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High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes

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Summary

Background: Vitamin D is important for bone health. An inadequate supply of vitamin D to the body is associated with a higher fracture risk in the elderly. Young adults with type 1 diabetes are reported to have a lower peak bone mass than healthy individuals, which could possibly lead to an increased fracture risk in the future. The prevalence of vitamin D deficiency in healthy young people is high. Thus, optimal supply of vitamin D may be of particular importance for bone health in children with type 1 diabetes.

Methods: In this prospective cross-sectional study we measured serum 25-hydroxy-vitamin D, iPTH, total and ionised calcium, phosphate, and alkaline phosphatase in 129 Swiss children and adolescents with type 1 diabetes.

Results: Of the 129 subjects 78 (60.5%) were vitamin D deficient, defined as a 25-hydroxy-vitamin-D level below 50 nmol/L. During the winter this number rose to 84.1%. 25-hydroxy-vitamin-D levels showed marked seasonal fluctuations, whereas there was no correlation with diabetes control. Despite the high prevalence of vitamin D deficiency, we found a low prevalence of secondary hyperparathyroidism in vitamin D deficient diabetic children and adolescents.

Conclusions: Prevalence of vitamin D deficiency in diabetic children and adolescents is high. Therefore, screening for vitamin D deficiency and supplementation in children with low vitamin D levels may be considered.

Key words: *vitamin D deficiency; type 1 diabetes, parathyroid hormone; 25-OH-cholecalciferol*

Introduction

Vitamin D has a major impact on bone health. Severe vitamin D deficiency is associated with rickets in the grow-

ing skeleton [1] and osteomalacia in adults [2]. In addition, low serum vitamin D levels are associated with a higher fracture risk in elderly people [3]. Furthermore, vitamin D deficiency is common in healthy children at a variety of different latitudes [4–18] and in children with type 1 diabetes mellitus (T1D), in whom the highly variable prevalence ranges between 15–65% at the end of winter [19–21].

Body vitamin D stores in an individual are assessed by measuring serum 25-hydroxy-vitamin D (25D) levels. However, there is no consensus on the 25D levels that are to be considered sufficient in children. The American Academy of Pediatrics (AAP) has defined vitamin deficiency as serum 25D below 27.5 nmol/L [22]. In infants and children, however, rickets is already seen at 25D levels below 37.5 nmol/L [8, 23]. In adolescents 25D levels below 40 nmol/L are associated with unphysiologically high intact parathyroid hormone (iPTH) levels and low mean forearm bone mineral density [17]. In adults the use of biomarkers such as serum PTH concentration or intestinal calcium absorption rate has recently been recommended to define biologically relevant circulating 25D levels [1]. This recommendation is based on the observation that both the intestinal calcium absorption rate increases significantly with rising 25D concentrations until a concentration of 75 nmol/L is reached and PTH levels also start to increase even at 25D concentrations below 75 nmol/L [24, 25]. Furthermore, 25D concentrations above 75 nmol/L appear to be important for optimal bone health, prevention of colorectal cancer and other health issues [1, 26, 27]. Hence, as stated, a 25D cut-off value of above 75 nmol/L for vitamin D sufficiency and a 25D cut-off value below 50 nmol/L for vitamin D deficiency were adopted in our study [1, 20, 25].

Clinical studies show that adolescents with T1D have a lower bone mass compared to their peers [28]. Similarly, adults with T1D are found to be at higher risk of osteoporosis and at higher risk of osteoporosis-related fractures [28].

In addition, some studies suggest a blunted response of the PTH to low levels of vitamin D in diabetic patients further deregulating calcium homeostasis [29–31]. Therefore, children with both conditions, T1D and vitamin D deficiency, may have an increased risk of bone fragility. Inad-

equate levels of PTH for a given low serum calcium value could be an aggravating factor in this setting.

The purpose of this study was to assess the prevalence of vitamin D deficiency in patients with T1D and to define factors such as duration of diabetes, HbA_{1c}, age, body mass index and seasonality which may possibly influence serum vitamin D levels. Additionally, we determined serum iPTH concentrations to assess secondary hyperparathyroidism in patients with vitamin D deficiency. For this purpose we conducted a prospective, cross-sectional study in children and adolescents with T1D seen at our outpatient clinic in Bern, Switzerland.

Subjects and methods

Study subjects

This study was approved by the institutional review board of the Inselspital at the University of Bern. The participants in this cross-sectional study were enrolled at the time of their regularly scheduled annual visit to our outpatient clinic. At study start, 130 patients with T1D previously diagnosed according to the diagnostic criteria of the American Diabetes Association [32] were examined at our outpatient clinic. All 130 patients agreed to participate and were thus included in the study. One patient was excluded from further analysis because of hyperparathyroidism (215 pg/ml) not explained by low vitamin D levels. We categorised each participant's study visit according to seasons as follows: Winter (22 December–21 March), spring (22 March–21 June), summer (22 June–22 September) and autumn (23 September–21 December). 44 participants were examined in winter, 20 in spring, 34 in summer and 31 in autumn.

A nutritional questionnaire enquiring into daily consumption of dairy products and calcium-rich mineral water was used to assess daily calcium intake (see appendix for details). All participants underwent a general physical examination. Standard auxological assessment was performed [33]. Measurements of weight were obtained to the nearest 0.1 kg using a Seca scale (Seca GmbH, Hamburg, Germany) and measurements of height were obtained to the nearest 0.1 cm using an Ulmer Stadiometer (Busse design, Ulm, Germany). Body mass index (BMI) was calculated as weight (kilograms) divided by height squared (square meters) [34].

Eight patients were excluded from evaluation of the PTH – vitamin D axis because measurements of ionised calcium concentration were not available for technical reasons.

Laboratory findings

Serum 25D was determined using a competitive chemiluminescence immunoassay (DPC Immulite 2500, Liaison, DiaSorin Inc., Minneapolis, USA). The between run CV was 8.4%. 25D levels below 50 nmol/L were defined as vitamin D deficient [1], 25D levels between 50 and 75 nmol/L were defined as vitamin D insufficient and 25D levels above 75 nmol/L were defined as vitamin D sufficient. Serum intact PTH (iPTH) was determined using an electro-chemiluminescent immune assay (Roche Modular E170, Roche Diagnostics Corp., Indianapolis, USA).

The reference values for iPTH were 9–59 pg/ml (6–9.9 years), 11–74 pg/ml (10–13.9 years), 9–69 pg/ml (14–17.9 years) and 10–65 pg/ml (adults). Serum ionised calcium was measured at a pH of 7.40 and 37 °C using an ion-selective electrode (Radiometer ABL800, Radiometer GmbH, Thalwil, Switzerland). Serum alkaline phosphatase was determined by a standard colorimetric assay on a Roche Modular P800 (Roche Diagnostics Corp.). HbA_{1c} was assessed using a latex immunoagglutination method standardised to the DCCT assay with a reference range of 4.5–5.6% (DCA 2000+, Siemens Co. Dublin, Ireland).

Statistical analysis

Results are expressed as mean ± 95% confidence interval (95% CI) unless otherwise stated. Statistical analysis was performed using the SPSS 17 (www.spss.com) statistics program. For detailed analysis, participants were divided into three groups according to their 25-OHD levels: >75 nmol/L: vitamin D sufficient, 50–75 nmol/L: vitamin D insufficient, <50 nmol/L: vitamin D deficient. Differences between groups were analysed by one-way ANOVA. Cross tabulation analysis with χ^2 – test was performed for analysis of frequencies and the results plotted in a 3x4 contingency table. Adjusted residuals were calculated to show which cell was over- or underrepresented.

Results

We assessed clinical and laboratory findings in 129 children and adolescents with T1D. None of the participants had bone deformities or a history of low intensity trauma fractures. 69 participants (53.5%) were males (60 (46.5%) were females. Most of the participants were white (123; 95.3%), the remaining being Indian (4; 3.1%), East-Asian (1; 0.8%) and black (1; 0.8%). The characteristics of the whole group and those of the three subgroups related to vitamin D levels are shown in table 1. No statistically significant differences in patients' characteristics between the

Figure 1

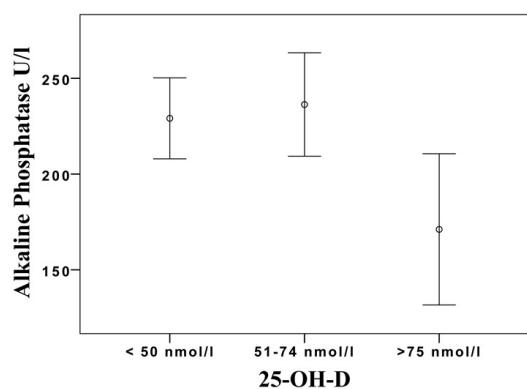


Figure 1

Alkaline phosphatase (mean±95% confidence intervals) according to 25-OH-D levels
One-way-ANOVA showed significant difference between the means ($p = 0.03$). The graph shows that alkaline phosphatase was significantly lower in participants with 25-OH-D levels above 75 nmol/L.

three subgroups were found by one-way ANOVA. 87% (n: 112) of the patients with T1D had 25D levels below 75 nmol/L, whereas 60.5% (n:78) were vitamin D deficient with 25D levels below 50 nmol/L. Calcium intake and the results of calcium and phosphate metabolism are shown in table 2. The only variable showing statistically significant differences between the three subgroups as tested by one-way-ANOVA was alkaline phosphatase ($F = 3.592$, $p = 0.03$). A plot for means with 95% confidence shows that alkaline phosphatase in both vitamin D deficient and vitamin D insufficient groups was significantly higher than in the vitamin D sufficient (fig. 1). It is important to stress that neither calcium intake, age, duration of diabetes, HbA1c nor serum magnesium concentration did explain any of the variance of serum 25D levels.

Cross tabulation revealed a highly significant effect of season on the levels of 25D ($\chi^2 = 38.9$, $p < 0.001$). 98% of patients seen during the winter period, 90% of patients seen during spring, and 90% of patients seen in the autumn presented with 25D levels below 75 nmol/L (table 3). By contrast, only 68% of patients seen in the summer presented with 25D levels below 75 nmol/L. Analysis of adjusted residuals showed that during winter and spring there were relatively more vitamin D deficient patients than in the yearly average, whereas in autumn the number of vit-

amin D deficient, insufficient and sufficient patients corresponded approximately to the yearly average. Furthermore, only during the months of July, August and September did more than 75% of the patients show 25D levels of above 50 nmol/L (fig. 2).

We found that iPTH was similar in all three subgroups (table 2). Three (3.8%) patients with vitamin D levels <50 nmol/l had hyperparathyroidism defined as iPTH above +2 SD for age. Eight patients (26%) with very low 25D levels (<25 nmol/L) were found to have iPTH levels above 50 pg/ml but only two were elevated above 2 SD for age qualifying for a diagnosis of secondary hyperparathyroidism.

Discussion

Our participants with T1D were found to have a high prevalence of vitamin D deficiency. Vitamin D levels showed a marked seasonal fluctuation. Additionally, vitamin D deficient children and adolescents with T1D have a low prevalence of secondary hyperparathyroidism.

It is of importance to state that the sample size is representative for a number of children and adolescents suffering from T1D in the area: the Canton of Bern has a population of 962 982. Considering the registered incidence of T1D in children and adolescents of 15/100 000 per year in

Table 1
Patient characteristics.

	Total (n = 129)		Vitamin D sufficient (n = 17) 25-OH-D >75 nmol/L		Vitamin D insufficient (n = 34) 25-OH-D 50–75 nmol/L		Vitamin D deficient (n = 78) 25-OH-D <50 nmol/L	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Age, years	11.6	11.0–12.3	12.5	10.5–14.5	10.9	9.6–12.2	11.7	10.8–12.6
Weight-SDS	0.13	-0.02–0.28	-0.04	-0.6–0.53	0.23	-0.02–0.48	0.12	-0.07–0.32
Height-SDS	0.06	-0.11–0.22	-0.10	-0.57–0.38	-0.02	-0.34–0.34	0.12	-0.1–0.33
BMI-SDS	0.14	0.0–0.29	-0.02	-0.47–0.43	0.31	0.06–0.56	0.11	-0.09–0.3
Duration of diabetes, years	4.9	4.3–5.5	5.8	4.0–7.7	4.0	3.0–4.9	5.1	4.3–6.6
HbA _{1c} %	8.1	7.9–8.4	8.1	7.7–8.6	7.9	7.7–8.2	8.2	7.9–8.6

Table 2
Results.

	Total (n = 129)		Vitamin D sufficient (n = 17) 25-OH-D >75 nmol/L		Vitamin D insufficient (n = 34) 25-OH-D 50–75 nmol/L		Vitamin D deficient (n = 78) 25-OH-D <50 nmol/L	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Calcium intake, mg/d ¹	845.3	760.6–930.0	822.4	672.6–972.1	920.9	738.1–1103.6	818.0	704.3–931.7
Calcium, mmol/L	2.36	2.34–2.37	2.35	2.31–2.38	2.37	2.34–2.4	2.35	2.34–2.37
Ca ²⁺ , mmol/L	1.22	1.22–1.23	1.22	1.21–1.23	1.24	1.22–1.25	1.22	1.21–1.23
Phosphate, mmol/L	1.41	1.38–1.44	1.41	1.34–1.49	1.38	1.31–1.44	1.43	1.38–1.47
Magnesium, mmol/L	0.81	0.80–0.82	0.80	0.78–0.83	0.81	0.79–0.82	0.82	0.80–0.83
Alk. Phosphatase U/l	223	208–239	171	132–211	236	209–263	230	208–250
iPTH, pg/ml ²	35.8	33.3–38.3	34.8	29.2–40.5	31.4	28.3–34.4	38.0	34.3–41.6
25-OH-D, nmol/L	45.7	41.2–50.3	92.5	83.4–101.5	61.3	58.6–63.9	28.8	26.0–31.6

¹ Calcium intake according to food frequency questionnaire

² only iPTH results of 121 participants were available (vitamin D sufficient n = 16, vitamin D insufficient n = 32, vitamin D deficient n = 73).

Table 3
Frequency of Vitamin D deficiency and insufficiency by season.

		Winter	Spring	Summer	Autumn	Total
Vitamin D deficient (25-OH-D >75 nmol/L)	N (%)	37 (84)	16 (80)	7 (21)	18 (58)	78 (61)
	adjusted residual	3.9	1.9	-5.5	-0.3	
Vitamin D insufficient (25-OH-D 50–75 nmol/L)	N (%)	6 (14)	2 (10)	16 (47)	10 (32)	34 (26)
	adjusted residual	-2.4	-1.8	3.2	0.9	
Vitamin D sufficient (25-OH-D <50 nmol/L)	N (%)	1 (2)	2 (10)	11 (32)	3 (10)	17 (13)
	adjusted residual	-2.6	-0.5	3.9	-0.7	
Chi-square-test: $\chi^2 = 38.9$ (6 df), $p < 0.001$						

2008 (data of the ongoing incidence study in Switzerland, personal communication: Prof. Eugen J. Schoenle, Zürich) we estimated a total of about 280 children between the ages of 0–16 years with T1D living in the Canton of Bern. We could therefore conclude that approximately 50% of the children with T1D living in the Canton of Bern participated in this study.

A most important finding is that the prevalence of vitamin D deficiency was higher in our cohort (60.5%) than in previous studies analysing subjects with T1D (15–43%) using the same cut-off values to define serum vitamin D status. The prevalence of vitamin D deficiency was 43% in an Australian study [19], about 25% in an Italian study [21] and 15% in a recent study in US East Coast youth [20]. However, when focusing on the prevalence of vitamin D sufficiency, the data were similar: Bern: 13%; Australia: 19%; US East coast: 25%. Thus the main difference is in the range of insufficiency (26–36%) [19–21]. These overall differences might be explained by the variability of geographical environment, the age of the subjects, duration of diabetes (diagnosis vs. follow-up), glycaemic control etc. as recently suggested [20]. In contrast, 25D levels were usually above 50 nmol/L in young adults at diagnosis of T1D in Sweden [35].

It is not surprising to find a marked seasonal variability of 25D levels since UV light exposure is essential for vitamin D biosynthesis in the skin [36]. Only during the months of July, August and September were 25D levels sufficient for most patients. This is in line with the Swedish study showing similar month-to-month fluctuations of the 25D levels although at a higher concentration [35]. In T1D children of the US East Coast vitamin D deficiency was found in 47% during winter and in only 5% during summer [20]. One might argue that the much higher prevalence of vitamin D deficiency found even during the summer in our study is due to absence of vitamin D supplementation in

many Swiss foods compared to other countries such as the US. Also, outdoor activities may play a key role when comparing other studies with Swiss data.

An additional finding was that the prevalence of secondary hyperparathyroidism was very low in vitamin D deficient patients. In contrast, PTH levels never fell below 20 pg/ml in our patients, which is clearly above the level of 10 pg/ml expected for the continuous tonic secretion of PTH [12, 37], which suggests that the continuous tonic PTH secretion might be elevated in diabetic subjects. In adults, PTH levels are expected to rise steeply above 40 pg/ml at 25D levels below 50 nmol/L and above 50 pg/ml at 25 D levels below 25 nmol/L [27]. This relationship seems to be less pronounced in healthy adolescents [12]. We have two possible explanations why PTH levels did not show the expected rise in diabetic children and adolescents. First, the PTH-vitamin D axis has a blunted response in diabetic patients, a finding supported by several studies [29, 31]. In one study a blunted response of PTH was associated with low magnesium levels and corrected after magnesium repletion [30]. Our patients, however, did not show any magnesium depletion. A blunted response of PTH would result in inadequately normal PTH levels with low ionised calcium. However, we did not find differences in ionised calcium levels between vitamin D deficient and vitamin D sufficient patients. Furthermore, the participants in our study had a mean calcium intake of 845 mg/day, close to the recommended daily intake for age of 1200 mg/day [38]. Since it is known that the clinical signs of vitamin D deficiency (rickets, hyperparathyroidism) are highly dependent on calcium intake [39], one can speculate that in our patients lacking PTH rise even in severely vitamin D insufficient individuals was due to near optimal oral calcium intake. However, no calcium balance studies were performed to investigate this hypothesis any further. In addition, increased tonic PTH secretion could have a protective effect on serum calcium concentrations. Second, hypocalcaemia was too mild to induce a rise in PTH in our patients. Physiologically the set-point in the inverse sigmoidal relationship between ionised calcium and PTH is around 1.22 mmol/L and PTH secretion reaches a plateau at ionised calcium concentrations below 1.15 mmol/L [37]. The concentration of ionised calcium in our patients, however, was mostly above 1.2 mmol/L and only three patients had values below 1.15 mmol/L. Hence, in our cohort we have not enough data to define both a top and bottom plateau of the sigmoidal relationship between iPTH and ionised calcium for a detailed description in comparison to a normal curve.

A clear limitation of our study is that no control group of non-diabetic children was included. However, similar studies in healthy children showed a prevalence of vitamin D deficiency in the same range (4–7, 10–12, 15–17, 40, 41). In the only existing Swiss study 15% of the participants aged between 11–16 years (29% if only adolescents of Tanner stage 4 and 5 were considered) had 25-OH levels below 30 nmol/L compared to 36% in our study [42]. Furthermore, a recent study in healthy German children and adolescents (who are in terms of customs and genetic background the closest to our study population) showed similar results, with 63% of the participants having 25-OH-D levels below 50 nmol/L [41]. Another limitation of our

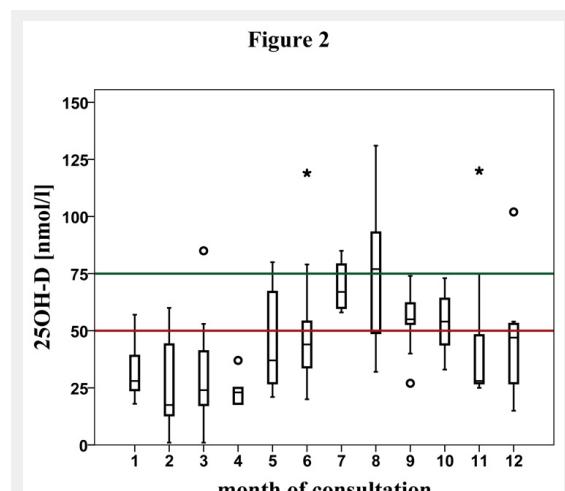


Figure 2

Boxplot of 25-OH-D by month of consultation
Circles indicate outliers (one and a half to three box-lengths outside the box), asterisks indicate extreme values (more than three box-lengths outside the box). The top line indicates the cut-off for vitamin D insufficiency (75 nmol/L), the bottom line indicates the cut-off for vitamin D deficiency (50 nmol/L). January corresponds to month 1, December to month 12.

study is that the effective time of sunshine exposure was not assessed for the individual patient.

In summary, we show that vitamin D deficiency is highly prevalent in young people with T1D attending our outpatient clinic, and that there is a marked seasonal fluctuation of serum 25D levels. Additionally, we found a low prevalence of secondary hyperparathyroidism in vitamin D deficient subjects. Knowing that vitamin D deficiency affects bone health, these findings raise the question whether vitamin D deficiency should be screened for and supplementation recommended in children with low levels. Since 25-OH-D levels above 37.5 nmol/L are necessary to prevent nutritional rickets, we suggest those children be treated according to AAP recommendations regardless of presence or absence of clinical symptoms and signs [43]. To prevent bone disease in adulthood, vitamin D supplementation may be considered even for children and adolescents with 25-OH-D levels between 37.5–75 nmol/L. Further studies are needed to solve these questions and to identify mechanisms responsible for the low prevalence of secondary hyperparathyroidism in T1D subjects with low vitamin D levels.

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Funding / potential competing interests

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Appendix

Calcium intake was assessed by asking the participants how many units of various dairy products they usually took during a week and by calculating the corresponding calcium intake by day. The sample units of dairy products were shown to the participants on a visual scale. Calcium intake through mineral water or calcium supplements was calculated separately and the result added to the intake resulting from dairy products. We based our calculations of calcium content of dairy products and mineral water on the following assumptions:

Product	Unit	Calcium content
Milk	1 dl	120 mg
Joghurt	150 g	215 mg
Cottage cheese	200 g	180 mg
Soft cheese	40 g	20 mg
Medium-hard cheese	40 g	28 mg
Hard cheese	40 g	48 mg
High Ca mineral water (i.e. Valser)	1 dl	55 mg
Medium Ca mineral water (i.e. Aproz)	1 dl	30 mg
Low Ca mineral water (i.e. Evian)	1 dl	10 mg