

ORIGINAL ARTICLE

Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease

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ABSTRACT

Background 25-hydroxyvitamin D (25(OH)D) may be associated with lung function through modulation of pulmonary protease-antiprotease imbalance, airway inflammation, lung remodelling and oxidative stress. We examined the association of plasma 25(OH)D levels with lung function, lung function decline and risk of chronic obstructive pulmonary disease (COPD).

Methods Plasma 25(OH)D was measured in 10 116 participants in the Copenhagen City Heart Study and in 8391 participants in the Copenhagen General Population Study. In the former study, up to three measurements of lung function spanning 20 years allowed analyses of lung function decline.

Results In both cohorts, forced vital capacity in % of predicted was 7% lower and forced expiratory volume in 1 s in % of predicted was 7–10% lower for lowest versus highest decile of 25(OH)D ($p_{\text{trend}} \leq 1 \times 10^{-28}$). In prospective analyses, participants in the lower versus higher 25(OH)D quintiles had a faster decline in forced expiratory volume in 1 s % predicted

($p_{\text{interaction}} = 1 \times 10^{-7}$) and forced vital capacity % predicted ($p_{\text{interaction}} = 8 \times 10^{-8}$). In cross-sectional analyses, multivariable adjusted ORs for COPD were 2.30 (95% CI 1.55 to 3.41) and 3.06 (1.97 to 4.76) for lowest versus highest quintile in the Copenhagen City Heart Study using Global Initiative for Chronic Obstructive Lung Disease (GOLD) and lower limit of normal criteria. The corresponding ORs were 1.82 (1.13 to 2.92) and 2.23 (1.35 to 3.69) in the Copenhagen General Population Study. In prospective analyses, corresponding multivariable adjusted HRs for developing COPD were 1.58 (1.05 to 2.40) and 2.00 (1.19 to 3.36).

Conclusions We observed a novel association of lower plasma 25(OH)D levels with faster decline in lung function and with a higher risk of COPD in prospective analyses.

INTRODUCTION

The most important pathogenic mechanisms involved in development of chronic obstructive pulmonary disease (COPD) are protease-antiprotease imbalance, inflammation, lung remodelling and oxidative stress.^{1–8} Interestingly, lower vitamin D levels have been related to regulation of each of these processes, that is, higher expression of proteases, modulation of inflammation, modulation of extracellular matrix turnover, and increased oxidative stress as reviewed elsewhere.^{9–13} Thus, one can speculate that lower vitamin D levels may be linked to increased risk of developing COPD.

Key messages

What is the key question?

- Do low vitamin D levels associate with lung function decline and future risk of chronic obstructive pulmonary disease (COPD).

What is the bottom line?

- Low plasma levels of 25-hydroxyvitamin D were associated with faster lung function decline and lower lung function. Furthermore, low plasma levels of 25-hydroxyvitamin D were also associated with higher risk of COPD in two separate large general population studies.

Why read on?

- This is the first study to show that low plasma levels of 25-hydroxyvitamin D are associated with faster lung function decline and future risk of COPD in the general population.

In some clinical studies, lower levels of vitamin D, measured as plasma 25-hydroxyvitamin D (25(OH)D), have been associated with lower lung function and faster lung function decline, but the results have been conflicting.^{14–17}

We tested the hypothesis that lower plasma 25(OH)D levels associate with lower lung function, faster lung function decline and increased risk of COPD. For this purpose, we studied 10 116 white individuals from the Copenhagen City Heart Study followed for up to 20 years, and 8391 white individuals from the Copenhagen General Population Study.

METHODS

Study design

The Copenhagen City Heart Study is a prospective cohort study of the Danish general population initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994 and 2001–2003.^{4 18} Individuals 20–100 years of age were drawn randomly from the national Danish Central Person Register to reflect the Danish general population and invited to participate. The present study included 10 116 participants from the 1981–1983 examination (70% participation rate), who had plasma samples available for 25(OH)D measurement and had valid lung function measurements.

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The Copenhagen General Population Study is a general population study initiated in 2003 with ongoing enrolment (participation rate 45%), recruited as the Copenhagen City Heart Study.¹⁸ The present study included 8391 participants from July 2004 to May 2005, who had plasma samples available for 25(OH)D measurement and had valid lung function measurements.

The Copenhagen City Heart Study and the Copenhagen General Population Study were approved by Danish ethical committees and Herlev Hospital. All participants gave written informed consent.

Measurements of 25-hydroxyvitamin D

Copenhagen City Heart Study plasma samples were collected in 1981–1983 and Copenhagen General Population Study plasma samples were collected in 2004–2005, and were stored at -20°C and -80°C , respectively, until 2009–2010 when 25(OH)D was measured using the DiaSorin Liaison 25(OH)D TOTAL assay.¹⁹ Assay precision was tested daily, while assay accuracy was tested monthly using an external quality control programme. The inter-assay coefficient of variance was 10% for low level controls (~ 40 nmol/L) and 8% for high level controls (~ 135 nmol/L).

Spirometry

In the Copenhagen City Heart Study, forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were determined using an electronic spirometer (Monaghan N 403; Monaghan, Littleton, Colorado, USA) at the baseline examination in 1981–1983 and with a dry wedge spirometer (Vitalograph; Maids Moreton,

Buckinghamshire, UK) at the 1991–1994 and 2001–2003 examinations. In the Copenhagen General Population Study FEV₁ and FVC were determined using the dry wedge spirometer.

Instruments were calibrated daily against a 1 L syringe. Each spirometry was performed in triplicate, and results were only accepted if variation between the two best-performing of these was less than 5%. Algorithms for calculation of FEV₁ % predicted and FVC % predicted were made using multiple regression with age and height as covariates on a subsample of never-smokers for men and women separately.

Covariates

Information on covariates was obtained from self-reported questionnaires reviewed together with an examiner at the day of attendance. Daily tobacco consumption (grams per day) was calculated for current smokers. A substantial proportion of smokers used other types of tobacco than cigarettes. Previous analyses of lung function decline showed that the approach of converting cigarettes, cigars, cheroots and pipe into gram of tobacco was useful as all types of smoking were associated with an increased decline of FEV₁ and FVC.²⁰ Cumulative tobacco consumption was calculated for former and current smokers in pack years; a pack year was defined as 20 g of tobacco per day for a year. Participants were also asked about duration and intensity of daily physical activities, and about their level of income. Body mass index (BMI) was calculated as measured weight (kilograms) divided by measured height (meters) squared.

Table 1 Baseline characteristics according to seasonally adjusted plasma 25-hydroxyvitamin D quintiles for the Copenhagen City Heart Study and the Copenhagen General Population Study

	Seasonally adjusted plasma 25-hydroxyvitamin D quintiles					Trend, p value*
	1st (lowest)	2nd	3rd	4th	5th (highest)	
Copenhagen City Heart Study	n=2024	n=2024	n=2022	n=2023	n=2023	
Men, %	46	45	43	44	41	0.001
Age, years	59 (50–65)	59 (50–65)	58 (49–65)	58 (48–65)	57 (47–64)	<0.001
Ever smokers, %	85	81	79	79	77	<0.001
Current smokers, %	69	61	56	52	52	<0.001
Former smokers, %	16	20	23	27	26	<0.001
Current tobacco consumption†, g/day	12 (8–16)	12 (8–16)	12 (8–16)	12 (8–16)	12 (8–16)	<0.001
Cumulative tobacco consumption, pack-years	24 (11–34)	20 (6–35)	19 (4–32)	18 (3–32)	17 (2–32)	<0.001
Body mass index, kg/m ²	25 (23–29)	25 (23–28)	25 (23–28)	25 (22–27)	24 (22–26)	<0.001
High physical activity, %	31	34	35	38	36	<0.001
High income‡, %	16	19	23	25	27	<0.001
Plasma 25-hydroxyvitamin D, nmol/L	16 (13–19)	29 (26–32)	41 (38–44)	54 (50–58)	75 (68–87)	<0.001
Copenhagen General Population Study	n=1679	n=1678	n=1678	n=1678	n=1678	
Men, %	50	53	48	42	42	<0.001
Age, years	55 (47–64)	55 (46–66)	56 (47–66)	57 (47–66)	55 (45–65)	0.44
Ever smokers, %	71	65	61	60	60	<0.001
Current smokers, %	42	28	24	23	23	<0.001
Former smokers, %	29	37	37	38	37	<0.001
Current tobacco consumption†, g/day	16 (10–18)	12 (8–16)	12 (6–16)	12 (6–16)	12 (8–16)	<0.001
Cumulative tobacco consumption, pack-years	13 (0–32)	6 (0–24)	3 (0–21)	4 (0–20)	3 (0–19)	<0.001
Body mass index, kg/m ²	27 (24–30)	26 (24–29)	26 (23–28)	25 (23–28)	24 (22–27)	<0.001
High physical activity, %	30	34	32	33	38	<0.001
High income‡, %	6	12	12	12	12	<0.001
Plasma 25-hydroxyvitamin D, nmol/L	20 (15–26)	33 (28–40)	43 (39–50)	55 (49–61)	76 (67–87)	<0.001

Note that continuous variables are summarised as median and IQR.

*p Values were calculated using Cuzick's non-parametric trend test.

†Summarised for current smokers only, not the whole population.

‡High income was defined differently in the two cohorts corresponding to approximately top 20% in Copenhagen City Heart Study and top 10% in the Copenhagen General Population Study.

Endpoints

In addition to analysis of a possible association of FEV₁ and FVC with plasma 25(OH)D, we also tested the association of plasma 25(OH)D levels with spirometric diagnosis of COPD

defined in two ways: (1) by the GOLD criteria demanding the presence of FEV₁/FVC < 0.7 and (2) using the lower limit of normal (LLN) definition for FEV₁/FVC calculated as the difference between the predicted value and 1.645 times the SE of the

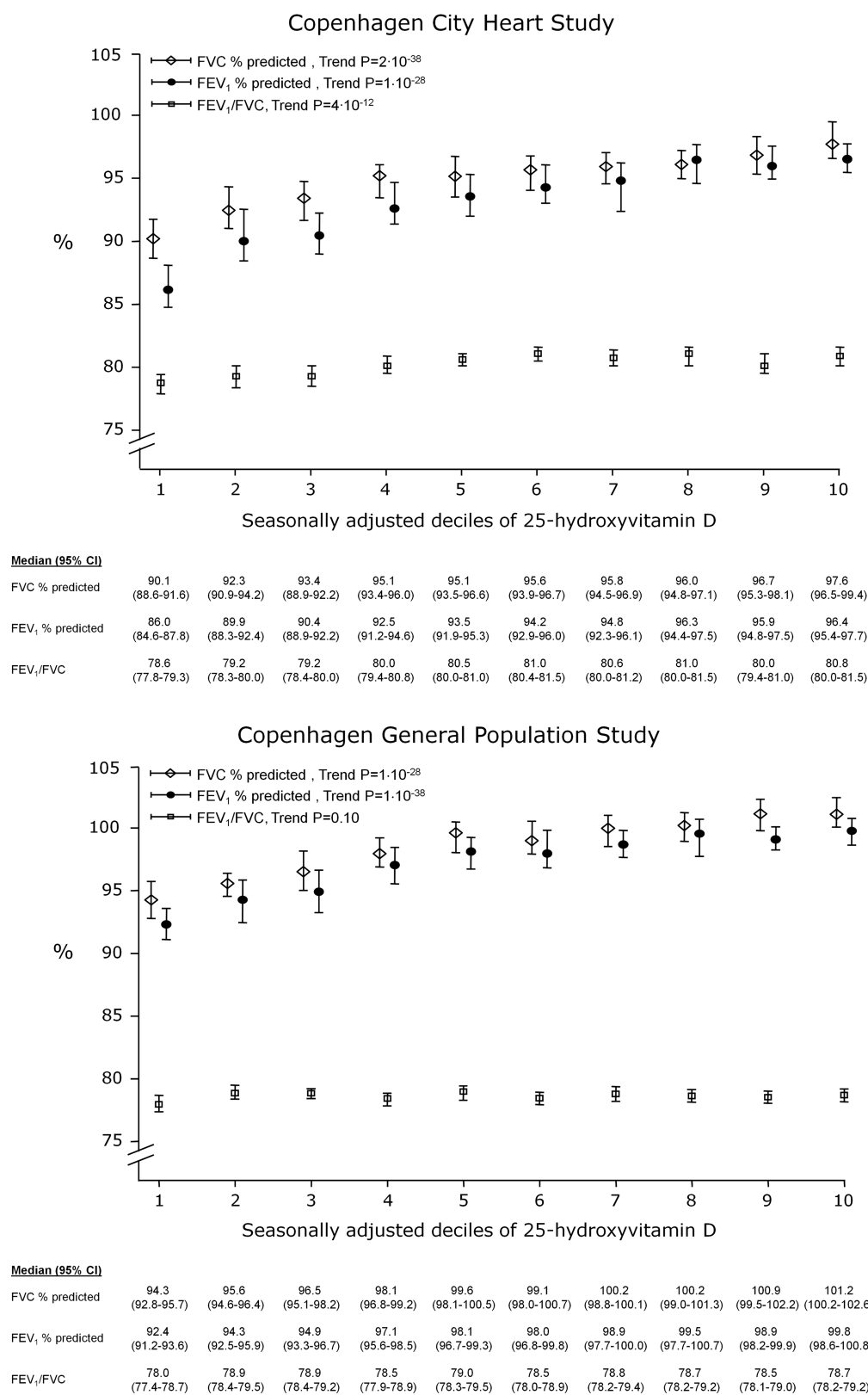


Figure 1 Association of FEV₁ % predicted, FVC % predicted and FEV₁/FVC with seasonally adjusted plasma 25-hydroxyvitamin D deciles. Based on 10 166 participants from the Copenhagen City Heart Study and 8391 participants from the Copenhagen General Population Study. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

estimate separately for men and women. The associations were evaluated cross-sectionally between spirometrically derived end-points in both populations. In the Copenhagen City Heart Study, we had up to three sets of spirometry measurements on each individual during a follow-up of up to 20 years. Hence, we also tested the association of baseline plasma 25(OH)D levels with lung function decline and prospective development of spirometrically defined COPD in this population. Participants with spirometrically defined COPD at baseline were excluded from the prospective analyses.

Statistical analysis

The levels of 25(OH)D was expected to vary according to time of year due to the high latitude geographical position of Denmark. Therefore, we used seasonally adjusted 25(OH)D percentiles in our analyses. We obtained season based percentiles for each population separately using locally weighted regression.²¹

The association of lung function with seasonally adjusted plasma 25(OH)D levels was evaluated using deciles, whereas to maximise statistical power, quintiles were used in the analyses concerning decline in lung function and risk of COPD. The cross-sectional association of FEV₁ % predicted, FVC % predicted and FEV₁/FVC with seasonally adjusted plasma 25(OH)D deciles was carried out using Cuzick's non-parametric trend test. We used quantile regression to test for interactions.

Triple measures of lung function were not available for all participants in the Copenhagen City Heart Study (26% had three measurements, 32% had two measurements, and 41% had one measurement), so lung function was analysed in a repeated measures linear mixed model. The number of examinations at the Copenhagen City Heart Study specified the repeated measurements of lung function; an unstructured covariance type for residuals was used as it places no restriction on structure. Identity of each participant was used as a random effect. The interaction term between plasma 25(OH)D percentiles and age was used to test the association of baseline plasma 25(OH)D percentile with prospective change in lung function. Sensitivity analyses using height adjusted FEV₁ and FVC were carried out in similar models.²² Model estimates were adjusted for age, sex, height, cumulative tobacco consumption and use of different spirometers between examinations.

Cross-sectional analyses concerning risk of COPD with decreasing levels of seasonally adjusted plasma 25(OH)D were

carried out using logistic regression. Cox proportional hazards regression was used for prospective analyses with age as time scale with delayed entry (left truncation). Thus, age differences were automatically adjusted for and referred to in text, tables and figures as age adjusted. Both models included the following covariates that were forced into the models: gender, smoking status (never or ever smoker), cumulative tobacco consumption (pack years), BMI, level of daily physical activity and level of income. Furthermore, age was also included as a variable in logistic regression analyses.

Relative risk estimates and CIs were corrected for regression dilution bias,²³ using plasma 25(OH)D from 400 individuals without COPD, cancer, cardiovascular disease and other chronic diseases participating in the 1981–1983, 1991–1994 and 2001–2003 examinations of the Copenhagen City Heart Study; the regression dilution ratio was 0.45.²⁴

The data was 99.8% complete in the Copenhagen City Heart Study and 99.3% complete in the Copenhagen General Population Study in relation to the covariates (see online supplementary table S1); the missing data were imputed using multivariable chained imputation, where age and gender were independent variables and variables with missing observations were dependent variables in the model. We analysed the data with the statistical package Stata V12.1 (College Station, Texas, USA).

RESULTS

Table 1 summarises baseline characteristics by plasma 25(OH)D levels in both cohorts. Lower levels of 25(OH)D were associated with male gender, current smoking, higher current tobacco consumption, higher cumulative tobacco consumption, higher BMI, lower physical activity and lower income. There was seasonal variation across the months of the year with high levels in August–September and low levels in January–February (see online supplementary table S2). The median 25(OH)D concentration was 41 nmol/L (IQR: 26–58 nmol/L) in the Copenhagen City Heart Study and 44 nmol/L (30–60 nmol/L) in the Copenhagen General Population Study.

Lung function: cross-sectional analyses

In both cohorts, FVC % predicted was 7% lower and FEV₁ % predicted was 7–10% lower for lowest versus highest decile of 25(OH)D ($P_{\text{trend}} \leq 1 \times 10^{-28}$) (figure 1). However, the association of plasma 25(OH)D with FEV₁/FVC was inconsistent. Similar results were seen when stratifying the populations into never

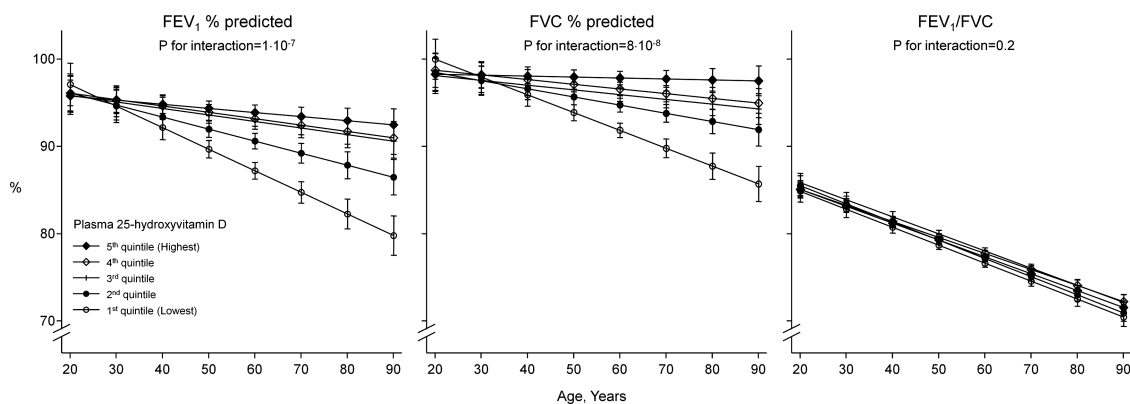


Figure 2 Relationship between age-related changes in FEV₁ % predicted, FVC % predicted, and FEV₁/FVC according to quintiles of seasonally adjusted plasma 25-hydroxyvitamin D. Adjusted for cumulative tobacco consumption updated at each examination. Based on one to three spirometries spanning up to 20 years in each of 10 166 participants from the Copenhagen City Heart Study. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

smokers, smokers who have smoked up to 20 pack years and smokers having smoked more than 20 pack years (see online supplementary figure S1). Tests for interaction showed a significant interaction of plasma 25(OH)D with cumulative tobacco consumption on FEV₁ % predicted (Copenhagen City Heart Study: $p=0.001$ and Copenhagen General Population Study: $p=0.009$), whereas the tests regarding FVC % predicted and FEV₁/FVC were non-significant. The results suggest that the association of 25(OH)D with FEV₁ % predicted is stronger with increasing levels of cumulative tobacco consumption.

Separate analyses for the subgroup of participants with spirometrically defined COPD at the baseline examination showed that FVC % predicted and FEV₁ % predicted were both 12% lower for lowest versus highest quintile of 25(OH)D in the pooled cohort (see online supplementary figure S2). Lower plasma 25(OH)D also weakly associated with lower FEV₁/FVC in participants with spirometrically defined COPD with FEV₁/FVC being 2% lower for lowest versus highest quintile of 25(OH)D. Furthermore, tests for interaction showed a significant interaction of plasma 25(OH)D with spirometrically defined COPD on FEV₁ % predicted ($p=2\times 10^{-6}$), FVC % predicted ($p=1\times 10^{-7}$) and FEV₁/FVC ($p=0.03$). The results suggest that the association is stronger in the subpopulation with COPD compared with the remaining population.

Lung function: prospective analysis

Prospective analysis of change in lung function in the Copenhagen City Heart Study over time showed a significant interaction of age with quintiles of plasma 25(OH)D levels on course of FEV₁ % predicted ($p=1\times 10^{-7}$) and FVC % predicted ($p=1\times 10^{-7}$) (figure 2). Participants in the lowest quintile of seasonally adjusted plasma 25(OH)D had a faster decline in lung function compared with participants in the highest quintile. Based on longitudinal models, average FEV₁ % predicted for participants of age 60 years and adjusted for cumulative tobacco consumption was 87% (95% CI 86% to 88%) in the lowest quintile compared with 94% (93% to 95%) in the highest quintile. The corresponding values for FVC % predicted were 92% (91% to 93%) and 98% (97% to 98%). The decline in FEV₁/FVC was similar for all five groups. The analyses were adjusted for cumulative tobacco consumption updated at the follow-up examinations.

Sensitivity analyses using height adjusted FEV₁ and FVC as the dependent variables showed similar results with a greater decline in lung function in the lowest quintile of seasonally adjusted plasma 25(OH)D compared with the highest quintile (see online supplementary figure S3). Based on the longitudinal models, for male participants of age 60 years, height 175 cm, and adjusted for cumulative tobacco consumption average FEV₁ was 2.79 L (95% CI 2.76 to 2.83) in the lowest quintile compared with 3.00 L (2.97 to 3.03) in the highest quintile. The corresponding values for FVC were 3.88 L (3.85 to 3.92) and 3.65 L (3.62 to 3.69).

Using data only from participants with more than one lung function measurement showed a similar trend, that is, higher decline in lung function within the lowest quintile of plasma 25(OH)D, when looking at all participants and at continuous smokers (table 2, see online supplementary figure S4). However, the results were not significant for never smokers or participants who quit smoking during follow-up. Analyses stratified according to spirometrically defined COPD at baseline showed that patterns of lung function decline were similar in both groups: in the lowest quintile of vitamin D, the decline of FEV₁ and FVC

Table 2 Decline in lung function in participants with more than one spirometric measurement using a non-parametric trend test

Smoking history	Lung function	Seasonally adjusted plasma 25-hydroxyvitamin D quintiles					P for trend
		1st (lowest)	2nd	3rd	4th	5th (highest)	
All participants (N=5819)	Decline in FEV ₁ % predicted/year	0.47 (0.38 to 0.56)	0.29 (0.23 to 0.27)	0.18 (0.11 to 0.26)	0.24 (0.19 to 0.33)	0.19 (0.13 to 0.28)	2×10^{-5}
	Decline in FVC % predicted/year	0.30 (0.18 to 0.38)	0.16 (0.05 to 0.22)	0.06 (0.00 to 0.14)	0.14 (0.08 to 0.20)	0.09 (0.02 to 0.16)	4×10^{-4}
Continuous smokers (N=2250)	Decline in FEV ₁ % predicted/year	0.69 (0.54 to 0.82)	0.61 (0.41 to 0.71)	0.32 (0.18 to 0.45)	0.59 (0.44 to 0.71)	0.43 (0.32 to 0.55)	0.02
	Decline in FVC % predicted/year	0.46 (0.33 to 0.56)	0.21 (0.12 to 0.31)	0.12 (0.00 to 0.24)	0.24 (0.11 to 0.40)	0.16 (0.02 to 0.24)	0.003
Quitters (N=2295)	Decline in FEV ₁ % predicted/year	0.26 (0.09 to 0.40)	0.22 (0.11 to 0.31)	0.14 (0.03 to 0.30)	0.19 (0.09 to 0.28)	0.13 (0.06 to 0.28)	0.4
	Decline in FVC % predicted/year	0.12 (0.02 to 0.29)	0.13 (−0.01 to 0.24)	0.02 (−0.07 to 0.16)	0.11 (0.02 to 0.21)	0.13 (0.02 to 0.22)	0.7
Never smokers (N=1274)	Decline in FEV ₁ % predicted/year	0.14 (−0.44 to 0.18)	0.06 (−0.24 to 0.08)	0.02 (−0.20 to 0.12)	0.06 (−0.18 to 0.12)	0.00 (−0.10 to 0.09)	0.3
	Decline in FVC % predicted/year	0.08 (−0.12 to 0.37)	−0.02 (−0.16 to 0.26)	0.06 (−0.05 to 0.15)	0.06 (−0.08 to 0.19)	0.00 (−0.09 to 0.11)	0.2

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

was faster than among individuals with higher vitamin levels (see online supplementary figure S5). However in the COPD group, only the association between decline in FEV₁ % predicted and vitamin D reached statistical significance.

Risk of spirometrically defined COPD

In the cross-sectional analysis, multivariable adjusted ORs for COPD defined by spirometry using GOLD criteria or LLN criteria increased with decreasing levels of 25(OH)D (figure 3).

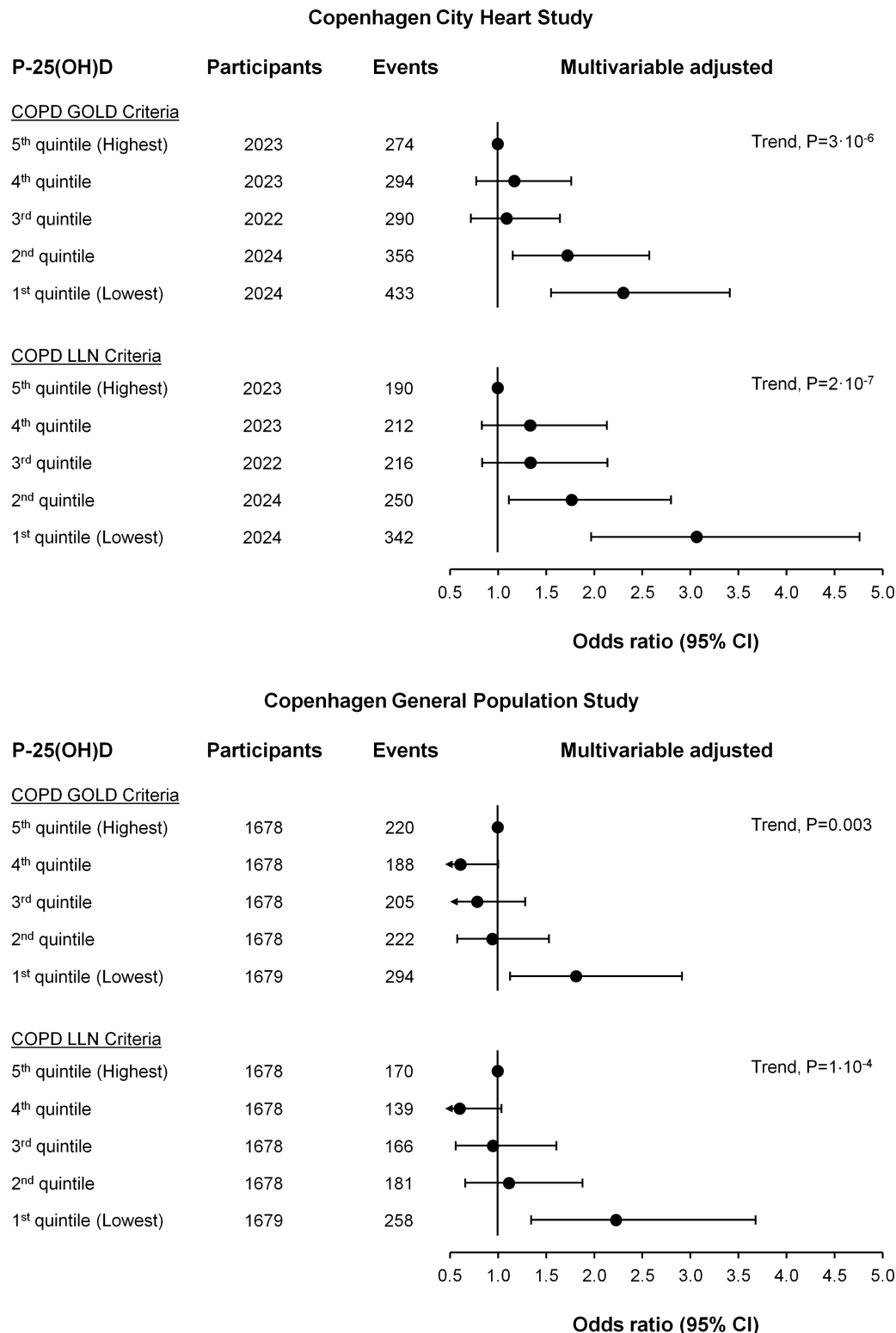


Figure 3 ORs for chronic obstructive pulmonary disease (COPD) by seasonally adjusted plasma 25-hydroxyvitamin D (25(OH)D) quintiles. Models were adjusted for gender, age, smoking status, cumulative tobacco consumption, body mass index, physical activity and income. Based on 10 166 participants from the Copenhagen City Heart Study and 8391 participants from the Copenhagen General Population Study.

The ORs for lowest versus highest quintile were 2.30 (95% CI 1.55 to 3.41) and 3.06 (1.97 to 4.76) in the Copenhagen City Heart Study for GOLD and LLN criteria, respectively. The corresponding ORs were 1.82 (1.13 to 2.92) and 2.23 (1.35 to 3.69) in the Copenhagen General Population Study.

In the Copenhagen City Heart Study many participants had follow-up spirometry carried out at 10-year (N=5819) and 20-year (N=2631) intervals, which were used for prospective development of COPD as a function of baseline 25(OH)D levels (figure 4). Longitudinal analyses restricted to these participants and excluding participants with spirometrically defined COPD at baseline comprised 5341 and 5527 individuals with at least two measurements for analyses when using GOLD criteria and LLN criteria, respectively. Multivariable adjusted HRs for COPD for lowest versus highest quintile were 1.58 (1.05 to 2.40) and 2.00 (1.19 to 3.36) for GOLD and LLN criteria, respectively.

DISCUSSION

In this study based on two independent samples of the general population, we present two novel findings showing that lower levels of plasma 25(OH)D were associated with a higher decline in lung function and with a higher risk of spirometrically defined COPD in prospective analyses. Furthermore, lower levels of plasma 25(OH)D were associated with lower FEV₁ and FVC and a higher risk of spirometrically defined COPD in cross-sectional analyses.

The latter finding is in accordance with the third National Health and Nutrition Examination Survey.¹⁴ One study has shown a similar association only among smokers and another study has shown no association at all.^{15 17} Compared with previous studies our study provides a larger total sample size and independent validation in a second data set that differs with regard to population structure and time of examination, thus extending the external validity of the results. Additionally, we have shown that the association is present among smokers and never smokers alike, although it is strongest among smokers regarding FEV₁ % predicted. Furthermore, the association seems to be strongest among the subgroup with spirometrically defined COPD, typically those that smoke the most.

The association of lower plasma 25(OH)D with faster decline of FEV₁ % predicted and FVC % predicted in the general population is a novel finding. Previously, the Normative Aging Study

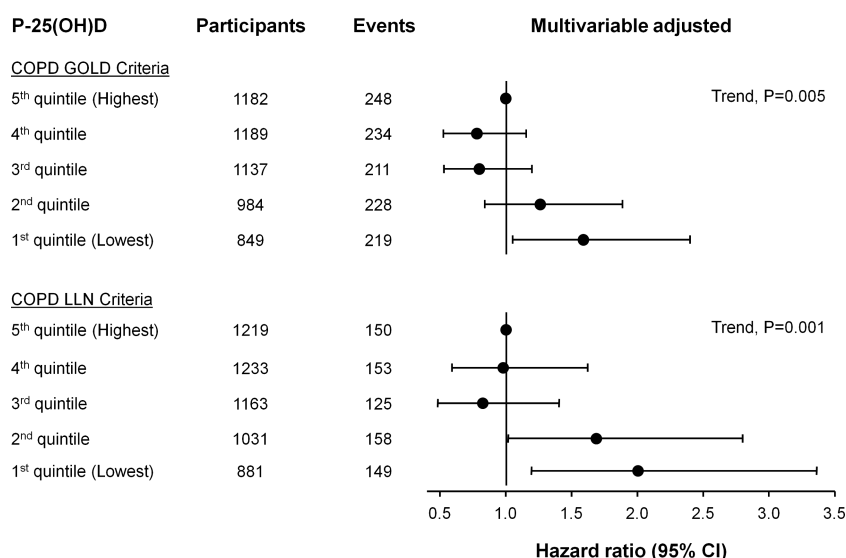
found a similar association in a smaller population consisting of men only¹⁶; however, the association was restricted to smokers only. Another study examining a cohort of smokers with COPD did not show any association between plasma 25(OH)D and rate of decline in lung function;¹⁵ that study was probably underpowered to detect an association given the relatively modest association of plasma 25(OH)D with lung function. In contrast to the latter study, we found that lower plasma 25(OH)D was associated with faster decline in FEV₁ % predicted in those with spirometrically defined COPD at baseline. In addition, studies of lung function decline in established COPD may show considerable variation depending on survival bias, regression to the mean and confounding by stage of COPD.²⁵

The prospective association of lower plasma 25(OH)D with increased risk of COPD is another novel finding. This study and previous cross-sectional analyses have shown that COPD patients have lower levels of plasma 25(OH)D.^{26 27} However, cross-sectional associations cannot show whether lower levels of plasma 25(OH)D precedes COPD, or vice versa. Our prospective analyses uniquely indicate that lower plasma 25(OH)D is associated with higher risk of future development of spirometrically defined COPD.

A somewhat puzzling finding was that lower 25(OH)D was associated with increased risk of spirometrically defined COPD, but not with decline in FEV₁/FVC. However, our interaction analyses suggest that the association is stronger with higher cumulative tobacco consumption and among those with spirometrically defined COPD, that is, low levels of vitamin D may play a stronger role among individuals who smoke more and/or have a lower lung function. Nevertheless, these observations do not fully explain our findings and additional research is required to answer this question.

A potential limitation of our study is that our cohort consists of only white individuals of Danish descent living in Denmark (55°–58° latitude North), with less sun exposure than closer to the equator; consequently, our findings would be most applicable to individuals with a similar skin colour and a similar level of sun exposure. The delay in measurement from 1981 to 1983 or 2004–2005 to 2009–2010 could raise concern of potential decay of plasma 25(OH)D, but this seems unlikely to have distorted our analyses for several reasons: we noticed the expected seasonal variation of 25(OH)D concentrations in both studies, median levels of plasma 25(OH)D across plasma samples from

Figure 4 HRs for chronic obstructive pulmonary disease (COPD) in participants with follow-up spirometry and without spirometrically defined COPD at baseline. Models were adjusted for gender, age, smoking status, cumulative tobacco consumption, body mass index, physical activity and income. Based on 5341 (COPD GOLD criteria) and 5527 (COPD lower limit of normal (LLN) criteria) participants from the Copenhagen City Heart Study.



three different examinations on the same healthy participants from the Copenhagen City Heart Study with storage times of 10 years, 20 years and 30 years were similar,^{24–28} previous studies have shown high stability during storage,²⁹ the median concentration observed in our studies were similar to those in comparable populations,³⁰ and a low sample quality for the 25(OH)D measurement would tend to weaken rather than inflate an association. A limitation is that we did not have information regarding intake of vitamin D supplement use at baseline or during follow-up. Furthermore, we only used one measurements of 25(OH)D, which could fluctuate with time; however, we and others have previously shown that 25(OH)D levels remain relatively stable with follow-up of up to 20 years.^{24–31}

Our study has several strengths: independent replication of the results in a second large general population study; up to three spirometry measurements spanning 20 years with no losses to follow-up; information on other major risk factors associated with COPD; and the highest statistical power to date to examine the associations of plasma 25(OH)D levels with lung function variables and risk of COPD. Also, in Denmark UV-B radiation from the sun is only adequate for endogenous vitamin D production in the skin during the summer months and food has never been fortified with vitamin D. Thus, this cohort from the Danish general population allows determination of the natural history of the association of vitamin D levels with lung function and risk of COPD.

In conclusion, we observed an association of lower plasma 25(OH)D levels with lower lung function, a faster decline in lung function and a higher risk of COPD.

Contributors SA designed the study, performed the data analysis and prepared the manuscript. SEB and JJF assisted with data analysis, were involved in data collection and in critical revision of the manuscript. PL designed the study, assisted with data analysis, and was involved in critical revision of the manuscript. BGN was involved in data collection, designed the study, assisted with data analysis, was involved in critical revision of the manuscript, and obtained funding.

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Competing interests PL received during the last 5 years pharmaceutical company grants from GSK in 2010 and 2011, Boehringer-Ingelheim and Almirall in 2012 via his home institution, and also participated in speaking activities, industry advisory committee or other related activities sponsored by Almirall, Boehringer-Ingelheim, Novartis, GSK, AstraZeneca, Pfizer, Mundipharma, UCB during the period 2008–2012.

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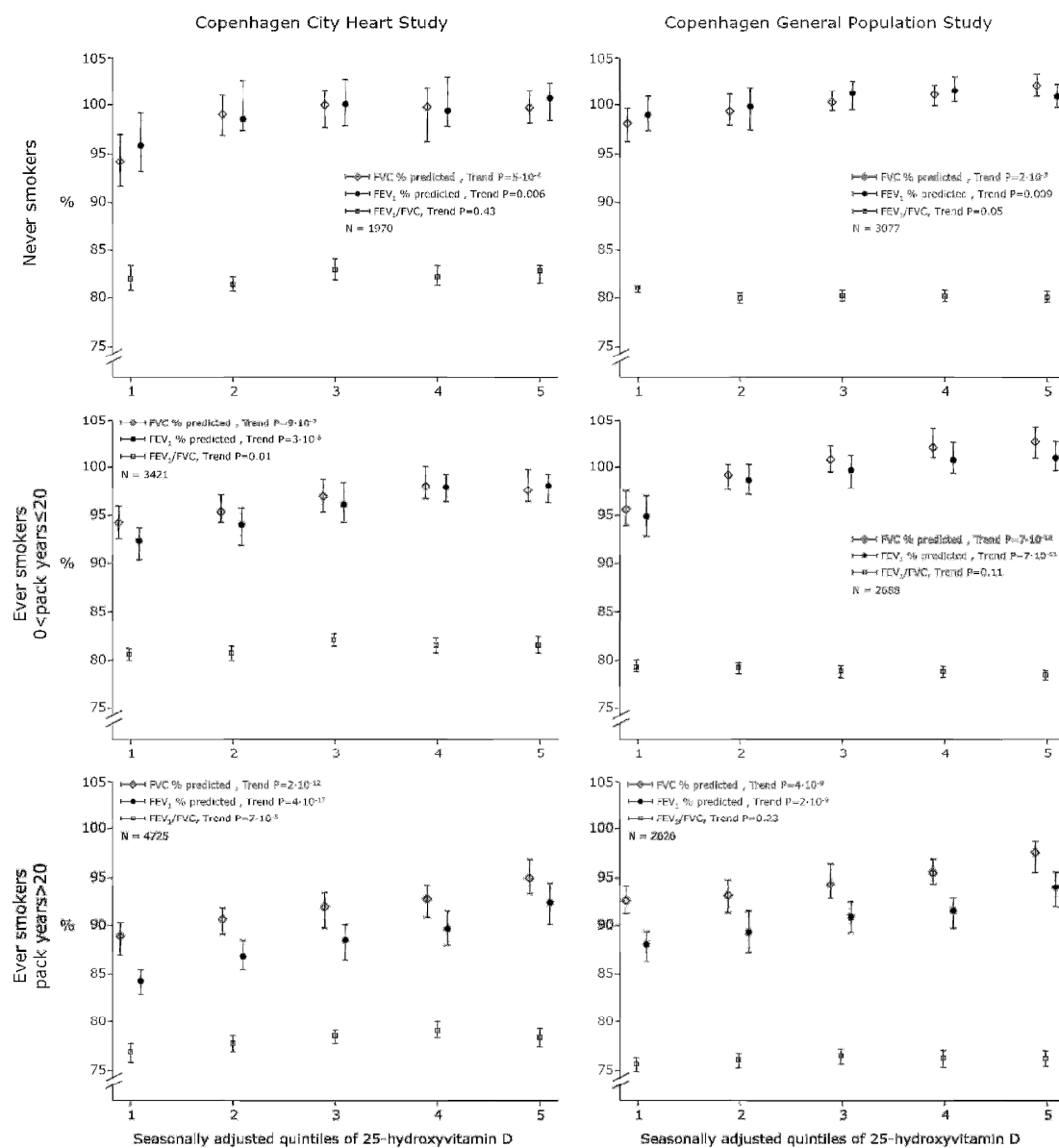
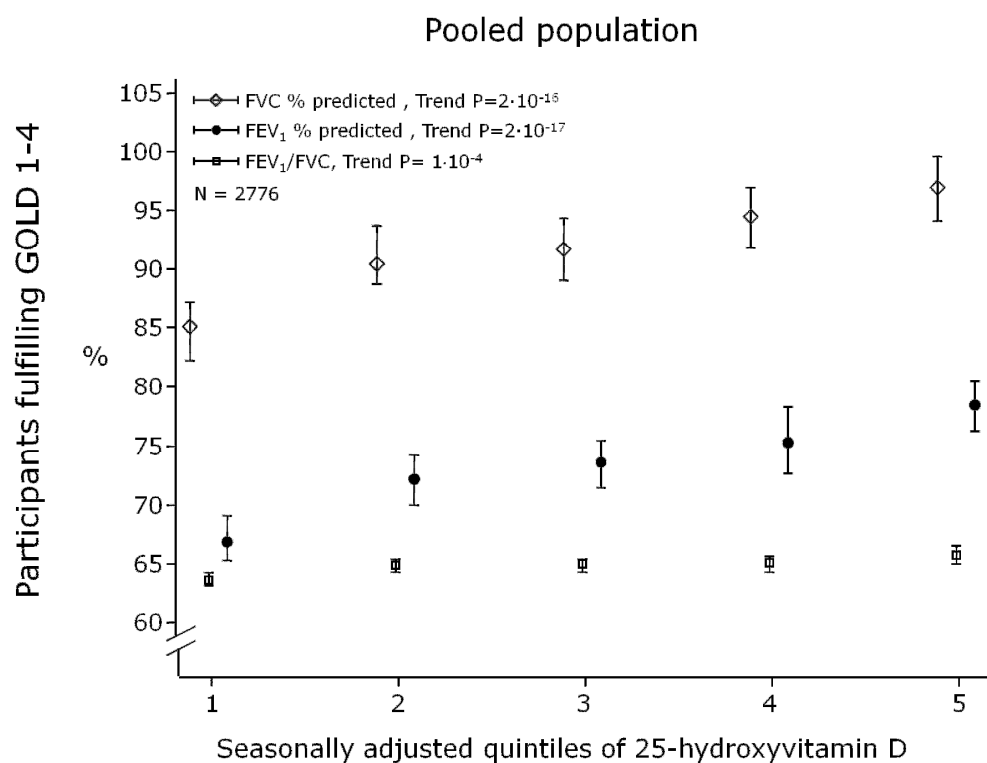


Figure S1. Lung function measures by seasonally adjusted 25-hydroxyvitamin D quintiles stratified by cumulative tobacco consumption in pack years. Quintiles were chosen due to the reduced number of participants in each subgroup.



Median (95% CI)					
FVC % predicted	85.0 (82.2-87.2)	90.5 (88.7-93.7)	91.7 (89.6-94.9)	94.5 (91.8-96.9)	97.0 (94.5-99.6)
FEV ₁ % predicted	66.8 (65.3-69.1)	72.3 (70.0-74.6)	73.7 (71.6-75.6)	75.2 (72.6-78.3)	78.4 (76.4-80.5)
FEV ₁ /FVC	63.6 (63.2-64.3)	64.9 (64.3-65.4)	65.0 (64.3-65.4)	65.1 (64.3-65.6)	65.7 (65.0-66.5)

Figure S2. Lung function measures by seasonally adjusted 25-hydroxyvitamin D quintiles in participants with spirometrically defined COPD. Quintiles were chosen due to the reduced number of participants in each subgroup.

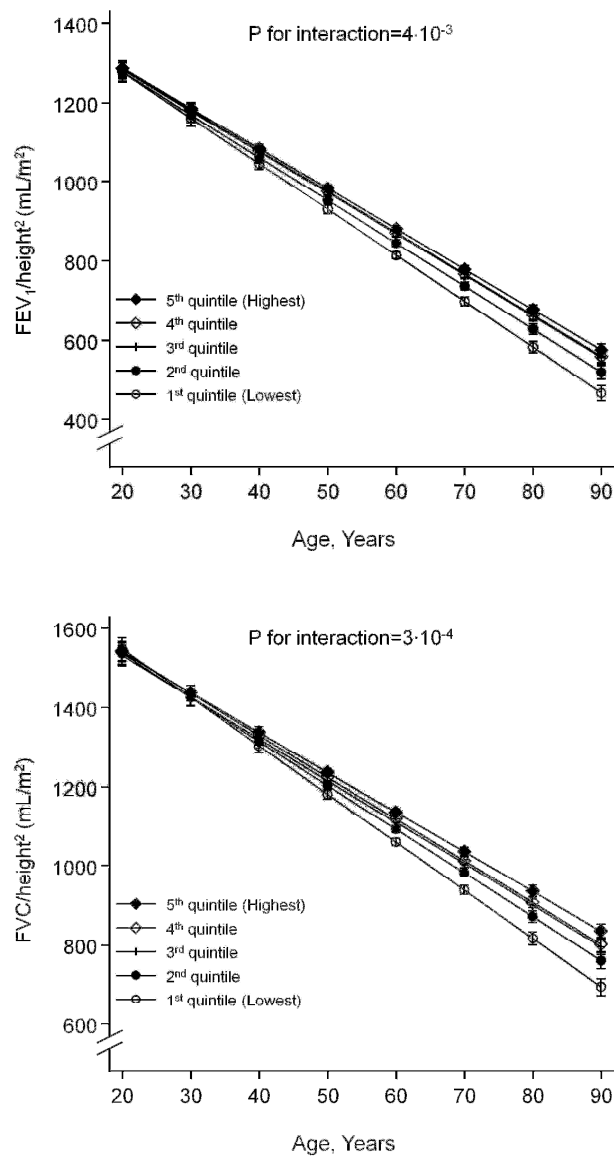


Figure S3. Age-related decline in lung function according to seasonally adjusted plasma 25-hydroxyvitamin D quintiles. Height adjusted FEV₁ and FVC decline was evaluated using repeated measures linear mixed models adjusted for gender and cumulative tobacco consumption.

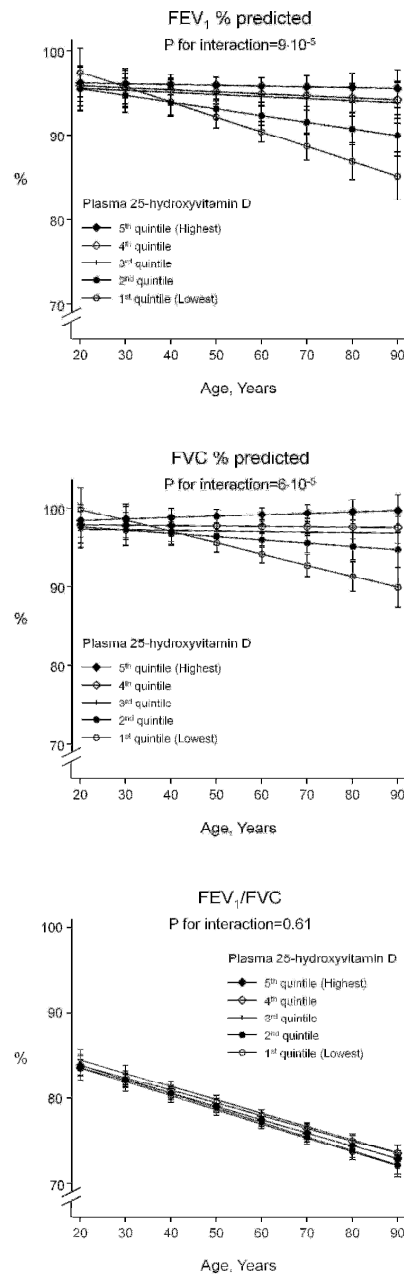


Figure S4. Analysis restricted to participants with 2 or more spirometries (N=5819). Relationship between age-related changes in FEV₁ % predicted, FVC % predicted, and FEV₁/FVC according to quintiles of seasonally adjusted plasma 25-hydroxyvitamin D. Based on 2 to 3 spirometries spanning up to 20 years in 5819 participants from the Copenhagen City Heart Study.

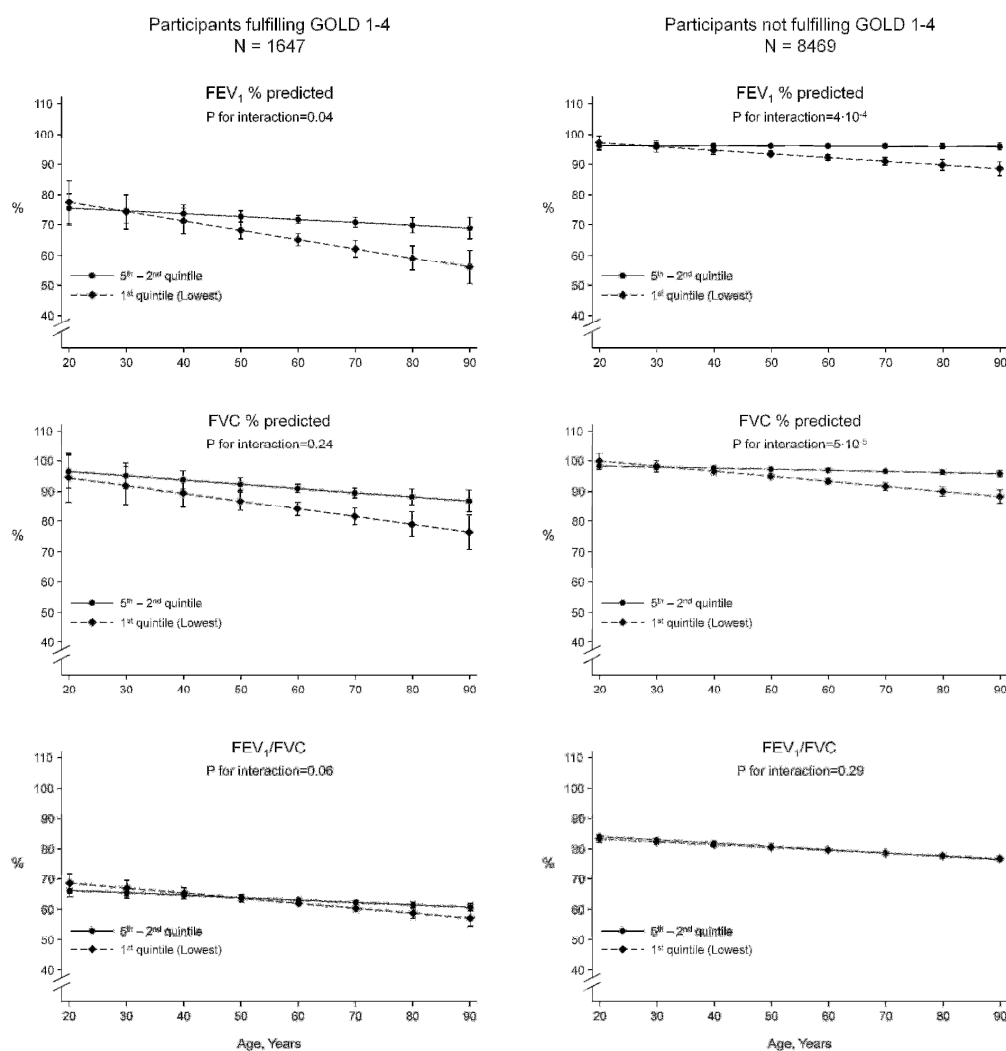


Figure S5. Analysis stratified according to spirometrically defined COPD at baseline (FEV₁/FVC <0.7). Relationship between age-related changes in FEV₁ % predicted, FVC % predicted, and FEV₁/FVC for bottom quintile vs. top 4 quintiles of seasonally adjusted plasma 25-hydroxyvitamin D. GOLD 1-4 categories were combined to increase power. Based on 1 to 3 spirometries spanning up to 20 years in 10116 participants from the Copenhagen City Heart Study.

Table S1. Distribution of missing information in baseline characteristics among the 10116 participants from the Copenhagen City Heart Study and 8391 participants from the Copenhagen General Population Study.

Copenhagen City Heart Study	Missing information, N(%)
Gender	0 (0)
Age	0 (0)
Smoking (Ever/Never)	0 (0)
Current tobacco consumption, g/day	0 (0)
Cumulative tobacco consumption, pack-years	0 (0)
Body mass index, kg/m ²	17 (0.2)
Physical activity	2 (0.02)
Income	123 (1.2)
<i>Total</i>	<i>142 (0.2)</i>
Copenhagen General Population Study	
Gender	0 (0)
Age	0 (0)
Smoking (Ever/Never)	0 (0)
Current tobacco consumption, g/day	164 (2.0)
Cumulative tobacco consumption, pack-years	191 (2.3)
Body mass index, kg/m ²	29 (0.3)
Physical activity	86 (1.0)
Income	92 (1.1)
<i>Total</i>	<i>562 (0.8)</i>

Table S2. Seasonal variation in 25-hydroxyvitamin D levels according to month.

Plasma 25-hydroxyvitamin D (nmol/L)								
Month	Copenhagen City Heart Study				Copenhagen General Population Study^a			
	Median	25th percentile	75th percentile	N	Median	25th percentile	75th percentile	N
January	33	20	50	669	37	26	50	754
February	32	20	48	987	35	23	47	828
March	35	20	54	849	36	23	50	876
April	35	21	53	892	41	28	56	836
May	37	24	52	1011	39	27	50	445
June	42	29	59	910	—	—	—	—
July	45	31	60	790	43	31	55	278
August	51	36	69	886	58	45	74	1012
September	57	40	75	975	55	43	70	908
October	47	32	63	685	50	38	64	810
November	39	24	54	924	46	33	62	1001
December	34	20	49	538	39	28	52	643
Total	41	26	58	10116	44	30	60	8391

^aNote that June was not represented in the Copenhagen General Population Study, since samples were only measured from July 2004 to May 2005