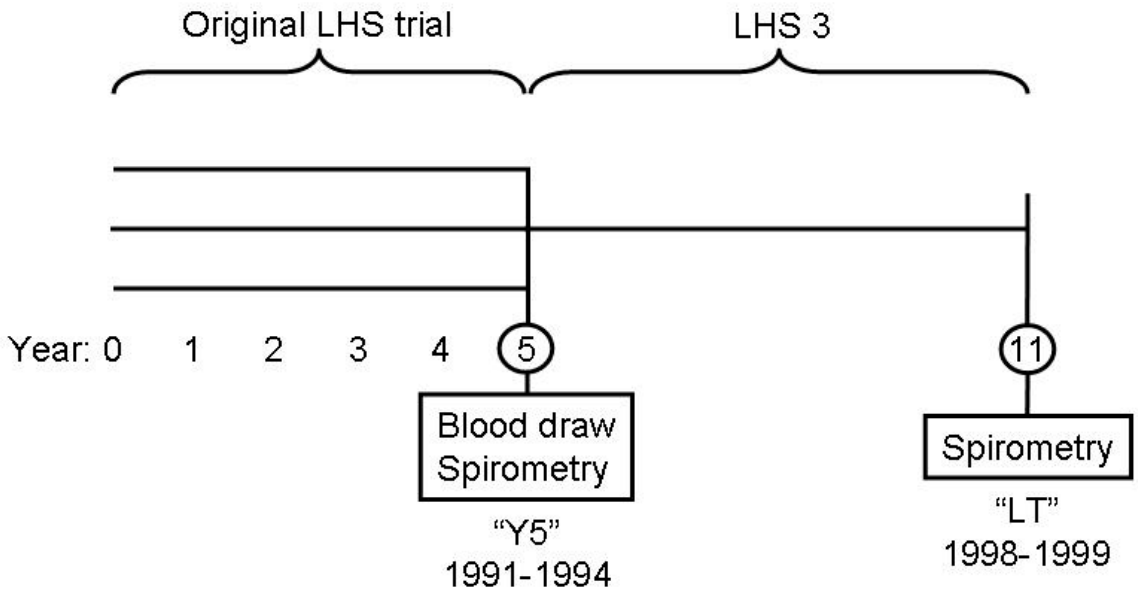
Manuscript: “Vitamin D in the Lung Health Study”

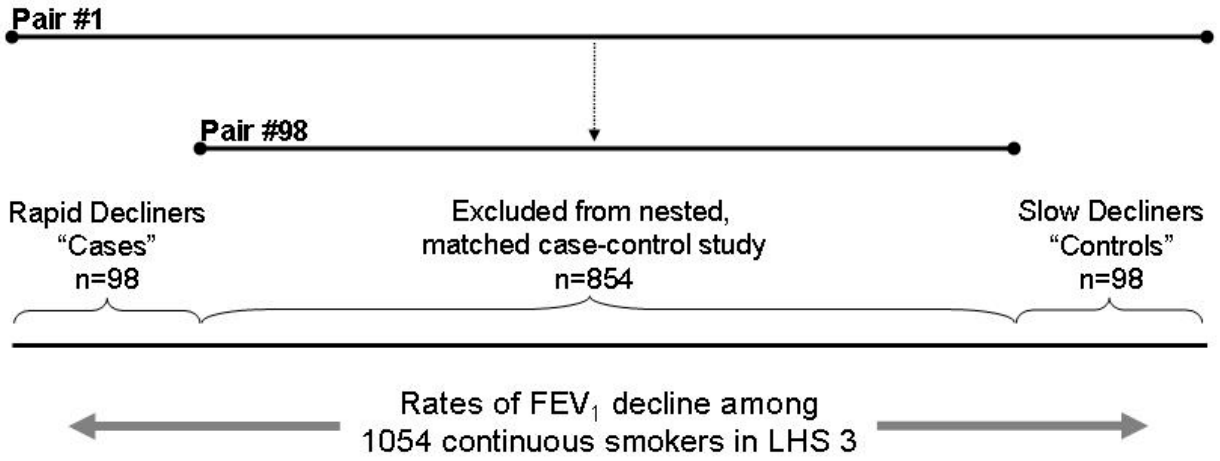
ONLINE DATA SUPPLEMENT

A. Figure of Study Design



The original Lung Health Study (LHS) trial was a 5-year, 3-arm randomized trail of patients with COPD, randomized to smoking intervention plus inhaled ipratropium, smoking intervention plus inhaled placebo, or usual care. At year 5 (Y5), the trial was completed and the LHS 3 observational cohort study was begun. LHS 3 only collected blood for future research at the Y5 visit. No blood was stored from the original LHS trial. Patients were seen again an average of 6 years after the Y5 visit. Spirometry was repeated at this long-term (LT) follow-up visit. There were no intermediate visits between Y5 and LT. LHS 3 served as the basis for this study of vitamin D and lung function decline.

B. Figure of Case-Control Selection Process



Among 1054 continuous smokers in LHS 3, samples from 98 matched pairs (198 patients) were selected for sample retrieval and analysis of baseline 25(OH)D levels. All pairs were matched on clinical center (to control for effects of latitude of 25(OH)D levels) and date of blood draw (to control for effects of seasonality on 25(OH)D levels). Of 100 paired samples sent for 25(OH)D assays, 2 pairs were incorrectly matched for clinical center and therefore not included in the final analysis.

C. 25(OH)D Assay Details

LC-MS/MS analysis provides values for both 25(OH)D2 (the form generated by ultraviolet irradiation of ergosterol from yeast and present in ergocalciferol-containing supplements) and 25(OH)D3 (the form generated by solar ultraviolet B exposure and present in cholecalciferol-containing supplements). These two results are summed to generate the total 25(OH)D level used in clinical assessments. For 25(OH)D2, intra-assay coefficients of variation (CV’s) are 4.4%, 3.3%, and 4.2% at 14, 41, and 124 ng/mL respectively; inter-assay CV’s are 6.1%, 6.2%, and 4.7% at 15, 43, and 128 ng/mL respectively. For 25(OH)D3, intra-assay CV’s are 3.8%, 2.4%, and 4.7% at 25, 54, and 140 ng/mL respectively; inter-assay CV’s are 6.4%, 6.8%, and 5.0% at 24, 52, and 140 ng/mL respectively.

D. Statistical Details

D1. Sample Size

Sample size is typically estimated using assumptions about the expected standard deviation (SD) and the minimal clinically important difference (MCID) of vitamin D levels, but neither of these measures has been well established in patients with mild to moderate COPD. The SD of blood vitamin D levels in patients with COPD are reported in only two published studies, which reported SD’s of 10.6 ng/mL[1] and 16.5 ng/mL[2]. The NHANES III study cited in the introduction section of the main paper[3] was a general population sample and reported a SD of 14.4 ng/mL for those 50-59 years old (the comparable age group to LHS participants). A significant shortcoming of all these studies is that none used our current LC-MS/MS assay methodology, thus potentially limiting the applicability of those SD data to our current study. Likewise, the MCID of 25(OH)D levels is not well established. Traditional definitions have considered 25(OH)D levels <30 ng/mL as indicative of vitamin D insufficiency, with levels <20 ng/mL as indicative of vitamin D deficiency[4]. Thus, a difference of 10 ng/mL is likely of clinical significance. However, even smaller changes may have significant clinical implications in terms of non-skeletal outcomes such as lung function decline. We assumed that a difference in 25(OH)D levels as small as 5 ng/mL might be considered clinically meaningful.

Due to our concern over the uncertainty of previous SD estimates using different assay methodology, we elected to begin with a preliminary analysis of the first 100 matched pairs (200 samples) for a determination of the SD of 25(OH)D levels of this pooled cohort, while remaining blinded to the case-control status of each result. With these SD data, we planned to re-calculate a definitive power analysis and possibly assay additional pairs if a larger sample size would be required to demonstrate our assumed MCID of 5 ng/mL.

This blinded SD analysis showed the pooled SD of 25(OH)D levels to be 10.3 ng/mL. An SD of 10.3 ng/mL provided 90% power (with a two-tailed alpha error rate of 0.05) to detect a difference of 2.4 ng/mL in baseline 25(OH)D levels between rapid and slow decliners using a paired t-test—pairing the differences between the fast decliners and slow decliners matched on date of blood draw and on clinical center. We therefore had excellent power to detect small differences in 25(OH)D levels. The blinded data were then integrated into the main

LHS database for unblinded data analysis. At this step, we identified 2 pairs (4 samples) that were not correctly matched on clinical center. These data were therefore excluded from analysis and all results reported are based on the 98 correctly matched pairs (196 samples).

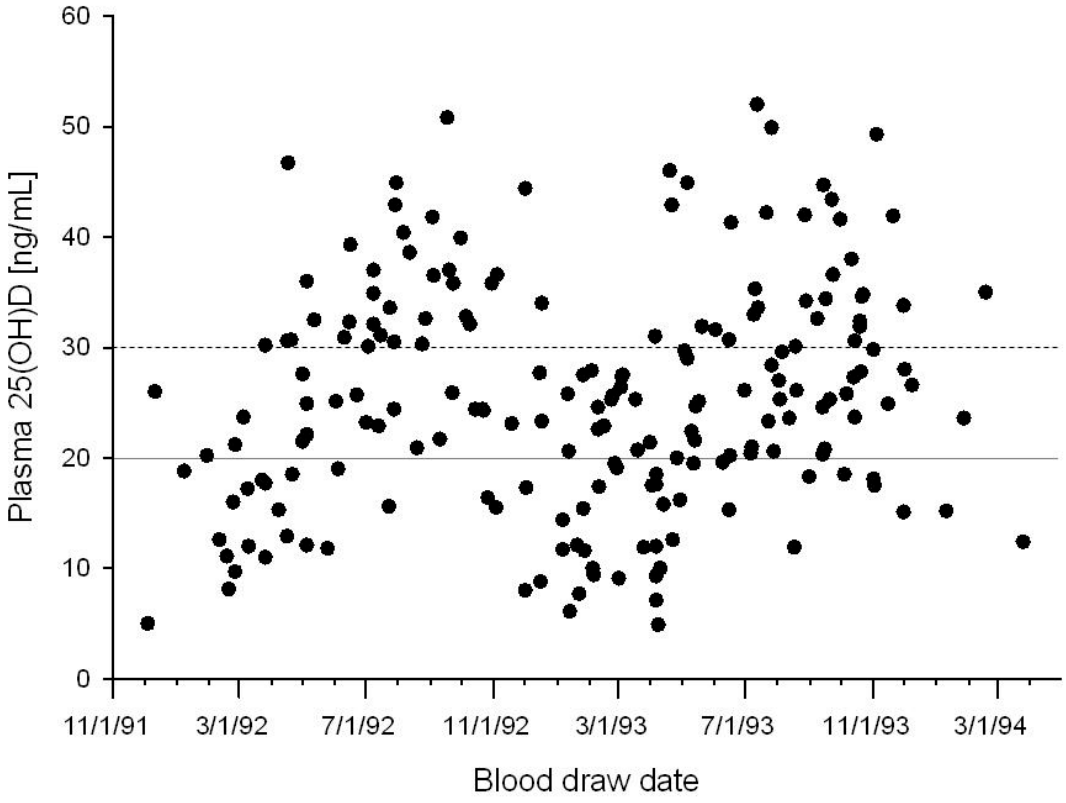
D2. Regression Analysis

Methods: We conducted a post-hoc regression analysis to explore whether or not other clinical factors could have affected the null findings of the primary analysis. We conducted a conditional (paired) logistic regression analysis in which the outcome was rapid vs. slow decline in FEV_1 and which included the following covariates from the Y5 visit (in addition to total vitamin D level): age, gender, number of cigarettes smoked per day, FEV_1 percent predicted, FVC percent predicted, bronchodilator response, and methacholine response. We also included time from the Y5 visit to the LT visit.

Results: The unadjusted model resulted in an odds ratio of 1.009 (95% confidence interval: 0.980—1.040; p=0.54); this reflects the effect of Y5 25(OH)D on the odds of being a case (rapid decliner) vs. being a control (slow decliner). The p-value was identical to that derived from the paired t-test used in the primary analysis (see Table 2 of main paper). The multivariate model with the above covariates remained statistically insignificant with an odds ratio of 0.993 (95% confidence interval: 0.951—1.038; p=0.77).

Discussion: This additional analysis did not alter our conclusions that baseline 25(OH)D is not associated with rates of lung function decline. In addition, the narrow confidence interval of this adjusted model suggest that power remained good and reliably excluded an effect size of an odds ratio of less than 0.95 or greater than 1.04. We doubt an effect size smaller than this would be considered clinically significant.

E. Figure of 25(OH)D Distribution by Date of Blood Draw



Scatterplot of date of Y5 blood draw and associated 25(OH)D levels. Dashed line at 30 ng/mL and solid line at 20 mg/mL indicate widely accepted cut-points for defining 25(OH)D levels as normal (≥ 30 ng/mL), insufficient (≥20 ng/mL, <30 ng/mL) and deficient (<20 ng/mL). 25(OH)D = 25-hydroxyvitamin D.

References

1. Shane E, Silverberg SJ, Donovan D, et al. Osteoporosis in lung transplantation candidates with end-stage pulmonary disease. Am J Med 1996; 101:262-269
2. Forli L, Halse J, Haug E, et al. Vitamin D deficiency, bone mineral density and weight in patients with advanced pulmonary disease. J Intern Med 2004; 256:56-62
3. Black PN and Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. Chest 2005; 128:3792-3798
4. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266-281