Meta-analysis across Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium provides evidence for an association of serum vitamin D with pulmonary function

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**Abbreviations:** 25(OH)D, 25-hydroxyvitamin D; 25(OH)2D, 25-dihydroxyvitamin 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; CHS, Cardiovascular Health Study; COPD, chronic obstructive pulmonary disease; EA, European ancestry; FEV1, forced expiratory volume in the 1st 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; PFT, pulmonary function test; RIA, radioimmunoassay.

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Abstract

The role that vitamin D plays in pulmonary function remains uncertain. Epidemiological studies reported mixed findings for serum 25-hydroxyvitamin D (25(OH)D)—pulmonary function association. 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 Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. Data were analysed 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 serum 25(OH)D was 68 (sd 29) nmol/l for EA and 49 (sd 21) nmol/l for AA. For each 1 nmol/l higher 25(OH)D, forced expiratory volume in the 1st second (FEV1) was higher by 1.1 ml in EA (95% CI 0.9–1.3; P<0.0001) and 1.8 ml (95% CI 1.1–2.5; P<0.0001) in AA (P_hetergeneity difference = 0.06), and forced vital capacity (FVC) was higher by 1.3 ml in EA (95% CI 1.0–1.6; P<0.0001) and 1.5 ml (95% CI 0.8–2.3; P=0.0001) in AA (P_hetergeneity difference = 0.56). Among EA, the 25(OH)D—FVC association was stronger in smokers: per 1 nmol/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 with 0.8 ml (95% CI 0.4–1.2) for never smokers. In summary, the 25(OH)D associations with FEV1 and FVC were positive in both ancestries. In EA, a stronger association was observed for smokers compared with never smokers, which supports the importance of vitamin D in vulnerable populations.

Key words: Vitamin D: Forced expiratory volume: Vital capacity: Respiratory function tests: Smoking: Whites: African Americans

Chronic obstructive pulmonary disease (COPD), the third leading cause of mortality in the USA(1) and among the top ten leading causes of total years of life lost in the world(2), is characterised by progressive airway obstruction. Pulmonary function tests (PFT), as performed by spirometry, are used to quantify pulmonary function parameters including forced expiratory volume in the 1st second (FEV1) 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 (1,25-(OH)2D), the active vitamin D metabolite, induced antimicrobial peptides for host defence in the lung and modulated airway remodelling(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 (DBP) (approximately 85–90%) and then is metabolised to 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 (approximately 3 weeks) and is less affected by Ca(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 evidence exists that AA populations have higher 1,25-(OH)\(_2\)D levels and greater bone mineral density compared with 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 18507), reported positive associations of 25(OH)D with pulmonary function\(^{19}\). 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 3957 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 harmonised analysis. In addition, we compared the association of serum 25(OH)D and pulmonary function across EA and AA groups and investigated effect modification by cigarette smoking.

**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 subcohorts analysed separately: the Offspring and the Third-Generation (Gen3) cohorts. Our analysis pipeline harmonised the outcome and exposure definitions, the units on all variables and the statistical modelling. The same exclusion criteria were applied to each cohort: missing PFT, unacceptable PFT using the American Thoracic Society and European Respiratory Society criteria for acceptability, missing serum 25(OH)D, serum 25(OH)D >374 nmol/l (or 150 ng/ml, leading to removal of a single outlier)\(^{25}\) or missing on other covariates (online Supplementary Table S1).

**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 standardised methods defined by the American Thoracic Society/European Respiratory Society criteria (online Supplementary Table S2). The methods used to measure 25(OH)D varied by cohort (online Supplementary Table S2). 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 (online Supplementary Table S3). Measurements of the outcome and exposure variables were planned for either the full cohort (ARIC, CHS, FHS, HABC and RS) or a subset of the cohort if the outcome or the exposure was only measured in an ancillary study (AGES and MESA)\(^{28–31}\) (online Supplementary Table S1). Continuous variables were used for serum 25(OH)D and pulmonary function to capture the association of 25(OH)D on PFT 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 <1 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 d 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 (online Supplementary Table S3). 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 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 centre (if applicable); for FVC, the model was further adjusted for weight. Residual outliers, identified using the studentised residuals of the linear models (online Supplementary methods for more details), were excluded from all models (about 0·3% of the total sample size). The model was extended to test the
interaction between 25(OH)D and smoking status (never (reference group), former and current smokers).

Meta-analysis

We tested the association of serum 25(OH)D on each PFT outcome among individuals in each ancestry group and each cohort, separately, and then combined the effect estimates (also referred to as two-stage meta-analysis(32)), using inverse variance weighting and assuming fixed-effects, with heterogeneity assessed via the \( I^2 \) statistic(33). Random-effects meta-analysis was performed as a sensitivity analysis if there was potential heterogeneity \( (I^2 > 30\%) \). The comparison of meta-analysed coefficients of the 25(OH)D–PFT associations for the two ancestry groups was conducted using a \( Z \) test(34). Meta-analysis of the interaction terms of 25(OH)D with smoking status was also performed (online Supplementary methods for more details, online Supplementary Tables S4 and S5 for cohort-specific findings and online Supplementary Table S6 for meta-analysed results).

Meta-regression was conducted to explore the potential causes of moderate heterogeneity in the meta-analysis of 25(OH)D on FEV\(_1\) and FVC in the EA cohorts. 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 1 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).

Regression coefficients (\( \beta \)) with their standard errors calculated within each cohort per 1 nmol/l 25(OH)D are presented in the figures. In addition, to put the magnitude of the 25(OH)D–PFT associations in terms relevant to public health, the meta-analysed regression coefficients were multiplied by 10 nmol/l 25(OH)D, which is about half of the standard deviation of the 25(OH)D distribution.

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, whereas FEV\(_1\)/FVC was similar across ancestry groups (Table 1 and online Supplementary Fig. S1). 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 mean serum 25(OH)D level was highest among never smokers (70 (sd 30) nmol/l), followed by former smokers (67 (sd 29) nmol/l) and current smokers (64 (29) nmol/l) in EAs, whereas the trend was less obvious in AA (49 (sd 21) nmol/l in current smokers, 50 (sd 21) nmol/l in former smokers and 48 (sd 21) nmol/l in never smokers). The mean of serum 25(OH)D for EA participants across nine cohorts was 68 (sd 29) nmol/l and for AA participants across five cohorts the mean was 49 (sd 21) nmol/l.

Fixed-effects meta-analysis (Fig. 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 (approximately 0·5 sd) higher 25(OH)D was associated with 11·1 ml higher FEV\(_1\) in EA \( (P=0·0001) \) and 17·9 ml higher FEV\(_1\) in AA \( (P=0·0001) \). Similarly, for a 10 nmol/l higher 25(OH)D, FVC was higher by 12·9 ml in EA \( (P=0·0001) \) and by 15·4 ml in AA \( (P=0·0001) \). 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 EA \( (P=0·0013) \), and the magnitude was negligible; a 10 nmol/l higher 25(OH)D was associated with a ratio being lower by 0·055% (online Supplementary Table S7 and Supplementary Fig. S2 for ancestry- and cohort-specific findings).

In the main-effect meta-analysis of serum 25(OH)D on pulmonary function, EA cohorts had low to moderate heterogeneity, whereas AA cohorts had low heterogeneity (Fig. 1, online Supplementary Fig. S2). We did a sensitivity analysis using random-effects meta-analysis among EA cohorts for the FEV\(_1\) and FVC outcomes, and no substantial change was found in the meta-analysed effect estimates and corresponding \( SE \) (coefficient of 1 nmol/l 25(OH)D on the FEV\(_1\) outcome was 1·11 (se 0·12) ml in the fixed-effects model and 1·21 (se 0·19) ml in the random-effects model; coefficient on the FVC outcome was 1·29 (se 0·14) ml in the fixed-effects model and 1·31 (se 0·20) ml in the random-effects model). Meta-regression was also performed in the EA cohorts and we found that among these cohorts, cohorts with lower mean 25(OH)D concentration had stronger 25(OH)D–PFT associations (Fig. 2). The proportion of ever smokers and of former smokers had significant linear associations with the 25(OH)D–PFT coefficients (online Supplementary Fig. S3), 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 \( P=0·0059) \); the association was attenuated in cohorts using RIA compared with cohorts using liquid chromatography in tandem with MS (pairwise \( P<0·005 \), online Supplementary Fig. S4). 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 (online Supplementary Fig. S3).

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). In addition, the meta-analysis findings were not sensitive to exclusion of residual outliers.

In the EA cohorts, 25(OH)D had a greater positive association with FVC in current smokers than in never smokers \( (\beta_{\text{current}}-25\text{(OH)D}=7·5\text{ ml for 10 nmol/l increment of 25\text{(OH)D}, } P=0·047) \). Similarly, 25(OH)D had a greater positive association with FVC
Table 1. Cross-sectional participant characteristics of each cohort in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (n 27 128)*  (Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>ARIC</th>
<th>CARDIA</th>
<th>CHS†</th>
<th>HABC‡</th>
<th>MESA</th>
<th>AGES</th>
<th>FHS (Offspring)</th>
<th>FHS (Gen3)</th>
<th>RS</th>
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<td></td>
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<tr>
<td>Number of participants</td>
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<td>1297</td>
<td>1411</td>
<td>1113</td>
<td>1685</td>
<td>1639</td>
<td>3610</td>
<td>3584</td>
</tr>
<tr>
<td>Males (%)</td>
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<td>58.7</td>
<td>30.2</td>
<td>53.3</td>
<td>41.5</td>
<td>50.1</td>
<td>48.1</td>
<td>47.3</td>
<td>44.6</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>23.4</td>
<td>11.6</td>
<td>9.4</td>
<td>6.5</td>
<td>8.4</td>
<td>9.8</td>
<td>14.3</td>
<td>15.3</td>
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<tr>
<td>Former smoker (%)</td>
<td>34.9</td>
<td>16.3</td>
<td>4.4</td>
<td>49.8</td>
<td>47.2</td>
<td>42.4</td>
<td>50.5</td>
<td>28.0</td>
<td>52.9</td>
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<td>Pack-years§</td>
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<td>20.9</td>
<td>6.2</td>
<td>7.2</td>
<td>28.1</td>
<td>25.3</td>
<td>36.4</td>
<td>29.6</td>
<td>24.6</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>5.7</td>
<td>34.8</td>
<td>31.7</td>
<td>73.7</td>
<td>4.4</td>
<td>73.7</td>
<td>28.6</td>
<td>66.3</td>
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<td>Height (m)</td>
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<td>0.09</td>
<td>1.73</td>
<td>0.09</td>
<td>1.63</td>
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<td>1.67</td>
<td>0.09</td>
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<tr>
<td>Weight (kg)‡‡</td>
<td>76.8</td>
<td>16.2</td>
<td>76.9</td>
<td>17.0</td>
<td>70.6</td>
<td>14.2</td>
<td>74.5</td>
<td>14.5</td>
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<tr>
<td>FEV1 (ml)‡‡</td>
<td>2946</td>
<td>767</td>
<td>3881</td>
<td>743</td>
<td>2010</td>
<td>611</td>
<td>2324</td>
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<td>FVC (ml)‡‡</td>
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<td>973</td>
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<td>999</td>
<td>2881</td>
<td>829</td>
<td>3118</td>
<td>810</td>
<td>3492</td>
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<tr>
<td>Serum 25(OH)D (nmol/l)¶¶</td>
<td>64.7</td>
<td>21.8</td>
<td>95.0</td>
<td>35.3</td>
<td>68.0</td>
<td>27.9</td>
<td>72.2</td>
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<td>95.4</td>
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<td>25.9</td>
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<td>43.0</td>
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<td>24.8</td>
<td>76.2</td>
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<tr>
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<td>23.1</td>
<td>92.7</td>
<td>29.5</td>
<td>65.4</td>
<td>33.2</td>
<td>65.0</td>
<td>28.1</td>
<td>66.9</td>
</tr>
<tr>
<td>Measurement method of 25(OH)D</td>
<td>LC-MS/MS</td>
<td>RIA</td>
<td>LC-MS/MS</td>
<td>RIA</td>
<td>LC-MS/MS</td>
<td>CLIA</td>
<td>RIA</td>
<td>RIA</td>
<td>Electro-CLIA</td>
</tr>
<tr>
<td>Time from 25(OH)D to PFT (d)‡‡</td>
<td>−1073</td>
<td>67</td>
<td>1122</td>
<td>89</td>
<td>363</td>
<td>29</td>
<td>−382</td>
<td>39</td>
<td>1765</td>
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<tr>
<td><strong>African ancestry cohort</strong></td>
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<td>30.5</td>
<td>20.5</td>
<td>30.5</td>
<td>22.4</td>
<td>29.0</td>
</tr>
<tr>
<td>Males (%)</td>
<td>35.3</td>
<td>51.6</td>
<td>25.6</td>
<td>44.5</td>
<td>30.1</td>
<td>18.1</td>
<td>22.2</td>
<td>12.4</td>
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<tr>
<td>Current smoker (%)</td>
<td>27.5</td>
<td>26.1</td>
<td>15.7</td>
<td>15.1</td>
<td>26.1</td>
<td>15.7</td>
<td>24.9</td>
<td>33.8</td>
<td>29.1</td>
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<tr>
<td>Former smoker (%)</td>
<td>23.9</td>
<td>9.6</td>
<td>42.9</td>
<td>38.3</td>
<td>23.9</td>
<td>9.6</td>
<td>31.4</td>
<td>30.7</td>
<td>19.4</td>
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<td>Pack-years§</td>
<td>21.4</td>
<td>20.7</td>
<td>5.3</td>
<td>4.6</td>
<td>21.9</td>
<td>18.3</td>
<td>29.4</td>
<td>23.4</td>
<td>23.6</td>
</tr>
<tr>
<td>Age (years)‡‡</td>
<td>53.3</td>
<td>5.7</td>
<td>33.9</td>
<td>3.2</td>
<td>71.9</td>
<td>4.5</td>
<td>73.4</td>
<td>2.9</td>
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<tr>
<td>Height (m)</td>
<td>1.68</td>
<td>0.09</td>
<td>1.71</td>
<td>0.10</td>
<td>1.63</td>
<td>0.08</td>
<td>1.66</td>
<td>0.09</td>
<td>1.68</td>
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<tr>
<td>Weight (kg)‡‡</td>
<td>83.5</td>
<td>17.1</td>
<td>82.2</td>
<td>16.9</td>
<td>75.7</td>
<td>13.3</td>
<td>78.2</td>
<td>15.1</td>
<td>84.3</td>
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<tr>
<td>FVC (ml)</td>
<td>3255</td>
<td>806</td>
<td>4077</td>
<td>920</td>
<td>2507</td>
<td>706</td>
<td>2594</td>
<td>712</td>
<td>2933</td>
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</table>

*Table for Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (COHAR-GEN) is provided as Supplemental Information.

†CHS: Cardiovascular Health Study.
‡‡FEV1 and FVC were measured using different techniques in different cohorts.
§Pack-years = smoking duration (years) x pack-years.
§§Serum 25(OH)D was measured using different techniques in different cohorts.
<table>
<thead>
<tr>
<th></th>
<th>ARIC</th>
<th>CARDIA</th>
<th>CHS†</th>
<th>HABC‡</th>
<th>MESA</th>
<th>AGES</th>
<th>FHS (Offspring)</th>
<th>FHS (Gen3)</th>
<th>RS</th>
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<tr>
<td>Mean</td>
<td>0.768</td>
<td>0.799</td>
<td>0.723</td>
<td>0.775</td>
<td>0.750</td>
<td>0.755</td>
<td>0.723</td>
<td>0.799</td>
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<td>s.d</td>
<td>0.077</td>
<td>0.070</td>
<td>0.076</td>
<td>0.090</td>
<td>0.070</td>
<td>0.070</td>
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<td>0.070</td>
<td>0.093</td>
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<td>FEV1/FVC</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Serum 25(OH)D (nmol/l)**</td>
<td>47.4</td>
<td>17.5</td>
<td>69.4</td>
<td>31.2</td>
<td>44.6</td>
<td>21.1</td>
<td>51.8</td>
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<td>Never smoker</td>
<td>46.8</td>
<td>16.7</td>
<td>71.3</td>
<td>30.1</td>
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<td>19.2</td>
<td>51.8</td>
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<td>Method of 25(OH)D measurement</td>
<td>LC-MS/MS</td>
<td>RIA</td>
<td>LC-MS/MS</td>
<td>RIA</td>
<td>LC-MS/MS</td>
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<tr>
<td>Time from 25(OH)D to PFT (d)‡‡</td>
<td>114</td>
<td>1101</td>
<td>104</td>
<td>350</td>
<td>26</td>
<td>−390</td>
<td>53</td>
<td>1719</td>
<td>115</td>
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<tr>
<td>Season of 25(OH)D measurement (%)§§</td>
<td>Spring</td>
<td>30.0</td>
<td>10.2</td>
<td>58.9</td>
<td>35.6</td>
<td>34.6</td>
<td></td>
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<tr>
<td></td>
<td>Summer</td>
<td>30.7</td>
<td>56.0</td>
<td>7.1</td>
<td>16.2</td>
<td>23.5</td>
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<td></td>
<td>Autumn</td>
<td>20.7</td>
<td>23.6</td>
<td>8.9</td>
<td>24.9</td>
<td>19.7</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Winter</td>
<td>18.6</td>
<td>10.2</td>
<td>29.0</td>
<td>23.3</td>
<td>22.3</td>
<td></td>
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</tbody>
</table>

ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; AGES, Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland; FHS (Offspring), Framingham Heart Study – Offspring Cohort; FHS (Gen3), Framingham Heart Study – Generation 3 Cohort; RS, Rotterdam Study (Netherlands); FEV1, forced expiratory volume in the 1st second; FVC, forced vital capacity; 25(OH)D, 25-hydroxyvitamin D; LC-MS/MS, liquid chromatography in tandem with MS; CLIA, chemiluminescence immunoassay; RIA, radioimmunoassay.

* AGES, RS and FHS only have participants of European ancestry; n = 22,838 for EA, n = 4,290 for AA, 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 EA and n = 6 for AA); whereas 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 = 1,385 for EA and n = 821 for AA; for the ratio outcome, n = 1,382 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.
‡‡ 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; whereas 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%).
in former smokers than in never smokers ($\beta_{\text{former}} \times 25\text{(OH)D} = 7.9 \text{ ml}$ for 10 nmol/l increment of 25\text{(OH)D}, $P = 0.0065$) (Fig. 3). For the FEV$_1$ outcome in the EA cohorts, the interaction coefficients for 25\text{(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 ($P = 0.14$). No statistical evidence of interaction of 25\text{(OH)D} and cigarette smoking was found in the AA cohorts for either outcome. To put the interaction finding into context, a 10 nmol/l higher serum 25\text{(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 ($\beta = 7.8 \text{ ml}$).

A similar trend was found for the FEV$_1$ outcome in the EA cohorts. For 10 nmol/l higher serum 25\text{(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 (Fig. 4).

### Discussion

This study investigated the association of serum 25\text{(OH)D} with pulmonary function using multiple cohorts of different ancestries. We found a consistently positive association of serum 25\text{(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 with never smokers.

A previous cross-sectional study in a EA population (two Copenhagen cohorts: $n = 10116$ and $n = 8391$, respectively) similarly reported positive associations of 25\text{(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, which may be due to the difference in the mean serum 25\text{(OH)D} (Danish median approximately 42 nmol/l vs. CHARGE median of approximately 65 nmol/l) given our finding that the 25\text{(OH)D}–PFT association was stronger in cohorts with lower serum 25\text{(OH)D}. Our finding for the serum 25\text{(OH)D}–FEV$_1$ association was similar in magnitude to the association reported in a British cohort of 6789 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 years is increased by approximately 15 ml/year in current smokers$^{35}$, we estimate that a 10 nmol/l higher 25\text{(OH)D} is similar to approximately 1 year of current smoking-related decline in FEV$_1$ for both ancestries, but in the opposite direction. Several putative
biological mechanisms may support a causal association between low 25(OH)D levels and worse pulmonary function. First, lung tissue cells can locally convert 25(OH)D to 1,25-(OH)2D, the active form of vitamin D, which could improve inflammation in lungs via gene regulation. If there is not enough circulating 25(OH)D, it is likely that the resolution of inflammation in lungs would be slower, which could have a negative impact on pulmonary function. In addition, 1,25-(OH)2D in lungs, converted locally to 25(OH)D, can regulate the extracellular matrix homeostasis via the EBP60-mediated pathway, and this is important for maintenance of lung structure. Furthermore, low vitamin D status could decrease circulating Ca status, which in turn can adversely affect thoracic skeleton mobility and respiratory muscle performance.

Our findings show that the association of serum 25(OH)D with FEV1 and FVC were stronger in magnitude in AA vs. EA participants, although the difference by race did not reach statistical significance. The finding may reflect the lower serum 25(OH)D levels in AA smokers and (b) former smokers. Our finding is consistent with a prior cross-sectional study and with a previous study reporting attenuated associations at higher serum 25(OH)D levels.

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 FEV1. The interaction finding is consistent with a prior cross-sectional National Heart and Nutrition Examination Survey (NHANES) study, which reported a stronger 25(OH)D–FEV1 association in current and former smokers than in never smokers that was
Serum vitamin D and pulmonary function

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 EA, 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. 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\(^{45}\), and 0.42–0.52 for measurements separated by 14 years\(^{45}\), 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)\(^{45}\); 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 \(O_2\) utilisation in lungs\(^{46}\), little evidence and no biological rationale exists for a causal association of physical activity with either FEV\(_1\) or FVC\(^{47}\), which are markers for airways obstruction and lung volume, respectively. Finally, even though three cohorts (AGES, CARDIA, MESA) had the outcome or the exposure only measured in an ancillary study (random subset of the entire cohort), we do not expect selection bias to affect the estimate of the serum vitamin D–PFT association; indeed, 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-analysed the serum 25(OH)D–PFT association across nine cohorts, according to a common pipeline that harmonised the variables and statistical analysis. The sample size comprised 17 569 EA participants from the USA;
5269 EA participants from Iceland and the Netherlands; and 4290 AA participants from the USA, all of whom were 19–95 years old. The sample provided excellent representation of the US population, based on comparisons of demographic factors including sex, height, weight, smoking status and COPD prevalence (about 6-1%) to national surveys\(^{(48-50)}\), 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 associations seen in current and former smokers. The observational design means we cannot infer a causal association, and future studies, such as randomised controlled trials or Mendelian randomisation studies, are needed to further investigate the causality of 25(OH)D on pulmonary function.

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P. A. C., D. B. H. and J. X. conceived and designed the study. R. G. B., J. L., J. D., S. A. G., L. L., S. J. L., K. E. N., A. V. S., B. M. P. and L. M. S. provided the data and supervised the data analysis in each cohort. J. X., T. M. B., R. R. R., A. V. S., A. W. M., F. S., N. T. and X. Z. analysed data within each cohort. J. X., P. A. C. and D. B. H. meta-analysed and interpreted the data, co-wrote and edited the first draft of the manuscript and 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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Supplementary material
For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518002180

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