

Vitamin D Status and Longitudinal Lung Function Decline in the Lung Health Study

A THESIS SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

Ken Kunisaki

IN PARTIAL FULFILLMENT OF THE REQUIERMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Advised by Dennis Niewoehner, MD

January 2014

© Ken Kunisaki 2014
ALL RIGHTS RESERVED

Acknowledgements

I would like to thank H. Blair (Mayo Clinic, Rochester, MN, USA) for technical assistance with the vitamin D assays, and H. Voelker and A. Boggess (both University of Minnesota, Minneapolis, MN, USA) for assistance with the identification and retrieval of samples. I also thank the Upper Midwest CTSA Consortium for fostering this study's institutional collaboration.

Abstract

Low vitamin D blood levels are postulated to be a risk factor for worse lung function, largely based on cross-sectional data. We sought to use longitudinal data to test the hypothesis that baseline plasma 25-hydroxyvitamin D [25(OH)D] is lower in subjects with more rapid lung function decline, compared to those with slow lung function decline.

We conducted a nested, matched case-control study in the Lung Health Study 3 cohort. Cases and controls were continuous smokers with rapid and slow lung function decline, respectively, over approximately 6 years of follow-up. We compared baseline 25(OH)D levels between cases and controls, matching on date of blood draw and clinical center.

Among 196 subjects, despite rapid and slow decliners experiencing strikingly and significantly different rates of decline of forced expiratory volume in one second (-152 vs. -0.3 mL/year; $p < 0.001$), there was no significant difference in baseline 25(OH)D levels (25.0 vs. 25.9 ng/mL; $p = 0.54$). There was a high prevalence of vitamin D insufficiency (35%) and deficiency (31%); only 4% had a normal 25(OH)D level in the winter.

Although vitamin D insufficiency and deficiency are common among continuous smokers with established mild to moderate COPD, baseline 25(OH)D levels are not predictive of subsequent lung function decline.

Table of Contents

Acknowledgements.....	i
Abstract.....	ii
List of Abbreviations.....	iv
List of Tables.....	v
List of Figures.....	vi
Introduction.....	1
Materials and Methods.....	2
Analysis.....	5
Results.....	7
Discussion.....	9
Bibliography.....	23

List of Abbreviations

25(OH)D = 25-hydroxyvitamin D

COPD = chronic obstructive pulmonary disease

FEV₁ = forced expiratory volume in one second

FVC = forced vital capacity

LHS 3 = Lung Health Study 3

LT = Long-term follow-up

NHANES III = Third National Health and Nutrition Examination Survey

SD = standard deviation

Y5 = Year-5 follow-up

List of Tables

Table 1: Characteristics of study participants.....	16
Table 2: Comparison of lung function decline and vitamin D status between rapid decliners and slow decliners, matched on date of blood draw (to within 60 days) and clinical center	17
Table 3: Year 5 plasma 25(OH)D levels by season	18

List of Figures

Figure 1: Figure of study Design	19
Figure 2: Figure of Case-Control Selection Process.....	20
Figure 3: Figure of 25(OH)D Distribution by Season.....	21
Figure 4: Figure of 25(OH)D Distribution by Date of Blood Draw.....	22

Introduction

Data from the Third National Health and Nutrition Examination Survey (NHANES III) showed that in a cross-sectional sample of a general U.S. population (n=14,076), lower serum vitamin D levels were associated with lower forced expiratory volume in one second (FEV₁) in a graded, “dose-dependent” fashion (Black & Scragg, 2005). The results of this report from Black and colleagues have spurred hypotheses that low vitamin D levels may be a modifiable risk factor for impaired lung function and chronic obstructive pulmonary disease (COPD).

Vitamin D has long been recognized for its effects on calcium homeostasis and skeletal health. However, its non-skeletal effects have recently received increasing scientific attention, including hypotheses on its potentially beneficial effects in patients with COPD (Janssens, Lehouck & Carremans et al., 2009). The mechanisms by which vitamin D levels might affect lung function are unclear. Potential explanations include effects on respiratory infection risk (via both innate and adaptive mechanisms) and lung tissue remodeling (via matrix metalloproteinases and other pathways) (Janssens, 2009; Koli & Keski, 2000; Bao, 2006).

We sought to build upon the cross-sectional data of Black and colleagues by using longitudinal data to further investigate vitamin D insufficiency as a risk factor for rapid lung function decline and COPD. We hypothesized that among persons with mild COPD, those with rapid declines in longitudinal lung function would have lower baseline vitamin D levels compared to persons with minimal declines in longitudinal lung function. We tested this hypothesis with a nested, matched case-control study in the Lung Health Study 3 cohort.

Materials and Methods

Study Subjects:

Participants in this nested, matched case-control study were selected from the Lung Health Study 3 (LHS 3), an observational follow-up study of participants in the Lung Health Study trial, a 5-year, 10-center, randomized trial of a smoking intervention and bronchodilator (Connett, Kusek & Bailey, 1993; Anthonisen, Connett & Kiley, 1994). Following the trial, study interventions were stopped, but most participants provided informed consent to participate in LHS 3 and agreed to return to study centers for a single long-term follow-up visit. 5,887 participants enrolled in the original LHS trial, 4,517 participants enrolled in LHS 3, and 4,194 completed spirometry at an average of 6 years after LHS 3 enrollment. Thus, the follow-up rate in LHS 3 was 93%. Detailed methods and results of LHS 3 have been previously published (Anthonisen, Connett & Murray, 2002; Anthonisen, Skeans & Wise et al., 2005).

Study Design:

We conducted a nested, matched case-control study within the LHS 3 cohort. Stored blood was only available at the end of the original trial, at the year 5 (Y5) visit; blood was not stored from other visits in the original trial or at the long-term (LT) follow-up visit. This Y5 specimen served as our baseline vitamin D assessment. Spirometry was available at the time of blood draw (at Y5) and at the LT visit. LHS 3 did not have any intermediate visits between the Y5 and LT visits (Figure 1). We restricted our analysis to the 1,054 LHS 3 participants who were biochemically validated continuous smokers throughout all visits in the original LHS trial and still smoking at the LT visit. Cases were continuous smokers with the most rapid declines in FEV₁ between the Y5

visit and the LT visit (rapid decliners). Controls were continuous smokers with the least decline in FEV₁ in the same time period (slow decliners).

The primary human source of vitamin D is ultraviolet sunlight exposure, which will vary by season and by latitude (LHS study centers varied from as far south as Birmingham, AL, USA [latitude 33° N] to as far north as Winnipeg, MB, Canada [latitude 49° N]). To control for these seasonal and latitude effects on vitamin D levels, we matched cases and controls on date of Y5 blood draw (to within 60 days) and on clinical center. We rationalized that if vitamin D affected rates of FEV₁ decline, then differences in vitamin D levels should be greatest between persons with the greatest differences in rates of lung function decline. Therefore, we constructed a LHS 3 database query, such that the matched case-control pair with the largest difference in rates of FEV₁ decline (as % of predicted) was selected as the first pair. This process was repeated sequentially and subsequent pairs had progressively smaller differences in rate of FEV₁ decline between the cases and controls, while still remaining matched on date and clinical center (Figure 2). This selection process was continued until the desired sample size was reached.

Methods:

Plasma was collected at the Y5 visit in standardized fashion, and shipped on dry ice to the LHS 3 data coordinating center, where samples have been continuously stored at -70°C. Once cases and controls were identified for this particular study, plasma samples were thawed and plasma 25-hydroxyvitamin D [25(OH)D] assays were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS;

ThermoFisher Scientific, Franklin, MA, USA and Applied Biosystems-MDS Sciex, Foster City, CA, USA) in the laboratory of R.J. Singh at the Mayo Clinic (Rochester, MN, USA) .

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.

Spirometry was performed in both the main trial and LT visit using the same rolling seal spirometers (Spirotech 500; Spirotech, Atlanta, GA, USA) and the same spirometry quality control program (Enright, Johnson & Connett et al., 1991). Measurements of FEV₁ and FVC were made before and at least 20 minutes after two puffs (200 mcg) of inhaled albuterol administered through a metered-dose inhaler. Our analysis was restricted to post-bronchodilator measures. The largest single FEV₁ and FVC were reported and converted to percentages of the predicted normal using the formulas of Crapo and colleagues (Crapo, Morris & Gardner, 1981).

Analysis

The primary outcome was the paired difference in the baseline exposure variable (Y5 plasma 25(OH)D level) between matched rapid and slow decliners. Annual rates of lung function decline were calculated by subtracting spirometry values at the LT visit from the values at the Y5 visit and dividing by time elapsed between the two measures.

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 (Black & Scragg, 2005) and 16.5 ng/mL (Janssens et al., 2009). The NHANES III study cited in the introduction section of the main paper (Koli & Keski, 2000) 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 (Bao et al., 2006). 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).

Our sample size of 196 (98 pairs) provided 90% power (two-tailed alpha = 0.05) to detect a difference of 2.4 ng/mL in baseline 25(OH)D levels between rapid and slow decliners using paired t-testing. We therefore had excellent power to detect small differences in 25(OH)D levels.

As secondary analyses, we also investigated seasonal variation in 25(OH)D levels. Seasons were defined as Winter=January-March, Spring=April-June,

Summer=July-September, Autumn=October-December. The seasonal 25(OH)D data were analyzed using one-way ANOVA, corrected for multiple comparisons with a Bonferroni-adjusted p-value significance level of 0.05.

We also conducted a post-hoc conditional (paired) logistic regression analysis in which the outcome was rapid vs. slow decline in FEV₁ 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, FEV₁ percent predicted, FVC percent predicted, bronchodilator response, and methacholine response. We also included time from the Y5 visit to the LT visit.

All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA) and STATA 9.2 (StataCorp, College Station, TX, USA). Figures were created using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

Results

Rapid and slow decliners (cases and controls) were similar in Y5 age, gender distribution, and smoking intensity (Table 1). Most participants were Caucasian, due to the sample recruited in the original Lung Health Study trial, and there was a statistically significant difference in ethnicity of rapid and slow decliners. Matching resulted in a mean difference in clinic visit days between rapid and slow decliners of 25 ± 16.5 days (range 0 – 60 days). The distribution of participants matched on clinical center was as follows: Baltimore, MD, USA (n = 24), Birmingham, AL, USA (n = 10), Cleveland, OH, USA (n = 14), Detroit, MI, USA (n= 24), Los Angeles, CA, USA (n = 20), Pittsburgh,

PA, USA (n = 24), Portland, OR, USA (n = 22), Rochester, MN, USA (n = 12), Salt Lake City, UT, USA (n = 18), and Winnipeg, MB, Canada (n = 28).

Our selection criteria for cases and controls resulted in clinically and statistically significant differences in the rate of FEV₁ decline between rapid and slow decliners. While FEV₁ was similar between rapid and slow decliners at the Y5 visit, rapid decliners had a mean FEV₁ that was >1 L worse than slow decliners at the LT visit (Table 1). This resulted in a rate of FEV₁ decline of -152 mL/year (-4.3% of predicted/year) in rapid decliners vs. -0.3mL/year (+0.7% of predicted/year) in slow decliners (p<0.001) (Table 2).

Despite the large differences in rate of FEV₁ decline, and appropriate control of latitude (through matching on clinical center) and time of year (through matching on date of blood draw), the difference in Y5 25(OH)D level between rapid decliners and slow decliners was not statistically significant (25.0 ng/mL vs. 25.9 ng/mL, respectively; p=0.54) (Table 2). Additional logistic regression analysis for baseline Y5 covariates also demonstrated no association between Y5 25(OH)D level and rapid or slow decliner status. 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. The multivariate adjusted model remained statistically insignificant with an odds ratio of 0.993 (95% confidence interval: 0.951—1.038; p=0.77).

We applied current widely accepted definitions of vitamin D insufficiency and deficiency (Holick, 2007), which classify patients as vitamin D deficient with 25(OH)D

levels <20 ng/mL and insufficient with levels ≥ 20 ng/mL but <30 ng/mL. Applying such criteria, we found 35% (n=69) of this LHS 3 sample was vitamin D insufficient and 31% (n=60) were vitamin D deficient. Only 34% (n=67) of the sample would be currently classified as sufficient in vitamin D status with levels ≥ 30 ng/mL. 14 participants (7%) had severe vitamin D deficiency, such that their 25(OH)D levels were ≤ 10 ng/mL.

There was also significant seasonal variation in 25(OH)D levels (Figures 3 and 4). As expected, 25(OH)D levels peaked in late summer, with nadir levels observed in the winter months. The magnitude of the seasonal variation was both clinically and statistically significant, with a mean winter 25(OH)D level of 18.3 ng/mL compared to 31.7 ng/mL in the summer (Bonferroni-corrected $p < 0.001$). Of 48 samples drawn in the winter months, 46 (96%) were under the recommended goal level of ≥ 30 ng/mL.

Discussion

We found no differences in baseline 25(OH)D levels between continuously smoking LHS 3 participants with rapid and slow declines in lung function over approximately 6 years of prospective follow-up. Therefore, our data do not support the notion that low 25(OH)D levels lead to faster rates of lung function decline.

Our study was primarily prompted by the study of Black and colleagues which examined cross-sectional data from 14,076 NHANES III participants (Black & Scragg, 2005). They demonstrated a graded relationship between lower 25(OH)D levels and lower lung function, such that those in the lowest 25(OH)D quintile (≤ 16.2 ng/mL) had a mean FEV₁ that was 126mL lower than those in the highest quintile (≥ 34.3 ng/ml), after adjusting for gender, age, ethnicity, body mass index, and cigarette smoking. Among a

small subgroup with self-reported emphysema (n=251), the differences were even greater, such that when comparing those with 25(OH)D \leq 16.2 ng/mL to those \geq 34.3 ng/mL, FEV₁ was 344mL worse in the low 25(OH)D group. The actual spirometry values from these 251 patients were not reported, so confirmation of COPD and assessment of COPD severity could not be made. While intriguing, a major limitation of these data is the cross-sectional nature of NHANES data. To our knowledge, ours is the first study examining relationships between baseline 25(OH)D levels and subsequent prospective, longitudinal rates of lung function decline.

Our study design allowed us to compare two groups of COPD patients of significant clinical interest—those who continuously smoke and have rapid lung function decline (rapid decliners) and those who continuously smoke, yet have preserved lung function over time (slow decliners). Because smoking is controlled for in both of these groups, we were able to investigate the hypothesis that the rapid decliners would have lower 25(OH)D levels as one potential mechanism by which their lung function rapidly declines. However, our data do not support this hypothesis.

Our study has several strengths. The longitudinal assessment of lung function was rigorously standardized with the same equipment and procedures used by experienced study staff (who had performed annual spirometry for 5 years prior to the Y5 measure in this study). Our matching criteria and the seasonal variation observed suggest that misclassification of 25(OH)D levels is unlikely. We had excellent power to detect small differences in 25(OH)D levels: 90% power to detect a difference as small as 2.4 ng/mL. It seems unlikely that a difference any smaller than this could explain differences in rates of subsequent lung function decline. Regression 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.

Our study has several important limitations. One limitation is that assessment of 25(OH)D levels was only possible from a single study visit. Therefore, this single assessment may not be fully reflective of an individual's overall vitamin D status. For example, a wintertime assessment could be a poor indicator of overall vitamin D status throughout the year, especially in more extreme latitudes. We attempted to correct for seasonal variation and latitude effects as best as we could by matching rapid and slow decliners on date of blood draw and clinical center, but this can not correct for seasonal changes which might vary significantly both within and between individuals. We were also unable to assess whether or not the presence of low 25(OH)D levels at Y5 were associated with persistent low levels at the LT follow-up visit, as there was no blood draw at the LT follow-up visit. It is possible that some participants might have begun activities during that time interval which could have affected their subsequent 25(OH)D levels. For example, participants could have begun vitamin D supplementation and subsequently increased their 25(OH)D levels after the Y5 visit. Conversely, they could have begun using sunscreen products which could have decreased their 25(OH)D levels after the Y5 visit.

Another limitation of our data is that these analyses were restricted to continuous smokers with evidence of mild to moderate COPD at baseline. Because smoking has

such a significant impact on rate of lung function decline, smoking is important to control for in a study such as ours. We chose to restrict our analysis to continuous smokers in order to focus on those COPD patients at greatest risk of progressive lung function decline and to reduce effects of variables other than vitamin D (such as intermittent smoking) that might also affect rate of lung function decline. Thus, we cannot extrapolate these findings to non-smokers or to intermittent smokers. We also cannot extrapolate these findings to persons without COPD confirmed by spirometry nor to persons with very advanced COPD.

We feel it important to highlight the high prevalence of vitamin D insufficiency and deficiency we found, such that only 34% of these LHS 3 participants had 25(OH)D levels that would currently be considered as adequate. In wintertime, we found only 2 of 48 25(OH)D measures to be in the accepted normal range. Riancho and colleagues studied 44 men with COPD (mean FEV₁ of 39% of predicted) between 1983-1985 and showed the mean 25(OH)D level was <10 ng/mL for most of the year, with peak mean 25(OH)D level in late summer still <20 ng/mL (Riancho, Gonzalez & Del Arco et al., 1987). They measured 25(OH)D using a competitive protein binding assay after HPLC purification—a method that is now rarely used, so a direct comparison to more current 25(OH)D assay methods may be limited. Shane and colleagues reported a mean 25(OH)D level of 20 ng/mL in 28 patients with COPD awaiting lung transplantation between 1993-1995 (Shane, Silverberg & Donovan et al., 1996). 10 of these patients (36%) had levels ≤10 ng/mL. Forli and colleagues reported vitamin D deficiency (<20 ng/ml) in over 50% of 71 consecutive non-smoking patients (of whom 46 had COPD)

undergoing lung transplantation evaluation between 1993 and 1998 (Forli, Halse & Haug et al., 2004).

These data are of particular concern in light of recent NHANES data demonstrating that between the surveys conducted in 1988-1994 and 2001-2004, the mean population 25(OH)D level decreased by 6 ng/mL and the percentage with inadequate 25(OH)D levels (<30 ng/mL) increased from 55% to 77% (Ginde, Liu & Camargo, 2009). Because our 25(OH)D data are based on samples collected between 1991-1994, it seems likely that the current prevalence of inadequate 25(OH)D levels in patients with mild to moderate COPD is even higher than 66% we found.

In support of this, Franco and colleagues recently reported a mean springtime of 2005 25(OH)D level of 20.8 ng/mL in a small cohort of 49 Brazilian patients with mostly mild and moderate COPD (Franco, Paz-Filho & Gomez et al., 2009). Of these 49 patients, only 3 (6%) had 25(OH)D levels ≥ 30 ng/mL; 29 (59%) were vitamin D insufficient, and 17 (35%) were vitamin D deficient. Janssens and colleagues also recently reported that among 262 Belgian patients with COPD, the mean 25(OH) D level was 19.9 ng/mL and 52% were vitamin D deficient with levels <20 ng/mL (Janssens, Bouillon & Claes et al., 2010).

Our cohort also demonstrated significant seasonal variation in 25(OH) D levels, which varied around the accepted cut-points of normal, insufficient, and deficient levels. As such, there was a substantial seasonal shift in the distribution of participants classified as normal or vitamin D deficient. While these blood samples from 1991-1994 are no longer a contemporary assessment, clinicians and researchers may need to consider the substantial effect of seasonality on 25(OH)D measures. It is important to note that LHS 3

participants were generally quite healthy with mostly mild COPD. One might hypothesize that in patients with more severe COPD, there may be less of a seasonal effect due to being more confined to the home and hence, less exposed to sunlight. However, we are unaware of any such contemporary data to either support or refute such a hypothesis. In addition, the mechanisms leading to vitamin D insufficiency/deficiency may be quite complex. Dietary vitamin D intake in patients with COPD has been shown to be low (De Batlle, Romieu & Anto et al., 2009), but multiple other mechanisms may lead to inadequate vitamin D status (Holick, 2007).

Although we found no association between 25(OH)D levels and subsequent rates of lung function decline, patients with COPD suffer from many co-morbidities potentially associated with low 25(OH)D levels. The one COPD co-morbidity with well-studied links to low 25(OH)D levels is osteoporosis (Cranney, Weiler & O'Donnell et al., 2008). Multiple other COPD complications and co-morbidities have been linked to vitamin D insufficiency, including respiratory infections (Ginde et al., 2009; Laaksi et al., 2007; Aloia & Li-Ng, 2007), cardiovascular disease (Kendrick et al., 2009; Lee et al., 2008) and muscle dysfunction (Bischoff-Ferrari et al., 2004; Sato et al., 2005). However, it is important to note that there are no clinical trial data to support to the hypothesis that improving 25(OH)D levels in patients with COPD will improve any of these COPD co-morbidities, but these remain topics requiring further investigation.

In conclusion, although we found a high prevalence of low 25(OH)D levels in continuous smokers with established mild and moderate COPD, we found no difference between baseline 25(OH)D levels among those with subsequent rapid declines in lung function and slow declines in lung function. Our data suggest that normalization of

25(OH)D levels is not likely to affect subsequent rates of lung function decline in such patients.

Table 1: Characteristics of study participants. All participants were continuous smokers from the first visit in the main Lung Health Study trial to the long-term follow-up visit. Data are presented as mean \pm standard deviation for continuous variables or as number (%) for categorical variables. p-values calculated using paired t-testing. FEV₁ = forced expiratory volume in one second, FVC = forced vital capacity, LT = Long-term follow-up visit, Y5 = Year-5 visit.

	Rapid Decliners (n=98)	Slow Decliners (n=98)	p-value
Male	62 (63%)	60 (61%)	0.77
Caucasian ethnicity	86 (88%)	97 (99%)	0.002
Age, years	53.2 \pm 6.9	52.2 \pm 6.6	0.31
Cigarettes/day reported at Y5	25.1 \pm 12.6	21.5 \pm 11.6	0.04
Y5 FEV ₁ (L)	2.35 \pm 0.63	2.49 \pm 0.65	0.09
Y5 FEV ₁ (% predicted)	71.0 \pm 12.1	73.2 \pm 11.5	0.15
Y5 FVC (L)	4.10 \pm 1.02	3.94 \pm 0.99	0.23
Y5 FVC (% predicted)	98.6 \pm 13.7	92.1 \pm 11.4	<0.001
Time between Y5 to LT spirometry visits (years)	6.0 \pm 0.64	5.8 \pm 0.66	0.06
LT FEV ₁ (L)	1.44 \pm 0.52	2.49 \pm 0.66	<0.001
LT FEV ₁ (% predicted)	45.2 \pm 13.3	77.2 \pm 11.6	<0.001
LT FVC (L)	3.31 \pm 1.01	3.92 \pm 1.05	<0.001
LT FVC (% predicted)	81.7 \pm 17.0	94.2 \pm 12.6	<0.001

Table 2: Comparison of lung function decline and vitamin D status between rapid decliners and slow decliners, matched on date of blood draw (to within 60 days) and clinical center. Data are presented as mean \pm standard deviation. p-values calculated using paired t-testing. 25(OH)D = 25-hydroxyvitamin D, FEV₁ = forced expiratory volume in one second, LT = Long-term follow-up visit, Y5 = Year-5 visit.

	Rapid Decliners (n=98)	Slow Decliners (n=98)	p-value
Rate of FEV ₁ decline from Y5 to LT (mL/year)	-151.6 \pm 47.7	-0.28 \pm 24.0	<0.0001
Rate of FEV ₁ decline from Y5 to LT (%predicted/year)	-4.3 \pm 1.2	+0.7 \pm 0.7	<0.0001
Y5 25(OH)D levels (ng/mL)	25.0 \pm 10.4	25.9 \pm 10.2	0.54

Table 3: Year 5 plasma 25(OH)D levels by season. Seasons were defined as:

Winter=January-March, Spring=April-June, Summer=July-September,

Autumn=October-December. Data are presented as mean \pm standard deviation for

25(OH)D and number (%) for the categorical data. 25(OH)D = 25-hydroxyvitamin D.

See Figure 3 for p-values from pairwise statistical testing of mean 25(OH)D levels by season.

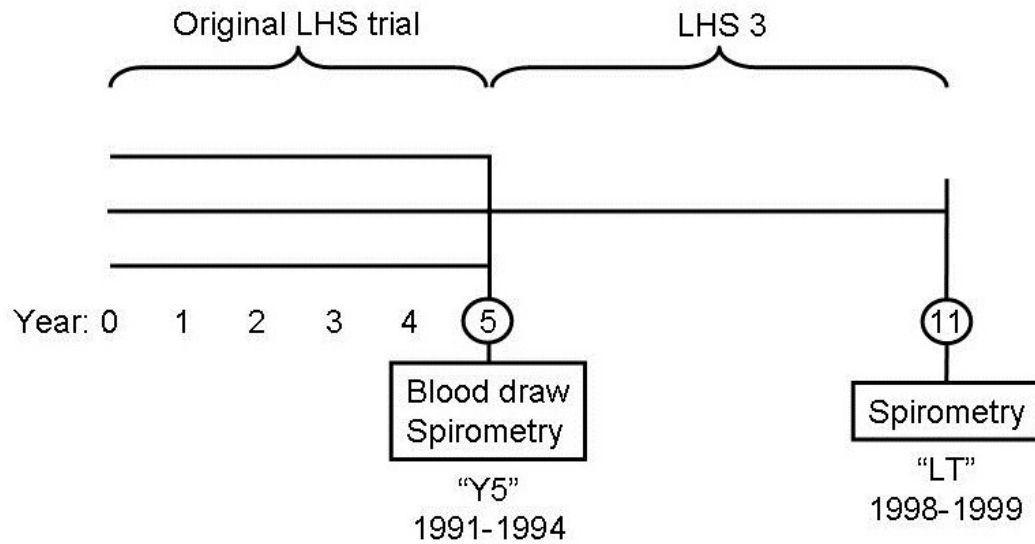
	Winter	Spring	Summer	Autumn
Participants	48	51	57	40
25(OH)D level [ng/mL]	18.3 \pm 7.0	24.1 \pm 10.3	31.7 \pm 9.2	26.8 \pm 9.6
Normal vitamin D level ^a	2 (4%)	16 (31%)	34 (60%)	15 (38%)
Vitamin D insufficient ^b	19 (40%)	15 (29%)	20 (35%)	15 (38%)
Vitamin D deficient ^c	27 (56%)	20 (39%)	3 (5%)	10 (25%)

a: Normal vitamin D level = \geq 30 ng/mL

b: Vitamin D insufficiency = 25(OH)D level \geq 20 ng/mL, <30 ng/mL

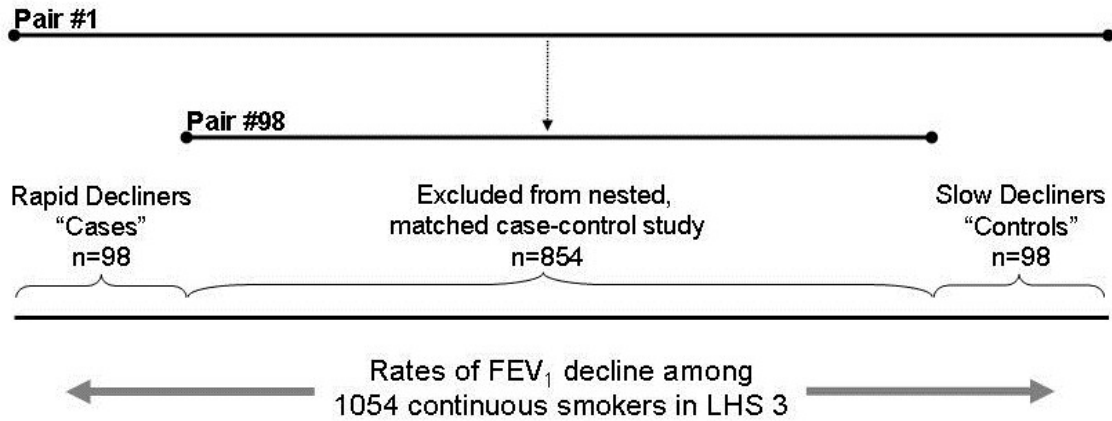
c: Vitamin D deficiency = 25(OH)D level <20 ng/mL

Figure 1: Design of the Lung Health Study 3



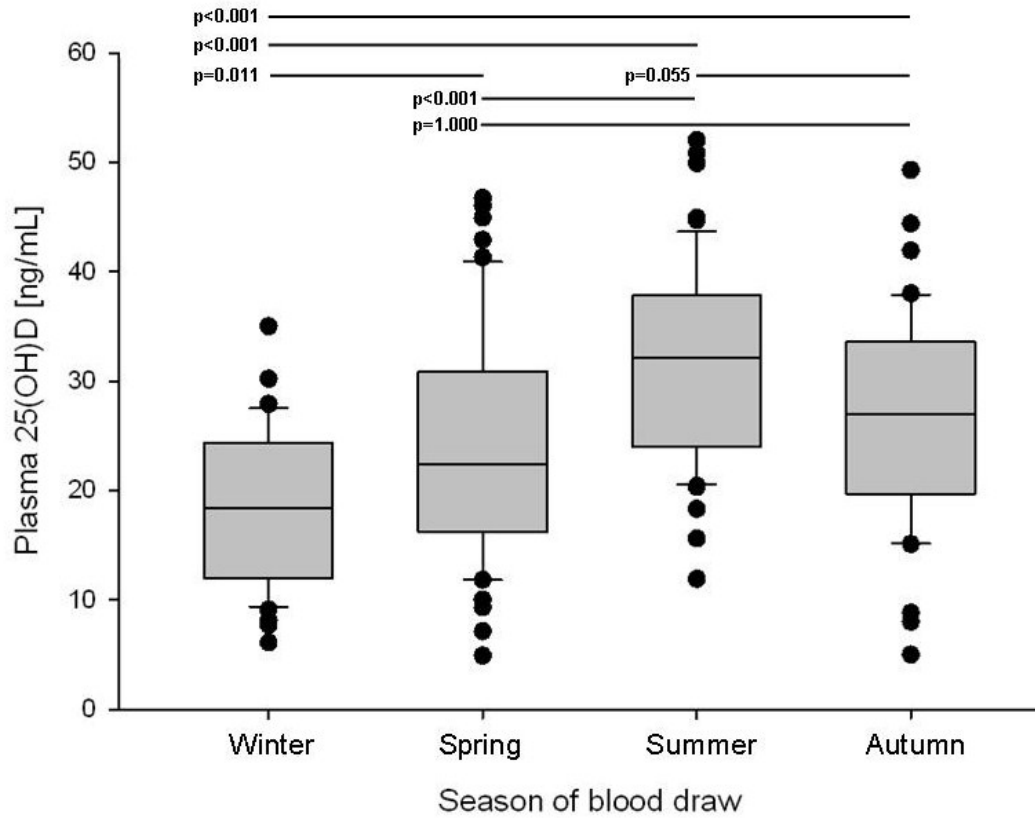
The original Lung Health Study (LHS) trial was a 5-year, 3-arm randomized trial of patients with COPD, randomized to smoking intervention plus inhaled ipratropium, smoking intervention plus inhaled placebo, or usual care. At year 5 (Y5), the trial was completed and the LHS 3 observational cohort study 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.

Figure 2: 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.

Figure 3: Boxplots of 25(OH)D levels by season.



Line=median, shaded box=interquartile range, whiskers=range, dots=outliers.

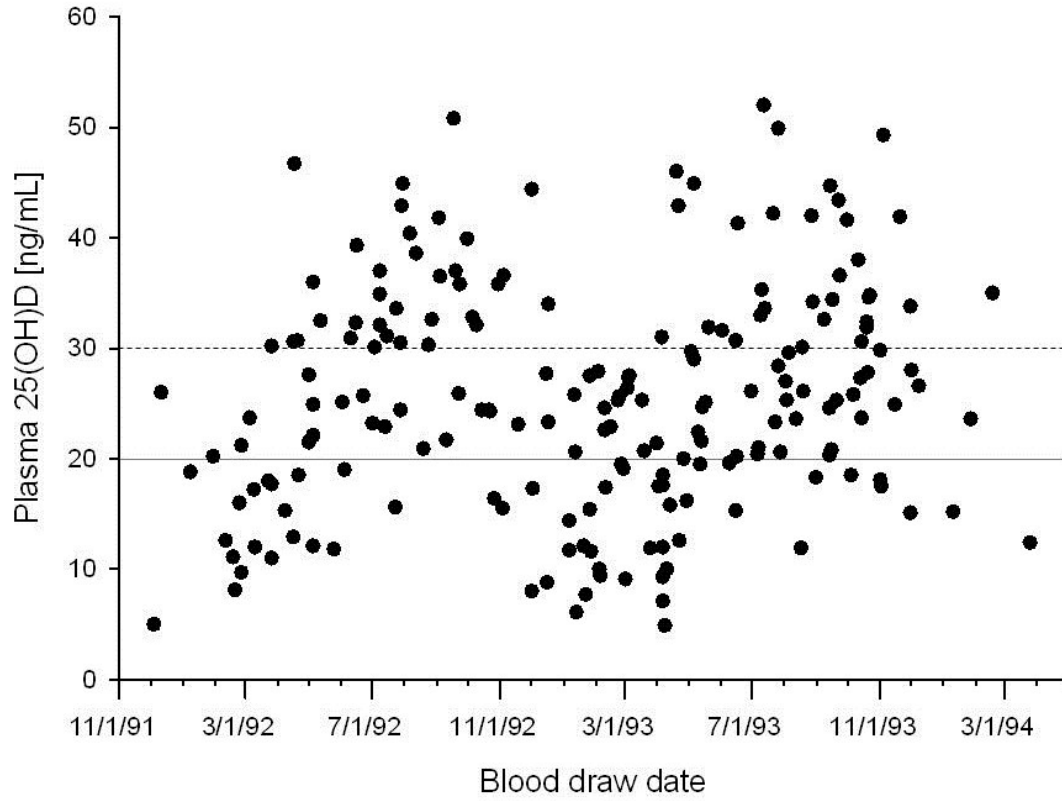
Winter=January-March, Spring=April-June, Summer=July-September,

Autumn=October-December. Results of statistical testing of means listed in Table 3

(one-way ANOVA with Bonferroni-corrected p-value) displayed at top with p-value and

corresponding line to indicate the comparison tested. 25(OH)D = 25-hydroxyvitamin D.

Figure 4: 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

- Aloia, J. F., & Li-Ng, M. (2007). Re: Epidemic influenza and vitamin D. *Epidemiology and Infection*, 135(7), 1095-6; author reply 1097-8.
doi:10.1017/S0950268807008308
- Anthonisen, N. R., Connett, J. E., Kiley, J. P., Altose, M. D., Bailey, W. C., Buist, A. S., Conway, W. A. Jr, Enright, P. L., Kanner, R. E., O'Hara P., et.al. (1994). Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. the lung health study. *JAMA: The Journal of the American Medical Association*, 272(19), 1497-1505. doi:10.1001/jama.272.19.1497
- Anthonisen, N. R., Connett, J. E., & Murray, R. P. (2002). Smoking and lung function of lung health study participants after 11 years. *American Journal of Respiratory and Critical Care Medicine*, 166(5), 675-679. doi:10.1164/rccm.2112096
- Anthonisen, N. R., Skeans, M. A., Wise, R. A., Manfreda, J., Kanner, R. E., Connett, J. E., for the Lung Health Study Research Group. (2005). The effects of a smoking cessation intervention on 14.5-year mortality: A randomized clinical trial. *Annals of Internal Medicine*, 142(4), 233-239.
- Bao, B. Y., Yeh, S. D., & Lee, Y. F. (2006). 1-alpha,25-dihydroxyvitamin D3 inhibits prostate cancer cell invasion via modulation of selective proteases. *Carcinogenesis*, 27(1), 32-42. doi:10.1093/carcin/bgi170
- Bischoff-Ferrari, H. A., Dietrich, T., Orav, E. J., Hu, F. B., Zhang, Y., Karlson, E. W., & Dawson-Hughes, B. (2004). Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged ≥ 60 y. *American Journal of Clinical Nutrition*, 80(3), 752-758.

- Black, P. N., & Scragg, R. (2005). Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. *Chest, 128*(6), 3792-3798. doi:10.1378/chest.128.6.3792
- Connett, J. E., Kusek, J. W., Bailey, W. C., O'Hara, P., & Wu, M. (1993). Design of the lung health study: A randomized clinical trial of early intervention for chronic obstructive pulmonary disease. *Controlled Clinical Trials, 14*(2 Suppl), 3S-19S.
- Cranney, A., Weiler, H. A., O'Donnell, S., & Puil, L. (2008). Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health. *Am J Clin Nutr, 88*(2), 513S-519.
- Crapo, R. O., Morris, A. H., & Gardner, R. M. (1981). Reference spirometric values using techniques and equipment that meet ATS recommendations. *The American Review of Respiratory Disease, 123*(6), 659-664.
- De Batlle, J., Romieu, I., Antó, J. M., Mendez, M., Rodríguez, E., Balcells, E., . . . Garcia-Aymerich, J. (2009). Dietary habits of firstly admitted Spanish COPD patients. *Respiratory Medicine, 103*(12), 1904-1910.
doi:10.1016/j.rmed.2009.06.001
- Enright, P. L., Johnson, L. R., Connett, J. E., Voelker, H., & Buist, A. S. (1991). Spirometry in the lung health study. 1. methods and quality control. *The American Review of Respiratory Disease, 143*(6), 1215-1223.
- Forli, L., Halse, J., Haug, E., Bjortuft, O., Vatn, M., Kofstad, J., & Boe, J. (2004). Vitamin D deficiency, bone mineral density and weight in patients with advanced pulmonary disease. *Journal of Internal Medicine, 256*(1), 56-62. doi:10.1111/j.1365-2796.2004.01337.x

- Franco, C. B., Paz-Filho, G., Gomes, P. E., Nascimento, V. B., Kulak, C. A., Boguszewski, C. L., & Borba, V. Z. (2009). Chronic obstructive pulmonary disease is associated with osteoporosis and low levels of vitamin D. *Osteoporosis International : A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, doi:10.1007/s00198-009-0890-5
- Ginde, A. A., Mansbach, J. M., & Camargo, C. A., Jr. (2009). Vitamin D, respiratory infections, and asthma. *Current Allergy and Asthma Reports*, 9(1), 81-87.
- Ginde, A. A., Liu, M. C., & Camargo, C. A., Jr. (2009). Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Archives of Internal Medicine*, 169(6), 626-632. doi:10.1001/archinternmed.2008.604
- Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine*, 357(3), 266-281. doi:10.1056/NEJMra070553
- Janssens, W., Bouillon, R., Claes, B., Carremans, C., Lehouck, A., Buyschaert, I., . . . Lambrechts, D. (2010). Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax*, 65(3), 215-220. doi:10.1136/thx.2009.120659
- Janssens, W., Lehouck, A., Carremans, C., Bouillon, R., Mathieu, C., & Decramer, M. (2009). Vitamin D beyond bones in chronic obstructive pulmonary disease: Time to act. *American Journal of Respiratory and Critical Care Medicine*, 179(8), 630-636. doi:10.1164/rccm.200810-1576PP

- Kendrick, J., Targher, G., Smits, G., & Chonchol, M. (2009). 25-hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the third national health and nutrition examination survey. *Atherosclerosis*, *205*(1), 255-260. doi:10.1016/j.atherosclerosis.2008.10.033
- Koli, K., & Keski-Oja, J. (2000). 1alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. *Cell Growth & Differentiation : The Molecular Biology Journal of the American Association for Cancer Research*, *11*(4), 221-229.
- Laaksi, I., Ruohola, J., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamaki, H., & Ylikomi, T. (2007). An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. *American Journal of Clinical Nutrition*, *86*(3), 714-717.
- Lee, J. H., O'Keefe, J. H., Bell, D., Hensrud, D. D., & Holick, M. F. (2008). Vitamin D deficiency: An important, common, and easily treatable cardiovascular risk factor? *Journal of the American College of Cardiology*, *52*(24), 1949-1956.
doi:10.1016/j.jacc.2008.08.050
- Riancho, J. A., Gonzalez Macias, J., Del Arco, C., Amado, J. A., Freijanes, J., & Anton, M. A. (1987). Vertebral compression fractures and mineral metabolism in chronic obstructive lung disease. *Thorax*, *42*(12), 962-966. doi:10.1136/thx.42.12.962
- Sato, Y., Iwamoto, J., Kanoko, T., & Satoh, K. (2005). Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: A randomized controlled trial. *Cerebrovascular Diseases*, *20*(3), 187-192.
doi:10.1159/000087203

Shane, E., Silverberg, S. J., Donovan, D., Papadopoulos, A., Staron, R. B., Adesso, V.,

Schulman, L. (1996). Osteoporosis in lung transplantation candidates with end-stage pulmonary disease. *The American Journal of Medicine*, 101(3), 262-269.

doi:10.1016/S0002-9343(96)00155-6

Timms, P. M., Mannan, N., Hitman, G. A., Noonan, K., Mills, P. G., Syndercombe-

Court, D., Boucher, B. J. (2002). Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: Mechanisms for inflammatory damage in chronic disorders? *QJM : Monthly Journal of the Association of Physicians*, 95(12), 787-796.