education

differences in 25(OH)D were observed depending on sampling season ( $p < 0.001$ ). Nevertheless, even in the autumn, when the levels are generally higher, altogether 44% of the children did not have the sufficiency level of 25(OH)D.

In this study, parents were asked several questions related to their child's sun exposure. Generally, the median serum concentrations of 25(OH)D were significantly higher in children who were frequently exposed to the sun in the last six weeks before sampling (median: 74.1 nmol/L versus 60.0 nmol/L;  $p < 0.001$ ) and in those who exposed a larger part of their body (arms and face versus most of the body) to the sun while spending time outdoors (median: 60.7 nmol/L versus 70.5 nmol/L;  $p < 0.001$ ). The level of 25(OH)D was also associated with using vitamin D dietary supplements within six weeks before sampling. Children who had used (according to the questionnaire) vitamin D-containing supplements had a median 25(OH)D concentration of 74.2 nmol/L compared with 62.0 nmol/L in non-users ( $p > 0.05$ ). In terms of skin types, we selected the first five categories according to the Fitzpatrick classification (9); however, this factor was not significant ( $p > 0.05$ ) in our study.

Differences in the 25(OH)D values on the borderline of significance ( $p=0.05$ ) were detected between children whose mothers have tertiary education (a slightly higher median of 67.6 nmol/L) and those whose mothers have primary or secondary education (median of 61.9 nmol/L). The maternal education level could also have an impact on food choices; more educated mothers might buy more marine oily fish, such as mackerel and salmon with increased content of vitamin D.

No statistical difference was found in the distribution by age, gender or place of residence between the study groups. Also, different lifestyle factors, such as the frequency of food consumption or time, in hours, spent outdoors during week or weekend, were not identified in this study as significantly influencing factors ( $p>0.05$ ).

## DISCUSSION

Results of this study confirmed that one of the main factors influencing vitamin D levels in children is season. Seasonal variation, with the lowest levels measured during October to March, has been observed in this study as well as worldwide among children and adults (10–15). It was shown that UV-B radiation is not strong enough during winter and spring time (October–March)

to ensure sufficient synthesis of vitamin D in the skin, partly due to solar zenith angle, controlling potential UV B radiation. The Czech Republic lies from  $\approx 48^\circ\text{N}$  to  $\approx 51^\circ\text{N}$  and belongs to many regions of the world where is not possible to synthesize significant amounts of vitamin D3 in realistic exposure times for several months of the year (16). Moreover, there is no mandatory and only minimal voluntary vitamin D food fortification in the Czech Republic.

Apart from the above, there are also many other factors potentially affecting the ability to synthesize vitamin D3, including age, skin pigmentation, amount of skin exposed, time of the day, use of sunscreen, lifestyle or even air quality (10).

Previous studies that assessed age-related differences identified that adults had lower vitamin D levels than children, as cutaneous synthesis of vitamin D is decreasing with declining capacity of the skin itself to make cholecalciferol and also varies depending on the type of behaviour. However, the age of individuals when this decline occurs is unknown (17, 18). Our investigation showed that children's age is not significantly affecting the 25(OH)D levels (5-year-olds: 66.3 nmol/L versus 9-year-olds: 62.5 nmol/L;  $p>0.05$ ), which is in agreement with the systematic literature review of 2014 (19).

The relationship between 25(OH)D and gender was not significant ( $p>0.05$ ) among our study subjects (boys 63.6 nmol/L versus

**Table 2.** Seasonal variation in serum 25(OH)D

	Total N=419	Spring n=124	Summer n=85	Autumn n=144	Winter n=66
GM (nmol/L)	61.3	48.0	69.8	73.9	54.6
Median (nmol/L)	63.0	49.8	67.8	79.0	56.0
P95 (nmol/L)	104	86.4	113	113	93.9
Range (nmol/L)	12.1–147	12.1–104	19.6–139	21.2–147	23.7–114
Sufficiency (%)	34	14	40	57	15
Insufficiency (%)	40	36	48	33	52
Deficiency (%)	24	44	11	10	32
Severe deficiency (%)	3	6	1	1	2

GM – geometric mean, P95 – 95th percentile. The calculated percentages were rounded off to the nearest whole number.

**Table 3.** Comparison of the available worldwide data with the current study

Country	N	Age (years)	Season	Method for vitamin D analysis	Severe deficiency (%)	Deficiency (%)	Insufficiency (%)	Sufficiency (%)	Reference
Czech Republic	419	5 and 9	All year	ECLIA	3	24	39	34	This study
Europe*	1,006	12–17	October–July	ELISA	15	27	39	19	(20)
Belgium	357	4–11	February–June	RIA	5	53	40	2	(21)
Greece	43	3–10	All year	RIA	40.3		35	24.7	(22)
Italy	652	2–21	All year	RIA	9.5	36.4	33.6	20.5	(25)
Norway	NA	15–18	September–April	LC-MS/MS	16.5	43.7	27.4	12.4	(23)
Netherlands	4,167	6	All year	LC-MS/MS	6.2	23.6	36.5	33.7	(24)
USA	1,855	6–11	All year	LC-MS/MS	0.7	9.7	56	33.6	(27)
Canada	1,898	1–5	All year	CLIA	0	6	24	70	(28)
South Korea	2,062	10–18	All year	RIA	13	55	28	4	(26)

\*HELENA study: Austria, Belgium, France, Germany, Greece, Hungary, Italy, Spain, and Sweden

girls 62.2 nmol/L) or in the sample populations of the eligible studies worldwide (19). However, in an Irish study of adolescents, girls had lower winter time mean serum 25(OH)D levels than boys although they had higher levels than boys during summer time. According to the authors, it might be related to the higher proportion of girls (72%) than boys (43%) being sampled in late summer (15). Gender as a significant predictor of the year-round vitamin D status was also observed in a nationally representative sample of New Zealand children and adolescents and in the US adolescents and adults (13, 20); nevertheless, the reason for this apparent gender difference remained unexplained.

As is known, vitamin D deficiency is a public health concern worldwide and is very common also in Europe (21, 22). The results of the current study were compared to selected available studies from Europe, North America and South Korea based on such parameters as the analytical method or sampling season. Nevertheless, it is important to note that there are many other potential factors which may influence the comparison across countries (23). As shown in Table 3, there are a few children-based European studies looking at the 25(OH)D levels according to the cut-off points used for evaluation. However, only a limited number of studies are available in children up to 10 years of age, which is also reflected in our comparison.

In the current study, we observed a high prevalence of 25(OH)D insufficiency or deficiency. Generally over half of the Czech children aged 5 and 9 years had serum 25(OH)D levels below 75 nmol/L and 27% of them even below 50 nmol/L, which might be explained by inadequate time spent outdoors and contemporary sedentary lifestyles. Similar results have been reported in 6-year-old children from the Netherlands, of whom 66% had 25(OH)D concentrations below the optimal levels and almost 30% of them were vitamin D deficient (24). In other European studies from Norway (22), Belgium (23), Greece (25), and Italy (26) there were even higher prevalence rates of vitamin D deficiency and insufficiency. The representative European study HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) included 1,006 children aged 12 to 17 years from nine European countries. The study showed that 81% of the children had 25(OH)D concentrations below 75 nmol/L (20). In our comparison, the lowest values were observed among older children from South Korea (27). In that study, deficiency was observed in 68% of children and insufficiency in 28% of them. On the other hand, vitamin D status in American studies showed relatively higher values of vitamin D, with only 10% of children from the USA (28) and 6% of the Canadian children (29) having the 25(OH)D levels below 50 nmol/L. These results are in accordance with vitamin D food fortification practices, which are on a voluntary basis. Food fortification differs between the USA and Canada; nevertheless both are similar in providing fortified foods with proven efficacy (30).

### Strengths and Limitations of the Study

The strength of this study is that it provides the first information on the vitamin D status of healthy Czech preschool and early school children and shows its seasonal variation.

The limitations are as follows: the respondents were not randomly selected, so the results cannot be extrapolated to the entire paediatric population. The serum 25(OH)D was only measured

once per participant. Time spent outdoors was only assessed subjectively by parents filling in the questionnaire. The questionnaire was incompletely filled by several subjects whose data concerning use of dietary supplements or maternal education were missing (Table 1). The type and quantity of dietary supplements used by children have not been specified. Differences in length of stay outdoors between girls and boys were not evaluated for reasons of the subjective nature of these data.

### CONCLUSIONS

Vitamin D deficiency is a public health concern in the Czech Republic as two thirds of the Czech children have a suboptimal vitamin D status during the whole year. The knowledge of possible determinants is of importance for the development of public health strategies for the prevention of vitamin D deficiency. As expected, this study indicated a significant contribution of the sampling month. In the period from October to April; it is essential to use vitamin D supplements for the reduction of risk for vitamin D deficiency, as the sun exposure is lower than during other months under the conditions of the Czech Republic. Children are a sub-population particularly vulnerable to low vitamin D status, thus we consider very important to monitor vitamin D saturation across the country also in the future as a part of the Czech Human Biomonitoring System. Further monitoring will be needed to characterize of vitamin D status in the Czech population.

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### Conflict of Interests

None declared

### Adherence to Ethical Standards

The study protocol was approved by the Ethical Committee of the National Institute of Public Health. Written informed consent was obtained from all parents.

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