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

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**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 (-151 vs. -0.28 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.

**Key Words:**

Pulmonary Disease, Chronic Obstructive

Smoking

Spirometry

Vitamin D

**Abbreviations**

25(OH)D = 25-hydroxyvitamin D

COPD = chronic obstructive pulmonary disease

$FEV_1$ = 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

## 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 ($FEV_1$) in a graded, "dose-dependent" fashion{{478 Black,P.N. 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{{509 Janssens,Wim 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){{495 Koli,K. 2000; 496 Bao,B.Y. 2006; 497 Timms,P.M. 2002; 509 Janssens,Wim 2009; }}.

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 {{488 Connett,J.E. 1993; 175 Anthonisen,N.R. 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{{489 Anthonisen,Nicholas R. 2002;508 Anthonisen,Nicholas R. 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 (see online supplement for

figure of design). 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 $FEV_1$ between the Y5 visit and the LT visit (rapid decliners). Controls were continuous smokers with the least decline in $FEV_1$ 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, Alabama [33° N latitude] to as far north as Winnepeg, Manitoba [49° N latitude]). 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 $FEV_1$ 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 $FEV_1$ 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 $FEV_1$ decline between the cases and controls, while still remaining matched on date and clinical center (see online supplement for figure of selection process). 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 and Applied Biosystems-MDS Sciex, Foster City, CA) in the laboratory of author RS at the Mayo Clinic (Rochester, MN) . Details of the 25(OH)D assays, including coefficients of variation, are provided in the online supplement.

Spirometry was performed in both the main trial and LT visit using the same rolling seal spirometers (Spirotech 500; Spirotech, Atlanta, GA) and the same spirometry quality control program{{494 Enright,P.L. 1991; }}. Measurements of $FEV_1$ 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 $FEV_1$ and FVC were reported and converted to percentages of the predicted normal using the formulas of Crapo and colleagues{{490 Crapo,R.O. 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.

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. Details of the sample size determination are provided in the online supplement.

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 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.

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

## 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 (n = 24), Birmingham, AL (n = 10), Cleveland, OH (n = 14), Detroit, MI (n= 24), Los Angeles, CA (n = 20), Pittsburgh, PA (n = 24), Portland, OR (n = 22), Rochester, MN (n = 12), Salt Lake City, UT (n = 18), and Winnipeg, MB (n = 28).

Our selection criteria for cases and controls resulted in clinically and statistically significant differences in the rate of $FEV_1$ decline between rapid and slow decliners. While $FEV_1$ was similar between rapid and slow decliners at the Y5 visit, rapid decliners had a mean $FEV_1$ that was over a liter worse than slow decliners at the LT visit (Table 1). This resulted in a rate of $FEV_1$ decline of -151.6 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 $FEV_1$ 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 multiple regression and paired logistic regression analysis for baseline Y5 covariates also demonstrated no association between Y5 25(OH)D level and rapid or slow decliner status (see online supplement for details).

We applied current widely accepted definitions of vitamin D insufficiency and deficiency{{428 Holick,Michael F. 2007; }}, which classify patients as vitamin D deficient with 25(OH)D levels <20 ng/mL and insufficient with levels ≥ 20ng/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 (Figure 1 and online supplement figure). 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{{478 Black,P.N. 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 $FEV_1$ 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 ≤16.2 ng/mL to those ≥ 34.3 ng/ml, $FEV_1$ 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.

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 can not extrapolate these findings to non-smokers or to intermittent smokers. We also can not 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 $FEV_1$ of 39% of predicted) between 1983-1985 and showed the mean 25(OH)D level was below 10 ng/mL for most of the year, with peak mean 25(OH)D level in late summer still below 20 ng/mL{{447 Riancho,J A. 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{{449 Shane,E. 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 1993-1998{{448 Forli,L. 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%{{501 Ginde,Adit A. 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{{504 Franco,C.B. 2009; }}. Of these 49 patients, only 3 (6%) had 25(OH)D levels ≥30ng/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{{518 Janssens,Wim 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{{513 de Batlle,Jordi 2009; }}, but multiple other mechanisms may lead to inadequate vitamin D status{{428 Holick,Michael F. 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{{469 Cranney,Ann 2008; }}. Multiple other COPD complications and co-morbidities have been linked to vitamin D insufficiency, including respiratory infections{{425 Ginde,A.A. 2009;441 Laaksi,Ilkka 2007; 450 Aloia,J.F. 2007; }}, cardiovascular disease{{512 Kendrick,J. 2009; ;426 Lee,John H. 2008; }}, and muscle dysfunction{{506 Bischoff-Ferrari,Heike A. 2004;507 Sato,Y. 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.

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**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 ± standard deviation for continuous variables or as number (%) for categorical variables. p-values calculated using paired t-testing. $FEV_1$ = 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 ± 6.9 | 52.2 ± 6.6 | 0.31 |
| Cigarettes/day reported at Y5 | 25.1 ± 12.6 | 21.5 ± 11.6 | 0.04 |
| Y5 $FEV_1$ (L) | 2.35 ± 0.63 | 2.49 ± 0.65 | 0.09 |
| Y5 $FEV_1$ (% predicted) | 71.0 ± 12.1 | 73.2 ± 11.5 | 0.15 |
| Y5 FVC (L) | 4.10 ± 1.02 | 3.94 ± 0.99 | 0.23 |
| Y5 FVC (% predicted) | 98.6 ± 13.7 | 92.1 ± 11.4 | <0.001 |
| Time between Y5 to LT spirometry visits (years) | 6.0 ± 0.64 | 5.8 ± 0.66 | 0.06 |
| LT $FEV_1$ (L) | 1.44 ± 0.52 | 2.49 ± 0.66 | <0.001 |
| LT $FEV_1$ (% predicted) | 45.2 ± 13.3 | 77.2 ± 11.6 | <0.001 |
| LT FVC (L) | 3.31 ± 1.01 | 3.92 ± 1.05 | <0.001 |
| LT FVC (% predicted) | 81.7 ± 17.0 | 94.2 ± 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 ± standard deviation. p-values calculated using paired t-testing. 25(OH)D = 25-hydroxyvitamin D, $FEV_1$ = 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 ± 47.7 | -0.28 ± 24.0 | <0.0001 |
| Rate of $FEV_1$ decline from Y5 to LT (%predicted/year) | -4.3 ± 1.2 | +0.7 ± 0.7 | <0.0001 |
| Y5 25(OH)D levels (ng/mL) | 25.0 ± 10.4 | 25.9 ± 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 ± standard deviation for 25(OH)D and number (%) for the categorical data. 25(OH)D = 25-hydroxyvitamin D. See Figure 1 for p-values from pairwise statistical testing of mean 25(OH)D levels by season.

| | Winter (n=48) | Spring (n=51) | Summer (n=57) | Autumn (n=40) |
|---|---|---|---|---|
| 25(OH)D level [ng/mL] | 18.3 ± 7.0 | 24.1 ± 10.3 | 31.7 ± 9.2 | 26.8 ± 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 = ≥ 30 ng/mL
b: Vitamin D insufficiency = 25(OH)D level ≥20 ng/mL, <30 ng/mL
c: Vitamin D deficiency = 25(OH)D level <20 ng/mL

**Figure 1:** 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.