Serum 25-hydroxyvitamin D level, smoking and lung function in adults: the HUNT Study

Tricia L. Larose*, Ben M. Brumpton, Arnulf Langhammer, Carlos A. Camargo Jr., Yue Chen, Pål Romundstad, and Xiao-Mei Mai

Correspondence to Tricia L Larose, Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway. Telephone: +47 7359 7529; Fax: +47 7359 7577 E-mail: tricia.larose@ntnu.no

25(OH)D	25-hydroxyvitamin D
ATS	American Thoracic Society
BMI	body mass index
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CV	coefficient of variation
FEV_1	forced expiratory volume in 1 second
FEV_1 % 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
HUNT	Nord-Trøndelag Health Study
IU	international units
LLN	lower limit of normal
LF	lung function
OR	odds ratio

ABSTRACT

The association between serum 25-hydroxyvitamin D (25(OH)D) level and lung function (LF) changes in the general population remains unclear.

We conducted cross-sectional (n=1,220) and follow-up (n=869) studies to investigate the interrelationship of serum 25(OH)D, smoking and LF changes in a random sample of adults from the Nord-Trøndelag Health Study (HUNT), Norway.

LF was measured by spirometry and included: forced expiratory volume in 1 second percent predicted (FEV_1 % pred.), forced vital capacity (FVC % pred.), and FEV_1/FVC ratio. Multiple linear and logistic regression models estimated the adjusted difference in LF measures or LF decline, adjusted odds ratios (OR) for impaired LF or development of impaired LF, and 95% confidence intervals (CI).

40% of adults had serum 25(OH)D level<50nmol/L. Overall, serum 25(OH)D level<50nmol/L showed worse LF, and increased odds for impaired LF compared to the ≥50nmol/L group. These associations tended to be stronger amongst ever smokers including more decline in FEV_1/FVC ratio and greater odds for development of impaired LF (FEV_1/FVC<70%: 2.4; 95%CI: 1.2-4.9). Associations amongst never smokers were null. Results from cross-sectional and follow-up studies were consistent. There were no associations between serum 25(OH)D levels and LF or LF changes in never smokers, whereas significant associations were observed in ever smokers.

INTRODUCTION

Increasing epidemiologic evidence on vitamin D and respiratory health has been reported[1]. Several cross-sectional studies have reported an association between low vitamin D status and lower lung function (LF) in a general adult population[2-4]. However, findings from prospective studies in the general population are not consistent with each other[5-7].

One Danish study by Thuesen et. al (n=4,999) found a significant cross-sectional association between low levels of serum 25-hydroxyvitamin D (25(OH)D) and a higher proportion of low forced expiratory volume in 1 second percent predicted (FEV_1 % pred.) defined as less than <80%, but a prospective association between high levels of serum 25(OH)D and adverse LF changes[7]. In contrast, a second Danish study by Afzal et. al, reported a prospective association between lower plasma 25(OH)D and more LF decline (FEV_1 % pred., forced vital capacity (FVC) % pred., but not FEV_1/FVC ratio)[5]. Results from the latter study were independently replicated in two general populations (n=10,116 and n=8,391)[5]. Finally, a smaller prospective study of elderly men (n=626) in the United States (US) observed a significant association between serum 25(OH)D level<50nmol/Land lower LF as well increased LF decline in current smokers[6]. In all three prospective studies, smoking status (daily, current or continuous vs. never) showed a tendency towards a larger effect-estimate of the association between serum 25(OH)D levels and LF changes[7], or modified the association between serum 25(OH)D level and LF decline[5, 6]. Taken together, the current state of evidence on the association between serum 25(OH)D levels and LF changes in the general population remains unclear, and the potential interrelationship of serum 25(OH)D levels, smoking and LF decline needs further investigation.

In the current study, we examined the interrelationship between serum 25(OH)D levels, smoking, LF and LF decline in a random sample of Norwegian adults (aged 19-55 years) using data from the Nord-Trøndelag Health Study (HUNT). We also estimated the odds for impaired LF and development of

impaired LF using two cut-points for FEV_1/FVC ratio (less than 70% and less than lower limit of normal (LLN)).

MATERIALS AND METHODS

Subjects and study design

HUNT is a longitudinal, population-based health study of Norwegian inhabitants at latitude 64° North. The HUNT study population consists of mostly Caucasian adults, aged 19 years or older, with socio-demographic characteristics, as well as mortality and morbidity profiles, considered generally representative of Norway[8, 9]. To date, three adult HUNT surveys are complete: HUNT1 (1984-1986), HUNT2 (1995-1997) and HUNT3 (2006-2008). The target population for HUNT2 (1995-1997) included 93,000 Norwegian adults aged 19 years and older living in Nord-Trøndelag County. The participation rate was 70%[9]. Among HUNT2 participants, approximately 57% (n=37,059) also took part in HUNT3 (2006-2008)[8].

We established a cohort of 25,616 adults aged 19-55 years at baseline who participated in both HUNT2 and HUNT3[10]. From this cohort, we selected a 10% random sample for measurement of serum 25(OH)D levels in blood samples collected during HUNT2[10]. A 5% random sample of all HUNT2 participants were selected as a sub-group for spirometry tests and followed up to HUNT3. The size of the random samples were based on available funding and capacity at spirometry stations. From these two random samples, a total of 1,293 subjects had complete data on both exposure (serum 25(OH)D) and outcome (LF) at HUNT2. Amongst whom, 922 subjects (71%) were followed-up with available LF data in HUNT3.

In the cross-sectional study, we included a total of 1,220 subjects to evaluate the interrelationship between serum 25(OH)D levels, smoking, and LF or impaired LF after missing data on smoking status was excluded (n=75, unknown for smoking). In the follow-up study, we included 869 subjects to study the interrelationship between serum 25(OH)D, smoking and LF decline or development of impaired LF after missing data on smoking status was excluded (n=53, unknown for smoking). Figure 1 shows a flow chart of the study population selection.

Serum 25(OH)D and LF measurements

Blood samples were collected in HUNT2 and stored at -70° C for later use. Serum 25(OH)D levels in HUNT2 were measured using DiaSorin Liaison 25(OH)D TOTAL assay with detection range 10-375nmol/L, intraassay coefficient of variation (CV) 4%, and interassay CV 8%. Serum 25(OH)D levels were categorised based on the most recent Institute of Medicine report (<50 nmol/L or ≥50 nmol/L)[11], or used as a continuous independent variable. As previously described[12], spirometry was performed by trained health professionals at screening stations. Instrument quality control was conducted once daily via staff LF assessment. Participants were made to sit upright and use a nose-clip[13].. Recommendations and criteria from the American Thoracic Society (ATS) were followed and applied[14]. Participants were required to give three to five acceptable and reproducible trials during which expiration continued for ≥6 seconds. The best trial was selected via identification of flow/volume curve using the highest sum of FEV_1 and FVC from all curves meeting acceptability criteria. LF measures included continuous values of FEV_1 % pred., FVC % pred. and FEV_1/FVC ratio. Reference values for calculating FEV_1 % pred., FVC % pred., and lower limit of normal (LLN), were based on the same HUNT population[13]. Variables included in prediction equations for LF parameters include age, sex, and height. Impaired LF was defined as FEV_1/FVC ratio <70% or FEV_1/FVC ratio<LLN based on prior literature and on recommendation by the ATS[15, 16].

Covariates

Data on all covariates was collected during HUNT2 in clinical examination and by questionnaire. Body weight and height were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Smoking status was categorised as never or ever (current or former). Other variables included age (continuous), sex (female or male), height (continuous), BMI (continuous), socio-economic status (SES) (high, low or unknown), season of blood sample collection (June-November or December-May), number of hours of light physical activity per week (<1, ≥1 or unknown), and pack-years (PY) of smoking as a categorical (<10 or ≥10) or continuous variable. We defined smoking status by participant's response to the following HUNT2 questions. Current smokers responded "yes" to "Do you smoke - cigarettes daily?" Former smokers respond "no" to "Do you smoke – cigarettes daily?" but did not indicate that they never smoked daily. Never smokers respond "no" to "Do you smoke –cigarettes daily?" and "yes" to "never smoked daily". In our current study, smoking status was dichotomized as never or ever where ever smokers included both current and former smokers. Participants with high SES included non-recipients of social benefits and/or persons with no economic difficulty in the last year. Low SES included recipients of social benefits and/or persons with economic difficulties in the last year. Social benefit recipients were those who reported receiving any public welfare benefits, such as sick pay/rehabilitation/retraining/unemployment/transitional benefits/retirement/widow's pension, and family supplement and/or other benefits. Participants with economic difficulty in the last year gave an affirmative response to the following question, "During the last year, has it at any time been difficult to meet the costs of food, transportation, housing and such?"

Statistical analysis

Descriptive statistics for both study populations are presented as numbers and percentages or means and standard deviations of key baseline characteristics (table 1). The statistical analyses were performed in the overall population and separately in never and ever smokers based on priori

information from the literature, and our hypothesis. Multiple linear (tables 2-3, online appendix 1) and logistic (table 4, online appendix 2) regression models were used to estimate adjusted differences in LF or LF decline, adjusted odds ratios (OR) for impaired LF or development of impaired LF, and 95% confidence intervals (CI). Analyses were conducted using serum 25(OH)D as a categorical (<50nmol/L compared to ≥50nmol/L), or continuous independent variable. All regression models included BMI, SES, season and physical activity as important covariates. Outcome measures for linear models included continuous values of FEV_1 % pred., FVC % pred., and FEV_1/FVC ratio (%)[15]. Outcome measures for logistic models included FEV_1/FVC<70% and FEV_1/FVC<LLN[15, 16]. FEV_1/FVC ratio models based on actual measurements rather than predicted equations were further adjusted for age, sex and height. To minimize possible residual confounding, we further controlled for smoking PY as a categorical (<10 or ≥10) or continuous variable amongst ever smokers. To test the robustness of our findings, we excluded subjects who reported ever having asthma or ever having chronic obstructive pulmonary disease (COPD), chronic bronchitis or emphysema, and repeated the analyses. Asthma status was determined by participant response to the following question, "Do you have or have you had asthma?" Whereas COPD was determined by participant response to the following question, "Do you have or have you had any of the following: COPD, chronic bronchitis or emphysema?" We used Stata, version 13.1 (StataCorp, College Station, Texas) for all statistical analyses.

Ethics

The Regional Committee for Medical Research Ethics granted ethics approval for this study. All participants gave informed written consent.

RESULTS

A comparison between participants in the initial (n=1293), cross-sectional (n=1220), and follow-up (n=869) study samples showed that the cross-sectional sample compared to the follow-up sample had slightly higher mean serum 25(OH)D level (58nmol/L vs. 57nmol/L), a slightly higher proportion of participants with serum 25(OH)D level ≥50nmol/L (60% vs 58%), and a higher proportion of participants with blood samples collected during summer months (44% vs 38%) at baseline (table 1). Mean LF measures, and the distribution of socio-demographic and lifestyle factors were similar in subjects from the original, cross-sectional and follow-up samples.

In the cross-sectional study, the overall adjusted difference in LF measures (table 2) revealed a significant association between serum 25(OH)D level <50nmol/L and FEV_1% pred. (-2.0, 95% CI: -3.7 to -0.4) compared to the ≥50nmol/L group. We also observed significant associations between each 25nmol/L reduction in 25(OH)D level and all three LF measures (FEV_1% pred., FVC % pred., and FEV_1/FVC ratio (%)) in the overall study population. After stratification by smoking status (table 2), the adjusted difference in FEV_1 % pred. and FVC % pred. appeared stronger amongst ever smokers with serum 25(OH)D level <50nmol/L compared to the ≥50nmol/L group (difference for FEV_1 % pred.: -3.0, 95% CI: -5.3 to -0.8; difference for FVC % pred.: -2.0, 95% CI: -4.0 to -0.1) (table 2). Serum 25(OH)D level as a continuous independent variable further supported these findings amongst ever smokers. Each 25nmol/L reduction in 25(OH)D level also showed a significant association with FEV_1/FVC ratio (%) (difference: -0.7, 95% CI: -1.2 to -0.1). However, amongst never smokers results showed non-significant associations between serum 25(OH)D level as a categorical or continuous variable in relation to all three LF measures ($P_{interaction}$= 0.03 for continuous 25(OH)D *and* smoking *for* FEV_1% pred. model).

In the follow-up study, the overall study population showed no clear associations between serum 25(OH)D levels and LF changes (table 3). After stratification by smoking status, serum 25(OH)D level <50nmol/L showed significantly more decline in FEV_1/FVC ratio (difference: 1.2, 95% CI: 0.1 to 2.2) compared to the ≥50nmol/L group in ever smokers. A sensitivity analysis that excluded subjects who reported ever asthma only (n=110), ever COPD, chronic bronchitis or emphysema only (n=10), or both ever asthma and ever COPD (n=12), provided additional evidence for significantly more decline in FEV_1/FVC ratio amongst ever smokers with serum 25(OH)D level<50nmol/L (difference: 1.3, 95% CI: 0.1 to 2.5) (online appendix 1). However, results amongst never smokers showed no associations between serum 25(OH)D level as a categorical or continuous independent variable in relation to all three LF measures.

In the follow-up study, the overall study population showed increased odds for the development of impaired LF, as measured by FEV_1/FVC ratio <70%, for both categorical (<50nmol/L group vs ≥50nmol/L group) and continuous (each 25nmol/L reduction) exposure variables (table 4). After stratification by smoking status, increased odds for the development of impaired LF remained amongst ever smokers when serum 25(OH)D level was analyzed as a categorical (OR: 2.4, 95% CI:1.2-4.9) or continuous (OR: 1.9, 95% CI: 1.2-3.0) variable (table 4). However, adjusted odds for the development of impaired LF in never smokers were null. Results from the cross-sectional study were supportive of the above findings (table 4) ($P_{interaction}$= 0.08 for categorical 25(OH)D *and* smoking *for* FEV_1/FVC ratio<70% model).

To better understand the possible impact of smoking burden on our results, we stratified by PY of smoking (<10 PY vs ≥10 PY) in the ever smoker group and further adjusted for continuous PY within each PY group to account for within-group residual confounding. We formally tested for interaction between serum 25(OH)D level as both a categorical and continuous exposure variable and PY of

smoking (<10 PY vs ≥10 PY) using the likelihood-ratio test after estimation where p<0.10 was considered statistically significant. We found no evidence for significant interaction between serum 25(OH)D and PY of smoking on lung function decline (data not presented). When LLN was used as the cut-point to define impaired LF, results showed a similar trend, although non-significant, in comparison to results from table 4 (online appendix 2).

DISCUSSION

In this general population of Norwegian adults, serum 25(OH)D levels <50nmol/L were associated with increased odds for development of impaired LF compared to the ≥50nmol/L group, after approximately 11-years of follow-up. This association appeared stronger in the ever smoker group, amongst whom more LF decline in FEV_1/FVC ratio was also observed. Associations in never smokers were null. Results from the cross-sectional and follow-up studies were consistent.

Our findings were supported by Afzal et. al who reported a prospective association between lowest plasma 25(OH)D quintile and higher decline in LF (decline in FEV_1 % predicted/year: 0.47, 95% CI: 0.38-0.56), as well as higher risk for development of COPD within two independent samples of the general population[5]. More interestingly, results from the above study were significant amongst smokers, whereas no significant associations were found amongst never smokers[5]. Our results are also consistent with those of a longitudinal cohort of elderly men in the US[6] which suggested that serum 25(OH)D level≥50nmol/L compared to <50nmol/L, may protect against lower LF and more rapid LF decline in smokers[6]. To be noted, this study was conducted amongst male only participants aged 21 to 80 years and included persons with chronic conditions such as COPD. Thus, generalizability of findings may be limited.

Serum 25(OH)D levels may influence LF or LF changes through modulation of fibroblasts in respiratory epithelial cells[17], and by mediating the contraction, inflammation and remodeling of airway smooth muscle function[18].The harmful consequences of smoking on lung health are well understood, and mechanisms by which cigarette smoke contributes to lung disease include oxidative stress and pro-inflammatory responses in lung cells[19], both of which may be modulated by vitamin D[20, 21]. Lung epithelial cells generate active vitamin D[22] and cigarette smoke extract has been

231 shown to decrease baseline conversion of inactive to active vitamin D in the lungs[23]. Low levels of
232 active vitamin D in the lungs may interfere with the regulation of prostaglandin E_2 to modulate lung
233 fibroblasts which play a critical role in lung tissue repair and modelling[24]. In addition to increased
234 local inflammation, cigarette smoke can also directly induce systemic inflammation[25] and
235 increased systemic inflammation has been linked to greater LF decline[26]. Higher serum 25(OH)D
236 levels may decrease circulating levels of cytokines to reduce systemic inflammation[27], whereas
237 attenuation of inflammation may be impaired in persons with lower serum 25(OH)D levels[28]. These
238 mechanisms may explain some of the interrelationship we observed between lower serum 25(OH)D
239 levels and adverse LF changes amongst ever smokers in our study population.

240

241 In contrast to our findings and to those of Afzal et. al[5], another prospective Danish study by
242 Thuesen et. al[7] found higher levels of serum 25(OH)D to be significantly associated with adverse LF
243 changes. Given that both Danish studies had large sample sizes derived from a general adult
244 population in Copenhagen, the prospective results in opposite directions are not easily explained.
245 Method-related differences based on different assays used for measurement of serum 25(OH)D
246 levels may be one explanation. Like our study, Afzal et. al[5] used DiaSorin radioimmunoassay for
247 serum 25(OH)D measurement, whilst Thuesen et. al[7] used liquid chromatography for serum
248 25(OH)D measurement. It has been suggested that results given by different assay methods lack
249 comparability, and that results from liquid chromatography are relatively high compared to those of
250 DiaSorin radioimmunoassay[29].

251

252 By using a standard cut-point to define airflow limitation (FEV_1/FVC ratio <70%)[30], our finding
253 amongst ever smokers suggests that lower serum 25(OH)D levels may indicate a potential risk for
254 future development of COPD. At least one prospective study reported an association between lowest
255 plasma 25(OH)D quintile and risk of COPD[5]. In addition, high doses of vitamin D supplementation

(100,000 IU every 4 weeks for 1 year) were reported to reduce COPD exacerbations in a randomized trial of participants (n=182) with moderate to very severe COPD and a history of recent exacerbations[31]. However, a longitudinal study found no association between baseline vitamin D status and rate of LF decline in slow versus rapid decliners amongst COPD patients[32]. Smoking is the main risk factor for COPD[33], and COPD is rarely reversible[34]. As such, further evidence is needed to fully elucidate the association between serum 25(OH)D levels, smoking and COPD. More evidence on vitamin D supplementation as an intervention strategy to mitigate the risk of disease onset amongst smokers or to reduce exacerbations amongst COPD patients is also needed.

Our study is one of few to investigate the association between serum 25(OH)D levels and LF changes in a general population, and also one of few studies to focus on the interrelationship between serum 25(OH)D levels, smoking and LF changes. Baseline serum 25(OH)D levels and LF were measured in a large random sample of adults. A broad range of serum 25(OH)D levels were captured and we also adjusted our models for seasonal variation. We followed up more than 70% of participants and characteristics between our cross-sectional and follow-up studies were similar (table 1). Thereby, we were able to minimize selection bias. Spirometry was quality controlled, and we based our reference values for prediction equations of spirometry on the same HUNT population. Trained health professionals objectively measured anthropometric data. We were able to control for a range of possible confounding factors which included a sensitivity analysis on smoking PY in the ever smoker group. Although residual confounding by smoking PY is not a major concern in this study, we cannot rule out the possibility that our results may be influenced by residual confounding due to unmeasured variables. A sensitivity analysis which excluded study participants who confirmed ever asthma or ever COPD status, further confirmed our results (online appendix 1). Finally, our use of LLN as an additional cut-point for development of impaired LF provided further information (online appendix 2), but the use of LLN may be more effective in studies with much larger sample sizes, a

broader age range of participants, or in diseased populations[30]. In our study, the use of LLN as a cut-point for the development of impaired LF separated study subjects into two groups with similar mean LF measures. Therefore, we were not able to discern strong statistical differences between these two groups. Still, LLN as a cut-point for impaired LF should be considered in future studies as recommended by expert committees[16, 30].

Although high intra-individual reproducibility of serum 25(OH)D levels over time has been reported[35], the use of single serum 25(OH)D levels is one limitation in our study. Regarding smoking status, although a direct measure can be obtained by measuring cotinine levels in biological fluids, most population studies, including the HUNT Study, rely on self-report. Analytical studies that measure the concordance between self-reported smoking status and measures of cotinine show a trend toward underestimation when smoking prevalence is based on self-report[36]. However, accurate estimates of the prevalence of cigarette smoking derived from self-report have also been observed[37].Regarding FVC measures, some participants may have been unable to fully exhale during trials which may have contributed to an underestimation of FVC and overestimation of FEV_1/FVC ratio. However, the change in LF was the main outcome in our study which may have minimized this measurement error. Potential COPD defined by pre- bronchodilator FEV_1/FVC ratio <70% was likely overestimated due to a lack of post-bronchodilator spirometry. Therefore, associations between low serum 25(OH)D level in ever smokers and increased odds for development of impaired LF, as it relates to potential future risk of COPD, must be interpreted with caution.

To summarize, in never smokers, our data showed no clear associations between categorical or continuous serum 25(OH)D levels and all LF measures in both cross-sectional and follow-up analysis. However, there seemed to be associations with lower serum 25(OH)D levels and more LF decline, as well as increased odds for development of impaired LF in ever smokers. These findings highlight the

306 need for continued research on serum 25(OH)D levels, smoking and LF changes in well designed
307 prospective and intervention studies.
308

309 ## ACKNOWLEDGEMENTS

310 This study was supported by the Research Council of Norway (project 201895/V50), ExtraStiftelsen
311 Helse og Rehabilitering and Landsforeningen for hjerte-og lungesyke (project 2011.2.0215), and
312 Liason Committee Central Norway Regional Health Authority – NTNU.

313

314 The Nord-Trøndelag Health Study (HUNT) is a collaboration between the HUNT Research Centre
315 (Faculty of Medicine, Norwegian University of Science and Technology), the Nord-Trøndelag County
316 Council, and the Norwegian Institute of Public Health. The authors especially thank the HUNT
317 Research Centre laboratory personnel for the measurement of serum 25(OH)D levels.

318

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

323

324

REFERENCES

1. Hughes, D.A. and R. Norton, *Vitamin D and respiratory health.* Clin Exp Immunol, 2009. **158**(1): p. 20-5.
2. Black, P.N. and R. Scragg, *Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey.* Chest, 2005. **128**(6): p. 3792-8.
3. Berry, D.J., et al., *Vitamin D status has a linear association with seasonal infections and lung function in British adults.* Br J Nutr, 2011. **106**(9): p. 1433-40.
4. Khan, S., X.M. Mai, and Y. Chen, *Plasma 25-hydroxyvitamin D associated with pulmonary function in Canadian adults with excess adiposity.* Am J Clin Nutr, 2013.
5. Afzal, S., et al., *Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease.* Thorax, 2014. **69**(1): p. 24-31.
6. Lange, N.E., et al., *Vitamin D deficiency, smoking, and lung function in the Normative Aging Study.* Am J Respir Crit Care Med, 2012. **186**(7): p. 616-21.
7. Thuesen, B.H., et al., *The association of serum 25-OH vitamin D with atopy, asthma, and lung function in a prospective study of Danish adults.* Clin Exp Allergy, 2015. **45**(1): p. 265-272.
8. Krokstad, S., et al., *Cohort Profile: The HUNT Study, Norway.* Int J Epidemiol, 2013. **42**(4): p. 968-77.
9. Holmen, J., K. Midthjell, and Ø. Kruger, *The Nord-Trøndelag Health Study 1995-97 (HUNT 2): Objectives, contents, methods and participation.* Norsk Epidemiologi, 2003. **13**: p. 19-32.
10. Mai, X.M., et al., *Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study.* Am J Epidemiol, 2012. **175**(10): p. 1029-36.
11. in *Dietary Reference Intakes for Calcium and Vitamin D*, A.C. Ross, et al., Editors. 2011: Washington (DC).
12. Larose, T.L., et al., *Serum 25-hydroxyvitamin D levels and lung function in adults with ashtma: the HUNT Study.* Eur Respir J, 2014.
13. Langhammer, A., et al., *Forced spirometry reference values for Norwegian adults: the Bronchial Obstruction in Nord-Trondelag Study.* Eur Respir J, 2001. **18**(5): p. 770-9.
14. Society, A.T., *Standardization of Spirometry - 1994 Update.* American Journal of Respiratory and Critical Care Medicine, 1995. **152**(3): p. 1107-1136.
15. Pellegrino, R., et al., *Interpretative strategies for lung function tests.* Eur Respir J, 2005. **26**(5): p. 948-68.
16. *Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society.* Am Rev Respir Dis, 1991. **144**(5): p. 1202-18.
17. Ramirez, A.M., et al., *Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells.* J Steroid Biochem Mol Biol, 2010. **118**(3): p. 142-50.
18. Banerjee, A. and R. Panettieri, Jr., *Vitamin D Modulates Airway Smooth Muscle Function*, in *Vitamin D and the Lung Mechanisms and Disease Associations*, A. Litonjua, Editor. 2012, Springer. p. 127-150.
19. Faux, S.P., et al., *The role of oxidative stress in the biological responses of lung epithelial cells to cigarette smoke.* Biomarkers, 2009. **14 Suppl 1**: p. 90-6.
20. Almerighi, C., et al., *1Alpha,25-dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in human monocytes.* Cytokine, 2009. **45**(3): p. 190-7.
21. Baeke, F., et al., *Vitamin D: modulator of the immune system.* Curr Opin Pharmacol, 2010. **10**(4): p. 482-96.
22. Hansdottir, S., et al., *Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense.* J Immunol, 2008. **181**(10): p. 7090-9.
23. Hansdottir S, M.M., Lovan N, Powers LS, Hunninghake GW., *Smoking disrupts vitamin D metabolism in the lungs.* Am J Respir Crit Care Med, 2008. **181**(A1425).

24. Liu, X., et al., *Vitamin D modulates prostaglandin E2 synthesis and degradation in human lung fibroblasts.* Am J Respir Cell Mol Biol, 2014. **50**(1): p. 40-50.
25. Bracke, K.R., et al., *Cigarette smoke-induced pulmonary inflammation and emphysema are attenuated in CCR6-deficient mice.* J Immunol, 2006. **177**(7): p. 4350-9.
26. Ahmadi-Abhari, S., et al., *Longitudinal association of C-reactive protein and lung function over 13 years: The EPIC-Norfolk study.* Am J Epidemiol, 2014. **179**(1): p. 48-56.
27. Schleithoff, S.S., et al., *Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial.* Am J Clin Nutr, 2006. **83**(4): p. 754-9.
28. Banerjee, A. and R. Panettieri, Jr., *Vitamin D modulates airway smooth muscle function in COPD.* Curr Opin Pharmacol, 2012. **12**(3): p. 266-74.
29. Carter, G.D., *Accuracy of 25-hydroxyvitamin D assays: confronting the issues.* Curr Drug Targets, 2011. **12**(1): p. 19-28.
30. Vestbo, J., et al., *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary.* Am J Respir Crit Care Med, 2013. **187**(4): p. 347-65.
31. Lehouck, A., et al., *High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial.* Ann Intern Med, 2012. **156**(2): p. 105-14.
32. Kunisaki, K.M., et al., *Vitamin D status and longitudinal lung function decline in the Lung Health Study.* Eur Respir J, 2011. **37**(2): p. 238-43.
33. Celli, B.R., W. MacNee, and A.E.T. Force, *Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper.* Eur Respir J, 2004. **23**(6): p. 932-46.
34. Raherison, C. and P.O. Girodet, *Epidemiology of COPD.* Eur Respir Rev, 2009. **18**(114): p. 213-21.
35. Sonderman, J.S., et al., *Reproducibility of serum 25-hydroxyvitamin d and vitamin D-binding protein levels over time in a prospective cohort study of black and white adults.* Am J Epidemiol, 2012. **176**(7): p. 615-21.
36. Connor Gorber, S., et al., *The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status.* Nicotine Tob Res, 2009. **11**(1): p. 12-24.
37. Wong, S.L., et al., *Assessment of validity of self-reported smoking status.* Health Rep, 2012. **23**(1): p. 47-53.

FIGURE 1 Selection of the study populations, the Nord-Trøndelag Health Study (HUNT), 1995-1997 to 2006-2008. HUNT 1 was conducted in 1984-1986, HUNT 2 in 1995-1997, and HUNT 3 in 2006-2008. (25(OH)D, 25-hydroxyvitamin D; LF, lung function).

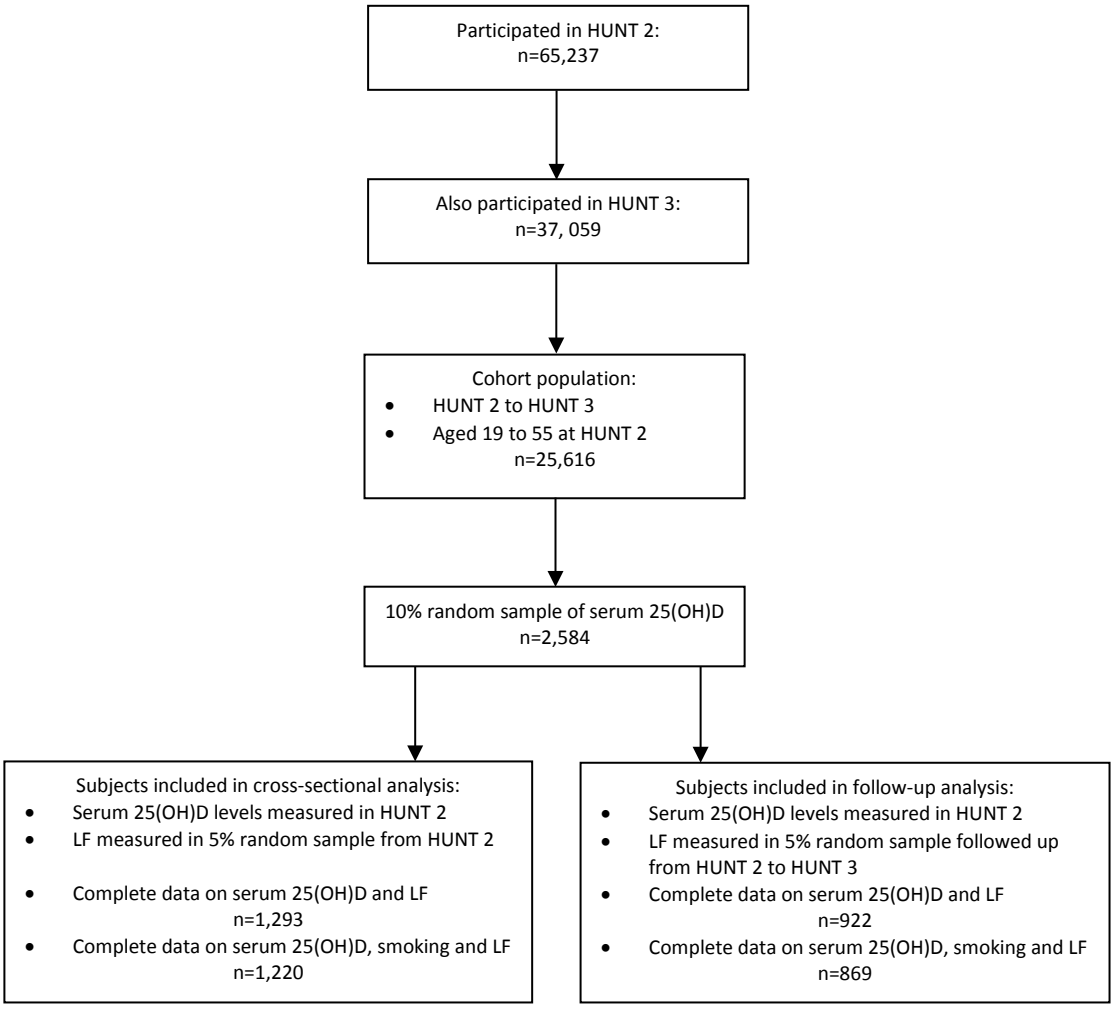

TABLE 1 Baseline characteristics in a random sample of Norwegian adults, Nord-Trøndelag Health Study, 1995-1997 to 2006-2008

	Cross-sectional study n=1,220	Follow-up study n=869
Age years	40±8.7	40±8.7
Sex		
Female	683 (56)	491 (56)
Male	537 (44)	378 (44)
25(OH)D level nmol·L^{-1}	58±23.2	57±22.4
≥50.0	734 (60)	505 (58)
<50.0	486 (40)	364 (42)
Body mass index kg·m^{-2}	26±3.7	26±3.7
Socio-economic status		
High	623 (51)	438 (50)
Low	462 (38)	330 (38)
Unknown	135 (11)	101 (12)
Season		
June-November	535 (44)	328 (38)
December-May	685 (56)	541 (62)
Physical activity h·wk		
≥1	798 (65)	569 (66)
<1	271 (22)	186 (21)
Unknown	151 (13)	114 (13)
Smoking status		
Never	525 (43)	370 (43)
Ever	695 (57)	499 (57)
FEV_1 % predicted	98±13.7	98±13.3
FVC % predicted	100±12.2	100±11.8
FEV_1/FVC ratio	0.8±0.1	0.8±0.1

Data are presented as mean±sd or n(%), unless otherwise stated. HUNT: Nord-Trøndelag Health Study; 25(OH)D: 25-hydroxyvitamin D; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity.

TABLE 2 Crude and adjusted differences in lung function measures by serum 25(OH)D levels and stratified by smoking status in a random sample of Norwegian adults, the HUNT Study, 1995-1997 (cross-sectional study)

25(OH)D (nmol/L)	N (%)	FEV_1 % pred.		FVC % pred.		FEV_1/FVC ratio (%)	
		Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Overall	1220						
<50.0*	487 (40)	-2.4 (-4.0, -0.8)	-2.0 (-3.7, -0.4)	-1.5 (-2.9, -0.1)	-1.3 (-2.8, 0.2)	-0.6 (-1.4, 0.2)	-0.6 (-1.4, 0.1)
Each 25nmol/L reduction		-1.8 (-2.6, -0.9)	-1.6 (-2.5, -0.7)	-1.1 (-1.8, -0.4)	-1.0 (-1.8, -0.2)	-0.5 (-0.9, -0.1)	-0.5 (-1.0, -0.1)
Never smoker	525						
<50.0*	185 (35)	-0.8 (-3.1, 1.5)	-0.6 (-3.2, 1.9)	-0.9 (-3.0, 1.3)	-0.6 (-2.9, 1.7)	0.1 (-0.9, 1.1)	0.1 (-1.0, 1.2)
Each 25nmol/L reduction		-0.6 (-1.8, 0.6)	-0.3 (-1.7, 1.1)	-0.4 (-1.5, 0.7)	0.0 (-1.3, 1.3)	-0.2 (-0.7, 0.4)	-0.2 (-0.8, 0.4)
Ever smoker	695						
<50.0*	302 (43)	-3.2 (-5.3, -1.1)	-3.0 (-5.3, -0.8)	-2.0 (-3.8, -0.1)	-2.0 (-4.0, -0.1)	-0.9 (-1.9, 0.2)	-0.9 (-2.0, 0.1)
Each 25nmol/L reduction		-2.4 (-3.5, -1.3)	-2.4 (-3.6, -1.2)	-1.6 (-2.6, -0.6)	-1.7 (-2.8, -0.7)	-0.5 (-1.1, 0.0)	-0.7 (-1.2, -0.1)

Data are presented as difference in lung function β(95% CI). *: Reference group was serum 25(OH)D level ≥50nmol/L; HUNT: Nord-Trøndelag Health Study; 25(OH)D: 25-hydroxyvitamin D; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity. Multiple linear regression models adjusted for body mass index, socio-economic status, season, and physical activity. Model for FEV_1/FVC ratio (%) adjusted for age, sex and height. Model for overall study population adjusted for smoking status.

TABLE 3 Crude and adjusted difference in lung function decline by serum 25(OH)D levels and stratified by smoking status in a random sample of Norwegian adults after an 11-year follow-up, the HUNT Study, 1995-1997 to 2006-2008 (follow-up study)

25(OH)D (nmol/L)	N (%)	FEV_1 % pred.		FVC % pred.		FEV_1/FVC ratio (%)	
		Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Overall	869						
<50.0*	365 (42)	0.6(-0.7, 1.9)	0.3(-1.0, 1.7)	0.3(-0.9, 1.6)	-0.4(-1.7, 1.0)	0.4(-0.4, 1.1)	0.7(-0.1, 1.5)
Each 25nmol/L reduction		0.3(-0.4, 1.0)	0.2(-0.6, 0.9)	0.4(-0.3, 1.1)	0.1(-0.7, 0.8)	0.0(-0.4, 0.4)	0.1(-0.3, 0.6)
Never smoker	370						
<50.0*	140 (38)	-0.3(-2.1, 1.4)	-1.0(-3.0, 0.9)	0.0(-1.8, 1.8)	-0.6(-2.7, 1.4)	-0.3(-1.3, 0.8)	-0.3(-1.5, 0.9)
Each 25nmol/L reduction		0.0(-1.0, 1.0)	-0.4(-1.5, 0.8)	0.4(-0.6, 1.5)	0.2(-1.0, 1.4)	-0.4(-1.0, 0.2)	-0.5(-1.2, 0.2)
Ever smoker	499						
<50.0*	225 (45)	1.0(-0.8, 2.7)	0.8(-1.0, 2.7)	0.4(-1.3, 2.1)	-0.4(-2.2, 1.4)	0.7(-0.3, 1.7)	1.2(0.1, 2.2)
Each 25nmol/L reduction		0.4(-0.6, 1.3)	0.3(-0.7, 1.3)	0.3(-0.6, 1.2)	-0.1(-1.1, 0.9)	0.2(-0.4, 0.7)	0.4(-0.2, 0.9)

Data are presented as difference in lung function decline β(95% CI). *: Reference group was serum 25(OH)D level ≥50nmol/L ; HUNT: Nord-Trøndelag Health Study; 25(OH)D: 25-hydroxyvitamin D; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity. Multiple linear regression models adjusted for body mass index, socio-economic status, season, and physical activity. Model for FEV_1/FVC ratio (%) adjusted for age, sex, and height. A negative coefficient indicates less decline in lung function. A positive coefficient indicates more decline in lung function. Model for overall study population adjusted for smoking status.

TABLE 4 Crude and adjusted odds ratios (OR) for the associations between serum 25(OH)D levels and impaired lung function or development of impaired lung function and stratified by smoking status in a random sample of Norwegian adults, the HUNT Study, 1996-1997 to 2006-2008

25(OH)D (nmol/L)	N(%)	FEV_1/FVC ratio <70% cross-sectional study n=1,220		N (%)	FEV_1/FVC ratio <70% follow-up study n=869	
		Crude	Adjusted		Crude	Adjusted
Overall	1220			816[¶]		
<50.0*	487 (40)	0.9 (0.6, 1.5)	1.0 (0.6, 1.6)	342 (42)	1.9 (1.0, 3.3)	2.2 (1.2, 4.2)
Each 25nmol/L reduction		1.2 (0.9, 1.6)	1.3 (1.0, 1.8)		1.6 (1.1, 2.3)	1.8 (1.2, 2.7)
Never smoker	525			352[¶]		
<50.0*	185 (35)	0.4 (0.2, 1.2)	0.5 (0.2, 1.5)	135 (38)	1.3 (0.3, 4.9)	0.9 (0.2, 3.9)
Each 25nmol/L reduction		0.9 (0.6, 1.4)	1.1 (0.6, 1.8)		1.0 (0.5, 2.2)	0.7 (0.3, 1.8)
Ever smoker				464[¶]		
<50.0*	695	1.2 (0.7, 2.1)	1.2 (0.6, 2.2)	207 (45)	1.9 (1.0, 3.7)	2.4 (1.2, 4.9)
Each 25nmol/L reduction	302 (43)	1.4 (1.0, 1.9)	1.4 (1.0, 2.0)		1.7 (1.1, 2.6)	1.9 (1.2, 3.0)

Data are presented as OR(95% CI). *: Reference category was serum 25(OH)D level ≥50nmol/L; [¶]: Subjects with FEV_1/FVC ratio<70% at baseline were excluded from longitudinal analysis; HUNT: Nord-Trøndelag Health Study; 25(OH)D: 25-hydroxyvitamin D; CI, confidence interval; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity. Logistic regression models for FEV_1/FVC ratio <70% adjusted for body mass index, socio-economic status, season, physical activity, age, sex, and height. Model for overall study population adjusted for smoking status.

ONLINE APPENDIX 1 Sensitivity analysis of Table 3 excluding ever asthma or ever COPD patients: adjusted difference in lung function decline by serum 25(OH)D levels and stratified by smoking status in a random sample of Norwegian adults after an 11-year follow-up, the HUNT Study, 1995-1997 to 2006-2008 (follow-up study)

25(OH)D (nmol/L)	N (%)	FEV_1 % pred.	FVC % pred.	FEV_1/FVC ratio (%)
		Adjusted	Adjusted	Adjusted
Overall	737			
<50.0*	309 (42)	0.4 (-1.1, 1.8)	-0.2 (-1.7, 1.2)	0.5 (-0.4, 1.4)
Each 25nmol/L reduction		0.2 (-0.6, 1.0)	0.1 (-0.7, 0.9)	0.1 (-0.4, 0.5)
Never smoker	326			
<50.0*	125 (38)	-1.4 (-3.4, 0.7)	-0.5 (-2.7, 1.7)	-0.8 (-2.0, 0.5)
Each 25nmol/L reduction		-0.4 (-1.6, 0.8)	0.2 (-1.1, 1.5)	-0.6 (-1.3, 0.2)
Ever smoker	411			
<50.0*	184 (45)	1.2 (-0.7, 3.2)	-0.3 (-2.2, 1.1)	1.3 (0.1, 2.5)
Each 25nmol/L reduction		0.4 (-0.7, 1.4)	-0.1 (-1.1, 0.9)	0.4 (-0.3, 1.0)

Data are presented as difference in lung function decline β (95% CI). *: Reference category was serum 25(OH)D level ≥50nmol/L; COPD: chronic obstructive pulmonary disease; HUNT: Nord-Trøndelag Health Study; 25(OH)D: 25-hydroxyvitamin D; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity. Multiple linear regression models adjusted for body mass index, socio-economic status, season, and physical activity. Model for FEV_1/FVC ratio (%) adjusted for age, sex, and height. A negative coefficient indicates less decline in lung function. A positive coefficient indicates more decline in lung function. Model for overall study population adjusted for smoking status. Subjects with ever asthma or ever COPD were excluded from analysis (n=132)

ONLINE APPENDIX 2 Crude and adjusted odds ratios (OR) for the associations between serum 25(OH)D levels and impaired lung function or development of impaired lung function using lower limit of normal (LLN) and stratified by smoking status in a random sample of Norwegian adults after an 11-year follow-up, the HUNT Study, 1995-1997 to 2006-2008

25(OH)D (nmol/L)	N(%)	FEV_1/FVC ratio <LLN cross-sectional study n=1,220		N (%)	FEV_1/FVC ratio <LLN follow-up study n=869	
		Crude	Adjusted		Crude	Adjusted
Overall	1220			707[¶]		
<50.0*	487 (40)	1.0 (0.7-1.3)	0.9 (0.7-1.3)	296 (42)	1.1 (0.6-1.9)	1.2 (0.7-2.1)
Each 25nmol/L reduction		1.0 (0.9-1.2)	1.0 (0.8-1.2)		1.1 (0.8-1.5)	1.1 (0.8-1.6)
Never smoker	525			308[¶]		
<50.0*	185 (35)	0.8 (0.5-1.3)	0.8 (0.5-1.4)	121 (39)	0.6 (0.2-1.8)	0.4 (0.1-1.2)
Each 25nmol/L reduction		0.6 (0.7-1.1)	0.9 (0.7-1.1)		0.8 (0.5-1.4)	0.6 (0.3-1.1)
Ever smoker	695			399[¶]		
<50.0*	302 (43)	1.1 (0.8-1.6)	1.0 (0.6-1.4)	175 (44)	1.3 (0.7-2.5)	1.7 (0.9-3.5)
Each 25nmol/L reduction		1.2 (1.0-1.4)	1.1 (0.9-1.4)		1.2 (0.8-1.8)	1.4 (0.9-2.0)

Data are presented as OR (95% CI). *: Reference category was serum 25(OH)D level ≥50nmol/L; [¶]: Subjects with FEV_1/FVC ratio<LLN at baseline were excluded from longitudinal analysis; HUNT: Nord-Trøndelag Health Study; 25(OH)D, 25-hydroxyvitamin D; LLN: lower limit of normal; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity. Logistic regression models for FEV_1/FVC ratio <LLN adjusted for body mass index, socio-economic status, season, and physical activity. Model for overall study population adjusted for smoking status.