Serum 25-hydroxyvitamin D levels and self-reported allergic rhinitis in Norwegian adults – The HUNT Study

Xiao-Mei Mai[1], Yue Chen[2], Carlos A. Camargo Jr[3], Arnulf Langhammer[1]

[1]Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Epidemiology and Community Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

[3]Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Author of correspondence:

Xiao-Mei Mai, PhD, Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway; Tel: +47 735 90598; E-mail: xiao-mei.mai@ntnu.no

Short title: Vitamin D and allergic rhinitis

Abstract

Background: The role of low vitamin D status in the development of allergic rhinitis is unclear. We aimed to investigate the relationship between serum 25-hydroxyvitamin D [25(OH)D] and incidence of allergic rhinitis in adults.

Methods: The study included a random sample from an adult population who participated in the second and third surveys of the Nord-Trøndelag Health Study (HUNT) in Norway (HUNT2, 1995-97 and HUNT3, 2006-08). Serum 25(OH)D levels were measured in blood samples collected at baseline. Among 1,351 adults who did not report allergic rhinitis at baseline, incident allergic rhinitis was identified by participant report of having or having had allergic rhinitis or hay fever at follow-up. Adjusted odds ratios (AOR) and 95% confidence intervals (CI) were calculated after adjustment for age, smoking, physical activity, socio-economic status, family history of allergy, body mass index, and season. The analyses were stratified by sex due to its significant interaction with 25(OH)D levels ($p < 0.02$).

Results: Over an average of 11 years, 9% of men and 15% of women developed allergic rhinitis. Among men, serum 25(OH)D level <50 nmol/L was associated with an increased risk of incident allergic rhinitis (AOR 2.55; 95% CI 1.01-6.49); each 25 nmol/L reduction of 25(OH)D level was associated with an AOR of 1.84 (95% CI 1.18-2.87). In women, however, the association was opposite, with AOR being 0.83 (95% CI 0.66-1.05) for each 25 nmol/L reduction of serum 25(OH)D level.

Conclusions: Vitamin D appears to play different roles in the development of allergic rhinitis among men and women.

Word count: 250

Keywords: allergic rhinitis, allergy, prospective study, serum 25(OH)D, vitamin D

Abbreviations used:

AOR: Adjusted odds ratio; **CI:** Confidence interval; **HUNT:** Nord-Trøndelag Health Study; **25(OH)D:** 25-hydroxyvitamin D; **OR:** Odds ratio

INTRODUCTION

The modulating effect of vitamin D on a variety of immune functions supports a possible causal link to allergic diseases. For example, vitamin D may affect regulatory T cells (Treg) (1, 2), which are known to control both T- helper 1 (Th1) and T-helper 2 (Th2) cell responses (3). Therefore, there are mechanistic reasons to implicate vitamin D with both Th1-related autoimmune diseases and Th2-related allergic diseases (4).

There have been contrasting hypotheses on the association of vitamin D status with allergic diseases: Wjst and Dold stated that *high* vitamin D status causes allergic diseases (5), whereas Litonjua and Weiss asserted that *low* vitamin D status causes allergic diseases (4). Epidemiologic studies on vitamin D intake and allergic diseases have shown inconsistent results (6-9). Studies investigating the associations of serum 25-hydroxyvitamin D [25(OH)D] levels, the best measure of body vitamin D status (10), with allergic diseases are also contradictory (11-18). These studies mostly focused on serum 25(OH)D levels in cord blood in relation to food allergy and sensitization to food or airborne allergens during childhood, and lower serum 25(OH)D levels were associated with an increased risk (11, 13, 14), a decreased risk (15) or no risk (12) for food allergy or sensitization.

There are sparse data on the role of serum 25(OH)D levels in the development of allergic diseases in adults. A study using the Third National Health and Nutrition Examination Survey (NHANES) from the U.S. reported no association of serum 25(OH)D with allergic sensitization in adults (14), whereas another study using an earlier survey from the NHANES showed a positive association of serum 25(OH)D levels with allergic rhinitis (16). In the current study, we aimed to investigate the association of serum 25(OH)D levels with incident allergic rhinitis in adults by using Norwegian population data from the Nord-Trøndelag

Health Study (HUNT). As our previous study demonstrated a sex difference in terms of the association between serum 25(OH)D levels and adult asthma (19), we also evaluated the possible modifying effect of sex on the association of 25(OH)D levels with allergic rhinitis.

MATERIALS AND METHODS

Study design

HUNT is the largest population-based health study in Norway (20). The adult part of the HUNT invited all inhabitants aged 19 years or older in the county of Nord-Trøndelag in 3 separate surveys: HUNT1 (1984-86), HUNT2 (1995-97) and HUNT3 (2006-08). In the present study, we used data from HUNT2 and HUNT3. In brief, HUNT2 invited 93,898 adults in 1995-97, and 65,233 people participated (response rate: 69.5%), among which 37,059 people took part in HUNT3 in 2006-08. We established a cohort population to study potential risk factors for asthma that included all subjects who participated in both HUNT2 and HUNT3 and were less than 65 years of age in HUNT3 (n=25,616) (21, 22). The age limit was set to minimize the possibility of comorbidities and misclassification of chronic obstructive pulmonary disease (COPD) with asthma.

A 10% random sample of the above cohort population (n=2,584) was selected for measurement of 25(OH)D levels in blood samples collected at baseline in HUNT2 (19, 23). Season for blood sampling varied among the subjects. The size of the random sample was decided upon economic and logistic reasons.

Serum 25(OH)D levels

Baseline serum 25(OH)D levels were measured by using LIAISON® 25-OH Vitamin D TOTAL (DiaSorin, Saluggia, Italy), a fully automated antibody-based chemiluminescence assay. The detection range of the assay is 10 to 375 nmol/L. The assay has an intra-assay coefficient of variation of 4% and an inter-assay coefficient of variation of 8%. This is a well-developed method and has been proven as a rapid, accurate and precise tool for the measurement of serum 25(OH)D levels (24).

Allergic rhinitis

Allergic rhinitis was ascertained according to responses to the following questions: “Do you have allergic rhinitis or hay fever?” in HUNT2 and “Do you have or have you had allergic rhinitis or hay fever?” in HUNT3. After exclusion of participants who reported allergic rhinitis in HUNT2 (n=442) and those with missing information on the question in HUNT2 or HUNT3 (n=751) or missing information on serum 25(OH)D levels (n=40), it yielded an analysis cohort with complete data for 1,351 adults who did not report allergic rhinitis at baseline. Among them, incident cases of allergic rhinitis were identified according to affirmative answer to the question in HUNT3.

Covariates

Several important baseline variables were collected in HUNT2, including age (19-29, 30-39, 40-49, and 50-55 years), daily smoker (yes, no, and unknown), average hours of light physical activity per week (<1, 1-2, ≥3 hours, and unknown), years of education (<10, 10-12, ≥13 years, and unknown), social benefit recipient (yes, no, and unknown), economic difficulties (yes, no, and unknown), family history of allergy (yes, no, and unknown), body mass index (BMI) (kg/m^2, <25.0, 25.0-29.9, and ≥30.0), and season of blood sampling (December to May, and June to November). Social benefit recipients were those who reported receiving any of the public welfare benefits. Subjects who had economic difficulties were identified by their affirmative answer to the question, “During the last year, has it at any time been difficult to meet the costs of food, transportation, housing and such?” Family history of allergy was defined as current or ever allergy in any of the family members (mother, father, brother and/or sister). Body weight and height in HUNT2 were measured by health professionals. People with missing information on smoking, physical activity, education, socioeconomic status, and

family history of allergy were regarded as an "unknown" category for each variable and included in the analyses. Multiple imputations for the missing data of these variables were performed, with no material change in our main findings (data not presented).

Statistical analysis

For comparisons, unpaired t-tests were used for continuous variables; Chi-square tests were used for categorical variables (Table 1). Baseline serum 25(OH)D levels were classified into 3 groups: <50.0, 50.0-74.9, and ≥75.0 nmol/L (equivalent to <20.0, 20.0-29.9, and ≥30.0 ng/ml), which are widely used cut-off points in scientific literature (10, 25). They were also treated as continuous values by using their actual levels. The association between 25(OH)D and incident allergic rhinitis was examined using logistic regression analyses, and odds ratios (OR) and 95% confidence intervals (CI) were calculated (Table 2). The interaction of serum 25(OH)D levels with each co-variable was tested and only sex was identified as a significant effect modifier for the association between serum 25(OH)D levels and incident allergic rhinitis ($p=0.017$ for 25(OH)D as a categorical variable; $p<0.001$ for 25(OH)D as a continuous variable). Therefore, the results were presented by men and women separately. The multivariable model included age, daily smoking, physical activity, education, social benefit, economic difficulties, family history of allergy, body mass index, and season of blood sampling at baseline. All statistical analyses were performed with STATA, release 12.0 (College Station, Texas, USA).

Ethics

The study was approved by the Regional Committee for Medical Research Ethics. All participants gave their informed consent.

RESULTS

Among the 1,351 adults, 9% of men (n=53) and 15% of women (n=119) developed allergic rhinitis during the 11-year follow-up period (p=0.001, Table 1). More women compared to men received social benefits (p<0.001) and had a family history of allergy at baseline (p<0.001). Other baseline characteristics, including serum 25(OH)D levels, did not differ significantly between sexes.

Men, with a serum 25(OH)D level <50 nmol/L, had a significantly higher risk of incident allergic rhinitis compared with those with a serum 25(OH)D level ≥75 nmol/L (AOR 2.55; 95% CI 1.01-6.49); each 25 nmol/L reduction of 25(OH)D level was associated with an AOR of 1.84 (95% CI 1.18-2.87) for allergic rhinitis (Table 2). Among women, however, there seemed to be the opposite association. For example, each 25 nmol/L reduction of 25(OH)D level was associated with about 17% reduced risk of allergic rhinitis (AOR 0.83; 95% CI 0.66-1.05, Table 2). In women whose age was <50 years at baseline (the approximate mean age of menopause among Norwegian women, n=669), each 25 nmol/L reduction of serum 25(OH)D level was significantly associated with a reduced risk of incident allergic rhinitis (AOR 0.74; 95% CI 0.57-0.96). However, in women of age ≥50 years (n=112), each 25 nmol/L reduction of serum 25(OH)D level seemed to be associated with an increased risk (AOR 3.06; 95% CI 0.94-9.91, p for interaction=0.089). The AORs for each 25 nmol/L reduction of 25(OH)D levels among women before and after menopause were 0.74 (95% CI 0.57-0.96, n=665) and 1.47 (95% CI 0.52-4.20, n=105), respectively.

Because asthma is often comorbid with allergic rhinitis, we performed sensitivity analyses to address this issue. Even after additional adjustment for self-reported asthma, or exclusion of

subjects who reported having asthma in HUNT2, similar results were obtained for both men and women (data not presented).

DISCUSSION

In this Norwegian adult population who reported no allergic rhinitis at baseline, we observed that lower serum 25(OH)D levels were associated with an increased risk of allergic rhinitis among men. By contrast, lower serum 25(OH)D levels seemed to be associated with a reduced risk of allergic rhinitis among women, especially women before menopause. This interaction of serum 25(OH)D levels with sex is unlikely to be due to chance ($p < 0.02$).

Previous studies showed varying results for the association of serum 25(OH)D levels with allergic diseases. A few pediatric studies demonstrated an increased risk of sensitization to food or airborne allergens among children with lower 25(OH)D levels (11, 13, 14). However, other studies of children showed an increased risk with higher 25(OH)D levels (15, 17) or no association between the two (12). To further complicate this relationship, a U-shaped association has been demonstrated with both low and high 25(OH)D levels associated with high allergen-specific IgE and total IgE levels in the same population (26, 27). A previous cross-sectional study of adults found that higher serum 25(OH)D levels were associated with higher prevalence of allergic rhinitis (16). Previous studies, however, did not evaluate the possible modifying effect of sex on the relationship between serum 25(OH)D levels and allergic diseases. Our large cohort study of Norwegian adults suggests that the association of 25(OH)D levels with allergic rhinitis is significantly modified by sex; while there was an inverse association in men, there was a positive association in women. This may help to explain the inconsistent findings in the literature where analyses were usually performed in the total population, with both sexes combined.

The observed interaction has biological plausibility. Optimal vitamin D levels enhance the production and function of regulatory T cells (1, 2) that can produce cytokines to suppress both Th1-mediated autoimmune responses and Th2-mediated allergic responses (3). Sex hormones can also regulate these immune responses. Both animal and human studies have demonstrated that female sex hormones promote Th1 immunity (28, 29), and women are thus more likely to suffer from autoimmune diseases (28). Male sex hormones decrease autoimmunity in animal studies (30) and in humans (28). Male sex hormones may also promote Th2 immunity and the subsequent allergic diseases (31). There is a possibility that low vitamin D status is associated with increased Th2 responses in males and increased Th1 responses (possibly reduced Th2 responses) in females. This would support a possible inverse association between serum 25(OH)D levels and allergic rhinitis in men, and a positive association in women, particularly before menopause. These mechanistic hypotheses merit further investigation.

There are several potential limitations to our study. Firstly, there were substantial number of individuals with missing data on allergic rhinitis at baseline or follow-up that had to be excluded. The group with missing data on allergic rhinitis differed from the rest of cohort on several baseline characteristics (online supplementary table 1). For example, the group with missing allergic rhinitis had less family history of allergy than the rest of the cohort. However, all of these baseline covariates were controlled for in the multivariable models. Besides, the missingness is likely to be at random, based on the similarity of allergic rhinitis prevalence at baseline or follow up in our study (20% and 27%, respectively), which is comparable with the prevalence from other European studies (17% in Italy to 29% in Belgium) (32). Secondly, we only had questionnaire information on allergic rhinitis or hay fever but no objective allergy markers such as skin prick tests or measures of antigen specific

IgE levels. In a previous study based on 22 population studies, the overall proportion of rhinitis cases that were atopic was 61% (33), and, as mentioned above, the prevalence of allergic rhinitis in our study was consistent with other European studies. Nevertheless, we cannot rule out over-report of allergic rhinitis that is associated with self-reporting. In addition, the question about allergic rhinitis or hay fever was, however, not exactly the same in HUNT2 or HUNT3 questionnaires. Both over-reporting and non-identical question in the two surveys may lead to misclassification of incident allergic rhinitis, although 76% of the cases confirmed their rhinitis symptoms during the past 12 months of HUNT3. Nevertheless, we assume that the misclassification was most likely non-differential (i.e. that misclassification did not differ by serum 25(OH)D levels). Under the presence of non-differential misclassification, the observed association is usually underestimated (34).

To our knowledge, we report the first prospective cohort study evaluating serum 25(OH)D levels and allergic rhinitis in adults, and it is also the first to formally evaluate the possible modifying effect of sex on the association. In addition, our study was strengthened by measurement of serum 25(OH)D levels, an objective measure of body vitamin D status, and our ability to control for a comprehensive panel of confounding factors including BMI. Overweight or obesity is associated with low body vitamin D status (35) and increased risk of asthma (36) that is often comorbid with allergic rhinitis. Nevertheless, our findings were derived from a random sample of young to middle aged adult population. This would limit the generalization of our results to populations with extended age, and we should be cautious when extrapolating these findings.

In summary, low vitamin D status was associated with an increased risk of allergic rhinitis in men and a seemingly reduced risk in women in this Norwegian adult population. Future

studies are warranted to confirm our results and further investigate the underlying mechanisms of how vitamin D affects immunity and its potential interaction with sex hormones.

ACKNOWLEDGMENTS

The Nord-Trøndelag Health Study (The HUNT Study) is collaboration between HUNT Research Centre (Faculty of Medicine, Norwegian University of Science and Technology NTNU), Nord-Trøndelag County Council and the Norwegian Institute of Public Health. We specially thank the HUNT Research Centre laboratory personnel for the measurement of serum 25(OH)D levels. This study was supported by the Research Council of Norway (project no. 201895/V50).

CONFLICT OF INTEREST STATEMENT

None declared.

STATEMENT ABOUT AUTHORS' CONTRIBUTION

Each author has contributed substantially to this paper. Xiao-Mei Mai was the principal investigator of the study. Xiao-Mei Mai, Yue Chen, Carlos A. Camargo Jr and Arnulf Langhammer contributed to the study design. Xiao-Mei Mai and Arnulf Langhammer were responsible for acquisition of data. Xiao-Mei Mai conducted statistical analysis, interpreted results and drafted the initial manuscript. Yue Chen, Carlos A. Camargo Jr and Arnulf Langhammer participated in the data interpretation and contributed to the final draft of the manuscript with important intellectual content.

WORD COUNT

2509

Table 1. Baseline characteristics and cumulative incidence of allergic rhinitis among men and women in a cohort population free of allergic rhinitis at baseline in the HUNT study, 1995-07 to 2006-08

	Men n=570		**Women** n=781	
	Mean (SD)	%[c]	Mean (SD)	%[c]
Age (years)[a]	41.2 (8.6)		39.8 (8.5)	
BMI (kg/m^2)[a]	26.4 (3.3)		25.3 (4.0)	
25(OH)D (nmol/L)[a]	59.0 (22.7)		60.2 (23.1)	
Daily smoking (yes)[b]		24.4		28.7
Physical activity (<1 hour/week)[b]		23.9		20.7
Education (<10 years)[b]		19.0		20.0
Social benefit recipient (yes)[b]		13.3		24.5
Economic difficulties (yes)[b]		30.0		31.2
Family history of allergy (yes)[b]		17.7		26.9
Self ever asthma (yes)[b]		4.2		3.2
Season for blood sampling (December - May)[b]		55.3		52.0
Cumulative incidence of allergic rhinitis[b]		9.3		15.2

Abbreviations: BMI, body mass index; HUNT, Nord-Trøndelag Health Study; 25(OH)D, 25-hydroxyvitamin D; SD, standard deviation.

[a]Unpaired t-tests were used for comparisons between men and women for continuous variables and [b]Chi-square tests for categorical variables. [c]The “unknown” categories for daily smoking, physical activity, education, socio-economic status, and family history of allergy are included when percentages are calculated.

Table 2. The association of serum 25(OH)D level with incident allergic rhinitis among men and women free of allergic rhinitis at baseline in the HUNT study, 1995-07 to 2006-08

Men (n=570)							
25(OH)D (nmol/L)	n	Cases	%	OR	95% CI	AOR	95% CI
≥75.0	128	8	6.3	1.00	Referent	1.00	Referent
50.0-74.9	221	16	7.2	1.17	0.49-2.82	1.23	0.49-3.10
<50.0	221	29	13.1	2.27	1.00-5.12	2.55	1.01-6.49
P for trend[a]				0.02		0.03	
Each 25 nmol/L reduction	570	53	9.3	1.69	1.16-2.45	1.84	1.18-2.87
Women (n=781)							
25(OH)D (nmol/L)	n	Cases	%	OR	95% CI	AOR	95% CI
≥75.0	191	40	20.9	1.00	Referent	1.00	Referent
50.0-74.9	311	40	12.9	0.56	0.34-0.90	0.55	0.33-0.92
<50.0	279	39	14.0	0.61	0.38-1.00	0.66	0.38-1.16
P for trend[a]				0.06		0.16	
Each 25 nmol/L reduction	781	119	15.2	0.82	0.67-1.01	0.83	0.66-1.05

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HUNT, Nord-Trøndelag Health Study; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio.

AOR derived after adjustment for age, daily smoking, physical activity, education, social benefit, economic difficulties, family history of allergy, body mass index, and season of blood sampling at baseline. [a]*P* for trend was tested by treating 25(OH)D categories as an ordinal variable.

References

1. Milliken SV, Wassall H, Lewis BJ, Logie J, Barker RN, Macdonald H, et al. Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function. *J Allergy Clin Immunol* 2012;129:1554-1561.

2. Smolders J, Thewissen M, Peelen E, Menheere P, Tervaert JW, Damoiseaux J, et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. *PLoS One* 2009;4:e6635.

3. Cohen JL, Trenado A, Vasey D, Klatzmann D, Salomon BL. CD4(+)CD25(+) immunoregulatory T Cells: new therapeutics for graft-versus-host disease. *J Exp Med* 2002;196:401-406.

4. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120:1031-1035.

5. Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? *Allergy* 1999;54:757-759.

6. Back O, Blomquist HK, Hernell O, Stenberg B. Does vitamin D intake during infancy promote the development of atopic allergy? *Acta Derm Venereol* 2009;89:28-32.

7. Erkkola M, Kaila M, Nwaru BI, Kronberg-Kippila C, Ahonen S, Nevalainen J, et al. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. *Clin Exp Allergy* 2009;39:875-882.

8. Hypponen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, 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.

9. Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium and vitamin D intake in pregnancy, and wheeze and eczema in infants. *Eur Respir J* 2010;35:1228-1234.

10. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-281.

11. Hollams EM, Hart PH, Holt BJ, Serralha M, Parsons F, de Klerk NH, et al. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. *Eur Respir J* 2011;38:1320-1327.

12. Jones AP, Palmer D, Zhang G, Prescott SL. Cord blood 25-hydroxyvitamin D3 and allergic disease during infancy. *Pediatrics* 2012;130:e1128-e1135.

13. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S, et al. Gene-vitamin D interactions on food sensitization: a prospective birth cohort study. *Allergy* 2011;66:1442-1448.

14. Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. *J Allergy Clin Immunol* 2011;127:1195-1202.

15. Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M, et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy* 2013;68:220-228.

16. Wjst M, Hypponen E. Vitamin D serum levels and allergic rhinitis. *Allergy* 2007;62:1085-1086.

17. Heimbeck I, Wjst M, Apfelbacher CJ. Low vitamin D serum level is inversely associated with eczema in children and adolescents in Germany. *Allergy* 2013;68:906-910.

18. Querfeld U, Keil T, Beyer K, Stock P, Pilz S, Marz W, et al. Vitamin D in early life: good or bad for food allergies? *Allergy* 2013;68:1081-1083.

19. Mai XM, Langhammer A, Camargo CA, Jr., Chen Y. Serum 25-Hydroxyvitamin D Levels and Incident Asthma in Adults: The HUNT Study. *Am J Epidemiol* 2012;176:1169-1176.

20. Krokstad S, Langhammer A, Hveem K, Holmen T, Midthjell K, Stene T, et al. Cohort Profile: The HUNT Study, Norway. *Int J Epidemiol* 2013;42:968-977.

21. Brumpton B, Langhammer A, Romundstad P, Chen Y, Mai XM. General and abdominal obesity and incident asthma in adults: the HUNT study. *Eur Respir J* 2013;41:323-329.

22. Mai XM, Langhammer A, Chen Y, Camargo CA, Jr. Cod liver oil intake and incidence of asthma in Norwegian adults--the HUNT study. *Thorax* 2013;68:25-30.

23. Mai XM, Chen Y, Camargo CA, Jr., Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study. *Am J Epidemiol* 2012;175:1029-1036.

24. Wagner D, Hanwell HE, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. *Clin Biochem* 2009;42:1549-1556.

25. Ford ES, Zhao G, Tsai J, Li C. Vitamin D and all-cause mortality among adults in USA: findings from the National Health and Nutrition Examination Survey Linked Mortality Study. *Int J Epidemiol* 2011;40:998-1005.

26. Hypponen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE - a significant but nonlinear relationship. *Allergy* 2009;64:613-620.

27. Rothers J, Wright AL, Stern DA, Halonen M, Camargo CA, Jr. Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen sensitization in children from Tucson, Arizona. *J Allergy Clin Immunol* 2011;128:1093-1099.

28. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Hum Reprod Update* 2005;11:411-423.

29. Gourdy P, Araujo LM, Zhu R, Garmy-Susini B, Diem S, Laurell H, et al. Relevance of sexual dimorphism to regulatory T cells: estradiol promotes IFN-gamma production by invariant natural killer T cells. *Blood* 2005;105:2415-2420.

30. Fijak M, Schneider E, Klug J, Bhushan S, Hackstein H, Schuler G, et al. Testosterone replacement effectively inhibits the development of experimental autoimmune orchitis in rats: evidence for a direct role of testosterone on regulatory T cell expansion. *J Immunol* 2011;186:5162-5172.

31. Townsend EA, Miller VM, Prakash YS. Sex differences and sex steroids in lung health and disease. *Endocr Rev* 2012; 33:1-47.

32. Cingi C, Catli T. Phenotyping of allergic rhinitis. *Curr Allergy Asthma Rep* 2012;12:115-119.

33. Zacharasiewicz A, Douwes J, Pearce N. What proportion of rhinitis symptoms is attributable to atopy? *J Clin Epidemiol* 2003;56:385-390.

34. Hernán MA, Robins JM. Causal inference, part I. September 27, 2013. http://www.hsph.harvard.edu/miguel-hernan/files/2013/09/hernanrobins_v1.10.23.pdf

35. Larose TL, Chen Y, Camargo CA, Jr., Langhammer A, Romundstad P, Mai X-M. Factors associated with vitamin D deficiency in a Norwegian population: the HUNT Study. *J Epidemiol Community Health* 2013;Nov 6. doi: 10.1136/jech-2013-202587. [Epub ahead of print]

36. Beuther DA, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am J Respir Crit Care Med* 2007;175:661-666.