

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; FEV₁, 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.

1 **ABSTRACT**

2 The role that vitamin D plays in pulmonary function remains uncertain. Epidemiological studies
3 reported mixed findings for the association of serum 25-hydroxyvitamin D [25(OH)D] and
4 pulmonary function. We conducted the largest cross-sectional meta-analysis of the 25(OH)D–
5 pulmonary function association to date, based on nine European ancestry (EA) cohorts
6 ($n=22,838$) and five African ancestry (AA) cohorts ($n=4,290$) in the CHARGE Consortium. Data
7 were analyzed using linear models by cohort and ancestry. Effect modification by smoking status
8 (current/former/never) was tested. Results were combined using fixed-effects meta-analysis.
9 Mean (SD) serum 25(OH)D was 68 (29) nmol/L for EAs and 49 (21) nmol/L for AAs. For each
10 1 nmol/L higher 25(OH)D, forced expiratory volume in the first second (FEV₁) was higher by
11 1.1 mL in EAs (95% CI: 0.9,1.3; $P=2.5\times 10^{-21}$) and 1.8 mL (95% CI: 1.1,2.5; $P=1.6\times 10^{-7}$) in AAs
12 ($P_{\text{race difference}}=0.06$), and forced vital capacity (FVC) was higher by 1.3 mL in EAs (95% CI:
13 1.0,1.6; $P=1.1\times 10^{-20}$) and 1.5 mL (95% CI: 0.8,2.3; $P=1.2\times 10^{-4}$) in AAs ($P_{\text{race difference}}=0.56$).
14 Among EAs, the 25(OH)D–FVC association was stronger in smokers: per 1nmol/L higher
15 25(OH)D, FVC was higher by 1.7 mL (95% CI: 1.1,2.3) for current smokers and 1.7 mL (95%
16 CI: 1.2,2.1) for former smokers, compared to 0.8 mL (95% CI: 0.4,1.2) for never smokers. In
17 summary, the 25(OH)D associations with FEV₁ and FVC were positive in both ancestries. In
18 EAs, a stronger association was observed for smokers compared to never smokers, which
19 supports the importance of vitamin D in vulnerable populations.

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

22 INTRODUCTION

23 Chronic obstructive pulmonary disease (COPD), the third leading cause of mortality in the
24 U.S.⁽¹⁾ and among the top 10 leading causes of total years of life lost in the world⁽²⁾, is
25 characterized by progressive airway obstruction. Pulmonary function tests (PFTs), as performed
26 by spirometry, are used to quantify pulmonary function parameters including forced expiratory
27 volume in the first second (FEV₁) and forced vital capacity (FVC). Pulmonary function increases
28 throughout childhood, plateaus in the 20s, and thereafter adults experience an age-related
29 decline⁽³⁾. The majority of COPD cases (85%) are related to smoking⁽⁴⁾, which alters the
30 trajectory in pulmonary function, by hindering growth, reducing peak function, and accelerating
31 age-related decline⁽⁵⁾.

32 Vitamin D is proposed to have protective effects in the lungs via gene regulation⁽⁶⁾. *In vitro*
33 studies found that 1,25-dihydroxyvitamin D, the active vitamin D metabolite, induced
34 antimicrobial peptides for host defense in the lung and modulated airway remodeling⁽⁷⁾. In
35 humans, 25-hydroxyvitamin D [25(OH)D] is the major vitamin D metabolite in serum, most of
36 which forms a complex with vitamin D binding protein (~85-90% is DBP-bound)⁽⁸⁾, and then is
37 metabolized to 1,25-dihydroxyvitamin D [1,25-(OH)₂D], the active steroid hormone form^(8, 9).
38 Total 25(OH)D is the commonly used biomarker of vitamin D status, and it is preferred to other
39 vitamin D metabolites, such as non-DBP-bound 25(OH)D and 1,25-(OH)₂D, given that it is a
40 comprehensive indicator for vitamin D stores, has a longer half-life (~3 weeks) and is less
41 affected by calcium^(10, 11). On average, African ancestry (AA) populations have lower serum
42 25(OH)D concentrations, due to multiple factors including genetics and skin pigmentation⁽⁷⁾, but
43 there is evidence that AA populations have higher 1,25-(OH)₂D levels and greater bone mineral
44 density compared to European ancestry (EA) populations⁽¹²⁾.

45 Previous observational cross-sectional studies of the vitamin D–pulmonary function association
46 in the general population reported mixed findings. Most of these studies reported a positive
47 association between 25(OH)D and pulmonary function⁽¹³⁻¹⁹⁾, although some reported a null or
48 inverse association⁽²⁰⁻²²⁾, and two others reported a positive association under certain conditions,
49 such as only in male current smokers⁽²³⁾ or only in overweight and obese males⁽²⁴⁾. The largest
50 previous cross-sectional study, which included two Danish cohorts (total $n = 18,507$), reported
51 positive associations of 25(OH)D with pulmonary function⁽¹⁶⁾. Only one prior cross-sectional

52 study investigated serum 25(OH)D and pulmonary function in an ancestry group other than
53 European, and it confirmed similar positive associations in the 3,957 AA participants studied⁽¹³⁾.

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

60 **MATERIALS AND METHODS**

61 **Cohorts and Participants**

62 Nine prospective cohorts in the CHARGE Consortium were included (**Table 1**). All cohorts had
63 EA participants, and five of the cohorts had AA participants. Only one cohort [Multi-Ethnic
64 Study of Atherosclerosis (MESA)] has participants with other ancestries, and these other
65 ancestries were not included in this study. Among the nine cohorts, the Framingham Heart Study
66 (FHS) had two sub-cohorts analyzed separately: the Offspring and the Third-Generation (Gen3)
67 cohorts. Our analysis pipeline harmonized the outcome and exposure definitions, the units on all
68 variables, and the statistical modeling. The same exclusion criteria were applied to each cohort:
69 missing PFTs, unacceptable PFTs using the American Thoracic Society and European
70 Respiratory Society criteria for acceptability, missing serum 25(OH)D, serum 25(OH)D > 374.4
71 nmol/L (or 150ng/mL, leading to removal of a single outlier)⁽²⁵⁾, or missing on other covariates
72 (**Supplemental Table 1**).

73 **Outcome and Exposure Assessment**

74 Pre-bronchodilator pulmonary function outcomes (FEV₁, FVC, and FEV₁/FVC), which have
75 similar accuracy as post-bronchodilator measures for long-term outcomes⁽²⁶⁾, were measured in
76 each cohort using standardized methods defined by the American Thoracic Society/European
77 Respiratory Society criteria (**Supplemental Table 2**). The methods used to measure 25(OH)D
78 varied by cohort (**Supplemental Table 2**). Three cohorts, including MESA, the Atherosclerosis
79 Risk In Communities (ARIC) study, and the Cardiovascular Health Study (CHS), used the
80 current reference method, liquid chromatography in tandem with mass spectrometry (LC-

81 MS/MS); three cohorts, including FHS, the Coronary Artery Risk Development in Young Adults
82 (CARDIA) study, and the Health, Aging, and Body Composition (HABC) study, used
83 radioimmunoassay (RIA); one cohort, the Age, Gene, Environment, Susceptibility Study—
84 Reykjavik, Iceland (AGES), used chemiluminescence immunoassay (CLIA); and one cohort [the
85 Rotterdam Study (RS)] used electro-CLIA. Only MESA calibrated the serum 25(OH)D
86 measurement against the standard reference material 972⁽²⁷⁾, which reflects the calendar time of
87 the measurements in the cohorts, most of which occurred before the availability of the standard
88 reference material (**Supplemental Table 3**). CARDIA measured serum vitamin D in a subset of
89 participants included in an ancillary study of bone mineral homeostasis⁽²⁸⁾. For the remaining
90 cohorts, measurements of the outcome and exposure variables were planned for either the full
91 cohort or a random sample (**Supplemental Table 1**). Continuous variables were used for serum
92 25(OH)D and pulmonary function to capture the association of 25(OH)D on PFTs across the
93 broad distribution of ranges in the cohorts.

94 As shown in **Table 1**, among nine cohorts, four [AGES, CHS, FHS-Offspring, and FHS-Gen3]
95 had a mean time difference of less than one year in the PFT measurements and the preceding
96 25(OH)D measurement, and the greatest mean time difference between 25(OH)D and PFT
97 measurement was < 5 years [MESA]. Participants in ARIC and HABC had blood drawn for
98 serum 25(OH)D after their PFT measure, but within 3 years.

99 Other covariates, including smoking status, pack-years (number of packs of cigarettes smoked
100 per day times the number of years smoked), height, weight, and age, were measured concurrently
101 with pulmonary function, except for CHS, which assessed covariates concurrent with the serum
102 25(OH)D measure, but within 1 year of the PFT measurement (**Supplemental Table 3**). All data
103 collection and analysis was approved by the Institutional Review Board at each cohort's
104 respective institution. Spirometry measures are available on the database of Genotypes and
105 Phenotypes via accession numbers as follows: ARIC (phs000280), CARDIA (phs000285), CHS
106 (phs000287), FHS (phs000007), and MESA (phs000209). Serum vitamin D measures are also
107 available at the same accession numbers for CHS, FHS, and MESA.

108 **Statistical Analysis in Individual Cohorts**

109 All analyses were first conducted independently in each cohort, stratified by ancestry, given the
110 lower mean serum 25(OH)D level in AA participants⁽⁷⁾. For FEV₁ and FEV₁/FVC, models were
111 adjusted for smoking status, pack-years, height, height squared, age, age squared, sex, season of
112 blood draw, and study center (if applicable); for FVC, the model was further adjusted for weight.
113 Residual outliers, identified using the studentized residuals of the linear models (**Supplemental**
114 **Methods** for more details), were excluded from all models. The model was extended to test the
115 interaction between 25(OH)D and smoking status [never (reference group), former, and current
116 smokers].

117 **Meta-Analysis**

118 Fixed-effects meta-analysis was conducted for the association of serum 25(OH)D on each PFT
119 outcome for each ancestry group, using inverse variance weighting, with heterogeneity assessed
120 via the I² statistic⁽²⁹⁾. The comparison of meta-analyzed coefficients of the 25(OH)D–PFT
121 associations for the two ancestry groups was conducted using a Z test⁽³⁰⁾. Meta-analysis of the
122 interaction terms of 25(OH)D with smoking status was also performed (**Supplemental Methods**
123 for more details).

124 Meta-regression was conducted to explore the potential causes of heterogeneity in the primary
125 meta-analysis of serum 25(OH)D on FEV₁ (or FVC) in EAs. Modifiers were tested individually
126 in the meta-regression models to investigate heterogeneity; modifiers included factors that could
127 vary between cohorts, such as proportion of ever, current, and former smokers, mean 25(OH)D
128 level, assay method for serum 25(OH)D, time between 25(OH)D and PFT measures, and mean
129 age of participants in each cohort. The two-sided type I error was examined at 0.05 for all
130 analyses. Meta-analysis and meta-regression were conducted using the metafor package (version
131 1.9-8) in R (version 3.2.3., R Foundation for Statistical Computing, Vienna, Austria).

132 **RESULTS**

133 We studied 22,838 EA and 4,290 AA participants. EA participants had higher FEV₁, FVC, and
134 serum 25(OH)D than AA participants in each cohort, while FEV₁/FVC was similar across
135 ancestry groups (**Table 1** and **Supplemental Figure 1**). CARDIA and FHS-Gen3 were younger
136 than the seven other cohorts, with consequently lower pack-years smoked in ever smokers.

137 Across all cohorts, among EA participants, 17% were current smokers and 40% were former
138 smokers; among AA participants, 22% were current smokers and 30% were former smokers. The
139 serum 25(OH)D level was highest among never smokers [mean(SD) = 70(30) nmol/L], followed
140 by former smokers [67 (29) nmol/L], and current smokers [64 (29) nmol/L] in EAs, while the
141 trend was less obvious in AAs [49 (21) nmol/L in current smokers, 50 (21) nmol/L in former
142 smokers, and 48 (21) nmol/L in never smokers]. The mean (SD) of serum 25(OH)D for EA
143 participants across nine cohorts was 68 (29) nmol/L and for AA participants across five cohorts
144 the mean (SD) was 49 (21) nmol/L.

145 Regression coefficients (β) and standard errors (SE) calculated within each cohort per 1 nmol/L
146 25(OH)D are presented in the figures. Additionally, to put the magnitude of the 25(OH)D–PFT
147 associations in terms relevant to public health, the meta-analyzed regression coefficients were
148 multiplied by 10 nmol/L 25(OH)D, which is about half of the standard deviation (SD) of the
149 25(OH)D distribution.

150 Meta-analysis (**Figure 1**) revealed a consistently positive association of serum 25(OH)D with the
151 PFT outcomes, FEV₁ and FVC, in both ancestry groups. To put these findings into context, a 10
152 nmol/L (~0.5 SD) higher 25(OH)D was associated with 11.1 mL higher FEV₁ in EAs ($P =$
153 2.5×10^{-21}) and 17.9 mL higher FEV₁ in AAs ($P = 1.6 \times 10^{-7}$). Similarly, for a 10 nmol/L higher
154 25(OH)D, FVC was higher by 12.9 mL in EAs ($P = 1.1 \times 10^{-20}$) and by 15.4 mL in AAs ($P =$
155 1.2×10^{-4}). The magnitudes of the 25(OH)D–PFT associations did not differ significantly between
156 the two ancestry groups ($P = 0.06$ and $P = 0.56$ for FEV₁ and FVC, respectively). The association
157 of serum 25(OH)D with FEV₁/FVC reached statistical significance only in EAs ($P = 0.0013$),
158 and the magnitude was negligible; a 10 nmol/L higher 25(OH)D was associated with a ratio
159 being lower by 0.0055% (**Supplemental Table 4 and Supplemental Figure 2** for ancestry- and
160 cohort-specific findings).

161 In the main-effect meta-analysis of serum 25(OH)D on pulmonary function, there was low to
162 moderate heterogeneity in the EA cohorts, and low heterogeneity in the AA cohorts (**Figure 1,**
163 **Supplemental Figure 2**). We used meta-regression to explore potential causes of moderate
164 heterogeneity in the meta-analysis of 25(OH)D on FEV₁ and FVC in the EA cohorts. Cohorts
165 with lower mean 25(OH)D concentration had stronger 25(OH)D–PFT associations (**Figure 2**).

166 The proportion of ever smokers and of former smokers had significant linear associations with
167 the 25(OH)D–PFT coefficients (**Supplemental Figure 3**), and these two variables were both
168 highly correlated with mean 25(OH)D levels (Pearson’s $r > 0.75$ for all pairwise correlations).
169 The 25(OH)D–PFT association in EA cohorts varied by 25(OH)D assay method (meta-
170 regression $p < 0.02$); the association was attenuated in cohorts using RIA compared to cohorts
171 using LC-MS/MS (pairwise $p < 0.005$, **Supplemental Figure 4**). Mean age of each cohort was a
172 significant positive modifier of the 25(OH)D–FEV₁ association, while time difference between
173 25(OH)D and spirometry measures did not affect the 25(OH)D–PFT association (**Supplemental**
174 **Figure 3**).

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

178 Cohort-specific findings (**Supplemental Table 5 and 6**) from models that included the 25(OH)D
179 \times smoking status interaction terms were combined in secondary meta-analyses (**Supplemental**
180 **Table 7**). In the EA cohorts, 25(OH)D had a greater positive association with FVC in current
181 smokers than in never smokers ($\beta_{\text{current} \times 25(\text{OH})\text{D}} = 7.5$ mL for 10 nmol/L increment of 25(OH)D, P
182 $= 0.047$). Similarly, 25(OH)D had a greater positive association with FVC in former smokers
183 than in never smokers ($\beta_{\text{former} \times 25(\text{OH})\text{D}} = 7.9$ mL for 10 nmol/L increment of 25(OH)D, $P =$
184 0.0065) (**Figure 3**). For the FEV₁ outcome in the EA cohorts, the interaction coefficients for
185 25(OH)D and smoking status had the same positive direction as the coefficients for FVC, but
186 were not statistically significant for either current ($P = 0.14$) or former smokers ($P = 0.14$). There
187 was no statistical evidence of interaction of 25(OH)D and cigarette smoking in the AA cohorts
188 for either outcome. To put the interaction finding into context, a 10 nmol/L higher serum
189 25(OH)D was associated with a 17.3 mL higher FVC in current smokers and a 16.6 mL higher
190 FVC in former smokers, which was more than double the association magnitude in never
191 smokers ($\beta = 7.8$ mL). A similar trend was found for the FEV₁ outcome in the EA cohorts. For
192 10 nmol/L higher serum 25(OH)D, FEV₁ was higher by 14.0 mL in current smokers, 12.0 mL in
193 former smokers, and 8.0 mL in never smokers (**Figure 4**).

194 **DISCUSSION**

195 This study investigated the association of serum 25(OH)D with pulmonary function using
196 multiple cohorts of different ancestries. We found a consistently positive association of serum
197 25(OH)D with FEV₁ and FVC across both EA and AA groups. In addition, in the EA group, a
198 significantly stronger association was observed for current and former smokers, compared to
199 never smokers.

200 A previous cross-sectional study in a European ancestry population (two Copenhagen cohorts: n
201 = 10,116 and n = 8,391 respectively) similarly reported positive associations of 25(OH)D with
202 FEV₁ percentage predicted and FVC percentage predicted, but not with FEV₁/FVC⁽¹⁶⁾. The
203 magnitude of the association was about four times greater in the Copenhagen study, which may
204 be due to the difference in the mean serum 25(OH)D (Danish median ~42 nmol/L vs. CHARGE
205 median of ~65 nmol/L) given our finding that the 25(OH)D–PFT association was stronger in
206 cohorts with lower serum 25(OH)D. Our finding for the serum 25(OH)D–FEV₁ association was
207 similar in magnitude to the association reported in a British cohort of 6,789 participants with an
208 average age of 45 years⁽¹⁷⁾, but weaker than a previous report from the FHS cohort⁽¹⁵⁾. Given that
209 the rate of decline in FEV₁ at age 45 is increased by ~15 mL/year in current smokers⁽³¹⁾, we
210 estimate that a 10 nmol/L higher 25(OH)D is similar to approximately 1 year of current
211 smoking-related decline in FEV₁ for both ancestries, but in the opposite direction. Potential
212 biological mechanisms for a causal association between low 25(OH)D levels and low pulmonary
213 function include an altered immune response that increases susceptibility to inflammation, a
214 reduction in pulmonary parenchyma related to extracellular matrix homeostasis important for
215 lung structure, and/or a decrease in serum calcium that could adversely affect thoracic skeleton
216 mobility and respiratory muscle performance⁽³²⁾.

217 Our findings show that the association of serum 25(OH)D with FEV₁ and FVC were stronger in
218 magnitude in AA versus EA participants, although the difference by race did not reach statistical
219 significance. The finding may reflect the lower serum 25(OH)D in AA participants, which is
220 consistent with the meta-regression finding and with a previous study reporting attenuated
221 associations at higher serum 25(OH)D (15). Future studies that investigate genetic variation in
222 EAs and AAs in the context of serum 25(OH)D may help explain the differences.

223 In EA participants, the positive interaction terms between serum 25(OH)D and smoking status
224 supported a stronger magnitude of association of serum 25(OH)D with FVC in current and
225 former smokers than in never smokers, with a consistent, but not statistically significant,
226 difference for FEV₁. The interaction finding is consistent with a prior cross-sectional National
227 Health and Nutrition Examination Survey (NHANES) study, which reported a stronger
228 25(OH)D–FEV₁ association in current and former smokers than in never smokers that was near
229 statistical significance ($P = 0.06$)⁽¹³⁾. Given smokers have a higher level of oxidative stress and
230 lower pulmonary function than never smokers, partly due to chronic inflammation in lung tissue,
231 the stronger protective association of 25(OH)D on pulmonary function in smokers suggests a
232 benefit for smokers. To explore this interaction, estimates of the 25(OH)D–PFT association were
233 computed within each smoking category. In EA participants, the 25(OH)D–FEV₁ (or FVC)
234 associations were statistically significant in all strata. Generally, in ever smokers of European
235 ancestry, the coefficients for 25(OH)D were greater for FVC than for FEV₁.

236 Meta-regression provided additional evidence for effect modification by smoking. The
237 proportion of ever smokers was a significant modifier of the association of serum 25(OH)D with
238 FEV₁ and FVC. The higher the proportion of ever smokers, the greater the 25(OH)D–PFT
239 association. More specifically, the proportion of former smokers explained the heterogeneity in
240 the 25(OH)D–PFT association across cohorts more fully than the proportion of current smokers;
241 this may be explained by a survival bias in older participants who were current smokers. The
242 meta-regression based on mean age of the cohorts yielded findings that were consistent with a
243 prior NHANES study that showed the association of 25(OH)D and FEV₁ was greater in people
244 over age 60 compared to younger individuals⁽¹³⁾.

245 There are several methodological considerations in interpreting the findings of this study. First,
246 the meta-regression showed stronger 25(OH)D–PFT associations in cohorts with lower mean
247 serum 25(OH)D, indicating a non-linear 25(OH)D–PFT association. This finding is consistent
248 with a prior study in the FHS cohort, which reported a non-linear association and a stronger
249 25(OH)D–FEV₁ association in participants at risk of vitamin D deficiency (< 30 nmol/L)⁽¹⁵⁾.
250 Second, serum 25(OH)D was measured by four different methods across the cohorts. For
251 example, two cohorts with high mean 25(OH)D (> 90 nmol/L) used RIA methods. These same
252 cohorts had a lower magnitude estimate of the 25(OH)D–PFT association; if the higher mean

253 represents the ‘truth’ (and is not caused by measurement error in the RIA assay), then the lower
254 25(OH)D–PFT association may be primarily driven by the vitamin D distribution and not by the
255 RIA method. Whether the assay method itself directly influences the estimate of the 25(OH)D–
256 PFT association requires further data. Third, in this cross-sectional meta-analysis, there were
257 minor differences in the time separation between the measurement of serum 25(OH)D and
258 pulmonary function, but the meta-regression test for heterogeneity confirmed that time
259 separation between measurements did not affect the 25(OH)D–PFT associations. Indeed, past
260 studies with longitudinal measurements of serum 25(OH)D reported a high correlation of
261 25(OH)D measurements over a long period of time, with a correlation coefficient of 0.7 for
262 measurements separated by 1 year, 0.5 for measurements separated by 5 years⁽³³⁾, and 0.42-0.52
263 for measurements separated by 14 years⁽³⁴⁾, which supports the use of a single 25(OH)D
264 measurement to represent usual level. Fourth, residual confounding was unlikely given the
265 consistent results across multiple cohorts in various settings. Weight was adjusted for the FVC
266 outcome, given that higher weight and adiposity negatively affects lung volume (i.e., FVC)⁽³⁵⁾;
267 weight was not adjusted in the FEV₁ models, given FEV₁ is a measure of airways obstruction
268 and not physical restriction of lung volume. Physical activity was not adjusted because it is not a
269 confounder in estimating the serum 25(OH)D–PFT association; while physical activity is known
270 to contribute to oxygen utilization in lungs⁽³⁶⁾ there is little evidence and no biological rationale
271 for a causal association of physical activity with either FEV₁ or FVC⁽³⁷⁾, which are markers for
272 airways obstruction and lung volume, respectively. Finally, we do not expect selection bias to
273 affect the estimate of the serum vitamin D–PFT association in this meta-analysis; the association
274 magnitude and direction was consistent across all cohorts, regardless of the proportion of the
275 original cohort contributing to the analysis. Thus, selection bias is expected to be negligible and
276 would likely lead to an underestimated association, given the participants retained in the cohorts
277 are expected to be, on average, healthier than those who were lost to follow-up.

278 This study meta-analyzed the serum 25(OH)D–PFT association across nine cohorts, according to
279 a common pipeline that harmonized the variables and statistical analysis. The sample size
280 comprised 17,569 EA participants from the United States; 5,269 EA participants from Iceland
281 and the Netherlands; and 4,290 AA participants from the United States, all of whom were 19 to
282 95 years old. The sample provided excellent representation of the U.S. population, based on
283 comparisons of demographic factors including sex, height, weight, smoking status, and COPD

284 prevalence (~6.1%) to national surveys⁽³⁸⁻⁴⁰⁾, which strengthens the external validity of the
285 study's findings.

286 In summary, using meta-analysis, we estimated a positive association of serum 25(OH)D with
287 the pulmonary function parameters FEV₁ and FVC in both EA and AA participants. Associations
288 varied by smoking status in the EA group, with stronger serum 25(OH)D–PFT associations seen
289 in current and former smokers. The observational design means we cannot infer a causal
290 association, and future studies, such as randomized controlled trials or Mendelian randomization
291 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
Summer	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
Summer	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; FEV₁, 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 FEV₁ 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 FEV₁ and FVC across cohorts in the CHARGE Consortium, stratified by participant ancestry. Associations are presented for serum 25(OH)D on (A) FEV₁ in European ancestry cohorts ($n = 22,787$). (B) FEV₁ 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; FEV₁, 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) FEV₁ outcome (coefficient unit: mL per 1 nmol/L 25(OH)D), and (B) FVC outcome (coefficient unit: mL per 1 nmol/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 FEV₁ 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; FEV₁, 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.

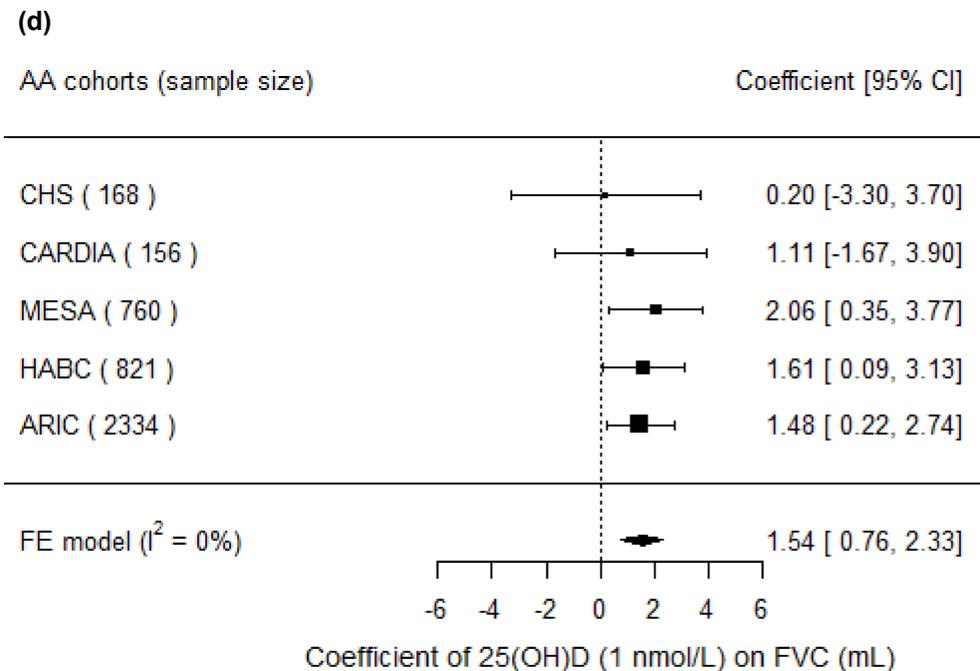
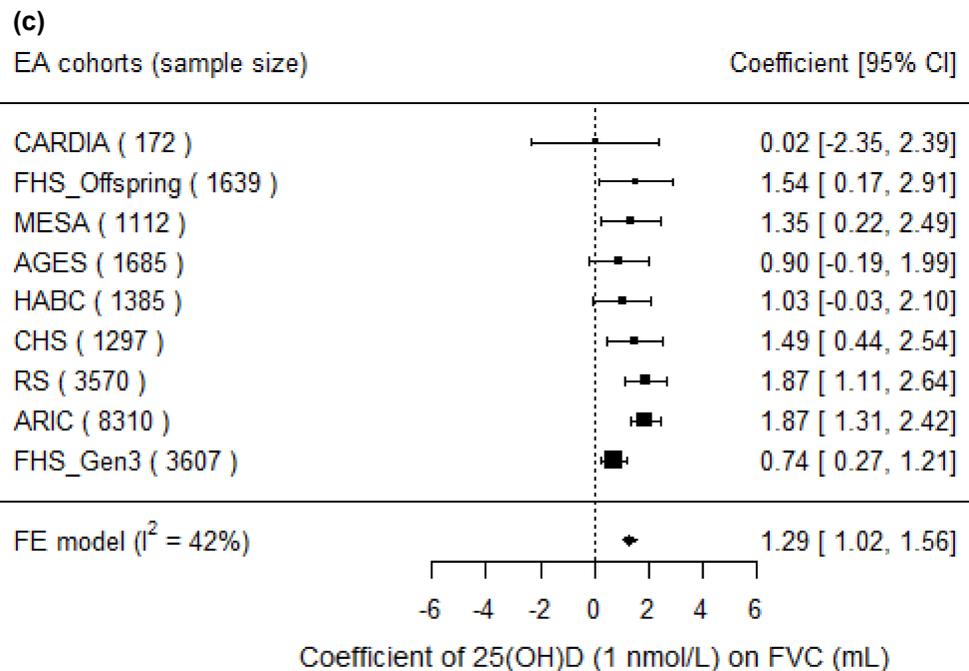
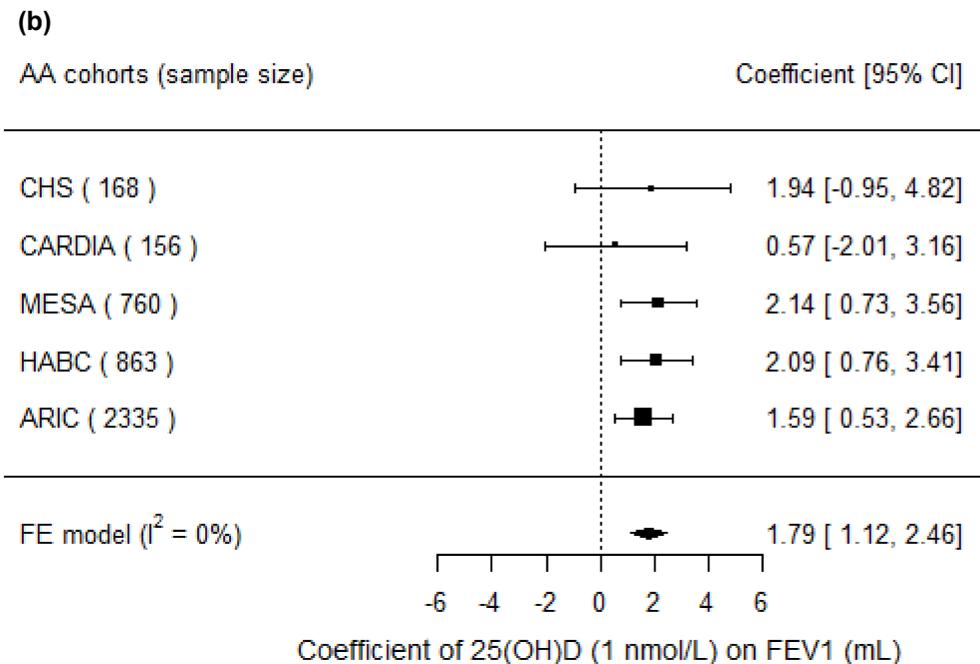
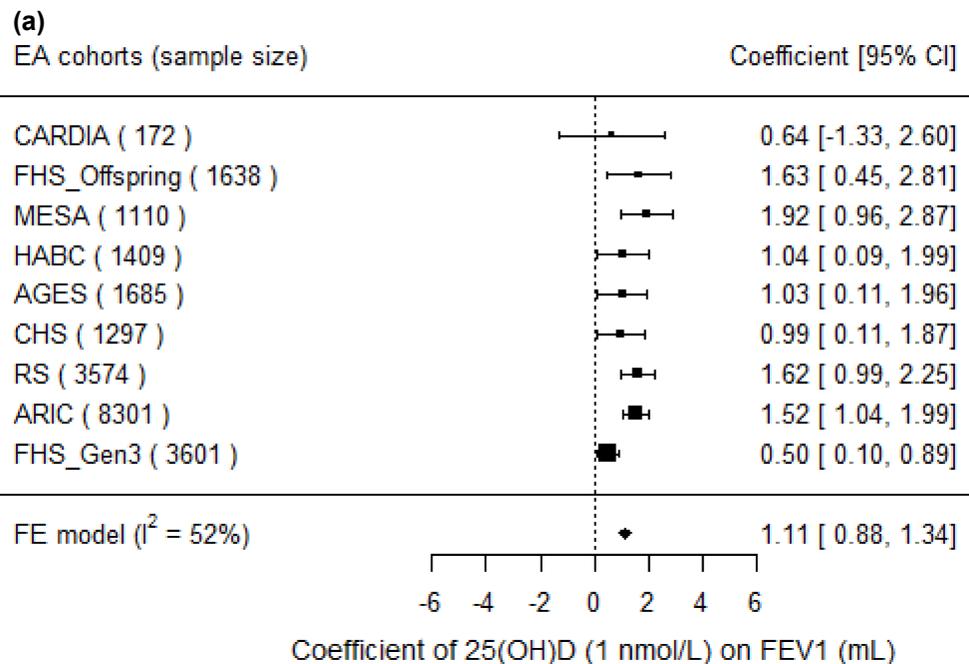
Figure 3. Forest plots of the interaction meta-analysis of serum 25(OH)D and smoking status on FVC in the European ancestry cohorts in the CHARGE Consortium ($n = 22,777$). (A) Current Smokers and (B) Former Smokers. β (unit: mL) denotes the interaction term coefficient of 25(OH)D and smoking status 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—

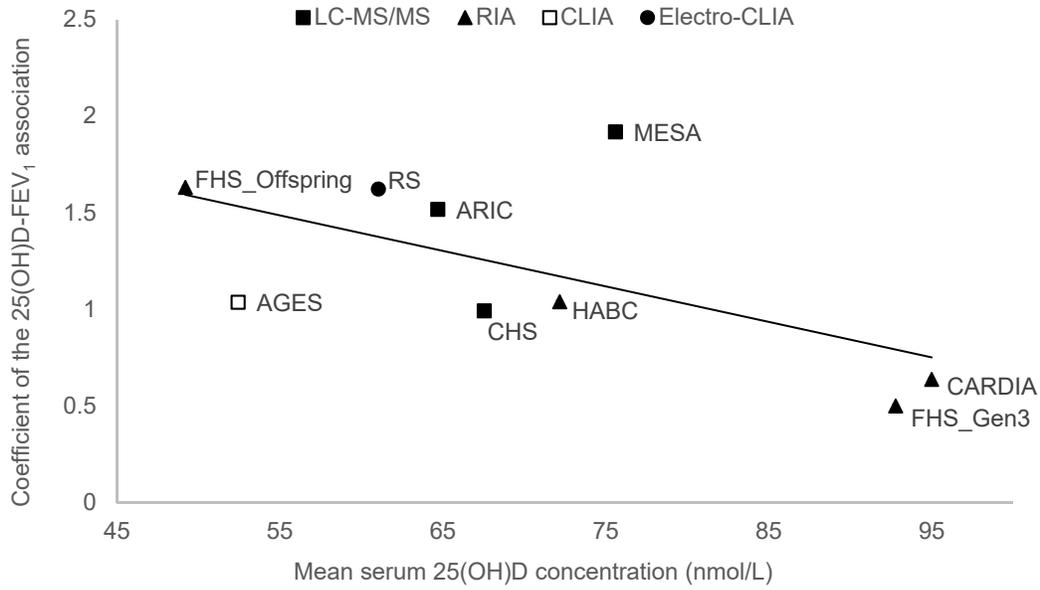
Generation 3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.

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. FEV₁ 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 FEV₁ (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 FEV₁ outcome and 22,777 EA participants for the FVC outcome.

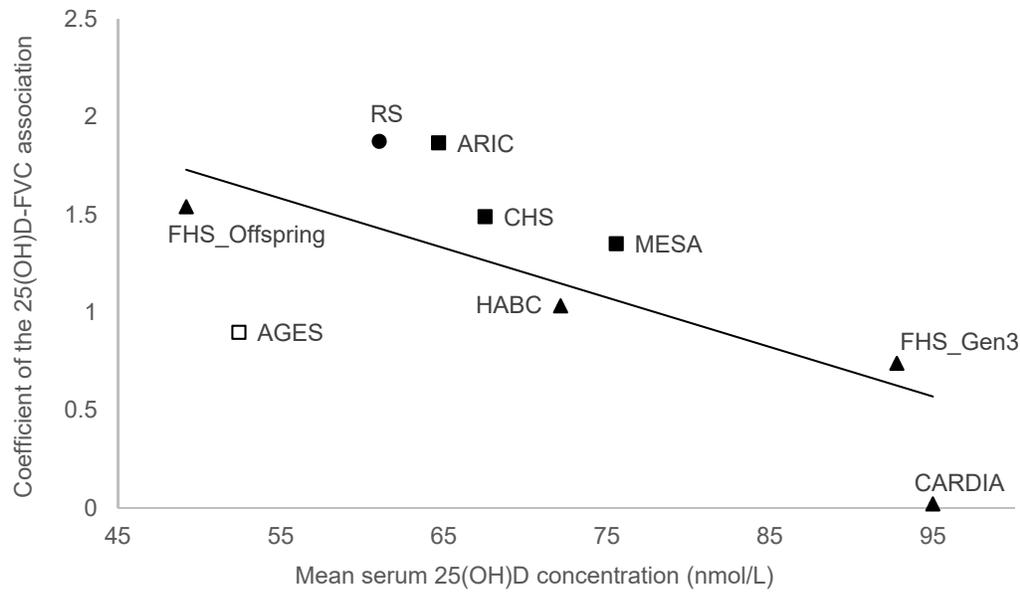
Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; EA, European Ancestry; FEV₁, Forced Expiratory Volume in the First Second; FVC, Forced Vital Capacity; PFT, Pulmonary Function Test.

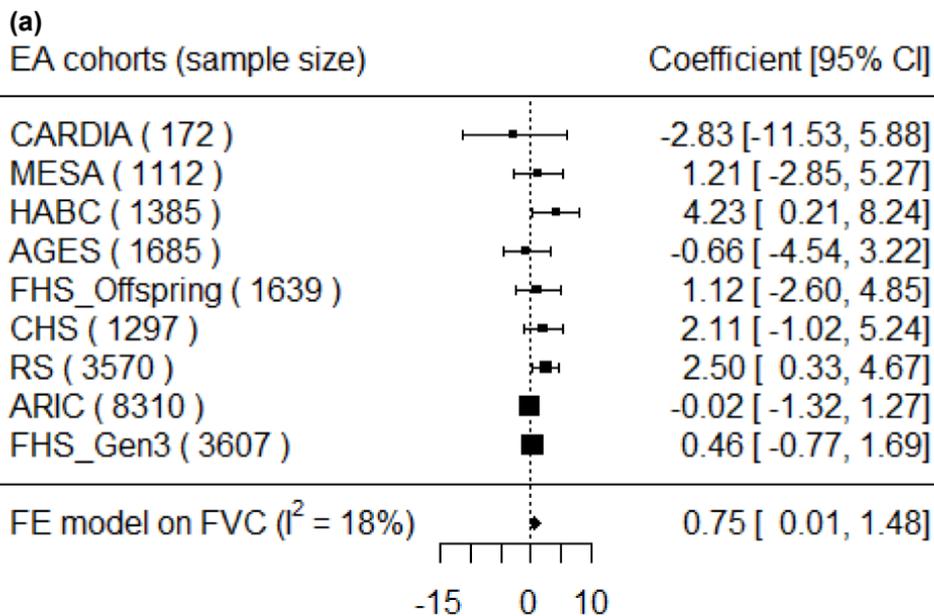


(a)

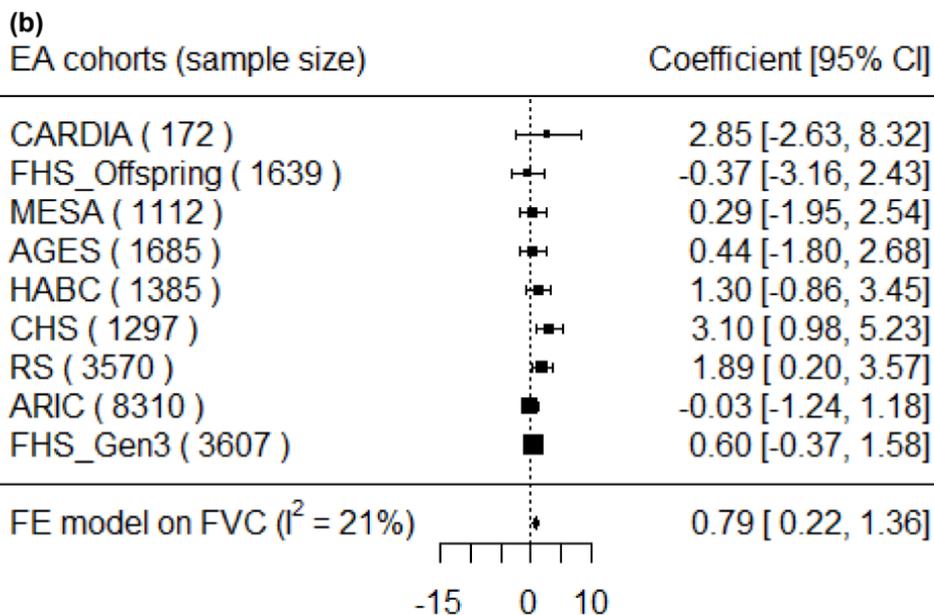


(b)





Interaction coefficient of 25(OH)D (1 nmol/L) with current smoking



Interaction coefficient of 25(OH)D (1 nmol/L) with former smoking

