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Obesity is associated with vitamin D deficiency in Danish children and adolescents

Abstract

Background: Sufficient serum concentrations of vitamin D are required to maintain bone health during growth. The aims of this study were to determine whether vitamin D deficiency is more prevalent among children and adolescents with obesity compared to their normal weight peers and to identify clinical and biochemical variables associated with vitamin D deficiency.

Methods: One thousand four hundred and eighty-four children and adolescents with overweight/obesity and 2143 population-based controls were recruited from the Danish Childhood Obesity Biobank. Anthropometric variables and fasting concentrations of serum 25-hydroxy vitamin D (25-OH-D), plasma parathyroid hormone (PTH), calcium and phosphate were assessed at baseline. Vitamin D deficiency was defined as serum 25-OH-D concentrations <30 nmol/L. Linear and logistic regressions were used to identify variables associated with vitamin D deficiency.

Results: A total of 16.5% of the children and adolescents with obesity (body mass index [BMI] standard deviation score [SDS] > 2.33) exhibited vitamin D deficiency, with an odds ratio (OR) 3.41 (confidence interval [CI]: 2.27–5.71; p < 0.0001) for being vitamin D deficient compared to their normal weight peers. BMI-SDS was independently and inversely associated with serum 25-OH-D concentrations. Other independent risk factors for vitamin D deficiency were being older than 14 years (OR: 2.39; CI: 1.28–4.48; p = 0.006), more than 4 daily hours of screen time (OR: 4.56; CI: 2.59–8.05; p < 0.0001) and blood sample assessment during winter-spring (OR: 6.44; CI: 4.47–9.26; p < 0.0001).

Conclusions: Vitamin D deficiency was common among Danish children and adolescents with obesity. The degree of obesity was independently associated with lower serum 25-OH-D concentrations.

Keywords: body mass index; calcium; childhood obesity; parathyroid hormone; vitamin D deficiency.

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Introduction

Childhood obesity has been associated with low circulating serum concentrations of 25-hydroxy vitamin D (25-OH-D), and vitamin D deficiency has been shown in the range of 17%–57%, depending on how vitamin D deficiency is categorized [1–4]. This might be associated with an unfavorable lifestyle [5]. It has been proposed that fat-soluble hormones, including vitamin D, are sequestered in the adipose tissue, resulting in a decreased bioavailability [6].

Sufficient serum concentrations of vitamin D and calcium are needed to maintain bone health and avoid childhood diseases such as rickets [7, 8]. Furthermore, epidemiologic studies suggest that vitamin D may protect against diseases such as colorectal cancer, type 2 diabetes and cardiovascular disease [9–11]. While a causal link between vitamin D deficiency and immune disorders is yet to be established, 1,25-dihydroxyvitamin D (1,25-OH-D_2) seems to be involved in the regulation of pathways that promote the innate immune response, while suppressing the adaptive immune response [12].

While serum 1,25-OH-D$_2$ is the active form of vitamin D, it is serum 25-OH-D, the major circulating form of vitamin D, that is considered the best indicator of vitamin D status [13].

Establishing an exact threshold for vitamin D deficiency has proved difficult and is further complicated by the vast array of potential health benefits of vitamin D. Focusing on bone health, it seems that the risk of developing nutritional rickets is greatly increased with 25-OH-D concentrations below 30 nmol/L, considering this threshold deficient and concentrations above 30 and below 50 nmol/L as insufficient [14, 15]. However, evidence from adult populations suggest that to obtain protective effects on cardiovascular health and colorectal cancer prevention, serum 25-OH-D concentrations should be maintained as high as 75–110 nmol/L [16]. Studies in children regarding long-term benefits of serum 25-OH-D concentrations of this magnitude are sparse. This is unfortunate as children and adolescents with obesity exhibit a higher risk of developing cardiovascular disease in adulthood [17] and cardiovascular risk markers are already present in childhood and adolescence [18–20].

The primary aim of this study was to determine the prevalence of vitamin D deficiency in a cohort of Danish children and adolescents and to investigate the possible associations between body mass index (BMI) standard deviation score (SDS), fasting concentrations of serum 25-OH-D and additional bone markers. Secondarily, we sought to identify anthropometric, biochemical and lifestyle variables associated with vitamin D deficiency.

Materials and methods

Subjects and study design

At the time of inclusion, 4909 children and adolescents from the Danish Childhood Obesity Biobank (2860 girls) were eligible for this study. The Danish Childhood Obesity Biobank consists of (i) children and adolescents with overweight/obesity enrolled into chronic care treatment at either the Children's Obesity Clinic, Copenhagen University Hospital Holbæk, or in eight Danish municipalities and (ii) a population-based group of children and adolescents of the same ages recruited from schools in 11 different Danish municipalities. All baseline visits took place in the time period between March 2007 and May 2015.

Exclusion criteria were (i) age below 6 or above 18 years, (ii) more than 60 days between baseline anthropometric measurements and fasting blood samples, (iii) intake of medications known to affect vitamin D metabolism [21] as well as intake of supplementary vitamin D or calcium, (iv) diseases affecting calcium or vitamin D metabolism or (v) ethnicity other than North European White. After applying these criteria, 3627 children and adolescents were found to be eligible for this study.

Anthropometric measurements

Weight was measured to the nearest 0.1 kg on a Tanita® BC418 scale (Tanita Corp., Tokyo, Japan) in the population-based cohort and on a Tanita® Digital Medical Scale, WB-110 MA (Tanita Corp., Tokyo, Japan) in the children and adolescents entering chronic care treatment. Height was measured using a stadiometer to the nearest 1 mm. Both measurements were performed in light indoor clothing BMI was calculated as weight divided by height squared (kg/m^2). The BMI-SDS was calculated using the least-mean-squares (LMS) method by converting BMI into a normal distribution by sex and age using the median coefficient of variation and a measure of the skewness [22], based on the Box-Cox power plot based on Danish BMI charts [23]. Waist circumference (WC) was measured to the nearest 1 mm at the level of the umbilicus with a non-elastic tape measure after a gentle expiration. Waist-height ratio (WHtR) was calculated as WC divided by height [24].

Assessment of serum 25-OH-D, plasma parathyroid hormone (PTH), calcium, phosphate and albumin concentrations

Fasting blood samples were performed in the morning after 8–10 h of fasting. Measurements of 25-OH-D serum concentrations were performed using an electrochemiluminescence-binding assay. The upper and lower detection limits were 175 nmol/L and 7.5 nmol/L, respectively. Plasma concentrations of PTH were measured using an electrochemiluminescent immunoassay for in vitro quantitative assessment of intact PTH (1-84). Both 25-OH-D and PTH were measured on a Cobas e (Roche Diagnostics GmbH, Mannheim, Germany). Calcium, phosphate and albumin were measured on a Dimension Vista/Centaur (Siemens Healthcare, Erlangen, Germany). Corrected calcium, which is total calcium adjusted for albumin, was calculated as: total plasma calcium + 0.020*(41.3 – plasma albumin), in accordance with the local laboratory guidelines.

Blood sample assessment was dichotomized according to season in winter/spring (November 1st–May 31st) and summer/fall (June 1st–October 31st).

Assessment of pubertal stages

Pubertal staging was performed according to the Tanner classification [25]. A trained pediatrician performed pubertal staging of children and adolescents from the Children's Obesity Clinic. Pubertal staging of the children and adolescents from the population-based group was self-reported, based on a questionnaire with illustrations and a supplementary text. Tanner stage 1 was considered prepubertal, while stages 2–5 were considered pubertal.

Assessment of daily screen time

Daily hours of screen time was defined as the daily number of hours that the child/adolescent spent in front of a TV or computer screen, a tablet or a cellphone. This was reported during a questionnaire-based

interview with children and adolescents entering chronic care treatment. Children and adolescents from the population-based group filled out a similar questionnaire.

Stratifications

Stratification based on BMI-SDS placed the children and adolescents in one of the three categories: obese (BMI-SDS ≥ 2.33 or above the 99th percentile and above), overweight (BMI-SDS ≥ 1.28 < 2.33) or lean (BMI-SDS < 1.28 or below the 90th percentile).

Vitamin D status was categorized as follows: vitamin D sufficiency (≥50 nmol/L), vitamin D insufficiency (<50 nmol ≥ 30 nmol/L) and vitamin D deficiency (<30 nmol/L) [15].

Statistical analyses

Mean values of continuous variables were compared using the one-way analysis of variance (ANOVA) with Tukey honest significant difference (HSD) post hoc comparisons (>2 groups) or Student's t-test (2 groups). Non-normally distributed variables were log-transformed prior to analyses. Categorical values were compared using the χ^2 test. Baseline continuous variables are stated as means with standard deviations (SD) or medians with interquartile ranges (IQR) and categorical variables as percentages. Associations of 25-OH-D and other variables were investigated with univariate linear regression using unstandardized (B) regression coefficients. Variables with univariate association were included in multivariate analyses. Additionally, multivariate multinomial logistic regression was performed to assess odds ratios (ORs) regarding vitamin D insufficiency and deficiency. Age, sex, Tanner stage, BMI-SDS, hours of daily screen time, season of blood sample assessment, PTH and albumin were included. A p-value below 0.05 was considered statistically significant. All analyses were performed using the statistical software SPSS (IBM SPSS Statistics for Windows, Version 23.0. IBM Corp., Armonk, NY, USA).

Ethical considerations

Study participants younger than 18 years provided informed assent to participation in the study and their parents gave informed written consent. Study participants aged 18 years provided informed assent and gave informed written consent. The study was approved by the Ethics Committee of Region Zealand, Denmark (ID: SJ-104) and the Danish Data Protection Agency (ID: REG-06-2014), and is registered at ClinicalTrials.gov (ID: NCT000928473).

Results

Clinical, biochemical and lifestyle variables of the children and adolescents with overweight/obesity entering chronic care treatment and the population-based controls are shown in Table 1. There were fewer boys in the population-based group, and they were older and more of them had reached puberty. The population-based group had higher serum concentrations of 25-OH-D, lower plasma concentrations of PTH, calcium and phosphate and fewer daily hours of screen time.

Vitamin D deficiency was detected in 16.5% of the children and adolescents with obesity, in 7.7% of the group with overweight and in 4.8% of the group with normal weight (p < 0.0001). Only 40.5% of the children and adolescents with obesity were considered vitamin D sufficient (Figure 1).

Serum 25-OH-D concentrations were inversely associated with BMI-SDS (p < 0.0001), age (p = 0.041), plasma concentrations of PTH (p < 0.0001), daily hours of screen time (p < 0.0001) and season of blood sample assessment

Table 1: Baseline variables of children and adolescents with overweight/obesity versus population-based control group.

	Children and adolescents with overweight/obesity (n = 1484)	Population-based control group (n = 2143)	p-Value
Male, n (%)	676 (45.6%)	856 (39.9%)	0.001
Age, years (SD)	11.8 (2.82)	12.1 (3.37)	0.001
Blood samples summer-fall, n (%)	749 (50.5%)	1034 (48.3%)	0.188
Tanner stage 1/2−5 (%)	38.7/58.3%	27.5/72.5%	<0.0001
BMI-SDS (SD)	2.87 (0.65)	0.31 (1.04)	<0.0001
WHtR (SD)	0.60 (0.06)	0.44 (0.05)	<0.0001
25-OH-D, nmol/L (SD)[b]	47.9 (21.8)	59.2 (23.0)	<0.0001
PTH, pmol/L[a,b]	4.73 (3.78−5.85)	4.15 (3.40−5.16)	<0.0001
Calcium, mmol/L (SD)[b]	2.53 (0.09)	2.48 (1.00)	<0.0001
Corrected calcium, mmol/L (SD)[b]	2.44 (0.11)	2.41 (0.13)	<0.0001
Phosphate, mmol/L (SD)[b]	1.49 (0.18)	1.44 (0.19)	<0.0001
Albumin, g/L (SD)[b]	45.0 (3.63)	43.1 (3.61)	<0.0001
Daily screen time, h[a]	3.5 (2.0−5.0)	2.0 (1.5−3.0)	<0.0001

BMI-SDS, body mass index-standard deviation score; WHtR, waist-height ratio; 25-OH-D, 25-hydroxy vitamin D; PTH, parathyroid hormone.
[a]Data are presented as medians (interquartile range) due to a non-normal distribution. [b]Fasting serum or plasma concentrations.
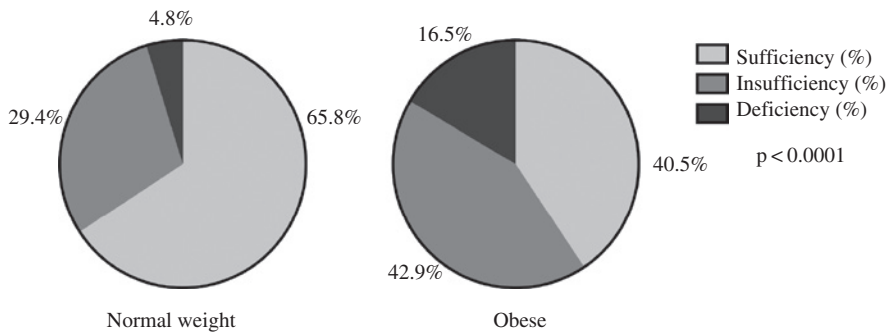
Figure 1: Categories of vitamin D status in children and adolescents with normal weight or obesity.
Vitamin D status categories: deficiency (25-OH-D <30 nmol/L), insufficiency (25-OH-D: 30–50 nmol/L) and sufficiency (25-OH-D >50 nmol/L). Normal weight was defined as body mass index (BMI) standard deviation score (SDS) <1.28, and obesity was defined as BMI SDS >2.33. p-Value was obtained using the χ^2 test.

Table 2: Linear regression analysis of 25(OH)-D serum concentrations and baseline variables.

	Univariate		Multivariate[c]	
	B (95% CI)	**p-Value**	**B (95% CI)**	**p-Value**
Age, years	−0.87 (−1.11; −0.63)	<0.0001	−0.25 (−0.49; −0.01)	0.041
Sex	1.71 (0.18; 3.24)	0.029	0.68 (−0.74; 2.11)	0.346
BMI-SDS	−3.96 (−4.43; −3.49)	<0.0001	−2.57 (−3.07; −2.07)	<0.0001
PTH, pmol/L[a, b]	−59.7 (−64.5; −54.8)	<0.0001	−45.8 (−50.9; −40.8)	<0.0001
Calcium, nmol/L[b]	1.09 (−6.62; 8.80)	0.782	–	–
Corrected calcium, nmol/L[b]	13.4 (7.16; 19.7)	<0.0001	8.20 (0.92; 15.5)	0.027
Phosphate, nmol/L[b]	−1.64 (−5.71; 2.44)	0.431	–	–
Albumin, g/L[b]	−0.68 (−0.88; −0.48)	<0.0001	−0.23 (−0.48; 0.01)	0.064
Daily screen time, h[a]	−20.6 (−23.2; −17.0)	<0.0001	−10.5 (−13.2; −7.74)	<0.0001
Season of blood sample	12.1 (10.6; 13.5)	<0.0001	12.4 (11.0; 13.8)	<0.0001

[a]Log-transformed due to a non-normal distribution. [b]Fasting plasma or serum concentrations. [c]Variables with a significant univariate association to vitamin D levels (age, sex, BMI-SDS, PTH, corrected calcium, albumin, daily hours of screen time and season of blood sample assessment) are included.

Table 3: Baseline variables after stratification based on BMI-SDS.

|	Obese group (n=1252)	Overweight group (n=609)	Lean group (n=1766)	p-Value a vs. b	p-Value a vs. c	p-Value b vs. c
Male, n (%)	602 (48.1%)	237 (38.9%)	693 (39.3%)	<0.0001	<0.0001	0.887
Age, years (SD)	11.9 (2.9)	11.7 (2.9)	12.2 (3.4)	0.766	0.024	0.015
Blood samples summer-fall, n (%)	628 (50.2%)	327 (53.7%)	828 (46.9%)	0.152	0.076	0.004
Tanner stage, 1/2−5 (%)	38.7/61.3	29.3/70.6	28.6/71.4	0.001	<0.0001	0.781
BMI-SDS (SD)	3.07 (0.53)	1.84 (0.31)	−0.03 (0.79)	<0.0001	<0.0001	<0.0001
WHtR (SD)	0.61 (0.06)	0.51 (0.05)	0.43 (0.03)	<0.0001	<0.0001	<0.0001
25-OHD, nmol/L (SD)[b]	46.6 (22.0)	54.5 (20.8)	60.2 (23.2)	<0.0001	<0.0001	<0.0001
PTH, pmol/L[a, b]	4.70 (3.79−5.87)	4.31 (3.49−5.43)	4.15 (3.40−5.17)	<0.0001	<0.0001	0.035
Calcium, mmol/L (SD)[b]	2.53 (0.09)	2.51 (1.00)	2.47 (0.09)	<0.0001	<0.0001	<0.0001
Corrected calcium, mmol/L (SD)[b]	2.46 (0.12)	2.45 (0.12)	2.44 (0.12)	0.557	<0.0001	0.035
Phosphate, mmol/L (SD)[b]	1.49 (0.18)	1.45 (0.19)	1.44 (0.18)	0.0002	<0.0001	0.281
Albumin, g/L (SD)[b]	45.0 (3.64)	44.1 (3.64)	43.1 (3.61)	<0.0001	<0.0001	<0.0001
Daily screen time, h[a]	4.00 (2.00−5.00)	3.00 (1.50−4.00)	2.00 (1.50−3.00)	<0.0001	<0.0001	<0.0001

BMI-SDS, body mass index-standard deviation score; WHtR, waist-height ratio; 25-OH-D, 25-hydroxy vitamin D; PTH, parathyroid hormone.
[a]Data are presented as medians (interquartile range) due to a non-normal distribution. [b]Fasting plasma or serum concentrations.

Table 4: Baseline variables after stratification based on vitamin D status.

	Vitamin D sufficiency (>50 nmol/L) (n=2025)	Vitamin D insufficiency (30–50 nmol/L) (n=1264)	p-Value	Vitamin D deficiency (<30 nmol/L) (n=338)	p-Value
Male, n (%)	840 (41.5%)	520 (41.1%)	0.846	172 (50.8%)	0.001
Age, years (SD)	11.6 (3.27)	12.2 (2.92)	<0.0001	13.5 (2.76)	<0.0001
Blood samples summer-fall, n (%)	1202 (59.4%)	489 (38.6%)	<0.0001	92 (27.3%)	<0.0001
Tanner stage 1/2–5 (%)	36.1/63.9	31.4/85.6	0.018	15.9/94.1	<0.0001
BMI-SDS (SD)	1.05 (1.45)	1.61 (1.56)	<0.0001	2.26 (1.52)	<0.0001
WHtR (SD)	0.48 (0.08)	0.52 (0.10)	<0.0001	0.57 (0.11)	<0.0001
PTH, pmol/L[a]	4.03 (3.32–4.96)	4.67 (3.87–5.82)	<0.0001	5.60 (4.55–6.95)	<0.0001
Calcium, mmol/L (SD)[b]	2.49 (0.09)	2.50 (0.09)	0.753	2.50 (0.12)	0.937
Corrected calcium, mmol/L (SD)[b]	2.43 (0.12)	2.42 (0.12)	0.078	2.42 (0.15)	0.811
Phosphate, mmol/L (SD)[b]	1.46 (0.18)	1.46 (0.18)	1.000	1.44 (0.20)	0.306
Albumin, g/L (SD)[b]	43.5 (3.63)	43.9 (3.64)	<0.0001	44.3 (3.52)	<0.0001
Daily screen time, h[a]	2.00 (1.50–3.50)	3.00 (2.00–4.50)	<0.0001	4.00 (4.55–6.95)	<0.0001

BMI-SDS, body mass index-standard deviation score; WHtR, waist-height ratio; PTH, parathyroid hormone. All p-values are generated with vitamin D sufficiency as reference category. Cases and population-based controls were pooled for these analyses. [a]Data are presented as medians (interquartile range) due to a non-normal distribution. [b]Fasting serum or plasma concentrations.
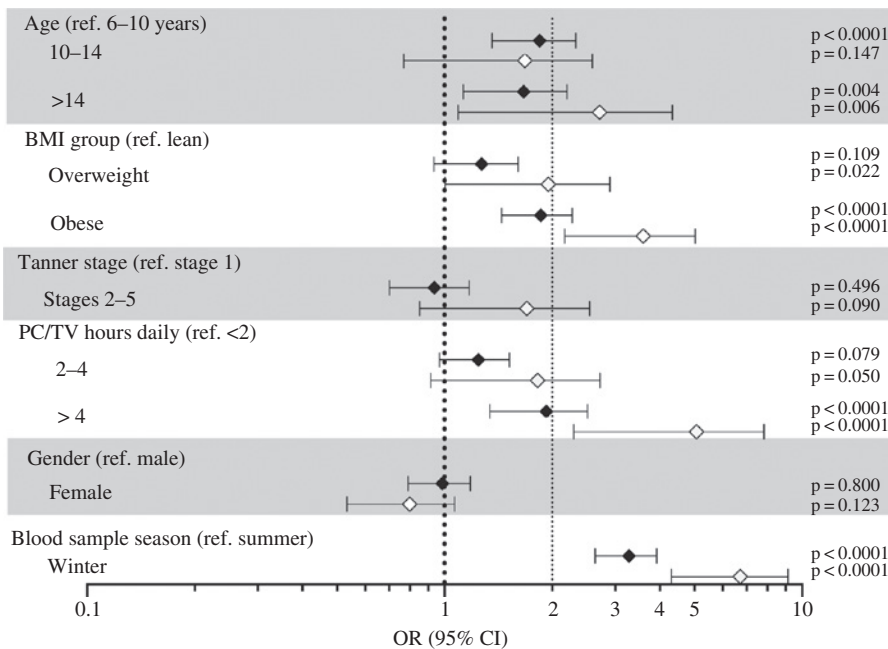
Figure 2: Odds ratios (ORs) of exhibiting vitamin D deficiency or vitamin D insufficiency.
White boxes represent ORs of exhibiting vitamin D deficiency (25-OH-D <30 nmol/L) and black boxes represent ORs of exhibiting vitamin D insufficiency (25-OH-D: 30–50 nmol/L). Cases and controls were pooled for these analyses.

(p < 0.0001) (Table 2). Serum 25-OH-D concentrations were associated with corrected plasma calcium concentrations (p = 0.027) (Table 2).

In the group with obesity, the mean serum concentration of 25-OH-D was lower and the mean plasma concentration of PTH was higher than in both the groups with overweight and normal weight. The mean plasma concentration of calcium was also higher among the children and adolescents with obesity compared to the two other groups. When investigating corrected plasma calcium concentration instead, the differences between the three groups were reduced, and the difference between the groups with obesity and overweight was no longer significant (Table 3).

Individuals with vitamin D deficiency had a higher BMI-SDS and WHtR and a higher mean plasma concentration of PTH, were older with more advanced Tanner stages and more often male compared with children and

adolescents who were vitamin D sufficient. Futhermore, vitamin D-deficient individuals had more daily hours of screen time. There were no differences in the mean total plasma calcium concentration, or the mean corrected calcium concentration, between those with sufficient concentrations of vitamin D compared with those exhibiting vitamin D insufficiency and deficiency, respectively (Table 4).

The highest ORs of exhibiting vitamin D deficiency were obesity (OR: 3.41; CI: 2.27–5.71; p < 0.0001), age above 14 years (OR: 2.39; CI: 1.28–4.48; p = 0.006), more than 4 daily hours of screen time (OR: 4.56; CI: 2.59–8.05; p < 0.0001) and blood sample assessment during winter-spring (OR: 6.44; CI: 4.47–9.26; p < 0.0001). Exhibiting overweight also increased ORs of vitamin D deficiency (Figure 2).

Finally, we investigated the prevalence of vitamin D insufficiency when the threshold was increased to 75 nmol/L. This left a majority of 88.5% of the group with obesity, 84.9% of the group with overweight and 76.1% of the group with normal weight vitamin D insufficient (p < 0.0001 for all).

Discussion

Vitamin D deficiency was common among children and adolescents with obesity compared to their normal weight peers, with children and adolescents with obesity exhibiting a doubled risk of having vitamin D deficiency.

Our results are comparable to previous findings, including a study investigating the association between BMI-SDS and vitamin D concentrations in 12,292 North American children and adolescents [1]. The authors found that 17% of those with a BMI above the 99th percentile exhibited vitamin D deficiency (25-OH-D < 30 nmol/L) [1]. The prevalence of vitamin D deficiency among the children and adolescents with normal weight in our study is in line with the findings of another Danish study on 782 children, where 2.4% of 8–11-year olds had serum concentrations of 25-OH-D below 25 nmol/L [26]. Evidence on the importance of vitamin D in regard to not only bone diseases, but also immune-mediated diseases, is emerging. It has been suggested to raise the threshold for vitamin D sufficiency in adults to 75 nmol/L in order to obtain the entire array of potential health benefits, considering the level of 50–75 nmol/L insufficient [13]. Applying the higher threshold on our cohort showed a majority of children and adolescents in all three BMI-SDS groups exhibiting vitamin D insufficiency, which again

is similar to the findings of Turer et al. [1]. While pediatric studies confirming the beneficial long-term effects of increasing vitamin D concentrations to such levels in children and adolescents are lacking, several studies have aimed to explore the associations between vitamin D deficiency and cardiovascular risk markers in children and adolescents. Cediel et al. [27] performed a study on 435 prepubertal Chilean children and found that serum 25-OH-D concentrations below 75 nmol/L increased the risk of insulin resistance 3 to 4 times, while an Italian study on 452 children showed an OR of 1.72 of exhibiting hypertension in the lowest 25-OH-D quartile (<42 nmol/L) compared to the highest quartile (>67 nmol/L) [28].

In line with Reinehr et al. [29], the present study demonstrated higher plasma concentrations of PTH and lower serum 25-OH-D concentrations in children and adolescents with overweight and obesity. Low serum 25-OH-D concentrations lead to reduced calcium concentrations, which is detected by the parathyroid glands. This induces an increased production of PTH and subsequently an increased activity of the enzyme responsible for 1-hydroxylation of 25-OH-D, and thus the biological activation of vitamin D [30].

Total concentrations of calcium and phosphate were higher in children and adolescents with obesity compared to the groups with overweight and normal weight, despite lower serum concentrations of 25-OH-D and higher plasma concentrations of PTH. Studies investigating the association of calcium and phosphate concentrations with childhood obesity are conflicting. Reinehr et al. [29] found no difference in concentrations of calcium and inorganic phosphate between 133 German children with obesity and 23 controls with normal weight, while a Polish study on 78 adolescents with obesity and 20 controls with normal weight found lower concentrations of calcium in obese adolescents [31]. Contrasting this, and supporting our findings, a large Norwegian population study on 8128 adults aged 30–89 years found that a high calcium concentration was a strong predictor of obesity in women [32]. As total calcium represents the free calcium fraction as well as the fraction bound to proteins such as albumin and globulins, total calcium concentrations are affected by plasma albumin concentrations [30]. In the present study, children and adolescents with obesity had slightly higher albumin concentrations, which could contribute to the increased total calcium. Few pediatric studies have investigated the association between serum albumin and obesity. One study on 331 Saudi Arabian children found that adolescents with obesity had lower serum albumin [33], while another study on 41 African-American children reported no difference [4]. In our cohort, the difference could, at

least in part, be explained by a higher protein intake in the children and adolescents with obesity, as dietary protein intake has been demonstrated to affect the fractional synthesis rate of serum albumin [34]. However, we do not have the dietary recordings to confirm this theory.

The physiological increase of PTH is driven by reduced concentrations of free calcium, which, due to essential neuromuscular functions, should be kept within narrow limits [30]. The high concentrations of PTH in children and adolescents with overweight and obesity suggest that, despite higher total concentrations, the free calcium fraction is not necessarily higher. Rather, the free calcium concentration may be kept within normal limits due to the physiological PTH surge caused by insufficient serum 25-OH-D concentrations. This could potentially result in bone loss, as bone serves as the major reservoir of calcium in the body. The impact of childhood obesity on bone mass is continually being debated. A study by Rocher et al. [35] comprising 43 prepubertal children has shown that bone mass in children with obesity, measured by dual-energy X-ray absorptiometry (DXA), was actually lower when adjusting for lean mass, despite higher unadjusted values. Cole et al. [36] found that fat mass negatively associated with the peripheral quantitative computed tomography (CT)-assessed true volumetric density. These findings are interesting, considering that forearm fractures resulting from ground-level falls as well as musculoskeletal disorders such as Blount disease and slipped capital femoral epiphysiolysis are more prevalent among children and adolescents with obesity compared to their peers with normal weight [37–39].

In the present study, increasing age was a significant risk factor of vitamin D deficiency, which is in line with the findings of other similar studies [1, 26, 40]. We anticipate that behavioral mechanisms are part of the reason for this. Our results have shown that hours spent in front of a screen are inversely associated with 25-OH-D concentrations. The number of hours spent in front of a screen could be considered an invert proxy of time spent outdoors. In a study recently published by Petersen et al. [26] on 782 Danish children, a higher age was associated with vitamin D deficiency. However, this association became insignificant when adjusting for moderate and vigorous physical activity, which could reflect that adolescents spend less time exercising outdoors. Supporting this, an American study on 2000 boys and girls aged 0–19 years demonstrated that adolescents had significantly lower exposure to ultraviolet (UV) radiation compared to younger children [41].

The present study also demonstrated that season was independently associated with vitamin D status, with the vast majority of cases of vitamin D deficiency being diagnosed during winter and spring. Exposure to UV-B-radiation, rather than diet, is the primary source of vitamin D [42], leaving both children and adults living closer to the polar parts of the world more susceptible to low concentrations of serum 25-OH-D during the winter. While it has been suggested that children require less sun exposure to obtain adequate vitamin D concentrations due to their greater surface area for size and greater capacity to produce vitamin D, this increased ability may still not be enough [43]. Besides seasonality, we found that daily hours of screen time were significantly associated with serum 25-OH-D concentrations, as more than 4 h was associated with a more than four-fold increase in the risk of vitamin D deficiency, independent of BMI-SDS. Increased screen time was also found to be associated with vitamin D deficiency by Turer et al. [1] and could reflect a lack of sun exposure.

Recent evidence has questioned whether low total concentrations of 25-OH-D in children with obesity are of clinical importance. Miraglia et al. [44] calculated the fraction of bioavailable 25-OH-D in 33 children with or without obesity and found it to be similar in the two groups, despite lower total concentrations of 25-OH-D in the group with obesity. This was probably due to lower concentrations of vitamin D-binding protein (DBP) in children with obesity, which might be a compensatory mechanism driven by increased insulin resistance [44]. However, the concentrations of PTH did not differ between the children with and without obesity [44]. It remains unclear whether this is an expression of a homeostatic calcium-PTH-vitamin D axis due to lower concentrations of DBP, and thus normal concentrations of bioavailable 25-OH-D. If so, increasing concentrations of PTH, as found in the present study, may be an important part of predicting a negative impact on bone mineral metabolism, rather than concentrations of total 25-OH-D alone.

The major strength of the present study is the large study population consisting of children and adolescents with obesity, overweight and normal weight of the same ethnicity, covering almost the entire pediatric age range. As sun exposure is the most important determinant of serum 25-OH-D concentrations, an exact measure of outdoor activity would have been preferred. Yet, we consider daily hours of screen time a reliable proxy measure and were also able to adjust for season of blood sample assessment. Diet is another, less important, source of vitamin D, but an important source of calcium. Unfortunately, we were not able to report on dietary components such as dairy intake. Finally, Tanner staging was self-reported in the population-based group, which means that the pubertal stage can be interpreted only as either prepubertal or pubertal.

Conclusions

Compared to about 5% of the group with normal weight, almost 17% of the children with obesity exhibited vitamin D deficiency and only around 40% were categorized as vitamin D sufficient. Independent risk factors of vitamin D deficiency were obesity, being 14 years or older, spending more than 4 daily hours in front of a screen and blood sample assessment performed during winter or spring. Clinicians should pay attention to vitamin D status, especially in adolescents with obesity during wintertime.

References

1. Turer CB, Lin H, Flores G. Prevalence of vitamin D deficiency among overweight and obese US children. Pediatrics 2013;131:e152–61.
2. Codoñer-Franch P, Tavárez-Alonso S, Simó-Jordá R, Laporta-Martín P, Carratalá-Calvo A, et al. Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. J Pediatr 2012;161:848–54.
3. Reyman M, Verrijn Stuart AA, van Summeren M, Rakhshandehroo M, Nuboer R, et al. Vitamin D deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. Int J Obes (Lond) 2014;38:46–52.
4. Rajakumar K, Fernstrom JD, Holick MF, Janosky JE, Greenspan SL. Vitamin D status and response to vitamin D 3 in obese vs. non-obese African American children. Obesity (Silver Spring) 2008;16:90–5.
5. Dong Y, Pollock N, Stallmann-Jorgensen IS, Gutin B, Lan L, et al. Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. Pediatrics 2010;125:1104–11.
6. Wortsman J, Matsuoka LY, Chen TC, Zhiren L, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2003;53:690–4.
7. Robinson PD, Högler W, Craig ME, Verge CF, Walker JL, et al. The re-emerging burden of rickets: a decade of experience from Sydney. Arch Dis Child 2006;91:564–8.
8. Martin AD, Bailey DA, McKay HA, Whiting S. Bone mineral and calcium accretion during puberty. Am J Clin Nutr 1997;66:611–5.
9. Touvier M, Chan DS, Lau R, Aune D, Vieira R, et al. Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2011;20:1003–16.
10. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2013;36:1422–8.
11. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008;117:503–11.
12. Wei R, Christakos S. Mechanisms underlying the regulation of innate and adaptive immunity by vitamin D. Nutrients 2015;7:8251–60.
13. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
14. Atapattu N, Shaw N, Högler W. Relationship between serum 25-hydroxyvitamin D and parathyroid hormone in the search for a biochemical definition of vitamin D deficiency in children. Pediatr Res 2013;74:552–6.
15. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, et al. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab 2016;101:394–415.
16. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, Hathcock J, Giovannucci E, et al. Benefit-risk assessment of vitamin D supplementation. Osteoporos Int 2010;21:1121–32.
17. Baker JL, Olsen LW, Sørensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. N Engl J Med 2007;357:2329–37.
18. Holm J, Gamborg M, Neland M, Ward L, Gammeltoft S, et al. Longitudinal changes in blood pressure during weight loss and regain of weight in obese boys and girls. J Hypertens 2012;30:368–74.
19. Nielsen TR, Gamborg M, Fonvig CE, Kloppenborg J, Hvidt KN, et al. Changes in lipidemia during chronic care treatment of childhood obesity. Child Obes 2012;8:533–41.
20. Bille D, Chabanova E, Gamborg M, Fonvig C, Nielsen T, et al. Liver fat content investigated by magnetic resonance spectroscopy in obese children and youths included in multidisciplinary treatment. Clin Obes 2012;2:41–9.
21. Gröber U, Kisters K. Influence of drugs on vitamin D and calcium metabolism. Dermatoendocrinol 2012;4:158–66.

22. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med 1992;11:1305–19.

23. Nysom K, Mølgaard C, Hutchings B, Michaelsen K. Body mass index of 0 to 45-y-old Danes: reference values and comparison with published European reference values. Int J Obes Relat Metab Disord 2001;25:177–84.

24. Ashwell M, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. Int J Food Sci Nutr 2005;56:303–7.

25. Tanner JM. Growth and maturation during adolescence. Nutr Rev 1981;39:43–55.

26. Petersen RA, Damsgaard CT, Dalskov SM, Sørensen LB, Hjorth MF, et al. Vitamin D status and its determinants during autumn in children at northern latitudes: a cross-sectional analysis from the optimal well-being, development and health for Danish children through a healthy New Nordic Diet (OPUS) School Meal Study. Br J Nutr 2016;115:239–50.

27. Cediel G, Corvalán C, Aguirre C, de Romaña DL, Uauy R. Serum 25-hydroxyvitamin D associated with indicators of body fat and insulin resistance in prepubertal chilean children. Int J Obes (Lond) 2016;40:147–52.

28. Pacifico L, Anania C, Osborn JF, Ferraro F, Bonci E, et al. Low 25(OH)D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. Eur J Endocrinol 2011;165:603–11.

29. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W. Vitamin D status and parathyroid hormone in obese children before and after weight loss. Eur J Endocrinol 2007;157:225–32.

30. Allgrove J. Physiology of calcium, phosphate, magnesium and vitamin D. Calcium Bone Disord Child Adolesc Second Ed 2015;28:7–32.

31. Suliburska J, Cofta S, Gajewska E, Kalmus G, Sobieska M, et al. The evaluation of selected serum mineral concentrations and their association with insulin resistance in obese adolescents. Eur Rev Med Pharmacol Sci 2013;17:2396–400.

32. Kamycheva E, Sundsfjord J, Jorde R. Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. Eur J Endocrinol 2004;151:167–72.

33. Al-Musharaf S, Al-Othman A, Al-Daghri NM, Krishnaswamy S, Yusuf DS, et al. Vitamin D deficiency and calcium intake in reference to increased body mass index in children and adolescents. Eur J Pediatr 2012;171:1081–6.

34. Thalacker-Mercer AE, Johnson CA, Yarasheski KE, Carnell NS, Campbell WW. Nutrient ingestion, protein intake, and sex, but not age, affect the albumin synthesis rate in humans. J Nutr 2007;137:1734–40.

35. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 2008;26:73–8.

36. Cole ZA, Harvey NC, Kim M, Ntani G, Robinson SM, et al. Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children. Bone 2012;50:562–7.

37. Manning Ryan L, Teach SJ, Searcy K, Singer SA, Wood R, et al. The association between weight status and pediatric forearm fractures resulting from ground-level falls. Pediatr Emerg Care 2015;31:835–8.

38. Scott AC, Kelly CH, Sullivan E. Body mass index as a prognostic factor in development of infantile Blount disease. J Pediatr Orthop 2007;27:921–5.

39. Compton S. Childhood obesity and slipped capital femoral epiphysis. Radiol Technol 2014;85:321–6.

40. Absoud M, Cummins C, Lim MJ, Wassmer E, Shaw N. Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. PLoS One 2011;6:e22179.

41. Godar DE. UV doses of American children and adolescents. Photochem Photobiol 2001;74:787–93.

42. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, et al. Molecular mechanisms of vitamin D action. Calcif Tissue Int 2013;92:77–98.

43. Munns C, Zacharin MR, Rodda CP, Batch JA, Morley R, et al. Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. Med J Aust 2006;185:268–72.

44. Miraglia del Giudice E, Grandone A, Cirillo G, Capristo C, Marzuillo P, et al. Bioavailable vitamin D in obese children: the role of insulin resistance. J Clin Endocrinol Metab 2015;100:3949–55.