

Article Type: Regular Article

Received date: 06/25/2014

Accepted date: 01/15/2015

Revised date: 01/09/2015

Wiley received date: 01/15/2015

No association between allergy and current 25-hydroxy vitamin D in serum or vitamin D intake

Malin Barman¹, M.Sc., Karin Jonsson¹, M.Sc., Bill Hesselmar², M.D., Ph.D., Agnes E. Wold³, M.D., Ph.D., Ann-Sofie Sandberg¹, Ph.D., and Anna Sandin⁴, M.D., Ph.D.

Affiliations: ¹Food and Nutrition Science, Department of Biology and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden; ²Department of paediatrics, Institute of Clinical Science, University of Gothenburg, Sweden; ³Institute of Biomedicine, Department of Infectious Diseases, Clinical Bacteriology section, University of Gothenburg, Göteborg, Sweden; ⁴Paediatrics, Department of Clinical Sciences, Umeå University, Umeå, Sweden.

Address reprint requests to: Malin Barman, Chalmers University of Technology, Department of Biology and Biological Engineering, Food and Nutrition Science, SE-412 96 Göteborg, Sweden. Telephone: +46(0)31-7723811; Fax: +46(0)31-7723830, or at malin.barman@chalmers.se.

Running header: 25-hydroxy vitamin D and allergy development

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/apa.12936

This article is protected by copyright. All rights reserved.

ABBREVIATIONS

25OHD 25-hydroxy vitamin D

1,25OH₂D 1,25-dihydroxy vitamin D

ABSTRACT

Aim: Vitamin D may be involved in allergy development, but there is conflicting evidence.

We investigated if dietary intake of vitamin D and levels of 25OHD in serum differed between allergic and non-allergic adolescents and if serum 25OHD correlated with dietary intake of vitamin D or season of blood sampling.

Methods: Serum 25-hydroxy vitamin D (25OHD) levels were analysed in 13-year-old subjects with atopic eczema (n=55), respiratory allergy (n=55) or no allergy (n=55). Intake of fat-containing foods was assessed by food frequency questionnaires and total daily vitamin D intake was calculated. Logistic regression was used to adjust for gender, parental allergy and time of blood sampling.

Results: Subjects with atopic eczema or respiratory allergy did not differ from non-allergic controls regarding serum 25OHD levels or calculated vitamin D intake. Subjects sampled in the autumn had significantly higher levels of serum 25OHD than subjects sampled in the winter or spring. Serum 25OHD levels correlated to consumption of vitamin D fortified lean milk (p=0.001).

Conclusion: The findings suggest no association between allergy and 25OHD levels in serum or vitamin D intake in adolescents. Serum 25OHD levels correlated to intake of vitamin D fortified lean milk.

Key words: Allergy, atopic eczema, asthma, 25-hydroxy vitamin D, vitamin D

KEY NOTES

- There is conflicting evidence about whether vitamin D is involved in allergy development.
- This study analysed serum 25-hydroxy vitamin D (25OHD) levels in 13-year-old subjects with atopic eczema (n=55), respiratory allergy (n=55) or no allergy (n=55).
- We found that serum 25OHD levels were not related to allergy diagnosis in adolescents, but they were associated with consumption of vitamin D fortified lean milk and to season of blood sampling.

INTRODUCTION

The major circulating form of vitamin D in serum, 25-hydroxyvitamin D (25OHD), is formed enzymatically in the body from vitamin D. The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25OH₂D), has immunoregulatory effects and two contradictory hypotheses have emerged regarding the association between allergy and vitamin D. The first one proposes that vitamin D is responsible for the rise in allergies (1). This theory was based on a concomitant increase in the habit of supplementing food products, for example dried milk with vitamin D to prevent rickets, and a rise in allergies. Accordingly, epidemiological studies have found higher serum levels of 25OHD in individuals with atopic rhinitis (2, 3) and eczema (4). Further, *in vitro* studies have shown that high 1,25OH₂D levels reduce the secretion of typical Th1-type cytokines, such as IL-12 and IFN- γ (5) from T cells, while increasing secretion of cytokines of the Th2-type, such as IL-4 and IL-13 (6).

According to the opposing theory, insufficient levels of vitamin D increase the risk of allergy (7). This theory was later supported by studies linking low serum 25OHD levels with asthma (8-10) and atopic dermatitis (11, 12). Keratinocytes express the enzyme 25OHD 1 α -

hydroxylase that converts 25OHD to 1,25OH₂D, which has been shown to enhance keratinocyte differentiation (13). Also, Vitamin D reduces epidermal permeability (12). Randomised control trials have evaluated the effect of supplementation with vitamin D on the severity of atopic dermatitis and all found a reduced severity of the disease in the supplemented group compared to placebo (14, 15). Regarding the mechanism for protection against asthma, vitamin D may decrease airway remodelling. Thus, the increased proliferation of airway smooth muscle cells exposed to serum from asthmatic patients, or to platelet-derived growth factor, is inhibited by 1,25OH₂D (16, 17). However, a recent randomised controlled trial failed to show an effect on airway hyper reactivity in asthmatic subjects of supplementation with vitamin D (18).

Vitamin D can be obtained from food, especially fatty fish, but also meat and eggs. Margarines and lean milk are fortified with vitamin D and constitute an important dietary source of vitamin D. However, the main source of vitamin D is sunlight, vitamin D being produced in the skin after UV-light exposure. In Sweden where sunlight is scarce in the dark season, vitamin D supplements (10 µg/day) are recommended for children up to two-years-of-age. Teenagers living in northern Scandinavia have higher serum levels of vitamin D during the sunny summer months and lower levels in the winter and spring (19).

The aim of this study was to investigate if levels of 25OHD in serum, intake of vitamin D containing food items and calculated total vitamin D intake differed between allergic and non-allergic adolescents living in northern Sweden, which has a Latitude of 63°. Individuals with atopic eczema or respiratory allergy, as well as non-allergic individuals, were selected from a birth cohort. Secondly we wished to examine whether serum 25OHD levels correlated with intake of vitamin D or to season of blood sampling.

METHODS

Study subjects

Cases and controls were selected from a birth cohort consisting of all 1,228 children born during one year between February 1996 and January 1997 in the County of Jämtland in Northern Sweden, Latitude 63°. The cohort was designed to assess the development of allergy and the children were followed with skin prick tests to measure sensitisation to common food and airborne allergens at one-year-of-age and four-years-of-age and questionnaires regarding allergic symptoms at one, four and seven-years-of-age. Socioeconomic, housing and lifestyle factors were recorded by questionnaires.

Allergy diagnosis at 13-years-of-age and sensitisation test

As previously described (20), all adolescents still living in the region (n=841) were invited to fill out a questionnaire regarding allergy symptoms, and other environmental factors known to influence allergy, and take part in skin prick tests at 13-years-of-age (Figure 1).

Based on the questionnaires and sensitisation tests at 13-years-of-age, we selected subjects representing three diagnostic groups including atopic eczema, respiratory allergy and no allergy. Atopic eczema without any other allergic disease manifestations was defined as a pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution, fulfilling three of the main criteria suggested by Hanifin and Rajka (21).

Respiratory allergy, but no atopic manifestations from other organs was defined as a positive skin prick test to an inhalant allergen, in combination with two or more of the following criteria: wheeze in the past year, doctor's diagnosed asthma, asthma inhalation treatment or a positive answer to the question: "Have you had any signs of pollen allergy or allergy to furry pets during the last 12 months?". No allergy was defined as having neither allergic symptoms,

nor any positive reaction in the skin prick test at this time or in any of the previous follow-ups.

Collection of serum

We drew 10 ml venous blood, allowed it to clot and centrifuged it. Serum was then separated, aliquoted, frozen within three hours and stored for a maximum of five years at -80 °C until analysed. An overview of the blood sampling is shown in Figure 1.

The season of blood sampling was considered when selecting subjects with or without allergy. We aimed to achieve similar numbers of subjects with blood samples in the autumn and in the winter and spring in the three groups: atopic eczema, respiratory allergy and no allergy, (Figure 1). Selected subjects were compared with all subjects in the group from which they were selected (Table 1). For the non-allergic group, having a dog in the family during the first year of life was significantly less common in the included subjects (16 %) than in the excluded subjects (30 %). No other differences were found between the selected and non-selected subjects (Table 1).

Analysis of 25-hydroxy vitamin D in serum

The concentration of serum 25OHD₃ was measured using liquid chromatography combined with mass spectrometry. Samples were prepared for the analysis according to Turpeinen *et al* (22) with some modifications. Serum (200 µl) was mixed in glass tubes with 150 µl methanol:iso-propanol (80:20), containing 10 ng internal standard (26, 26, 26, 27, 27, 27-D₆-25-OH Vitamin D₃, Cerilliant, Round Rock, TX, USA). Samples were then vortex mixed for one minute and left to rest for 20 minutes. After extraction with 2x2 ml hexane, organic phases were combined, evaporated under nitrogen flow at 30°C and dissolved into 100 µl of

methanol. After five minutes, samples were vortexed gently and transferred into vials. A control serum sample was extracted and analysed in every run. Study samples and control samples were prepared and analysed in duplicates. 25OHD₃ and D6-25OHD₃ were analysed using a liquid chromatography mass spectrometry system (Agilent 1260 Infinity Binary LC and Agilent 6120 Quadrupole LC/MS, Agilent Technologies, Santa Clara, California, USA). Metabolites were separated on a Luna 3μ C18(2) 10A column (250 x 4.60 mm, Phenomenex) thermostated at 50°C using an isocratic mobile phase consisting of 98% methanol with 0.1% of formic acid. The mobile phase flow rate was 0.7 ml/min and the sample injection volume 25 μl. The total chromatographic run time for each sample was 30 minutes. The mass spectrophotometer was equipped with an atmospheric pressure chemical ionisation source (Agilent Technologies, USA) and was operated in the positive ionisation mode with the following source parameters: drying gas flow rate 4.0 L/min; gas temperature 250°C; pressure of nebulizer gas 25 psi; Vcap 1500 V; and fragmentor 100 V. The instrument was operating in selected-ion monitoring mode: 384 for 25OHD₃ and 389 for D6-25OHD₃, respectively. Data acquisition was conducted with LC/MSD ChemStation B.04.03 (Agilent Technologies, USA). 25OHD₃ and D6-25OHD₃ were identified based on retention time of pure standard of 25OHD₃ and D6-25OHD₃. Typical elution times for both 25OHD₃ and D6-25OHD₃ were 6.1 minutes.

Linearity, reproducibility and sample stability

The deviation between each of the two duplicate samples differed less than 10% from the mean of the two duplicates ($(a-b)/[\text{duplicate mean}]$). The relative standard deviation for the intra-assay precision was 3% for a serum sample with normal concentration of serum 25OHD (66 nmol/L) and 2% for a serum sample with high concentration of serum 25OHD (200 nmol/L). Control samples (mean of 47 nmol/L) were analysed every day and the relative

standard deviation was 3% for between day variability between the mean of the duplicates. The method was tested for linearity between 6 nmol/L and 500 nmol/L using the internal standard diluted in methanol. The method was found to be linear ($R^2 = 0.9996$) from 12 nmol/L to 500 nmol/L. As the lowest serum 25OHD concentration in a sample was 19 nmol/L, which is in the linear range of the method, no lower limit of quantification or lower limit of detection were determined as it was deemed unnecessary. The samples were tested for short-term stability by letting four control samples sit in room temperature for 24 hours. Four control samples were also tested for freeze-and-thaw stability by repeating three freeze-thaw cycles, i.e. thawed in room temperature and frozen for 24 hours.

Dietary assessment

On the same occasion as the blood sampling, the adolescents were asked to fill out a food frequency questionnaire regarding food consumption at present. The questionnaire contained questions about how often selected food items were consumed, i.e. butter, margarine and vegetable oils, marine foods, lean/light and full fat dairy products, dressings, meat and meat products, eggs, snacks, and french fries. Questions regarding butter/margarine and some types of meat and seafood concerned both their use as sandwich spreads and as components of main meals. Options for intake frequencies included never, once a month, once a week and daily. Respondents were instructed to report frequencies in detail in an open commentary field, if deviating from the options. The intake frequencies of the different food items were compiled by one food scientist and one nutritionist and converted to grams using the latest weight table from the Swedish National Food Agency, dated 2001. Ice cream and pastries were excluded in the nutrient calculations, since their fat composition varies widely. The vitamin D intake was analysed by a nutritionist using the software Diet32 (Aivo AB, Stockholm, Sweden, 2008-04-11).

Statistical analysis

Chi-square test and Student's t-test were used to analyse differences in background variables between the selected individuals and the entire group. Student's t-test was used to compare group means of serum 25OHD. For difference in food intake and total weekly intake of vitamin D, which was not normally distributed, the Mann Whitney U-test was used. Correlations between intake of vitamin D-rich food items and total daily intake of vitamin D and serum 25OHD levels were done using Spearman's correlation test. Multiple logistic regression analysis was used to analyse the association between allergy diagnosis and serum 25OHD as well as calculated dietary intake of vitamin D, taking the following potential confounders into consideration: for serum 25OHD, gender, month of sampling (January, April or November) and maternal, as well as paternal allergy, for vitamin D intake, gender and maternal and paternal allergy. Post hoc power analysis was used to calculate effect size in our study. With the sample size used in the present study it is possible to detect mean differences down to 0.5 standard deviations with 80 % power, i.e. a medium effect. Hence, it is possible to detect a mean difference of 6-7 nmol/L with 80 % power between our groups with a standard deviation of 14. If a mean difference of +/- 10 nmol/L is assumed to be clinically relevant for an effect on allergy, we would have 98 % power to detect this difference. Power analyses were performed using GPower 3.1.9.2 (Franz Faul, Universität Kiel, Germany). The other statistical analyses were performed using IBM SPSS Statistics version 19 (IBM Corporation, New York), two-tailed p-value ≤ 0.05 was considered significant.

The study was approved by the local ethical committee in Umeå, Sweden (Dnr 09-110M) and was conducted according to the Declaration of Helsinki. At 13-years-of-age, the adolescents verbally approved their participation in skin prick tests, food registration and collection and analysis of blood samples for vitamin D and their parents provided written consent on behalf

of the children. Participation was voluntary, and the adolescents were free to decide not to participate further at any time, without any stated reason. The participants were informed that they would not be disadvantaged in any way as a result of a potential decision to decline further participation.

RESULTS

The mean serum 25OHD level in the entire group of 165 individuals was 51 ± 14 nmol/L. Only three were defined as vitamin D deficient, i.e. had levels below 25 nmol/L, 79 subjects had insufficient 25OHD levels (30-50 nmol/L), 83 subjects had sufficient 25OHD levels (50-100 nmol/L), and no subjects had high 25OHD levels (>100 nmol/L).

Serum levels of 25OHD were significantly higher in samples collected in autumn ($n = 80$, mean 54 nmol/L) compared to samples collected in winter/spring ($n = 85$, mean 49 nmol/L, $P = .009$). Adolescents with respiratory allergy, atopic eczema and non-allergic controls were distributed equally over the seasons of sampling (Table 2).

No differences in serum 25OHD levels were found between individuals with atopic eczema and non-allergic controls, or between individuals with respiratory allergy and controls, regardless of season of sampling (Table 2).

Multiple logistic regression analysis was used to analyse the association between allergy diagnosis and serum 25OHD adjusted for: gender, month of sampling (January, April or November) and maternal, as well as paternal allergy. As seen in Table 3, serum 25OHD levels did not differ between the clinical groups in either the unadjusted logistic regression models or in the adjusted logistic regression models. Skin colour and ethnicity may affect the ability

to produce, metabolise and respond to Vitamin D. In our study, ethnicity was not recorded, but all of the individuals had fair skin (study nurse, personal communication).

Dietary intake of selected food items and vitamin D

The adolescents filled out a food-frequency questionnaire focusing on fat-containing items, considered significant sources of Vitamin D. Table 4 shows the intake of such food items in the three clinical groups. No significant differences were found between the three groups, except that adolescents with respiratory allergy consumed less egg than did non-allergic controls. The estimated total daily intake of vitamin D, as calculated from the response to the questionnaires, was 3.8 µg/day for the whole group ($n = 163$) and did not differ significantly between any of the two allergic groups and the non-allergic controls (Table 4).

Multiple logistic regression analysis was also used to analyse the association between allergy diagnosis and the calculated total daily dietary intake of vitamin D adjusted for gender and maternal and paternal allergy. As seen in Table 3, calculated intake of vitamin D did not differ between the clinical groups in either the unadjusted logistic regression models or in the adjusted logistic regression models.

Serum levels of 25OHD correlated to intake of vitamin D-fortified lean milk but not to fatty fish or other food items known to contain considerable amounts of vitamin D (Table 5). Serum 25OHD did not correlate to calculated total intake of vitamin D (Table 5).

DISCUSSION

Adolescents with respiratory allergy or atopic eczema at 13-years-of-age differed neither in serum levels of 25OHD nor in dietary intake of vitamin D from non-allergic and non-sensitised controls of the same age.

Previous studies have yielded inconsistent results regarding the relation between vitamin D levels in serum and allergy. Similar to our results, Samochocki *et al* (15) and Chiu *et al* (23) found no association between serum concentration of 25OHD and atopic dermatitis, while Gergen *et al* (24) found no association between serum concentration of 25OHD and asthma. A large study comprising almost 10,000 German children and adolescents aged one to 17 reported higher levels of serum 25OHD in individuals with eczema compared with those without eczema (4). In contrast, other studies have found lower levels of serum 25OHD in individuals with asthma (8, 9) or atopic dermatitis (11, 12).

The mean level of serum 25OHD (51 nmol/L) in the entire group was barely above the limit defining sufficient levels (50 nmol/L). Only three subjects had serum 25OHD levels below the limit defined as deficiency (25 nmol/L). Hence, our study does not permit conclusions regarding any potential effects of vitamin D deficiency on allergy.

No differences were found in the calculated intake of food items containing vitamin D among allergic and non-allergic adolescents, except a significantly higher intake of eggs in subjects with no allergy than in subjects with respiratory allergy. However, the general intake of egg was low (4.8 g/day) and the difference between the groups corresponds only to approximately one egg per month.

The currently recommended intake of vitamin D for children and adults (<75 yr) in the Nordic countries is 10 µg/day, but the average intake in Sweden is 5.7 µg/day in the 18 to 30 year age group (25). Relatively few foods contain vitamin D; fish and egg are the best sources, per 100 g, fatty fish contains approximately 12-16 µg vitamin D, lean fish 0.1-2 µg and eggs 1.4 µg; according to the Food Database of the Swedish National Food Agency, consulted on 12

November 2014. Although fatty fish is one of the best food sources of vitamin D, intake of this food item did not correlate to serum 25OHD levels in our study. This is likely explained by the very low intake of fatty fish, 12 g/day in average, in this group.

In Sweden, and many other countries, margarine and lean milk are fortified with vitamin D, 10 µg/100 g and 0.45 µg/100 g, respectively. Hence, one standard portion of 200 g lean milk contributes to 0.9 µg vitamin D, i.e. 16% of the mean daily vitamin D intake of 5.7 µg in 18-30-year-olds (25). To estimate vitamin D intake, the diet was registered by food frequency questionnaire, comprising fat-containing foods and foods known to contain vitamin D.

Vitamin D fortified lean milk was the only food item whose intake correlated significantly with serum levels of 25OHD, suggesting that fortified food items may be a major dietary source of vitamin D in the investigated group of adolescents. Milk products are generally consumed regularly and in substantial amounts. The mean daily intake by the participants in this study was 230 g/day, which was lower than expected. However, apart from the open commentary field, the questionnaire did not have options to register intake frequencies more frequent than once a day. Underestimation of the milk consumption is therefore likely, since milk may be consumed during several meals throughout the day. Furthermore, the food-frequency questionnaire did not include questions regarding sour milk or yoghurt products, which are often consumed for breakfast in Sweden.

As calculated from the food frequency questionnaires, the teens in this study consumed an estimated average of 3.8 µg/day of vitamin D. However, the true intake is likely to be higher due to the probable underestimation of vitamin-D fortified milk and sour milk/yoghurt products.

The intake of vitamin D fortified lean milk correlated significantly with 25OHD levels in serum, while total intake of vitamin D, assessed via the food frequency questionnaire, did not. In addition to a possible underestimation of D-vitamin containing foods, intake of vitamin D supplements was not included in the food frequency questionnaire, which was initially designed to measure intake of different types of fatty acids. The probable underestimations of calculated total vitamin D intake may well explain the lack of correlation to serum 25OHD. Thus, no firm conclusions can be drawn regarding the effect of vitamin D intake and allergy in our study.

The importance of sunlight for serum 25OHD levels was shown by the fact that serum samples collected in the autumn had higher levels of serum 25OHD than samples collected in winter and spring. Vitamin D is lipophilic and is stored in adipose tissue, where it has a slow turnover, with a half-life of approximately two months (26). A drawback of this study is the lack of registration of outdoor activities, foremost time spent on vacations in sunny countries.

The allergy diagnoses in this study were based on questionnaires and skin prick tests when the children were 13-years-of-age. Due to the long follow-up we had an ability to select three very clearly defined clinical groups; i.e. those with atopic eczema, but no other allergic symptoms, those with respiratory allergy and sensitisation to common aeroallergens, but no other allergic symptoms, as well as non-allergic and non-sensitised controls who had not been allergic or sensitised at any of the follow-ups. A total of 300 subjects were invited to give a blood sample and participate in the study. Blood was drawn from 274 subjects on three different occasions from November 2009 to January 2011. The selected subjects did not differ from the groups from which they derived, except that it was less common among selected non-allergic subjects to have a dog during the first year of life.

Our study was not prospective in design and we cannot rule out that difference in serum 25OHD levels or vitamin D intake might have preceded sensitisation and allergy development. Hyppönen *et al* (27) found that infants receiving supplementation with vitamin D during the first year of life had a higher risk for allergy at 31-years-of-age, suggesting an early life Th2 switch in infants receiving vitamin D supplementation. Contrary to that, Litonjua *et al* (7) suggested that vitamin D deficiency, primarily in pregnant mothers, is involved in the allergic epidemic. The last theory is in line with several epidemiological studies linking a higher vitamin D intake in pregnant women with lower risk of allergic disease, as reviewed by Rueter *et al* (28). However, since dietary sources generally contribute to less than 10 % of serum vitamin D levels (28), measuring maternal serum 25OHD levels during pregnancy is a better reflection of vitamin D exposure to the developing fetus *in utero*. According to the review by Rueter *et al* (28) only a few observational studies have evaluated this association with inconsistent results: two studies found an association between high maternal serum 25OHD levels and allergy in the offspring, while three studies found no association. Studies associating cord serum 25OHD levels with later allergy development, also reviewed by Reuter *et al*, have mostly failed to find any associations (28). Two studies found a negative association with eczema, one study found a positive association with food allergy, while no studies found cord serum 25OHD levels to be associated with asthma or allergic rhinitis. Two studies reported a negative association between cord serum 25OHD and wheezing, but early wheezing may reflect airway infections, rather than allergy.

CONCLUSION

The results from this study suggest that adolescents with allergy do not differ from non-allergic adolescents regarding levels of 25OHD in serum. Although the incidence of allergy is high in Sweden, and particularly high in the north of the country, we did not find any

evidence that insufficient 25OHD levels in serum were more prevalent among subjects with respiratory allergies or atopic eczema. However, prospective studies are needed to rule out if low serum 25OHD levels increase the risk of allergy development.

ACKNOWLEDGEMENT

We thank Nils-Gunnar Carlsson and Otto Savolinen for technical assistance, Anna Bernholm for blood sampling and performing skin prick tests, and Mads Vendelbo Lind and Staffan Nilsson for consultation regarding statistical calculations.

Finance statement

The work was supported by grants from: the Swedish Research Council for Environmental, Agricultural Sciences and Spatial Planning (FORMAS), Stockholm, Sweden, project number 216-2009-752; the Swedish Research Council (VR grant no 521-2013-3154), the West Gothia Region, Sweden; the Centre for Environment and Sustainability, GMV, Gothenburg, Sweden; the Research and Development Departments of the Jämtland and Norrbotten County Councils, Sweden; the Th. C. Bergh Foundation, and the Olle Engkvist Foundation, Sweden.

Conflicts of interest statement

The authors declare no financial or commercial conflicts of interest.

REFERENCES

1. Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? *Allergy* 1999; 54:757-9
2. Jung JW, Kim JY, Cho SH, Choi BW, Min KU, Kang HR. Allergic rhinitis and serum 25-hydroxyvitamin D level in Korean adults. *Ann Allergy Asthma Immunol* 2013;

3. Wjst M, Hypponen E. Vitamin D serum levels and allergic rhinitis. *Allergy* 2007; 62:1085-6
4. Heimbeck I, Wjst M, Apfelbacher CJ. Low vitamin D serum level is inversely associated with eczema in children and adolescents in Germany. *Allergy* 2013; 68:906-10
5. Reichel H, Koeffler HP, Tobler A, Norman AW. 1 alpha,25-Dihydroxyvitamin D3 inhibits gamma-interferon synthesis by normal human peripheral blood lymphocytes. *PNAS* 1987; 84:3385-9
6. Matheu V, Bäck O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: Enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol* 2003; 112:585-92
7. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007; 120:1031-5
8. Bener A, Ehlayel MS, Tulic MIK, Hamid Q. Vitamin D Deficiency as a Strong Predictor of Asthma in Children. *Int Arch Allergy Immunol* 2012; 157:168-75
9. Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DYM. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *J Allergy Clin Immunol* 2010; 125:995-1000
10. Bonanno A, Gangemi S, La Grutta S, Malizia V, Riccobono L, Colombo P, et al. 25-Hydroxyvitamin D, IL-31, and IL-33 in children with allergic disease of the airways. *Mediators Inflamm* 2014; 2014:520241
11. Baek JH, Shin YH, Chung IH, Kim HJ, Yoo E-G, Yoon JW, et al. The Link between Serum Vitamin D Level, Sensitization to Food Allergens, and the Severity of Atopic Dermatitis in Infancy. *J Pediatr* 2014; 165:849-54.e1

12. Peroni DG, Piacentini GL, Cametti E, Chinellato I, Boner AL. Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in children. *Br J Dermatol* 2011; 164:1078-82
13. Gurlek A, Pittelkow MR, Kumar R. Modulation of growth factor/cytokine synthesis and signaling by 1alpha,25-dihydroxyvitamin D(3): implications in cell growth and differentiation. *Endocr rev* 2002; 23:763-86
14. Amestejani M, Salehi BS, Vasigh M, Sobhkhiz A, Karami M, Alinia H, et al. Vitamin D supplementation in the treatment of atopic dermatitis: A clinical trial study. *J Drugs Dermatol* 2012; 11:327-30
15. Samochocki Z, Bogaczewicz J, Jeziorkowska R, Sysa-Jędrzejowska A, Glińska O, Karczmarewicz E, et al. Vitamin D effects in atopic dermatitis. *J Am Acad Dermatol* 2013; 69:238-44
16. Damera G, Fogle HW, Lim P, Goncharova EA, Zhao H, Banerjee A, et al. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *Br J Pharmacol* 2009; 158:1429-41
17. Song Y, Qi H, Wu C. Effect of 1,25-(OH)₂D₃ (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. *Respirology* 2007; 12:486-94
18. Bar Yoseph R, Livnat G, Schnapp Z, Hakim F, Dabbah H, Goldbart A, et al. The effect of vitamin D on airway reactivity and inflammation in asthmatic children: A double-blind placebo-controlled trial. *Pediatr Pulmonol* 2014: "Early view". Article first published online: 2 JUL 2014
19. Oren E, Banerji A, Camargo Jr CA. Vitamin D and atopic disorders in an obese population screened for vitamin D deficiency. *J Allergy Clin Immunol* 2008; 121:533-4
20. Barman M, Jonsson K, Sandin A, Wold AE, Sandberg A-S. Serum Fatty Acid

Profile Does Not Reflect Seafood Intake In Adolescents With Atopic Eczema. *Acta Paediatr* 2014; 103: 968-76

21. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980; Suppl. 92:44-7
22. Turpeinen U, Hohenthal U, Stenman U-H. Determination of 25-Hydroxyvitamin D in Serum by HPLC and Immunoassay. *Clin Chem* 2003; 49:1521-4
23. Chiu YE, Havens PL, Siegel DH, Ali O, Wang T, Holland KE, et al. Serum 25-hydroxyvitamin D concentration does not correlate with atopic dermatitis severity. *J Am Acad Dermatol* 2013; 69:40-6
24. Gergen PJ, Teach SJ, Mitchell HE, Freishtat RF, Calatroni A, Matsui E, et al. Lack of a relation between serum 25-hydroxyvitamin D concentrations and asthma in adolescents. *Am J Clin Nutr* 2013; 97:1228-34
25. Riksmaten - adults 2010-11, Food and nutrition intake among adults in Sweden. Uppsala, Sweden: The Swedish National Food Administration; 2012
26. Jones G. Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr* 2008; 88:582S-6S
27. Hyppönen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen A-L, et al. Infant Vitamin D Supplementation and Allergic Conditions in Adulthood: Northern Finland Birth Cohort 1966. *Ann N Y Acad Sci* 2004; 1037:84-95
28. Rueter K, Siafarikas A, Prescott SL, Palmer DJ. In utero and postnatal vitamin D exposure and allergy risk. *Expert Opin Drug Saf* 2014; 13:1601-11

Table 1 Characteristics of the study population

	No allergy (<i>n</i> = 332)			Respiratory allergy ¹ (<i>n</i> = 130)			Atopic eczema ² (<i>n</i> = 79)		
	Entire group (<i>n</i> = 262)	Selected (<i>n</i> = 55)	<i>P</i> ³	Entire group (<i>n</i> = 130)	Selected (<i>n</i> = 55)	<i>P</i> ³	Entire group (<i>n</i> = 79)	Selected (<i>n</i> = 55)	<i>P</i> ³
Female gender, %	55	60	.48	32	29	.72	70	73	.70
Have older siblings ⁴ , %	60	69	.23	63	60	.70	62	63	.90
Exclusive breast feeding ^{4,5} , %	74	73	.86	74	68	.40	80	83	.68
Maternal allergic heredity ⁶ , %	32	32	.95	55	49	.43	52	53	.93
asthma, %	6.1	3.6	.47	17	16	.93	23	26	.72
rhinitis, %	11	15	.49	33	36	.67	24	29	.52
eczema, %	23	20	.65	32	22	.18	38	40	.81
Paternal allergic heredity ⁶ , %	32	39	.35	48	52	.64	37	35	.80
asthma, %	11	17	.21	16	15	.91	7.6	7.3	.95
rhinitis, %	15	20	.27	32	37	.49	19	15	.50
eczema, %	16	11	.35	19	24	.48	25	26	.97
Cat during first year in life ⁴ , %	19	20	.91	12	9	.53	24	24	.96
Dog during first year in life ⁴ , %	30	16	.04*	16	13	.55	29	29	1.0
Positive SPT ⁷ , %	0	0	-	100	100	-	14	9.3	.39

Body mass index ⁷ , mean (SD)	19 (3.0)	20 (3.4)	.11	19 (3.0)	18 (2.4)	.38	20 (3.5)	20 (2.8)	.94
---	----------	----------	-----	-------------	----------	-----	----------	----------	-----

¹Fulfilling ≥ 1 of the following criteria: wheeze in the past year, asthma inhalation treatment, or a positive answer to the question “Have you had any signs of pollen allergy or allergy to furred pets during the last 12 months?”; diagnosed at 13-years-of-age

²Defined as a pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution three of the main criteria suggested by Hanifin and Rajka[18], diagnosed at 13-years-of-age

³Chi-Square test for dichotomous variables and students t-test for continuous variables between the included and the excluded subjects.

⁴Answered at 1-year-of-age

⁵Exclusively breast feeding at 4-month-of-age

⁶Defined as an affirmative answer to the question “Has the child's mother/father ever had asthma, hay fever or eczema?”; answered at 13-years-of-age

⁷Measured at 13-years-of-age

Table 2 Serum levels of 25OHD in non-allergic subjects and subjects with atopic eczema or respiratory allergy at 13-years-of-age.

	<i>n</i>	mean	<i>standard deviation</i>	<i>mean difference</i>	<i>95 % CI for the difference</i>	<i>P</i> ¹
<i>All subjects</i>						
No allergy	55	50	13			
Respiratory allergy	55	51	15	-0.8	-6.2 – 4.6	.77
Atopic eczema	55	53	13	-3.4	-8.4 – 1.7	.19
<i>Autumn samples</i>						
No allergy	26	51	13			
Respiratory allergy	27	54	12	-3.0	-10 – 4.0	.39
Atopic eczema	27	57	13	-5.4	-13 – 1.8	.14
<i>Winter/spring samples</i>						
No allergy	29	49	14			
Respiratory allergy	28	47	17	1.5	-6.6 – 9.6	.72
Atopic eczema	28	50	13	-1.4	-8.6 – 5.7	.69

¹*P* obtained by Student's t-test compared to non-allergic controls

Table 3 Estimated odds ratios and 95% CIs of association between serum 25OHD or calculated intake of vitamin D and atopic eczema or respiratory allergy in 13-year-old adolescents.

	Cases/controls	Unadjusted OR (95% CI)	P	Cases/controls	Adjusted OR (95% CI)	P
<i>Serum levels of 25OHD</i>						
Respiratory allergy	55/55	1.044 (0.978- 1.031)	.77	52/54	0.998 (0.968- 1.028)	.87 ¹
Atopic eczema	55/55	1.020 (0.991- 1.049)	.19	55/54	1.016 (0.986- 1.046)	.31 ¹
<i>Calculated daily intake of vitamin D</i>						
Respiratory allergy	53/55	0.889 (0.668- 1.184)	.42	52/54	0.828 (0.601- 1.142)	.81 ²
Atopic eczema	55/55	1.135 (0.866- 1.049)	.36	55/54	1.235 (0.921- 1.656)	.16 ²

¹Adjusted for gender, maternal allergy, paternal allergy, and time of blood sampling

²Adjusted for gender, maternal allergy and paternal allergy

Table 4 Intake of vitamin D containing food items¹ in the three groups

	No allergy (<i>n</i> = 55) ²	Respiratory allergy (<i>n</i> = 53) ²	Atopic eczema (<i>n</i> = 55) ²	<i>P</i> (resp vs ctrl) ³	<i>P</i> (ecz vs ctrl) ³
Butter	5.2 (5.0-5.9)	5.4 (3.2-10)	5.7 (1.4-10)	.462	.205
Margarine	5.2 (0.71-6.4)	5.0 (0.18-6.4)	5.0 (0.71-8.5)	.625	.681
Lean fish	18 (4.9-20)	8 (4.9-20)	20 (4.9-20)	.362	.569
Fatty fish	8.0 (4.9-22)	7 (4.9-20)	10 (4.9-20)	.296	.969
Total seafood intake ⁴	25 (12-39)	25 (9.8-36)	28 (18-39)	.226	.722
Total meat intake ⁵	84 (69-100)	89 (75-110)	90 (66-110)	.517	.872
Lean milk	250 (36-250)	250 (250- 250)	250 (250- 260)	.909	.090
Egg	3.6 (1.8-8.9)	1.8 (0.00-3.6)	3.6 (0.71-8.9)	.002	.134
Vitamin D	3.3 (2.7-4.9)	3.5 (2.6-4.5)	3.8 (3.0-5.0)	.616	.260

¹Data are presented as medians (interquartile range), in g/day for food items and µg/day for vitamin D

²Number of subjects who filled in a complete food frequency questionnaire in each group. Two questionnaires were discarded as more than eight questions were left unanswered, leaving 163 food assessments for analysis

³*P* obtained by non-parametric Mann-Whitney U-test (intake pattern were not normally distributed)

⁴Including fatty and lean fish, as well as shellfish

⁵Including prepared meat, red meat and chicken

Abbreviations: resp=respiratory allergy, ctrl=non-allergic controls, ecz=atopic eczema

Table 5 Correlations between amount of serum 25OHD and intake of selected food items and calculated daily intake of vitamin D

All subjects (<i>n</i> = 163)		
	Correlation coefficient ¹	<i>P</i>
Butter	.002	.982
Margarine	-.015	.849
Lean fish	.064	.416
Fatty fish	-.024	.746
Total fish intake ²	-.013	.871
Total meat intake ³	.024	.759
Lean milk (vitamin D fortified)	.267	.001
Egg	-.018	.818
Total daily vitamin D intake	.114	.148

¹Correlation were performed with Spearman's correlation test since intake patterns were not normally distributed

²Including fatty and lean fish, as well as shellfish

³Including prepared meat, red meat and chicken

Figure legends

Figure 1: Flow chart over the cohort in the follow up at 13-years-of-age.

¹ Subjects who were not included in any of the three above mentioned groups were either sensitised but did not show any allergic symptoms or had more than one allergic symptom (food allergy, respiratory allergy or eczema)

²Number of subjects that gave blood in November 2009

³Number of subjects that gave blood in April 2010

⁴Number of subjects that gave blood in January 2011

Figure 1

