Respiratory Medicine (2013) 107, 1578–1588

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Vitamin D, vitamin D binding protein, lung function and structure in COPD

Isaac Berg [a,1], Corrine Hanson [b,1], Harlan Sayles [c], Debra Romberger [d], Amy Nelson [a], Jane Meza [c], Bruce Miller [e], Emiel F.M. Wouters [f], William MacNee [g], E.P.A. Rutten [h], Elisabeth A.P.M. Romme [i], Jørgen Vestbo [j,k], Lisa Edwards [l], Stephen Rennard [a,*]

[a] *Division of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198-5300, United States*
[b] *Division of Medical Nutrition Education, School of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE, United States*
[c] *College of Public Health, University of Nebraska Medical Center, Omaha, NE, United States*
[d] *VA Nebraska Western Iowa Healthcare System, Omaha, NE, United States*
[e] *GlaxoSmithKline, King of Prussia, PA, United States*
[f] *Department of Pulmonary Diseases, University Hospital Maastricht, The Netherlands*
[g] *University of Edinburgh, Scotland, UK*
[h] *Program Development Centre, Centre of Expertise for Chronic Organ Failure (CIRO), The Netherlands*
[i] *Department of Respiratory Medicine, Catharina Hospital Eindhoven, The Netherlands*
[j] *Manchester Academic Health Sciences Centre, South Manchester University Hospital NHS Foundation Trust, Manchester, United Kingdom*
[k] *Odense University Hospital and University of Southern Denmark, Odense, Denmark*
[l] *GlaxoSmithKline, Research Triangle Park, NC, United States*

Received 16 November 2012; accepted 27 May 2013
Available online 1 July 2013

KEYWORDS
Emphysema;
25(OH)D;
FEV1;
Free vitamin D

Summary
Rationale: Vitamin D and vitamin D binding protein (DBP) have been associated with COPD and FEV1. There are limited data regarding emphysema and vitamin D and DBP.
Objective: This is a pilot study of a portion of the subjects in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study designed to examine the relationship between vitamin D status, DBP, FEV1 and emphysema in COPD patients.
Methods: We measured serum 25(OH)D and DBP in 498 ECLIPSE subjects. Subjects were distributed amongst smoker controls, non-smoker controls, and GOLD stages 2, 3 and 4. Within each

* Corresponding author.
E-mail addresses: srennard@unmc.edu, lrichard@unmc.edu (S. Rennard).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.rmed.2013.05.010

GOLD stage, the subjects were equally divided amongst high and low emphysema burden. The associations between 25(OH)D, DBP, and free vitamin D with FEV1, CT-defined emphysema, biomarkers and clinical data including CT-measured bone attenuation were assessed.
Measurements: 25(OH)D and DBP were measured using tandem mass spectroscopy and competitive enzyme-linked immunosorbent assay, respectively,
Main result: 25(OH)D was correlated with FEV1 ($p = 0.01$) and with severity of emphysema ($p < 0.01$). 25(OH)D was also associated with six-minute walk ($p = 0.02$), bronchodilator response ($p = 0.04$), and Clara cell secretory protein (CC-16) ($p = 0.01$). 25(OH)D levels were not associated with CT-measured bone attenuation, however DBP was associated with bone attenuation in subjects with emphysema. DBP was not associated with FEV1 or emphysema. 25(OH)D and DBP were inversely associated ($p = 0.01$).
Conclusion: This is the first study to demonstrate a relationship between emphysema and vitamin D. We also provide further evidence for a relationship between vitamin D and FEV1.

At a glance commentary: scientific knowledge on the subject

Vitamin D deficiency is recognized as a widespread problem among COPD patients, and has been associated with FEV1 and several co-morbid conditions of COPD.

What this study adds to the field

This study demonstrates a relationship between vitamin D status and emphysema, and confirms associations between vitamin D status with FEV1, co-morbid conditions, and inflammatory biomarkers of COPD.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is presently the third leading cause of death in the United States [1]. COPD, and many of its co-morbid conditions, are associated with vitamin D status. Vitamin D deficiency is recognized as a widespread problem among adults in general, and in COPD patients specifically [1,2]. The association of vitamin D deficiency with poor pulmonary health is becoming more well-defined. Studies such as NHANES have identified a positive relationship between vitamin D status and pulmonary function [3,4]. Whether Vitamin D is associated with emphysema remains unknown. Vitamin D deficiency is also associated with the extra-pulmonary features of COPD. For example, vitamin D deficiency can impair host defense, and vitamin D replacement can improve innate immunity [5,6]. Vitamin D deficiency has also been associated with other clinical features of COPD, such as 6-min walking distance, and co-morbidities, including osteoporosis [7–9] as well as poor adherence and outcome of pulmonary rehabilitation [10]. While one recent study did not demonstrate an effect of vitamin D supplementation on exacerbations in a population of 182 COPD patients, supplementation in a subset of patients with severe vitamin D deficiency was associated with a reduction in the rate of exacerbations [11].

In contrast to the direct relationship of 25(OH)D levels with lung function, vitamin D binding protein (DBP) levels have been reported to be inversely related to FEV1 in patients with alpha-1 anti-trypsin deficiency [12]. DBP has also been implicated as a candidate in the pathogenesis of COPD due to its role in macrophage activation and neutrophil chemotaxis [13]. Serum DBP circulates in concentrations that are 20-fold higher than 25(OH)D concentrations, and bind more than 88% of 25(OH)D. Owing to the avid binding affinity of DBP to vitamin D, a molar concentration ratio can be calculated to represent the "free vitamin D" metabolite concentration [14–17]. Free vitamin D levels may be of interest, as protein-bound vitamin D metabolites may have limited access to target cells, and free levels may provide a better estimate of the biologically active component of vitamin D.

The current study was designed to address several key questions exploring the role of vitamin D measures in outcomes related to lung function, lung structure, and COPD related conditions. First, we addressed the question of whether vitamin D is associated with emphysema as well as with FEV1 in COPD patients. Second, we assessed if DBP levels were associated with FEV1 or with emphysema and, using DBP levels, we assessed the relationships with calculated free Vitamin D. Finally, we assessed the relationship of these measures of Vitamin D with selected clinical features of COPD.

Methods

Study population

This analysis is based on data from 498 subjects from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study (ECLIPSE). A visual breakdown of the study subjects is provided in Fig. 1. Demographic features of these subjects are presented in Table 1. Subjects included 75 smoker and 75 non-smoker controls and three groups of 116 subjects each with GOLD airflow limitation severity stage 2, 3 and 4. The latter three groups included the 58 subjects with the highest and the 58 subjects with the lowest CT emphysema scores (% of lung voxels below −950 Hounsfield units) in each GOLD stage. Stored serum samples from visit 7 of the ECLIPSE study, which corresponded to 2½ years after study entry, were assessed.

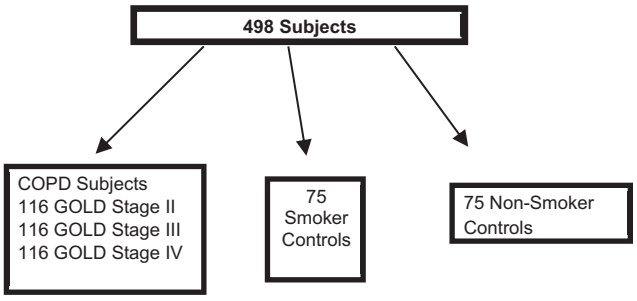

Figure 1 Subject distribution. Subjects were selected from the entire Eclipse cohort to have a range of FEV1 values (GOLD 2, 3 and 4) and to have a range of emphysema severities within each GOLD group stage. Both smoking and non-smoking controls were included.

Complete details of ECLIPSE study participants and study design have been previously described [18].

Measurements

25(OH)D was measured with liquid—liquid extraction, tandem mass spectroscopy (ABSciex 4000 tandem mass spectrophotometer, ABSciex, Massachusetts). Values lower than the minimal reporting threshold (<10 ng/mL) were given a value of 5 ng/ml to facilitate analysis. DBP was measured with an internally validated competitive enzyme linked immunoabsorbant assay (ELISA) using commercially available reagents and optimized as described [19]. Pre- and post-bronchodilator FEV1 and FVC were measured concurrently with the serum sampling. CT-scan defined emphysema was assessed at year 3, the nearest assessment point to serum data for smoker controls and COPD subjects, and at study entry in non-smoker controls. Quantitative assessment of emphysema was performed by attenuation mask analysis using a threshold of −950 Hounsfield Units (Pulmonary Workstation 2.0, VIDA Diagnostics, Iowa City, IA, USA) and expressed as the percent of low attenuation areas (%LAA). Serum free vitamin D was estimated by molar concentration ratio index of 25(OH)D:DBP.

Additional biomarker and clinical data evaluated for their relationship with vitamin D measures are given in Table 2, and were measured at study baseline. Six-minute walk data were only available on COPD subjects, and were taken from the year 3 visit. SGRQ data were used from year 3 visit. GOLD grouping (A—D) was performed and characterized as noted in most recent GOLD document [20] with Modified Medical Research Council dyspnea score (MMRC) as the symptomatic measure.

Low-dose CT scans of the chest were performed as described [18]. Bone attenuation was measured on CT using the software VOXAR 3D version 16.0 as described previously [21]. Briefly, the mean bone attenuation of thoracic vertebrae 4, 7 and 10 (T4, T7 and T10) were determined by placing circular regions of interest in the central parts of the vertebral bodies. The average bone attenuation was calculated and expressed as a single unit expressed in Hounsfield Units.

Statistical analysis

Associations between pairs of variables of interest were evaluated using Spearman correlation coefficients. Linear regression models were used to further examine possible associations and to test for interactions across groups of subjects. ANOVA models were used to test for differences in means of various measures across groups based on level of 25(OH)D. Multivariate models for each of the primary outcome measures were run controlling for the confounders of age, sex, BMI, smoking status, and season. A fixed effects

Table 1 Population demographics.

% or mean (SD)	Non-smoking controls (n = 75)	Smoking controls (n = 75)	COPD cases		
			GOLD 2 (n = 116)	GOLD 3 (n = 116)	GOLD 4 (n = 116)
Age (years)	54.8 (8.7)	54.8 (8.9)	62.6 (8.1)	62.2 (7.5)	61.5 (7.5)
Male	40.0	56.0	53.5	69.0	74.1
Race					
White/Caucasian/European	96.0	100.0	98.3	97.4	96.6
White/Arabic/North African	1.3	0.0	0.0	0.0	0.9
African American/African	2.7	0.0	1.7	1.7	2.6
Body mass index (kg/m^2)	26.8 (4.8)	27.1 (4.3)	25.5 (4.2)	25.6 (6.0)	25.2 (6.0)
Smoking status					
Current	0.0	56.0	32.8	39.7	31.9
Former	13.3	44.0	67.2	60.3	68.1
Never	86.7	0.0	0.0	0.0	0.0
Lifetime smoking (pack years)	0.4 (1.4)	32.7 (23.7)	41.1 (20.7)	43.4 (23.2)	48.8 (28.3)
FEV_1 pre-dose (L)	3.1 (0.7)	3.1 (0.8)	1.5 (0.5)	1.0 (0.4)	0.7 (0.3)
FEV_1 post-dose (L)	3.2 (0.7)	3.2 (0.8)	1.7 (0.5)	1.1 (0.4)	0.8 (0.3)
% Predicted FEV_1 Pre- Bronchodilator	107.8 (13.7)	99.1 (13.4)	55.9 (13.6)	36.1 (11.1)	23.8 (7.6)
% Predicted FEV_1 post-dose	110.7 (13.3)	103.6 (13.7)	61.7 (14.2)	39.3 (11.4)	25.6 (7.9)
Forced vital capacity pre-dose (L)	4.0 (1.0)	4.1 (1.1)	3.1 (0.9)	2.6 (0.8)	2.3 (0.7)
Forced vital capacity post-dose (L)	4.0 (1.0)	4.2 (1.1)	3.3 (0.9)	2.9 (0.8)	2.5 (0.7)
% Low attenuation area (>950HU)	3.9 (4.5)	3.3 (3.2)	18.4 (17.9)	25.1 (21.2)	30.4 (14.8)

Table 2 Vitamin D, biomarkers, and clinical characteristics.

Spearman correlation coefficients *P*-value Number of observations	25(OH)D (ng/mL)	Vitamin D binding protein (mg/dL)	Free vitamin D ratio
SGRQ score	0.183 <0.001 477	0.023 0.621 482	−0.158 <0.001 477
6-min walk	0.136 0.02 313	−0.034 0.55 316	0.156 0.01 313
Bronchodilator reversibility	0.095 0.04 488	0.058 0.20 493	0.042 0.36 488
Fibrinogen (mg/dL)	0.064 0.16 491	0.066 0.14 496	0.020 0.66 491
Clara cell secretory protein (ng/mL)	0.181 <0.01 485	−0.036 0.43 490	0.164 <0.01 485
C-reactive protein (mg/L)	−0.080 0.08 478	0.046 0.32 482	−0.083 0.07 478
Surfactant protein D (ng/mL)	0.094 0.04 485	0.003 0.95 490	0.068 0.13 485
Total neutrophils	−0.070 0.13 482	0.075 0.10 487	−0.092 0.04 482
White blood cell count	−0.041 0.36 482	0.100 0.03 487	−0.086 0.06 482
Body mass index (kg/m^2)	−0.052 0.25 492	−0.019 0.67 497	−0.022 0.63 492
Fat free mass	−0.023 0.61 484	−0.085 0.06 489	0.037 0.42 484
CT-measured bone attenuation (mean of T4, T7, T10	−0.033 0.50 407	0.053 0.28 411	−0.052 0.30 407

analysis examining GOLD subject group based on mMRC, exacerbation history and FEV1 was performed using Group D (high risk, more symptoms) as a reference group. All analyses were conducted using SAS v9.2 (The SAS Institute, Cary, NC).

Results

Serum 25(OH)D

The mean 25(OH)D level of the cohort, including COPD subjects and controls, was 24.0 ng/mL. Vitamin D insufficiency, defined as 25(OH)D levels of <30 ng/mL, was present in 69% of the cohort, and deficiency, defined as 25(OH)D levels of <20 ng/mL, was present in 39% of the population. Severe deficiency (<10 ng/mL) occurred in 8.7% of subjects. Among the groups, the only mean 25(OH)D levels that differed significantly from each other were GOLD 4 group compared to smoking controls (22.18 vs. 27.99 ng/ml, respectively), and GOLD 3 group vs. smoking controls (21.9 vs. 27.99 ng/mL, respectively).

As expected, 25(OH)D levels were significantly associated with FEV1 (Fig. 2a, $r = 0.116$, $p = 0.01$). The effect was primarily driven by individuals with the lowest levels of Vitamin D, which is better visualized when Vitamin D levels

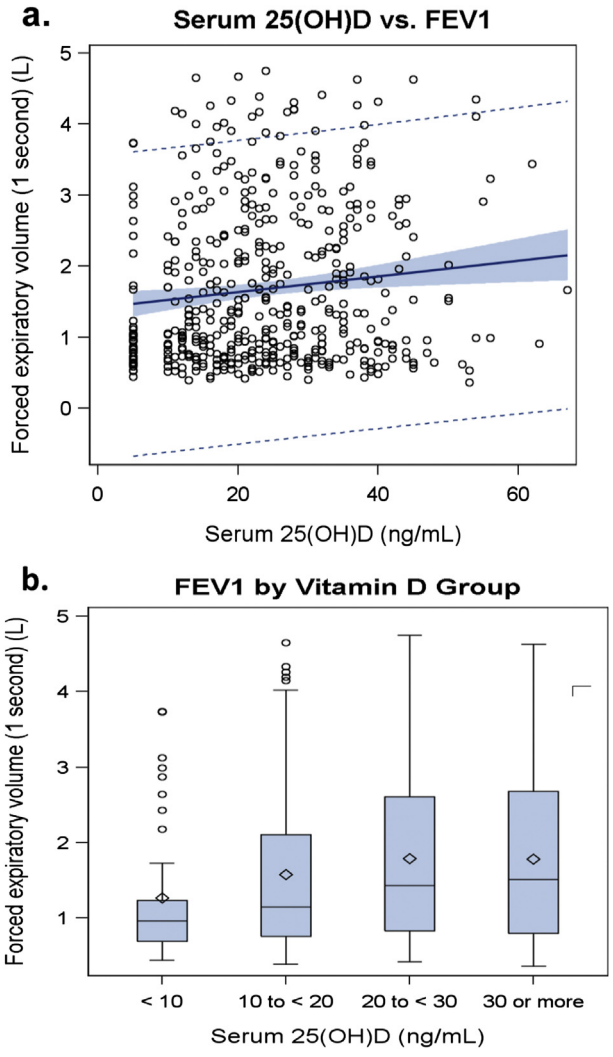

Figure 2 Relationship between FEV1 and Serum 25(OH)D. 25(OH)D was measured as described in methods and related to FEV1 measured at the same visit. Panel A: direct relationship between the variables. The relationship was significant ($r = 0.116$, $p = 0.01$). Panel B: Subjects were grouped by vitamin D level (<10, severely deficient, 10–20, deficient; 20–30; insufficient, and >30; sufficient. There were reductions in FEV1 associated with vitamin D status that were most marked in the severely deficient group. (Group comparison to sufficient group (>30) $p = 0.0062$).

are grouped (Fig. 2b). The association remained significant after adjustment for confounders ($p = 0.006$). Lower 25(OH)D levels were also significantly associated with emphysema ($r = -0.141$, $p < 0.01$) (Fig. 3). As with FEV1, the relationship between 25(OH)D and emphysema was most marked for individuals with lower 25(OH)D levels, and this is better visualized when the vitamin D levels are grouped (Fig. 3b). Similar to FEV1, the relationship remained significant after adjustment for confounders ($p = 0.007$). When only the COPD subjects were assessed, the relationship between 25(OH)D and FEV1 and emphysema was no longer significant (Supplementary Table E1). Lung capacity measures were also assessed. After controlling for confounders, both post-dose FVC and predicted normal FVC were positively associated with 25(OH)D levels ($p = 0.024$ and 0.009, respectively).

Vitamin D binding protein

DBP was not associated with either FEV1 or emphysema, (Supplemental Figure E1). However, DBP was negatively associated with serum 25(OH)D level (Fig. 4, $r = -0.122$, $p = 0.01$).

Free vitamin D

Direct measurement of free Vitamin D is problematic. However, as the dissociation constant of Vitamin D from DBP is well established, it is possible to estimate free vitamin D from the concentration of total Vitamin D and DBP. Free vitamin D was associated with 25(OH)D ($r = 0.81$, $p < 0.0001$) and with DBP ($r = -0.63$, $p < 0.0001$). Using this calculated estimate, no association was observed between either FEV1 ($r = 0.079$, $p = 0.08$) or emphysema ($r = -0.082$, $p = 0.07$, Supplemental figure E2).

Clinical features and selected biomarkers

Because vitamin D has been previously associated with certain systemic effects of COPD, the relationships between vitamin D, DBP, free vitamin D and selected clinical features and biomarkers were assessed in order to provide additional information on vitamin D and extra-pulmonary features of COPD (Table 2). There were significant associations between 25(OH)D and SGRQ, Clara cell secretory protein-16, surfactant protein D, 6 min walking distance and bronchodilator reversibility. For 6 min walking distance and SGRQ, where the associations were somewhat stronger, a disproportionate association of impairment was associated with very severe depletion of vitamin D (Fig. 5). This was much less apparent for bronchodilator responsiveness (Fig. 5). 25(OH)D levels were not associated with CT measured bone attenuation (Supplemental Figure E3, Supplemental Table E2). After adjustment for BMI and smoking status, the association between bone attenuation and 25(OH)D remained non-significant. However, in subjects with emphysema, DBP levels were associated with bone attenuation measurements ($r = 0.20$, $p = 0.02$) (Fig. 6).

A significant relationship was observed between DBP and total WBC count ($p = 0.03$), and free vitamin D with SGRQ ($p < 0.001$), bronchodilator response ($p = 0.05$), 6 min walk

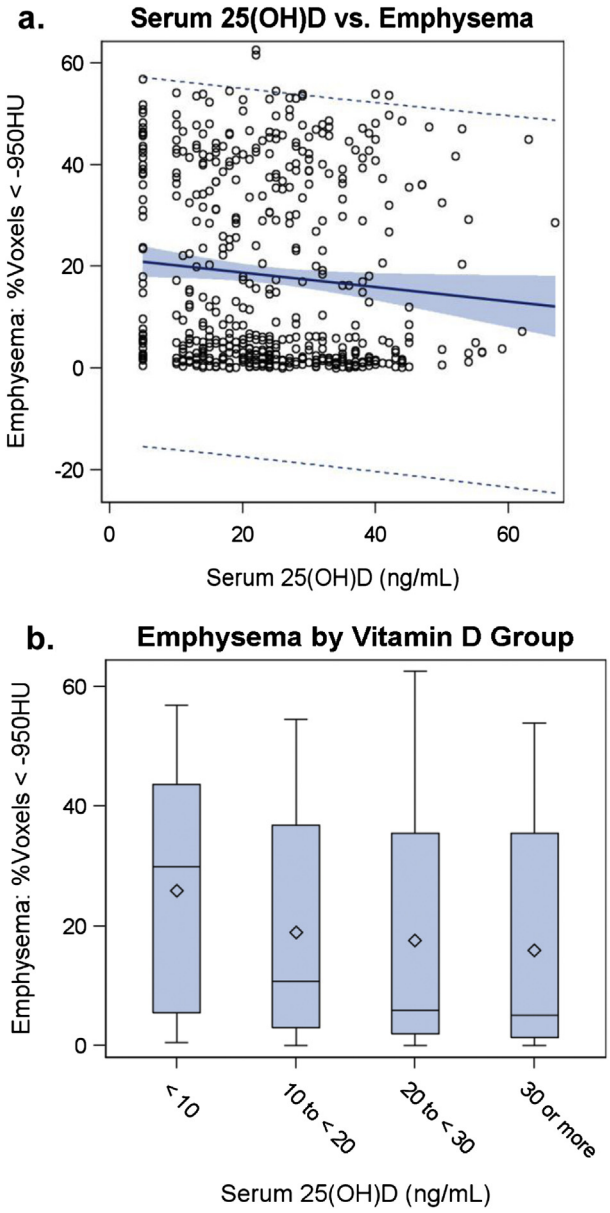

Figure 3 Relationship between emphysema and Serum 25(OH)D. 25(OH)D was measured as described in methods and related to emphysema assessed by CT scan (see methods), which was performed on a visit 6 months later. Panel A: direct relationship between the variables. The relationship was significant ($r = 0.141$, $p = <0.01$). Panel B: Subjects were grouped by vitamin D level (<10, severely deficient, 10–20, deficient; 20–30; insufficient, and >30; sufficient. There was increased emphysema severity associated with vitamin D status that was most marked in the severely deficient group. (Group comparison to replete group (>30) $p = 0.0018$).

($p = 0.01$), SGRQ ($p \leq 0.001$), clara cell secretory protein ($p \leq 0.001$) and total neutrophil count ($p = 0.04$) (Table 2). It was also of specific interest to assess the relationship between Vitamin D and body composition. Interestingly, neither fat free mass nor body mass index was significantly related to 25(OH)D, DBP or free Vitamin D (Supplementary Table E3).

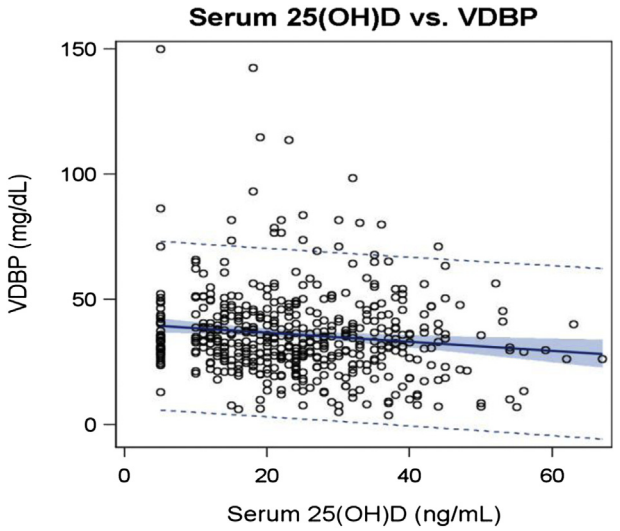

Figure 4 Relationship between Vitamin D Binding Protein and 25(OH)D. Vitamin D binding protein (DBP) and 25(OH)D were measured as described. Values of 25(OH)D below the limit of detectability (10 ng/ml) were assigned a value of 5 ng/ml. Vertical axis: DBP, horizontal axis: 25(OH)D. The relationship was significant ($r = -0.122$, $p = 0.01$).

Vitamin D and GOLD severity

The most recent GOLD Strategy document has suggested that COPD patients should be classified into 4 groups based on presence of low lung function, frequent exacerbations, and severity of symptoms [20]. Using the mMRC to gauge symptoms, subjects were classified into 4 groups corresponding to the current GOLD strategy. 25(OH)D and free vitamin D levels were significantly lower in group D when compared to controls. DBP was not different among the groups. (Fig. 7, see also Supplementary Figures E4, Tables E4 and E5).

Discussion

In addition to confirming the previously established relationship between 25(OH)D with FEV1 [3,4] and 6-min walk distance [7,9], we provide new evidence establishing a relationship between 25(OH)D and emphysema. Interestingly, the relationship with emphysema was seen in the entire population, but was not seen quantitatively in the COPD subject group. This resembles the effect of ever smoking vs. amount smoked [22]. In that setting, smoking has been suggested to initiate a process that leads to

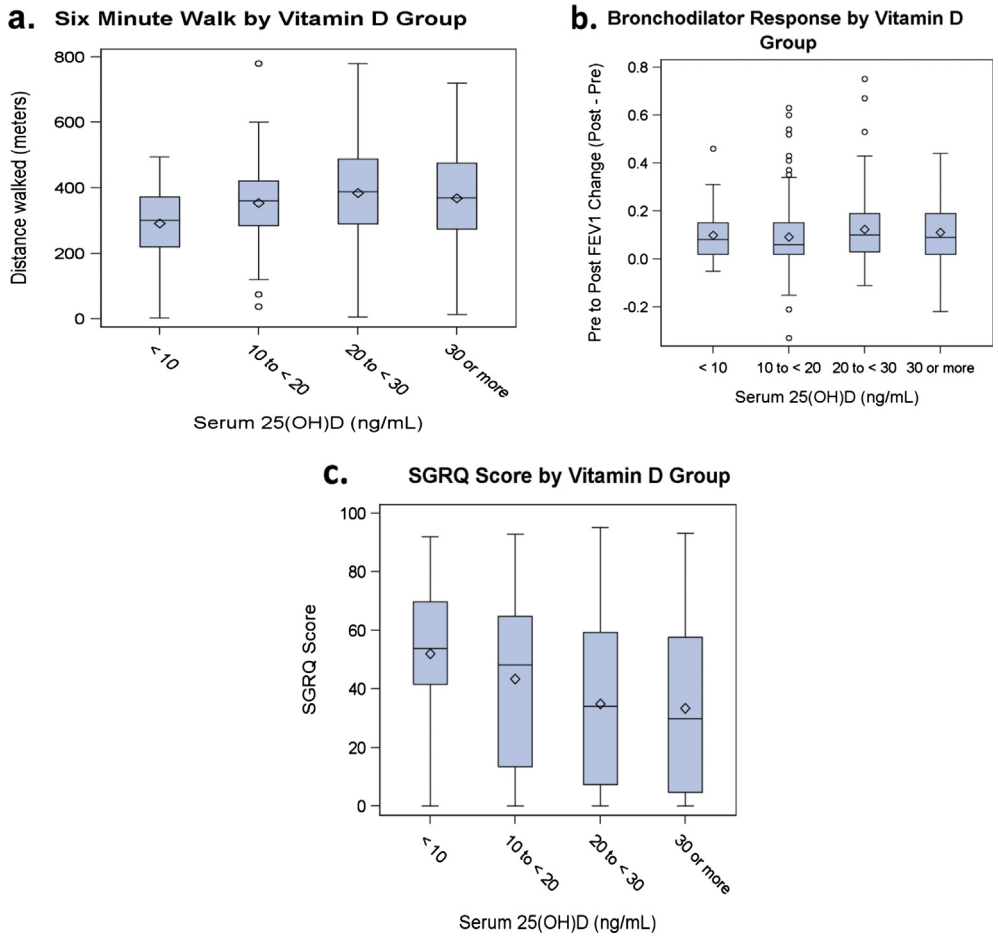

Figure 5 Relationship between 6 min walk distance, bronchodilator responsiveness, SGRQ and Serum 25(OH)D. 25(OH)D was measured as described in methods. Subjects were grouped by vitamin D level (<10, severely deficient, 10–20, deficient; 20–30; insufficient, and >30; intact. Panel A: 6 min walking distance; Panel B: Bronchodilator Respose; Panel C: SGRQ

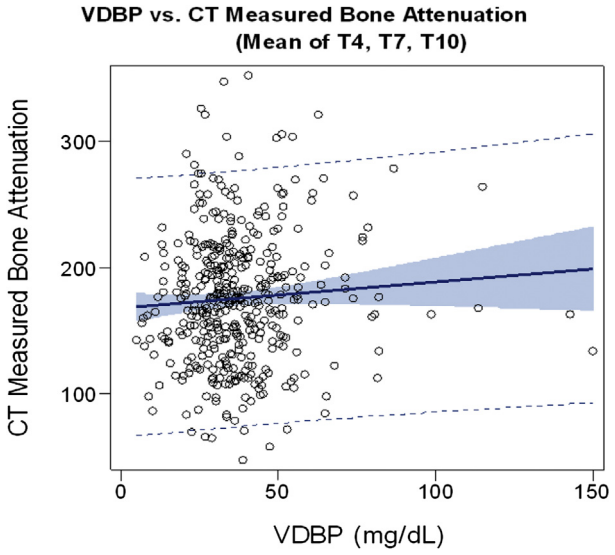

Figure 6 Relationship between VDBP and CT measured bone attenuation emphysema is defined here as those GOLD subjects with above-median measures for emphysema within their respective GOLD classes, DPB levels were associated with CT measured bone attenuation (mean of T4, T7, and T10) ($r = 0.20$, $p = 0.02$).

emphysema, but continued smoking is not required for its emphysema progression. We also demonstrate a significant relationship between vitamin D with SGRQ, bronchodilator responsiveness and selected biomarkers of COPD.

Consistent with previous studies [1,2], this study documents a high prevalence of vitamin D deficiency and insufficiency in a population of COPD patients and controls. Reduced FEV1 has been associated with lower 25(OH)D levels in both the general population and COPD patients [3,4,12]. However, these findings were not replicated by Shaheen and colleagues [23]. Our results continue to provide evidence of a relationship between lung function and 25(OH)D in the general population, but, like Shaheen we did not find a significant correlation between 25(OH)D and FEV1 in the COPD population. There are some important differences among these studies. Janssens, who found a relationship between FEV1 and 25(OH)D in a COPD population, it included mostly elderly men, while both the current study and that of Shaheen included more women. In addition, Janssens excluded subjects taking vitamin D replacement. This was not a requirement in either the Shaheen study or the current study, although Shaheen noted only a weak relationship between supplementation and 25 (OH)D levels. The studies also differ in their geography. ECLIPSE recruited subject in many locations, while Janssens evaluated subjects in Belgium. In addition, Janssens included subjects with FEV1 severity stage 1, while these milder subjects were excluded from the ECLIPSE population. Whether the association noted by Janssens would be present in GOLD 2—4 only was not reported. Studies assessing the relationship between vitamin D and severity of emphysema in humans are limited to date. Animal models have shown that 25(OH)D deficiency is associated with alterations in lung structure [24], but this has not been demonstrated in humans. Dairy intake, but not specifically vitamin D intake, has been inversely associated with severity of emphysema [25].

It is possible the relationship between FEV1 and emphysema with vitamin D may be driven by individuals with very marked deficiency. Stratifying our subjects into groups based on severity of vitamin D deficiency suggests a

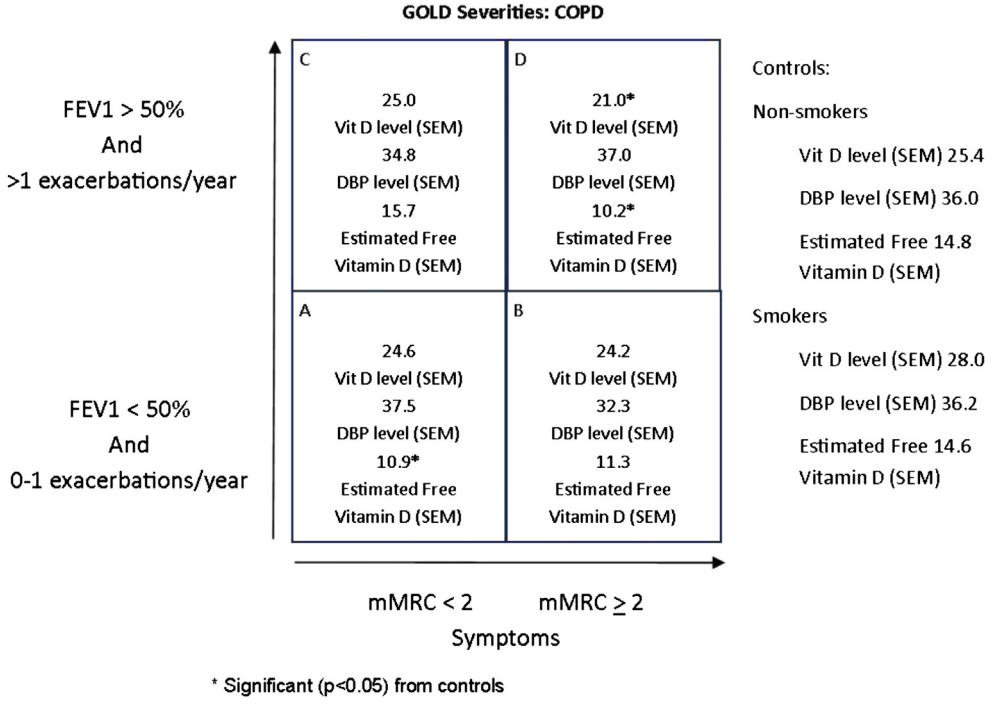

Figure 7 Vitamin D and GOLD severity.

disproportionate association of the severely deficient (<10 ng/mL) subjects with reduced FEV1 and with more severe emphysema. This raises the possibility that the association of low 25(OH)D with COPD may not correspond to the current cut-off levels of deficiency (<20 ng/mL), but rather that the association is driven by the severely deficient as recently suggested by Lehouck and colleagues [11].

Our study did not demonstrate a relationship between DBP levels and either FEV1 or emphysema. Previous studies have shown a relationship between various DBP alleles and COPD [26,27], and several potential mechanisms that might explain this relationship [28—31]. DBP can directly bind to leukocytes and modulate macrophage activation and neutrophil chemotaxis [13]. In this regard, DBP has been described as enhancing the chemotactic potency of C5a, one of the key active peptides generated during complement activation. Interestingly, upon binding Vitamin D, DBP may be less potent in this regard, providing a potential mechanism for Vitamin D to have an "anti-inflammatory" effect independent of binding to its canonical receptor. Another potential mechanism could be the ability of DBP to sequester Vitamin D. For this reason, we used standard methods to estimate free vitamin D, based on concentrations of Vitamin D and DBP. These estimates did not correlate better with FEV1, emphysema or clinical parameters than the measures of Vitamin D. Although this does not provide support for a role for free Vitamin D, direct measurement of free vitamin D or of 1,25 (OH)D, the biologically active form of vitamin D, which was beyond the scope of this project, may have stronger associations. The most widely used laboratory methods do not delineate free versus bound vitamin D, and, to our knowledge free vitamin D has not been directly measured in COPD. In our study, calculated free vitamin D was not associated with primary end points of lung function or emphysema, although associations neared significance. Significant associations, however, were noted with conditions related to COPD, including Clara cell secretory protein, 6 min walk distance, SGRQ score and GOLD grouping.

Our study did not demonstrate a relationship between 25(OH)D levels and CT measured bone attenuation. An association between 25(OH)D levels and bone density has been identified in many populations [32], including populations of COPD specifically [8,9]. Indeed, the presence of vitamin D deficiency at baseline increased the risk of the development of osteoporosis over a 3-year period by 7.5-fold in COPD patients [33]. Supplementation with the active form of vitamin D, calcitriol, showed a beneficial effect on bone density in pulmonary patients with osteoporosis [34]. However, other studies have failed to confirm the association between 25(OH)D levels and bone density in COPD patients [35]. Interestingly, our study did find an association between DBP and bone attenuation measurements in the subjects with emphysema. Recent studies in osteoporotic males have shown increased VDBP levels as compared to non-osteoporotic controls [15].

In the current study, the relationship between vitamin D and change in clinical status was not assessed. An analysis of the Lung Health Study rapid decliners vs. slow decliners found no relationship between vitamin D levels and rate of change in FEV1 [36]. The current study was designed primarily to assess a relationship between vitamin D and emphysema, which we demonstrated. Subjects were selected to demonstrate a range of emphysema levels for this reason. The severity of emphysema has been related to the rate of lung function decline in the ECLIPSE cohort [37]. An analysis of the full ECLIPSE cohort will have more power to explore a relationship between vitamin D and change in measures of severity of COPD.

Our study demonstrates a relationship between vitamin D and 6 min walking distance and disease related health status assessed by the SGRQ. Both the SGRQ and 6 min walking distance have been related to increased mortality in COPD independently of FEV1, which is also related to mortality [38,39]. Whether correction of severe vitamin D deficiency with therapy is possible in COPD was not tested in the current study. However, both the SGRQ and 6 min walking distance can be improved by specific interventions in COPD. Thus it is reasonable to propose that these parameters may respond to vitamin D augmentation. No pharmacotherapy has yet been definitively demonstrated to improve survival in COPD. Whether vitamin D supplementation would be useful in this regard is untested, but may be reasonable, particularly among patients who are severely deficient.

Our study also demonstrates a relationship between vitamin D and bronchodilator responsiveness. Prior studies in asthma have demonstrated that lower vitamin D levels are associated with increased airway responsiveness on methacholine challenge [40]. An inverse relationship is seen between bronchodilator response and 25(OH)D levels in asthmatic children [41,42]. Our study demonstrates the opposite correlation of 25(OH)D and bronchodilator response, as we found higher levels of 25(OH)D were associated with increased bronchodilator response. This raises the possibility that repletion of vitamin D stores may improve treatment responsiveness. Bronchodilator response in COPD, however, is directly related to lung function [43]. Thus the association of vitamin D with responsiveness may be secondary to the relationship between vitamin D and lung function.

Vitamin D status has also been shown to be inversely related with biomarkers of inflammation [44—46], which may play a role in the pathogenesis of COPD. Clara cell secretory protein (CCSP), the most abundant secretory protein in the airway, has been shown to play a role in inflammation, infection, and oxidative stress [47]. Serum CCSP is reduced in COPD patients and smokers, and significantly higher in former smokers with airflow obstruction reversibility [48]. Our study shows increased serum CCSP levels associated with higher levels of 25(OH)D as well as free vitamin D. Whether or not there is a mechanistic relationship between these measures is unclear. CCSP is produced by the epithelium of the airways. Dysfunction of airway epithelium is thought to lead to reduced production. A role for vitamin D in directly modulating epithelial function is plausible as several types of epithelium have been demonstrated to express vitamin D receptors and to respond to vitamin D [49,50]. Alternatively, vitamin D may modulate epithelial cell function in the lung indirectly through an effect on inflammatory cells.

It was of interest to assess vitamin D levels with respect to the new GOLD classification. In this, a significantly lower 25(OH)D was observed in group D, which has both more severely impaired FEV1 (stages 3—4) and/or more frequent exacerbation history as well as more symptoms. These

levels were lower than Group C, which had impaired FEV1 (stages 3—4) and/or more frequent exacerbation history but less dyspnea on the mMRC scale, suggesting that vitamin D deficiency may be related to symptoms independent of the association with lung function and structure. The use of oral corticosteroids, which may play a role in this association, was not included in the analysis.

While there are some reasons to suggest vitamin D deficiency may contribute to the pathogenesis of COPD, it is also possible that there is a cause and effect relationship in the opposite direction. Specifically, patients with COPD are less active than individuals without COPD and spend less time in outdoor activities. Whether the behavioral impact of COPD affects diet is relatively unexplored and could also, conceptually, lead to a decrease in vitamin D intake in COPD. COPD as a cause of reduced vitamin D levels is a plausible and untested possibility. The relationships of vitamin D levels with dietary intake, metabolism and supplementation are complex and may account for the relatively weak relationships that have been observed between vitamin D and FEV1 in prior studies [4,23] and with the clinical and biochemical measures the current study. Our study, however, confirms the relationship between COPD and vitamin D levels and specifically establishes a relationship between emphysema and COPD clinical features with vitamin D.

Our study has several weaknesses. Although we adjusted for several confounders, information on other known confounders of vitamin D status such as race and place of living was not available, and therefore were not accounted for. Information on vitamin D supplementation was not available. Also, CT scans were not performed at the exact time as the serum used in this study due to availability of stored samples, although CT scans for quantification of emphysema were performed 6 months after serum collection. The CT scans used to assess bone density were done 2 years prior to the serum collection used in this study. Clinical classification of the subjects and biomarker data were not available at the time of the serum collection, but were available at study baseline, 2½ years prior to serum measurement. The fact that significant associations were observed suggests that these relationships may be very robust. Assessment at similar time points may have revealed stronger relationships or relationships in addition to those observed. Finally, the associations observed in the current study between vitamin D and features of COPD do not establish a causal relationship.

Conclusion

This study provides evidence supporting the role of vitamin D in COPD by confirming the relationship between vitamin D and airflow. The association between emphysema and 25(OH)D levels is a novel finding, suggesting the possibility of a modulating effect of vitamin D on lung structure. A relationship between vitamin D and biomarkers that are believed to reflect disease activity in COPD merit further exploration. Further studies to elucidate the pathogenic mechanisms and interaction between vitamin D and DBP, including the DBP genotype, and their effect on existing therapies may characterize a subset of COPD patients that may benefit from vitamin D supplementation.

Sources of support

ECLIPSE study is funded by GlaxoSmithKline Clinical Trials.gov identifier NCT00292552 and GSK No. SCO104960.

Robert E. Kuhl Testamentary Trust Fund through the University of Nebraska Foundation, Clinical Research Center, Nebraska Medical Center. Larson Endowment, University of Nebraska Medical Center.

Authors contribution

IB, CH, DR, SR, JM, HS, designed the study, conducted the analysis, and prepared the manuscript. AN performed DBP studies and critically revised the manuscript. BM provided human tissue and conceptual advice, EW, BMcN, EPR, EAR, JV, LE provided guidance, expertise and critical manuscript revision.

Conflict of interest

None.

Acknowledgments

We gratefully acknowledge the administrative support of Lillian Richards, Internal Medicine, University of Nebraska Medical Center, and Fran Higgins, School of Allied Health Professions, University of Nebraska Medical Center.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2013.05.010.

References

[1] Hughes DA, Norton R. Vitamin D and respiratory health. Clin Exp Immunol 2009;158(1):20—5.
[2] Franco CB, Paz-Filho G, Gomes PE, et al. Chronic obstructive pulmonary disease is associated with osteoporosis and low levels of vitamin D. Osteoporos Int 2009;20(11):1881—7.
[3] Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. Chest 2005; 128(6):3792—8.
[4] Janssens W, Bouillon R, Claes B, et al. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax 2010;65(3):215—20.
[5] Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173(5):2909—12.
[6] Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J 2005;19(9): 1067—77.

[7] 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(1):56—62.
[8] Forli L, Bjortuft O, Boe J. Vitamin D status in relation to nutritional depletion and muscle function in patients with advanced pulmonary disease. Exp Lung Res 2009;35(6):524—38.
[9] Romme EA, Rutten EP, Smeenk FW, Spruit MA, Menheere PP, Wouters EF. Vitamin D status is associated with bone mineral density and functional exercise capacity in patients with chronic obstructive pulmonary disease. Ann Med 2013;45(1):91—6.
[10] Ringbaek T, Martinez G, Durakovic A, et al. Vitamin d status in patients with chronic obstructive pulmonary disease who participate in pulmonary rehabilitation. J Cardiopulm Rehabil Prev 2011;31(4):261—7.
[11] Lehouck A, Mathieu C, Carremans C, et al. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. Ann Intern Med 2012; 156(2):105—14.
[12] Wood AM, Bassford C, Webster D, et al. Vitamin D-binding protein contributes to COPD by activation of alveolar macrophages. Thorax 2011;66(3):205—10.
[13] Robbins RA, Hamel FG. Chemotactic factor inactivator interaction with gc-globulin (vitamin D-binding protein). A mechanism of modulating the chemotactic activity of C5a. J Immunol 1990;144(6):2371—6.
[14] White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. Trends Endocrinol Metab 2000;11(8):320—7.
[15] Al-oanzi ZH, Tuck SP, Raj N, et al. Assessment of vitamin D status in male osteoporosis. Clin Chem 2006;52(2):248—54.
[16] Bouillon R, Van Assche FA, Van Baelen H, Heyns W, De Moor P. Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D3. significance of the free 1,25-dihydroxyvitamin D3 concentration. J Clin Invest 1981;67(3):589—96.
[17] Nyomba BL, Bouillon R, Bidingija M, Kandjingu K, De Moor P. Vitamin D metabolites and their binding protein in adult diabetic patients. Diabetes 1986;35(8):911—5.
[18] Agusti A, Calverley PM, Celli B, et al. Characterisation of COPD heterogeneity in the ECLIPSE cohort. Respir Res. 2010; 11:122.
[19] Rennard SI, Berg R, Martin GR, Foidart JM, Robey PG. Enzyme-linked immunoassay (ELISA) for connective tissue components. Anal Biochem 1980;104(1):205—14.
[20] Vestbo J, Hurd SS, Agusti AG, et al. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease, GOLD executive summary. Am J Respir Crit Care Med 2012.
[21] Romme EA, Murchison JT, Phang KF, et al. Bone attenuation on routine chest CT correlates with bone mineral density on DXA in patients with COPD. J Bone Miner Res. 2012.
[22] Grydeland TB, Dirksen A, Coxson HO, et al. Quantitative computed tomography measures of emphysema and airway wall thickness are related to respiratory symptoms. Am J Respir Crit Care Med 2010;181(4):353—9.
[23] Shaheen SO, Jameson KA, Robinson SM, et al. Relationship of vitamin D status to adult lung function and COPD. Thorax 2011;66(8):692—8.
[24] Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH. Vitamin D deficiency causes deficits in lung function and alters lung structure. Am J Respir Crit Care Med 2011;183(10): 1336—43.
[25] Jiang R, Jacobs DR, He K, et al. Associations of dairy intake with CT lung density and lung function. J Am Coll Nutr 2010; 29(5):494—502.
[26] Lehouck A, Wauters E, Mathieu C, et al. Vitamin D binding protein phenotypes have an impact on vitamin D substitution in COPD. Am J Respir Crit Care Med 2011;183(1).
[27] Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr 2009;89(2):634—40.
[28] Lauridsen AL, Vestergaard P, Hermann AP, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of gc (vitamin D-binding protein): A cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005;77(1):15—22.
[29] Ito I, Nagai S, Hoshino Y, et al. Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. Chest 2004;125(1):63—70.
[30] Schellenberg D, Pare PD, Weir TD, Spinelli JJ, Walker BA, Sandford AJ. Vitamin D binding protein variants and the risk of COPD. Am J Respir Crit Care Med 1998;157(3 Pt 1):957—61.
[31] Sandford AJ, Silverman EK. Chronic obstructive pulmonary disease. 1: Susceptibility factors for COPD the genotype-environment interaction. Thorax 2002;57(8):736—41.
[32] Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: A population-based study of younger and older adults. Am J Med 2004;116(9):634—9.
[33] Graat-Verboom L, Smeenk FW, van den Borne BE, et al. Progression of osteoporosis in patients with COPD: A 3-year follow up study. Respir Med 2012;106(6):861—70.
[34] Mirzaei S, Zajicek HK, Knoll P, et al. Effect of rocaltrol on bone mass in patients with pulmonary disease treated with corticosteroids. J Asthma 2003;40(3):251—5.
[35] Duckers JM, Evans BA, Fraser WD, Stone MD, Bolton CE, Shale DJ. Low bone mineral density in men with chronic obstructive pulmonary disease. Respir Res. 2011;12:101.
[36] Kunisaki KM, Niewoehner DE, Singh RJ, Connett JE. Vitamin D status and longitudinal lung function decline in the lung health study. Eur Respir J 2011;37(2):238—43.
[37] Vestbo J, Edwards LD, Scanlon PD, et al. Changes in forced expiratory volume in 1 second over time in COPD. N Engl J Med 2011;365(13):1184—92.
[38] Sin DD, Wu L, Man SF. The relationship between reduced lung function and cardiovascular mortality: A population-based study and a systematic review of the literature. Chest 2005; 127(6):1952—9.
[39] Hole DJ, Watt GC, Davey-Smith G, Hart CL, Gillis CR, Hawthorne VM. Impaired lung function and mortality risk in men and women: Findings from the renfrew and paisley prospective population study. BMJ 1996;313(7059):711—5. discussion 715—6.
[40] Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med 2010;181(7):699—704.
[41] Gupta A, Sjoukes A, Richards D, et al. Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. Am J Respir Crit Care Med 2011; 184(12):1342—9.
[42] Brehm JM, Celedon JC, Soto-Quiros ME, et al. Serum vitamin D levels and markers of severity of childhood asthma in costa rica. Am J Respir Crit Care Med 2009;179(9):765—71.
[43] Albert P, Agusti A, Edwards L, et al. Bronchodilator responsiveness as a phenotypic characteristic of established chronic obstructive pulmonary disease. Thorax 2012;67(8):701—8.
[44] Timms PM, Mannan N, Hitman GA, et al. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: Mechanisms for inflammatory damage in chronic disorders? QJM 2002;95(12):787—96.
[45] Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: A double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006;83(4):754—9.

[46] Bellia A, Garcovich C, D'Adamo M, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern Emerg Med 2011.
[47] Wong AP, Keating A, Waddell TK. Airway regeneration: The role of the clara cell secretory protein and the cells that express it. Cytotherapy 2009;11(6):676—87.
[48] Lomas DA, Silverman EK, Edwards LD, et al. Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort. Thorax 2008;63(12):1058—63.
[49] Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: Potential effects on host defense. J Immunol 2008;181(10):7090—9.
[50] Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. J Immunol 2010;184(2):965—74.