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No association between vitamin D and atopy, asthma, lung function or atopic dermatitis: a prospective study in adults

Short title

No association of vitamin D with atopic diseases in adults

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Abstract

Studies suggest that vitamin D may be involved in the pathogenesis of allergic disorders, asthma and decreased lung function. However, results are inconsistent and only few prospective studies have examined adults. The purpose of this study was to investigate the association of serum 25-hydroxy vitamin D (s25(OH)D) with atopy, atopic dermatitis (AD), asthma, wheezing, and impaired lung function in a prospective study of Danish adults. A random sample of 3,471 persons was examined in 2006-2008. Of these, 2,308 were reexamined 5 years later. s25(OH)D and specific IgE against four common inhalant allergens were measured by standard procedures. Wheezing, asthma and AD were assessed from questionnaires and lung function was measured by spirometry. We found no statistically significant associations between s25(OH)D and prevalence or incidence of atopy, AD, asthma or wheezing. Associations with lung function were inconsistent. We conclude that vitamin D status does not influence these conditions in adults.

Introduction

Vitamin D has been suggested as a potential etiological factor affecting both incidence and severity of asthma and allergic diseases including atopic dermatitis (AD)(1-3). As recently reviewed by Bozzetto et al.(1), the predominant hypothesis suggests a protective action of vitamin D. In contrast, some studies indicate that high levels of vitamin D may instead increase the risk of asthma and

atopic diseases(2;4). Studies investigating the relation between vitamin D status and lung function has also been inconclusive. Several studies have associated lower vitamin D status with decreased lung function(5;6). Recently, we however reported an association between low levels of serum 25-hydroxy vitamin D (s25(OH)D) and restricted lung function among adults in a cross-sectional setting(7). In contrast, longitudinal analyses showed an association between high levels of s25(OH)D and adverse changes in lung function indicating a complex relation between vitamin D and lung function that calls for further investigation(7).

The purpose of this study was to investigate the association between 25(OH)D and asthma, atopy, AD and lung function in a population of Danish adults – cross-sectionally as well as longitudinally. Furthermore, we investigated effect modification by loss-of-function mutations in the filaggrin gene (*FLG*) since *FLG* mutations are common and associated with both higher levels of s25(OH)D(8) and increased risk of atopic diseases(9).

Methods

The population-based Health2006 cohort is conducted at the Research Centre for Prevention and Health(10). Study participants (N=3,471, participation rate: 44.7%) were recruited between June 2006 and May 2008 from the Danish Central Personal Register, as a random sample from persons living in the western area of the Capital Region of Denmark. Five-year follow-up examinations were conducted between November 2011 and November 2012 and included 2,308 participants(11). Written informed consent was obtained from all participants. Both the baseline (KA-20060011) and 5-year follow-up (H-3-2011-081) studies were approved by the regional Ethical Committee and the Danish Data Protection Agency.

At baseline examination, fasting venous blood samples were drawn from all participants.

Concentrations of s25(OH)D were measured with the chemiluminescent Vitamin D3 immunoassay on a Cobas e411 system (Roche Diagnostics GmbH, Mannheim, Germany) at University Medicine Greifswald, Germany. Measurements of s25(OH)D were successfully completed for 3,409 participants (98 %) at baseline.

Atopy was defined as a positive test for specific immunoglobulin E (sIgE) (≥ 0.35 kU/L) to at least one of the four most common and clinically important inhalant allergens in Denmark (house dust mite (*Dermatophagoides pteronyssinus*), cat, grass, and birch). At baseline serum samples were analysed using the ADVIA Centaur Specific IgE assay (Siemens, Deerfield, Ill). Serum samples collected at follow-up examination were screened for sIgE to a panel of inhalant allergens by using Phadiatop® on a Phadia 250 system at Thermo Scientific (ImmunoDiagnostics, Allerød, Denmark).

Phadiatop®-positive (≥ 0.35 kU/L) samples were further analysed for sIgE to the four allergens mentioned above. Incident atopy was defined as atopy at follow-up among participants with no prevalent atopy at baseline. A history of AD was defined by the U.K. Working Party's diagnostic criteria as a history of an itchy skin condition plus a minimum of two of four minor criteria(12).

Asthma and wheezing without a cold were defined as previously described(13). Spirometry was performed according to international standards(14) with the Spiro USB Spirometer (MicroMedical Limited, Rochester, Kent, United Kingdom). Measurements of forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) without use of bronchodilators were recorded. Percent predicted FEV₁ was calculated from sex, age, and measured height(15). Incident AD, asthma and obstructive/restrictive lung function were defined similarly to the definition of incident atopy.

Genotyping for the most common *FLG* mutations R501X, 2282del4 and R2447X was performed as previously described(9). *FLG* genotype was noted as wild type, heterozygous, or homozygous/compound heterozygous-

All statistical analyses were performed in SAS, version 9.3 (SAS Institute Inc. Cary, NC USA).

Multiple logistic regression models were used to estimate odds ratios for associations between s25(OH)D and dichotomous outcomes. Linear regression models were used to evaluate associations between s25(OH)D and continuous measurements of lung function and changes in lung function. Statistical models were adjusted for confounding by age, sex, body mass index (BMI), smoking, and month of examination. Longitudinal linear regression models were further adjusted for the baseline value of the outcome to adjust for regression to the mean. Potential effect modification of s25(OH)D by smoking or *FLG* mutations were evaluated by assessing the *P* values of the relevant interaction term (e.g. 25(OH)D**FLG* mutation) in the regression models. P-values (two-tailed) of likelihood ratio tests were used to test for statistical significance defined as $p < 0.05$.

Results

Study population characteristics are shown in Table 1. Participants with follow-up examination were older and had higher levels of s25(OH)D at baseline when compared with those not participating at follow-up. Also, atopy was more prevalent among participants with follow-up whereas AD, asthma and impaired lung function (restrictive and obstructive) were more prevalent among participants without follow-up.

No statistically significant associations were observed between s25(OH)D and atopy, AD, asthma, wheezing or reduced lung function ($FEV_1\%pred < 80\%$), respectively, in cross-sectional analyses after adjustment for potential confounders (Table 2). However, the prevalence of obstructive lung function ($FEV_1/FVC < 70\%$) increased significantly with increasing levels of s25(OH)D in crude

analyses ($p=0.029$). Furthermore, no statistically significant associations were found in longitudinal analyses – except for an association between decreasing risk of obstructive lung function with increasing levels of s25(OH)D ($p=0.013$, adjusted model). Moreover, we found no statistically significant associations between s25(OH)D and lung function in linear regression models (data not shown). None of the considered interactions terms were statistically significant and restriction of analyses to only include participants not reporting use of vitamin D supplements ($N=2,680$) or never smokers ($N=1409$) did not change the results. However, the statistical power was reduced by the restrictions and no associations were significant in subgroup analyses. Adjustment for *FLG* genotype did not affect the results.

Discussion

We found no associations between s25(OH)D and prevalence or incidence of atopy, AD, asthma or wheezing. Neither did we observe any robust associations with lung function.

Results from this study are in line with our previous findings from another Danish cohort study reporting no statistical associations between s25(OH)D and prevalence or incidence of asthma and atopy(7). In our previous study we also found contradictory associations between s25(OH)D and lung function. However, the present study indicates an adverse influence of high s25(OH)D levels on lung function in a cross-sectional setting and a positive effect of s25(OH)D in a longitudinal setting which is opposite to our previous findings. Thus, our studies in combination do not lend consistent support to the hypothesis that s25(OH)D affects lung function.

Asthma and atopic diseases often starts in childhood and many previous studies reporting significant associations between vitamin D and atopic diseases have focused on vitamin D exposure in early life. A recent study showed increased risk of sensitization to food allergens and increased severity of AD in infants with vitamin D deficiency(16). Also low maternal vitamin D intake during

pregnancy has been associated with increased risk of early childhood wheezing(17), asthma and allergic rhinitis(18). Our study only relates to the possible role of vitamin D in adults and associations among adults are less frequently reported. Some propose that the mechanisms behind the potential association of vitamin D with asthma and atopic diseases mainly operate in early life and therefore vitamin D status in adults may be less important to asthma and atopic disease(7). However, a recent study with over 20 years of follow-up did not provide support for a long-term protective effect of high maternal s25(OH)D levels on allergic airway disease and lung function in offspring(19). In contrast, the study indicates an adverse effect of high maternal s25(OH)D on risk of allergic diseases in offspring, and thus illustrates that also studies on early life exposure to high levels of s25(OH)D yield contradictory findings. Inflammatory processes associated with active atopic disease may negatively affect s25(OH)D(20) and thus partly account for the conflicting results from previous studies.

A potential limitation to this study is the relatively low participation rates at 5 year follow-up. Since participants with follow-up are healthier than those without follow-up, the longitudinal analyses may be influenced by selection bias. The large number of participants, the prospective study design, and the use of objective measures of lung function and atopy are strengths of this study. In addition, the inclusion of *FLG* genotype in the analyses improves this study since unknown *FLG* genotype might have been an important confounder in previous studies. Adjustment for *FLG* genotype did however not affect our results and we did not find evidence of interactions between genotype and vitamin D status. The great number of different outcome measures also strengthens this study since more aspects of pulmonary and atopic diseases are considered. It is however important to be aware that the great number of outcome measures imply an equivalent great number of statistical analyses that may increase the risk of type I errors. Thus the contradictory findings in relation to lung function may be explained by multiple testing.

In conclusion, our study indicates that vitamin D status does not influence the prevalence or development of atopy, AD, asthma, wheezing, or impaired lung function among adults. Despite its relevance in relation to s25(OH)D as well as atopic diseases, *FLG* genotype does not seem to be an important confounder of the relations between s25(OH)D and the considered outcome variables.

Author Contributions

BHT and AL designed the study and were together with NF responsible for conduction of data. BHT, NGH and LT performed the data analyses and all authors contributed substantially to interpretation of data. The paper was written by BHT and LT and was critically revised by NGH, TS, JPT, NF and AL. All authors approved the final version of the manuscript before submission.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Table 1: Baseline characteristics of participants in the Health2006 cohort with complete measurement of serum 25-hydroxy-vitamin D (s25(OH)D)

Health2006	All	Participants with follow-up	Participants without follow-up
Age (mean (SD)), years	49.4 (13.0) (N=3409)	50.0 (12.5) (N=2272)	48.0 (14.0)* (N=1137)
Men, prevalence	44.9 % (N=3409)	46.0 % (N=2272)	42.3 % (N=1137)
s25(OH)D, nmol/l (median (25th/75th percentile))	41.5 (29.5-55.5) (N=3409)	42.3 (30.3-55.8) (N=2272)	40.8 (27.3-55.38)* (N=1137)
Filaggrin gene mutation carriers	9 % (N=3316)	8 % (N=2204)	11 %* (N=1112)
Atopy, prevalence	23.2 % (N=3406)	24.3 % (N=2271)	21.0 %* (N=1135)
Atopic dermatitis, prevalence	10.3 % (N=3319)	9.5 % (N=2217)	11.9 %* (N=1102)
Asthma, prevalence	11.1 % (N=3491)	10.5 % (N=2264)	12.2 % (N=1127)
Wheezing without a cold, prevalence	15.5% (N=3385)	13.2% (N=2248)	19.5%* (N=1110)
FEV ₁ /FVC<70%, prevalence	11.3 % (N=3395)	10.1 % (N=2262)	13.7 %* (N=1133)
FEV ₁ %pred<80%, prevalence	10.2 % (N=3393)	7.7 % (N=2260)	15.3 %* (N=1133)

*Statistically significant difference (P<0.05) between participants and non-participants in the follow-up examination.

Table 2: Results from logistic regression models evaluating the associations between continuous measures of serum 25-hydroxy-vitamin D (s25(OH)D) at baseline and binary outcome variables at baseline (cross-sectional analyses of prevalence) and follow-up (longitudinal analyses of incidence).

	Baseline (Cross-sectional)		Follow-up (Longitudinal)	
	Crude OR(95%CI)*	Adjusted** OR(95%CI)*	Crude OR(95%CI)*	Adjusted** OR(95%CI)*
Atopy	1.002 (0.998-1.005) p=0.314	1.002 (0.998-1.006) p=0.269	1.000 (0.991-1.009) p=0.967	0.997 (0.988-1.007) p=0.603
Atopic dermatitis	0.999 (0.994-1.004) p=0.759	0.998 (0.993-1.004) p=0.542	0.999 (0.991-1.008) p=0.902	0.998 (0.989-1.008) p=0.707
Asthma	0.998 (0.997-1.003) p=0.527	0.997 (0.992-1.003) p=0.347	0.998 (0.989-1.008) p=0.701	0.996 (0.985-1.007) p=0.500
Wheezing without a cold	1.000 (0.996-1.004) p=0.917	0.998 (0.994-1.003) p=0.499	1.003 (0.997-1.009) p=0.394	1.002 (0.995-1.009) p=0.567
FEV₁/FVC<70 %	1.005 (1.000-1.010) p=0.029	1.002 (0.997-1.007) p=0.522	0.997 (0.989-1.004) p=0.368	0.989 (0.981-0.998) p=0.013
FEV₁%pred<80 %	1.000 (0.995-1.005) p=0.943	0.996 (0.990-1.002) p=0.171	1.000 (0.989-1.012) p=0.938	1.000 (0.987-1.014) p=0.944

*OR(95%CI): Odds Ratios with 95% confidence interval pr nmol/l increase in s25(OH)D.

** Adjusted for potential confounding by age, gender, BMI, smoking status, month of blood sampling and filaggrin genotype