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ORIGINAL CONTRIBUTION

Vitamin D status of 3-year-old children in Denmark: determinants and associations with bone mineralisation and blood lipids

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Abstract

Purpose Low vitamin D status is a global problem and has been associated with reduced skeletal and cardiometabolic health. However, evidence in young children is lacking. We, therefore, aimed to characterise vitamin D status in toddlers, identify its determinants, and explore if vitamin D status was associated with bone mineralisation and lipid profile.

Methods We used cross-sectional data from 3-year-old children ($n = 323$) living in Denmark (latitude: 55°N). Bone mineralisation ($n = 108$) was measured by DXA. Blood samples were analysed for serum 25-hydroxyvitamin D (s-25(OH)D) by LC–MS/MS, triacylglycerol, and total, low- and high density lipoprotein cholesterol.

Results Mean ± SD s-25(OH)D was 69 ± 23 nmol/L, but varied with season. During winter, 38% had inadequate s-25(OH)D (< 50 nmol), whereof 15% had deficiency (< 30 nmol/L); these numbers were only 7 and 1% during summer. In terms of status determinants, supplement use (66% were users) was associated with s-25(OH)D ($P < 0.001$), whereas dietary vitamin D intake (median [25–75th percentile] of 1.3 [0.9–1.9] µg/d), sex, parental education, BMI, and physical activity were not. There were no associations between s-25(OH)D and blood lipids or bone measurements, using either unadjusted or adjusted regression models.

Conclusion More than 1/3 of Danish toddlers had inadequate vitamin D intake during winter, but acceptable mean vitamin D status. In addition to season, supplement use was the main determinant of vitamin D status, which was, however, not associated with bone mineralisation or lipid profile. The results support recommendations of vitamin D supplements during winter at northern latitudes, but potential health effects need further investigation.

Keywords Cholecalciferol · Predictors · DXA · Fat mass · BMI · Cardiometabolic markers

Introduction

Vitamin D has received much attention during the last decade due to widespread vitamin D deficiency globally and its association with both skeletal and extra-skeletal health [1–3]. Early childhood is a vulnerable period of profound growth and development during which maintenance of sufficient vitamin D status may be particularly important for health outcomes later in life. However, evidence on the prevalence and predictors of low vitamin D status, as well as its effects on health, is lacking in young children.

The prevalence of vitamin D insufficiency, defined as serum 25-hydroxyvitamin D (s-25(OH)D) < 50 nmol/L, has been reported to be as high as 20–70% in children and adolescents living at northern latitudes [1, 4–10]. Yet, this is not well described in toddlers, but has been reported to be around 20–30% among 2- to 6-year-olds living in Denmark, Sweden, and Ireland across seasons, but with a much higher prevalence during winter than summer months [11–13]. Since vitamin D occur naturally in only few foods, vitamin D intakes are low in most countries worldwide [3], especially in those with limited fortification, such as Denmark [14, 15]. All European countries recommend vitamin D supplementation during infancy [3], but until December 2020, supplements were not recommended in the Nordic countries past the first 2 years of life [14].

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Identification of determinants of vitamin D status is important for preventing low vitamin D status. Aside from sun exposure [4, 10, 13, 16–21] and dietary vitamin D intake [6, 13, 16, 18–20, 22], age [10, 18–20], body mass index (BMI) [22, 23], and time spend on physical activity (PA) [4, 19, 20] have previously been associated with vitamin D status in children. Most of the existing literature is, however, based on children above 4 years of age, whereas determinants of vitamin D status in toddlers are not well characterised.

Vitamin D is important for optimal bone mineralisation via effects on intestinal absorption of calcium [3]. Some [5, 7, 24, 25] but not all studies [8, 9, 21, 26] in children have shown a positive association between s-25(OH)D and measures of bone mineralisation. Further, we recently found that vitamin D supplementation during winter increased bone mineralisation in 6- to 8-year-old Danish children [27]. Apart from bone, vitamin D receptors are also located in cells of the cardiovascular and immune system [3], and in children, s-25(OH)D has been associated with a more favourable blood lipid profile [28–32], although not consistently [33]. In our previous study in 6- to 8-year-olds, vitamin D supplementation reduced low-density lipoprotein cholesterol (LDL-C) [34], but other studies showed no effects on blood lipids [35, 36] or even increased LDL-C [37]. Thus, evidence on vitamin D and health-related outcomes in children is conflicting, and we lack studies in the younger age groups.

Thus, the aims of the present cross-sectional study were to (1) characterise vitamin D status as reflected by s-25(OH)D in 3-year-old Danish children, (2) identify determinants of vitamin D status at this age, and (3) explore possible associations between vitamin D status and bone mineralisation as well as lipid profile in these children. We hypothesised that there would be seasonal differences in vitamin D status, and that any associations between vitamin D intake and use of supplements, respectively, and vitamin D status would differ according to season. Further, we hypothesised that vitamin D status would be positively associated with measures of bone mineralisation and with a more favourable blood lipid profile. In order to base this on a considerable and broad sample of Danish toddlers, we pooled data from two cohorts that included children of mothers with mainly normal weight and obesity, respectively, that were examined using similar protocols and across all seasons. Secondarily, we explored whether any of the results were cohort-specific.

Methods

Study design and population

The study included children from two Danish child cohorts, SKOT-I [38] and SKOT-II [39]. The overall aim of both cohorts was to describe how complementary feeding influences growth and later disease risk, and inclusion criteria were term singletons aged 9 months ± 2 weeks without illnesses known to affect diet or growth and living in Copenhagen or Frederiksberg (latitude: 55°N). In SKOT-I, infants were recruited from the National Danish Civil Registry [38]. In SKOT-II, infants were recruited among pregnant women participating in the intervention study 'Treatment of Obese Pregnant Women' at Hvidovre Hospital (Hvidovre, Denmark) [40], and thus had a pre-pregnancy BMI > 30 kg/m^2. The two cohorts followed the same measurement protocol and together represent a wide range of maternal weight statuses and social classes [39]. SKOT-I was conducted from April 2007 to October 2010 and SKOT-II from January 2011 to March 2015. The studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark (SKOT-I: H-KF-2007-0003; SKOT-II: H-3-2010-122). Written informed consent was obtained from all custody holders of the children.

In SKOT-I and SKOT-II, respectively, 329 and 183 infants were enrolled, and examinations took place at age 9, 18, and 36 months. The present study uses cross-sectional data from the 3-year examination and includes only children with available s-25(OH)D, corresponding to 67% (n = 220) and 56% (n = 103) of the children in the original SKOT-I and SKOT-II cohorts, respectively. Bone outcomes were only measured in SKOT-I (n = 108).

Background information and season

Parental education was obtained from an interview at the 36-month visit, defined as the highest completed education by any parent, and categorised as short (ranging from ≤ lower secondary education to short higher education < 3 years), medium (3–4 years), or long (> 4 years). We considered children's skin pigmentation due to its influence on dermal vitamin D synthesis; however, this was only registered for those with a dual-energy X-ray absorptiometry (DXA) scan (n = 166), where 98% were characterised as "white". Season of examination was categorised as spring (March–May), summer (June–August), autumn (September–November), and winter (December–February). As vitamin D was not a focus of the cohorts at their planning stages, there were no data on use of sunscreen, time spend outdoor, or sunny vacations. Almost all children (94% of children in SKOT-I and all in SKOT-II) had access to own or shared garden or court, so this was not meaningful to include in the analyses.

Dietary intake and use of supplements

Dietary vitamin D intake was assessed from 7-day (SKOT-I) or 4-day (SKOT-II) validated, pre-coded dietary records [41]. The intake was calculated by the National Food Institute, Technical University of Denmark, using the GIES software (version 1.000 d; National Food Institute, Søborg, Denmark), which uses data from the Danish food composition database [42]. Parents further recorded time and content of the child's last meal before the examination visit, from which energy and macronutrient intake was calculated. Frequency of supplement use was obtained from interview at the visit, and since the majority of multivitamins in Denmark contain vitamin D, multivitamins were categorised as vitamin D containing supplements along with vitamin D drops and vitamin D tablets. Use of vitamin D supplements was categorised as "yes" when the frequency was ≥ 1/week, and as "no" when < 1/week. Data regarding the dose of vitamin D supplied by the supplementation was not available and thus total vitamin D intake could not be estimated.

Physical activity

As previously described [43, 44], time spend on PA was measured during 7 days and nights by tri-axis accelerometer (Actigraph GT3X), which was placed on the right hip by an elastic waistband. Parents were instructed only to take it off when the child was showering or swimming and to note any non-wear time. The data was analysed using the Actilife software version 6.4.5 (Actigraph, Pensacola, Florida) and expressed as mean counts per minute (CPM) per day from at least four valid days (> 8 h wear time). Data were available from 197 and 79 children from SKOT-I and SKOT-II, respectively.

Anthropometry and body composition

Measurements of anthropometrics and body composition are described in detail elsewhere [45, 46]. In brief, body weight was measured once without clothes to the nearest 0.1 kg using a Tanita WB-100MA digital scale (Tanita, Amsterdam, Netherlands). Height was measured in triplicate to the nearest 0.1 cm using a 235 Heightronic Digital Stadiometer (Quick Medical and Measurement Concepts, USA) and the mean was used in the analyses. BMI was calculated as weight divided by height squared, and age- and sex-specific z-scores were calculated by using the software WHO Anthro 2005 [45].

Fat mass and fat free mass were obtained by bioelectrical impedance using a Quantum III (RJL Systems, USA). Measurements were performed twice and mean resistance values used in the equation to predict fat free mass. Because equations for children in early childhood was scarce, the equation was developed in SKOT-I and validated by cross-referencing with DXA data, as previously described [46]. Data from bioelectrical impedance was chosen over estimates derived from DXA in the present study because DXA scans were only performed in SKOT-I [45]. Fat mass index (FMI) and fat free mass index (FFMI) were calculated as fat mass and fat free mass, respectively, divided by height squared.

Bone mineralisation

Bone mineral content (BMC), bone area (BA), and bone mineral density (BMD) were obtained in SKOT-I using a Lunar Prodigy Advance with enCore software version 12.3 (GE Healthcare, USA). Whole body and regional measures were obtained and total body less head (TBLH) calculated as the sum of arms, legs, and trunk. Results were available from 166 children. Due to their young age, some children shifted position during the scan, which was categorised as "perfect scan", "good scan with minor irregularities", "scan with several irregularities", or "useless scan" [45]. Only "perfect" ($n = 74$) and "good" ($n = 34$) scans were included in the present study.

Blood sampling and analyses

As previously described [38], 5 mL venous blood samples were drawn from the forearm in lithium-heparinized tubes after an average ± SD of 2.9 ± 0.7 h of fasting. Time since and composition of the last meal were recorded. Serum triacylglycerol and total (total-C), high-density lipoprotein cholesterol (HDL-C), and LDL-C were analysed using a Pentra 400 (HORIBA ABX) with intra- and inter-assay CV < 4 and ≤ 2%, respectively. Serum 25(OH)D were analysed by LC–MS/MS at University College Cork, Ireland, as described elsewhere [47]. Total s-25(OH)D was calculated as the sum of s-25(OH)D_2 and 25(OH)D_3, and the intra- and inter-assay CVs for both metabolites were < 5 and < 6%, respectively. Vitamin D sufficiency, insufficiency and deficiency were defined as s-25(OH)D ≥ 50 nmol/L, < 50 and ≥ 30 nmol/L, and < 30 nmol/L, respectively, as suggested by the IOM [48].

Statistical analyses

Data are presented as mean ± SD, median [25–75th percentile], or *n* (%), as appropriate. All analyses were carried out in Stata version 16.1 and $P < 0.05$ was considered statistically significant. Differences between cohorts and between included and excluded children were tested with Pearson's chi-squared test, two-sample t-test, or Wilcoxon rank-sum test.

To identify determinants of vitamin D status, we performed ANCOVA of s-25(OH)D as a function of potential

explanatory variables. These were sex (male/female), parental education (short/medium/long), BMI z-score, weight category (under-/normal-/overweight and obesity), FMI, FFMI, season of blood sampling (summer/autumn/winter/spring), dietary vitamin D intake, use of vitamin D supplements (yes/no), and PA. Further, we assessed whether interactions with season existed within the associations between s-25(OH)D and vitamin D intake (season × intake) and supplement use (season × supplements), as has been reported by others [13, 49, 50]. First, these potential determinants were tested one at a time with cohort as a fixed effect and s-25(OH)D as the outcome variable. Then, sex, parental education, FMI, FFMI, season, vitamin D intake, use of supplements, and PA were all introduced in the same, mutually adjusted model. Finally, we explored whether associations were cohort-specific by including determinant × cohort for all potential determinants in the mutually adjusted model, and stratified the analyses by cohort if the interaction term was significant. Since all but two children were recorded as "white", and since all children were 3.0 ± 0.1 years old, we did not evaluate skin pigmentation or age as determinants.

To explore associations between s-25(OH)D and bone mineralisation as well as blood lipids, we ran linear regression models with the outcomes as dependent variables and s-25(OH)D as explanatory. The crude models included cohort as a fixed factor, and all adjusted models included sex, parental education, and PA, which were pre-specified potential confounders. Additional data-specific potential confounders were included in the relevant models when these were associated with both s-25(OH)D and the outcome in the ANCOVAs described above. As season was associated with both s-25(OH)D and BMC, BMD, and HDL, all adjusted models were adjusted for season. Due to short fasting before blood sampling, blood lipids were further adjusted for fat content (gramme) of last meal, and BMI z-score was investigated as a mediator in the blood lipid models. Bone outcomes were analysed both with and without size-adjustment for height, weight, and BA (BMC only), as recommended by Prentice et al. [51]. Finally, the interaction term s-25(OH)D × cohort was included in each adjusted model to assess whether associations were cohort specific. Model assumptions were checked using residual and Q–Q plots, and plasma triacylglycerol was log-transformed to fulfil these.

Results

Children's characteristics

The 323 children included in the present study had slightly higher PA compared to those not included (1316 vs 1242 CPM/d, $P = 0.040$), but did not differ in age, sex, parental education, vitamin D intake or supplement use, anthropometrics, body composition or bone measures ($P > 0.1$). The included children had a mean ± SD age of 3.0 ± 0.1 years, with an equal number of boys and girls (Table 1), and examinations were evenly distributed among the four seasons (Table 2). Children in SKOT-II had parents with lower parental education level, as previously reported [39], and had higher weight, BMI z-score [52], and FMI, as well as lower total-C than children in SKOT-I (Supplemental Table 1).

Table 1 Characteristics of included children

	All	SKOT-I	SKOT-II	P^a
n^b	323	220	103	
Sex (% girls/boys)	48/52	50/50	45/55	0.41
Age (years)[c]	3.0 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	0.21
Parental education (% short/medium/long)	20/31/49	15/28/57	31/39/30	< 0.001
Height (cm)[c]	96.1 ± 3.5	95.8 ± 3.4	96.6 ± 3.7	0.076
Weight (kg)[c]	14.9 ± 1.7	14.7 ± 1.6	15.3 ± 1.9	0.003
BMI Z-score	0.44 ± 0.93	0.29 ± 0.90	0.74 ± 0.92	< 0.001
Fat mass index (kg/cm^2)	0.28 ± 0.09	0.28 ± 0.08	0.30 ± 0.09	0.021
Fat free mass index (kg/cm^2)	1.33 ± 0.07	1.33 ± 0.07	1.34 ± 0.07	0.33
Physical activity (CPM/d)[c]	1316 ± 242	1329 ± 226	1284 ± 278	0.17

Values are mean ± SD unless otherwise stated. Abbreviations: s-25(OH)D, serum 25-hydroxyvitamin D; BMI, body mass index; CPM, counts per minute

[a]Differences between cohorts were tested with Pearson's chi-squared test, two-sample *t* test, or Wilcoxon rank-sum test, as appropriate. The differences in parental education and sex distribution at 9 months [39] as well as BMI Z-score at 36 months [52] have been reported previously

[b]*n* Represent maximal numbers; for all, SKOT-I and SKOT-II, respectively, $n = 309$, $n = 214$, and $n = 95$ for fat and fat free mass index, and $n = 276$, $n = 197$, and $n = 79$ for physical activity

[c]Have been reported previously for SKOT-I ($n = 208$) and SKOT-II ($n = 79$) [44]

Vitamin D intake and status

The children had a very low dietary vitamin D intake (Table 2), which was slightly higher in SKOT-I (1.3 [1.0–2.0] µg/d) than SKOT-II (1.0 [0.8–1.7] µg/d) ($P = 0.003$). Fewer than 1% of the children had dietary vitamin D intakes reaching the recommended intake of 10 µg/d, but 66% received vitamin D supplements at least once per week, with no difference between the cohorts (65% and 70% in SKOT-I and SKOT-II, respectively, $P = 0.34$). Mean ± SD s-25(OH)D was 68.6 ± 23.0 nmol/L, and the majority of the children had s-25(OH)D ≥ 50 nmol/L, whereas only 5% ($n = 17$) had values < 30 nmol/L (Table 2). As expected, there were substantial seasonal differences, as vitamin D status was highest during summer, lower during autumn and spring, and lowest during winter. The prevalence of s-25(OH)D ≥ 50 and < 30 nmol/L, respectively, were 93 and 1% during summer and 62% and 15% during winter (Table 2). Four children who all received supplements and were measured during summer ($n = 3$) or spring ($n = 1$) had high s-25(OH)D ≥ 125 nmol/L, of which one had a very high value of 190 nmol/L.

Determinants of vitamin D status

Apart from season of examination, use of vitamin D supplements was positively associated with s-25(OH)D in both the crude and mutually adjusted models (Table 3). Also, most (82%) of the cases of vitamin D deficiency (s-25(OH)D < 30 nmol/L) were found among children that did not receive supplements, comprising 13% of the unsupplemented group. Supplement users had higher s-25(OH)D than non-users regardless of season ($P_{\text{season×supplements}} = 0.13$) (Supplemental Figure 1). However, whereas 80% of the supplement users had s-25(OH)D ≥ 50 nmol/L during winter, sufficiency was only found in 23% of the children who did not receive supplements at this time of year. During summer, the corresponding numbers were 97 and 86%. Sex, parental education, BMI, body composition, and PA were not associated with s-25(OH)D, nor was dietary vitamin D intake (Table 3), and this did not depend on season ($P_{\text{season×intake}} = 0.44$). Compared to children with normal weight, those with overweight and obesity had lower s-25(OH)D in the crude model ($P = 0.019$), but this association disappeared after adjustment for sex, parental education, season, vitamin D intake, use of supplements, and PA ($P = 0.98$) (data not shown). Cohort-specific associations were seen for FMI ($P_{\text{FMI×cohort}} = 0.029$) and PA ($P_{\text{PA×cohort}} = 0.039$), due to an inverse association between FMI and s-25(OH)D and an indication of a positive association between PA and s-25(OH)D, respectively, in SKOT-II only (Supplemental Table 2).

Associations between vitamin D status and bone mineralisation and blood lipids

None of the bone outcomes or blood lipids were associated with s-25(OH)D, neither in the crude nor adjusted regression models (Table 4), and there were no cohort-specific associations (data not shown).

Discussion

In this population of toddlers living at 55°N and measured across the year, 81% had sufficient vitamin D status, whereas only 14 and 5% had insufficiency and deficiency. Season and supplement use were important determinants of s-25(OH)D, which was more than 20 nmol/L higher during summer than

Table 2 Vitamin D status, intake, and use of supplements in the children throughout the year and by season

	All year	Summer	Autumn	Winter	Spring
n[a]	323	98	72	81	72
S-25(OH)D (nmol/L)	68.6 ± 23.0	77.0 ± 22.7	68.6 ± 19.6	58.9 ± 23.7	68.2 ± 21.6
S-25(OH)D					
< 30 nmol/L	17 (5)	1 (1)	1 (1)	12 (15)	3 (4)
≥ 30 and < 50 nmol/L	45 (14)	6 (6)	10 (14)	19 (23)	10 (14)
≥ 50 and < 75 nmol/L	144 (45)	43 (44)	39 (54)	30 (37)	32 (44)
≥ 75 and < 125 nmol/L	113 (35)	45 (46)	22 (31)	20 (25)	26 (36)
≥ 125 nmol/L	4 (1)	3 (3)	0 (0)	0 (0)	1 (1)
Vitamin D intake from diet (µg/d)	1.3 [0.9–1.9]	1.5 [0.9–2.1]	1.3 [0.9–1.7]	1.3 [1.0–2.0]	1.3 [0.9–1.7]
Use of vitamin D supplements, yes	214 (66)	63 (64)	46 (64)	55 (68)	50 (69)

Values are mean ± SD, number (%), or median [25–75th percentile]

s-25(OH)D serum 25-hydroxyvitamin D

[a]n represent maximal numbers; for vitamin D intake from diet, $n = 279$, $n = 88$, $n = 67$, $n = 67$, $n = 57$ for all year, summer, autumn, winter, and spring, respectively

Table 3 Potential determinants of s-25(OH)D in the children ($n = 323$)

	Crude[a]				Mutually adjusted[b]		
Determinant	*n*	*β*	95% CI	*P*	*β*	95% CI	*P*
Cohort							
SKOT-I	220						
SKOT-II	103	− 3.2	− 8.6, 2.2	0.25	− 3.3	− 9.8, 3.2	0.32
Sex							
Girls	155	Reference			Reference		
Boys	168	1.3	− 3.8, 6.3	0.62	− 0.7	− 7.7, 6.4	0.85
Parental education				0.28			0.9329
Short	64	Reference			Reference		
Medium	101	− 0.3	− 7.5, 7.0	0.95	− 1.4	− 9.5, 6.7	0.74
Long	156	4.0	− 2.8, 10.7	0.25	− 1.3	− 9.0, 6.4	0.73
FMI (kg/m^2)	309	− 21.9	− 51.8, 8.0	0.15	− 19.8	− 57.0, 17.5	0.30
FFMI (kg/m^2)	309	24.2	− 12.7, 61.2	0.20	24.9	− 26.0, 75.8	0.34
Season of examination				< 0.001			< 0.001
Summer	98	Reference			Reference		
Autumn	72	− 8.5	− 15.2, − 1.7	0.014	− 8.5	− 15.8, − 1.2	0.022
Winter	81	− 18.1	− 24.6, − 11.6	< 0.001	− 14.2	− 21.5, − 6.9	< 0.001
Spring	72	− 8.9	− 15.6, − 2.1	0.010	− 12.5	− 19.8, − 5.1	0.001
Vitamin D supplements[c]							
Yes	214	Reference			Reference		
No	109	− 19.4	− 24.3, − 14.5	< 0.001	− 19.6	− 25.1, − 14.2	< 0.001
Vitamin D intake (µg/d)[d]	279	0.3	− 1.5, 2.1	0.73	0.4	− 1.4, 2.3	0.65
PA (per 100 CPM/d)	276	0.8	− 0.3, 2.0	0.16	− 0.2	− 1.4, 1.0	0.77

s-25(OH)D serum 25-hydroxyvitamin D, *FMI* fat mass index, *FFMI* fat free mass index, *PA* physical activity, *CPM* counts per minute

[a]Regression estimates with 95% CI and corresponding *P* values from one-way ANOVA only adjusted for cohort

[b]Regression estimates with 95% CI and corresponding *P* values from one-way ANCOVA where all predictors were mutually adjusted for one-another ($n = 239$)

[c]Vitamin D supplementation ≥ once per week

[d]Vitamin D intake from diet, not including supplements

winter and among supplement users than non-users, and this was also reflected in the prevalence of deficiency. FMI was a negative determinant of s-25(OH)D in the cohort of children with obese mothers. Nevertheless, vitamin D status was not associated with bone health or blood lipids in the toddlers.

The finding of noteworthy seasonal differences in vitamin D status was likely due to fluctuations in sun-induced dermal vitamin D synthesis. This is in line with observations in other paediatric populations [6, 10, 17–22, 53], including pre-school children in Denmark, Sweden, and Ireland [11–13]. This indicates that deficiencies may be overlooked when vitamin D status is evaluated as a mean across the year, and should instead, or at least, be evaluated during the vitamin D winter. We hypothesised that the importance of supplement use for vitamin D status would depend on season, but even during summer months, s-25(OH)D was higher in those receiving supplements, thus supporting the new Danish recommendation of year-round supplementation to toddlers [54]. On the contrary, supplementation during summer may lead to high levels of vitamin D [55], and s-25(OH)D > 125 nmol/L was seen in four children in the present study, although this was not likely caused by the typical supplementation dose of just 10 µg/day. Further, there appeared to be larger differences between supplement users and non-users in the prevalence of deficiency and insufficiency during winter/spring than during summer/autumn, which has also been shown in previous studies among children [11, 13, 49, 50]. Thus, the results underline the importance of vitamin D supplements being particularly important for maintaining vitamin D sufficiency during the vitamin D winter at these northern latitudes, i.e. during October to March when negligible amounts of vitamin D is synthesised in the skin [1].

Our finding of a mean s-25(OH)D of 69 nmol/L and 19% having insufficiency or deficiency corresponds well with findings in other paediatric populations below the age of 6 years [2, 11, 13, 16–18, 22, 35, 53, 56, 57], although a lower proportion may have insufficiency at lower latitudes

Table 4 Associations between s-25(OH)D and bone mineralisation and blood lipids in the children

	Crude[a]				Adjusted[b]			
	n	*β*	95% CI	*P*	*n*	*β*	95% CI	*P*
Bone mineralisation of TBLH[c]								
Bone mineral content (g)	108	− 1.8	− 5.0, 1.5	0.28	101	− 1.8	− 5.0, 1.4	0.26
Bone area (cm^2)	108	0.4	− 0.3, 1.0	0.26	101	0.2	− 0.5, 0.8	0.57
Bone mineral density (g/cm^2)	108	− 3.2	− 7.6, 1.2	0.15	101	− 3.1	− 7.5, 1.3	0.16
Blood lipids[d]								
Total cholesterol (mmol/L)	319	− 0.0003	− 0.04, 0.04	0.99	262	0.004	− 0.04, 0.05	0.85
HDL cholesterol (mmol/L)	319	0.005	− 0.007, 0.02	0.44	262	0.005	− 0.008, 0.02	0.47
LDL cholesterol (mmol/L)	319	− 0.009	− 0.04, 0.02	0.60	262	− 0.0009	− 0.04, 0.03	0.96
Triacylglycerol (mmol/L)[e]	319	0.005	− 0.03, 0.02	0.63	262	− 0.002	− 0.02, 0.02	0.83

s-25(OH)D serum 25-hydroxyvitamin D, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TBLH* total body less head

[a]Regression estimates and 95% CI per 10 nmol/L increase in s-25(OH)D from one-way ANOVA. Cohort was included as fixed factor in blood lipid models, but not in bone models, as bone mineralisation was only measured in SKOT-I

[b]Regression estimates and 95% CI per 10 nmol/L increase in s-25(OH)D from one-way ANCOVA adjusted for sex, parental education, physical activity, and season

[c]Bone mineralisation was only measured in SKOT-I, and only perfect and good scans of TBLH are included. The adjusted models were further size-adjusted for height, weight, and BA (BMC only)

[d]The adjusted blood lipid models were further adjusted for fat content of last meal

[e]Triacylglycerol were log-transformed in the models

[2, 16, 22]. In older children, s-25(OH)D is generally lower and the prevalence of insufficiency higher [2, 5–11, 19, 20, 32, 50, 53, 57]. This may be due to less time spent outdoor as well as fewer supplement users among the older age groups [11, 58], as use of vitamin D supplements has been shown to be a major determinant of vitamin D status both in the present and in most observational studies and supplementation trials in children [13, 16, 19, 20, 22, 59, 60]. In our population, 2/3 of the children received vitamin D supplements, which shows that many Danish parents continued supplementing their children past the age of 2 years despite no official recommendation to do so at that time. In contrast, dietary vitamin D intake was not associated with s-25(OH)D, probably because of the low intake [4, 19, 20], which may in part be due to the fact that food fortification is rare in Denmark. In e.g. Ireland, Finland, and Canada, milk and/or other foods are commonly fortified with vitamin D, and intake of fortified milk is a major determinant of children's vitamin D status in these countries [6, 13, 22], together with season and supplement use [13, 22].

Although body composition was not a determinant of vitamin D status in the overall population, higher FMI was linked to lower s-25(OH)D in SKOT-II, where the children had higher FMI and BMI Z-score than in SKOT-I. This may indicate that adipose tissue affects vitamin D status only above a certain amount of fat mass and supports the speculated depot effect or sequestration of vitamin D in adipose tissue [61]. In line with this, a recent review in adults showed the strongest association between measures of adiposity and s-25(OH)D in persons with obesity [61]. Further, a cross-sectional analysis in Dutch children showed a 1.03 (1.01, 1.05) higher OR of s-25(OH)D < 50 nmol/L for each kg/m^2 higher maternal BMI [10], and although we did not have information on maternal BMI at 3 years, the cohort-specific finding for FMI could perhaps be linked to maternal BMI. Socioeconomic differences between the cohorts could also play a role for s-25(OH)D, but we tried to minimise this potential confounding by adjusting for parental education. Vitamin D status has been shown to be higher in more physically active children [4, 6, 19, 20], but this may be due to more time spend outdoor and not PA per se, as these are usually difficult to distinguish. Time spend outdoor has indeed been related to vitamin D status in some [10, 22], although not all [4, 18] studies in pre-school and school-children. Yet, it is unclear why PA was only associated with s-25(OH)D in SKOT-II.

To our knowledge, there is no reference data for bone-related outcomes in this young age group, and it is difficult to relate DXA numbers to those of previous studies, when these are not comparable regarding both age group, type of DXA scanner, and reporting of TBLH results. However, as the included children were healthy, we expect them to have numbers within the normal range for their age group. Due to vitamin D's known effects on calcium homeostasis [3], we hypothesised that vitamin D status would be associated with measures of bone mineralisation, as seen in some previous studies among children at different ages, who has measurements performed throughout the year or during winter [5,

7, 24, 25]. However, bone mineralisation was not associated with vitamin D status in the present study, which may be due to the year-round cross-sectional design and the low proportion (5%) of children with s-25(OH)D < 30 nmol/L, above which calcium absorption is considered sufficient by the IOM and risk of rickets is minimal [48]. Although this prevalence was higher during winter, the lower s-25(OH)D during winter may not result in health consequences when the period of deficiency is short and sufficiency is restored during summer. Further, there may be a lag from changes in vitamin D status to changes in bone mineralisation due to the rate of bone growth, and the cross-sectional design may therefore not be optimal to investigate this association. Finally, any potential haltering in bone mineralisation that may be induced during winter may be counteracted during summer. However, in a strict winter design, we recently found smaller increases in bone mineralisation of the whole body and lumbar spine when s-25(OH)D decreased during winter compared to when it was upheld among 6- to 8-year-olds [27]. Although some trials showed higher bone mineralisation after vitamin D supplementation [62, 63], the lack of association in the present study are in line with the results of other observational studies [8, 9, 21, 26] and trials [64–70] across the year or during winter in children of different age groups.

Vitamin D status was not associated with blood lipids in the present study. This is in contrast with our group's previous finding that s-25(OH)D was inversely associated with total-C, HDL-C, and triacylglycerol in SKOT-I at 9 months of age [28]. However, these results were not adjusted for parental education. Comparable associations were reported in some other child populations [29–31], and in our recent trial, vitamin D supplementation of 20 ug/d reduced LDL-C compared to placebo [34]. Vitamin D may affect blood lipids by increasing catabolism and/or decreasing synthesis of cholesterol upon activation of the vitamin D receptor, as has been shown in rodents [71, 72]. However, numerous observational studies [33, 73, 74] and randomised trials [35–37] has not been able to link vitamin D and blood lipids in children and it is possible that vitamin D may not be a major regulator of blood lipids at normal blood lipid concentrations.

Strengths of our study include the investigation of determinants and associations in a broad study population of toddlers, which gave a considerable sample size and represented the general population better than either cohort alone. Further, s-25(OH)D was analysed by certified LC–MS/MS [47] and bone outcomes by DXA, which are considered the gold standard methods for assessing vitamin D status as well as BMC and areal BMD in children [75], respectively. Although parents with long education were somewhat over-represented compared to national numbers [76], all education levels were represented, and the analyses were carefully adjusted for potential confounders. Limitations of our study include the low number of acceptable DXA scans, which may have resulted in false negative findings. Further, blood samples were collected after a short fasting period, which introduces meal-dependent variation in the blood lipids. However, non-fasting mainly affects plasma triacylglycerol, not LDL and HDL cholesterol, and a longer fasting period would have been practically and ethically difficult in this age group. We had no data on current growth or growth spurts, which are likely to affect DXA results, but we conducted size-adjusted analyses. It would have been valuable to have had information about dose of supplementation and children's sun behaviour, intake of ultra-processed foods, and structured physical activity, but such data were not considered during the time of data collection, and the latter is rare in Denmark in this age group.

In conclusion, in this population of Danish toddlers, the prevalence of year-round vitamin D deficiency and insufficiency, respectively, was only 5% and 14%, but these numbers were twice as high during winter and much lower during summer. Besides season of examination, use of vitamin D supplements was a strong determinant of vitamin D status in the total population, and FMI a negative determinant in the cohort of children with obese mothers. These findings support recommendations of vitamin D supplements especially during winter in order to maintain sufficient vitamin D status among young children living at northern latitudes. However, vitamin D status was not associated with measures of bone mineralisation or blood lipids, so the clinical significance needs further investigation in randomised trials among children below 5 years of age.

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Author contributions NGS formulated the research questions, analysed the data, interpreted the findings, and wrote the paper; CM interpreted the findings; KDC analysed and interpreted s-25(OH) D; KFM designed the study and interpreted the findings; CTD formulated the research questions, interpreted the findings, and helped revise the manuscript. All authors reviewed, provided input to, and approved the final manuscript.

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Availability of data and materials Upon request, the research data can be shared with editors and reviewers in a de-identified form for verification of the research results.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The studies were conducted in accordance with the Declaration of Helsinki and approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark: SKOT I: H-KF-2007–0003; SKOT II: H-3–2010-122.

Informed consent Written informed consent was obtained from all custody holders of the children.

Study registration The studies were registered at clinicaltrials.gov as NCT02170428 and NCT02377973.

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