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

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Online Supporting Material

Supplemental table, figures, and methods are available.

Abbreviation Footnote

25(OH)D, 25-Hydroxyvitamin D; AA, African Ancestry; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium; CHS, Cardiovascular Health Study; CLIA, Chemiluminescence Immunoassay; COPD, Chronic Obstructive Pulmonary Disease; EA, European Ancestry; FEV_1, Forced Expiratory Volume in the First 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 Study; LC-MS/MS, Liquid Chromatography in Tandem with Mass Spectrometry; MESA, Multi-Ethnic Study of Atherosclerosis; NHANES, National Health and Nutrition Examination Survey; PFT, Pulmonary Function Test; RIA, Radioimmunoassay; RS, Rotterdam (Netherlands) Study.

ABSTRACT

The role that vitamin D plays in pulmonary function remains uncertain. Epidemiological studies reported mixed findings for the association of serum 25-hydroxyvitamin D [25(OH)D] and pulmonary function. We conducted the largest cross-sectional meta-analysis of the 25(OH)D–pulmonary function association to date, based on nine European ancestry (EA) cohorts (*n*=22,838) and five African ancestry (AA) cohorts (*n*=4,290) in the CHARGE Consortium. Data were analyzed using linear models by cohort and ancestry. Effect modification by smoking status (current/former/never) was tested. Results were combined using fixed-effects meta-analysis. Mean (SD) serum 25(OH)D was 68 (29) nmol/L for EAs and 49 (21) nmol/L for AAs. For each 1 nmol/L higher 25(OH)D, forced expiratory volume in the first second (FEV_1) was higher by 1.1 mL in EAs (95% CI: 0.9,1.3; $P=2.5\times10^{-21}$) and 1.8 mL (95% CI: 1.1,2.5; $P=1.6\times10^{-7}$) in AAs ($P_{race\ difference}=0.06$), and forced vital capacity (FVC) was higher by 1.3 mL in EAs (95% CI: 1.0,1.6; $P=1.1\times10^{-20}$) and 1.5 mL (95% CI: 0.8,2.3; $P=1.2\times10^{-4}$) in AAs ($P_{race\ difference}=0.56$). Among EAs, the 25(OH)D–FVC association was stronger in smokers: per 1nmol/L higher 25(OH)D, FVC was higher by 1.7 mL (95% CI: 1.1,2.3) for current smokers and 1.7 mL (95% CI: 1.2,2.1) for former smokers, compared to 0.8 mL (95% CI: 0.4,1.2) for never smokers. In summary, the 25(OH)D associations with FEV_1 and FVC were positive in both ancestries. In EAs, a stronger association was observed for smokers compared to never smokers, which supports the importance of vitamin D in vulnerable populations.

Keywords: 25-hydroxyvitamin D; vitamin D; forced expiratory volume; vital capacity; respiratory function tests; smoking; human; adult; whites; African Americans.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), the third leading cause of mortality in the U.S.[1] and among the top 10 leading causes of total years of life lost in the world[2], 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 thereafter adults experience an age-related decline[3]. The majority of COPD cases (85%) are related to smoking[4], which alters the trajectory in pulmonary function, by hindering growth, reducing peak function, and accelerating age-related decline[5].

Vitamin D is proposed to have protective effects in the lungs via gene regulation[6]. *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[7]. In humans, 25-hydroxyvitamin D [25(OH)D] is the major vitamin D metabolite in serum, most of which forms a complex with vitamin D binding protein (~85-90% is DBP-bound)[8], and then is metabolized to 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], the active steroid hormone form[8, 9]. Total 25(OH)D is the commonly used biomarker of vitamin D status, and it is preferred to other vitamin D metabolites, such as non-DBP-bound 25(OH)D and 1,25-$(OH)_2D$, given that it is a comprehensive indicator for vitamin D stores, has a longer half-life (~3 weeks) and is less affected by calcium[10, 11]. On average, African ancestry (AA) populations have lower serum 25(OH)D concentrations, due to multiple factors including genetics and skin pigmentation[7], but there is evidence that AA populations have higher 1,25-$(OH)_2D$ levels and greater bone mineral density compared to European ancestry (EA) populations[12].

Previous observational cross-sectional studies of the vitamin D–pulmonary function association in the general population reported mixed findings. Most of these studies reported a positive association between 25(OH)D and pulmonary function[13-19], although some reported a null or inverse association[20-22], and two others reported a positive association under certain conditions, such as only in male current smokers[23] or only in overweight and obese males[24]. 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[16]. 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[13].

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. Only one cohort [Multi-Ethnic Study of Atherosclerosis (MESA)] has participants with other ancestries, and these other ancestries were not included in this study. 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 and European Respiratory Society 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)[25], or missing on other covariates (**Supplemental Table 1**).

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[26], were measured in each cohort using standardized methods defined by the American Thoracic Society/European Respiratory Society criteria (**Supplemental Table 2**). The methods used to measure 25(OH)D varied by cohort (**Supplemental Table 2**). Three cohorts, including MESA, the Atherosclerosis Risk In Communities (ARIC) study, and the Cardiovascular Health Study (CHS), used the current reference method, liquid chromatography in tandem with mass spectrometry (LC-

MS/MS); three cohorts, including FHS, the Coronary Artery Risk Development in Young Adults (CARDIA) study, and the Health, Aging, and Body Composition (HABC) study, used radioimmunoassay (RIA); one cohort, the Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland (AGES), used chemiluminescence immunoassay (CLIA); and one cohort [the Rotterdam Study (RS)] used electro-CLIA. Only MESA calibrated the serum 25(OH)D measurement against the standard reference material 972[27], which reflects the calendar time of the measurements in the cohorts, most of which occurred before the availability of the standard reference material (**Supplemental Table 3**). CARDIA measured serum vitamin D in a subset of participants included in an ancillary study of bone mineral homeostasis[28]. For the remaining cohorts, measurements of the outcome and exposure variables were planned for either the full cohort or a random sample (**Supplemental Table 1**). Continuous variables were used for serum 25(OH)D and pulmonary function to capture the association of 25(OH)D on PFTs across the broad distribution of ranges in the cohorts.

As shown in **Table 1**, among nine cohorts, four [AGES, 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, and the greatest mean time difference between 25(OH)D and PFT measurement was < 5 years [MESA]. Participants in ARIC and HABC had blood drawn for serum 25(OH)D after their PFT measure, but within 3 years.

Other covariates, including smoking status, pack-years (number of packs of cigarettes smoked per day times the number of years 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 (**Supplemental Table 3**). 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 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 lower mean serum 25(OH)D level in AA participants[7]. 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. Residual outliers, identified using the studentized residuals of the linear models (**Supplemental Methods** for more details), were excluded from all models. The model was extended to test the interaction between 25(OH)D and smoking status [never (reference group), former, and current smokers].

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[29]. The comparison of meta-analyzed coefficients of the 25(OH)D–PFT associations for the two ancestry groups was conducted using a Z test[30]. Meta-analysis of the interaction terms of 25(OH)D with smoking status was also performed (**Supplemental Methods** for more details).

Meta-regression was conducted to explore the potential causes of heterogeneity in the primary meta-analysis of serum 25(OH)D on FEV_1 (or FVC) in EAs. Modifiers were tested individually in the meta-regression models to investigate heterogeneity; modifiers included factors that could vary between cohorts, such as proportion of ever, current, and former smokers, mean 25(OH)D level, assay method for serum 25(OH)D, time between 25(OH)D and PFT measures, and mean age of participants in each cohort. The two-sided type I error was examined at 0.05 for all analyses. Meta-analysis and meta-regression were conducted using 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. EA participants had higher FEV_1, FVC, and serum 25(OH)D than AA participants in each cohort, while FEV_1/FVC was similar across ancestry groups (**Table 1** and **Supplemental Figure 1**). 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 highest among never smokers [mean(SD) = 70(30) nmol/L], followed by former smokers [67 (29) nmol/L], and current smokers [64 (29) nmol/L] in EAs, while the trend was less obvious in AAs [49 (21) nmol/L in current smokers, 50 (21) nmol/L in former smokers, and 48 (21) nmol/L in never smokers]. The mean (SD) of serum 25(OH)D for EA participants across nine cohorts was 68 (29) nmol/L and for AA participants across five cohorts the mean (SD) was 49 (21) nmol/L.

Regression coefficients (β) and standard errors (SE) calculated within each cohort per 1 nmol/L 25(OH)D are presented in the figures. Additionally, to put the magnitude of the 25(OH)D–PFT associations in terms relevant to public health, the meta-analyzed regression coefficients were multiplied by 10 nmol/L 25(OH)D, which is 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. To put these findings into context, a 10 nmol/L (~0.5 SD) higher 25(OH)D was associated with 11.1 mL higher FEV_1 in EAs ($P = 2.5\times10^{-21}$) and 17.9 mL higher FEV_1 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 only in EAs (P = 0.0013), and the magnitude was negligible; a 10 nmol/L higher 25(OH)D was associated with a ratio being lower by 0.0055% (**Supplemental Table 4 and Supplemental Figure 2** for ancestry- and cohort-specific findings).

In the main-effect meta-analysis of serum 25(OH)D on pulmonary function, there was low to moderate heterogeneity in the EA cohorts, and low heterogeneity in the AA cohorts (**Figure 1, Supplemental Figure 2**). We used meta-regression to explore potential causes of moderate heterogeneity in the meta-analysis of 25(OH)D on FEV_1 and FVC in the EA cohorts. Cohorts with lower mean 25(OH)D concentration had stronger 25(OH)D–PFT associations (**Figure 2**).

The proportion of ever smokers and of former smokers had significant linear associations with the 25(OH)D–PFT coefficients (**Supplemental Figure 3**), and these two variables were both highly correlated with mean 25(OH)D levels (Pearson's $r > 0.75$ for all pairwise correlations). The 25(OH)D–PFT association in EA cohorts varied by 25(OH)D assay method (meta-regression $p < 0.02$); the association was attenuated in cohorts using RIA compared to cohorts using LC-MS/MS (pairwise $p < 0.005$, **Supplemental Figure 4**). Mean age of each cohort was a significant positive modifier of the 25(OH)D–FEV_1 association, while time difference between 25(OH)D and spirometry measures did not affect the 25(OH)D–PFT association (**Supplemental Figure 3**).

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 (**Supplemental Table 5 and 6**) from models that included the 25(OH)D × smoking status interaction terms were combined in secondary meta-analyses (**Supplemental Table 7**). 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 3**). For the FEV_1 outcome 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 = 0.14) or former smokers (P = 0.14). There was no statistical evidence of interaction of 25(OH)D and cigarette smoking in the AA cohorts for either outcome. To put the interaction finding into context, a 10 nmol/L higher serum 25(OH)D was associated with a 17.3 mL higher FVC in current smokers and a 16.6 mL higher FVC in former smokers, which was more than double the association magnitude in never smokers (β = 7.8 mL). 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 14.0 mL in current smokers, 12.0 mL in former smokers, and 8.0 mL in never smokers (**Figure 4**).

DISCUSSION

This study investigated the association of serum 25(OH)D with pulmonary function using multiple cohorts of different ancestries. We found a consistently positive association of serum 25(OH)D with FEV_1 and FVC across both EA and AA groups. In addition, in the EA group, a significantly stronger association was observed for current and former smokers, compared to never smokers.

A previous cross-sectional study in a European ancestry population (two Copenhagen cohorts: *n* = 10,116 and *n* = 8,391 respectively) similarly reported positive associations of 25(OH)D with FEV_1 percentage predicted and FVC percentage predicted, but not with FEV_1/FVC[16]. The magnitude of the association was about four times greater in the Copenhagen study, which may be due to the difference in the mean serum 25(OH)D (Danish median ~42 nmol/L vs. CHARGE median of ~65 nmol/L) given our finding that 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 in magnitude to the association reported in a British cohort of 6,789 participants with an average age of 45 years[17], but weaker than a previous report from the FHS cohort[15]. Given that the rate of decline in FEV_1 at age 45 is increased by ~15 mL/year in current smokers[31], 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 causal association between low 25(OH)D levels and low pulmonary function include an altered immune response that increases susceptibility to inflammation, a reduction in pulmonary parenchyma related to extracellular matrix homeostasis important for lung structure, and/or a decrease in serum calcium that could adversely affect thoracic skeleton mobility and respiratory muscle performance[32].

Our findings show that the association of serum 25(OH)D with FEV_1 and FVC were stronger in magnitude in AA versus EA participants, although the difference by race did not reach statistical significance. The finding may reflect the lower serum 25(OH)D in AA participants, which is consistent with the meta-regression finding and with a previous study reporting attenuated associations at higher serum 25(OH)D (15). 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 cross-sectional 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 = 0.06)[13]. 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 more fully than the proportion of current smokers; this may be explained by a survival bias in older participants who were current smokers. The meta-regression based on mean age of the cohorts yielded findings that were consistent with a prior NHANES study that showed the association of 25(OH)D and FEV_1 was greater in people over age 60 compared to younger individuals[13].

There are several methodological considerations in interpreting the findings of this study. First, the meta-regression showed stronger 25(OH)D–PFT associations in cohorts with lower mean serum 25(OH)D, indicating a non-linear 25(OH)D–PFT association. This finding is consistent with a prior study in the FHS cohort, which reported a non-linear association and a stronger 25(OH)D–FEV_1 association in participants at risk of vitamin D deficiency (< 30 nmol/L)[15]. Second, serum 25(OH)D was measured by four different methods across the cohorts. For example, two cohorts with high mean 25(OH)D (> 90 nmol/L) used RIA methods. These same cohorts had a lower magnitude estimate of the 25(OH)D–PFT association; if the higher mean

represents the 'truth' (and is not caused by measurement error in the RIA assay), then the lower 25(OH)D–PFT association may be primarily driven by the vitamin D distribution and not by the RIA method. Whether the assay method itself directly influences the estimate of the 25(OH)D–PFT association requires further data. Third, in this cross-sectional meta-analysis, there were minor differences in the time separation between the measurement of serum 25(OH)D and pulmonary function, but the meta-regression test for heterogeneity confirmed that time separation between measurements did not affect the 25(OH)D–PFT associations. Indeed, 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[33], and 0.42-0.52 for measurements separated by 14 years[34], which supports the use of a single 25(OH)D measurement to represent usual level. Fourth, residual confounding was unlikely given the consistent results across multiple cohorts in various settings. Weight was adjusted for the FVC outcome, given that higher weight and adiposity negatively affects lung volume (i.e., FVC)[35]; weight was not adjusted in the FEV_1 models, given FEV_1 is a measure of airways obstruction and not physical restriction of lung volume. Physical activity was not adjusted because it is not a confounder in estimating the serum 25(OH)D–PFT association; while physical activity is known to contribute to oxygen utilization in lungs[36] there is little evidence and no biological rationale for a causal association of physical activity with either FEV_1 or FVC[37], which are markers for airways obstruction and lung volume, respectively. Finally, we do not expect selection bias to affect the estimate of the serum vitamin D–PFT association in this meta-analysis; the association magnitude and direction was consistent across all cohorts, regardless of the proportion of the original cohort contributing to the analysis. Thus, selection bias is expected to be negligible and would likely lead to an underestimated association, given the participants retained in the cohorts are expected to be, on average, healthier than those who were lost 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[38-40], 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 and former 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. PAC, DBH and JX had primary responsibility for final content. All authors provided data, analytic support and/or study design suggestions at all stages, critically reviewed the manuscript, and read and approved the final version.

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Table 1. Cross-sectional participant characteristics of each cohort in the CHARGE Consortium (*n* = 27,128)[*]

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)
Method of 25(OH)D measurement	LC-MS/MS	RIA	LC-MS/MS	RIA	LC-MS/MS	CLIA	RIA	RIA	Electro-CLIA
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)
Method of 25(OH)D measurement	LC-MS/MS	RIA	LC-MS/MS	RIA	LC-MS/MS
Time from 25(OH)D to PFT, days[‡‡]	-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; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CLIA, Chemiluminescence Immunoassay; FEV_1, Forced Expiratory Volume in the First 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 Study; LC-MS/MS, Liquid Chromatography in Tandem with Mass Spectrometry; MESA, Multi-Ethnic Study of Atherosclerosis; RIA, Radioimmunoassay; RS, Rotterdam (Netherlands) Study.

[*] Data are presented as mean (SD) unless otherwise indicated; AGES, RS, and FHS only have participants of European ancestry; n = 22,838 for EAs, n = 4,290 for AAs, total n = 27,128.

[†] The number of participants used to compute descriptive statistics in CHS excluded those who had residual outliers based on the preliminary models (n = 8 for EAs and n = 6 for AAs); 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 = 1385 for EAs and n = 821 for AAs; for the ratio outcome, n = 1382 for EAs and n = 817 for AAs). The numbers of participants for the FEV_1 outcome are used. However, the descriptive statistics is similar across different outcomes.

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

[‖] 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.

[¶] 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.

[††] 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.

[‡‡] 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%).

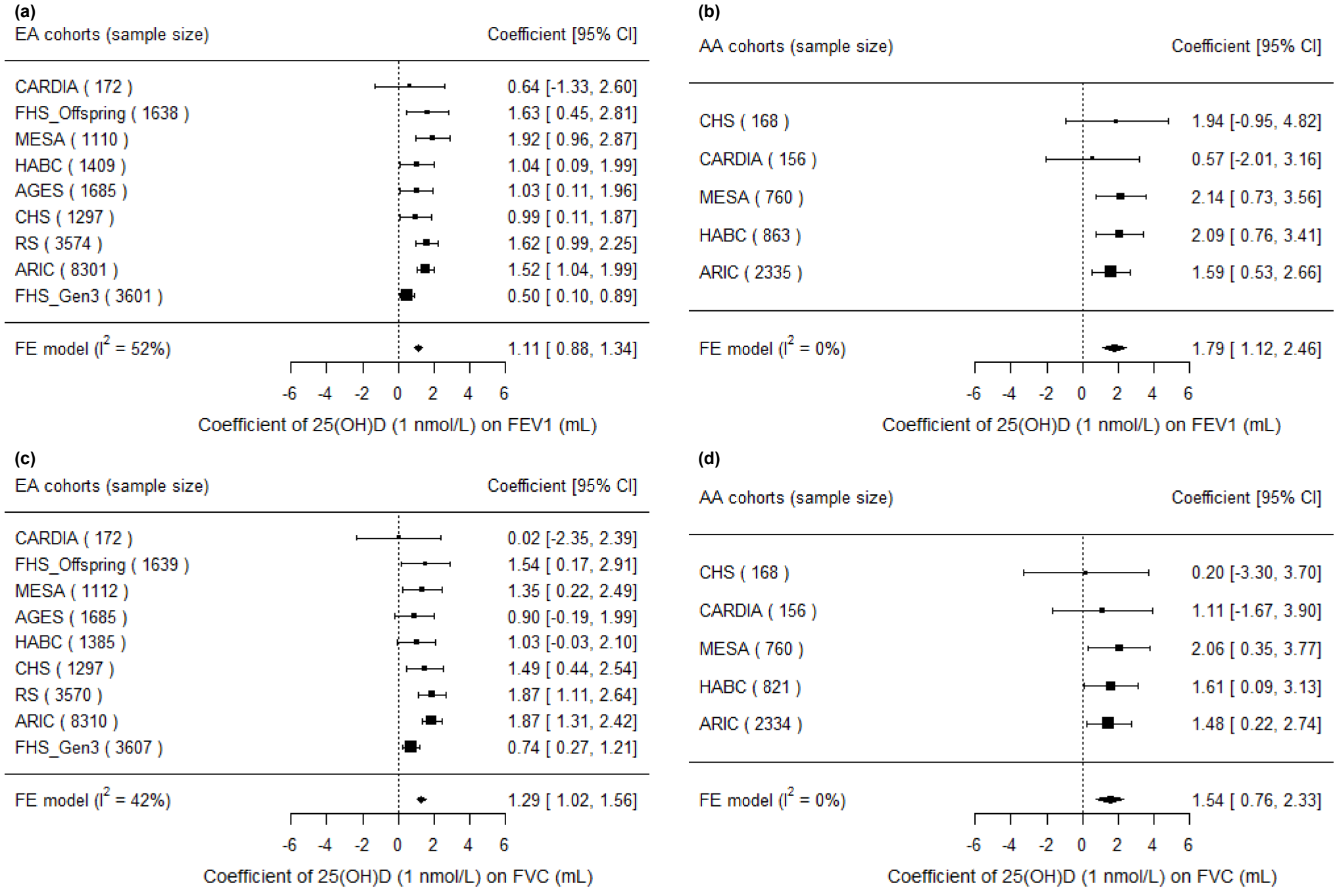
Figure 1. Forest plots of the meta-analysis of serum 25(OH)D on FEV_1 and FVC across cohorts in the CHARGE Consortium, stratified by participant ancestry. Associations are presented for serum 25(OH)D on (A) FEV_1 in European ancestry cohorts (*n* = 22,787). (B) FEV_1 in African ancestry cohorts (*n* = 4,282). (C) FVC in European ancestry cohorts (*n* = 22,777). (D) FVC in African ancestry cohorts (*n* = 4,239). β (unit: mL) denotes the coefficient from the fixed-effects 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 least to the most precise, and heterogeneity is presented (I^2).
Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; AA, African Ancestry; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CI, Confidence Interval; EA, European Ancestry; FE, Fixed-Effects; FEV_1, Forced Expiratory Volume in the First 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 Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.

Figure 2. Meta-regression of mean serum 25(OH)D levels against the association estimates of 25(OH)D with PFT in nine European ancestry cohorts in the CHARGE Consortium. (A) FEV_1 outcome (coefficient unit: mL per 1 nmol/L 25(OH)D), and (B) FVC outcome (coefficient unit: mL per 1nmol/L 25(OH)D). The modifier is mean serum 25(OH)D level of each nine cohorts. A linear regression line is present for each sub-figure, with a meta-regression p-value of 0.0006 for the FEV_1 outcome, and 0.005 for the FVC outcome. The figure also shows the measurement method for the serum 25(OH)D assay (legend shows symbols for each of the 4 assay methods).
Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CLIA, Chemiluminescence Immunoassay; FEV_1, Forced Expiratory Volume in the First 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 Study; LC-MS/MS, Liquid Chromatography in Tandem with Mass Spectrometry; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, Pulmonary Function Test; RIA, Radioimmunoassay; RS, Rotterdam (Netherlands) Study.

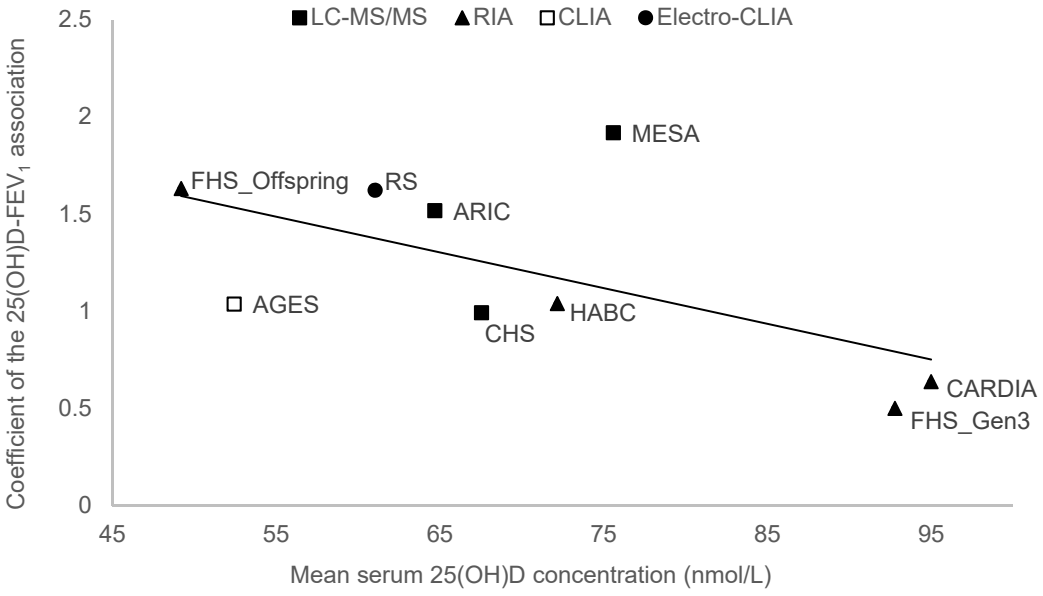
Figure 3. Forest plots of the interaction meta-analysis of serum 25(OH)D and smoking status on FVC in the European ancestry cohorts in the CHARGE Consortium (*n* = 22,777). (A) Current Smokers and (B) Former Smokers. β (unit: mL) denotes the interaction term coefficient of 25(OH)D and smoking status on FVC from the fixed effects meta-analysis, per 1 nmol/L increment of 25(OH)D, with its 95% confidence interval. Cohorts were ordered from the least to the most precise, and heterogeneity is presented (I^2).
Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CI, Confidence Interval; EA, European Ancestry; FE, Fixed-Effects; 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 Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.

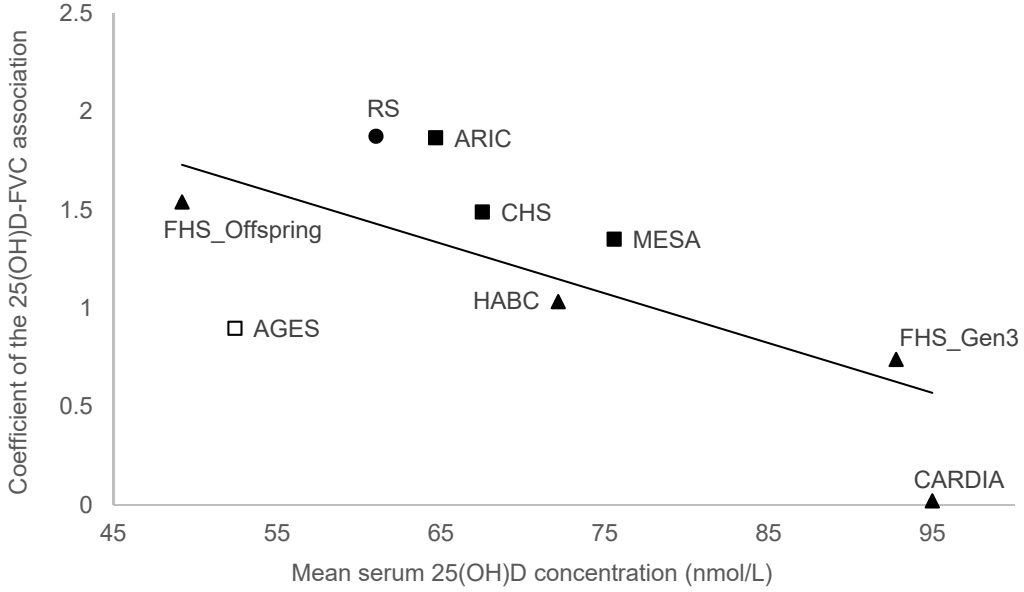
Figure 4. Meta-analysis of the association of serum 25(OH)D–PFT outcomes among current, former, and never smokers in the European ancestry cohorts in the CHARGE Consortium. FEV_1 and FVC are presented for each smoking status. β (unit: mL) denotes that 1 nmol/L higher serum 25(OH)D was associated with 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.
Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; EA, European Ancestry; FEV_1, Forced Expiratory Volume in the First Second; FVC, Forced Vital Capacity; PFT, Pulmonary Function Test.


(a)
EA cohorts (sample size)
Coefficient [95% CI]
CARDIA (172) 0.64 [-1.33, 2.60]
FHS_Offspring (1638) 1.63 [0.45, 2.81]
MESA (1110) 1.92 [0.96, 2.87]
HABC (1409) 1.04 [0.09, 1.99]
AGES (1685) 1.03 [0.11, 1.96]
CHS (1297) 0.99 [0.11, 1.87]
RS (3574) 1.62 [0.99, 2.25]
ARIC (8301) 1.52 [1.04, 1.99]
FHS_Gen3 (3601) 0.50 [0.10, 0.89]
FE model (I^2 = 52%) 1.11 [0.88, 1.34]
-6 -4 -2 0 2 4 6
Coefficient of 25(OH)D (1 nmol/L) on FEV1 (mL)
(b)
AA cohorts (sample size)
Coefficient [95% CI]
CHS (168) 1.94 [-0.95, 4.82]
CARDIA (156) 0.57 [-2.01, 3.16]
MESA (760) 2.14 [0.73, 3.56]
HABC (863) 2.09 [0.76, 3.41]
ARIC (2335) 1.59 [0.53, 2.66]
FE model (I^2 = 0%) 1.79 [1.12, 2.46]
-6 -4 -2 0 2 4 6
Coefficient of 25(OH)D (1 nmol/L) on FEV1 (mL)
(c)
EA cohorts (sample size)
Coefficient [95% CI]
CARDIA (172) 0.02 [-2.35, 2.39]
FHS_Offspring (1639) 1.54 [0.17, 2.91]
MESA (1112) 1.35 [0.22, 2.49]
AGES (1685) 0.90 [-0.19, 1.99]
HABC (1385) 1.03 [-0.03, 2.10]
CHS (1297) 1.49 [0.44, 2.54]
RS (3570) 1.87 [1.11, 2.64]
ARIC (8310) 1.87 [1.31, 2.42]
FHS_Gen3 (3607) 0.74 [0.27, 1.21]
FE model (I^2 = 42%) 1.29 [1.02, 1.56]
-6 -4 -2 0 2 4 6
Coefficient of 25(OH)D (1 nmol/L) on FVC (mL)
(d)
AA cohorts (sample size)
Coefficient [95% CI]
CHS (168) 0.20 [-3.30, 3.70]
CARDIA (156) 1.11 [-1.67, 3.90]
MESA (760) 2.06 [0.35, 3.77]
HABC (821) 1.61 [0.09, 3.13]
ARIC (2334) 1.48 [0.22, 2.74]
FE model (I^2 = 0%) 1.54 [0.76, 2.33]
-6 -4 -2 0 2 4 6
Coefficient of 25(OH)D (1 nmol/L) on FVC (mL)

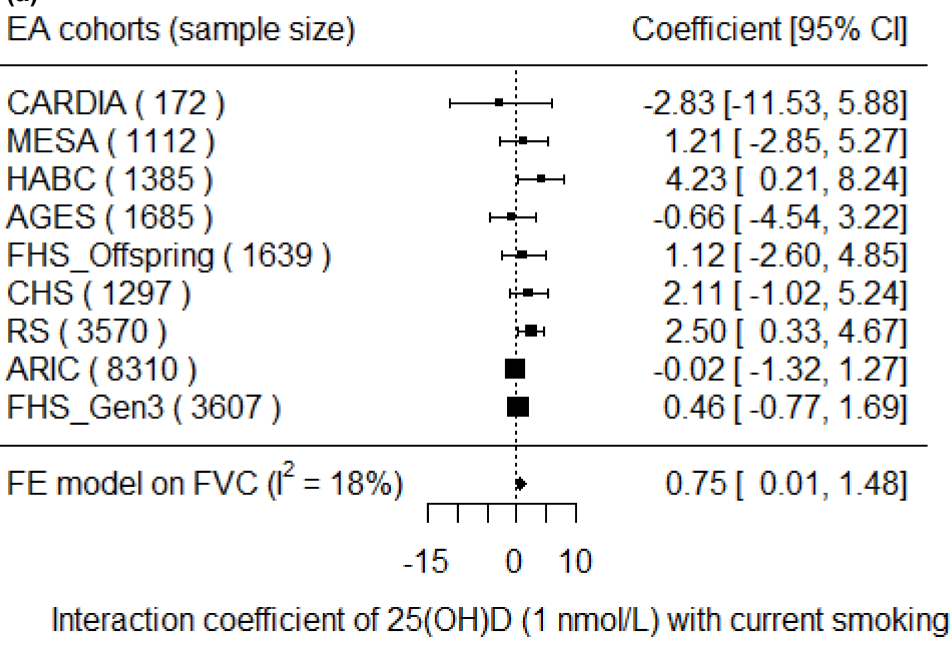
(a)


LC-MS/MS
RIA
CLIA
Electro-CLIA
Coefficient of the 25(OH)D-FEV1 association
2.5
2
1.5
1
0.5
0
45
55
65
75
85
95
Mean serum 25(OH)D concentration (nmol/L)
MESA
FHS_Offspring
RS
ARIC
AGES
HABC
CHS
CARDIA
FHS_Gen3

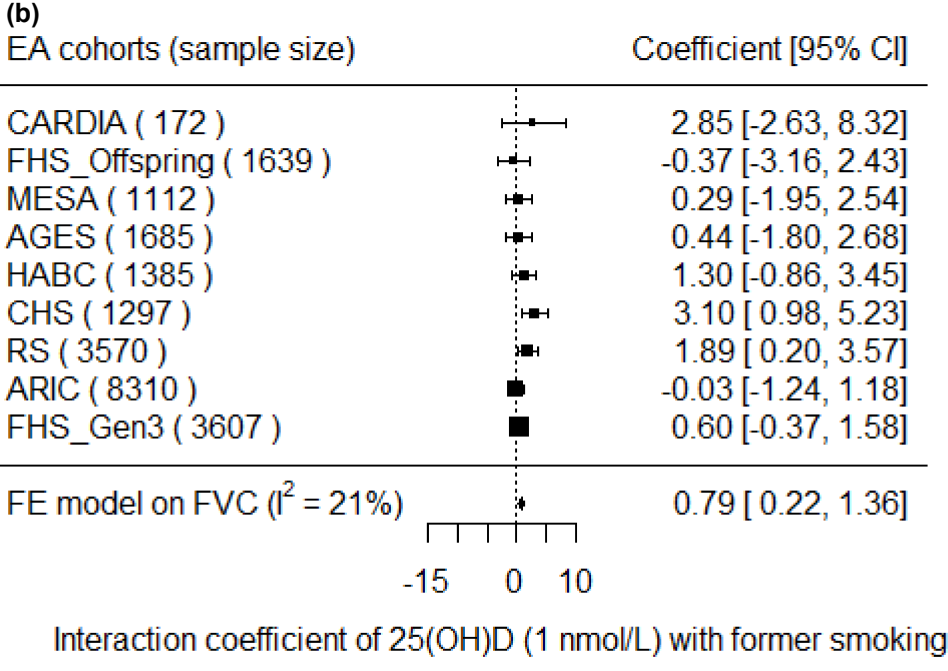
(b)

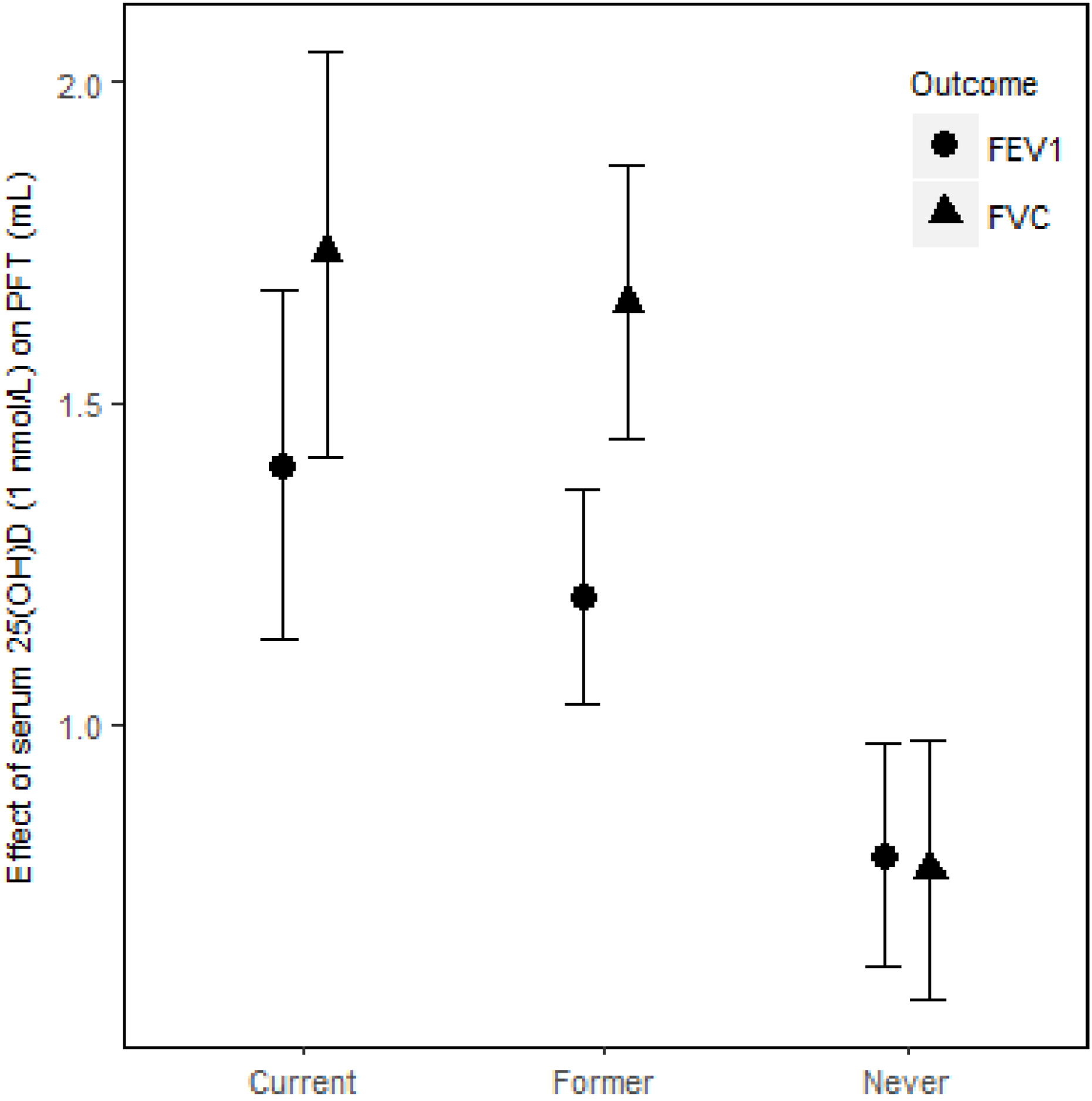

Coefficient of the 25(OH)D-FVC association
2.5
2
1.5
1
0.5
0
45
55
65
75
85
95
Mean serum 25(OH)D concentration (nmol/L)
RS
ARIC
CHS
FHS_Offspring
MESA
HABC
AGES
FHS_Gen3
CARDIA

(a)


EA cohorts (sample size)
Coefficient [95% CI]
CARDIA (172)
-2.83 [-11.53, 5.88]
MESA (1112)
1.21 [-2.85, 5.27]
HABC (1385)
4.23 [0.21, 8.24]
AGES (1685)
-0.66 [-4.54, 3.22]
FHS_Offspring (1639)
1.12 [-2.60, 4.85]
CHS (1297)
2.11 [-1.02, 5.24]
RS (3570)
2.50 [0.33, 4.67]
ARIC (8310)
-0.02 [-1.32, 1.27]
FHS_Gen3 (3607)
0.46 [-0.77, 1.69]
FE model on FVC (I2 = 18%)
0.75 [0.01, 1.48]
-15
0
10
Interaction coefficient of 25(OH)D (1 nmol/L) with current smoking

(b)


EA cohorts (sample size)
Coefficient [95% CI]
CARDIA (172)
2.85 [-2.63, 8.32]
FHS_Offspring (1639)
-0.37 [-3.16, 2.43]
MESA (1112)
0.29 [-1.95, 2.54]
AGES (1685)
0.44 [-1.80, 2.68]
HABC (1385)
1.30 [-0.86, 3.45]
CHS (1297)
3.10 [0.98, 5.23]
RS (3570)
1.89 [0.20, 3.57]
ARIC (8310)
-0.03 [-1.24, 1.18]
FHS_Gen3 (3607)
0.60 [-0.37, 1.58]
FE model on FVC (I2 = 21%)
0.79 [0.22, 1.36]
-15
0
10
Interaction coefficient of 25(OH)D (1 nmol/L) with former smoking


Effect of serum 25(OH)D (1 nmol/L) on PFT (mL)
2.0
1.5
1.0
Current
Former
Never
Outcome
FEV1
FVC