

REGULAR ARTICLE

Vitamin D deficiency at the Arctic Circle – a study in food-allergic adolescents and controls

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ABSTRACT

Aim: At the extremes of latitude, UVB intensity is insufficient for adequate vitamin D synthesis in winter. Fatty fish, vitamin D enriched milk, margarine and eggs are main dietary sources of vitamin D. Their elimination may increase the risk of vitamin D deficiency. The aim was to assess vitamin D status in food-allergic adolescents eliminating milk, egg and/or fish compared with adolescents on normal diets.

Methods: In winter, vitamin D intake was assessed by a food frequency questionnaire in 20 food-allergic adolescents and 42 controls in the population-based Obstructive Lung Disease In Northern Sweden (OLIN) cohort studies. Vitamin D supplementation was queried. Serum 25-hydroxyvitamin D [S-25(OH)D] and S-parathormone (S-PTH) levels were determined.

Results: Mean (SD) dietary vitamin D intake was 7.9 (3.6) µg/day in allergic adolescents and 7.8 (3.4) in controls ($p > 0.05$). Mean (SD) S-25(OH)D levels in supplement consumers were 44 (18) nmol/L compared with 35 (10) in non-consumers ($p = 0.03$). S-25(OH)D and S-PTH levels were similar in food-allergic adolescents and controls ($p > 0.05$). Eighty-two percentage had deficient S-25(OH)D levels <50 nmol/L, and none reached levels >75 nmol/L.

Conclusion: Vitamin D deficiency was as common in food-allergic adolescents as in controls although the vitamin D intake met national recommendations. Large-scale studies on the prevalence of vitamin D deficiency in this region are needed.

INTRODUCTION

Vitamin D is currently a topic of great interest for the public health sector, not only for the well-known function of mineralization of skeleton and teeth but also for the suggested role in extra-skeletal health. Suboptimal vitamin D status has been proposed in the pathophysiology of immune disorders (asthma, allergy, diabetes type 1 and coeliac disease) (1). Over the last decades, the prevalence of allergic diseases, such as asthma and eczema, has increased among children and several studies now report an increase in the prevalence of food allergy (2,3). Recently, we

reported an increase in allergic sensitization among children in northern Sweden even though the increase in asthma seems to have levelled off (4). Several environmental factors have been implicated in the allergy epidemic including life style changes, reduced exposure to microbial organisms and suboptimal levels of a number of dietary factors including vitamin D (5). Observational studies have reported an association between increasing latitude and proxies of food allergy prevalence such as higher frequencies of food allergy related hospital admissions and

Abbreviations

BMI, Body Mass Index; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence; HPLC-APCI-MS, High-pressure liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry; IgE, Immunoglobulin E; ISAAC, International Study of Asthma and Allergies in Childhood; OLIN, Obstructive Lung Disease In Northern Sweden; S-25(OH)D, Serum 25-hydroxyvitamin D; S-PTH, Serum-parathormone; SPT, Skin prick test; Tregs, CD4+ CD25+ regulatory T cells; WHO, World Health Organization.

Key notes

- Vitamin D deficiency was as common in food-allergic adolescents eliminating vitamin D containing foods (milk, egg and/or fish) as in controls.
- Eight of 10 adolescents living near the Arctic Circle were vitamin D deficient in winter although the vitamin D intake met current recommendations.
- The prevalence of vitamin D deficiency in this region, and its relation to health outcomes need further investigation into a larger study population.

epinephrine auto-injector prescriptions in regions with less sun exposure in the United States and Australia [reviewed in (6)].

Vitamin D is a modulator of innate and adaptive immune system functions [reviewed in (1)]. There is an increasing evidence that the immunomodulatory effect of vitamin D acts not only through modulation of T-helper cell function, but also through induction of CD4⁺ CD25⁺ regulatory T cells (Tregs)(7). Tregs play a critical role in the maintenance of peripheral tolerance, and the active form of vitamin D, 1 α ,25(OH)₂D₃, has been demonstrated to enhance Treg differentiation (8). Due to these immune-modulating functions, vitamin D deficiency has been suggested to predispose to and aggravate manifestations of allergic disease, including food allergies.

To define vitamin D status, plasma or serum 25-hydroxyvitamin D [S-25(OH)D] are analysed. Optimal levels are debated but based on measures of bone health, deficiency is considered at levels <50 nmol/L (9,10). The major source of vitamin D is dermal biosynthesis catalysed by ultraviolet B sunlight (11). During winter, northern Sweden has limited hours of daylight leading to reduced sun exposure. Consequently, the dietary source of vitamin D is of specific importance in this region (12,13). Fatty fish, eggs, vitamin D fortified milk and margarines are the main sources, foremost the active form D₃. These important basic foods also contain common food allergens. Thus, children with food allergies towards these foods can be at increased risk of vitamin D deficiency.

Vitamin D intake amongst Swedish adolescents has been shown to be below recommended intake (14), and a recent study reported inadequate vitamin D intake in children with coeliac disease (15). However, there is a paucity of studies investigating vitamin D status in food-allergic adolescents eliminating vitamin D containing foods. Therefore, the main objective of this study was to assess whether vitamin D intake and status in food-allergic adolescents eliminating milk, egg and/or fish from their diet differ compared with controls on a normal diet.

MATERIAL AND METHODS

The paediatric cohort II within the Obstructive Lung Disease In Northern Sweden studies

Clinical and epidemiological research on asthma and allergy is performed among children in the OLIN studies since 1996 (16). This study is based on the second paediatric cohort within the OLIN studies recruited in 2006 (4). In brief, this paediatric cohort consisted of 2704 participants aged 7–8 years in Luleå, Kiruna and Piteå. Of these, 2585 (96%) participated in a questionnaire based primarily on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Participants from Kiruna and Luleå were also invited to a skin prick test (SPT) to common airborne allergens and 1700 (90%) participated. The cohort was followed up in 2010, including a new questionnaire, SPT and analysis of specific immunoglobulin E (IgE) antibodies towards cow's milk, cod, egg

and wheat. In the follow-up study in 2010, when participants were 12–13 years old, 125 (5%) reported hypersensitivity reactions towards one or several of the basic food items (i.e. milk, egg, fish and wheat) and had eliminated them from their diet.

Participants

This study was conducted between February 2011 and March 2011. All participants were recruited from the paediatric OLIN cohort. All participants were of Caucasian origin and were living in Norrbotten County, latitude 65–67° north. Of 125 participants who reported hypersensitivity reactions towards cow's milk, egg or fish and complete avoidance of the offending food or foods, 27 were diagnosed as food allergic (based on clinical history, analysis of specific IgE to the offending foods and oral food challenges). Of these, 21 agreed to participate in this study. A control group of randomly selected 42 adolescents from the same OLIN cohort was included. Participants in the control group had no diagnosed food allergy, no previous hypersensitivity reactions towards any foods, and were on a normal diet. The study was approved by the local ethics committee of Umeå University, and written consent was collected before participation in the study.

Questionnaires

As dietary assessment must cover a relatively long time period to capture less commonly consumed foods containing vitamin D, a short food frequency questionnaire was chosen (17). The questionnaire included 17 food items known to be important food sources of vitamin D among children and adolescents in Sweden (14). Food frequency was queried as numbers of servings per day, week or month. The Swedish Food Database (version 2011-06-21) was used for estimations of vitamin D intake from foods. A question on the use (brand and dose) of supplements containing vitamin D was also included. To assess sunlight exposure, questions on hours spent outdoors, trips to sunnier locations, use of sunscreen and protective clothes were included. Hours spent outdoors were calculated as a mean value per day [(week-days*5 + weekends*2)/7].

Anthropometric measurements and biochemistry

Weight was measured to nearest 0.1 kg. Height and waist circumference were measured to nearest 0.5 cm. Body mass index (BMI, kg/m²) and BMI-Z scores were calculated using the definition by World Health Organization (WHO) (18).

Anaesthetic cream (EMLA) was applied before venous blood was drawn. Blood samples were immediately centrifuged and kept in a freezer at –20°C until analysis. S-25(OH)D was analysed according to the most valid method, high-pressure liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (HPLC–APCI–MS) (Vitas, Oslo, Norway) (19). Serum-parathormone (S-PTH) was analysed by routine analysis at the Department of

Clinical Chemistry, Umeå University Hospital, the same day as blood was drawn.

Statistics

This study has 80% power ($\alpha = 0.05$) to detect a 30% reduction in the frequency of S-25(OH) D levels >50 nmol/L in the allergic group ($n = 20$), assuming that 97.5% of the participants in the control group ($n = 40$) attain S-25(OH) D levels >50 nmol/L. Statistical analyses were performed using PASW 18.0 (SPSS, Chicago, IL, USA). Descriptive data are presented as mean (SD), number and frequency (%) as appropriate. Independent samples *t*-test and Fisher's exact test were used to analyse differences between the two groups. Spearman's correlation test was used to analyse associations between S-25(OH)D and S-PTH.

RESULTS

Descriptives of the study population

Although 21 allergic adolescents agreed to participate, one of them was not able to participate fully in the investigations that were completed during a short time period in winter (February–March). Therefore, 20 adolescents remained for analysis in the allergic group. Of these, 6 were diagnosed with cow's milk protein allergy, 6 with fish allergy, 3 with egg allergy and 5 with multiple food allergy (including diagnosed allergy to at least one of the allergens cow's milk, fish and or/egg). Descriptive characteristics of the participants are displayed in Table 1. Allergic boys, but not girls, were significantly shorter compared with controls, and the BMI-Z score of allergic boys was below that of controls (Table 1).

Table 1 Age and anthropometric data in the allergic and control groups

	Girls			Boys		
	Allergic ($n = 11$)	Controls ($n = 19$)	p-Value*	Allergic ($n = 9$)	Controls ($n = 23$)	p-Value*
Age (year) [†]	13.3 (0.6)	13.3 (0.6)	0.980	13.0 (0.6)	13.4 (0.6)	0.106
Weight (kg) [†]	51.8 (8.7)	53.5 (8.6)	0.490	42.4 (5.1)	54.4 (10.2)	0.001
Height (cm) [†]	156.6 (5.5)	161 (9.6)	0.079	153.9 (9.3)	166 (2.0)	0.004
Waist circumference (cm) [†]	78.2 (9.0)	70.2 (4.8)	0.040	68.4 (4.7)	70.7 (8.7)	0.352
BMI [†]	21.1 (3.2)	20.9 (3.2)	0.847	17.9 (1.6)	19.4 (3.2)	0.061
BMI-Z score [‡]	0.68 (1.0)	0.73 (1.1)	0.918	−0.34 (0.72)	0.01 (0.8)	0.268
Height-Z score [‡]	−0.96 (0.7)	0.4 (1.1)	0.173	−0.4 (0.9)	1.1 (1.3)	0.003

*Independent samples *t*-test was used for the assessment of differences between girls and boys in the allergic and control group.

[†]Mean (SD).

[‡]WHO Child growth standards.

Bold font indicates statistically significant difference.

Table 2 Descriptive and biochemistry data of the study population of allergic adolescents and controls

Variables measured and reported	Allergic ($n = 20$)	Controls ($n = 42$)	p-Value*
Serum-25(OH) D (nmol/L) [†]	35 (13)	38 (13)	0.38
Serum-PTH (mmol/L) [†]	5.0 (2.3)	4.9 (1.7)	0.77
Dietary vitamin D intake ($\mu\text{g/day}$) [†]	7.9 (3.6)	7.8 (3.4)	0.89
Total vitamin D intake ($\mu\text{g/day}$) ^{†,‡}	9.9 (5.4)	9.1 (7.3)	0.64
Vitamin D intake from the respective food			
Milk ($\mu\text{g/day}$) [†]	2.3 (3.6)	3.6 (2.3)	0.05
Fish ($\mu\text{g/day}$) [†]	2.2 (3.0)	2.0 (0.7)	0.72
Spreads ($\mu\text{g/day}$) [†]	1.3 (0.9)	1.0 (0.9)	0.73
Eggs ($\mu\text{g/day}$) [†]	0.1 (0.2)	0.1 (0.3)	0.61
Cow's milk protein free products ($\mu\text{g/day}$) [†]	1.1 (0.2)	0.0 (0.01)	<0.001
Other foods ($\mu\text{g/day}$) [†]	0.8 (0.9)	0.7 (0.8)	0.59
Using vitamin D supplements [n (%)]	6 (30)	5 (12)	0.15
Reported hours outdoors weekdays [†]	1.8 (1.0)	1.9 (1.2)	0.51
Reported hours outdoors weekends [†]	2.6 (1.4)	2.9 (2.2)	0.81
Travel [n (%)]	5 (25)	15 (36)	0.56
Sunscreen [n (%)]	13 (65)	36 (86)	0.09
Other kind of sun protection [n (%)]	10 (50)	26 (62)	0.42

*Independent samples *t*-test was used for assessing differences between allergic adolescents and controls.

[†]Mean (SD).

[‡]Intake from diet and vitamin D containing supplements.

Bold font indicates statistically significant difference.

All adolescents had suboptimal S-25(OH)D levels

Dietary and total vitamin D intakes are displayed in Table 2. In summary, adolescents in both groups met the recommended intake (14). Thirty percentage in the allergic and 11% in the control group consumed vitamin D containing supplements, in the form of multivitamin or vitamin D supplements ($p > 0.05$). In these, mean (SD) supplemental vitamin D intake was 8.6 (11.5) $\mu\text{g/day}$. Total mean (SD) vitamin D intake was 16.3 (11.8) $\mu\text{g/day}$ in adolescents consuming vitamin D supplements, compared with 7.8 (3.8) $\mu\text{g/day}$ in non-consumers ($p < 0.001$). This was reflected as higher mean (SD) S-25(OH)D levels, 44 (18) nmol/L compared with 35 (10) nmol/L in non-consumers ($p = 0.03$). S-25(OH)D levels did not differ between the allergic and control groups (Table 2). Overall, 82% of all adolescents had levels below the cut-off S-25(OH)D of 50 nmol/L; 80% of food allergic versus 90% of controls ($p = 0.4$). In the whole study population, median (range) S-25(OH)D concentration was 36 (14–69) nmol/L, and consequently, none reached levels >75 nmol/L. The HPLC–APCI–MS method separates D_2 and D_3 , but only three of 64 samples had D_2 levels above the limit of detection (all of them in the allergic group). D_2 and D_3 were therefore not analysed separately.

Serum-parathormone was measured as a marker of bone health, and levels did not differ between the two groups (Table 2). In total, six adolescents (10%) had elevated S-PTH levels ≥ 6.9 nmol/L. Next, we investigated the

association between S-25(OH)D and S-PTH levels. As depicted in Figure 1, there was a weak inverse correlation between S-25(OH)D and S-PTH levels ($r_s -0.390$, $p = 0.02$).

DISCUSSION

Our main findings are that vitamin D deficiency appears to be as common in food-allergic adolescents as in the general population in this setting. We observed a high prevalence of vitamin D deficiency in winter in adolescents living near the Arctic Circle, latitude 65–67° north. In total, 82% of the participating adolescents were vitamin D deficient, defined as S-25(OH) <50 nmol/L, despite an adequate vitamin D intake according to current national recommendations.

Although we expected vitamin D intake to be lower in allergic adolescents eliminating milk, egg and/or fish, this could not be confirmed. Consequently, our hypothesis that food-allergic adolescents on elimination diets would be at increased risk of vitamin D deficiency was not supported as similar deficient S-25(OH)D levels were observed in the control group. The prevalence of vitamin D deficiency in the present study is higher than previously reported. In Boston, USA, 42% of healthy adolescents had 25(OH)D levels <50 nmol/L (20). In Calgary, Canada, 37% of healthy children and adolescents had levels below <75 nmol/L (21). In the European Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, 80% had 25(OH)D levels <75 nmol/L (22). For comparison, none of the participants in the present study attained 25(OH)D levels >75 nmol/L. Less sun hours in northern Sweden plausibly contribute to the higher frequency of vitamin D deficiency in the present study.

Mean (SD) vitamin D intake was 7.9 (3.6) and 7.8 (3.4) $\mu\text{g/d}$ in the allergic and control group, respectively, which meets the current recommended dietary intake in Sweden; 7.5 $\mu\text{g/day}$ for adults and children over 2 years of age (23). Overall, the major source of dietary vitamin D was primarily dairy products and fish, but for some allergic adolescents, it was cow's milk protein free products fortified with vitamin D. Many of these products are fortified with D_2 and not D_3 . D_2 is not as bioactive as D_3 , and this discrepancy is important to keep in mind when evaluating the vitamin D intake (24). The use of vitamin D containing supplements was more frequent in the allergic (30%) compared with the control group (12%), although the difference was not statistically significant ($p > 0.05$). However, this might have levelled a potential difference in vitamin D status between the two groups. In Canada and the United States, the national recommendation on vitamin D intake was changed in 2010 to 15 $\mu\text{g/day}$ for adults and children >1 year old (9). Still, there is debate if the recommendations should have been even further increased (25). In Finland, the recommended intakes and recommendations for fortification have been revised with vitamin D fortification in all liquid dairy products and margarines. Only margarine and liquid dairy products with a fat content

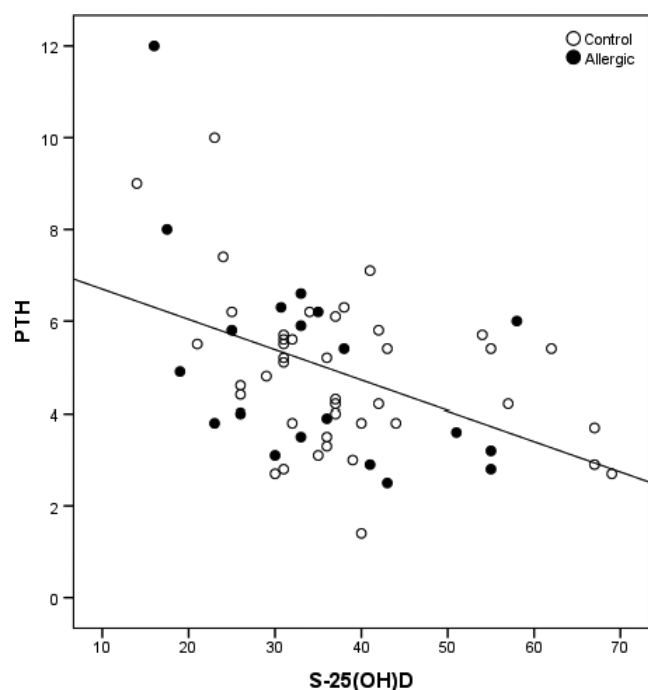


Figure 1 Serum 25-hydroxyvitamin D [S-25(OH)D] levels were inversely associated with serum parathyroid hormone (S-PTH) levels ($r_s -0.390$, $p = 0.02$) in the whole study population ($n = 62$). Filled circles represent allergic adolescents and unfilled circles controls.

≤ 1.5% are fortified in Sweden. Finland also has twice the amount of vitamin D in their fortified foods; 1.0 mg/100 g versus 0.45 mg/100 g in Sweden (26). However, our results suggest that current national recommendations on vitamin D intake do not meet the requirements, at least not in the studied area.

As expected (27), there was a negative correlation between S-25(OH)D and S-PTH. Six adolescents (10%) had elevated PTH levels. Bone mineral density (BMD) was not investigated in the present study. However, in a previous study, adolescent girls with higher 25(OH)D (≥ 74.1 nmol/L) had significantly greater forearm BMD and lower S-PTH levels compared with adolescents girls with lower 25(OH)D (≤ 46.3 nmol/L) as well as moderate (46.4–74.0 nmol/L) 25(OH)D levels (28). As several studies, including the present study, suggest that vitamin D deficiency is common in children and adolescents (22,28–30), further studies are needed to evaluate the possible health consequences. Even though vitamin D deficiency has been implicated in non-bone disease, including allergic disease (1), a casual role has not been clearly demonstrated.

In conclusion, vitamin D deficiency appears to be as common in food-allergic adolescents as in the general population in this setting. The majority of the participating adolescents (82%) living near the Arctic Circle, latitude 65–67° north, were vitamin D deficient in winter despite a dietary vitamin D intake meeting current national recommendations. This calls for large-scale studies on the prevalence of vitamin D deficiency in this region and its possible relation to health outcomes.

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CONFLICT OF INTEREST

None of the authors report any personal or financial conflict of interest.

References

- Jones AP, Tulic MK, Rueter K, Prescott SL. Vitamin D and allergic disease: sunlight at the end of the tunnel? *Nutrients* 2012; 4: 13–28.
- Östblom E, Lilja G, Pershagen G, van Hage M, Wickman M. Phenotypes of food hypersensitivity and development of allergic diseases during the first 8 years of life. *Clin Exp Allergy* 2008; 38: 1325–32.
- Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Lowe AJ, Matheson MC, et al. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol* 2011; 127: 668–76 e1–2.
- Rönmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundbäck B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *J Allergy Clin Immunol* 2009; 124: 357–63, 63 e1–15.
- West CE, D'Vaz N, Prescott SL. Dietary immunomodulatory factors in the development of immune tolerance. *Curr Allergy Asthma Rep* 2011; 11: 325–33.
- Mullins RJ, Camargo CA. Latitude, sunlight, vitamin d, and childhood food allergy/anaphylaxis. *Curr Allergy Asthma Rep* 2012; 12: 64–71.
- Gorman S, Judge MA, Burchell JT, Turner DJ, Hart PH. 1,25-dihydroxyvitamin D3 enhances the ability of transferred CD4+ CD25+ cells to modulate T helper type 2-driven asthmatic responses. *Immunology* 2010; 130: 181–92.
- Chambers ES, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. *Curr Allergy Asthma Rep* 2011; 11: 29–36.
- Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; 96: 53–8.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab* 2012; 97: 1153–8.
- Cashman KD, FitzGerald AP, Viljakainen HT, Jakobsen J, Michaelsen KF, Lamberg-Allardt C, et al. Estimation of the dietary requirement for vitamin D in healthy adolescent white girls. *Am J Clin Nutr* 2011; 93: 549–55.
- Brustad M, Edvardsen K, Wilsgaard T, Engelsen O, Aksnes L, Lund E. Seasonality of UV-radiation and vitamin D status at 69 degrees north. *Photochem Photobiol Sci* 2007; 6: 903–8.
- Edvardsen K, Brustad M, Engelsen O, Aksnes L. The solar UV radiation level needed for cutaneous production of vitamin D3 in the face. A study conducted among subjects living at a high latitude (68 degrees N). *Photochem Photobiol Sci* 2007; 6: 57–62.
- Enghardt B, Pearson M, Becker W. *Dietary habits and nutrient intake in Swedish children 4 year old and school children in grade 2 and 5 (Riksmaten - barn 2003)*. Uppsala: National Food Administration (Livsmedelsverket), 2006.
- Öhlund K, Olsson C, Hernell O, Öhlund I. Dietary shortcomings in children on a gluten-free diet. *J Hum Nutr Diet* 2010; 23: 294–300.
- Rönmark E, Jönsson E, Platts-Mills T, Lundbäck B. Different pattern of risk factors for atopic and nonatopic asthma among children—report from the Obstructive Lung Disease in Northern Sweden Study. *Allergy* 1999; 54: 926–35.
- Millen AE, Bodnar LM. Vitamin D assessment in population-based studies: a review of the issues. *Am J Clin Nutr* 2008; 87: 1102S–5S.
- WHO. *The WHO child growth standards*. www.who.int/childgrowth/standard/en/: WHO, 2009.
- Snellman G, Melhus H, Gedeberg R, Byberg L, Berglund L, Wernroth L, et al. Determining vitamin D status: a comparison between commercially available assays. *PLoS One* 2010; 13: 5: e11555.

20. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* 2004; 158: 531–7.
21. Stoian CA, Lyon M, Cox RG, Stephure DK, Mah JK. Vitamin D concentrations among healthy children in Calgary, Alberta. *Paediatr Child Health* 2011; 16: 82–6.
22. Gonzalez-Gross M, Valtuena J, Breidenassel C, Moreno LA, Ferrari M, Kersting M, et al. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr* 2012; 107: 755–64.
23. NNR. *Nordic Nutrition recommendations 2004*, 4th ed. Copenhagen: Nordic council of ministers, 2004.
24. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, et al. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr* 2012; 95: 1357–64.
25. Heaney RP, Holick MF. Why the IOM recommendations for vitamin D are deficient. *J Bone Miner Res* 2011; 26: 455–7.
26. Finland's National Nutrition Council webpage. Message 25.5. 2010, www.ravitsemusneuvottelukunta.fi/portal/se/naringsrekommendationer/sarskilda_anvisningar_och_restriktioner (accessed on 02 May 2012).
27. Willett AM. Vitamin D status and its relationship with parathyroid hormone and bone mineral status in older adolescents. *Proc Nutr Soc* 2005; 64: 193–203.
28. Cashman KD, Hill TR, Cotter AA, Boreham CA, Dubitzky W, Murray L, et al. Low vitamin D status adversely affects bone health parameters in adolescents. *Am J Clin Nutr* 2008; 87: 1039–44.
29. Öhlund I, Silfverdal SA, Hernell O, Lind T. Serum 25-hydroxyvitamin D levels in pre-school children in Northern Sweden are inadequate after the summer season and diminishes further during winter. *J Pediatr Gastroenterol Nutr* 2012 DOI: 10.1097/MPG.0b013e3182838e5b.
30. Whiting SJ, Langlois KA, Vatanparast H, Greene-Finestone LS. 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* 2011; 94: 128–35.