Applied nutritional investigation

Vitamin D status in preschool children and its relations to vitamin D sources and body mass index—Fish Intervention Studies-KIDS (FINS-KIDS)

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ABSTRACT

Objectives: The aims of this study were to determine vitamin D status (serum 25-hydroxyvitamin D_3 [s-25(OH)D_3]) and examine possible associations between vitamin D status and vitamin D−rich dietary sources, sun exposure, and body mass index in preschool children ages 4 to 6 y.

Methods: This is a cross-sectional study based on baseline data (collected in January-February 2015) from the two-armed randomized controlled trial Fish Intervention Studies-KIDS (FINS-KIDS) conducted in Bergen, Norway. S-25(OH)D_3 concentration was determined by liquid chromatography−tandem mass spectrometry. Information regarding habitual dietary intake, recent sun vacations, and body mass index were assessed with questionnaires answered by the children's caregivers.

Results: The children (n = 212) had a mean (standard deviation) s-25(OH)D_3 of 60.7 (13.8) nmol/L; 18.9% had s-25(OH)D_3 ≤50 nmol/L. In logistic regression models, non-overweight versus overweight status was inversely associated with s-25(OH)D_3 ≤50 nmol/L (odds ratio: 0.41; 95% confidence interval, 0.18−0.95; $P = 0.037$). Non-sun versus sun vacations were associated with s-25(OH)D_3 ≤75 nmol/L (odds ratio: 5.33; 95% confidence interval, 1.93−14.77; $P = 0.001$).

Conclusions: The majority of the preschool children (81%) had s-25(OH)D_3 >50 nmol/L. Children with overweight status had an increased risk of s-25(OH)D_3 ≤50 nmol/L, and children who had not been on sun vacations were at a greater risk of s-25(OH)D_3 ≤75 nmol/L.

Introduction

Vitamin D deficiency is a global problem across all age spans [1] and is specifically a challenge in Europe [2]. Approximately one billion people worldwide are estimated to have vitamin D deficiency or insufficiency [3]. Two distinct forms of vitamin D exist. Vitamin D_3 (cholecalciferol) is synthesized cutaneously in the skin from ultraviolet (UV) radiation and is the predominant form found in the diet. Vitamin D_2 (ergocalciferol) is found in some plants, but largely in fungi [4,5]. Both vitamins D_2 and D_3 are available from fortified foods and supplements [6].

A circulating concentration of serum 25-hydroxyvitamin D_3 (s-25[OH]D_3) is the most reliable marker for vitamin D status [7]. Traditionally a cutoff of s-25(OH)D <25 nmol/L has been used in Europe to define vitamin D deficiency [2], whereas the U.S. Health and Medicine Division (previously the Institute of Medicine) defines deficiency as s-25(OH)D <30 nmol/L [8]. Furthermore, there are discussions regarding optimal s-25(OH)D concentrations for bone health, and whether the cutoff should be 50 nmol/L [8,9] or 75 nmol/L, as suggested by the Endocrine Society [7].

Although vitamin D is important throughout life, an adequate status is particularly crucial early in life. Vitamin D is necessary for calcium absorption and normal bone health [4], and deficiency in utero and during childhood may lead to growth retardation and

skeletal deformities [1]. Vitamin D is also important for the immune system, and a sufficient vitamin D status has been suggested to be protective against different diseases, including cancer, autoimmune disease, infection, and cardiovascular disease [10–12]. In addition, vitamin D can be important for brain development and mental health [1].

Owing to the limitations of UV-B solar radiation, there is almost no epidermal formation of vitamin D between September and May at latitudes of Nordic countries [10,13]. Hence, dietary sources of vitamin D are pivotal to maintain a sufficient vitamin D status during the winter time [13,14]. National recommendations for daily vitamin D intake in Norway is 10 μg for children [15]. If the daily intake of vitamin D rich sources is low during the winter time, a supplement is recommended [16]. Dietary sources that are rich in vitamin D include fatty fish [12], cod liver oil (liquid or capsulated) [10], and fortified foods [17,18] or supplements. Despite the higher latitudes and less sunshine, vitamin D status is usually better in Nordic countries compared with countries in the Mediterranean. Contributing factors may be the high intake of omega-3 supplements that contain vitamin D, as well as a lighter skin tone and more sun bathing during sun vacations [19].

Excess bodyweight is a risk factor for decreased vitamin D status. Overweight and obesity have been linked previously to vitamin D deficiency [20–23], probably through reduced bioavailability of vitamin D_3 from cutaneous and dietary sources because of its deposition in body fat compartments [24].

The results from earlier studies on vitamin D status in European children vary. Concentrations >50 nmol/L are often reported [25–30], although there are reports that a considerable number of children have concentrations <50 nmol/L (between 22–41%) [28,31,32]. Results from studies conducted in children in Greece, Belgium, and the UK showed, on average, concentrations <50 nmol/L (41–47 nmol/L) [33–36]. In Dutch and German children, mean concentrations were >50 nmol/L, and measured to 64 nmol/L [30] and 54 nmol/L [2], respectively. In Nordic countries, Sweden, Finland, Iceland, and Denmark, mean concentrations from 55 to 76 nmol/L have been reported, depending on the country and season [25–29]. There is a lack of Norwegian studies assessing vitamin D status in children [14] and relatively few studies on the determinants of children's vitamin D status [37].

The aims of the present study were to determine vitamin D status and investigate possible associations between vitamin D status and vitamin D-rich dietary sources, sun exposure, and body mass index (BMI) in Norwegian preschool children ages 4 to 6 y.

Methods

Ethical statement

Informed consent was obtained from all caregivers. The procedures were in accordance with the Declaration of Helsinki, and the trial was approved by the Regional Committees for Medical and Health Research Ethics West (2014/1396) and registered in ClinicalTrials.gov (NCT02331667).

Study design and population

This is a cross-sectional study based on baseline data (collected from January–February 2015) from the two-armed randomized controlled trial, Fish Intervention Studies-KIDS (FINS-KIDS), conducted in Bergen, Norway [38]. In this study, 232 children participated, and vitamin D metabolites were analyzed from 212 participants. Dietary data and information on recent sun vacations were available from 194 children, and information on birth place was available from 188 children or caregivers.

Biochemical analyses

Non-fasting blood sampling was conducted in kindergarteners by two authorized biomedical laboratory scientists. Venous blood samples from the elbow cavity were collected in 3.5 mL BD Vacutainer SST II Advance by using BD Vacutainer

Safety-Lok Blood Collection Set (Becton, Dickinson and Company, Plymouth, UK). The blood was centrifuged (10 min, 1000 g, 20°C) after 30 min. Serum was transferred to 1.8 mL Cryotube (Nunc, Roskilde, Denmark), and transported on dry ice for storage at −80°C until analysis.

Serum 25-hydroxyvitamin D_3 (25[OH]D_3), 25-hydroxyvitamin D_2 (25[OH]D_2), 1,25-dihydroxyvitamin D_3 (1,25[OH]$_2D_3$), and 3-*epi*-25-hydroxyvitamin D_3 (3-epi-25 [OH]D_3) concentrations were determined by standardized procedures at the Institute of Marine Research [39,40], using a liquid chromatographic−tandem mass spectrometric assay adding acetonitrile and internal standard (^{2}H 25[OH]D_3) to the samples. The total 25(OH)D in the circulation was accounted for by 25(OH)D_3 only.

Questionnaire

A food-frequency questionnaire (FFQ) to assess food intake during the 3 mo before blood sampling was answered electronically by the caregivers. The questionnaire was modified from an earlier validated FFQ [41–43]. Questions from the FFQ regarding vitamin D-rich sources were used to compare against vitamin D status in the present study and included seafood as dinner (fish, fish products, and other types of seafood), seafood as spreads (spread, salads, and snacks), dairy products (milk, yoghurt, and cheese), eggs (fried, boiled, scrambled, and omelet), butter/margarine, and vitamin D supplements (cod liver oil, omega-3 capsules, Sana-sol, Biovit, and vitamin D tablets). The questionnaire also included questions regarding anthropometric data (body weight and height), socioeconomic status (family income and caregiver's education), birth place of child and caregivers, and information regarding sun vacations (at least 1 wk during the last 3 mo before study testing). A sun vacation includes exposure to short-term, high-dose, solar UV radiation [44]. The FFQ did not contain questions regarding skin color. BMI was calculated as weight (kg) divided by height squared (m^2). Age and sex were adjusted according to BMI calculations based on BMI curves on the basis of the growth study from Bergen, Norway [45]. A BMI calculator with age and sex adjustments was used for these calculations [46]. The BMI cutoffs for boys and girls ages 4 to 6 y are similar, and the following was used: underweight ≤14 kg/m^2; normal weight 14 to 17 kg/m^2; overweight 17 to 19 kg/m^2; and obese ≥19 kg/m^2.

Statistical analyses

Categorical variables are summarized as numbers with percentage and continuous variables as mean with standard deviation (SD). Independent samples t test (age, weight, height, parental education, birth place and s-25[OH]D_3), and Pearson χ^2 test (family income, BMI) were used to compare between boys and girls. Unadjusted and adjusted (sex, parental education or family income) logistic regression analyses were used to investigate possible associations between s-25(OH)D_3 (≤50 nmol/L and ≤75 nmol/L) and vitamin D-rich sources and BMI. The cutoff values used for the different sources are shown in Table 1. Two-tailed *P* values <0.05 were considered statistically significant. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Statistics, version 24).

Results

Study population

The families of 232 children agreed to participate in the study, and vitamin D metabolites (25[OH]D_3, 25[OH]D_2, 1,25[OH]$_2D_3$, and 3-epi-25[OH]D_3) were analyzed from 212 participants (91%; 101 boys and 111 girls). Furthermore, the dietary data and information of recent sun vacations were available from 194 children (84%; 92 boys and 102 girls).

The baseline characteristics of the participants are shown in Table 2. Mean (SD) age was 5.2 (0.6) y, and mean BMI was 15.6 (1.9) kg/m^2, including 58% of children with normal BMI and 21% who were overweight or obese. The boys' caregivers had a higher education and income compared with those of the girls.

Table 1
Serum 25(OH)D_3 in all participants and by sex

nmol/L	All (n = 212)	Boys (n = 101)	Girls (n = 111)
25(OH)D_3	60.7 (13.8)	60.6 (13.5)	60.8 (14.2)
Minimum-maximum	23.7-93.7	23.7-90.8	29.4-93.7
≤50, n (%)	40 (18.9)	16 (15.8)	24 (21.6)
≤75, n (%)	179 (84.4)	85 (84.2)	94 (84.7)

Data are shown as mean (standard deviation) if not otherwise indicated.
25(OH)D_3, 25-hydroxyvitamin D_3.

Vitamin D metabolites

The results from the analyses of s-25(OH)D$_3$ are provided in Table 1. The mean (SD) concentration of s-25(OH)D$_3$ was 60.7 (13.8) nmol/L for all participants, and there were no sex differences. The lowest and highest concentrations detected were 23.7 nmol/L and 93.7 nmol/L, respectively. One child (0.5%) had s-25(OH)D$_3$ ≤25 nmol/L.

Furthermore, 39 children (18.4%) had s-25(OH)D$_3$ between 25 nmol/L and 49 nmol/L, 139 (65.6%) between 50 nmol/L and 75 nmol/L, and 33 (15.6%) >75 nmol/L (Fig. 1). Concentrations of 25(OH)D$_2$, 1,25(OH)$_2$D$_3$, and 3-epi-25(OH)D$_3$ were under the limit of quantification for all participants (data not shown).

Dietary intake

Dietary intake of different foods rich in vitamin D are listed in Table 3. Of all participants, 93 children (48%) consumed seafood for dinner ≥2 times per week. Sixty-two children (32%) ate seafood as a spread ≥2 times per week. Dairy products ≥2 times per day were consumed by 93 children (48%), and 78 children (40%) had eggs ≥2 times per week. Furthermore, 173 children (89%) had butter or margarine on their bread/roll/crispbread. Vitamin D supplements were consumed by 115 participants (59%).

Vitamin D status in relation to vitamin D−rich sources and body mass index

The odds ratio (OR) was calculated for two different cutoffs for vitamin D status. First, the OR for s-25(OH)D$_3$ ≤50 nmol/L in relation to seafood, dairy products, eggs, vitamin D supplements, recent sun vacations, and BMI were investigated (Table 3). Non-overweight (BMI <17 kg/m^2) versus overweight (BMI ≥17 kg/m^2) status was inversely associated with s-25(OH)D$_3$ ≤50 nmol/L (OR: 0.41; 95% confidence interval, 0.18−0.95; *P* = 0.037). In analyses stratified for sex, similar results were observed for girls but not boys, which indicates that girls with overweight status had a higher risk of s-25(OH)D$_3$ ≤50 nmol/L compared with those with non-overweight status. No other associations were observed (Table 3).

Second, the OR for s-25(OH)D$_3$ ≤75 nmol/L in relation to vitamin D−rich dietary sources, recent sun vacations, and BMI are

shown in Table 4. No-sun versus sun vacations were associated with s-25(OH)D$_3$ ≤75 nmol/L (OR: 5.33; 95% confidence interval, 1.93−14.77; *P* = 0.001). In the analyses stratified for sex, intake of dairy products <2 times per day versus ≥2 times per day was associated with s-25(OH)D$_3$ ≤75 nmol/L in girls, whereas the use of vitamin D supplements was associated with s-25(OH)D$_3$ ≤75 nmol/L in boys.

The analyses categorizing BMI according to underweight and obesity status in addition to overweight and non-overweight status showed similar results as for the presented results in Tables 3 and 4 (data not shown).

Adjustments for sex, parental education or family income did not substantially alter the estimates or *P*-values (data not shown). There were no associations between birth place of the child or the caregivers and vitamin D status in this study (data not shown).

Discussion

In this study, vitamin D status was investigated among children ages 4 to 6 y in Bergen, Norway. Our findings demonstrated that the majority of participants had s-25(OH)D$_3$ >50 nmol/L during winter time. However, 18.9% of the children had s-25(OH)D$_3$ ≤50 nmol/L. Children with an overweight status had an increased risk of s-25(OH)D$_3$ ≤50 nmol/L, and children who had not been on recent sun vacations were at a greater risk of s-25(OH)D$_3$ ≤75 nmol/L.

There are relatively few other Norwegian studies where children's vitamin D status has been determined; however, Ungkost 3 (dietary survey conducted in 2016) revealed that 4-y-old children in Norway had an average intake of vitamin D below the national recommendations [47]. In a study from Bergen, Norway in 1982, 191 children ages 8 to 12 y had a mean plasma 25(OH)D$_3$ concentration of 80 nmol/L [48], which was higher than the mean serum concentration of 61 nmol/L observed in the present study.

In preschool children from northern Sweden, mean s-25(OH)D$_3$ concentration was 60 nmol/L during summer time and 55 nmol/L during winter time, and 75% and 60% of the children had s-25(OH)D$_3$ >50 nmol/L measured during summer and winter time, respectively [26]. Our measurements during winter time were similar to the summer measures in Swedish preschoolers. Mean s-25(OH)D$_3$ concentration among 6 y old children from Iceland was 57 nmol/L, which is similar to the results of the preschoolers in the present

Table 2
Characteristics of participants

	n	All (n = 212)	Boys (n = 101)	Girls (n = 111)	*P*-value
Demographic characteristics					
Age (y)	212	5.2 (0.6)	5.1 (0.6)	5.2 (0.5)	0.175
Body weight (kg)	172	20.2 (3.2)	20.5 (3.1)	19.9 (3.2)	0.189
Body height (cm)	174	113.7 (6.2)	114.1 (7.3)	113.5 (5.2)	0.543
BMI (kg/m^2)*	170	15.6 (1.9)	15.7 (1.7)	15.5 (2.1)	0.398
BMI (kg/m^2) categories, n (%)					
≤14 (underweight)		35 (20.6)	14 (18.2)	21 (22.6)	0.102
14−17 (normal weight)		99 (58.2)	42 (54.5)	57 (61.3)	
17−19 (overweight)		29 (17.1)	19 (24.7)	10 (10.8)	
≥19 (obesity)		7 (4.1)	2 (2.6)	5 (5.4)	
Education parents (y)	188	15.3 (1.7)	15.6 (1.6)	15.1 (1.7)	0.027
Family income (NOK[†]), n (%)	187				
<200,000−549,999		23 (12.3)	3 (3.5)	20 (19.8)	0.001
550,000−999,999		79 (42.2)	37 (43.0)	42 (41.6)	
1,000,000−>2,000,000		85 (45.5)	46 (53.5)	39 (38.6)	

Data are shown as mean (standard deviation) if not otherwise indicated.
*BMI was calculated as weight (kg) divided by height squared (m^2), and adjusted for age and sex.
[†]100 NOK = approximately €10 or $11.
P-value for comparison between boys and girls is given with the independent samples *t* test (demographic characteristics and serum vitamin D), and Pearson χ^2 test (family income).
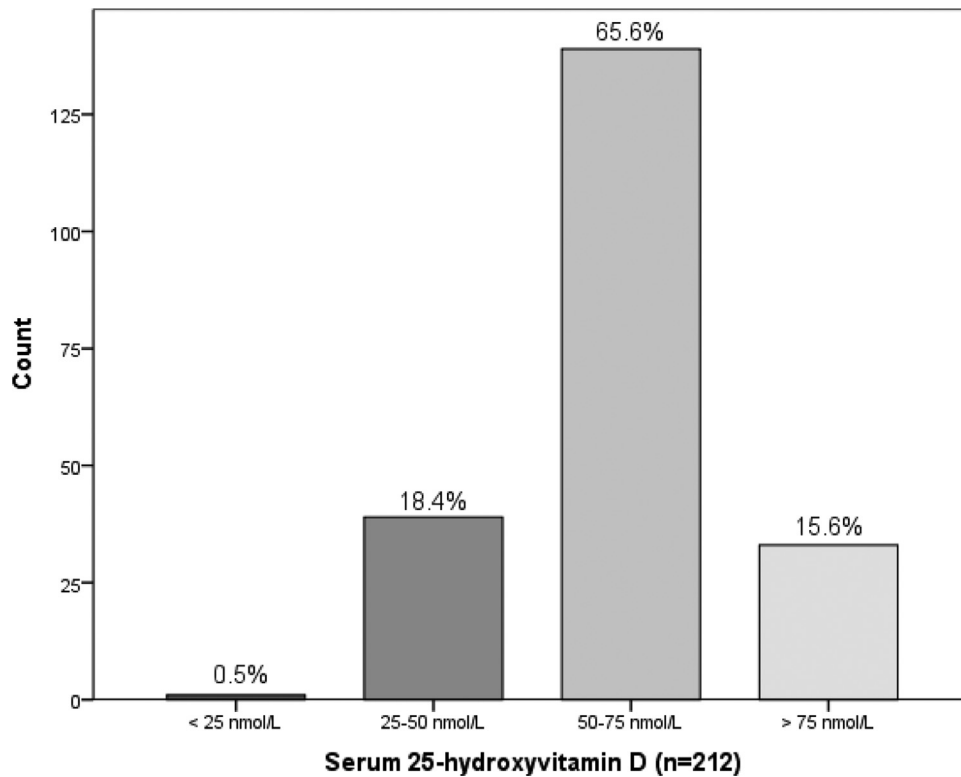BMI, body mass index.

Fig. 1. Serum 25-hydroxyvitamin D_3 concentrations in all participants.

study, and 64% had s-25(OH)D_3 >50 nmol/L measured from June to December [28]. A study of 4 to 8 y old Danish children revealed a mean s-25(OH)D_3 of 57 nmol/L [49], supported by the results of another study in Danish children (age <18 y) in 2018 where the same concentration was detected [50].

In addition, similar concentrations were observed in Finish children (55 nmol/L) [27], which also aligns with our findings.

Somewhat lower concentrations were detected in two studies including children from Greece (41 nmol/L and 46 nmol/L) [33,34] and one study from Belgium (47 nmol/L) [35]. A study in Croatia revealed a mean s-25(OH)D of 47 nmol/L [51] whereas in children from the Netherlands, the results were similar to ours (64 nmol/L) [30]. Thus, these results indicate that the children from our study have a similar vitamin D status compared with children from other

Table 3
OR for s-25(OH)D_3 status ≤50 nmol/L according to intake of seafood, dairy products, eggs, vitamin D supplements, sun vacations, and BMI

Dietary products		All (n = 194)			Boys (n = 92)			Girls (n = 102)	
	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value
Seafood dinner (all)									
<2 times/week	101	0.79 (0.39−1.61)	0.519	48	0.90 (0.31−2.65)	0.848	53	0.72 (0.28−1.85)	0.491
≥2 times/week	93	1 (ref)		44	1 (ref)		49	1 (ref)	
Seafood (spreads)									
<2 times/week	132	0.76 (0.36−1.60)	0.472	63	0.72 (0.24−2.23)	0.572	69	0.80 (0.30−2.14)	0.650
≥2 time/week	62	1 (ref)		29	1 (ref)		33	1 (ref)	
Dairy products (all)									
<2 times/d	101	1.03 (0.51−2.09)	0.938	52	0.99 (0.33−2.93)	0.981	49	1.11 (0.43−2.84)	
≥2 times/d	93	1 (ref)		40	1 (ref)		53	1 (ref)	0.835
Eggs									
<2 times/week	116	1.37 (0.65−2.89)	0.402	57	2.07 (0.61−7.00)	0.244	59	1.07 (0.41−2.78)	0.894
≥2 time/week	78	1 (ref)		35	1 (ref)		43	1 (ref)	
Butter/margarine									
No	21	1.76 (0.64−4.90)	0.277	13	1.52 (0.37−6.31)	0.562	8	2.37 (0.52−10.8)	0.265
Yes	173	1 (ref)		79	1 (ref)		94	1 (ref)	
Vitamin D supplements									
No	79	1.83 (0.89−3.73)	0.098	39	1.45 (0.49−4.28)	0.499	40	2.23 (0.86−5.80)	0.101
Yes	115	1 (ref)		53	1 (ref)		62	1 (ref)	
Recent sun vacations									
No	175	4.83 (0.62−37.34)	0.132	87	0.83 (0.09−7.99)	0.874	88	-*	
Yes	19	1 (ref)		5	1 (ref)		14		
BMI (kg/m^2)									
<17	134	0.41 (0.18−0.95)	0.037	56	0.61 (0.18−2.10)	0.436	78	0.25 (0.08−0.80)	0.020
≥17	36	1 (ref)		21	1. (ref)		15	1 (ref)	

BMI, body mass index; CI, confidence interval; OR, odds ratio; ref, reference.
*To few numbers of participants.

Table 4
OR for s-25(OH)D$_3$ status ≤75 nmol/L according to intake of seafood, dairy products, eggs, vitamin D supplements, sun vacations, and BMI

Dietary products		All (n = 194)			Boys (n = 92)			Girls (n = 102)	
	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value
Seafood dinner (all)									
<2 times/week	101	0.86 (0.39−1.91)		48	0.93 (0.29−3.00)	0.896	53	0.82 (0.28−2.39)	0.709
≥2 times/week	93	1 (ref)	0.716	44	1 (ref)		49	1 (ref)	
Seafood (spreads)									
<2 times/week	132	0.95 (0.41−2.23)	0.908	63	0.96 (0.27−3.42)	0.950	69	0.94 (0.30−2.97)	0.918
≥2 time/week	62	1 (ref)		29	1 (ref)		33	1 (ref)	
Dairy products (all)									
<2 times/d	101	1.66 (0.75−3.69)	0.215	52	0.53 (0.15−1.87)	0.324	49	4.98 (1.33−18.75)	0.018
≥2 times/d	93	1 (ref)		40	1 (ref)		53	1 (ref)	
Eggs									
<2 times/week	116	1.25 (0.56−2.77)	0.583	57	1.48 (0.45−4.82)	0.517	59	1.08 (0.37−3.17)	0.888
≥2 time/week	78	1 (ref)		35	1 (ref)		43	1 (ref)	
Butter/margarine									
No	21	3.86 (0.50−29.96)	0.196	13	2.15 (0.26−18.10)	0.482	8	-*	
Yes	173	1 (ref)		79	1 (ref)		94		
Vitamin D supplements									
No	79	2.43 (0.99−6.01)	0.054	39	11.12 (1.38−89.67)	0.024	40	1.09 (0.36−3.28)	0.878
Yes	115	1 (ref)		53	1 (ref)		62	1 (ref)	
Sun vacations									
No	175	5.33 (1.93−14.77)	0.001	87	11.55 (1.72−77.74)	0.012	88	3.89 (1.10−13.75)	0.035
Yes	19	1 (ref)		5	1 (ref)		14	1 (ref)	
BMI (kg/m^2)									
<17	134	0.82 (0.29−2.35)	0.713	56	0.87 (0.21−3.58)	0.848	78	0.77 (0.16−3.82)	0.748
≥17	36	1 (ref)		21	1 (ref)		15	1 (ref)	

BMI, body mass index; CI, confidence interval; OR, odds ratio; ref, reference
*To few numbers of participants.
All P values are given with unadjusted logistic regression.

Nordic countries; however, the present study had a higher number of participants (81%) with s-25(OH)D$_3$ >50 nmol/L.

In a previous study, we investigated vitamin D status among adolescents from Bergen, Norway during winter time, and participants had a mean s-25(OH)D$_3$ concentration of 49 nmol/L, and 46% of participants had s-25(OH)D$_3$ >50 nmol/L [52]. These results are somewhat lower compared with those of the preschool children in the present study, which compares with the results of other cross-sectional studies reporting higher concentrations in children than in adolescents [20,53]. Furthermore, results from the present study indicate that preschool children had a higher adherence to Norwegian dietary recommendations [38] than adolescents [43], which may have influenced their vitamin D status. On the other hand, in the present study, no significant associations were found between vitamin D−rich dietary sources and s-25(OH)D$_3$ >50 or 75 nmol/L. However, these results are limited by the fact that the FFQ used in the present study did not include all vitamin D−rich food products available and did not take the amount of food eaten into account.

Our results indicate that overweight status was related to s-25(OH)D$_3$ ≤50 nmol/L for all children. Vitamin D is fat soluble and stored in adipose tissue. Hence, overweight- and obesity-related vitamin D deficiency might be due to the reduced bioavailability of vitamin D$_3$ from cutaneous and dietary sources because of its deposition in body fat compartments [24]. When we stratified our analyses on sex, an association between overweight status and s-25(OH)D$_3$ ≤50 nmol/L was seen among girls but not boys. However, BMI was similar among the sexes, but fat and lean mass were not measured. In general, girls have a higher fat mass than boys, independent of BMI [54]. This may explain why we found a significant association between overweight status and s-25(OH)D$_3$ ≤50 nmol/L among girls only.

Vitamin D deficiency has previously been found in overweight and obese children [20−23], and in the study by Turer et al. [22], vitamin D deficiency increased with an increasing BMI in children ages 6 to 18 y. We did not observe any significant association between s-25(OH)D$_3$ ≤50 nmol/L and obesity, but this may be due to the lack of power for this hypothesis (7 children in the study sample were obese).

Children who had not been exposed to sun through sun vacations at least 1 wk during the last 3 mo before study testing were at a greater risk of s-25(OH)D$_3$ ≤75 nmol/L. Only 10% of children had been on sun vacations during the winter time, which illustrates the impact of sun exposure on vitamin D status. Of note, the use of vitamin D supplements and s-25(OH)D$_3$ ≤75 nmol/L was borderline significant for the total group, and negatively associated for boys but not girls. For dairy products, a negative association with vitamin D ≤75 nmol/L was seen for girls only. In Norway, some dairy products are fortified with vitamin D. For example, low-fat milk (0.5% fat) is fortified with 0.4 μg vitamin D per 100 g. However, the questions from the FFQ did not include all dairy products enriched with vitamin D; hence, we do not know whether girls ate more vitamin D-enriched dairy products compared with boys.

Although 48% of participants reported that they ate fish or seafood for dinner ≥2 times per week, we did not observe any association between fish intake and vitamin D status. However, as previously mentioned, the FFQ in the present study did not take amount into account, so even if children appeared to eat fish frequently, the actual amount of intake may still be small. More importantly, the amounts of vitamin D differ in different fish types, and lean fish does not contribute considerably with vitamin D unless the meals also include fish liver. Owing to a considerable change in fish feed composition toward plant-based ingredients, the amount of vitamin D in farmed salmon fillet is relatively low [55]. This may be reflected in the current results where children consumed more farmed salmon or trout than other fatty fish types [38], and vitamin D intake from fish was still relatively low.

There are discussions on whether vitamin D status should be >50 nmol/L [8,9] or 75 nmol/L to secure optimal health outcomes, including bone health [7]. In the age group of children ages 1 to 18 y, at least 600 IU/d (15 μg/d) of vitamin D is suggested to maximize bone health [8,9], but whether this is sufficient for all health benefits

of vitamin D is not known. To increase the cutoff to >75 nmol/L, at least 1000 IU/d (25 μg/d) of vitamin D is necessary [7]. However, there is no evidence to how much vitamin D is required to prevent vitamin D deficiency in children ages 1 to 9 y [7]. Only 16% of children in the present study had s-25(OH)D$_3$ >75 nmol/L, but as many as 81% had s-25(OH)D$_3$ >50 nmol/L. According to the evidence so far, most children appeared to have s-25(OH)D$_3$ >50 nmol/L during winter time, but more research is needed to identify optimal vitamin D status for different health outcomes in children and adolescents.

The main limitation of the present study is that the FFQ did not include vitamin D−fortified products for children (e.g., vitamin D-fortified milk [0.4 μg/100 g] and yogurt [1.4 μg/100 g]) because this was planned to primarily study fish consumption and cognitive outcomes [38]. The number of meals per week was evaluated, but the amount of different food sources and total energy intake could not be calculated. In addition, body weight and height was self-reported by the children's caregivers, and information regarding skin color was not obtained; however, few children (2.1%) were born in countries other than Norway. Furthermore, we measured total s-25(OH)D$_3$ and were unable to distinguish between bioavailable 25(OH)D$_3$ (free or bound to albumin) compared with 25(OH)D$_3$ tightly bound to vitamin D binding protein [56].

A strength of our study is the method used to analyze vitamin D where the metabolites could be separated. In other methods, the separation of 25(OH)D$_3$ and 3-epi-25(OH)D$_3$ is not possible; thus, 25(OH)D$_3$ might be overestimated [57]. In addition, vitamin D metabolites were analyzed from as many as 91% of participants.

Conclusions

Our results suggest that the majority of children ages 4 to 6 y from Bergen, Norway had s-25(OH)D$_3$ >50 nmol/L during winter time. Children with an overweight status had an increased risk of s-25(OH)D$_3$ ≤50 nmol/L, and children who had not been on recent sun vacations were at a greater risk of s-25(OH)D$_3$ ≤75 nmol/L.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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