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Determinants of serum 25-hydroxyvitamin D concentration in Finnish children: the Physical Activity and Nutrition in Children (PANIC) study

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Abstract

We studied vitamin D intake, serum 25-hydroxyvitamin D (S-25(OH)D) concentration, determinants of S-25(OH)D and risk factors for S-25(OH)D <50 nmol/l in a population sample of Finnish children. We studied 184 girls and 190 boys aged 6–8 years, analysed S-25(OH)D by chemiluminescence immunoassay and assessed diet quality using 4-d food records and other lifestyle factors by questionnaires. We analysed the determinants of S-25(OH)D using linear regression and risk factors for S-25(OH)D <50 nmol/l using logistic regression. Mean dietary intake of vitamin D was 5·9 (SD 2·1) μg/d. Altogether, 40·8 % of children used no vitamin D supplements. Of all children, 82·4 % did not meet the recommended total vitamin D intake of 10 μg/d. Milk fortified with vitamin D was the main dietary source of vitamin D, providing 48·7 % of daily intake. S-25(OH)D was <50 nmol/l in 19·5 % of children. Consumption of milk products was the main determinant of S-25(OH)D in all children (standardised regression coefficient $\beta = 0{\cdot}262$; $P < 0{\cdot}001$), girls ($\beta = 0{\cdot}214$; $P = 0{\cdot}009$) and boys ($\beta = 0{\cdot}257$; $P = 0{\cdot}003$) in multivariable models. Vitamin D intake from supplements ($\beta = 0{\cdot}171$; $P = 0{\cdot}035$) and age ($\beta = -0{\cdot}198$; $P = 0{\cdot}015$) were associated with S-25(OH)D in girls. Children who drank ≥450 g/d of milk, spent ≥2·2 h/d in physical activity, had ≥13·1 h/d of daylight time or were examined in autumn had reduced risk for S-25(OH)D <50 nmol/l. Insufficient vitamin D intake was common among Finnish children, one-fifth of whom had S-25(OH)D <50 nmol/l. More attention should be paid to the sufficient intake of vitamin D from food and supplements, especially among children who do not use fortified milk products.

Key words: Vitamin D: 25-Hydroxyvitamin D: Children: Determinants

Vitamin D is a pro-hormone that is converted in the liver to 25-hydroxyvitamin D (25(OH)D) and then in the kidney to 1,25-dihydroxyvitamin D, the active metabolite that regulates Ca, P and bone metabolism[1]. Vitamin D can be obtained from foods and supplements or synthesised endogenously in the skin in response to the UVB radiation of the sun. The major circulating form of vitamin D in serum is 25(OH)D, which is commonly used as an indicator of vitamin D status.

Knowledge of the health effects of vitamin D is increasing. In addition to the well-known beneficial effect of vitamin D on bone health, there is some evidence that higher serum levels of 25(OH)D are associated with better muscle strength[2] and decreased risk of several diseases such as type 1 diabetes and other autoimmune diseases, cancer and infections[1].

The recommendations of the Institute of Medicine in the USA for serum 25(OH)D concentration and vitamin D intake are mainly based on the effects of vitamin D on bone health, because evidence on its effects on other outcomes is still not strong enough to inform the recommendations[3]. There is no consensus on the optimal serum level of 25(OH)D. The limit of serum 25(OH)D concentration for vitamin D deficiency varies between 25 and 50 nmol/l, and the lower limit for the sufficient serum 25(OH)D concentration is suggested by some authors to be as high as 75 nmol/l[1–8]. There is some evidence that serum 25(OH)D concentration above 125 nmol/l may increase the risk of vitamin D intoxication followed by hypercalcaemia, hypercalciuria and premature death[3]. Serum 25(OH)D concentrations vary depending on the laboratory assays used[9,10] that makes defining the optimal serum level of 25(OH)D even more difficult.

The Institute of Medicine in the USA has recommended the intake of vitamin D from food and supplements of 10 μg/d for

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.

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infants and 15 μg/d for other individuals[3]. The Nordic and Finnish experts recently recommended the intake of vitamin D from food and supplements of 10 μg/d for all children[11,12]. Moreover, all children in Finland are recommended to use 7·5 μg/d of vitamin D supplements year round, regardless of their dietary intake of vitamin D[12]. In other Nordic countries, the use of supplements is generally recommended for infants but not for older children, unless the dietary intake of vitamin D is insufficient[11].

The insufficient intake of vitamin D is common among children. In Finland, the average intake of vitamin D has been above the previous recommendation of 7·5 μg/d in 1-year-old children[13], whereas in older children and adolescents the average intake has been lower than the recommended levels[14]. In one Finnish study, 71 % of children and adolescents had serum 25(OH)D concentrations <50 nmol/l, although the intake of vitamin D was below the recommendation only in 34 % of the children and adolescents[15].

Older age, more advanced puberty, female sex, lower socio-economic position, non-Caucasian race and higher BMI and waist circumference have been associated with vitamin D deficiency in children and adolescents[16–18]. Serum 25(OH)D concentration has also been inversely associated with the development of adiposity in school-aged children[19]. Vitamin D deficiency has been found to be more common in winter than in other seasons among children and adolescents[16,18,20]. Moreover, the use of vitamin D supplements has been related to higher serum 25(OH)D concentrations in children and adolescents[17,21] and has been reported to blunt seasonal variation in serum 25(OH)D levels[22].

Consensus regarding the optimal serum concentration of 25(OH)D is not yet obtained. Therefore, more information on the distribution and determinants of serum 25(OH)D concentration in different age groups and geographic areas is needed. However, there are a few studies on serum 25(OH)D concentration and its determinants, including the intake of vitamin D from food and supplements, among children from Nordic countries and other countries located at the same latitude who are at increased risk of vitamin D deficiency due to long and dark winters. We therefore investigated the distribution and determinants of serum 25(OH)D concentration and the risk factors for low serum 25(OH)D concentration (<50 nmol/l) in a population sample of Finnish children by assessing a number of factors that could be related to vitamin D status.

Methods

Study design and study population

The present analyses are based on the baseline data of the Physical Activity and Nutrition in Children (PANIC) study, which is an ongoing physical activity and diet intervention study in a population sample of 6–8-year-old children from the city of Kuopio, Finland (ClinicalTrials.gov registration number NCT01803776). Altogether, 736 children from the primary schools of Kuopio were invited to participate in the baseline examinations between 2007 and 2009. Of the invited children, 512 (70 %) participated in the baseline examinations. Altogether, 374 children (184 girls, 190 boys) had complete data on serum 25(OH)D concentration and its determinants and were included in the present study sample. Of these children, 99·1 % were Caucasian. The study was conducted according to the ethical guidelines laid down in the Declaration of Helsinki. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. Both children and their parents gave their written informed consent.

Assessment of food consumption and nutrient intake

The consumption of foods, energy intake and the dietary intake of vitamin D were assessed using food records of 4 consecutive days that consisted of 2 weekdays and 2 weekend days (95·5 %) or 3 weekdays and 1 weekend day (0·5 %)[23]. A clinical nutritionist instructed the parents to record all food and drinks consumed by their child using household or other measures, such as tablespoons, decilitres and centimetres. The parents were also instructed to ask their child about food eaten outside home. Moreover, a clinical nutritionist collected information about the details of menus and recipes of food served at schools and afternoon daycare centres from the catering company that provided food for the schools. A clinical nutritionist used all this information and also a picture booklet of portion sizes when reviewing and completing the food records at return, if needed. Food consumption and nutrient intake were assessed using Micro Nutrica dietary analysis software, version 2.5 (The Social Insurance Institution of Finland). We estimated that 22 % of the girls and 24 % of the boys may have under-reported their total energy intake comparing it with energy expenditure estimated by BMR calculated using Schofield's equation and using the cut-offs for under-reporting suggested by Torun *et al.*[24]. We studied the proportion of food groups for vitamin D intake and selected the food groups that contributed more than 5 % to vitamin D intake for further analyses. Milk products included milk, sour milk products and other dairy products. Milk was generally fortified with vitamin D with a maximum of 0·5 μg/100 g, whereas only some of the sour milk products including mainly sour milk and yoghurts were fortified with vitamin D in Finland with a maximum of 0·5 μg/100 g at the time of data collection. Other dairy products included mainly cheese, creams and ice cream, which are generally not fortified with vitamin D in Finland apart from single products. Margarines included table margarines, which were generally fortified with vitamin D in Finland with a maximum of 10 μg/100 g at the time of data collection. Other fat products included mainly baking margarines, many of which are fortified with vitamin D in Finland, and coconut butter which is not fortified with vitamin D. Other food groups selected for further analyses were fish products including fresh fish, shellfish and processed fish, meat products including red meat, sausage and poultry and grain products including bread, rice, pasta, flours, muesli and ready-to-eat cereals. Vitamin and mineral supplements were not included in the food records. The mean daily intake of vitamin D was compared with the Finnish nutrition recommendations released in 2005[25] and in 2014[12].

Assessment of supplement use

The use of vitamin and mineral supplements was assessed by a questionnaire administered by the parents. The questionnaire included questions on the brand and dosage of the

supplements and the frequency of supplement use. Vitamin D supplements and multivitamin products containing vitamin D were combined into a single variable for the analyses, and the use of supplements containing vitamin D was classified by one researcher into six categories (no, ≤1 tablet/week, 2–4 tablets/week in series, ≥5 tablets/week in series, 2–4 tablets/week year round, ≥5 tablets/week year round) according to the combined answers (frequency, dosage and other given information). The average daily dose during a year was calculated by combining the information on the frequency of supplement use, the estimated number of weeks per year of supplement use if the supplement was used in series and the dosage. The average daily dose was also calculated for the 1-month period before blood sampling, considering that many of the children used supplements in series only in winter. The dose of vitamin D in most of the common supplement products used in Finland in 2007–2009 was confirmed from the manufacturers. If the name or dose of the supplement was not mentioned, the dose was assumed to be 7·5 μg for vitamin D supplements and 5 μg for multivitamin products containing vitamin D.

Assessment of physical activity and sedentary behaviour

Physical activity and sedentary behaviour were assessed by the PANIC Physical Activity Questionnaire administered by the children together with their parents[26]. Physical activity included organised sports, organised exercise other than sports, unsupervised physical activity, physically active school transportation such as walking and bicycling, physical activity during recess and physical education. Physical activities performed outdoors and indoors were not separated. Sedentary behaviour included screen-based sedentary behaviour (watching TV and videos, using the computer, playing video games, using a mobile phone and playing mobile games), sedentary behaviour related to music (listening to music, playing music), sedentary behaviour related to academic skills (reading, writing), sedentary behaviour related to arts, crafts and games (drawing, doing arts and crafts, playing board and card games), and sitting and lying for a rest. Physical activity and sedentary behaviour were expressed in hours per day.

Assessment of body size and composition

Body size and composition were assessed by trained research personnel as explained previously[27]. Body height was measured to accuracy of 0·1 cm using a wall-mounted stadiometer in the Frankfurt plane without shoes. Body weight was measured to accuracy of 0·1 kg after overnight fasting, empty-bladdered and in light underwear using a calibrated InBody 720 device (Biospace). BMI was calculated by dividing body weight (kg) by body height squared (m^2). BMI-standard deviation score was calculated using national references[28]. Waist circumference was measured to accuracy of 0·1 cm after expiration at mid-distance between the bottom of the rib cage and the top of the iliac crest with an unstretchable measuring tape. Body fat percentage was measured in the supine position, empty-bladdered and in light clothing with all metal objects removed by dual-energy X-ray absorptiometry (DXA) using the Lunar DXA device (Lunar Prodigy Advance; GE Medical Systems).

Assessment of other determinants of serum 25-hydroxyvitamin D concentration

Daylight time from sunrise to sunset in Kuopio, Finland, at latitude 62·89°N, was calculated as the average during 3 months before the blood sampling. The daylight time was provided by the Almanac Office, University of Helsinki. No blood samples were collected in July when most Finns have vacation. The season of blood sampling was determined as winter (December, January, February), spring (March, April, May), summer (June, August) and autumn (September, October, November). Travels to sunny countries within 3 months before the blood sampling (no, yes), sunscreen use (no, occasionally or frequently), skin colour type (four categories according to Fitzpatrick[29] from light to dark; I = always burns, never tans; II = often burns, sometimes tans; III = sometimes burns, often tans; IV = never burns, always tans), race (Caucasian, non-Caucasian), parental education and household income were assessed using questionnaires administered by the parents. Parental education was defined as the highest completed or ongoing degree of the parents (vocational school or less, vocational high school, university). Annual household income was reported to accuracy of 10 000€ and was categorised as ≤30 000€ or >30 000€. A research physician classified the boys as having entered clinical puberty if their testicular volume assessed by an orchidometer was >3 ml and the girls if their breast development in scales described by Tanner was > B1[30,31].

Measurement of serum 25-hydroxyvitamin D concentration

Venous blood samples were collected after a 12-h overnight fast. Blood samples were immediately centrifuged and stored at a temperature of −75°C until further analyses. Serum 25(OH)D concentration was analysed by a chemiluminescence immunoassay called the LIAISON® 25 OH Vitamin D TOTAL Assay (DiaSorin Inc.) using an automatic immunoanalyser (DiaSorin S.p.A.). Total variation, including intra-assay and inter-assay variation, for the assay is 8·2–11·0 % in the concentration range of 21–123 nmol/l.

Statistical methods

IBM SPSS Statistics for Windows software, version 19.0 (IBM Corp.), was used for statistical analyses. The normality of distributions of the variables was verified visually and by the Kolmogorov–Smirnov test. Before the statistical analyses, logarithmic transformation was performed for body weight, waist circumference, body fat percentage and serum 25(OH)D because of the skewed distributions of these variables. The *t* test for independent samples, the Mann–Whitney *U* test and the Pearson χ^2 test were used to examine differences in the basic characteristics between sexes. Linear regression analysis was used to investigate the determinants of serum 25(OH)D concentration. Food groups that provided at least 5 % of dietary intake of vitamin D were used in the linear regression models. Because of overlapping between the total intake of vitamin D from diet and supplements and the dietary sources of vitamin D, total vitamin D intake was not used in the linear regression models. The variables were first entered one by one into the models and were then entered stepwise into

the model to study whether they were independently associated with serum 25(OH)D concentration. Risk factors of having serum 25(OH)D concentration below 50 nmol/l were studied using the logistic regression analysis. Milk consumption was divided into five classes to accuracy of 150 g that corresponded to one glass of milk and all other continuous variables were categorised in thirds for the logistic regression models. The data were first adjusted for age and sex and were then additionally adjusted for other statistically significant determinants of serum 25(OH)D concentration by entering each of them separately as a continuous variable into the models. Associations with a *P*-value of <0·05 were considered statistically significant.

Results

Characteristics of children

The boys were taller, had a higher waist circumference and a lower body fat percentage, were physically more active, were less likely to use sunscreen and consumed more skimmed milk, fat products and red meat and sausages than girls (Table 1). The boys had a higher absolute intake of vitamin D from food than the girls, but the energy-adjusted intake of vitamin D did not differ between sexes (Table 1).

Vitamin D intake from food and supplements

The mean intake of vitamin D from food but not supplements was 5·9 μg/d in all children, 5·4 μg/d in the girls and 6·4 μg/d in the boys (Table 1). Altogether, 40·8 % of all children, 36·6 % of the girls and 45·1 % of the boys, did not use vitamin D supplements at all, and 45·6 % of all children, 43·4 % of the girls and 47·9 % of the boys, did not use them during the month before the blood sampling. The total intake of vitamin D from food and supplements was on average 7·7 μg/d in all children, 7·4 μg/d in the girls and 8·1 μg/d in the boys, including both supplement consumers and non-consumers. Total vitamin D intake from food and supplements was below the earlier Finnish recommendation of 7·5 μg/d[25] in 60·2 % of all children, in 66·3 % of the girls and in 54·2 % of the boys. As many as 82·4 % of all children, 84·8 % of the girls and 80·0 % of the boys, did not meet the current Nordic recommendation for total vitamin D intake of 10 μg/d[11]. As many as 95·8 % of all children, 95·2 % of the girls and 96·5 % of the boys, did not meet the current Finnish recommendation for vitamin D supplement use of 7·5 μg/d[12].

Serum 25-hydroxyvitamin D concentration

Serum 25(OH)D concentration varied between 19·4 and 199·0 nmol/l. The mean serum 25(OH)D concentration was 68·5 nmol/l in all children, 66·5 nmol/l in the girls and 70·4 in the boys (Table 1). Only 0·5 % of the children, 0·5 % of the girls and 0·5 % of the boys, had serum 25(OH)D concentrations below 25 nmol/l (Fig. 1). Altogether, 19·5 % of all children, 17·9 % of the girls and 21·1 % of the boys, had serum 25(OH)D concentrations below 50 nmol/l, and 69·0 % of all children, 76·1 % of the girls and 62·1 % of the boys, had serum 25(OH)D concentrations below 75 nmol/l. Serum 25(OH)D concentration was above 125 nmol/l in 2·4 % of all children, in 1·6 % of the girls and in 3·2 % of the boys. Serum 25(OH)D levels did not differ significantly across the calendar months (Fig. 2).

Dietary sources of vitamin D

The main dietary sources of vitamin D among children were milk and other milk products, fat products and fish products, which accounted for 88·4 % of vitamin D obtained from food (Table 2). The children received 51·7 % of their vitamin D from milk products. The mean intake of vitamin D from milk was higher in the boys than in the girls (3·05 (SD 1·37) *v.* 2·68 (SD 1·12) μg/d; $P = 0·004$). There were no other sex differences in dietary sources of vitamin D.

Determinants of serum 25-hydroxyvitamin D concentration

A higher dietary intake of vitamin D was associated with a higher serum 25(OH)D concentration in all children ($\beta = 0·205$; $P < 0·001$), in the girls ($\beta = 0·172$; $P = 0·020$) and in the boys ($\beta = 0·211$; $P = 0·004$), without adjustments.

In all children, higher consumption of milk products, higher levels of physical activity and younger age were associated with higher serum 25(OH)D concentrations without adjustments (Table 3, model 1). Only higher consumption of milk products was related to a higher serum 25(OH)D concentration when all the variables listed in Table 3 were entered simultaneously in the stepwise model (Table 3, model 2).

Among girls, higher consumption of milk products, higher intake of vitamin D from supplements and younger age were associated with higher serum 25(OH)D concentrations without adjustments (Table 3, model 1). These relationships remained statistically significant when all the variables listed in Table 3 were entered simultaneously in the stepwise model (Table 3, model 2).

Among boys, higher consumption of milk products, sunscreen use and a higher household income were associated with higher serum 25(OH)D concentrations without adjustments (Table 3, model 1). Only higher consumption of milk products was related to higher serum 25(OH)D concentrations when all the variables listed in Table 3 were entered simultaneously in the stepwise model (Table 3, model 2).

Risk factors of having serum 25-hydroxyvitamin D concentration below 50 nmol/l

Children who drank at least 450 g/d of milk had a 72–74 % lower risk of having serum 25(OH)D concentration <50 nmol/l than those who drank <300 g/d of milk adjusted for age and sex (Table 4). Children who spent at least 2·2 h/d in physical activity had a 59 % lower risk for low serum 25(OH)D levels (<50 nmol/l) than those with less than 1·5 h/d of physical activity. Children with average daylight time of at least 13·1 h/d during 3 months before blood sampling had a 50 % lower risk, and children whose blood samples were collected in autumn had a 57 % lower risk of having serum 25(OH)D concentration <50 nmol/l than those in the reference groups. Children who used sunscreen had a 58 % lower risk of having low serum 25(OH)D levels than those with no

Table 1. Characteristics of children*
(Medians and interquartile ranges (IQR); mean values and standard deviations; numbers and percentages of children)

	All†		Girls†		Boys†		
	Median	IQR	Median	IQR	Median	IQR	*P*
Age (years)							0·270
Mean	7·6		7·6		7·6		
SD	0·4		0·4		0·4		
Parental education							0·410
Vocational school or less							
n	67		30		37		
%	18·0		16·3		19·6		
Vocational high school or university							
n	306		154		152		
%	82·0		83·7		80·4		
Household income							0·176
≤30 000€/year							
n	74		42		32		
%	20·2		23·1		17·4		
>30 000€/year							
n	292		140		152		
%	79·8		76·9		82·6		
Body height (cm)							0·005
Mean	128·9		128·1		129·7		
SD	5·5		5·6		5·3		
Body weight (kg)							0·205
Mean	27·0		26·7		27·2		
SD	4·8		5·1		4·5		
BMI-SDS							0·738
Mean	−0·16		−0·14		−0·18		
SD	1·05		1·07		1·04		
Waist circumference (cm)							0·043
Mean	56·7		56·2		57·3		
SD	5·6		5·9		5·1		
Body fat percentage							<0·001
Mean	19·8		22·6		17·0		
SD	8·2		7·7		7·6		
Pubertal status							0·117
Pre-pubertal							
n	357		174		183		
%	97·0		95·6		98·4		
Pubertal							
n	11		8		3		
%	3·0		4·4		1·6		
Skin type							0·751
I always burns, never tans							
n	5		2		3		
%	1·7		1·4		2·1		
II often burns, sometimes tans							
n	78		43		35		
%	27·2		29·7		24·6		
III sometimes burns, often tans							
n	193		94		99		
%	67·2		64·8		69·7		
IV never burns, always tans							
n	11		6		5		
%	3·8		4·1		3·5		
Physical activity (h/d)	1·8	1·3–2·4	1·7	1·2–2·2	2·0	1·5–2·6	<0·001
Sedentary behaviour (h/d)	3·3	2·4–4·4	3·4	2·5–4·7	3·2	2·3–4·2	0·059
Average daylight time during 3 months before blood sampling (h/d)	10·98	7·12–13·85	11·04	7·21–14·01	10·92	7·06–13·84	0·567
Season of blood sampling							0·390
Winter (from December to February)							
n	109		52		57		
%	29·1		28·3		30·0		
Spring (from March to May)							
n	121		54		67		
%	32·4		29·3		35·3		
Summer (from June to August)							
n	27		13		14		
%	7·2		7·1		7·4		

Table 1. *Continued*

	All†		Girls†		Boys†		
	Median	IQR	Median	IQR	Median	IQR	*P*
Autumn (from September to November)							
n	117		65		52		
%	31·3		35·3		27·4		
Travels to sunny countries							0·459
Yes							
n	23		10		13		
%	8·1		6·9		9·3		
No							
n	262		135		127		
%	91·9		93·1		90·7		
Sunscreen use							<0·001
Occasionally or frequently							
n	230		129		101		
%	80·1		89·0		53·2		
No							
n	57		16		41		
%	19·9		11·0		21·6		
Food consumption (g/d)							
Milk products	742	567–921	708	523–862	804	612–982	<0·001
Milk	588	416–751	556	404–693	614	452–795	0·004
Skimmed milk (<1 % of fat)	387	93·4–610	359	90·2–588	450	115–650	0·042
Milk (≥1 % of fat)	95·5	33·3–264	94·1	30·6–262	99·6	37·5–272	0·643
Sour milk products	93·8	37·5–150	89·2	41·9–149	93·8	37·5–163	0·470
Other dairy products	48·8	25·0–75·6	47·8	26·1–73·3	51·0	24·2–76·4	0·664
Fat products	26·0	19·2–33·3	24·4	19·0–30·8	28·7	19·9–36·4	0·003
Margarine	9·4	4·4–16·1	9·7	5·9–14·8	9·1	3·8–18·4	0·997
Other fat products	5·2	2·7–8·9	5·1	2·7–8·9	5·5	2·7–9·3	0·723
Butter and butter-oil mixtures	2·9	0·8–7·7	2·9	1·0–6·4	2·8	0·7–9·5	0·647
Oil and fluid margarines	2·9	1·2–5·6	2·5	1·2–5·3	3·1	1·2–6·6	0·126
Fish products	6·9	0·0–24·6	6·3	0·0–22·8	7·5	0·0–27·5	0·442
Meat products	88·4	64·1–119	78·2	59·6–105	104	69·8–132	<0·001
Red meat and sausage	73·7	49·2–103	63·9	45·7–90·0	81·2	53·8–81·2	<0·001
Poultry	10·3	0·0–10·3	10·3	0·0–23·5	10·2	0·0–28·6	0·904
Egg	10·0	5·6–19·2	9·6	5·7–18·1	11·0	5·6–19·8	0·367
Grain products	180	146–223	179	141–213	181	147–233	0·128
Vitamin D intake from food (μg/d)	5·9	2·1	5·4	1·7	6·4	2·4	<0·001
Vitamin D intake from food (μg/MJ)	0·87	0·29	0·85	0·25	0·88	0·31	0·319
Vitamin D intake from supplements (μg/d)	0·36	0·00–4·29	0·36	0·00–4·29	0·36	0·00–4·42	0·536
Serum 25(OH)D (nmol/l)	68·5	23·5	66·5	21·1	70·4	25·5	0·186

BMI-SDS, BMI-standard deviation score calculated using Finnish reference values[28]; 25(OH)D, 25-hydroxyvitamin D.
* Differences between girls and boys were tested with independent samples *t* test for normally distributed variables, Mann–Whitney's *U* test for skewed variables and Pearson's χ^2 test for categorical variables. Logarithmic transformation was performed for body weight, waist circumference, body fat percentage and serum 25(OH)D before analyses.
† *n* varies from 285 to 374 in different variables: *n* 374, 184 girls and 190 boys: age, sex, physical activity, sedentary behaviour, average daylight time, season of blood sampling, dietary factors; *n* =373, 184 girls and 189 boys: parental education; *n* 368, 182 girls and 186 boys: pubertal status; *n* 366, 182 girls and 184 boys: household income; *n* 364, 181 girls and 183 boys: body fat percentage; *n* 287, 145 girls and 142 boys: vitamin D intake from supplements, skin type, sunscreen use; *n* 285, 145 girls and 140 boys: travels to sunny countries.

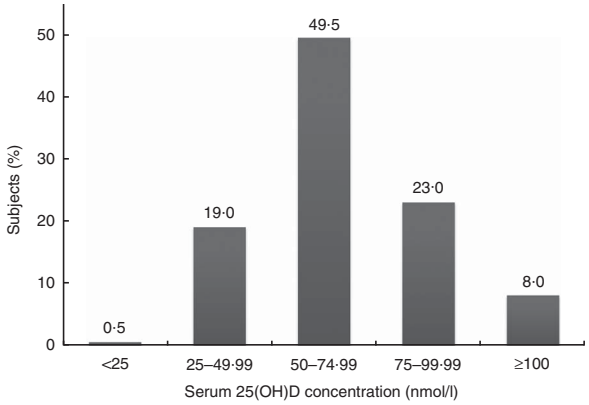

Fig. 1. Distribution of serum 25-hydroxyvitamin D (25(OH)D) concentration (nmol/l) among all children.

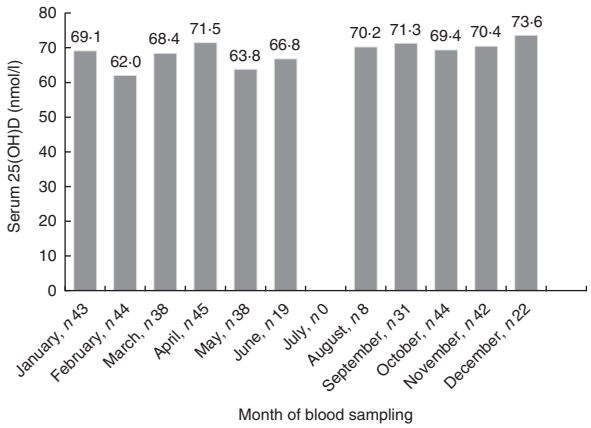

Fig. 2. Serum 25-hydroxyvitamin D (25(OH)D) concentrations across the calendar months among children.

Table 2. Main dietary sources of vitamin D in all children
(Mean values and standard deviations; percentages and standard deviations)

	Dietary intake of vitamin D			
Foods	Mean (μg/d)	SD (μg/d)	Percentage	SD
Milk products*	3·05	1·35	51·7	16·1
Milk	2·87	1·27	48·7	16·8
Skimmed milk (<1 % of fat)	1·92	1·47	31·8	21·8
Milk (≥1 % of fat)	0·95	1·13	16·9	19·1
Sour milk products	0·10	0·55	1·6	5·5
Other dairy products	0·07	0·17	1·4	3·6
Fat products*	1·53	0·91	26·6	14·3
Margarines	1·02	0·88	17·5	14·4
Other fat products	0·38	0·32	6·9	6·2
Butter and butter-oil mixtures	0·10	0·07	1·5	4·3
Oil and fluid margarines	0·03	0·13	0·6	2·3
Fish products	0·72	1·22	10·1	14·9
Meat products	0·35	0·24	6·6	5·4
Red meat and sausage	0·28	0·18	5·4	4·2
Poultry	0·07	0·20	1·2	3·6
Egg	0·17	0·14	3·1	2·8
Grain products	0·05	0·07	1·0	1·5

* In Finland, most of the milk products and margarines, many other fat products and some of the sour milk products are generally fortified with vitamin D, whereas in other dairy products and butter and butter-oil mixtures as well as oil and fluid margarines vitamin D fortification is less common.

sunscreen use. The associations of physical activity (OR 0·51; 95 % CI 0·23, 1·13 for children who spent at least 2·2 h/d in physical activity) and season of blood sampling (OR 0·49; 95 % CI 0·21, 1·15 for autumn) with the risk of having serum 25(OH)D concentration <50 nmol/l weakened slightly after further adjustment for sunscreen use. Further adjustments for milk consumption, physical activity or average daylight time during the 3 months before blood sampling had a weak if any effect on other relationships in Table 4 (data not shown). Other possible determinants of serum 25(OH)D concentration were not related to the risk of having serum 25(OH)D concentration <50 nmol/l (data not shown).

Discussion

The results of our study in a population sample of Finnish children showed that about 60 % of the children did not meet the previous Nordic recommendation for vitamin D intake from food and supplements of 7·5 μg/d, and over 80 % of the children did not meet the current Nordic recommendation for total vitamin D intake of 10·0 μg/d. Almost 20 % of the children had serum 25(OH)D concentration below 50 nmol/l, which some authors regard as vitamin D deficiency, and about 70 % of the children had serum 25(OH)D concentration below 75 nmol/l, which is considered by some authors to be an insufficient level. Milk was the main dietary source of vitamin D, and milk products were the strongest determinants of serum 25(OH)D concentration in children. Three glasses (450 g) of milk per d was sufficient to reduce the risk of having serum 25(OH)D concentration below 50 nmol/l.

In our study, serum 25(OH)D levels were not as low as expected given the northern latitude of Finland where cutaneous synthesis of vitamin D induced by the sun is limited especially during winter. Some authors have suggested that the lower limit for sufficient serum 25(OH)D concentration could be as high as 75 nmol/l[1,7]. However, the Institute of Medicine in the USA concluded that serum 25(OH)D levels of 50 nmol/l cover the requirements of at least 97·5 % of the people[3]. About one-third of the children in our population sample had serum 25(OH)D levels of at least 75 nmol/l. About 20 % of the children had serum 25(OH)D levels below 50 nmol/l, and only 0·5 % of the children had serum 25(OH)D concentration below 25 nmol/l. The Diasorin LIAISON® 25 OH Vitamin D TOTAL Assay is commonly used to measure serum 25(OH)D concentration, but this assay has been reported to provide slightly lower serum 25(OH)D concentrations than other assays, which suggests that the serum 25(OH)D levels in our study may even be slightly underestimated[10]. Serum 25(OH)D levels among children in our study were higher than that among children in many other countries, even in those located in more southern latitudes[32]. The intake of vitamin D among children in our study was at the same level as among children in Sweden and Norway but higher than that among children in many other European countries[33,34], which is the most feasible explanation for the relatively high serum 25(OH)D levels in our study. The main dietary source of vitamin D in our study population was milk fortified with vitamin D, which is commonly used in Finland. Another reason for the relatively high serum 25(OH)D levels in our study could due to a more common vitamin D supplement use in Finland than in other countries. In all, when comparing serum 25(OH)D levels between studies, the differences in age, ethnic background and other characteristics of study populations, season of blood sampling, latitude and serum 25(OH)D assays must be taken into account.

Girls have had lower serum 25(OH)D concentrations than boys in several studies among children and adolescents[16–18]. The explanation for this sex difference may be that girls have a lower intake of vitamin D from food and supplements, lower levels of physical activity outdoors, a higher body fat content

Table 3. Determinants of serum 25-hydroxyvitamin D concentration in children*
(Regression coefficients and *P*-values from linear regression models)

	All children†				Girls†				Boys†			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
Sex‡	0·068	0·186										
Age (years)	−0·125	0·016			−0·161	0·029	−0·198	0·015	−0·103	0·157		
Parental education§	0·019	0·721			−0·091	0·221			0·110	0·131		
Household income‖	0·027	0·606			−0·108	0·148			0·147	0·046		
Body fat percentage	−0·036	0·490			−0·030	0·684			0·005	0·949		
Skin type¶	0·034	0·561			−0·087	0·298			0·138	0·102		
Physical activity(h/d)**	0·102	0·049			0·127	0·085			0·061	0·403		
Sedentary behaviour (h/d)††	−0·033	0·519			−0·030	0·691			−0·025	0·735		
Average daylight time (h/d)‡‡	0·079	0·125			0·087	0·507			0·078	0·655		
Travels to sunny countries§§	−0·021	0·718			0·052	0·537			−0·086	0·314		
Sunscreen use‖‖	0·098	0·099			−0·046	0·584			0·222	0·008		
Milk products (g/d)	0·256	<0·001	0·262	<0·001	0·166	0·024	0·214	0·009	0·306	<0·001	0·257	0·003
Fat products (g/d)	−0·021	0·692			−0·049	0·506			−0·019	0·798		
Fish products (g/d)	0·043	0·406			0·065	0·382			0·019	0·797		
Meat products (g/d)	0·007	0·886			−0·097	0·192			0·054	0·456		
Vitamin D intake from supplements (μg/d)¶¶	0·059	0·320			0·171	0·040	0·171	0·035	−0·041	0·624		

* The values are standardised regression coefficients and *P*-values from linear regression models by entering each variable separately in model 1 and by entering all the variables stepwise in model 2.
† *n* varies from 285 to 374 in different variables: *n* 374, 184 girls and 190 boys: age, sex, physical activity, sedentary behaviour, average daylight time, dietary factors; *n* 373, 184 girls and 189 boys: parental education; *n* 366, 182 girls and 184 boys: household income; *n* 364, 181 girls and 183 boys: body fat percentage; *n* 287, 145 girls and 142 boys: vitamin D intake from supplements, skin type, sunscreen use; *n* 285, 145 girls and 140 boys: travels to sunny countries.
‡ Sex; 1 = girl, 2 = boy.
§ Parental education; 1 = vocational school or less, 2 = vocational high school or university.
‖ Household income: 1 = ≤30 000€/year, 2 = >30 000€/year.
¶ Skin types according to Fitzpatrick[29], combined in 2 classes: skin colour type I or II = 1, III or IV = 2.
** Physical activity includes organised sports, organised exercise other than sports, unsupervised physical activity, physically active school transportation (such as walking and bicycling), physical activity during recess and physical education.
†† Sedentary behaviour includes screen-based sedentary behaviour (watching TV and videos, using the computer and playing video games, using a mobile phone and playing mobile games), sedentary behaviour related to music (listening to music, playing music), sedentary behaviour related to academic skills (reading, writing), sedentary behaviour related to arts, crafts and games (drawing, doing arts and crafts, playing board and card games) and sitting and lying for a rest.
‡‡ Average daylight time from sunrise to sunset during 3 months before blood sampling.
§§ Travels to sunny countries within 3 months before blood sampling; 1 = no, 2 = yes.
‖‖ Sunscreen use; 1 = no sunscreen use, 2 = sunscreen use occasionally or frequently.
¶¶ Daily intake of vitamin D from supplements on average during a month before blood sampling.

Table 4. Odds ratios (95 % CI) of having serum 25-hydroxyvitamin D concentration below 50 nmol/l* (Odds ratios and 95 % confidence intervals)

	OR	95 % CI
Milk consumption (g/d)		
0–299	1·00	Ref.
300–449	0·47	0·21, 1·09
450–599	0·27	0·12, 0·61
600–749	0·26	0·11, 0·61
≥750	0·28	0·12, 0·63
$P_{\text{for linear trend}}$	0·001	
Physical activity (h/d)		
<1·5	1·00	Ref.
1·5–2·1	0·61	0·33, 1·12
≥2·2	0·41	0·21, 0·80
$P_{\text{for linear trend}}$	0·008	
Average daylight time during 3 months before blood sampling (h/d)		
<8·4	1·00	Ref.
8·4–13·0	0·83	0·46, 1·51
≥13·1	0·50	0·26, 0·96
$P_{\text{for linear trend}}$	0·039	
Season of blood sampling		
Winter (December, January, February)	1·00	Ref.
Spring (March, April, May)	0·64	0·33, 1·23
Summer (June, August)†	0·42	0·13, 1·36
Autumn (September, October, November)	0·43	0·22, 0·87
$P_{\text{for linear trend}}$	0·014	
Sunscreen use		
No	1·00	Ref.
Occasionally or frequently	0·42	0·21, 0·84
$P_{\text{for linear trend}}$	0·014	

Ref., referent value.
* The values are from logistic regression models adjusted for age and sex.
† No subjects studied in July.

and earlier maturation, which have been associated with lower serum 25(OH)D levels compared with boys. We found a lower dietary intake of vitamin D in girls than in boys that was mainly due to a lower consumption of skimmed milk among girls. However, no sex differences in the energy-adjusted intake of vitamin D or in the serum 25(OH)D concentration were observed. Some other studies in children have also reported no difference in serum 25(OH)D concentration between sexes[35,36]. However, there are a few European studies on the association between vitamin D intake and serum 25(OH)D concentration among school-aged children[15,16]. Consistent with those studies, we observed a direct relationship between the dietary intake of vitamin D and serum 25(OH)D concentration.

Age has been inversely associated with serum 25(OH)D concentration among children in some previous studies[16,35]. The explanation for this relationship could be that older children have a lower intake of vitamin D from food and supplements, lower levels of physical activity outdoors, a higher body fat content and more advanced maturation than younger children. However, in some studies among children, age has not been related to serum 25(OH)D concentration or has even been directly associated with serum 25(OH)D levels[18,37]. We found an inverse association between age and serum 25(OH)D concentration in girls, but the relationship was much weaker in boys.

An important finding of our study is that only 20 % of vitamin D was obtained from its natural dietary sources such as fish, meat and eggs in the population sample of Finnish children. Fish is easily available in Finland and it is rich in vitamin D, but the consumption of fish was very low among children in our study. This may be one of the reasons why we did not find an association between fish consumption and serum 25(OH)D concentration in children.

In order to increase the intake of vitamin D at the population level, most fluid milk products, spreads and some other single food products have been fortified with vitamin D in Finland after the recommendation of the Ministry of Social Affairs and Health in 2003. In 2010, the Finnish recommendation for vitamin D fortification was increased from 0·5 to 1 μg/100 g for fluid milk products and from 10 to 20 μg/100 g for spreads[38]. The Nordic and Finnish recommendations for vitamin D intake were increased in 2014 to reach sufficient serum levels of vitamin D in these populations[11,12].

A Finnish study assessed the impacts of the initiation of fortification of fluid milk products and margarines with vitamin D and found a higher vitamin D intake and a higher mean serum 25(OH)D concentration after fortification in 4-year-old children[39]. In Canadian and US studies among children, a higher intake of milk fortified with vitamin D has been associated with a higher serum 25(OH)D concentration[40] and with a lower risk of having vitamin D deficiency[41]. A recent British study also concluded that the fortification of food with vitamin D could be the most effective way to improve vitamin D status in children[42].

The main dietary sources of vitamin D among children in our study were milk products that accounted for about half of vitamin D intake from food and were the strongest determinants of serum 25(OH)D concentration. Our study also showed that daily use of at

least 450 g of milk mostly fortified with 0·5 μg of vitamin D/100 g was sufficient to reduce the risk of having serum 25(OH)D concentration below 50 nmol/l among children. These findings are expected, given that the consumption of milk fortified with vitamin D is high among Finnish children. The level of vitamin D fortification for fluid milk products is currently higher than that at the time when we collected the food records. Nowadays, even a lower consumption of milk fortified with vitamin D than 450 g/d could be adequate to reduce the risk of having serum 25(OH)D concentration below 50 nmol/l.

The results of some previous studies in adults suggest that increased Ca intake, even without vitamin D supplementation, can increase serum 25(OH)D concentration, but the combination of Ca and vitamin D supplementation may be more effective in increasing serum 25(OH)D concentration than either of them alone[43,44]. However, not all studies have found this effect[45,46]. Although these findings have been inconsistent, variability in serum 25(OH)D response to vitamin D intake may be partly due to individual differences in Ca status. In our study, milk was the main dietary source of Ca and vitamin D, and therefore we were not able to study the associations of Ca and vitamin D intake with serum 25(OH)D concentration separately.

In previous studies among children, the use of vitamin D supplements has been associated with higher serum 25(OH)D levels and a lower risk of vitamin D deficiency[17,21,47]. Although we found no marked difference in the intake of vitamin D from supplements between sexes, the use of vitamin D supplements was a stronger determinant of serum 25(OH)D concentration in girls than in boys. This observation suggests that the lower dietary intake of vitamin D was compensated for the higher use of vitamin D supplements in girls. However, supplement use was still rather low compared with the national recommendation. This is consistent with the results of a previous Finnish study where 86 % of children aged 1 year and only 21 % of children aged 6 years used vitamin D supplements[13]. This finding indicates that the recommendation for vitamin D supplement use is followed better by younger children. Our findings also support the idea that the use of vitamin D supplements according to the current Finnish nutrition recommendations is an important way to increase serum 25(OH)D concentration, particularly if the dietary intake of vitamin D is insufficient.

Obesity has been associated with low serum 25(OH)D levels or vitamin D deficiency in some previous studies among children[17–19]. However, none of the measures of adiposity was independently related to serum 25(OH)D concentration in our population sample of Finnish children where, 15 % of the girls and 11 % of the boys were overweight or obese at baseline[48]. One reason for this inconsistency could be that lifestyle factors partly explain the association of adiposity with low serum 25(OH)D levels. It is also possible that obesity but not less-severe adiposity is related to vitamin D status in children. However, there was no difference in the risk of vitamin D deficiency between normal weight and obese children in a recent study[35], which is in line with the results of our population-based study.

Vigorous physical activity was directly associated with serum 25(OH)D concentration in one study among children[18]. However, it remained unclear whether the relationship was independent of some confounding factors such as time spent outdoors and the dietary intake of vitamin D because of lack of data on these factors. We also found a direct association between physical activity and serum 25(OH)D concentration in children, but it was explained by other determinants of vitamin D status. This observation is consistent with the results of one previous study in children[16]. The direct association between physical activity and serum 25(OH)D concentration may reflect the exposure to sunlight due to physical activity outdoors, although we were not able to distinguish between physical activity indoors and outdoors.

We found that daylight time as a continuous variable was not associated with serum 25(OH)D concentration in children. However, the risk of having low serum 25(OH)D concentration was lower in autumn than in winter possibly due to the longer daily exposure to sunlight during months preceding autumn. Because of the northern latitude of Kuopio, Finland (62·89°N), the daylight time varied between 4·8 h/d in December and 20·2 h/d in June. One reason for observing no clear difference in the risk of having low serum 25(OH)D levels between summer and winter may be that we had a few study visits in summer, because most of Finns have vacation in summer. However, we observed that an average of at least 13·1 h of daylight time/d during 3 months before blood sampling was associated with a reduced risk of having serum 25(OH)D concentration below 50 nmol/l among children. These findings suggest that the time spent outdoors, particularly when the daylight time is long, should be increased in Finland and other Northern countries to promote the cutaneous synthesis of vitamin D and to prevent vitamin D deficiency among children. We also found that sunscreen use was associated with higher serum 25(OH)D levels in boys but not in girls. The reason for this may be that boys who used sunscreen spent more time outdoors than girls who used sunscreen or that girls used sunscreen more frequently than boys. Therefore, sunscreen use was sufficient to reduce the cutaneous synthesis of vitamin D induced by sun in girls but not in boys. Our observations suggest that the intake of vitamin D from food and supplements is a much more important determinant of serum 25(OH)D concentration than daylight time among children in Finland, where the cutaneous synthesis of vitamin D induced by sun is limited.

The strengths of our study include the population-based sample of girls and boys, the assessment of dietary vitamin D intake and other dietary factors using 4-d food records and the assessment of a number of other relevant determinants of serum 25(OH)D concentration. A weakness of the study is that the food record method is subject to errors related to inaccuracy in estimating portion sizes and tendency not to follow a normal diet during reporting. We calculated that the parents under-reported the energy intake in 22 % of the girls and in 24 % of the boys, suggesting that they may also have under-reported their children's dietary intake of vitamin D. Another weakness of our study is the assessment of vitamin D intake from supplements by a questionnaire that may have underestimated or overestimated the use of vitamin D supplements. Moreover, the number of children who were examined during summer months and who had travelled to sunny countries was low, which diminished the statistical power of the analyses related to these variables.

Finally, we did not collect data on time spent outdoors and on sunlight exposure behaviour. However, the levels of physical activity to some extent reflect time spent outdoors.

Conclusions

Our study shows that about four-fifths of Finnish children did not meet the current recommendation for vitamin D intake from food and supplements. One-fifth of children had serum 25(OH)D concentration below 50 nmol/l that some authors regard as vitamin D deficiency. These findings suggest that many children need more vitamin D from food or supplements to reach sufficient serum 25 (OH)D levels in countries located in high latitudes where the cutaneous synthesis of vitamin D induced by sunlight is limited. The consumption of milk was the strongest determinant of serum 25(OH)D concentration among girls and boys in Finland followed by the intake of vitamin D from supplements in girls. These observations emphasise that more attention should be paid to the sufficient intake of vitamin D from food and supplements, especially among children who do not use fortified milk products.

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The authors' contributions are as follows: S. S. participated in the collection of data, conducted the statistical analyses and wrote the draft of the manuscript. A.-M. E. and V. L. participated in data collection and statistical analyses and contributed to the critical revision of the manuscript. T. V. and N. Z. participated in the collection of data and contributed to the critical revision of the manuscript. A. M. contributed to the interpretation of the data, critical revision of the manuscript and provided funding for the study. T. A. L. was responsible for planning the study, funding, statistical analyses and the interpretation of the data, and also contributed to the critical revision of the manuscript. All the authors read and approved the final version of the manuscript.

The authors declare that there are no conflicts of interest.

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