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REGULAR ARTICLE

Vitamin D deficiency is associated with prediabetes in obese Swedish children

Kerstin Ekbom (kerstin.ekbom@karolinska.se), Claude Marcus

Division of Pediatrics, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

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Correspondence
Kerstin Ekbom, Karolinska University Hospital Huddinge, B62, SE-141 86 Stockholm, Sweden.
Tel: +46 8 585 87 357 |
Fax: +46 8 585 873 70 |
Email: kerstin.ekbom@karolinska.se

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ABSTRACT

Aim: Low vitamin D levels have been associated with obesity and living in areas that lack sunshine, such as northern Europe. The aim of this study was to investigate the vitamin D status of a group of obese children in Sweden and to investigate the associations between vitamin D status and markers of glucose metabolism and metabolic risk markers.

Methods: This was a prospective cross-sectional study of 202 obese children between 4.5 and 17.9 years of age who had been referred to the National Childhood Obesity Centre at Karolinska University Hospital, Stockholm. We examined age, gender, 25-hydroxyvitamin D (25(OH)D), f-glucose, f-insulin and metabolic risk markers. Vitamin D deficiency was defined as less than 30 25(OH)D nmol/L. Children with and without a vitamin D deficiency were compared.

Results: Just over a third (33.2%) of our study population had vitamin D levels <30 nmol/L 25(OH)D. A significant interaction effect was found between age and 25(OH)D. An association was also found between low 25(OH)D levels and impaired fasting glycaemia (IFG) independent of age and season.

Conclusion: Low vitamin D levels were common among obese adolescents living in Sweden and were strongly associated with age and associated with a higher risk of IFG.

INTRODUCTION

The biological effect of vitamin D is traditionally related to bone health, but it may also have important and more general effects when it comes to regulating metabolism and inflammation (1). It has also been questioned whether low 25-hydroxyvitamin D (25(OH)D) is the cause of various diseases or merely a result (2). In adults, inadequate levels of 25(OH)D are considered to be independently associated with increased risks of cancer, cardiovascular disease, impaired glucose tolerance and metabolic syndrome (1). Vitamin D deficiency during childhood and adolescence has been associated with infections and respiratory and autoimmune diseases and with an increased risk of developing type 1 diabetes, multiple sclerosis and Crohn's disease later in life (3).

It has been well established that obesity in humans leads to lower serum levels of 25(OH)D, the main circulating vitamin D metabolite, which is commonly used as the marker of vitamin D status (4). It has been suggested that levels are low, owing to increased accumulation in the adipose tissue, and that these low levels of 25(OH)D do not reflect a true vitamin D deficiency (5). But it has also been suggested that the metabolism of vitamin D is disturbed in obese subjects (6). Increased fat mass has been associated with increased bone size, but reduced volumetric density in children with obesity. These observations, combined with a positive relationship between obesity and fracture risks, may indicate that vitamin D deficiency is definitely associated with obesity (7).

The link between obesity and cardiometabolic diseases has been well established, as has the link between obesity and vitamin D deficiency, but the significance of vitamin D as a link between obesity and cardiometabolic disorders is unclear (4). In obese adults, low 25(OH)D levels have been shown to be associated with inflammatory and car-

Abbreviations
25(OH)D, 25-Hydroxyvitamin D; BMI SDS, BMI standard deviation scores; HOMA, Homeostasis model assessment; IFG, Impaired fasting glycaemia.

Key notes

- Low vitamin D levels have been associated with obesity and living in areas that lack sunshine, such as northern Europe.
- Our study of 202 obese children between 4.5 and 17.9 years of age who had been referred to a national obesity centre found that 33.2% had low vitamin D levels
- Low vitamin D levels were also strongly associated with age and associated with a higher risk of impaired fasting glycaemia.

diometabolic risk markers (8). In children, it has been questioned whether there is an association between vitamin D status and cardiometabolic risk (9) and the results of a mini-review were inconclusive (10). Lower levels of 25 (OH)D have been associated with risk factors for type 2 diabetes in obese children in some observational studies, but not in others (10). Intervention studies have also yielded conflicting results (11).

The major sources of vitamin D are endogenous synthesis in the skin after sun exposure and food intake (1). However, in Scandinavia, as well as in other regions close to the earth's poles, the endogenous synthesis of vitamin D is limited from October to April, increasing the risk of developing a low vitamin D status (1).

We hypothesised that the combination of obesity and low sunshine exposure during winter in Sweden may have severely affected the vitamin D status of obese children and adolescents. Therefore, the aim of this study was to investigate vitamin D status in a Swedish population of obese children and to determine whether there was an association between vitamin D status and markers of glucose metabolism in patients referred to a childhood obesity centre.

MATERIAL AND METHODS

In this prospective cross-sectional study, children newly referred to the National Childhood Obesity Centre at Karolinska University Hospital in Stockholm were consecutively included. Data were collected between January 2011 and January 2014, and a total of 202 obese children between 4.5 and 17.9 years of age were studied. Obesity was defined by sex-specific and age-specific cut-off points for children. The exclusion criteria were children who were younger than four, older than 18 and those who had a chronic disease other than obesity.

This study was approved by the Central Ethical Review Board, Sweden (Dnr O 23-2013).

Procedure

Each child underwent a physical examination by a physician at the registration visit. Blood samples were obtained after an overnight fast. Height was measured to the nearest 0.1 cm with a Ulmer stadiometer (Busse Design and Engineering, Elchingen, Germany). Body weight was measured to the nearest 0.1 kg using the Vetek TI-1200S OIML (Vetek, Vaddo, Sweden), and body mass index (BMI) was calculated by dividing weight by height in metres squared (kg/m^2). The subjects were weighed in light clothing and without shoes.

Outcome measurements

Data were stored in, and later extracted from, the National Health Care Quality Register for Childhood Obesity in Sweden (BORIS), supervised by the National Board of Health and Welfare. The database includes data from each patient's first visit to the National Childhood Obesity Centre at Karolinska University Hospital, Stockholm.

For this study, fasting insulin, fasting glucose, glycosylated haemoglobin, cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, aspartate aminotransferase, alanine aminotransferase, apolipoprotein A-I, apolipoprotein B, gamma-glutamyltransferase and 25(OH)D were extracted. Complementary data were extracted from the medical files. Routine laboratory analyses were performed by Karolinska University Laboratory.

Analyses of 25(OH)D were performed in serum with a Liaison chemiluminescence immunoassay (DiaSorin, Dietzenbach, Germany), coefficient of variation (CV), 8–11% and reference range (range) 75–250 nmol/L. S-insulin was analysed with a method based on Modular E electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA), CV 5% and range 2–25 mIE/L. P-glucose was analysed with a DXC800 Beckman enzymatic potentiometric reaction (Beckman Coulter, Brea, CA, USA), CV 4% and range 4–6 mmol/L. Glycosylated haemoglobin was analysed by photometric chromatography using the VARIANT II Hemoglobin Testing System (Bio-Rad, Stockholm, Sweden), CV 3% and range 27–42 mmol/mol. Plasma lipids were analysed with an enzymatic reaction by photometry with the DXC800 Beckman (Beckman Coulter), cholesterol, CV 4% and range <5.2 mmol/L, triglyceride, CV 6% and range <1.8 mmol/L, high-density lipoprotein cholesterol, CV 5% and range female 1.0–2.7/male 0.8–2.1 mmol/L. Low-density lipoprotein cholesterol was calculated (low-density lipoprotein cholesterol = cholesterol − high-density lipoprotein cholesterol − (0.45 × triglyceride). Turbidimetric immunoassay DXC800 Beckman (Beckman Coulter) was used for analysing apolipoprotein A-I, CV 8% and range female 1.1–2.1/male 1.1–1.8 g/L, apolipoprotein B, CV 8% and range 0.5–1.5 g/L, high-sensitivity C-reactive protein, CV 7% and range <3 mg/L. Enzymatic activity by photometry DXC800 Beckman (Beckman Coulter) was used for analysing aspartate aminotransferase, CV 6% and range <0.70 microkat/L, alanine aminotransferase, CV 5–10% and range female <0.75/male < 1.1 microkat/L and gamma-glutamyltransferase, CV 2.5–10% and range <0.76 microkat/L. The methods were not changed during the time period when our data were collected and analysed.

Both a family history of diabetes and the presence of acanthosis nigricans were documented, as a clinical sign of insulin resistance.

The homeostasis model assessment (HOMA) was calculated to obtain a measure of insulin sensitivity: (fasting glucose × fasting insulin)/22.5. Impaired fasting glucose (IFG), defined according to the American Diabetes Association definition as a fasting glucose ≥ 5.6 mmol/L, was used as a marker for disturbed glucose metabolism (12), and insulin resistance was defined as a HOMA IR of >2.1 predefined as insulin resistance (13). In this study, we used the definition from the US Institute of Medicine (IOM) for adequate levels: deficiency <30 nmol/L 25(OH)D, insufficiency 30–50 nmol/L 25(OH)D and adequate >50 nmol/L 25(OH)D. These were based on the facts that rickets and osteomalacia are expected to occur when

25(OH)D is below recommended levels (14). Each individual child was categorised as either vitamin D deficient <30 nmol/L 25(OH)D or vitamin D nondeficient >30 nmol/L 25(OH)D.

The patients were divided into three age groups: 4 < 9.9, 10–13.9 and > 14–17.9 years. BMI standard deviation scores (BMI SDS) (15) were used in the statistical analyses to investigate how the level of obesity was associated with vitamin D and other health markers. The study period was divided into two seasons, winter and summer, based on dates of blood sampling. Children referred and examined during the period October to April were categorised as winter, and children referred and examined during the period May to September were categorised as summer.

Statistics

Descriptive statistics were expressed in means, SDs, numbers and percentages. Independent *t*-tests and ANOVA were used to evaluate the difference in the mean of 25(OH)D, glucose and risk markers. Frequencies were presented in percentages and cases. A chi-square test was used to compare the prevalence of vitamin D deficiency between groups. General linear models were performed to examine the associations between 25(OH)D and metabolic risk markers while controlling for potential covariates, such as age, gender and BMI SDS.

A logistic regression model was used to calculate independently factors related to IFG. All tests were two-sided, and p values of <0.05 were regarded as significant. Statistical analyses were performed using SPSS for windows version 22 (SPSS, IL, USA).

RESULTS

The clinical characteristics of the 202 children in the study population, divided into predefined vitamin D categories and age groups, are presented in Table 1. The prevalence of vitamin D deficiency was 33.2% for the whole study group. An interaction effect was found between age and 25(OH)D ($p < 0.001$), with a significantly lower prevalence of vitamin D deficiency in the youngest children ($p < 0.001$). The mean 25(OH)D levels were higher in the youngest age group than in the oldest age group ($p = 0.005$).

Girls had significantly higher mean 25(OH)D levels than boys ($p < 0.03$), but no differences were seen in the proportion of vitamin D-deficient and vitamin D-nondeficient children when comparing gender. We found no associations between the degree of obesity and 25(OH)D when the whole study population was investigated. However, a post hoc analysis showed a significant correlation between BMI SDS and 25(OH)D in children 10–13 years old (data not shown).

For the whole group, 25(OH)D levels differed between summer and winter ($p = 0.02$). This was primarily because children of 10–14 years of age had significantly higher 25(OH)D levels during summer than winter ($p < 0.03$), whereas the youngest children and adolescents had similar levels during summer and winter. But no differences were seen in the proportion of vitamin D-deficient and vitamin D nondeficient children when comparing seasons.

Children born in Sweden had significantly higher levels of 25(OH)D than children born outside Sweden ($p < 0.001$) in all age groups. The proportion of vitamin D deficiency was significantly higher in children not born in Sweden. We found that 84.1% of the children had HOMA levels >2.1

Table 1 Clinical and metabolic characteristics categorising VD-deficient and nondeficient children

	All subjects	25(OH)D nmol/L	Deficient <30	Nondeficient >30	
	n = 202		n = 67	n = 135	p
VD groups %			33.2	76.8	
Age mean (SD)	12.8 (3.1)		14.0 (2.2)	12.2 (3.3)	
Age groups† (1–3)%					
1	19.8		7.5	92.5	
2	35.1		29.8	70.2	
3	45.1		62.7	37.3	<0.001*
Gender F/M %	49.5/50.5		43.3/56.7	52.6/47.4	0.2*
BMI SDS mean (SD)	3.5 (0.7)		3.45 (0.6)	3.49 (0.7)	0.7
Place of birth %					
Born in Sweden/not born in Sweden	74.5/25.5		57.6/42.4	82.8/17.2	<0.001*
Season %					
Winter/summer	70.3/29.7		76.1/23.9	67.4/32.6	0.2*
Prevalence of IFG %					
(Impaired fasting glucose ≥ 5.6)	9.1		16.7	5.3	0.01*
Prevalence of HOMA ≥ 2.1%	84.1		91.8	80.5	0.046*

p-value; difference between deficient and nondeficient; independent *t*-test.
*Chi-square test.
†Year 1; 4 – 9.9, 2; 10 – 13.9 3; 14 – 17.9.

(13), with a higher frequency in the vitamin D-deficient group ($p = 0.05$).

Table 2 shows the laboratory characteristics of the study population divided into age groups and vitamin D categories. F-glucose, f-insulin and HOMA increased with age ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively). We found no differences in the glucose homeostasis when comparing children born in Sweden with children born elsewhere.

A family history of diabetes and the presence of acanthosis nigricans were documented in 66.8% and 34.2%, respectively. Both were present concurrently in 26.4% of the study population, although neither was significantly more prevalent in children with vitamin D deficiency. Cholesterol, triglyceride and gamma-glutamyltransferase levels differed between vitamin D groups, with significantly higher levels in vitamin D-deficient children ($p = 0.05$, $p = 0.003$ and $p = 0.003$, respectively). No associations were found for other risk makers (data not shown).

The level of 25(OH)D was inversely related to f-glucose ($r = -0.15$, $p < 0.05$), f-insulin ($r = -0.18$, $p < 0.01$) and HOMA ($r = -0.19$, $p < 0.01$). When adjusting for age, however, only the association between 25(OH)D and f-glucose levels remained statistically significant ($p = 0.02$).

The prevalence of IFG was 9.1% in this cohort of obese children and adolescents. No difference was seen when comparing the proportion of IFG in children born in Sweden with that of children born elsewhere. The prevalence of IFG was 16.7% among patients with vitamin D deficiency, compared with 5.3% among nondeficient children ($p = 0.01$).

Logistic regression models were applied to assess the associations between the factors independently related to IFG. We found vitamin D deficiency to be an independent factor of IFG (OR 2.3, 95 CI 1.0–7.9, $p < 0.05$) (Table 3).

DISCUSSION

In our cohort of obese Swedish children, 33.2% had 25(OH)D < 30 nmol/L, defined as vitamin D deficiency by the IOM's classification. The prevalence of vitamin D deficiency was twice as high as that found in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, an examination of European adolescents' vitamin D status (16). Thus, our results clearly show that obese children living in northern Europe have a high risk of vitamin D deficiency regardless of the season. There seems also to be a wide seasonal variation among nonobese adolescents and among younger children living in Sweden (16).

In the present study, we found no associations between 25(OH)D and BMI SDS. Similar findings were observed by Weng et al. (17), whereas others have reported significant and inverse relationships in children and adolescents (18). The most likely explanation is that all of our studied children were obese or extremely obese and had a mean

Table 2 25(OH)D and metabolic markers divided into groups by age and VD level

	Total		1	2	3			Deficient	Nondeficient	
			4–9.9	10–13.9	14–17.9		25(OH)D	<30	>30	p**
	n = 202		n = 40	n = 71	n = 91		nmol/L	n = 67	n = 135	adjusted
BMI SDS	3.5 (0.7)	Year	4 (0.87)	3.3 (0.66)	3.6 (0.52)	p*		3.45 (0.6)	3.49 (0.7)	for age
25(OH)D, nmol/L	37.1 (14.4)		42.5 (13.9)	37.9 (13.7)	34 (14.4)	<0.001		22.0 (45)	44.6 (11.4)	<0.001
Missing										
Glucose, mmol/L	5.0 (0.4)		4.7 (0.4)	5.1 (0.4)	5.1 (0.5)	<0.001		5.2 (0.5)	4.9 (0.4)	0.021
Missing	3		1	1	1			1	2	
Insulin, mIE/L	24.4 (14.4)		16.5 (10.2)	23.5 (15)	28.3 (14)	<0.001		27.7 (13.7)	22.8 (14.7)	0.4
Missing	9			6	3			2	7	
HOMA	5.5 (3.6)		3.5 (2.4)	5.4 (3.8)	6.5 (3.5)	<0.001		6.5 (3.6)	5.1 (3.6)	0.24
Missing	12		1	7	4			3	9	
Glycosylated haemoglobin, mmol/mol	35.3 (3.6)		34.6 (3.1)	35.6 (3.7)	35.4 (3.6)	0.3		35.6 (4.0)	35.2 (3.4)	0.68
Missing	8		2	1	5			1	7	
High-sensitivity C-reactive protein, mg/L	4.1 (4.0)		3.8 (3.9)	4.0 (4.4)	4.4 (4.0)	0.7		4.1 (4.0)	4.2 (4.0)	0.36
Missing	10		1	4	5			1	9	
Cholesterol, mmol/L	4.3 (0.9)		4.5 (0.8)	4.2 (0.9)	4.2 (0.9)	0.3		4.4 (0.9)	4.2 (0.8)	0.05
Missing	2			1	1				2	
Triglyceride, mmol/L	1.2 (0.8)		1.2 (0.7)	1.2 (0.7)	1.3 (0.9)	0.7		1.5 (0.9)	1.1 (0.7)	0.009
Missing	4		1	1	2				4	
Gamma-glutamyltransferase, mikrokat/L	0.3 (0.25)		0.27 (0.14)	0.27 (0.17)	0.38 (0.33)	0.008		0.40 (0.37)	0.28 (0.18)	0.02
Missing	12		2	4	6			3	9	

*One-way ANOVA.
**Independent *t*-test.

Table 3 Logistic regression analysis: odds ratio of having IFG for potential risk factors

	Odds ratio	95% CI	p
Model 1			
Variable			
Age	1.18	0.95–0.47	0.14
Season	1.1	0.35–3.2	0.9
VD group	2.3	1.0–7.9	0.049
Model 2			
Born in Sweden/not born in Sweden	0.5	0.18–1.47	0.22
Season	0.9	0.31–2.9	0.9
VD group	3.1	1.1–8.7	0.033

BMI SDS of 3.5 units. Thus, within this group of obese children, other factors predominated.

Age was negatively correlated with vitamin D. These results aligned with some (19), but not all, of the published data that showed a positive correlation (16), or no association, between age and vitamin D (20). One explanation may be that in Sweden, older children are indoors more than younger children are. Similarly, boys may engage in less outdoor activity than girls do (16), possibly explaining the lower 25(OH) levels in boys.

Our secondary aim was to examine a possible association between glucose metabolism and vitamin D status. It has been suggested that low vitamin D may reflect a risk factor in the development of insulin resistance and the pathogenesis of type 2 diabetes, as it affects either insulin sensitivity or beta-cell function (21). We found an inverse association between 25(OH)D and f-glucose when adjusted for age, and this agreed with other observational and intervention studies that demonstrated associations between vitamin D and f-glucose (10). In our study population, 9.1% had IFG and the prevalence was significantly higher among children with vitamin D deficiency. There have been discrepancies with this issue as some studies have reported such relationships (22) and others have not (23).

IFG is common among obese children and adolescents (24) and our results indicate that vitamin D deficiency was associated with an increased risk of a disturbed glucose metabolism. We found no associations between HOMA levels and vitamin D deficiency when adjusted for age. This does not align with studies comparing obese and nonobese children (22,25). It is possible that adjustments for BMI were insufficient and that, therefore, the associations found in these studies were primarily between BMI and vitamin D. Thus, our results support an association between low vitamin D levels and disturbed glucose homeostasis in obese children. It is possible that low vitamin D levels are important for glucose homeostasis, because vitamin D receptors are present in adipose tissues as well as in beta cells and vitamin D up-regulates glucose transporter 4 (26).

Our study indicates that higher 25(OH)D levels were related to a decreased lipid profile in obese children and adolescents. This accords with previous results regarding the association between vitamin D and lipid profiles (27). However, the causative relationship was unclear, as inflammation and disturbed glucose metabolism may well affect vitamin D metabolism and intervention studies have so far shown inconclusive results (11).

No association between 25(OH)D and high-sensitivity C-reactive protein was observed. This was in line with previous reports and may be explained by the difficulty in separating the effects of vitamin D and adiposity. However, there are discrepancies regarding this issue (10).

Surprisingly, a difference was observed in gamma-glutamyltransferase levels but not in aspartate aminotransferase or alanine aminotransferase levels, when comparing vitamin D-deficient children with their nondeficient counterparts. While all gamma-glutamyltransferase levels were within the normal range, suggesting clinical nonrelevance, a low vitamin D level has been associated with an elevated risk for fatty liver disease in nonobese adults, albeit not in children (28).

Our study was a prospective cross-sectional examination of a relatively large number of obese children and adolescents. The children were consecutively included over a period of three years, and seasonal variations were covered. Nevertheless, our study had several limitations. A potential weakness in our study was that parathyroid hormone was not included in the blood tests at the patient's first visit to the clinic. As ethnicity was frequently missing from the medical files or BORIS register, we used the readily available data on birthplace – in or outside Sweden – as a surrogate method of stratification. The data were cross-sectional, so no causal relationships could be determined. No control group of normal weight, healthy children was included and so the comparisons were made in relation to reports from other populations living in Sweden (16,29).

The variability between the methods used for analysing 25(OH)D (30) may have influenced our comparisons marginally, but the methods used at the Karolinska University Laboratory were not changed during the time period when our data were collected and analysed. No information on dietary intake, outdoor activities or sedentary behaviour – such as hours watching TV – were included in this study, and these factors may have affected the vitamin D status.

CONCLUSION

This study shows that one-third of the severely obese children and adolescents living in Stockholm, Sweden, had vitamin D deficiency. We conclude that vitamin D supplementation should be considered for obese children and adolescents, given that disturbed bone metabolism has been observed among obese children (7) and there have been associations reported between vitamin D and glucose homeostasis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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