

Low Vitamin D blood levels with significant differences between the sexes in Viennese school children – the EDDY study

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ZUSAMMENFASSUNG

Hintergrund Die Bestimmung des Serumspiegels von 25 (OH)-Vitamin D wird für die Erfassung des Vitamin-D-Status einer Person empfohlen. Vitamin-D-Mangel ist weit verbreitet unter europäischen Jugendlichen. Die Bedeutung von Vitamin D für die Mineralisierung der Knochen und für die Risikoreduktion von Atemwegsinfekten ist gesichert.

Material und Methodik Im Rahmen der EDDY-Studie wurden 25(OH) Vitamin-D-Spiegel bei 138 Kindern (47,8% weiblich) im Alter von 11–13 Jahren in 4 verschiedenen Wiener Schulen bestimmt. Drei Blutabnahmen wurden zu verschiedenen Jahreszeiten durchgeführt. 25(OH)-Vitamin D wurde im Serum mittels CLIA (Chemiluminescent Immunoassay) bestimmt. Dieselben Vitamin-D-Kategorien wie in der HELENA-Studie wurden verwendet.

Ergebnisse Im September und Oktober 2013 hatten lediglich 36,9% der Kinder 25(OH)-Vitamin-D-Spiegel > 50 nmol/l (untere Grenze für eine adäquate Vitamin-D-Versorgung) und nur 9,4% wiesen Werte ≥ 75 nmol/l auf. Von den Kindern

mit unzureichender Versorgung wiesen 20,3% 25(OH)-Vitamin-D-Spiegel < 27,5 nmol/l auf. Es bestand ein signifikanter Unterschied in mittleren Vitamin-D-Spiegeln zwischen Mädchen und Jungen (♀ 40,9 ± 20,2 nmol/l versus ♂ 49,9 ± 22,1 nmol/l, p = 0,015), welcher sich auch in den Ergebnissen der zweiten und dritten Blutabnahme präsentierte. In den Wintermonaten hatten nur 0,9% der Kinder Vitamin-D-Werte > 75 nmol/l.

Schlussfolgerung Vitamin-D-Mangel scheint unter Wiener Jugendlichen im Alter zwischen 11 und 13 Jahren weit verbreitet zu sein. 9,4% der Kinder hatten im Herbst „optimale“ 25(OH)-Vitamin-D-Spiegel ≥ 75 nmol/l, in den Wintermonaten nur noch 0,9%.

ABSTRACT

Background The serum level of 25(OH) vitamin D is the best indicator for a person's vitamin D status. Vitamin D deficiency is widely spread among European adolescents. The importance of vitamin D in bone mineralization and in risk reduction of respiratory tract infections is indisputable. **Material and Methods** During the EDDY study 25(OH) vitamin D levels in 138 children (47.8% female) aged 11–13 years attending four Viennese schools were assessed. Three blood tests have been performed in different seasons. 25 (OH) vitamin D was determined in serum via CLIA (Chemiluminescent Immunoassay). The classification of vitamin D levels was the same that was used in the HELENA study.

Results In September and October 2013, 36.9% of all children had 25(OH) vitamin D levels > 50 nmol/l (lower limit for adequate vitamin D levels) and only 9.4% had levels ≥ 75 nmol/l. Of the children with inadequate vitamin D levels 20.3% had 25(OH) vitamin D levels < 27.5 nmol/l. We found a significant difference in mean 25(OH) vitamin D levels between females and males (♀ 40.9 ± 20.2 nmol/l versus ♂ 49.9 ± 22.1 nmol/l, p = 0.015), which persisted throughout the second and third blood tests. In the winter months, only 0.9% of all children achieved optimal levels > 75 nmol/l.

Conclusion Vitamin D deficiency seems to be very prevalent among Viennese adolescents aged 11 to 13 years. 9.4% of the tested Viennese children had “optimal” 25 (OH) vitamin D levels ≥ 75 nmol/l in autumn, in winter only 0.9%.

Introduction

Vitamin D has been a controversially discussed topic over the past few years. Serum level of 25(OH) vitamin D (25-hydroxy vitamin D) has been suggested as the best predictor for a person's vitamin D status as it not only represents nutritional intake but also cutaneous synthesis [1, 2].

Internationally standardized cut-off levels for adults and children have not yet been defined [3–5]. Each organization has its own recommendations regarding 25(OH) vitamin D cut-off levels. Since 2011, the Endocrine Society advocates vitamin D deficiency as levels below 50 nmol/l, insufficiency as levels between 50 and 75 nmol/l, and sufficiency as above 75 nmol/l [6]. The 2012 European HELENA study defined optimal levels as ≥ 75 nmol/l, insufficiency as 50–75 nmol/l, deficiency as 27.5–49.99 nmol/l, and severe deficiency as < 27.5 nmol/l [2]. The American Academy of Pediatrics recommends serum levels ≥ 50 nmol/l for infants and children [7]. 50 nmol/l as the cut-off level for deficiency is also approved by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition [8]. On the other hand, long-term levels above 125 nmol/l may result in adverse effects due to vitamin D toxicity [9].

Consequently, prevalence and incidence of vitamin D deficiency differ depending on the cut-off levels used. Further research is needed to establish universal cut-off levels for both optimal skeletal and non-skeletal health effects of vitamin D. However, studies have shown that vitamin D deficiency is widespread among European adolescents [2, 10, 11]. The HELENA study reported deficiency rates of 42% across nine European countries [2].

More and more health effects of vitamin D are being discovered. Skeletal effects of vitamin D in children have long been known. Most forms of rickets are caused by severe vitamin D deficiency. In adults, osteomalacia, osteopenia, osteoporosis and a higher risk of fracture can occur [4, 12]. Studies have found that elderly patients who received long-term calcium and vitamin D supplementation had a decreased risk of fractures [13, 14]. Additionally, vitamin D levels might affect the achievement of peak bone mass in adolescence and therefore influence the risk for osteoporosis in adulthood [5, 11]. Viljakainen et al. reported that vitamin D supplementation in adolescent girls increased their bone mineral content augmentation by 14–17% compared to a placebo group [5]. Mølgaard et al. also found that supplementation increased bone mineral density and bone mineral content, but only in girls with a specific genotype (FF VDR) [11].

Various non-skeletal effects of vitamin D have been discussed in the past decades. Vitamin D metabolites seem to act as hormones as receptors for $1\alpha,25(\text{OH})_2$ Vitamin D₃ have been found in many different types of tissue [15]. Martineau et al. reported that supplementation of vitamin D was effective in preventing respiratory tract infections [16].

This study investigated the prevalence of vitamin D deficiency in Viennese adolescents. Literature on vitamin D levels in healthy Viennese adolescents is rare. Our results differ from those reported previously in the HELENA study and suggest higher rates of vitamin D deficiency.

Patients and methodology

The EDDY study – ‘Effect of sports and diet trainings to prevent obesity and secondary diseases and to influence young children's lifestyle’ – is an interventional cohort study. The four Viennese schools which participated were randomized into intervention and control group, two schools in each group. The intervention group received dietary and sports trainings throughout the school year, which have been discussed in detail elsewhere [17]. There have been three blood tests for both groups. This paper will concentrate on the baseline results of 25(OH) vitamin D testing and repeated vitamin D measurements. At baseline, there have been 139 children, which participated in the blood test. In one child 25(OH) vitamin D could not be measured due to laboratory difficulties, so for vitamin D we have 138 values at baseline, 72 males and 66 females.

Baseline 25(OH) vitamin D was assessed in September and October 2013, T₁. The second blood drawing took place between December 2013 and April 2014, T₂. 58 males and 58 females, all in all 116 children participated. The third blood test was performed one year after baseline in September and October 2014, T₃, with 101 children, 52 boys and 49 girls.

All participants were apparently healthy school children aged 11–13 years and therefore representative of the Austrian and Viennese adolescent population.

Fasting blood samples were taken directly at the schools, centrifuged to obtain serum and brought to the laboratory within two hours. 25(OH) vitamin D has been determined in serum using a method called CLIA (Chemiluminescent Immunoassay). The device used was called Liaison XL by the company DiaSorin. This method has also been used in the German National Health Interview and Examination Survey for Children and Adolescents (KiGGS) [10].

The categories for vitamin D were grouped as follows: *optimal levels* ≥ 75 nmol/l, *insufficiency* 50–75 nmol/l, *deficiency* 27.5–49.99 nmol/l, and *severe deficiency* < 27.5 nmol/l. The same classification was used in the HELENA study [2].

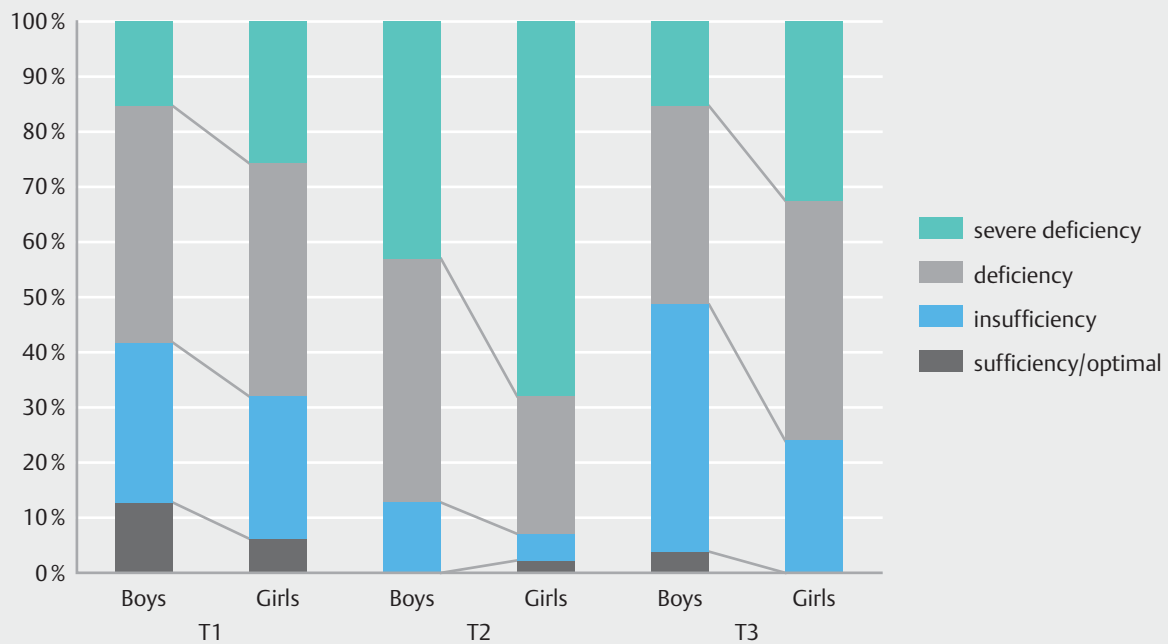
Descriptive statistics and further analyses have been performed with SPSS Version 21. Means, medians, 25th and 75th percentiles have been calculated. Furthermore, frequencies of the different vitamin D categories in girls and boys have been reported. To test significant differences in 25(OH) vitamin D levels in girls and boys Wilcoxon-Mann-Whitney tests have been performed. ■ p-Values < 0.05 were considered statistically significant.

Informed consents of all participants and their parents was obtained and the EDDY study was approved by the ethics committee of the City of Vienna (EK_13_070_0413).

Results

At baseline 138 children (47.8% female) aged 11–13 years participated. The mean BMI was 20 kg/m² with a mean weight of 49.7 kg and a mean height of 156 cm. 63% of all children had a *normal* BMI [17].

In autumn 2013 (T₁), 9.4% of the children had optimal levels of 25(OH) vitamin D, 27.5% were insufficient, 42.8% deficient



► **Figure 1** Comparison of sex distribution to 25(OH) vitamin D categories at T₁, T₂, T₃.

► **Table 1** Mean levels, standard deviations, medians, minimum, maximum, and percentiles of 25(OH) vitamin D in nmol/l.

	All			Female				Male			
	Mean (SD)	25 th /75 th perc.	Median	Mean (SD)	25 th /75 th perc.	Min./Max.	Median	Mean (SD)	25 th /75 th perc.	Min./Max.	Median
T ₁	45.6 (21.6)	27.5/60.0	42.4	40.9 (20.2)	25.0/54.9	10.0/92.4	39.9	49.9 (22.1)	33.1/67.4	12.5/97.3	44.9
T ₂	27.0 (15.3)	15.0/36.2	22.5	23.9 (15.1)	12.5/34.9	10.0/84.9	17.5	30.4 (14.9)	17.5/42.4	10.0/67.4	27.5
T ₃	42.2 (18.4)	28.7/57.4	39.9	37.7 (17.0)	22.5/51.2	10.0/74.9	37.4	47.0 (18.4)	30.0/59.9	10.0/104.8	49.9

and 20.3% exhibited severe deficiency. In the following winter 0.9% of the children had optimal levels, 8.5% were insufficient, 35.0% deficient, and 55.6% exhibited severe deficiency. The results for autumn 2014 were similar to those of autumn 2013. Detailed mean values, standard deviation, percentiles and medians of 25(OH) vitamin D can be found for all time points in ► **table 1**. Detailed distribution of all children and girls and boys separately to the four categories can be found in ► **table 2**.

There was a significant difference in mean 25(OH) vitamin D levels between girls and boys in T₁, T₂ and T₃ (T₁ p = 0.022; T₂ p = 0.004; T₃ p = 0.012). The difference in 25(OH) vitamin D levels dependent on the sex was consistent throughout the seasons. At T₁ the mean 25(OH) vitamin D level of the girls was 40.9 ± 20.2 and of the boys 49.9 ± 22.1 nmol/l. For T₂ the mean 25(OH) vitamin D levels of the girls was 23.9 ± 15.1 nmol/l, whereas the boys had a mean of 30.4 ± 14.9 nmol/l. For T₃ the mean level of the girls was 37.7 ± 17.0 nmol/l and of the boys

47.0 ± 18.4 nmol/l. The results from all three blood tests comparing girls and boys are provided in ► **figure 1**.

We also observed slight differences between the intervention and the control group – the control group constantly showed higher results in vitamin D levels, though not statistically significant at two out of three measurements.

Discussion

Our results suggest a higher prevalence of vitamin D deficiency than previously reported for Vienna in the HELENA study [2]. Notably was a consistent gender difference throughout T₁ to T₃ – girls had significantly lower 25(OH) vitamin D levels than boys.

Mean 25(OH) vitamin D level at baseline was 45.6 nmol/l (SD 21.6) and the median 42.4 nmol/l, which are much lower results than those obtained in the HELENA study, where they found the median to be 57.1 nmol/l for the whole study population and

► **Table 2** Distribution of 25(OH) vitamin D blood levels according to different categories at all three measurements (T₁, T₂, T₃).

		Sufficiency/optimal levels	Insufficiency	Deficiency	Severe deficiency	Total
T ₁	Total % (n)	9.4 (13)	27.5 (38)	42.8 (59)	20.3 (28)	100 (138)
	Female % (n)	6.1 (4)	25.8 (17)	42.4 (28)	25.8 (17)	100 (66)
	Male % (n)	12.5 (9)	29.2 (21)	43.1 (31)	15.3 (11)	100 (72)
T ₂	Total % (n)	0.9 (1)	8.5 (10)	35.0 (41)	55.6 (65)	100 (117)
	Female % (n)	1.7 (1)	5.2 (3)	25.9 (15)	67.2 (39)	100 (58)
	Male % (n)	0 (0)	12.1 (7)	44.8 (26)	43.1 (25)	100 (58)
T ₃	Total % (n)	2.0 (2)	34.3 (35)	40.2 (41)	23.5 (24)	100 (102)
	Female % (n)	0 (0)	24.5 (12)	44.9 (22)	30.6 (15)	100 (49)
	Male % (n)	3.8 (2)	44.2 (23)	36.5 (19)	15.4 (8)	100 (52)

the mean value for Viennese adolescents to be 63.7 nmol/l (SD 31.7) [2]. The HELENA study collected the Viennese blood samples in November (34%), February (17%), March (13%), and May (36%). Thus, they reported on mean values of different seasons. This might explain the difference in mean 25(OH) vitamin D levels, but not the discrepancy comparing both sexes as described in the following paragraph.

Interestingly the HELENA study group reported slightly higher mean levels of vitamin D in girls than in boys, but the results of the EDDY study suggest the opposite. Specifically, for Vienna the HELENA study indicated mean values for females at 67.2 nmol/l (SD 31.9) and for males at 58.2 nmol/l (SD 31.0) [2]. In all three blood tests the girls in our cohort had significantly lower 25(OH) vitamin D levels than the boys. In another study, Kumar et al. also came to the conclusion that girls were more likely to be vitamin D deficient (OR: 1.9 [1.6 to 2.4]) [18].

At baseline 9.4% of the children had *optimal levels* of 25(OH) vitamin D, 27.5% were *insufficient*, 42.8% *deficient*, and 20.3% exhibited *severe deficiency*. 90.6% of the whole population of children did not achieve optimal vitamin D levels, whereas the HELENA study found 80% to have suboptimal levels [2]. Hintz-peter et al. analyzed 25(OH) vitamin D concentrations of 10 015 German children and adolescents aged 3–17 years and found that more than 90% of immigrant girls and boys had suboptimal levels <75 nmol/l compared to 87% of nonimmigrants. They also reported that 29% of immigrant boys and 31% of immigrant girls compared with 18% of nonimmigrant boys and 17% of nonimmigrant girls to have 25(OH) levels <25 nmol/l [10].

During the winter months of the second blood drawing, 25 (OH) vitamin D levels were even lower. Only one out of 117 children had optimal levels ≥75 nmol/l. 55.6% had a severe deficiency according to the classification used.

One year after baseline we came to similar results as previously at baseline, possibly due to effects of the seasons on vitamin D. The average vitamin D levels in September and October 2014 were just slightly lower than those in 2013, see

► **table 2**. However, only 2 of 102 children had sufficient levels compared to 13 of 138 at baseline.

The differences between intervention and control group might be due to socioeconomic differences between those two groups, as both schools in the control group were so called *Gymnasien*, whereas the intervention group consisted of another type of school *Mittelschule*. Socioeconomic differences between those types of schools have been described in the 2015 national report on education for Austria [19, 20]. An association between higher prevalence of vitamin D deficiency and immigrant background has already been reported by Hintz-peter et al. in German children and adolescents [10].

The typical Austrian diet is not very rich in vitamin D, as Austrians in general do not tend to eat a lot of fish [21]. In addition, few foods are fortified with vitamin D [4]. Therefore, the population is highly dependent on vitamin D synthesis in the skin, which is hardly available during winter in Austria. According to Godar et al., the major source of vitamin D is synthesis in the skin, which is why deficiencies are frequent in winter [22]. In a study by Koenig et al., 68% of the tested Austrian population could not meet the recommendations for dietary intake of vitamin D [21]. Also covering up the skin for religious purposes or sun protective measure, e.g. sunscreen, decreases cutaneous synthesis of vitamin D [12].

Until today, there is no consensus on optimal serum levels of 25(OH) vitamin D and there is lack of evidence that levels >75 nmol/l are beneficial compared to levels >50 nmol/l. However, this study used >75 nmol/l as cut-off for optimal 25(OH) vitamin D serum levels for better comparability as the same cut-offs have been used in the HELENA study [2].

The main limitation of our study is lack of information regarding the application of sunscreens and the children's skin types. Vitamin D supplementation and immigrant background have not been evaluated either. The drop-out rate from 138 at T₁ to 101 children at T₃ has to be acknowledged, too.

Conclusion

Our results suggest that vitamin D deficiency is widely spread among Austrian adolescents and should be a matter of public health concern. Especially female adolescents seem to be at higher risk of vitamin D deficiency. Health benefits of vitamin D supplementation should be considered. In addition, the work on international, or at least European-wide, cut-off levels for 25(OH) vitamin D should be continued.

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Conflict of interest

The authors declare that they have no conflict of interest.

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