Large Meta-Analysis Provides Evidence for an Association of Serum Vitamin D with Pulmonary Function

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ABSTRACT

Background Epidemiological studies have reported mixed cross-sectional findings for the association of serum 25-hydroxyvitamin D [25(OH)D] and pulmonary function in the general population. We conducted the largest meta-analysis of the serum 25(OH)D–pulmonary function association to date.

Methods Data on 25(OH)D and pulmonary function from nine European ancestry (EA) cohorts (N=22,838) and five African ancestry (AA) cohorts (N=4,290) in the CHARGE Consortium was analyzed using linear models by cohort and ancestry group. Interaction terms tested effect modification of the 25(OH)D–pulmonary function association by smoking status (current/former/never). Results were combined using inverse variance weighted fixed-effects meta-analysis.

Findings Mean (SD) serum 25(OH)D was 68 (29) nmol/L for EAs and 49 (21) nmol/L for AAs. For each 10 nmol/L higher 25(OH)D (~0.5 SD), forced expiratory volume in 1 second (FEV_1) was higher by 11.1 mL ($P=2.5\times10^{-21}$) in EAs and 17.9 mL ($P=1.6\times10^{-7}$) in AAs. Forced vital capacity (FVC) was higher by 12.9 mL ($P=1.1\times10^{-20}$) in EAs and 15.4 mL ($P=1.2\times10^{-4}$) in AAs. The 25(OH)D–FEV_1/FVC associations were negligible. Among EAs, the magnitude of the 25(OH)D–FVC association was stronger in smokers, as evidenced by association estimates of 17.3 mL for current smokers, 16.6 mL for former smokers, compared to 7.8 mL for never smokers, per 10 nmol/L higher 25(OH)D.

Interpretation The 25(OH)D associations with FEV_1 and FVC were consistently positive in both EAs and AAs, and the stronger magnitudes of the 25(OH)D–pulmonary function association among smokers support the importance of vitamin D in vulnerable populations.

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Keywords: 25-hydroxyvitamin D; FEV_1; FVC; pulmonary function; smoking; ancestry

Summary:

1) What is the key question?

What is the association of serum vitamin D with pulmonary function in European Ancestry population and African Ancestry population, and is this association modified by smoking status?

2) What is the bottom line?

Serum vitamin D was consistently positively associated with pulmonary function (FEV_1 and FVC) in each ancestry group (European and African), and the interaction meta-analysis revealed a stronger association in cigarette smokers in the European ancestry group.

3) Why read on?

Existing evidence for the serum vitamin D—pulmonary function association is from single-cohort studies and findings are mixed; this meta-analysis over multiple cohort studies provides a clearer picture of the serum vitamin D—pulmonary function association, including effect modification by smoking.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), the third leading cause of mortality in the United States,[1] is characterized by progressive airway obstruction. Pulmonary function tests (PFTs), as performed by spirometry, are used to quantify pulmonary function parameters including forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC). Pulmonary function increases throughout childhood, plateaus in the 20s, and remains stable until about the mid-40s; thereafter, adults experience an age-related decline.[2] The majority (85%) of COPD cases are related to smoking,[3] which alters the trajectory in pulmonary function, by hindering growth, reducing peak function, and accelerating age-related decline.[4]

Vitamin D is proposed to have protective effects in the lungs via gene regulation.[5] *In vitro* studies found that 1,25-dihydroxyvitamin D, the active vitamin D metabolite, induced antimicrobial peptides for host defense in the lung and modulated airway remodeling.[6] In humans, 25-hydroxyvitamin D [25(OH)D], the most widely used biomarker of vitamin D status, is the major vitamin D metabolite in serum. Levels of 25(OH)D are higher in European ancestry (EA) populations than in African ancestry (AA) populations, regardless of life stage, presumably reflecting differences in UV-mediated vitamin D production in skin.[6]

Previous observational cross-sectional studies of the vitamin D–pulmonary function association in the general population have reported mixed findings. Most of these studies have reported a positive association between 25(OH)D and pulmonary function;[7-13] some have reported a null or inverse association;[14-16] and some have reported a positive association under certain conditions, such as only in male current smokers[17] or only in overweight and obese males.[18] The largest previous cross-sectional study, which included two Danish cohorts (total N=18,507), reported positive associations of 25(OH)D with pulmonary function.[10] Only one prior cross-sectional

study investigated serum 25(OH)D and pulmonary function in an ancestry group other than European, and it confirmed similar positive associations in the 3,957 AA participants studied.[7]

The current study investigated the hypothesis that serum 25(OH)D level is positively associated with pulmonary function. We leveraged the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium to include population-based data on serum 25(OH)D and pulmonary function in a harmonized analysis. Additionally, we compared the association of serum 25(OH)D and pulmonary function across EA and AA groups and investigated effect modification by cigarette smoking.

MATERIALS AND METHODS

Cohorts and Participants

Nine prospective cohorts in the CHARGE Consortium were included (Table 1). All cohorts had EA participants, and five of the cohorts had AA participants. Participants with other ancestry groups were not included in this study given limited and small sample sizes. Among the nine cohorts, the Framingham Heart Study (FHS) had two sub-cohorts analyzed separately: the Offspring and the third-generation (Gen3) cohorts. Our analysis pipeline harmonized the outcome and exposure definitions, the units on all variables, and the statistical modeling. The same exclusion criteria were applied to each cohort: missing PFTs, unacceptable PFTs using the American Thoracic Society (ATS) and European Respiratory Society (ERS) criteria for acceptability, missing serum 25(OH)D, serum 25(OH)D > 374.4 nmol/L (or 150ng/mL, leading to removal of a single outlier),[19] or missing on other covariates (see supplement pp. 3–4).

Table 1. Cross-sectional description of the characteristics of each cohort*

European Ancestry Cohort	ARIC	CARDIA	CHS[††]	HABC[‡‡]	MESA	AGES	FHS (Offspring)	FHS (Gen3)	RS
Number of participants	8,327	172	1,297	1,411	1,113	1,685	1,639	3,610	3,584
Males, percentage	46.0	58.7	30.15	53.3	49.2	40.8	48.1	47.3	44.6
Current Smoker, percentage	23.4	11.6	9.4	6.5	8.4	9.8	14.3	15.3	16.0
Former Smoker, percentage	34.9	16.3	44.9	49.8	47.2	42.4	50.5	28.0	52.9
Pack-years[†]	28.0 (20.9)	6.2 (7.2)	28.1 (25.3)	36.4 (32.0)	30.1 (29.6)	24.6 (21.9)	26.5 (22.8)	12.4 (13.4) [§§]	22.9 (21.6)
Age, year	54.2 (5.7)	34.8 (3.1)	73.7 (4.4)	73.7 (2.8)	66.3 (9.9)	76.2 (5.6)	59.4 (9.3)	40.2 (8.7)	64.4 (9.7)
Height, m	1.69 (0.09)	1.73 (0.09)	1.63 (0.09)	1.67 (0.09)	1.69 (0.10)	1.67 (0.09)	1.68 (0.09)	1.71 (0.09)	1.69 (0.09)
Weight, kg[‡]	76.8 (16.2)	76.9 (17.0)	70.6 (14.2)	74.5 (14.5)	79.7 (17.3)	75.4 (14.7)	79.4 (17.2)	78.6 (18.4)	79.5 (14.6)
FEV_1, mL	2,946 (767)	3,881 (743)	2,010 (611)	2,324 (649)	2,556 (768)	2,142 (670)	2,724 (757)	3,592 (787)	2848 (866)
FVC, mL	3,987 (973)	4,967 (999)	2,881 (829)	3,118 (810)	3,492 (995)	2,877 (837)	3,711 (950)	4,621 (999)	3692 (1063)
FEV_1/FVC	0.739 (0.077)	0.785 (0.060)	0.700 (0.095)	0.745 (0.078)	0.734 (0.087)	0.744 (0.087)	0.733 (0.078)	0.779 (0.063)	0.771 (0.082)
Serum 25(OH)D, nmol/L[§]	64.7 (21.8)	95.0 (35.3)	68.0 (27.9)	72.2 (25.6)	75.6 (28.2)	52.4 (23.5)	49.2 (18.9)	92.8 (36.0)	61.0 (27.4)
Never smoker	64.3 (21.0)	95.4 (34.4)	67.1 (25.1)	73.7 (25.9)	76.5 (27.7)	54.1 (22.8)	49.6 (18.6)	93.2 (35.4) [‖‖]	59.7 (25.9)
Former smoker	67.1 (21.5)	94.5 (43.0)	69.4 (29.4)	71.7 (24.8)	76.2 (28.5)	52.3 (24.1)	49.8 (18.6)	93.5 (37.0)	62.3 (27.7)
Current smoker	61.8 (23.1)	92.7(29.5)	65.4 (33.2)	65.0 (28.1)	66.9 (28.2)	44.5 (22.7)	45.9 (20.6)	89.9 (36.3)	59.5 (29.4)
Time from 25(OH)D to PFT, days [‖]	-1,073 (67)	1,122 (89)	363 (29)	-382 (39)	1,765 (112)	1 (5)	133 (377)	2 (61)	846 (808)
Season of 25(OH)D measurement, percentage [**]									
Spring	31.2	8.1	20.5	30.5	29.0	22.4	29.2	26.8	29.6
Sumer	26.1	36.1	30.1	18.1	22.2	12.4	11.0	29.6	18.9
Fall	23.3	34.3	29.6	22.8	24.9	33.8	29.1	24.1	30.0
Winter	19.5	21.5	19.8	28.6	23.9	31.4	30.7	19.4	21.5

African Ancestry Cohort	ARIC	CARDIA	CHS[††]	HABC[‡‡]	MESA
Number of participants	2,339	157	168	863	763
Males, percentage	35.3	51.6	25.6	44.5	47.4
Current Smoker, percentage	27.5	26.1	10.7	15.8	15.7
Former Smoker, percentage	23.9	9.6	42.9	39.3	38.3
Pack-years[†]	21.4 (20.7)	5.3 (4.6)	21.9 (18.3)	29.4 (23.4)	23.6 (21.8)
Age, year	53.3 (5.7)	33.9 (3.2)	71.9 (4.5)	73.4 (2.9)	65.6 (9.7)
Height, m	1.68 (0.09)	1.71 (0.10)	1.63 (0.08)	1.66 (0.09)	1.68 (0.10)
Weight, kg[‡]	83.5 (17.1)	82.2 (16.9)	75.7 (13.3)	78.2 (15.1)	84.3 (16.8)
FEV_1, mL	2,495 (638)	3,237 (709)	1,801 (508)	1,958 (566)	2,200 (667)
FVC, mL	3,255 (806)	4,077 (920)	2,507 (706)	2,594 (712)	2,933 (869)
FEV_1/FVC	0.768 (0.077)	0.799 (0.070)	0.723 (0.076)	0.757 (0.090)	0.755 (0.093)
Serum 25(OH)D, nmol/L[§]	47.4 (17.5)	69.4 (31.2)	44.6 (21.1)	51.8 (22.4)	47.9 (22.3)
Never smoker	46.8 (16.7)	71.3 (30.1)	43.7 (19.2)	51.8 (22.7)	49.1 (22.3)
Former smoker	48.5 (18.0)	69.2 (35.6)	47.2 (24.2)	52.3 (21.8)	49.3 (22.6)
Current smoker	47.5 (18.4)	64.8 (32.4)	38.3 (14.9)	50.4 (23.2)	40.9 (20.0)
Time from 25(OH)D to PFT, days[ll]	-1,054 (114)	1,101 (104)	350 (26)	-390 (53)	1,719 (115)
Season of 25(OH)D measurement, percentage[**]					
Spring	30.0	10.2	58.9	35.6	34.6
Sumer	30.7	56.0	7.1	16.2	23.5
Fall	20.7	23.6	8.9	24.9	19.7
Winter	18.6	10.2	25.0	23.3	22.3

Abbreviation: 25(OH)D = 25-hydroxyvitamin D; AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; EA = European ancestry; FEV_1 = forced expiratory volume in 1 second; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; FVC = forced vital capacity; HABC = Health, Aging, and Body Composition; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.

[*] Data are presented as mean (SD) unless otherwise indicated; AGES, RS, and FHS only have participants of European ancestry; N_{EA} = 22,838, N_{AA} = 4,290, N_{total} = 27,128.

[†] Pack-years is calculated only among current and former smokers in each cohort.

[‡] The number of participants who have weight data is slightly different from the total number of participants in each cohort. However, the descriptive statistics of weight stays similar.

[§] Mean (SD) of serum 25(OH)D level for all the participants in each cohort, and mean (SD) of 25(OH)D level in participants with each smoking status are shown here, stratified by ancestry.

[ll] The time difference is the interval between the time when pulmonary function was measured and the time when serum vitamin D was measured. The difference is positive, if the serum vitamin D was measured before the pulmonary function test; while the value is negative, if the serum vitamin D was measured after the pulmonary function test.

[**] The proportion of participants in each season when their serum was measured was rounded (thus rounding errors mean sums may not be exactly 100%).

[††] The number of participants used to compute descriptive statistics in CHS excluded those who had residual outliers based on the preliminary models (N_{EA} = 8 and N_{AA}= 6); while other cohorts used the number of participants before applying residual exclusion for the descriptive statistics.

[‡‡] Numbers vary slightly for different outcomes in HABC (For the FVC outcome, N_{EA}=1385 and N_{AA}=821; for the ratio outcome, N_{EA}=1382 and N_{AA}=817). The numbers of participants for the FEV_1 outcome are used. However, the descriptive statistics is similar across different outcomes.

[§§] We used 1,554 ever smokers here, instead of a total of 1,561 ever smokers in the Gen3 cohort, because the pack-years of seven ever smokers were so small that they were coded as 0. Therefore, these seven ever smokers do not contribute to the pack-years descriptive statistics here.

[lll] We used 2,046 never smokers, rather than a total of 2,049 never smokers in the Gen3 cohort, to compute the 25(OH)D level in never smokers.

Outcome and Exposure Assessment

Pre-bronchodilator pulmonary function outcomes (FEV_1, FVC, and FEV_1/FVC), which have similar accuracy as post-bronchodilator measures for long-term outcomes,[20] were measured in each cohort using standardized methods (see supplement pp. 5–8). The methods used to measure 25(OH)D varied by cohort (see supplement pp. 5–8), reflecting the calendar year of assay in relation to the standardization efforts for vitamin D assays. The Coronary Artery Risk Development in Young Adults (CARDIA) study measured serum vitamin D in a subset of participants included in an ancillary study of bone mineral homeostasis.[21] For the remaining cohorts, measurements of the outcome and exposure variables were planned for either the full cohort or a random sample (see supplement pp. 3, pp. 9). The 25(OH)D-pulmonary function association was estimated using continuous variables for serum 25(OH)D and pulmonary function.

As shown in Table 1, among nine cohorts, four [Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland (AGES), Cardiovascular Health Study (CHS), FHS-Offspring, and FHS-Gen3] had a mean time difference of less than one year in the PFT measurements and the preceding 25(OH)D measurement; the maximum mean time difference between 25(OH)D and PFT measurement was less than 5 years [Multi-Ethnic Study of Atherosclerosis (MESA)]. Participants in the Atherosclerosis Risk In Communities (ARIC) study and the Health, Aging, and Body Composition (HABC) study had blood drawn for serum 25(OH)D after their PFT measure, but within 3 years. The 25(OH)D concentration is expected to be representative of usual levels given that repeated measurements of serum 25(OH)D taken over a 5-year period were reasonably stable, and repeated measurements of serum 25(OH)D levels over 14 years had moderate to high correlations.[22, 23]

Other covariates, including smoking status, pack-years (number of packs of cigarettes smoked per day times the number of years the person has smoked), height, weight, and age, were measured concurrently with pulmonary function, except for CHS, which assessed covariates concurrent with the serum 25(OH)D measure, but within 1 year of the PFT measurement (see supplement pp. 9). All data collection and analysis was approved by the Institutional Review Board at each cohort's respective institution. Spirometry measures are available on the database of Genotypes and Phenotypes (dbGaP) via accession numbers as follows: ARIC (phs000280), CARDIA (phs000285), CHS (phs000287), FHS (phs000007), and MESA (phs000209). Serum vitamin D measures are also available at the same accession numbers for CHS, FHS, and MESA.

Statistical Analysis in Individual Cohorts

All analyses were first conducted independently in each cohort, stratified by ancestry, given the higher prevalence of risk of vitamin D deficiency and a lower mean serum 25(OH)D level in AA participants.[6] For FEV_1 and FEV_1/FVC, models were adjusted for smoking status, pack-years, height, height squared, age, age squared, sex, season of blood draw, and study center (if applicable); for FVC, the model was further adjusted for weight. The first analysis of serum 25(OH)D on each pulmonary outcome was conducted in each ancestry group, adjusting for all the covariates, after excluding residual outliers, which were identified using the studentized residuals of the linear models (see supplement pp. 1). A second analysis including interaction between 25(OH)D and smoking status [never (reference group), former, and current smokers] was also conducted in each ancestry group, adjusting for covariates.

Meta-Analysis

Fixed-effects meta-analysis was conducted for the association of serum 25(OH)D on each PFT outcome for each ancestry group, using inverse variance weighting, with heterogeneity assessed

via the I^2 statistic.[24] The comparison of meta-analyzed coefficients of the 25(OH)D-PFT associations for the two ancestry groups was conducted using a Z test.[25] Meta-analysis of the interaction terms of 25(OH)D with smoking status was also performed (see supplement pp. 1).

Meta-regression was conducted to explore the potential causes of heterogeneity (see supplement pp. 1). In the main-effect meta-analysis of serum 25(OH)D on pulmonary function, there was little to moderate heterogeneity in the EA cohorts (I^2, 6 to 52%); and little heterogeneity in the AA cohorts (I^2, 0 to 27%). The two-sided type I error was set at 0.05 for all analyses. Meta-analysis and meta-regression used the metafor package (version 1.9-8) in R (version 3.2.3., R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We studied 22,838 EA and 4,290 AA participants (see Table 1**)**. EA participants had higher FEV_1, FVC, and serum 25(OH)D (see supplement pp. 14) than AA participants in each cohort, while FEV_1/FVC was similar across ancestry groups. CARDIA and FHS-Gen3 were younger than the seven other cohorts, with consequently lower pack-years smoked in ever smokers. Across all cohorts, among EA participants, 17% were current smokers and 40% were former smokers; among AA participants, 22% were current smokers and 30% were former smokers. The serum 25(OH)D level was similar among never [EA: 68 nmol/L, AA: 49 nmol/L] and former smokers [EA: 69 nmol/L, AA: 50 nmol/L], with consistently lower mean 25(OH)D in the current smokers [EA: 64 nmol/L, AA: 47 nmol/L]. The mean (SD) of serum 25(OH)D for EA

participants across nine cohorts was 68 (29) nmol/L and it was 49 (21) nmol/L for AA participants across five cohorts.

Regression coefficients (β) and standard errors (SE) calculated within each cohort per 1 nmol/L 25(OH)D are presented. Additionally, to convey the magnitude of the 25(OH)D-PFT associations, the meta-analyzed regression coefficients were multiplied by 10 nmol/L 25(OH)D, which was about half of the standard deviation (SD) of the 25(OH)D distribution.

Meta-analysis (Figure 1) revealed a consistently positive association of serum 25(OH)D with the PFT outcomes, FEV_1 and FVC, in both ancestry groups. For a 10 nmol/L higher 25(OH)D, FEV_1 was higher by 11.1 mL in EAs ($P = 2.5\times10^{-21}$) and by 17.9 mL in AAs ($P = 1.6\times10^{-7}$). Similarly, for a 10 nmol/L higher 25(OH)D, FVC was higher by 12.9 mL in EAs ($P = 1.1\times10^{-20}$) and by 15.4 mL in AAs ($P = 1.2\times10^{-4}$). The magnitudes of the 25(OH)D-PFT associations did not differ significantly between the two ancestry groups (P = 0.06 and P = 0.56 for FEV_1 and FVC, respectively). The association of serum 25(OH)D with FEV_1/FVC reached statistical significance, but the magnitude was negligible; a 10 nmol/L higher 25(OH)D was associated with a ratio being lower by 0.0055% in EAs (P = 0.0013) and greater by 0.0035% in AAs (P = 0.5492) (see Figure E2 and supplement pp. 10 for cohort- and ancestry-specific findings).

We used meta-regression to explore six potential causes of moderate heterogeneity in the meta-analysis of 25(OH)D on FEV_1 ($I^2 = 51.95\%$) and FVC ($I^2 = 41.93\%$) in the EA cohorts. The six variables were proportion of ever smokers, current smokers, and former smokers; mean serum 25(OH)D level; time from 25(OH)D to PFT; and mean age. Three highly correlated variables (Pearson's r > 0.75 for all pairwise correlations) had significant linear relationships with the

association of 25(OH)D on FEV_1 and FVC: proportion of ever smokers, proportion of former smokers, and mean 25(OH)D in a cohort (see supplement pp. 16-17).

To examine the potential impact of family relatedness between the FHS-Gen3 and the FHS-Offspring cohorts on the meta-analysis, sensitivity analysis confirmed that the findings were unchanged when either cohort was excluded (results not shown).

Cohort-specific findings (see supplement pp. 11–12) from models that included the 25(OH)D × smoking status interaction terms were combined in a second meta-analyses (see supplement pp. 13). In the EA cohorts, 25(OH)D had a greater positive association with FVC in current smokers than in never smokers ($\beta_{current \times 25(OH)D}$ = 7.5 mL for 10 nmol/L increment of 25(OH)D, P = 0.047). Similarly, 25(OH)D had a greater positive association with FVC in former smokers than in never smokers ($\beta_{former \times 25(OH)D}$ = 7.9 mL for 10 nmol/L increment of 25(OH)D, P = 0.0065) (Figure 2). For FEV_1 in the EA cohorts, the interaction coefficients for 25(OH)D and smoking status had the same positive direction as the coefficients for FVC but were not statistically significant for either current (P = 0.138) or former smokers (P = 0.137). There was no statistical evidence of interaction of 25(OH)D and cigarette smoking in the AA cohorts for either outcome. To provide further interpretation of the interaction, the meta-analyzed β estimate (sum of $\beta_{reference} + \beta_{interaction\ term}$) for the association of 25(OH)D with FVC was 17.3 mL for current smokers and 16.6 mL for former smokers, which were more than double the estimate for never smokers (β = 7.8 mL), per 10 nmol/L higher serum 25(OH)D. A similar trend was found for the FEV_1 outcome in the EA cohorts. For 10 nmol/L higher serum 25(OH)D, FEV_1 was higher by 8.0 mL in never smokers, 12.0 mL in former smokers, and 14.0 mL in current smokers (see Figure 3).

DISCUSSION

This study showed a consistently positive association of serum 25(OH)D with FEV_1 and FVC across EA and AA groups. A small magnitude of association was observed for FEV_1/FVC, which may result from the similar associations of 25(OH)D with FEV_1 and FVC. The previous largest cross-sectional study (including two Copenhagen cohorts: N=10,116 and N=8,391 respectively) also reported a consistently positive association of 25(OH)D with FEV_1 percentage predicted and FVC percentage predicted but not with FEV_1/FVC.[10] The magnitude of the association was about four times greater in the Copenhagen study; this may be explained by the difference in the distribution of serum 25(OH)D (Danish median ~42 nmol/L vs. CHARGE median of ~65 nmol/L) given the 25(OH)D–PFT association was stronger in cohorts with lower serum 25(OH)D. Our finding for the serum 25(OH)D–FEV_1 association was similar to the association reported in a British cohort of 6,789 participants with an average age of 45 years,[11] but weaker than that observed in a previous study in the FHS.[9] Given that the rate of decline in FEV_1 at age 45 is increased by ~15 mL/year in current smokers,[26] we estimate that a 10 nmol/L higher 25(OH)D is similar to approximately 1 year of current smoking-related decline in FEV_1 for both ancestries, but in the opposite direction. Potential biological mechanisms for a decrease in pulmonary function due to low 25(OH)D levels in adults include an altered immune system that increases susceptibility to inflammation, a reduction in pulmonary parenchyma related to extracellular matrix homeostasis important for lung structure, or a decrease in serum calcium that could adversely affect thoracic skeleton mobility and respiratory muscle performance.[27]

The associations of serum 25(OH)D with FEV_1 and FVC had stronger, yet not statistically significant, magnitudes in AA than in EA participants, which may be explained by the lower serum 25(OH)D level in AA participants and is consistent with a previously reported non-linearity in the serum 25(OH)D–pulmonary function association—attenuated at higher 25(OH)D.[9] Future studies that investigate genetic variation in EAs and AAs in the context of serum 25(OH)D may help explain the differences.

In EA participants, the positive interaction terms between serum 25(OH)D and smoking status supported a stronger magnitude of association of serum 25(OH)D with FVC in current and former smokers than in never smokers, with a consistent, but not statistically significant, difference for FEV_1. The interaction finding is consistent with a prior National Health and Nutrition Examination Survey (NHANES) study, which reported a stronger 25(OH)D–FEV_1 association in current and former smokers than in never smokers that was near statistical significance ($P = 0.059$).[7] Given smokers have a higher level of oxidative stress and lower pulmonary function than never smokers, partly due to chronic inflammation in lung tissue, the stronger protective association of 25(OH)D on pulmonary function in smokers suggests a benefit for smokers. To explore this interaction, estimates of the 25(OH)D–PFT association were computed within each smoking category. In EA participants, the 25(OH)D–FEV_1 (or FVC) associations were statistically significant in all strata. Generally, in ever smokers of European ancestry, the coefficients for 25(OH)D were greater for FVC than for FEV_1.

Meta-regression provided additional evidence for effect modification by smoking. The proportion of ever smokers was a significant modifier of the association of serum 25(OH)D with FEV_1 and FVC. The higher the proportion of ever smokers, the greater the 25(OH)D–PFT association. More specifically, the proportion of former smokers explained the heterogeneity in

the 25(OH)D–PFT association across cohorts better than the proportion of current smokers did; this may be explained by a survival bias in older participants who were current smokers. Mean serum 25(OH)D also explained a significant portion of the heterogeneity, with smaller magnitudes of 25(OH)D–PFT association being observed in cohorts that had higher mean 25(OH)D. However, given the high correlation between proportion of smokers and 25(OH)D level, we were unable to distinguish a unique cause of heterogeneity. By comparing the cohort-level 25(OH)D–PFT association magnitudes based on mean age of the cohorts, our study findings were consistent with a prior NHANES study showing that the association of 25(OH)D and FEV_1 was greater in people over age 60 than in younger individuals.[7]

There are several limitations of this study. First, serum 25(OH)D was measured via different methods across the cohorts; three cohorts (ARIC, CHS, MESA) used the current reference method, liquid chromatography in tandem with mass spectrometry (LC-MS/MS); three cohorts (CARDIA, FHS, HABC) used radioimmunoassay (RIA); one cohort (AGES) used chemiluminescence immunoassay (CLIA); and one cohort [Rotterdam Study (RS)] used electro-CLIA. Only MESA calibrated the serum 25(OH)D measurement against the current gold standard (standard reference material 972),[28] reflecting the calendar time of the measurements in the cohorts, some of which occurred before availability of the standard reference material. Previous studies reported a regression slope of 0.87 and 0.88 for the RIA method and the CLIA method, respectively, when compared with the LC-MS/MS (slope=1, perfect agreement),[29, 30] suggesting a high level of agreement among the three methods. Any error in serum vitamin D measurement is expected to be non-differential with respect to pulmonary function, which leads to an underestimate of the true association. Second, in this cross-sectional meta-analysis, serum 25(OH)D was measured concurrently with pulmonary function in two cohorts (AGES and FHS-

Gen3), and, about 1 year prior (CHS and FHS-Offspring), about 3 years prior (CARDIA, and RS) and 5 years prior (MESA) in five cohorts. HABC measured serum vitamin D after spirometry by 1 year, and ARIC measured it after spirometry by 3 years. However, meta-regression tests for heterogeneity confirmed that time difference between 25(OH)D and PFT measurements did not significantly affect the 25(OH)D–PFT associations. In addition, past studies with longitudinal measurements of serum 25(OH)D reported a high correlation of 25(OH)D measurements over a long period of time, with a correlation coefficient of 0.7 for measurements separated by 1 year, 0.5 for measurements separated by 5 years,[22] and 0.42-0.52 for measurements separated by 14 years,[23] indicating that it is reasonable to use a single 25(OH)D measurement to represent usual level in association studies. Third, although physical activity was not adjusted in models, to the extent that it is a determinant of serum 25(OH)D through outdoor sun exposure, the adjustment for season of blood draw will partly account for this confounding. Finally, if the serum vitamin D—PFT association in cohort members with all the required data differed from the association in participants missing data on GWAS, PFTs, and/or serum vitamin D, this would lead to selection bias. The subset of the cohort with all required data varied; in most instances missing data was related to practical issues (for example, an ancillary study assayed serum vitamin D only a subset of the full cohort) and not to dropout or loss to follow-up.

This study meta-analyzed the serum 25(OH)D–PFT association across nine cohorts, according to a common pipeline that harmonized the variables and statistical analysis. The sample size comprised 17,569 EA participants from the United States; 5,269 EA participants from Iceland and the Netherlands; and 4,290 AA participants from the United States, all of whom were 19 to 95 years old. The sample provided excellent representation of the U.S. population, based on

comparisons of demographic factors including sex, height, weight, smoking status, and COPD prevalence (~6.1%) to national surveys,[31, 32, 33] which strengthens the external validity of the study's findings.

In summary, using meta-analysis, we estimated a positive association of serum 25(OH)D with the pulmonary function parameters FEV_1 and FVC in both EA and AA participants. Associations varied by smoking status in the EA group, with stronger serum 25(OH)D–PFT associations seen in current smokers. The observational design means we cannot infer a causal association, and future studies, such as randomized controlled trials or Mendelian Randomization studies, are needed to further investigate the causality of 25(OH)D on pulmonary function.

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CONTRIBUTORS

PAC, DBH, and JX conceived and designed the study. RGB, JL, JD, SAG, LL, SJL, KEN, AVS, BMP, and LMS provided the data and supervised the data analysis in each cohort. JX, TMB, RRR, AVS, AWM, FS, NT, and XZ analyzed data within each cohort. JX, PAC and DBH meta-analyzed and interpreted the data. JX, PAC and DBH co-wrote and edited the first draft of the manuscript. All authors provided support and suggestions at all stages, critically reviewed the manuscript, and approved the final version.

AUTHOR DISCLOSURE

Dr. Psaty serves on the DSMB of a clinical trial funded by the manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. All other authors have no competing interests. There is no commercial support or financial interest from the tobacco industry for the research presented.

The study sponsors were not involved in study design, data collection, data analysis, data interpretation, report writing, or decisions to submit the paper for publication. PAC and DBH had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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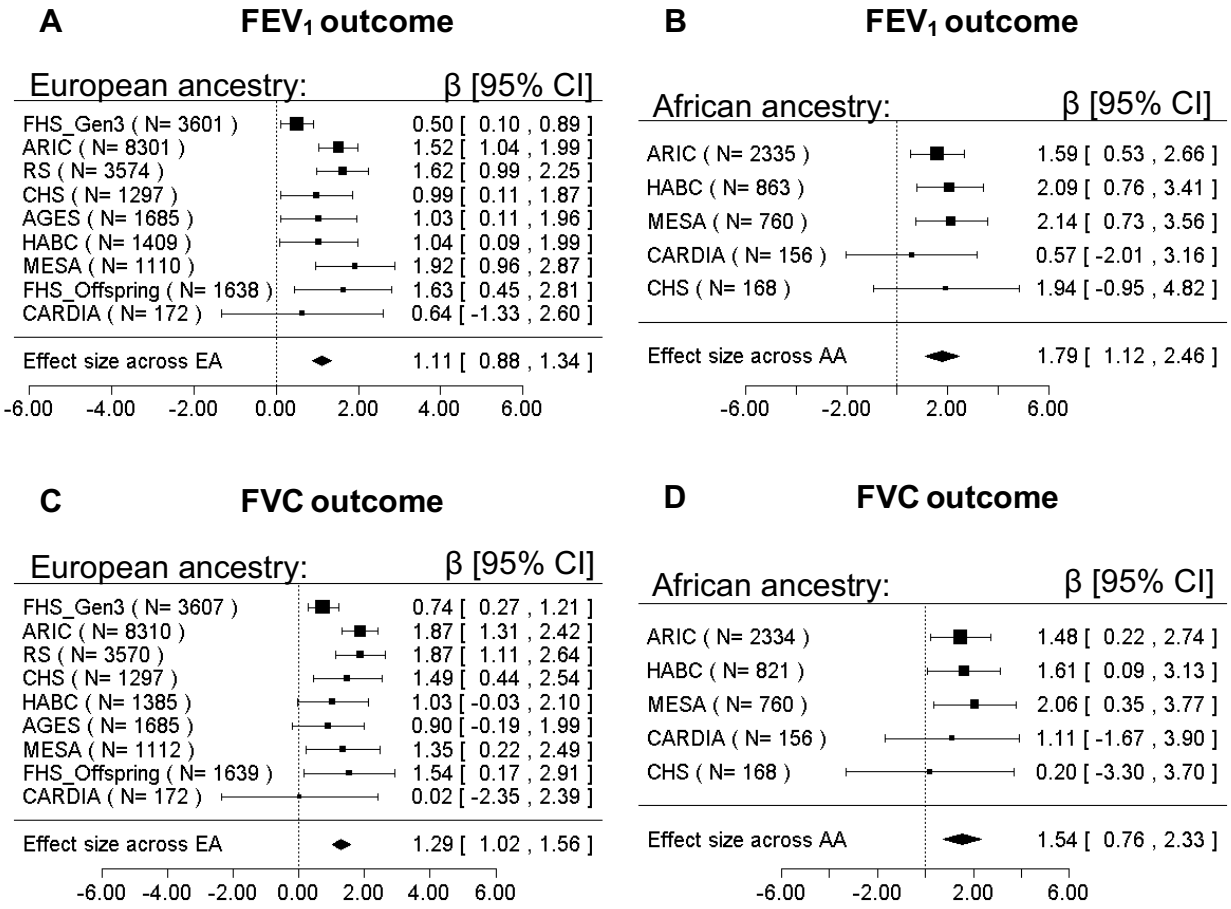
LEGENDS

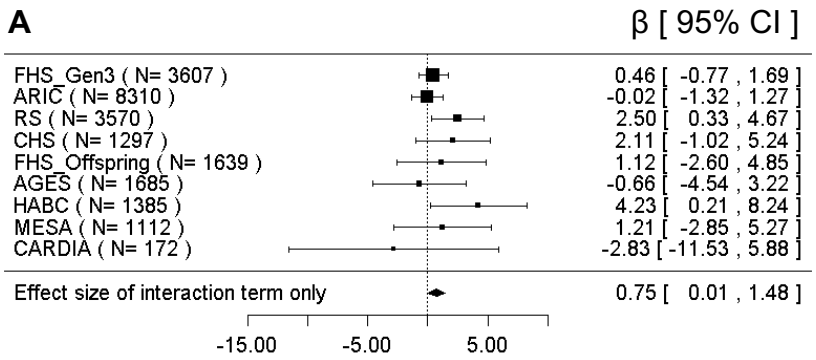
Figure 1. Forest plots of the meta-analysis of serum 25(OH)D on FEV_1 and FVC, stratified by ancestry. Associations are presented for serum 25(OH)D on (A) FEV_1 in European ancestry cohorts. (B) FEV_1 in African ancestry cohorts. (C) FVC in European ancestry cohorts. (D) FVC in African ancestry cohorts

Figure 2. Forest plots of the interaction meta-analysis of serum 25(OH)D and smoking status on FVC in the European ancestry cohorts (A) Current Smokers (B) Former Smokers

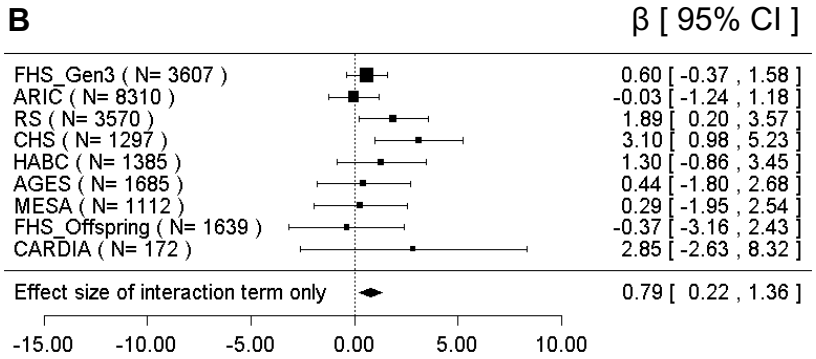
Figure 3. Meta-analysis of the association of serum 25(OH)D–PFT outcomes in the European ancestry cohorts, by smoking status; Smoking status includes never, former, and current smoker, and FEV_1 and FVC are presented for each smoking status.



β (unit: mL) denotes the coefficient from the fixed-effect meta-analysis for serum 25(OH)D on the pulmonary function outcome per 1 nmol/L increment of 25(OH)D, with its 95% confidence interval. Cohorts findings were ordered from the most precise to the least.
Abbreviations: 25(OH)D = 25-hydroxyvitamin D; AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; CI = Confidence Interval; EA = European ancestry; FEV_1 = forced expiratory volume in 1 second; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; FVC = forced vital capacity; HABC = Health, Aging, and Body Composition; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.

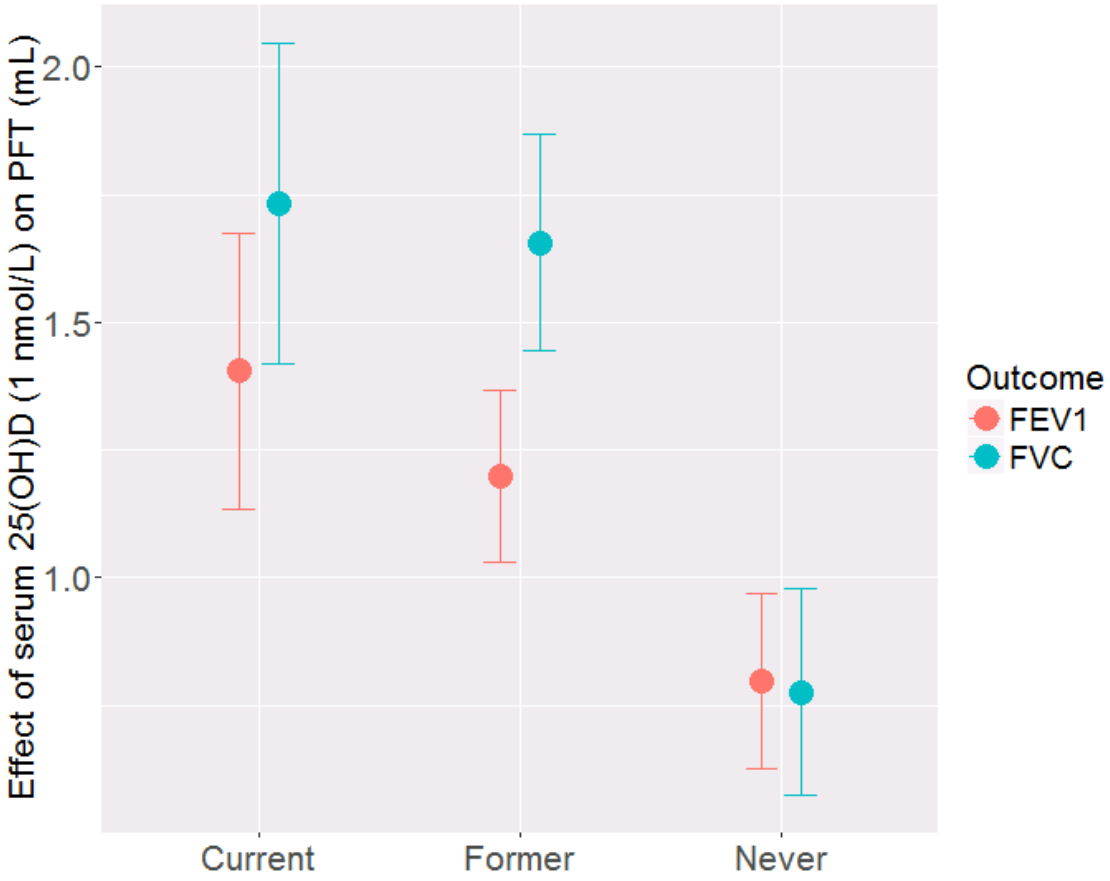


Interaction coefficient of serum 25(OH)D and current smoker on FVC



Interaction coefficient of serum 25(OH)D and former smoker on FVC

β (unit: mL) denotes the interaction term coefficient of 25(OH)D and smoking status on FVC from the fixed effect meta-analysis, per 1 nmol/L increment of 25(OH)D, with its 95% confidence interval. Cohorts were ordered from the most precise to the least.
Abbreviations: 25(OH)D = 25-hydroxyvitamin D; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; CI = Confidence Interval; FHS Offspring = Framingham Heart Study—Offspring Cohort; FHS_Gen3 = Framingham Heart Study—Generation 3 Cohort; FVC = forced vital capacity; HABC = Health, Aging, and Body Composition; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.



β (unit: mL) denotes that 1 nmol/L higher serum 25(OH)D was associated with a β mL higher FEV_1 (or FVC), calculated from an analysis including the interaction of serum 25(OH)D and smoking status. The error bar represents ± 1 standard error. We used 22,787 EA participants for the FEV_1 outcome and 22,777 EA participants for the FVC outcome.
Abbreviations: 25(OH)D = 25-hydroxyvitamin D; EA = European ancestry; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PFT = pulmonary function test.