Serum 25-hydroxyvitamin D levels and lung function in adults with asthma: the HUNT Study

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Take home message: Low 25(OH)D levels were not associated with airway obstruction in most asthma adults except for men with no allergy.

25(OH)D	25-hydroxyvitamin D
ASM	airway smooth muscle
BMI	body mass index
COPD	chronic obstructive pulmonary disease
HUNT	Nord-Trøndelag Health Study
FEV_1	forced expiratory volume in 1 second
FEV1 % pred.	forced expiratory volume in 1 second percent predicted
FVC	forced vital capacity
FVC % pred.	forced vital capacity percent predicted
FEV_1/FVC ratio	ratio of FEV_1 to FVC (actual)
PEFR	peak expiratory flow rate

ABSTRACT

The association between vitamin D status and lung function (LF) in adults with asthma remains unclear.

We studied this cross-sectional association and possible modification by sex and allergic rhinitis in 760 adults (19-55 years) with self-reported asthma in the Nord-Trøndelag Health Study. Serum 25-hydroxyvitamin D (25(OH)D) level <50 nmol/L was considered deficient. LF measures included forced expiratory volume in 1 second percent predicted (FEV_1 % pred.) forced vital capacity percent predicted (FVC % pred.) and FEV_1/FVC ratio. Multiple linear regression models were used to estimate adjusted regression coefficients (β) and 95% confidence intervals (CI).

44% of asthma adults had serum 25(OH)D level <50 nmol/L. Its associations with LF measures seemed to be modified by sex and allergic rhinitis (P<0.03 for 3-way interaction term). Overall, serum 25(OH)D level <50 nmol/L was not associated with LF measures in women or in men with allergic rhinitis in this asthma cohort. In men with asthma but without allergic rhinitis, however, serum 25(OH)D level <50 nmol/L was significantly associated with lower FEV_1/FVC ratio (β= -8.60%; 95% CI: -16.95% to -0.25%).

Low serum 25(OH)D level was not associated with airway obstruction in most asthma adults with the exception of men with asthma but without allergic rhinitis.

Key words: 25-hydroxyvitamin D; adults; allergy; asthma; HUNT study; sex; spirometry.

INTRODUCTION

Successful treatment and prevention of rickets during the first half of the 20th century led to universal acceptance that optimal vitamin D status is required for good bone health in children and adults (1). Recently, the relationships between vitamin D status and various non-skeletal health outcomes including respiratory disorders (2), cardiovascular disease (3), cancer (4), and all-cause mortality (5), have been addressed. Vitamin D deficiency (defined as 25-hydroxyvitamin D [25OHD] <50 nmol/L) is prevalent worldwide (6, 7). The global burden of obstructive airway diseases, such as asthma, is high (8).

In our previous study, we observed an association between vitamin D deficiency and incident asthma in adults, particularly in men without allergy status (9). In addition, several studies have shown an association between vitamin D deficiency and lower lung function in general adult populations (10-14), among which, two studies suggested a potentially stronger association in men compared to women (11, 14). Most of these previous studies found a significant association between serum 25(OH)D at the <50 nmol/L level and lower forced expiratory volume in 1 second (FEV_1), and forced vital capacity (FVC), but not FEV_1/FVC ratio in the general population. To date, there are few published studies on vitamin D status and lung function in adults with asthma. A recent cross-sectional study of Chinese adults with asthma showed significantly lower FEV_1 percent predicted (FEV_1 % pred.) and significantly lower FEV_1/FVC ratio in participants who were vitamin D deficient (<50 nmol/L) (15).

In addition to our previous finding of an association between low serum 25(OH)D and incident asthma in men without allergy, a cross-sectional study using data from the National Health and Nutrition Examination Survey (NHANES 2005-2006) reported an association between lower serum 25(OH)D levels and greater odds of asthma diagnosis in non-atopic individuals (16). These previous findings suggest that vitamin D status may influence asthma via a non-allergic rather than an allergic pathway. However, the biological pathway by which vitamin D may influence lung function parameters in asthma patients, remains unclear.

In this current cross-sectional study we aimed to assess the association between serum 25(OH)D and lung function in adults with asthma. We also aimed to examine possible interactions by sex and allergy status. We hypothesized that low serum 25(OH)D levels would be associated with lower lung function, and that this association would most likely be present in men with asthma and without allergy status.

MATERIALS AND METHODS

Study design

This is a cross-sectional study using the second survey of the Nord-Trøndelag Health Study (HUNT2). HUNT is a large population health survey of Norwegian inhabitants at latitude 64° North (17). Three adult surveys have been conducted to date: HUNT1 to HUNT3 (1984-86 to 2006-08). The study population consisted of Norwegian adults aged 19 years or older, with socio-demographic characteristics considered generally representative of Norway (18).

The target population for HUNT2 (1995-97) consisted of approximately 93,000 Norwegian adults living in Nord-Trøndelag County. The participation rate was 70% (n=65,237) (17) from which we established an asthma cohort of adults aged 19-55 who provided an affirmative response to both of the following two questions, “Have you had attacks of wheezing or breathlessness during the last 12 months?” and “Do you have or have you had asthma?” The asthma cohort also confirmed their asthma status in HUNT3 with an affirmative response to the question as above, “Do you or have you had asthma?” (n=898).The current study was based on 760 asthma cases with complete data on both exposure (serum 25(OH)D) and outcome (lung function); 40 subjects were excluded due to missing data on 25(OH)D, and 98 subjects were excluded due to missing data on lung function.

Serum 25 (OH) D measurements

Blood samples were collected in HUNT2 and stored at -70° C for later use. Serum 25(OH)D levels were measured using LIASON 25-OH Vitamin D TOTAL (DiaSorin, Saluggia, Italy); a fully automated antibody-based chemiluminescence assay with detection range 10-375 nmol/L, intraassay coefficient of variation (CV) 4%, and interassay CV 8%. Serum 25(OH)D levels are considered the best marker for body vitamin D status (19) and were categorized according to widely used and accepted cut-points (<50 nmol/L, 50-74.9 nmol/L or ≥ 75 nmol/L) (7). Serum 25(OH)D levels were also analyzed as a continuous independent variable.

Lung function measures

Two MasterScope Jaeger v.5.1 spirometers were used to measure lung function by trained professionals at screening stations. Instrument quality control included twice daily calibration. Biological control was conducted once daily via staff lung function assessment. Participants were made to sit upright and use a nose-clip (20). Recommendations and criteria from the American Thoracic Society (ATS) were followed and applied (21). Participants were required to give three to five acceptable and reproducible trials during which expiration continued for at least six seconds. The best trial was determined by identification of the flow/volume curve using the highest sum of FEV_1 and FVC. The acceptability and reproducibility of results were reviewed by expert technicians. In the HUNT surveys, the highest sum of FEV_1 and FVC, and the best FEV_1/FVC ratio were used. Predicted reference values were derived from the

prediction equations of spirometry based on the same HUNT population (20), and these predicted values were used to calculate FEV_1 % pred., and FVC % pred.

Other variables

Sex and allergy status were considered potentially important modifiers of the association between serum 25(OH)D and lung function. Allergic rhinitis was used as a proxy for allergy status (yes, no or unknown) based on participant response to the question: "Do you have or have you had allergic rhinitis or hay fever?" Other important variables including body mass index (BMI), socio-economic status (education, receipt of social benefit and economic difficulties), season of blood sample collection, lifestyle factors (physical activity and smoking status), and asthma medication or corticosteroid use, were collected in HUNT2. Body weight and height were measured in HUNT2 by trained professionals whilst participants wore light clothing. Body mass index (BMI, kg/m^2) was calculated and included in the analysis as a continuous variable. The other covariates were categorized as years of education (<10, ≥ 10 or unknown), receipt of social benefits (yes, no or unknown), economic difficulties in the past year (yes, no or unknown), season of blood sample collection (December-May or June-November), number of hours of light physical activity per week (<1, ≥ 1 or unknown), smoking status (never, former, current or unknown), ever use of asthma medication (yes, no or unknown), and regular use of inhaled corticosteroids in the last 6 months (yes or no).

123 **Statistical analysis**

124 The statistical analyses were performed separately in women (*n*=446) and men (*n*=314), and
125 further stratified by allergic rhinitis based on our prior hypothesis and a significant 3-way
126 interaction of categorical serum 25(OH)D with sex and allergic rhinitis on lung function
127 parameters (P<0.03). Baseline characteristics were compared between women and men (Table
128 1). Linear regression analysis was used to estimate the association between serum 25(OH)D
129 level and lung function measures (FEV_1 % pred., FVC % pred., and FEV_1/FVC ratio) (Tables
130 2-4). Analyses were conducted using serum 25(OH)D as a categorical (<50 nmol/L, 50-74.9
131 nmol/L or $\geq$ 75 nmol/L), or continuous independent variable. Crude and adjusted regression
132 coefficients (β) and 95% confidence intervals (CI) were estimated. Multiple linear regression
133 models included BMI, education, receipt of social benefits, economic difficulties in the last
134 year, season of blood sample collection, physical activity, smoking status, ever use of asthma
135 medication, and regular use of inhaled corticosteroids in the last six months as important
136 covariates. Missing data on education, social benefits, economic difficulties, physical activity,
137 smoking status, and ever asthma medication, were categorized as "unknown" and included in
138 the multiple linear regression analysis; multiple imputations of missing data on the above
139 covariates and missing on allergic rhinitis were performed. To minimize possible
140 misclassification of reported asthma, we excluded those who reported having chronic
141 obstructive pulmonary disease (COPD), chronic bronchitis or emphysema and repeated the
142 analyses. All statistical analyses were performed using Stata, version 12.1 (StataCorp, College
143 Station, Texas).

144

Ethics

This study received ethics approval from the Regional Committee for Medical Research Ethics. All study participants gave informed written consent.

RESULTS

A comparison between participants in the analysis group (n=760) and those excluded due to missing information on either exposure or outcome (n=138) showed that the analysis group had higher serum 25(OH)D levels, a higher proportion of never smokers, were less likely to report regular use of ICS, and had better lung function (online Appendix 1).

Table 1 shows the characteristics of the study sample by sex. Overall, 44% of study participants had serum 25(OH)D level <50 nmol/L with no substantial difference between sexes. The mean level of serum 25(OH)D in all adults with asthma was 57 nmol/L. Women with asthma were more likely than men with asthma to receive social benefits, be physically active, use asthma medication, and have allergic rhinitis. Women and men were similar in age, BMI, education, season of blood sample collection, and smoking status. Men with asthma had lower FEV_1 % pred. and FEV_1/FVC ratio compared to women with asthma, whereas FVC % pred. showed no difference between sexes.

The 3-way interaction term (categorical 25(OH)D x sex x allergic rhinitis) was significant for the FEV_1/FVC ratio (P=0.023) and FEV_1 (P=0.017) models. After stratification by sex (Table 2), the adjusted regression coefficients for women with asthma revealed non-significant associations between serum 25(OH)D as a categorical or continuous variable and all three lung function measures. However, men with asthma and with serum 25(OH)D level <50 nmol/L showed a significantly lower FEV_1/FVC ratio (β= -4.31%, 95% CI: -7.25% to -1.38%), and FEV_1 % pred. (β= -8.44%, 95% CI: -13.78% to -3.11%) compared to the ≥ 75

nmol/L group (Table 2). Men with asthma also showed a lower FEV_1/FVC ratio and FEV_1 % pred. for each 25 nmol/L reduction of 25(OH)D, but we found no substantial associations between serum 25(OH)D and FVC % pred. in men with asthma.

After further stratification by allergic rhinitis, neither categorical nor continuous serum 25(OH)D levels were significantly associated with lung function measures in women with asthma, and with or without allergic rhinitis (Table 3). We did not observe a significant association of serum 25(OH)D <50 nmol/L with FEV_1/FVC ratio among men with asthma and with allergic rhinitis, but a substantial association was observed among men with asthma but without allergic rhinitis (adjusted β = -8.60%, CI: -16.95% to -0.25% for 25(OH)D as a categorical variable) (Table 4).

When participants with reported COPD, chronic bronchitis or emphysema were excluded, the association between categorical serum 25(OH)D and lung function measures in women with asthma and with or without allergic rhinitis remained null. The association between categorical serum 25(OH)D and FEV_1/FVC ratio was still more obvious in men with asthma but without allergic rhinitis (online Appendix 2).

Multiple imputations of missing data on allergic rhinitis and other adjusted covariates were performed, and similar analytical results were obtained (data not presented).

DISCUSSION

We found that 44% of adults with asthma had deficient serum 25(OH)D levels (<50 nmol/L), which was slightly higher than the prevalence of vitamin D deficiency (40%) in the general HUNT population (22). We observed no association between serum 25(OH)D and lung function among women with asthma and with or without allergy status. However, we did find a significant association in a subgroup of men. In men with asthma but without allergic rhinitis, low serum 25(OH)D level was associated with a considerably reduced FEV_1/FVC ratio.

Studies on vitamin D status and lung function in asthma populations are scarce. A cross-sectional study of Puerto Rican children with asthma (n=287) reported a significant association between vitamin D insufficiency (<75 nmol/L) and lower FEV_1/FVC ratio (23). A cross-sectional study of 54 US adults with persistent asthma observed an association between reduced continuous serum 25(OH)D and impaired FEV_1 after adjustment for age, sex, and BMI (24). To be noted, this study did not evaluate other lung function measures except for FEV_1. A Chinese study of 435 adults with asthma found a significant association between vitamin D deficiency (<50 nmol/L) and low values for FEV_1/FVC ratio and FEV_1 (15). However, this study did not report sex-specific results.

Regarding a sex difference, a most recent report in children provided consistent results of an association between low plasma 25(OH)D levels and low FEV_1 and FEV_1/FVC ratio in boys with asthma (25). A study of 3359 Canadian adults observed an association between vitamin

212 D deficiency (<50 nmol/L) and lung function (FEV_1 and FVC, but not FEV_1/FVC ratio) in
213 men (14). In the Longitudinal Aging Study Amsterdam, a strong association between serum
214 25(OH)D and peak expiratory flow rate (PEFR) was observed in older men but not in older
215 women (11). Although both adult studies were performed in a general population, these
216 findings do provide some support to our sex specific finding in adults with asthma. Our
217 observation in asthmatic men but not women does not seem to be explained by type 2 error in
218 women (false negative finding) due to a comparable number of women (n=446) and men
219 (n=314) in our analyses. It may be explained by lower lung function in asthmatic men
220 compared with asthmatic women (Table 1). Women with asthma in our study were more
221 likely than men with asthma to report use of asthma medication, which may indicate greater
222 compliance with recommended treatment for asthma and thus better lung function. However,
223 a previous Canadian study indicated that sex may modify the association between asthma and
224 lung function, i.e. the association of asthma with lower lung function was stronger in men
225 than in women (26). Even though the explanation seems plausible, a sex-specific association
226 of serum 25(OH)D with lung function in adults with asthma warrants further investigation
227 and confirmation.

228

229 Our finding of an association between low serum 25(OH)D level and reduced FEV_1/FVC
230 ratio in men with asthma but without allergic rhinitis is consistent with our earlier study in
231 which an association between low serum 25(OH)D and incident asthma was demonstrated
232 only among men with no allergy status (9). In support of our previous finding, Keet et al (16)
233 found an association between low serum 25(OH)D levels and ever asthma in non-atopic
234 subjects. According to a recent genome-wide association study composed of Euro-American

subjects with asthma, T_H1 non-allergic pathway genes are associated with lung function in asthmatic subjects (27). Lower serum 25(OH)D_3 levels have also been associated with thicker airway smooth muscle (ASM) mass in children with severe asthma (28). Serum 25(OH)D levels modulate the contraction, inflammation and remodeling of ASM function (29) which may be a possible mechanism for airway obstruction in asthma subjects. Taken together, our current data extends our previous findings to generate the hypothesis that low serum 25(OH)D levels associated with airway obstruction may influence asthma via a non-allergic pathway, not only on asthma onset but also on asthma severity and control, particularly in men.

Our study is one of few to investigate the relationship between serum 25(OH)D and lung function in adults with asthma, and the first to explore the potential modification of this association by sex and allergy status. Our study has several strengths including a large sample of adults with asthma who contributed complete data on both serum 25(OH)D and lung function measures. Serum 25(OH)D, spirometric and anthropometric data were objectively measured by trained health professionals. Blood samples were collected across all four seasons with a large variation in serum 25(OH)D levels. We were able to control for a range of potential confounding factors in an adult asthma cohort of participants who reported current asthma (wheeze plus ever asthma) in HUNT2 and who further confirmed their asthma status in HUNT3. Multiple imputations of missing data and a sensitivity analysis which excluded potential COPD participants were conducted to strengthen our results.

We acknowledge several limitations to this study including the use of single serum 25(OH)D measurements which may have contributed to measurement error. However, results from a recent prospective study in the US suggested high intra-individual reproducibility over time (30).We excluded 15% of asthma cases due to missing data on exposure and/or outcomes which may lead to selection bias. Nevertheless, persons included in the analysis cohort seemed to have better serum 25(OH)D levels and better lung function which may have resulted in an underestimation of the association (online Appendix 1). Residual confounding may exist due to lack of more complete and/or precise information on doses of, and adherence to asthma medication or regular use of inhaled corticosteroids. Due to the cross-sectional design of this study, it was not possible to infer causality.

In conclusion, we found no association between serum 25(OH)D and lung function in most adults with asthma, with the exception of men with asthma but without allergic rhinitis. The observed interactions by sex and allergy status warrant further investigation and replication. Previous longitudinal work has looked at serum 25(OH)D and lung function decline in continuous smoking COPD patients (31), a prospective study on serum 25(OH)D and lung function changes in an asthma cohort or a general adult population, would be of high interest.

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Author contributions were as follows: all authors contributed to the study design; AL and XMM contributed to data collection; TLL conducted statistical analyses, interpreted results and wrote the initial draft of the manuscript; all authors participated in the data interpretation and helped to write the final draft of the manuscript.

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374 **Table 1** Baseline characteristics in an adult asthma cohort, the HUNT Study, 1995-1997

	Women n=446				Men n=314				
	No.	(%)	Mean	(SD)	No.	(%)	Mean	(SD)	P Value[1]
Age (years)			37.35	0.44			38.54	0.51	0.08
25(OH)D (nmol/L)			56.87	1.12			57.28	1.28	0.81
<50.0	195	43.72			138	43.95			0.95
≥50.0	251	56.28			176	56.05			
Body mass index (kg/m^2)			26.86	0.26			26.80	0.22	0.86
Education (years)									0.23
<10	84	18.83			70	22.29			
≥10	354	79.37			242	77.07			
Unknown	8	1.79			2	0.64			
Social benefit recipient									<0.001
Yes	166	37.22			62	19.75			
No	201	45.07			175	55.73			
Unknown	79	17.71			77	24.52			
Economic difficulties									0.94
Yes	179	40.13			117	37.26			
No	207	46.41			137	43.63			
Unknown	60	13.45			60	19.11			
Season									0.73
December-May	223	50.00			161	51.27			
June-November	223	50.00			153	48.73			
Physical activity (hrs/wk)									0.003
<1	99	22.20			95	30.25			
≥1	310	69.51			180	57.32			
Unknown	37	8.30			39	12.42			
Smoking status									0.08
Never	161	36.10			123	39.17			
Current	154	34.53			84	26.75			
Former	118	26.46			96	30.57			
Unknown	13	2.91			11	3.50			
Asthma medication (ever)									0.002
Yes	424	95.07			279	88.85			
No	22	4.93			34	10.83			
Unknown	0	0.00			1	0.32			
Inhaled corticosteroids (last 6 months)									0.06
Yes	170	38.12			99	31.53			
No	276	61.88			215	68.47			
Allergic rhinitis (ever)									0.03
Yes	270	60.54			172	54.78			
No	85	19.06			81	25.80			
Unknown	91	20.40			61	19.43			
FEV_1 % pred.			90.38	0.74			88.06	0.94	0.05
FVC % pred.			95.77	0.59			95.58	0.75	0.84
FEV_1/FVC ratio			78.40	0.39			75.01	0.53	<0.001

375
376 [1] A t-test was performed to analyze the difference between women and men for continuous variables, and a
377 chi-squared test was applied for categorical variables (missing data was excluded).
378
379 25(OH)D, 25-hydroxyvitamin D; FEV_1 % pred., forced expiratory volume in 1 second percent predicted; FVC %
380 pred., forced vital capacity percent predicted; FEV_1/FVC ratio, ratio of FEV_1 to FVC (actual); SD, standard deviation.

Table 2 Crude and adjusted regression coefficients (β) for the associations between serum 25(OH)D and lung function measures in an adult asthma cohort, the HUNT Study, 1995-1997

25(OH)D (nmol/L)	FEV_1 % pred.				FVC % pred.				FEV_1/FVC ratio			
	Crude β	95% CI	Adjusted β	95% CI	Crude β	95% CI	Adjusted β	95% CI	Crude β	95% CI	Adjusted β	95% CI
Women (n=446)												
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent
50.0-74.9	-1.92	-5.91, 2.06	-1.25	-5.27, 2.77	-0.89	-4.08, 2.30	-0.10	-3.36, 3.16	-1.35	-3.46, 0.76	-0.82	-2.89, 1.25
<50.0	-4.46	-8.29, -0.64	-2.16	-6.22, 1.90	-2.44	-5.50, 0.62	-0.30	-3.59, 2.99	-1.88	-3.91, 0.14	-1.41	-3.49, 0.67
Each 25-nmol/L reduction	-1.51	-3.04, 0.02	-0.69	-2.32, 0.95	-0.81	-2.03, 0.42	0.02	-1.30, 1.35	-0.62	-1.43, 0.19	-0.53	-1.37, 0.31
Men (n=314)												
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent
50.0-74.9	-5.66	-10.66, -0.66	-6.31	-11.39, -1.24	-3.48	-7.50, 0.54	-3.48	-7.61, 0.66	-1.96	-4.78, 0.88	-2.39	-5.18, 0.39
<50.0	-7.78	-12.58, -2.98	-8.44	-13.78, -3.11	-4.30	-8.16, -0.45	-4.17	-8.52, 0.17	-3.34	-6.06, -0.63	-4.31	-7.25, -1.38
Each 25-nmol/L reduction	-2.76	-4.77, -0.75	-3.05	-5.31, -0.79	-1.20	-2.81, 0.42	-1.21	-3.05, 0.62	-1.39	-2.53, -0.26	-1.73	-2.96, -0.49

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; FEV_1 % pred., forced expiratory volume in 1 second percent predicted; FVC % pred., forced vital capacity percent predicted; FEV_1/FVC ratio, ratio of FEV_1 to FVC (actual).

Multiple linear regression models adjusted for body mass index, education, social benefits, economic difficulties, season, physical activity, smoking status, asthma medication, inhaled corticosteroid.

Multiple linear regression models for FEV_1/FVC ratio were also adjusted for age and height.

Table 3 Crude and adjusted regression coefficients (β) for the associations between serum 25(OH)D and lung function measures stratified by allergic rhinitis in an adult asthma cohort, the HUNT Study, 1995-1997 (women only)

25(OH)D (nmol/L)	FEV_1 % pred.				FVC % pred.				FEV_1/FVC ratio			
	Crude β	95% CI	Adjusted β	95% CI	Crude β	95% CI	Adjusted β	95% CI	Crude β	95% CI	Adjusted β	95% CI
Allergic Rhinitis Yes	(*n*=270)											
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent
50.0-74.9	0.87	-3.85, 5.59	0.63	-4.16, 5.42	0.96	-3.07, 5.00	1.29	-2.87, 5.45	-0.16	-2.62, 2.31	-0.32	-2.73, 2.09
<50.0	-1.26	-5.90, 3.38	1.01	-3.80, 5.82	-1.98	-5.95, 1.98	-0.11	-4.29, 4.06	0.32	-2.10, 2.75	0.83	-1.57, 3.24
Each 25-nmol/L reduction	-0.60	-2.50, 1.31	0.47	-1.53, 2.47	-0.80	-2.43, 0.84	0.10	-1.64, 1.83	0.07	-0.93, 1.06	0.24	-0.76, 1.24
Allergic Rhinitis No	(*n*=85)											
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent
50.0-74.9	-6.41	-17.13, 4.32	-5.64	-17.09, 5.80	-4.36	-12.07, 3.35	-2.99	-11.27, 5.28	-3.21	-9.61, 3.19	-1.95	-8.25, 4.36
<50.0	-6.19	-15.73, 3.34	-0.96	-12.33, 10.41	-1.88	-8.73, 4.97	1.64	-6.58, 9.86	-4.29	-9.98, 1.41	-1.67	-7.85, 4.51
Each 25-nmol/L reduction	-2.02	-5.62, 1.58	0.17	-3.96, 4.31	-0.55	-3.15, 2.05	0.72	-2.27, 3.72	-1.40	-3.55, 0.75	-0.12	-2.36, 2.11

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; FEV_1 % pred., forced expiratory volume in 1 second percent predicted; FVC % pred., forced vital capacity percent predicted; FEV_1/FVC ratio, ratio of FEV_1 to FVC (actual).

Multiple linear regression models adjusted for body mass index, education, social benefits, economic difficulties, season, physical activity, smoking status, asthma medication, inhaled corticosteroid.

Multiple linear regression models for FEV_1/FVC ratio were also adjusted for age and height.

Table 4 Crude and adjusted regression coefficients (β) for the associations between serum 25(OH)D and lung function measures stratified by allergic rhinitis in an adult asthma cohort, the HUNT Study, 1995-1997 (men only)

25(OH)D (nmol/L)	FEV_1 % pred.				FVC % pred.				FEV_1/FVC ratio			
	Crude β	95% CI	Adjusted β	95% CI	Crude β	95% CI	Adjusted β	95% CI	Crude β	95% CI	Adjusted β	95% CI
Allergic Rhinitis Yes	(*n*=172)											
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent
50.0-74.9	-5.46	-11.44, 0.52	-5.38	-11.69, 0.94	-4.30	-9.42, 0.81	-4.14	-9.51, 1.22	-1.21	-4.72, 2.30	-1.11	-4.80, 2.57
<50.0	-8.82	-14.67, -2.97	-7.67	-14.84, -0.50	-6.02	-11.02, -1.01	-4.46	-10.56, 1.63	-2.92	-6.36, 0.52	-3.23	-7.44, 0.97
Each 25-nmol/L reduction	-3.76	-6.10, -1.42	-3.40	-6.31, -0.48	-2.36	-4.37, -0.34	-1.98	-4.47, 0.50	-1.37	-2.75, 0.01	-1.34	-3.05, 0.37
Allergic Rhinitis No	(*n*=81)											
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent	0.00	Referent
50.0-74.9	-5.58	-18.62, 7.46	-10.43	-25.10, 4.23	-1.63	-11.25, 7.99	-2.00	-13.45, 9.44	-2.12	-9.46, 5.23	-6.18	-14.01, 1.67
<50.0	-11.85	-24.15, 0.46	-17.56	-33.20, -1.93	-6.57	-15.65, 2.50	-7.91	-20.11, 4.29	-4.69	-11.62, 2.24	-8.60	-16.95, -0.25
Each 25-nmol/L reduction	-3.46	-8.80, 1.89	-5.28	-11.86, 1.31	-1.44	-5.38, 2.51	-1.37	-6.49, 3.75	-1.79	-4.77, 1.19	-3.06	-6.58, 0.45

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; FEV_1 % pred., forced expiratory volume in 1 second percent predicted; FVC % pred., forced vital capacity percent predicted; FEV_1/FVC ratio, ratio of FEV_1 to FVC (actual).

Multiple linear regression models adjusted for body mass index, education, social benefits, economic difficulties, season, physical activity, smoking status, asthma medication, inhaled corticosteroid.

Multiple linear regression models for FEV_1/FVC ratio were also adjusted for age and height.

Online Appendix 1 Baseline characteristics in analysis and missing data in an adult asthma cohort, the HUNT Study, 1995-1997

	Analysis cohort (n=760)				Missing cohort (n=138)				
	No.	(%)	Mean	(SD)	No.	(%)	Mean	(SD)	p-Value[1]
Age (years)			37.84	0.35			38.47	0.77	0.47
Sex (Men)	314	41.32			61	44.20			0.52
25(OH)D (nmol/L)			57.04	0.86			50.93	2.11	0.01
<50.0	333	43.82			56	40.58			0.01
≥50	427	56.18			42	30.43			
Unknown	0	0			40	28.99			
Body mass index (kg/m^2)			26.84	0.18			27.62	0.45	0.09
Education (years)									0.71
<10	154	20.26			26	18.84			
≥10	596	78.42			110	79.71			
Unknown	10	1.32			2	1.45			
Social benefit recipient									0.80
Yes	228	30.00			41	29.71			
No	376	49.47			64	46.38			
Unknown	156	20.53			33	23.91			
Economic difficulties									0.45
Yes	296	38.95			47	34.06			
No	344	45.26			64	46.38			
Unknown	120	15.79			27	19.57			
Season									0.14
December-May	384	50.53			77	55.78			
June-November	376	49.47			57	41.30			
Unknown	0	0			4	2.90			
Physical activity (hrs/wk)									0.88
<1	194	25.53			36	26.09			
≥1	490	64.47			88	63.77			
Unknown	76	10.00			14	10.14			
Smoking status									0.043
Never	284	37.37			40	28.99			
Current	238	31.32			54	39.13			
Former	214	28.16			29	21.01			
Unknown	24	3.16			15	10.87			
Asthma medication (ever)									0.59
Yes	703	92.50			126	91.30			
No	56	7.37			12	8.70			
Unknown	1	0.13			0	0.00			
Inhaled corticosteroids (last 6 months)									<0.001
Yes	269	35.39			16	11.59			
No	491	64.61			24	17.39			
Unknown	0	0			98	71.01			
Allergic rhinitis (ever)									0.32
Yes	442	58.16			70	50.72			
No	166	21.84			33	23.91			
Unknown	152	20.00			35	25.36			
FEV_1 % pred.			89.42	0.58			77.67	3.20	<0.001
FVC % pred.			95.69	0.46			89.74	2.37	0.01
FEV_1/FVC ratio			76.99	0.32			70.88	2.04	<0.001

[1] A t-test was performed to analyze the difference between women and men for continuous variables, and a chi-squared test was applied for categorical variables (missing data was excluded).

25(OH)D, 25-hydroxyvitamin D; FEV_1 % pred., forced expiratory volume in 1 second percent predicted; FVC % pred., forced vital capacity percent predicted; FEV_1/FVC ratio, ratio of $FEV_1$to FVC (actual); SD, standard deviation.

Online Appendix 2 Sensitivity analysis excluding ever COPD participants: Adjusted regression coefficients (β) for the associations between serum 25(OH)D and lung function measures stratified by allergic rhinitis in an adult asthma cohort, the HUNT Study, 1995-1997

25(OH)D (nmol/L)	FEV_1 % pred.		FVC % pred.		FEV_1/FVC ratio	
	Adjusted β	95% CI	Adjusted β	95% CI	Adjusted β	95% CI
Women						
Allergic Rhinitis Yes (n=233)						
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent
≤74.9	1.88	-2.78, 6.54	1.24	-2.84, 5.32	0.63	-1.67, 2.93
Allergic Rhinitis No (n=62)						
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent
≤74.9	3.29	-7.66, 14.24	1.26	-7.41, 9.94	3.50	-2.58, 9.58
Men						
Allergic Rhinitis Yes (n=145)						
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent
≤74.9	-8.45	-14.37, -2.52	-4.74	-9.81, 0.33	-3.59	-7.12, -0.06
Allergic Rhinitis No (n=65)						
≥75.0	0.00	Referent	0.00	Referent	0.00	Referent
≤74.9	-13.52	-24.18, -2.86	-5.13	-15.28, 5.01	-6.98	-12.88, -1.06

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; FEV_1 % pred., forced expiratory volume in 1 second percent predicted; FVC % pred., forced vital capacity percent predicted; FEV_1/FVC ratio, ratio of $FEV_1$to FVC (actual)

Multiple linear regression models adjusted for body mass index, education, social benefits, economic difficulties, season, physical activity, smoking status, asthma medication, inhaled corticosteroid.

Multiple linear regression models for FEV_1/FVC ratio were also adjusted for age and height.