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Vitamin D status and its determinants in children and adults among families in late summer in Denmark

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Abstract

The impact of the familial relationship on vitamin D status has not been investigated previously. The objective of the present cross-sectional study was to assess serum 25-hydroxyvitamin D (25(OH)D) concentration and its determinants in children and adults among families in late summer in Denmark (56°N). Data obtained from 755 apparently healthy children (4–17 years) and adults (18–60 years) recruited as families (*n* 200) in the VitmaD study were analysed. Blood samples were collected in September–October, and serum 25(OH)D concentration was measured by liquid chromatography–tandem MS. Information on potential determinants was obtained using questionnaires. The geometric mean serum 25(OH)D concentration was 72·1 (interquartile range 61·5–86·7) nmol/l (range 9–162 nmol/l), with 9 % of the subjects having 25(OH)D concentrations <50 nmol/l. The intra-family correlation was 0·27 in all subjects, 0·24 in the adults and 0·42 in the children. Serum 25(OH)D concentration was negatively associated with BMI ($P<0·001$) and positively associated with dietary vitamin D intake ($P=0·008$), multivitamin use ($P=0·019$), solarium use ($P=0·006$), outdoor stay ($P=0·001$), sun preference ($P=0·002$) and sun vacation ($P<0·001$), but was not associated with lifestyle-related factors in the adults when these were assessed together with the other determinants. In conclusion, the majority of children and adults among the families had serum 25(OH)D concentrations >50 nmol/l in late summer in Denmark. Both dietary and sun-related factors were determinants of vitamin D status and the familial component was stronger for the children than for the adults.

Key words: **Serum 25-hydroxyvitamin D: Determinants: Families**

The importance of vitamin D in bone health is recognised with rickets in children and osteomalacia and osteoporosis in adults being the traditional clinical conditions associated with vitamin D deficiency[1,2]. Furthermore, the expression of vitamin D receptors in different tissues[3] indicates additional biological functions of the vitamin. Low vitamin D status has been reported to be associated with a range of health outcomes[4,5], including an increased risk of cardiometabolic disorders[6], some cancers[7], autoimmune diseases[8] and mortality[9]. However, it is still unclear whether these associations are causal. Studies have found vitamin D status to be associated with sociodemographic and lifestyle-related factors[10,11]; thus, vitamin D status may serve as an indicator of general health and/or lifestyle.

The accepted biomarker of vitamin D status is 25-hydroxyvitamin D (25(OH)D) concentration in the blood[12]. In Denmark, the following values are used to define deficient, insufficient and sufficient serum 25(OH)D concentrations: <25; 25–50; >50 nmol/l[13]. The Institute of Medicine (USA) has defined 30 nmol/l as the limit beyond which adverse effects on bone might occur[14]. Serum 25(OH)D concentrations of 40 and 50 nmol/l have been considered to represent the estimated average requirement and the recommended daily allowance, which are assumed to meet the requirement in the average population and the majority of the population for bone health. A threshold value >125 nmol/l has been considered to be the at-risk value for harm by the Institute of Medicine (USA).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; DEQAS, Vitamin D External Quality Assessment Scheme; IQR, interquartile range; LC–MS/MS, liquid chromatography–tandem MS; NIST, National Institute of Standards and Technology; PTH, parathyroid hormone.

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The use of cut-off values and the comparison of vitamin D status between studies are complicated due to variations in measurements between the methods as well as among the laboratories using the same 25(OH)D assay[15–17]. In 2010, the National Institute of Standards and Technology (NIST) introduced a standard reference material for the measurement of vitamin D concentrations in human serum[18], which is expected to improve the analytical performance of 25(OH)D measurements and to facilitate harmonisation across 25(OH)D assays[15].

Thus, there is a need for comparative data on vitamin D status yielded by standardised and calibrated methods to better compare vitamin D status between population groups and evaluate the current situation of vitamin D deficiency. Vitamin D status has been measured in different population groups in Denmark[10,19–23]. None of the previous studies has assessed a broad range of age and sex groups in both children and adults. Especially, there is a lack of data on vitamin D status in young boys. One of the studies has reported vitamin D status in men, women and girls from Danish immigrant families[23]. However, to our knowledge, no previous studies have quantified the impact of the familial relationship on vitamin D status. This knowledge on vitamin D status within families will be helpful when considering strategies to improve vitamin D status.

The objective of the present study was to assess serum 25(OH)D concentration and its determinants in children and adults among families in late summer in Denmark.

Subjects and methods

Study population

The present cross-sectional study used baseline data obtained from the VitmaD study[24] conducted in Denmark (56°N). Children and adults were recruited as families randomly drawn from the Danish Civil Registration System. Inclusion criteria were age between 4 and 60 years and a permanent address in Gladsaxe Municipality. Exclusion criteria were pregnancy and disease or use of medication influencing vitamin D metabolism (including dietary supplements with vitamin D levels >10 μg/d for children and >5 μg/d for adults, which correspond to the typical levels in multivitamin supplements in Denmark). Of the 782 recruited children and adults, 755 (representing 200 families) had their serum 25(OH)D concentration measured and complete questionnaire data at baseline. The present analyses were conducted on these subjects. Written informed consent was obtained from all the adult subjects and from the guardians of the children. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Research Ethics Committee of the Capital Region of Denmark (record no. H-4-2010-020) and registered in ClinicalTrials.gov (NCT01184716).

Methods

The subjects were examined and their blood samples were collected in September–October 2010 in an authorised laboratory (Copenhagen's General Practitioners Laboratory, Søborg, Denmark). The weight of the subjects was measured to the nearest 0·1 kg in normal clothes without shoes (1 kg was subtracted from the measured weight) with a body composition analyser (Tanita BC-418; Tanita Europe B.V.). Height was measured to the nearest centimetre without shoes with an ultrasonic height measure (Soehnle 5001; Soehnle Professional GmbH & Company). BMI was calculated based on the measured weight and height, and the subjects were categorised into normal-weight, overweight and obese classes according to the standards for children[25] and the WHO International standard for adults[26]. Non-fasting venous blood samples were drawn from the subjects, and serum and plasma were collected and stored at −80°C until analysis.

Serum 25(OH)D concentration was measured at the Clinical Biochemical Department, Holbæk Hospital, Denmark, using isotope dilution liquid chromatography–tandem MS (LC–MS/MS) according to the principles described elsewhere[27]. The method was calibrated against the NIST standard for the analysis of vitamin D in human serum (standard reference material 972)[16], and the inter-assay CV for the method used in the present study were 2·2 and 2·8 % at 30 and 180 nmol/l, respectively, for 25(OH)D_3 and 7·6 and 4·6 % at 43 and 150 nmol/l, respectively, for 25(OH)D_2. The analytical quality of this method was ensured through participation in the Vitamin D External Quality Assessment Scheme (DEQAS). In this validation scheme, the mean bias for our method compared with the mean of the DEQAS LC–MS group during the period the present analyses were carried out was −3·2 %. Plasma parathyroid hormone (PTH) concentration was measured using an immunology analyser (Cobas e601; Roche Diagnostics), according to the standard procedures of the manufacturer (CV = 3·4 %).

Information on background, health, sun exposure and lifestyle, including the use of multivitamin and vitamin D supplements, of the subjects was obtained using detailed self-administered web-based questionnaires. Dietary vitamin D intakes were recorded using a semi-quantitative FFQ adapted from a FFQ used in the European union project Towards a strategy for Optimal Vitamin D Fortification (OPTIFORD)[23]. The vitamin D intakes were calculated based on the reported consumption frequencies and the vitamin D concentrations in the food items given in the Danish Food Composition Databank[28].

Statistical analyses

Data were analysed using the SPSS statistical software (version 20.0, IBM SPSS, Inc.), and statistical significance was evaluated at a level of $P<0·05$ (two-sided). Linear mixed models with family as a random variable were used in all the analyses to account for the non-independency of the subjects. Before analysis, serum 25(OH)D and PTH concentrations were logarithmically transformed to meet the model requirements. Trend analyses were carried out to test for linear relationships between PTH concentrations and 25(OH)D groups. Univariate models were used to assess the association between serum 25(OH)D concentration and each of the following sun-related variables: outdoor transport to school/work (<15, 15–30,

Table 1. Characteristics of the study population (*n* 755)
(Number of subjects and percentages)

	All subjects		Children		Adults	
	n	%	*n*	%	*n*	%
Sex						
Female	386	51	178	52	208	50
Male	369	49	162	48	207	50
Age (years)						
4–10	179	24	179	53	–	–
11–17	161	21	161	47	–	–
18–40	198	26	–	–	198	48
41–60	217	29	–	–	217	52
BMI (kg/m^2)						
Obese	54	7	1	<1	53	13
Overweight	156	21	20	6	136	33
Normal weight	545	72	319	94	226	55
Dietary vitamin D* (μg/d)						
Q1: <1·7	186	25	85	25	101	24
Q2: 1·7–2·4	193	25	83	24	110	27
Q3: 2·5–3·3	186	25	93	27	93	22
Q4: >3·3	190	25	79	23	111	27
Multivitamin use						
Yes	244	32	140	41	104	25
No	511	68	200	59	311	75
Outdoor transport to school/work (min/d)						
< 15	369	50	162	48	207	51
15–30	236	32	132	39	104	26
31–60	95	13	36	11	59	15
> 60	43	6	10	3	33	8
Solarium use						
Yes	12	2	2	<1	10	2
No	743	98	338	99	405	98
Outdoor stay in light clothes						
Most of the time	408	54	199	59	209	50
Often	273	36	116	34	157	38
Sometimes	62	8	18	5	44	11
Seldom/never	12	2	7	2	5	1
Sun preference						
Prefer sun	246	33	103	30	143	35
Sometimes in sun	467	62	222	65	245	59
Avoid sun	42	6	15	4	27	7
Sunscreen use						
Always	68	9	47	14	21	5
Most of the time	274	36	169	50	105	25
Sometimes	224	30	80	24	144	35
Seldom/never	189	25	44	13	145	35
Sun vacation						
Yes	363	48	168	49	195	47
No	392	52	172	51	220	53

*Quartiles (Q) for the whole study population.

31–60, or >60 min/d); solarium use at least once a week (yes or no); sun preference (prefer sun, sometimes in sun, or avoid sun); outdoor stay in light clothes (most of the time, often, sometimes, or seldom/never); sunscreen use (always, most of the time, sometimes, or seldom/never); sun vacation the preceding summer in June–September (yes or no). Sun-related variables with $P<0·05$ significance in their univariate model were included in a multiple analysis (linear mixed model) together with the following categorical variables: age (4–10, 11–17, 18–40, or 41–60 years); sex (female or male); BMI (normal weight, overweight, or obese); dietary vitamin D intake (quartiles: <1·7, 1·7–2·4, 2·5–3·3, or >3·3 μg/d); multivitamin use (yes or no). The interaction between age and sex was tested. This multiple analysis was carried out for all subjects and for children and adults separately. The strength of the familial component was considered by calculating the intra-class correlation for each model:

$$\rho = \frac{\omega^2}{(\omega^2 + \sigma^2)},$$

where ρ is the intra-family correlation, σ is the within-family standard deviation and ω is the between-family standard deviation. The lower the variation within the classes, the higher the intra-class correlation, which in this case means that the closer the correlation is to 1, the more alike the subjects are within a family with respect to their vitamin D status.

The relationship between serum 25(OH)D concentration and each of the following lifestyle-related variables was explored in univariate models only in adults: smoking status (current, former, or never); alcohol consumption (never, <1 time/month, 1–3 times/month, 1 time/week, 2–4 times/week, 5–6 times/week, or daily); leisure-time physical activity (mainly sedentary, light-to-moderate activity, regular sport and exercise, or athletic training); self-rated physical shape (really good, good, fairly good, bad, or really bad); self-rated health

Table 2. Lifestyle-related characteristics of adults in the study population (*n* 415)
(Number of subjects and percentages)

	n	%
Education		
None/technical	115	28
< 3 years of higher education	59	14
3–4 years of higher education	133	32
> 4 years of higher education	108	26
Smoking status		
Current	77	19
Former	106	26
Never	232	56
Alcohol consumption		
Never	19	5
< 1 time/month	61	15
1–3 times/month	103	25
1 time/week	67	16
2–4 times/week	124	30
5–6 times/week	27	7
Daily	14	3
Leisure-time physical activity		
Mainly sedentary	44	11
Light-to-moderate activity	155	37
Regular sports and exercise	168	41
Athletic training	48	12
Self-rated physical shape		
Really good	29	7
Good	154	37
Fairly good	167	40
Bad	53	13
Really bad	12	3
Self-rated health		
Excellent	51	12
Really good	167	40
Good	170	41
Less good/bad	27	7
Effort to eat healthily		
Very often	125	30
Often	211	51
Sometimes	65	16
Seldom/never	14	3

(excellent, really good, good, or less good/bad); effort to eat healthily (very often, often, sometimes, or seldom/never); education after state and/or upper secondary school (none or technical education, <3 years of higher education, 3–4 years of higher education, or >4 years of higher education). Lifestyle-related variables with $P<0·05$ significance in their univariate model were included in the multiple model described above.

Results

The characteristics of the study population and the lifestyle-related characteristics of adults in the study population are given in Tables 1 and 2, respectively. The median ages of the youngest children (4–10 years), the oldest children (11–17 years), the youngest adults (18–40 years) and the oldest adults (41–60 years) were 7 (interquartile range (IQR) 6–9), 13 (IQR 12–15), 37 (IQR 33–39) and 45 (IQR 43–48) years, respectively. The sexes were evenly distributed among both the children and adults, and the majority of the subjects were of normal weight (Table 1). The median dietary vitamin D intakes were similar across the age groups (range 2·3–2·6 μg/d). The total median vitamin D intake of the multivitamin users (41 % of the children and 25 % of the adults) was 6·7 (IQR 4·5–10·2) μg/d. Approximately half of the adults had a medium-long- to long-duration higher education, and their lifestyles were generally healthy (Table 2).

The individual serum 25(OH)D concentrations (sum of 25(OH)D_2 and 25(OH)D_3 concentrations) ranged from 9·3 to 161·9 nmol/l, with an overall geometric mean of 72·1 (IQR 61·5–86·7) nmol/l. Serum 25(OH)D_2 was found in 11 % of the samples in the range of 3–29 nmol/l. The serum 25(OH)D concentration of the different age and sex groups and its distribution across the ranges are summarised in Table 3. The overall distribution of serum 25(OH)D concentrations <30, <40 and <50 nmol/l was 1, 2 and 9 %, with no children being found to have serum 25(OH)D concentration <30 nmol/l. In the adults, the geometric mean PTH concentrations in the 25(OH)D concentration <25, 25–49, 50–75 and >75 nmol/l groups were 59·8 (95 % CI 43·2, 82·8), 39·9 (95 % CI 35·8, 44·3), 36·2 (95 % CI 34·3, 38·2) and 32·7 (95 % CI 31·1, 34·4) ng/l, respectively (P for trend <0·001). The same trend was observed in children aged 4–10 years (P for trend=0·012), but not in children aged 11–17 years (P for trend=0·067).

No differences were found in serum 25(OH)D concentration among the age (P=0·190), sex (P=0·332) or age, and sex groups (P=0·223) in the multiple analysis of all subjects (Table 4). When the children were analysed separately, serum 25(OH)D concentration was found to be associated with sex (P=0·034) and so 25(OH)D concentration was estimated to be 5 % lower in the girls than in the boys. In the univariate models, outdoor transport to work/school (P=0·972) and sunscreen use (P=0·154) were not associated with 25(OH)D concentration and thus not included in the multiple models. In the multiple analysis of all subjects, serum 25(OH)D concentration was found to be negatively associated with BMI ($P<0·001$) and positively associated

Table 3. Serum 25-hydroxyvitamin D (25(OH)D) concentration and distribution by age and sex groups among the families in late summer in Denmark (56°N)
(Geometric mean values and interquartile ranges; number of subjects and percentages)

	Girls		Boys		Women		Men				
	4–10 years	11–17 years	4–10 years	11–17 years	18–40 years	41–60 years	18–40 years	41–60 years	All children	All adults	All subjects
n	94	84	85	77	102	106	96	111	340	415	755
Serum 25(OH)D (nmol/l)*											
Geometric mean	71·7	70·3	76·0	72·2	74·1	70·7	71·2	71·0	72·5	71·7	72·1
Interquartile range	64·1–82·0	59·9–82·0	65·1–89·5	63·1–84·6	60·7–87·7	60·0–88·5	61·0–87·4	61·3–87·2	62·7–84·9	60·8–88·0	61·5–86·7
Distribution (%)											
< 25 nmol/l	0	0	0	0	0	1	2	1	0	1	1
25–50 nmol/l	5	8	5	8	7	15	6	9	7	9	8
51–74 nmol/l	55	55	46	48	45	35	40	42	51	41	45
75–125 nmol/l	39	35	49	44	44	47	51	48	42	48	45
> 125 nmol/l	0	2	1	0	5	2	1	0	1	2	2

* No overall differences in serum 25(OH)D concentration among the age (P=0·190), sex (P=0·332), and the combined age and sex groups (P=0·223) as analysed in the linear mixed model with family as a random variable; sex, age, BMI, dietary vitamin D, multivitamin use, solarium use, outdoor stay in light clothes, sun preference and sun vacation as the categorical variables; and the logarithm of the serum 25(OH)D concentration as the dependent variable.

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Table 4. Associations between potential determinants and serum 25-hydroxyvitamin D (25(OH)D) concentration*
(Ratio of means and 95 % confidence interval)

	All subjects (*n* 755)			Children (*n* 340)			Adults (*n* 415)		
Variables	Ratio of means†	95 % CI	*P*	Ratio of means†	95 % CI	*P*	Ratio of means†	95 % CI	*P*
Sex			0·332			0·034			0·792
Female	0·98	0·95, 1·02		0·95	0·91, 1·00		1·01	0·96, 1·06	
Male	1	1		1	1		1	1	
Age (years)			0·190			0·823			0·937
4–10	0·99	0·94, 1·05	0·778	1·01	0·96, 1·06		–	–	
11–17	0·95	0·91, 1·01	0·082	1	1		–	–	
18–40	1·01	0·96, 1·07	0·653	–	–		1·00	0·94, 1·06	
41–60	1	1		–	–		1	1	
BMI (kg/m^2)			<0·001			0·348			0·001
Obese	0·83	0·77, 0·90	<0·001	0·97	0·61, 1·54	0·908	0·84	0·77, 0·92	<0·001
Overweight	0·97	0·92, 1·02	0·173	0·93	0·85, 1·03	0·148	0·98	0·92, 1·04	0·519
Normal weight	1	1		1	1		1	1	
Dietary vitamin D‡ (μg/d)			0·008			0·065			0·034
Q1: <1·7	0·92	0·87, 0·97	0·002	0·94	0·88, 1·00	0·068	0·91	0·84, 0·98	0·015
Q2: 1·7–2·4	0·97	0·92, 1·02	0·284	0·95	0·88, 1·01	0·104	1·00	0·93, 1·08	0·982
Q3: 2·5–3·3	1·00	0·95, 1·05	0·927	1·01	0·95, 1·08	0·741	0·99	0·92, 1·08	0·899
Q4: >3·3	1	1		1	1		1	1	
Multivitamin use			0·019			0·066			0·045
Yes	1·06	1·01, 1·10		1·05	1·00, 1·11		1·07	1·00, 1·14	
No	1	1		1	1		1	1	
Solarium use			0·006			0·199			0·007
Yes	1·2	1·06, 1·43		0·82	0·61, 1·11		1·29	1·07, 1·55	
No	1	1		1	1		1	1	
Outdoor stay in light clothes			0·001			0·013			<0·001
Most of the time	1·30	1·11, 1·51	0·001	1·20	1·01, 1·43	0·034	1·58	1·20, 2·08	0·001
Often	1·29	1·11, 1·50	0·001	1·25	1·05, 1·49	0·011	1·51	1·15, 2·00	0·003
Sometimes	1·16	0·99, 1·36	0·063	1·32	1·10, 1·59	0·004	1·29	0·97, 1·71	0·083
Never/seldom	1	1		1	1		1	1	
Sun preference			0·002			0·621			0·001
Prefer sun	1·14	1·04, 1·25	0·004	0·96	0·85, 1·09	0·570	1·24	1·09, 1·40	0·001
Sometimes in sun	1·07	0·98, 1·17	0·116	0·95	0·84, 1·07	0·393	1·14	1·01, 1·29	0·034
Avoid sun	1	1		1	1		1	1	
Sun vacation			<0·001			<0·001			0·021
Yes	1·09	1·04, 1·15		1·11	1·06, 1·17		1·07	1·01, 1·14	
No	1	1		1	1		1	1	

* Analysed in the linear mixed models with family as a random variable; sex, age, BMI, dietary vitamin D, multivitamin use, solarium use, outdoor stay in light clothes, sun preference and sun vacation as the categorical variables; and the logarithm of the serum 25(OH)D concentration as the dependent variable.
† The regression coefficients were exponentially transformed (10^{β}).
‡ Quartiles (Q) for the whole study population.

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with dietary vitamin D intake ($P=0·008$), multivitamin use ($P=0·019$), solarium use ($P=0·006$), outdoor stay in light clothes ($P=0·001$), sun preference ($P=0·002$) and sun vacation ($P<0·001$) (Table 4). When the children and adults were analysed separately, serum 25(OH)D concentration was found to be associated with BMI and sun preference in the children. In the adults, the associations remained the same as in the model with all subjects, but the significance for dietary vitamin D intake, multivitamin use and sun vacation was weakened.

From these multivariate models, it was found that the variations in serum 25(OH)D concentration were higher within the families than between the families with an intra-family correlation of 0·27 in all subjects. The intra-family correlation was higher in the children than in the adults (Table 5).

In a further analysis of the adults, serum 25(OH)D concentration was found to be associated with leisure-time physical activity ($P<0·001$), self-rated physical shape ($P=0·001$) and self-rated health ($P=0·003$) in their univariate models. When these variables were included in the multiple analysis together with the vitamin D source-related variables, none remained significant, although leisure-time physical activity was borderline significant ($P=0·054$). Serum 25(OH)D concentration was not associated with smoking status ($P=0·722$), alcohol consumption ($P=0·070$), effort to eat healthily ($P=0·193$) or education ($P=0·219$).

Discussion

The overall geometric mean serum 25(OH)D concentration among the families in the present study was 72·1 nmol/l, with no differences being observed between the age and sex groups in the analysis of all subjects. The distribution of 25(OH)D concentrations <25, <50 and <75 nmol/l was 1, 8 and 54 %, with no children being found with 25(OH)D concentration <25 nmol/l. A novelty of the present study was that the familial component was quantitatively assessed by calculating the intra-class correlation. The intra-family correlation for all subjects was 0·27, which indicates that a subject's vitamin D status is not strongly related to the familial relationship. However, to our knowledge, no previous studies have quantified the familial component for vitamin D status and thus we do not have a value for comparison of the familial relationship. The intra-family correlation was almost double in the children (0·42) as in the adults (0·24), indicating that the children within a family were more alike than the adults within a family with respect to their vitamin D status. This might be an indication of the influence of genetic factors on vitamin D status or more similar habits in children than in adults from the same family. It is likely that children within a family share activities and dietary/supplementation habits to a greater extent than adults within a family, e.g. outdoor stay, sun protection, multivitamin use and lunch in school.

Table 5. Standard deviation for the within-family and between-family effects and the intra-family correlation*

Parameters	All subjects (*n* 755)	Children (*n* 340)	Adults (*n* 415)
Between-family standard deviation	0·060	0·063	0·061
Within-family standard deviation	0·098	0·074	0·11
Intra-family correlation†	0·27	0·42	0·24

* Derived from the linear mixed models with family as a random variable; sex, age, BMI, dietary vitamin D, multivitamin use, solarium use, outdoor stay in light clothes, sun preference, and sun vacation as the categorical variables; and the logarithm of the serum 25-hydroxyvitamin D concentration as the dependent variable.

† Calculated as follows: between-family standard deviation^2/(between-family standard deviation^2 + within-family standard deviation^2).

The serum 25(OH)D concentrations found in the present study were higher and the distribution of 25(OH)D concentrations <50 nmol/l was lower than that reported in previous studies among similar age and ethnicity groups in Denmark[10,20–23], other European countries[11,23,29–33], the USA[34,35] and Canada[36]. Most of these studies measured vitamin D status across different seasons or in the winter as opposed to late summer in the present study; however, the distribution of 25(OH)D concentrations <50 nmol/l found in the present study was also lower than that found during summer. Most of the participants of the present study were of normal weight, which could favourably affect the serum 25(OH)D concentration compared with, for example, the high rate of obesity in the USA[37]. Nevertheless, the studies should be compared with caution as differences may also depend on the laboratory and method used for the measurement of serum 25(OH)D concentrations[17]. In the present study, we used the LC–MS/MS method that might be considered the gold standard[38,39], and our method was standardised and calibrated against the international reference material of the NIST[18]. The chromatographic methods are more specific compared with the frequently used immunoassays that are limited in their ability to detect vitamin D_2[38,40]. In the DEQAS, the LC–MS method is positively biased for the all-laboratory trimmed mean, whereas the immunoassays are mostly negatively biased[17]. Several studies have also found the LC–MS method to yield better results than some other 25(OH)D assays[15,16,39–41]. In a study of the German population, standardisation to the LC–MS/MS method has been found to reduce the prevalence of vitamin D deficiency (<30 nmol/l) from approximately 48 to 16 %[42]. This might partly explain the higher serum 25(OH)D concentrations found in the present study compared with previous studies in similar population groups.

Similar to the common finding of an inverse relationship between 25(OH)D and PTH concentrations[43], we observed a negative trend between PTH and 25(OH)D groups in the adults and in children aged 4–10 years. In present study, relatively few subjects had a low serum 25(OH)D concentration (<25 nmol/l), but the PTH concentration was markedly higher in this group than in the other 25(OH)D groups. Elevated PTH concentrations may result in increased bone resorption in adults, whereas the implication for bone health in children is unclear[44].

For all subjects, outdoor stay in light clothes and sun vacation were major determinants of serum 25(OH)D concentration in late summer. In the adults, sun preference, solarium use and BMI were also strong determinants. We expected

vitamin D status to be related to sun exposure as cutaneous vitamin D synthesis is considered to be the major source of vitamin D during summer[45]. It is interesting though that several expressions of sun exposure were related to serum 25(OH)D concentrations at the same time. Most suggestive may be that vitamin D status was associated with sun vacation (abroad), despite that the hours of sunshine the preceding summer (2010) in Denmark were only slightly less than (3 %) the average hours of sunshine in the preceding 10 years[46]. To our knowledge, this association between vitamin D status and sun vacation during the summer season has not been shown previously. The dietary vitamin D intake was also associated with serum 25(OH)D concentration in the present study, despite the median intake (2·5 μg/d) being much lower than the new recommended intake proposed by the Nordic Nutrition Recommendation. The recommended intake has recently been increased from 7·5 to 10 μg/d for 2- to 60-year-olds[47]. This makes room for an even greater improvement in vitamin D intake, and our finding suggests that dietary vitamin D intake is also important during summer even in a group of children and adults frequently staying outdoors.

In the present study, no association between vitamin D status and age was found. Some previous studies have shown an association between vitamin D status and age in both children[35] and adults[11], whereas others did not find an association between 25(OH)D concentration and age[10,31,32]. The observed higher vitamin D status in boys than in girls has been reported previously among similar age groups[48]. In the present study, this sex difference was not attributable to differences in dietary vitamin D intakes or multivitamin use. An explanation might be the higher level of physical activity in the boys than in the girls (56 % of the boys compared with 35 % of the girls reported to be involved in sports and physically active play activities in their leisure time; results not shown), assuming that these activities were primarily outdoor activities. Serum 25(OH)D concentration was strongly inversely related to BMI in the adults, whereas there was no association in the children. This might be because the children were not categorised as obese and only a few were overweight. Another study in healthy-weight children with a broad age span did not find an association between 25(OH)D concentration and BMI or fat mass either[35]. The association between 25(OH)D concentration and obesity is a common finding[11,34,49], and one explanation could be the sequestration of vitamin D in fat tissue[50]. In a cross-sectional study of 686 adults, adjusting for body weight eliminated the obesity-related component of variability in serum 25(OH)D concentrations[51]. This indicates that the lower 25(OH)D concentration could be due to dilution in the large fat mass of obese subjects rather than sequestration and that vitamin D requirements could be based on body weight.

In the adults, serum 25(OH)D concentration was not related to lifestyle when assessed together with the influence of vitamin D source-related factors, except for a borderline relationship with leisure-time physical activity. It might be that physical activity acts like a surrogate marker for sun exposure, assuming that the activities are mainly outdoor activities. Previous studies have found an association between 25(OH)D concentration and various lifestyle-related factors[10,11]. In one study, an overall lifestyle index was used and vitamin D concentrations were found to be substantially higher in those with the healthiest lifestyle than in those with a less-healthy lifestyle and this difference was found be substantially higher than that between the single components of the lifestyle index[11]. This suggests that a high vitamin D concentration may serve as an indicator of a generally healthy lifestyle.

The strength of the present study was the random and population-based inclusion of families, which made it possible to compare vitamin D status across the age and sex groups. This has not been done previously in studies of the Danish population. Another strength was the use of detailed information on vitamin D sources including several variables for sun exposure, dietary vitamin D intake and supplement use. A limitation of the present study was that it was conducted at a single site in Denmark. However, the sample size of the present study was large and the subjects were randomly selected with few exclusion criteria, and we believe that the results are likely to be generalisable.

We assessed vitamin D status in a representative sample of Danish families using a standardised and calibrated method, and thus the results are useful for future comparisons of vitamin D status between populations. In conclusion, the majority of children and adults among the families had serum 25(OH)D concentrations >50 nmol/l in late summer in Denmark. Vitamin D status was associated with BMI, dietary vitamin D intake, multivitamin use, solarium use, outdoor stay in light clothes, sun preference and sun vacation, but was not associated with lifestyle-related factors in the adults when these were assessed together with the other determinants. Children within a family appeared to be more alike than the adults within a family with respect to their vitamin D status.

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wrote the first draft of the manuscript, which was critically reviewed and approved by all authors.

None of the authors has any conflicts of interest to declare.

References

1. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
2. Sahota O (2000) Osteoporosis and the role of vitamin D and calcium–vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency. *Age Ageing* **29**, 301–304.
3. Wang Y, Zhu J & DeLuca HF (2012) Where is the vitamin D receptor? *Arch Biochem Biophys* **523**, 123–133.
4. Grant WB (2006) Epidemiology of disease risks in relation to vitamin D insufficiency. *Prog Biophys Mol Biol* **92**, 65–79.
5. Wacker M & Holick MF (2013) Vitamin D – effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients* **5**, 111–148.
6. Parker J, Hashmi O, Dutton D, *et al.* (2010) Levels of vitamin D and cardiometabolic disorders: systematic review and meta-analysis. *Maturitas* **65**, 225–236.
7. Gandini S, Boniol M, Haukka J, *et al.* (2011) Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer* **128**, 1414–1424.
8. Kriegel MA, Manson JE & Costenbader KH (2011) Does vitamin D affect risk of developing autoimmune disease?: a systematic review. *Semin Arthritis Rheum* **40**, 512–531.
9. Zittermann A, Iodice S, Pilz S, *et al.* (2012) Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am J Clin Nutr* **95**, 91–100.
10. Thuesen B, Husemoen L, Fenger M, *et al.* (2012) Determinants of vitamin D status in a general population of Danish adults. *Bone* **50**, 605–610.
11. Jaaskelainen T, Knekt P, Marniemi J, *et al.* (2013) Vitamin D status is associated with sociodemographic factors, lifestyle and metabolic health. *Eur J Nutr* **52**, 513–525.
12. Seamans KM & Cashman KD (2009) Existing and potentially novel functional markers of vitamin D status: a systematic review. *Am J Clin Nutr* **89**, 1997S–2008S.
13. National Board of Health (2010) *Forebyggelse, diagnostik og behandling af D-vitaminmangel* (*Prevention, Diagnostics and Treatment of Vitamin D Deficiency*). National Board of Health. Copenhagen: National Board of Health. http://www.sst.dk/~/media/Sundhed%20og%20forebyggelse/Ernaering/D-vitamin/D-vitamin%20baggrundsnotat%2027052010.ashx (accessed April 2013).
14. Food and Nutrition Board, Institute of Medicine (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press.
15. Farrell CL, Martin S, McWhinney B, *et al.* (2012) State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography–tandem mass spectrometry methods. *Clin Chem* **58**, 531–542.
16. Lai JKC, Lucas RM, Banks E, *et al.* (2012) Variability in vitamin D assays impairs clinical assessment of vitamin D status. *Intern Med J* **42**, 43–50.
17. Carter GD (2011) Accuracy of 25-hydroxyvitamin D assays: confronting the issues. *Curr Drug Targets* **12**, 19–28.
18. Phinney KW, Bedner M, Tai SS, *et al.* (2012) Development and certification of a standard reference material for vitamin D metabolites in human serum. *Anal Chem* **84**, 956–962.
19. Brot C, Vestergaard P, Kolthoff N, *et al.* (2001) Vitamin D status and its adequacy in healthy Danish perimenopausal women: relationships to dietary intake, sun exposure and serum parathyroid hormone. *Br J Nutr* **86**, Suppl. 1, S97–S103.
20. Mølgaard C, Larnkjær A, Cashman KD, *et al.* (2010) Does vitamin D supplementation of healthy Danish Caucasian girls affect bone turnover and bone mineralization? *Bone* **46**, 432–439.
21. Skaaby T, Husemoen LL, Pisinger C, *et al.* (2013) Vitamin D status and incident cardiovascular disease and all-cause mortality: a general population study. *Endocrine* **43**, 618–625.
22. Andersen R, Mølgaard C, Skovgaard LT, *et al.* (2005) Teenage girls and elderly women living in northern Europe have low winter vitamin D status. *Eur J Clin Nutr* **59**, 533–541.
23. Andersen R, Mølgaard C, Skovgaard LT, *et al.* (2008) Pakistani immigrant children and adults in Denmark have severely low vitamin D status. *Eur J Clin Nutr* **62**, 625–634.
24. Madsen KH, Rasmussen LB, Andersen R, *et al.* (2013) Randomized controlled trial of the effects of vitamin D-fortified milk and bread on serum 25-hydroxyvitamin D concentrations in families in Denmark during winter: the VitmaD study. *Am J Clin Nutr* **98**, 374–382.
25. Cole TJ, Bellizzi MC, Flegal KM, *et al.* (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* **320**, 1240–1243.
26. World Health Organization (2000) *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation (WHO Technical Report Series 894)*. Geneva: WHO.
27. Blomberg Jensen M, Bjerrum PJ, Jessen TE, *et al.* (2011) Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Hum Reprod* **26**, 1307–1317.
28. National Food Institute, Technical University of Denmark (2009) *Danish Food Composition Databank, Version 7.01*. Mørkhøj: National Food Institute. http://www.foodcomp.dk/v7/fvdb_search.asp (accessed April 2012).
29. Gonzalez-Gross M, Valtuena J, Breidenassel C, *et al.* (2012) Vitamin D status among adolescents in Europe: the healthy lifestyle in Europe by nutrition in adolescence study. *Br J Nutr* **107**, 755–764.
30. Cashman KD, Muldowney S, McNulty B, *et al.* (2013) Vitamin D status of Irish adults: findings from the National Adult Nutrition Survey. *Br J Nutr* **109**, 1248–1256.
31. Hirani V, Mosdol A & Mishra G (2009) Predictors of 25-hydroxyvitamin D status among adults in two British national surveys. *Br J Nutr* **101**, 760–764.
32. Hintzpeter B, Mensink GBM, Thierfelder W, *et al.* (2008) Vitamin D status and health correlates among German adults. *Eur J Clin Nutr* **62**, 1079–1089.
33. Hill TR, Cotter AA, Mitchell S, *et al.* (2008) Vitamin D status and its determinants in adolescents from the Northern Ireland Young Hearts 2000 cohort. *Br J Nutr* **99**, 1061–1067.
34. Forrest KYJ & Stuhldreher WL (2011) Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res* **31**, 48–54.
35. Weng FL, Shults J, Leonard MB, *et al.* (2007) Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. *Am J Clin Nutr* **86**, 150–158.
36. Whiting SJ, Langlois KA, Vatanparast H, *et al.* (2011) The vitamin D status of Canadians relative to the 2011 Dietary Reference Intakes: an examination in children and adults with and without supplement use. *Am J Clin Nutr* **94**, 128–135.
37. OECD (2011) *Health at a Glance 2011: OECD Indicators, OECD Publishing*. Paris: OECD. http://dx.doi.org/10.1787/health_glance-2011-en (accessed October 2013).

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38. Fraser WD & Milan AM (2013) Vitamin D assays: past and present debates, difficulties, and developments. *Calcif Tissue Int* **92**, 118–127.
39. Roth HJ, Schmidt-Gayk H, Weber H, *et al.* (2008) Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography–tandem mass spectrometry as a reference. *Ann Clin Biochem* **45**, 153–159.
40. van den Ouweland JMW, Vogeser M & Baecher S (2013) Vitamin D and metabolites measurement by tandem mass spectrometry. *Rev Endocr Metab Disord* **14**, 159–184.
41. Moon H, Cho J, Hur M, *et al.* (2012) Comparison of four current 25-hydroxyvitamin D assays. *Clin Biochem* **45**, 326–330.
42. Perna L, Haug U, Schoettker B, *et al.* (2012) Public health implications of standardized 25-hydroxyvitamin D levels: a decrease in the prevalence of vitamin D deficiency among older women in Germany. *Prev Med* **55**, 228–232.
43. Sai AJ, Walters RW, Fang X, *et al.* (2011) Relationship between vitamin D, parathyroid hormone, and bone health. *J Clin Endocrinol Metab* **96**, E436–E446.
44. Cashman KD (2007) Vitamin D in childhood and adolescence. *Postgrad Med J* **83**, 230–235.
45. Macdonald HM (2013) Contributions of sunlight and diet to vitamin D status. *Calcif Tissue Int* **92**, 163–176.
46. The Danish Institute of Meteorology (2010) *Vejret i Danmark – sommeren 2010* (*The Weather in Denmark – The Summer of 2010*). Copenhagen: The Danish Institute of Meteorology. http://www.dmi.dk/dmi/vejr_i_danmark_-_sommeren_2010 (accessed April 2013).
47. Nordic Council of Ministers (2014) *Nordic Nutrition Recommendations 2012 - Integrating Nutrition and Physical Activity*. Copenhagen: Nordic Council of Ministers.
48. Rockell JE, Green TJ, Skeaff CM, *et al.* (2005) Season and ethnicity are determinants of serum 25-hydroxyvitamin D concentrations in New Zealand children aged 5–14 y. *J Nutr* **135**, 2602–2608.
49. Bertrand KA, Giovannucci E, Liu Y, *et al.* (2012) Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. *Br J Nutr* **108**, 1889–1896.
50. Wortsman J, Matsuoka L, Chen T, *et al.* (2000) Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* **72**, 690–693.
51. Drincic AT, Armas LA, Van Diest EE, *et al.* (2012) Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity* **20**, 1444–1448.