Annals of Internal Medicine | ORIGINAL RESEARCH

High Doses of Vitamin D to Reduce Exacerbations in Chronic Obstructive Pulmonary Disease

A Randomized Trial

An Lehouck, PhD; Chantal Mathieu, MD, PhD; Claudia Carremans, MS; Femke Baeke, PhD; Jan Verhaegen, MD, PhD; Johan Van Eldere, MD, PhD; Brigitte Decallonne, MD, PhD; Roger Bouillon, MD, PhD; Marc Decramer, MD, PhD; and Wim Janssens, MD, PhD

Background: Low serum 25-hydroxyvitamin D (25-[OH]D) levels have been associated with lower FEV_1, impaired immunologic control, and increased airway inflammation. Because many patients with chronic obstructive pulmonary disease (COPD) have vitamin D deficiency, effects of vitamin D supplementation may extend beyond preventing osteoporosis.

Objective: To explore whether supplementation with high doses of vitamin D could reduce the incidence of COPD exacerbations.

Design: Randomized, single-center, double-blind, placebo-controlled trial. (ClinicalTrials.gov registration number: NCT00666367)

Setting: University Hospitals Leuven, Leuven, Belgium.

Patients: 182 patients with moderate to very severe COPD and a history of recent exacerbations.

Intervention: 100 000 IU of vitamin D supplementation or placebo every 4 weeks for 1 year.

Measurements: The primary outcome was time to first exacerbation. Secondary outcomes were exacerbation rate, time to first hospitalization, time to second exacerbation, FEV_1, quality of life, and death.

Results: Mean serum 25-(OH)D levels increased significantly in the vitamin D group compared with the placebo group (mean between-group difference, 30 ng/mL [95% CI, 27 to 33 ng/mL]; $P < 0.001$). The median time to first exacerbation did not significantly differ between the groups (hazard ratio, 1.1 [CI, 0.82 to 1.56]; $P = 0.41$), nor did exacerbation rates, FEV_1, hospitalization, quality of life, and death. However, a post hoc analysis in 30 participants with severe vitamin D deficiency (serum 25-[OH]D levels <10 ng/mL) at baseline showed a significant reduction in exacerbations in the vitamin D group (rate ratio, 0.57 [CI, 0.33 to 0.98]; $P = 0.042$).

Limitation: This was a single-center study with a small sample size.

Conclusion: High-dose vitamin D supplementation in a sample of patients with COPD did not reduce the incidence of exacerbations. In participants with severe vitamin D deficiency at baseline, supplementation may reduce exacerbations.

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For author affiliations, see end of text.

Chronic obstructive pulmonary disease (COPD) is characterized by an abnormal inflammatory response of the airways to the inhalation of noxious particles or gases, such as cigarette smoke. With disease progression, marked by a decline in FEV_1, patients develop systemic consequences and become prone to infectious exacerbations (1). Of note, low serum 25-hydroxyvitamin D (25-[OH]D) levels, reflecting vitamin D status, are associated with impaired FEV_1 (2), and we demonstrated that vitamin D deficiency (defined as serum 25-[OH]D levels <20 ng/mL by the Institute of Medicine [3]), is present in 60% to 75% of patients with severe COPD (4). Whether such deficiency is only the consequence of COPD or may causally contribute to the pathogenesis of COPD is unclear.

Traditionally, vitamin D is associated with bone health (5), but large epidemiologic studies have associated low serum 25-(OH)D levels with autoimmune diseases; cancer; cardiovascular diseases; and infections, including respiratory tract infections and tuberculosis (6–10). However, few randomized, controlled trials have examined the effects of vitamin D supplementation on these important health outcomes. Moreover, recent studies of vitamin D supplementation in patients with multiple sclerosis (11), diabetes (12), influenza (13), and tuberculosis (14) have reported disappointing results. A reason for these negative results may be found in insufficient vitamin D supplementation, because no clear evidence nor consensus exists on what minimum serum levels are needed to achieve these "extraskeletal" effects. On the basis of observational data, experts have suggested that in contrast to obtaining beneficial effects on the bone, higher serum levels (30 to 50 ng/mL) are needed, requiring more aggressive supplementation regimens (15–17). One recent intervention study in tuberculosis corroborates with this idea (18). Overall, an

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Context

The association between low serum vitamin D levels and more severe chronic obstructive pulmonary disease (COPD) suggests that vitamin D supplementation might be beneficial for COPD treatment.

Contribution

In this randomized trial, supplementation with vitamin D did not reduce the number of acute exacerbations of COPD nor improve lung function, compared with placebo. A post hoc analysis suggested possible benefit in patients with the lowest baseline vitamin D levels.

Implication

Vitamin D supplementation does not seem to be beneficial for COPD, although further study may be warranted to assess whether it might help certain patient groups.

—The Editors

extensive expert analysis of the potential effects of vitamin D supplementation on the health outcomes of North American participants concluded that evidence for extraskeletal benefits of vitamin D therapy is insufficient and that only new randomized, controlled trials can define such effects (19).

Particularly for COPD, the vitamin D pathway is an attractive target for intervention studies because vitamin D deficiency may enhance chronic airway and systemic inflammation, reduce bacterial clearance, and increase the risk for infectious exacerbations at the same time (20). Therefore, we aimed to explore the effect of adequate vitamin D supplementation on exacerbations in patients with moderate to very severe COPD. We report the efficacy and safety of long-term, high-dose vitamin D supplementation in patients with COPD prone to exacerbations.

METHODS

Study Design and Participants

Our study was a single-center, double-blind, randomized, placebo-controlled intervention trial. Patients were screened for eligibility at the University Hospitals Leuven, Leuven, Belgium, over a 1.5-year recruitment period in 2008 and 2009. Eligible patients were current or former smokers, were older than 50 years, had a diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) definition (postbronchodilator FEV_1–FVC ratio <0.7), and had an FEV_1 less than 80% predicted. Patients were excluded if they had a history of hypercalcemia, sarcoidosis, or active cancer. Treatment with vitamin D supplements for newly discovered symptomatic osteoporosis and long-term azithromycin treatment, with antibacterial and anti-inflammatory functions, were additional exclusion criteria, because they could interfere with 25-(OH)D dosages and exacerbation analyses (21). All participants provided written informed consent. The study was approved by the local ethics review committee of the University Hospitals Leuven and was registered with ClinicalTrials.gov (NCT00666367).

Randomization and Masking

Randomization was performed in 2 strata: one group of vitamin D–naive participants and one group of participants receiving low-dose vitamin D supplements (400 to 880 IU/d) for osteoporosis at baseline. We randomly assigned participants in blocks of 20 to overcome seasonal influences on baseline characteristics. In each consecutive block, participants were allocated in a 1:1 ratio to receive a monthly oral dose of 100 000 IU of vitamin D (4 mL of D-Cure [manufactured and provided by Laboratoires SMB, Brussels, Belgium]) or placebo (4 mL of arachidis oleum) in addition to their regular treatment. Pharmacists of the University Hospitals Leuven, who were independent from the clinical study team, randomly assigned participants by using a computer-generated randomization list and prepared the study medication. Vitamin D and placebo were prepared in oral syringe dispensers, were identical in appearance and taste, and were numbered according to the randomization schedule. After the last participant completed the trial, masking continued until all data were entered in a database, which was verified and locked before unblinding in July 2010.

Procedures

Patients were screened during hospitalization for an exacerbation or before referral for respiratory rehabilitation. Randomization occurred 5 to 6 weeks after screening if the participant had convalesced. If not, randomization was postponed until steroid, antibiotic, or combination treatment was completed and spirometric values were similar to preexacerbation values. Baseline characteristics included the Body-Mass Index, Airflow Obstruction, Dyspnea, and Exercise Capacity (BODE) Index, a multidimensional, COPD-specific, 10-point scale in which higher scores indicate a higher risk for death (22), and the Charlson comorbidity index, a non–COPD-specific but validated comorbidity index (23). After randomization, follow-up visits occurred every 4 months (at 4, 8, and 12 months). The primary end point was the time to first exacerbation. Secondary end points were exacerbation rate; time to first hospitalization; time to second exacerbation; FEV_1 (24); quality of life, as measured with the Chronic Respiratory Questionnaire (CRQ) (scores for dyspnea, emotion, fatigue, and mastery) (25); and death. In addition to these clinical end points, bacterial presence in morning sputa, plasma cathelicidin levels, serum 25-(OH)D levels, and blood monocyte capacities for phagocytosis were determined in a blinded manner (**Appendix**, available at www.annals.org).

The clinical study team remained blinded to these results until the database was locked. We defined COPD exacerbations as sustained worsening of respiratory symp-

toms during 48 hours and requiring oral corticosteroid, antibiotic, or combination treatment that was initiated by a physician. Respiratory symptoms included at least 1 of the Anthonisen criteria (26) (increased dyspnea, sputum volume, or sputum purulence) with or without minor symptoms, such as cough, fever, common cold, wheezing, or sore throat. Time to first exacerbation was assessed by quantifying the days between randomization and the first exacerbation. To obtain data on exacerbations, we asked participants to complete diaries every 2 weeks that detailed respiratory tract symptoms, visits to health care providers, hospitalizations, and changes in medication. At each visit, diaries were reviewed in the participant's presence and the general practitioner was contacted in case of doubt, missing data, or suspicion of self-medication.

Spirometry was repeated at each visit by using standard equipment (CareFusion, Vilvoorde, Belgium) and was performed according to American Thoracic Society/European Respiratory Society guidelines (27). To monitor safety, we collected blood samples every 4 months to measure serum calcium and phosphate levels. After a protocol amendment halfway through the trial, we collected urine samples to better document the safety profile of the study drug. From that point on, all participants ($n = 79$) collected 24-hour urine the day before the last study visit to measure urinary calcium level, the calcium–creatinine ratio, and the glomerular filtration rate.

Statistical Analysis

The study was designed to demonstrate a minimum delay of 25% in the time to first exacerbation when comparing the vitamin D group with the placebo group. As we enrolled patients who were recently treated for an exacerbation, we based our assumptions on the MOSAIC (Moxifloxacin Compared to Standard Antibiotics for Acute Exacerbations in Chronic Bronchitis) trial (28), which best resembled our study sample and showed a mean time to next exacerbation of 130 days (SD, 70) after a first exacerbation. Based on *t* test statistics, a sample size of 57 participants in each group was needed to demonstrate a 25% delay in time to first exacerbation, with 80% power at 5% significance. Because approximately 20% of our participants were receiving low-dose vitamin D supplements for osteoporosis prevention (which was not an exclusion criterion), and given an estimated maximum rate of 15% of participant withdrawals without follow-up, 180 participants had to be randomly assigned to end up with at least 120 vitamin D–naive participants at inclusion. All analyses for exacerbations, deaths, and serum 25-(OH)D levels used the intention-to-treat (ITT) population, defined as all randomly assigned participants who received at least 1 dose of study medication. All available data were used for the ITT analysis. On-treatment analyses for these variables are reported in the **Appendix**.

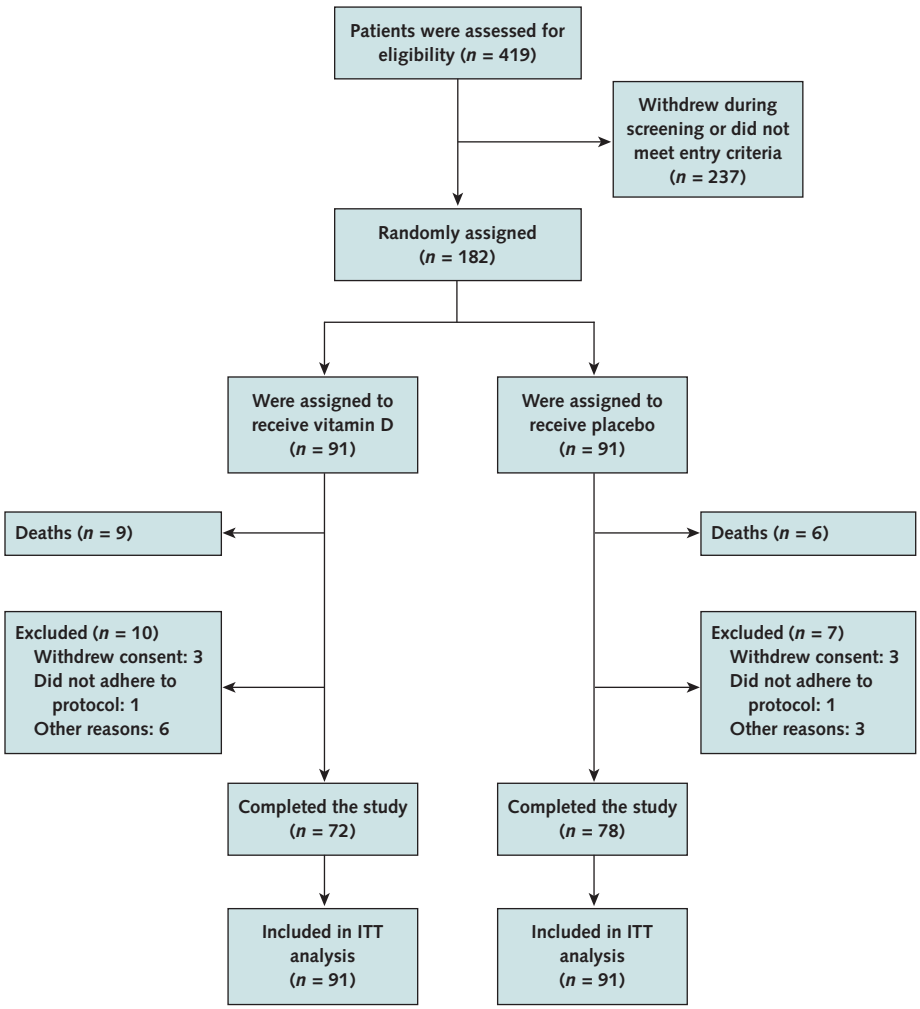
Time to first or second exacerbation and time to first hospitalization in both groups in the ITT population were compared by using Kaplan–Meier curves and log-rank tests. We calculated the mean number of exacerbations per patient-year by dividing the total number of exacerbations by the total years of follow-up in the ITT population. Exacerbation rate in the ITT population was analyzed with a generalized linear model for a Poisson distribution, correcting for duration of treatment exposure and overdispersion. Because age, FEV_1, GOLD stage, and smoking status did not have a statistically significant influence on our model, we did not include these covariates in the final analysis (29). In a post hoc analysis for exacerbation rate, a similar generalized linear model for a Poisson distribution was applied in the ITT population but with correction for baseline serum 25-(OH)D levels and for the interaction of these levels with treatment. All further subgroup analyses (time to first exacerbation, exacerbation rate, and serum 25-[OH]D levels) in participants receiving low-dose supplements, vitamin D–naive participants at baseline, or participants with severe vitamin D deficiency (defined as having serum 25-[OH]D levels <10 ng/mL) were post hoc.

Serial FEV_1, CRQ scores, plasma cathelicidin levels, serum 25-(OH)D levels, and bacterial presence in morning sputa over 1 year were predefined secondary end points. They were compared in a linear mixed-model analysis, with visit number as the repeated measure and the respective marker as the dependent variable, in the ITT population. Two-way analysis-of-variance *P* values of treatment-by-visit interaction are reported. Posttests to compare differences per visit were performed by using *t* test statistics with unadjusted *P* values. Chi-square statistics were used to compare proportions of participants with hypercalcemia (during the study) and hypercalciuria (at the end of the study) between the groups at every visit. To compare the monocyte capacity for phagocytosis between the groups at the end of the study, we used *t* test statistics. Subgroup analysis of monocyte capacity for phagocytosis and plasma cathelicidin levels in participants with severe vitamin D deficiency at baseline were post hoc analyses. Effect sizes between groups of primary or secondary outcomes are given with *P* values and 95% CIs. *P* values less than 0.05 are considered statistically significant. Analyses were performed with SAS software, version 9.1 (SAS Institute, Cary, North Carolina), and GraphPad Prism 4.01 for Windows (GraphPad Software, La Jolla, California).

Role of the Funding Source

The Applied Biomedical Research Program, Agency for Innovation by Science and Technology (IWT-TBM), provided funding for the study, and Laboratoires SMB provided the study medication. These sources were not involved in the study design; collection, analysis, or interpretation of data; or in prepartion or submission of the manuscript for publication.

Figure 1. **Study flow diagram.**



ITT = intention-to-treat.

RESULTS

Study Recruitment and Follow-up

Figure 1 shows the study flow diagram. Of 419 screenings, 340 patients were eligible for inclusion; 182 (54%) were randomly assigned. One hundred fifty (82%) participants completed the study, 15 (8%) died, and 17 (9%) were classified as withdrawals with no differential dropout between the 2 groups. Sixteen participants completed follow-up but were removed from a prespecified on-treatment analysis for serum 25-(OH)D levels because they had started vitamin D supplementation or long-term azithromycin treatment during the study (**Appendix Figure 1**, available at www.annals.org). Among the 17 withdrawals, medication was stopped, but the study team continued collecting data on exacerbations and deaths. From 10 of the 17 participants, we were able to contact the general practitioner and collected information on deaths and exacerbations (time to first exacerbation and exacerbation rate). We did not have follow-up data on exacerbations for the remaining 7 participants, but we still had access to their files to complete data on deaths. Overall, we collected information on exacerbations and survival in 175 (96%) and 182 (100%) participants, respectively.

Baseline Characteristics

The **Table** shows the baseline characteristics of the participants. Study inclusion required that the participant not be receiving antibiotic and oral steroid treatment for an acute exacerbation. However, 14% of the participants were considered to be steroid-dependent, and the stable pretrial maintenance dose (4 mg of methylprednisolone) was continued during the study. Eighty-two percent of the participants were receiving maximum inhalation therapy (inhaled corticosteroid plus long-acting β-agonist plus long-acting anticholinergic drug therapy) before inclusion; this proportion did not change during or at the end of the study (84%). Forty (22%)

participants were receiving vitamin D supplements (800 IU/d) for bone protection before randomization, but they were equally distributed between the groups (20 persons each).

Exacerbations

A total of 468 exacerbations occurred: 229 in the vitamin D group and 239 in the placebo group. Kaplan–Meier survival analysis showed no significant difference in the median time to first exacerbation between the vitamin D and placebo groups (84 days [interquartile range {IQR}, 29 to 200 days] vs. 56 days [IQR, 21 to 200 days]) (hazard ratio [HR], 1.1 [95% CI, 0.82 to 1.56]; $P = 0.41$) (**Figure 2**, *A*). Median time to second exacerbation also did not differ between the groups (204 days [IQR, 123 to 329 days] vs. 201 days [IQR, 113 to 333 days]) (HR, 1.02 [CI, 0.72 to 1.47]; $P = 0.88$) (**Figure 2**, *B*). The annual rate of exacerbations was 2.8 per patient-year in the vitamin D group and 2.9 in the placebo group, resulting in a nonsignificant rate ratio of 0.94 (CI, 0.76 to 1.16; $P = 0.57$). We did not find a significant difference in the median time to first hospitalization for an exacerbation (HR, 0.84 [CI, 0.50 to 1.40]; $P = 0.50$) (**Figure 2**, *C*). A total of 152 exacerbations resulted in hospitalization: 79 in the vitamin D group and 73 in the placebo group (rate ratio, 1.13 [CI, 0.70 to 1.82]; $P = 0.62$).

Death, FEV_1, and Quality of Life

Fifteen deaths occurred within 1 year after randomization: 11 of respiratory disease, 1 of lung cancer, 2 of cardiac disease, and 1 of unknown cause. The proportions of deaths from any cause were 10% in the vitamin D group and 7% in the placebo group (rate ratio, 1.5 [CI, 0.56 to 4.04]; $P = 0.42$). Kaplan–Meier survival analysis showed no significant difference in survival between the groups (HR, 0.69 [CI, 0.25 to 1.90]; $P = 0.47$) (**Figure 2**, *D*). Linear mixed-model analysis showed no significant differences in CRQ dyspnea, emotional, mastery, and fatigue scores and in FEV_1 between the group at any time during follow-up (**Appendix Figure 2** and **Appendix Table 1**, available at www.annals.org).

Efficacy of Supplementation

Serum 25-(OH)D levels in the ITT population were measured during 90% of the visits. At baseline, mean serum 25-(OH)D levels did not differ between the vitamin D group (20 ng/mL [SD, 12]) and the placebo group (20 ng/mL [SD, 11]) (**Figure 3**, *A*; and **Appendix Table 2**, available at www.annals.org). Vitamin D supplementation resulted in a steep and significant increase in serum 25-(OH)D levels that remained stable during the study (52 ng/mL [SD, 16]) (mean between-group difference, 30 ng/mL [CI, 27 to 33 ng/mL]; $P < 0.001$) (**Figure 3**, *A*, and **Appendix Table 2**). A post hoc analysis in the subgroup of vitamin D–naive participants ($n = 142$) demonstrated that mean serum 25-(OH)D levels at baseline were lower than those of the ITT population, but that the subgroup received equal, effective supplementation. However, the time to first exacerbation and annual rate of exacerbations or other outcomes did not differ in this subgroup (**Figure 3**, *B*, and **Appendix Table 2**). Data from the subgroup of participants receiving low-dose supplements at baseline are not reported.

Interaction Between Vitamin D Supplementation and Baseline Serum 25-(OH)D Levels

Because a post hoc analysis on the rate of exacerbations in the ITT population demonstrated a significant interaction in treatment by baseline serum 25-(OH)D level ($P = 0.027$), we performed a further subgroup analysis for participants with severe vitamin D deficiency at baseline (serum 25-[OH]D levels <10 ng/mL). Among these 30 participants, 15 were randomly allocated to receive vitamin D supplementation, which resulted in a significant increase in serum 25-(OH)D levels (from 8 ng/mL [SD, 2] to 50 ng/mL [SD, 15]; mean between-group difference, 38 ng/mL [CI, 33 to 44 ng/mL]; $P < 0.001$). Although the time to first exacerbation did not differ in this subgroup, the rate of exacerbations per patient-year decreased by 43% (rate ratio, 0.57 [CI, 0.33 to 0.98]; $P = 0.042$) (**Figure 3**, *C*).

Table. **Baseline Characteristics***

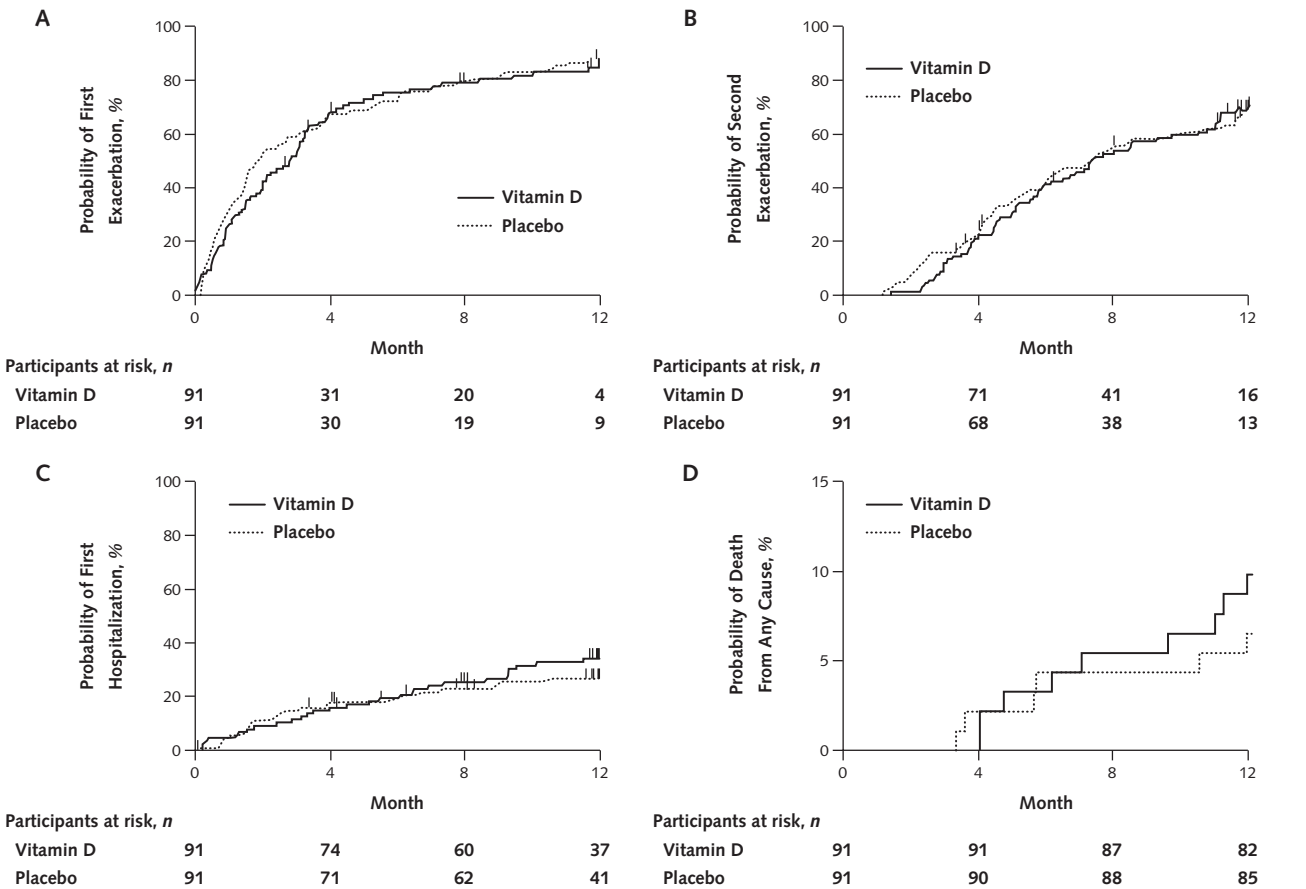
Characteristic	Vitamin D Group ($n = 91$)	Placebo Group ($n = 91$)
Men, *n (%)*	72 (79)	73 (80)
Mean age (SD), *y*	68 (9)	68 (8)
Mean BMI (SD), *kg/m²*	25 (5)	24 (5)
Smoking status		
Current smokers, *n (%)*	13 (14)	19 (21)
Mean pack-years smoked (SD)	51 (23)	53 (32)
Mean FEV_1 (SD), *L*	1.22 (0.45)	1.17 (0.43)
Mean FEV_1 (SD), *% predicted*	44 (16)	42 (14)
Mean FVC (SD), *L*	2.78 (0.78)	2.88 (0.83)
Mean FEV_1–FVC ratio (SD), %	44 (12)	41 (11)
Mean DLCO (SD), *% predicted*	46 (16)	49 (16)
GOLD stage, *n (%)*		
II	25 (28)	24 (26)
III	43 (47)	48 (53)
IV	23 (25)	19 (21)
Mean 25-(OH)D level (SD), *ng/mL*	20 (12)	20 (11)
Mean Charlson index score (SD)	3.48 (2.54)	3.52 (2.44)
Mean BODE Index score (SD)	3.82 (2.28)	3.55 (2.03)
Medication, *n (%)*		
LABA	2 (2)	4 (4)
LABA plus ICS	81 (89)	79 (87)
Long-acting anticholinergic drugs	81 (89)	84 (92)
Short-acting bronchodilators	64 (70)	72 (79)
Steroids†	11 (12)	15 (16)
Long-term oxygen therapy	11 (12)	10 (11)
Started with rehabilitation at first visit	25 (27)	25 (27)

25-(OH)D = 25-hydroxyvitamin D; BMI = body mass index; BODE = Body-Mass Index, Airflow Obstruction, Dyspnea, and Exercise Capacity; DLCO = diffusing capacity of lung for carbon monoxide; GOLD = Global Initiative for Chronic Obstructive Lung Disease; ICS = inhaled corticosteroid; LABA = long-acting β-agonist.

* Baseline characteristics of vitamin D and placebo groups at randomization. Both groups were matched for all given variables.

† ≤4 mg of methylprednisolone.

Figure 2. **Kaplan–Meier plots of time to first exacerbation (*A*), time to second exacerbation (*B*), time to first hospitalization (*C*), and death from any cause (*D*) in the intention-to-treat population.**



Safety

Blood samples were available in 90% of the study visits for the ITT population. Mean serum calcium and phosphate levels did not differ between the groups at any time during follow-up (mean between-group difference in calcium levels, 0.02 mmol/L [0.08 mg/dL] [CI, −0.03 to 0.04 mmol/L {−0.01 to 0.16 mg/dL}; $P = 0.061$]; mean between-group difference in phosphate levels, −0.006 mmol/L [−0.02 mg/dL] [CI, −0.04 to 0.03 mmol/L {−0.13 to 0.09 mg/dL}; $P = 0.69$]) (**Appendix Table 3**, available at www.annals.org). At 4 months, 4 cases of mild and asymptomatic hypercalcemia (defined as serum calcium levels between 2.63 and 2.75 mmol/L [10.5 and 11.0 mg/dL]) were detected in the vitamin D group compared with 0 cases in the placebo group ($P = 0.043$). Despite continuation of the study medication, hypercalcemia spontaneously resolved with normal serum calcium levels at 8 and 12 months. No cases of hypercalciuria (defined as urinary calcium levels >7.5 mmol/d [>300 mg/d]) were detected at the end of the study (**Appendix Table 3**).

Bacteriology, Plasma Cathelicidin Levels, and Monocyte Phagocytosis

Plasma cathelicidin levels were not affected by serum 25-(OH)D levels or supplementation (**Appendix Figure 3**, available at www.annals.org). Monocyte capacity for phagocytosis in the vitamin D group was significantly better than that in the placebo group ($P = 0.002$); this difference was more pronounced in the subgroup of participants with severe vitamin D deficiency at baseline (**Appendix Figure 4**, available at www.annals.org). No effect of vitamin D on the rate of detecting pathogenic strains or on the total number of colony-forming units was observed (**Appendix Table 4**, available at www.annals.org).

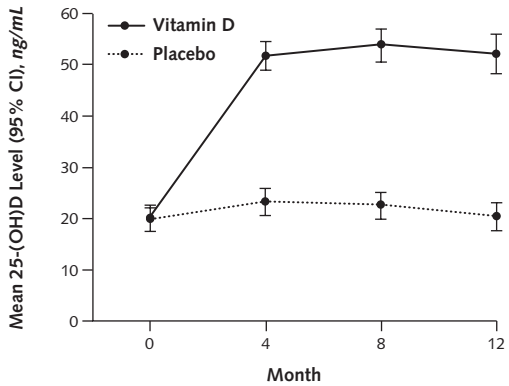
DISCUSSION

This study is, to our knowledge, the first randomized, placebo-controlled trial to examine the efficacy and safety of long-term and high-dose vitamin D supplementation in COPD. The main finding is that a monthly dose of 100 000 IU of vitamin D in addition to regular therapy

Figure 3. **Mean serum 25-(OH)D levels and COPD exacerbation rates among the ITT population (*A*), vitamin D–naive subgroup (*B*), and subgroup with severe vitamin D deficiency at baseline (*C*).**

A. ITT Population

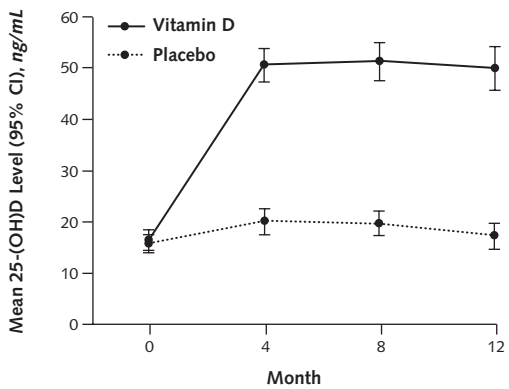
Variable	Vitamin D (*n* = 91)	Placebo (*n* = 91)	Difference or RR (95% CI)	*P* Value
Mean 25-(OH)D level (SD), *ng/mL*				
At baseline	20 (12)	20 (11)	0.2 (–3 to 4)	0.90
During study	52 (16)	22 (13)	30 (27 to 33)	<0.001
COPD exacerbations per patient-year, *n*	2.8	2.9	0.94 (0.76 to 1.16)	0.57



Participants, *n*				
Vitamin D	91	83	79	70
Placebo	91	86	81	74

B. Vitamin D–Naive Subgroup

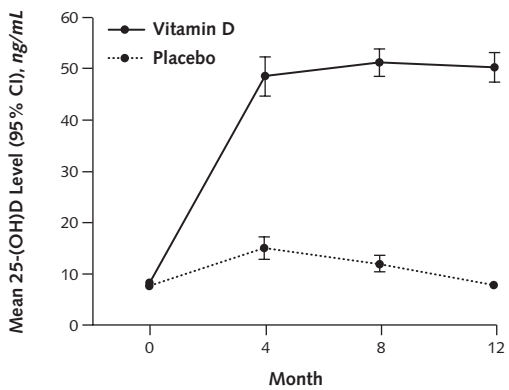
Variable	Vitamin D (*n* = 71)	Placebo (*n* = 71)	Difference or RR (95% CI)	*P* Value
Mean 25-(OH)D level (SD), *ng/mL*				
At baseline	17 (8)	16 (8)	0.75 (–2 to 3)	0.58
During study	51 (16)	19 (11)	32 (29 to 34)	<0.001
COPD exacerbations per patient-year, *n*	2.69	2.99	0.88 (0.69 to 1.12)	0.30



Participants, *n*				
Vitamin D	71	65	62	56
Placebo	71	68	67	61

C. Subgroup With Severe Vitamin D Deficiency

Variable	Vitamin D (*n* = 15)	Placebo (*n* = 15)	Difference or RR (95% CI)	*P* Value
Mean 25-(OH)D level (SD), *ng/mL*				
At baseline	8 (2)	7 (2)	0.57 (–1 to 2)	0.36
During study	50 (15)	12 (8)	38 (33 to 44)	<0.001
COPD exacerbations per patient-year, *n*	1.84	3.45	0.57 (0.33 to 0.98)	0.042



Participants, *n*				
Vitamin D	15	13	12	12
Placebo	15	15	14	13

Two-way analysis-of-variance statistics for between-group differences in serum 25-(OH)D levels and Poisson regression statistics for exacerbation rates are given. *P* values are unadjusted. Severe vitamin D deficiency was defined as having serum 25-(OH)D levels <10 ng/mL. To convert values to nmol/L, multiply by 2.5. 25-(OH)D = 25-hydroxyvitamin D; COPD = chronic obstructive pulmonary disease; ITT = intention-to-treat; RR = rate ratio.

does not reduce the time to first exacerbation or the rate of exacerbations in patients with moderate to very severe COPD. Secondary outcomes, such as FEV_1, quality of life, and death, were also not affected.

The absence of a vitamin D–mediated effect in our study sample contrasts with indirect evidence from most association studies in COPD (2, 4, 6), but is consistent with recent data from the Lung Health Study (30), which showed that vitamin levels did not determine the rate of decline in FEV_1 in a limited subgroup. In addition, most intervention trials of vitamin D supplementation targeting extracalcemic effects in other chronic diseases have shown disappointing results (12, 14, 31). Because the absence of clinically significant effects in such trials may relate to a lack of power, insufficient supplementation, or insensitive end points, we specifically designed our study to overcome some of these concerns. We carefully assessed the effect of a monthly dose of 100 000 IU of vitamin D on serum 25-(OH)D levels at several time points during the trial. Active treatment resulted in mean serum 25-(OH)D levels of 52 ng/mL (SD, 16), which is within the therapeutic range to obtain the hypothesized extracalcemic effects (15). In addition, we selected a study sample prone to severe exacerbations (32) by recruiting most (81%) participants during a hospitalization for an acute exacerbation. Consequently, the average number of exacerbations observed (2.9 per patient-year) was higher than that usually found in large intervention trials of inhalation therapy in participants from an outpatient clinic, thereby enhancing the power to detect significant differences (33, 34). However, the original power calculations, based on a *t* test to evaluate the difference in the mean time to first exacerbation, did not consider the skewed distribution of the data on exacerbations. Although the limited sample size provided insufficient power to demonstrate a clinically significant delay in time to first exacerbation, the survival curves for the time to first exacerbation almost completely overlapped (HR, 1.1 [CI, 0.82 to 1.56]) and the exacerbation rate did not differ (rate ratio, 0.94 [CI, 0.76 to 1.16]). Thus, we think that the probability of making a type II error in our study was rather small.

The absence of therapeutic effect of vitamin D in our study sample may relate to the fact that most of our participants presented with severe COPD and were receiving maximum inhalation therapy. As all of these treatments are known to reduce exacerbations, any additional effect of vitamin D in addition to regular treatment is probably more difficult to obtain (33, 34). Intervention in the earlier stages of COPD when patients receive fewer medications might therefore be more effective, which is consistent with the idea that such milder stages are also more sensitive to disease modification (35). Second, a post hoc analysis showed a significant interaction of baseline serum 25-(OH)D levels with intervention for exacerbation rates in the ITT population. Subsequently, our intervention in participants with severe vitamin D deficiency (<10 ng/mL) showed a statistically significant reduction in exacerbations of 43% over 1 year. In this subgroup, vitamin D treatment was associated with a significant increase in the monocyte capacity for phagocytosis. Considering the post hoc nature of the analysis and the small size of the subgroup, therapy should not be altered owing to these hypothesis-generating data. However, because 1 out of 6 participants in the trial presented with such asymptomatic, low serum 25-(OH)D levels at baseline that persisted during the study, further focus and future studies on this important subgroup may be warranted, eventually resulting in better patient-tailored interventions. Recently, a frequent exacerbator phenotype was identified suggesting that individualized therapy, including appropriate vitamin D supplementation, may become important (32). Finally, we should note that the lack of an overall effect may be explained by local insensitivity to vitamin D because of smoking or chronic inflammation. Epigenetic silencing of vitamin D signaling has been described in several types of cancer, and similar mechanisms may apply to chronic inflammatory diseases, such as COPD (36–38). Of note, we could not detect any differential effect of the treatment in former smokers or current smokers, with the limitation that the latter group was very small.

Our study also assessed the potential toxicity of prolonged and high-dose supplementation in patients with COPD, although we acknowledge that it was underpowered to determine long-term safety. Our data demonstrate that an average daily dose of more than 3200 IU of vitamin D during 1 year given as once-monthly 100 000 IU, which is more than 4 times the recommended dose for bone protection (39), introduced a small and transient risk for asymptomatic hypercalcemia. Because our blood samples were collected independently at drug intake, we recognize that other transient peaks of hypercalcemia in the days after ingestion may have been missed in some participants. This indicates that the upper limit of tolerability of 4000 IU/d, which is newly recommended and defined by the Institute of Medicine, may still be too high in elderly, sick patients (19).

Given the wide interest for vitamin D intervention trials in other chronic diseases, these findings may help to guide the design of and dosage in future trials. Although our results demonstrate that supplementation beyond what is recommended for bone health does not reduce exacerbations in patients with moderate to very severe COPD, they corroborate the suggestion that vitamin D deficiency is a potential risk in some patients. The dose used in our study was beyond the currently recommended daily requirements but yielded reliable levels of serum 25-(OH)D in all participants without any observed symptomatic calcium-related toxicity. More studies in other chronic diseases are needed to further explore the need and safety for recommending these higher doses of vitamin D to obtain potential beneficial effects beyond bone health, particularly in vitamin D–deficient patients with immune-related diseases.

From University Hospitals Leuven, Leuven, Belgium.

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Requests for Single Reprints: Wim Janssens, MD, PhD, Respiratory Medicine, University Hospitals Leuven, Herestraat 49, Bus 7003, 3000 Leuven, Belgium; e-mail, wim.janssens@uz.kuleuven.be.

Current author addresses and author contributions are available at www.annals.org.

References

1. **Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA.** Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax. 2002;57:847-52. [PMID: 12324669]
2. **Black PN, Scragg R.** Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. Chest. 2005;128:3792-8. [PMID: 16354847]
3. **Ross AC.** The 2011 report on dietary reference intakes for calcium and vitamin D [Letter]. Public Health Nutr. 2011;14:938-9. [PMID: 21492489]
4. **Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buysschaert I, et al.** Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax. 2010;65:215-20. [PMID: 19996341]
5. **Lips P.** Vitamin D physiology. Prog Biophys Mol Biol. 2006;92:4-8. [PMID: 16563471]
6. **Ginde AA, Mansbach JM, Camargo CA Jr.** Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2009;169:384-90. [PMID: 19237723]
7. **Kriegel MA, Manson JE, Costenbader KH.** Does vitamin D affect risk of developing autoimmune disease?: a systematic review. Semin Arthritis Rheum. 2011;40:512-531.e8. [PMID: 21047669]
8. **Krishnan AV, Trump DL, Johnson CS, Feldman D.** The role of vitamin D in cancer prevention and treatment. Endocrinol Metab Clin North Am. 2010;39:401-18. [PMID: 20511060]
9. **Nnoaham KE, Clarke A.** Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol. 2008;37:113-9. [PMID: 18245055]
10. **Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al.** Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008;117:503-11. [PMID: 18180395]
11. **Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, et al.** A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. Neurology. 2010;74:1852-9. [PMID: 20427749]
12. **von Hurst PR, Stonehouse W, Coad J.** Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient—a randomised, placebo-controlled trial. Br J Nutr. 2010;103:549-55. [PMID: 19781131]
13. **Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H.** Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr. 2010;91:1255-60. [PMID: 20219962]
14. **Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al.** Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med. 2009;179:843-50. [PMID: 19179490]
15. **Holick MF.** Vitamin D deficiency. N Engl J Med. 2007;357:266-81. [PMID: 17634462]
16. **Vieth R.** What is the optimal vitamin D status for health? Prog Biophys Mol Biol. 2006;92:26-32. [PMID: 16766239]
17. **Dawson-Hughes B, Mithal A, Bonjour JP, Boonen S, Burckhardt P, Fuleihan GE, et al.** IOF position statement: vitamin D recommendations for older adults. Osteoporos Int. 2010;21:1151-4. [PMID: 20422154]
18. **Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, et al.** High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet. 2011;377:242-50. [PMID: 21215445]
19. **The National Academies.** IOM report sets new dietary intake levels for calcium and vitamin D to maintain health and avoid risks associated with excess [press release]. Washington, DC: The National Academies; 30 November 2010. Accessed at www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=13050 on 5 November 2011.
20. **Janssens W, Lehouck A, Carremans C, Bouillon R, Mathieu C, Decramer M.** Vitamin D beyond bones in chronic obstructive pulmonary disease: time to act. Am J Respir Crit Care Med. 2009;179:630-6. [PMID: 19164701]
21. **Seemungal TA, Wilkinson TM, Hurst JR, Perera WR, Sapsford RJ, Wedzicha JA.** Long-term erythromycin therapy is associated with decreased chronic obstructive pulmonary disease exacerbations. Am J Respir Crit Care Med. 2008;178:1139-47. [PMID: 18723437]
22. **Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al.** The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004;350:1005-12. [PMID: 14999112]
23. **Charlson M, Szatrowski TP, Peterson J, Gold J.** Validation of a combined comorbidity index. J Clin Epidemiol. 1994;47:1245-51. [PMID: 7722560]
24. **Roca J, Burgos F, Sunyer J, Saez M, Chinn S, Antó JM, et al.** References values for forced spirometry. Group of the European Community Respiratory Health Survey. Eur Respir J. 1998;11:1354-62. [PMID: 9657579]
25. **Puhan MA, Behnke M, Laschke M, Lichtenschopf A, Brändli O, Guyatt GH, et al.** Self-administration and standardisation of the Chronic Respiratory Questionnaire: a randomised trial in three German-speaking countries. Respir Med. 2004;98:342-50. [PMID: 15072175]
26. **Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA.** Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med. 1987;106:196-204. [PMID: 3492164]
27. **Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al; ATS/ERS Task Force.** Standardisation of spirometry. Eur Respir J. 2005;26:319-38. [PMID: 16055882]
28. **Wilson R, Allegra L, Huchon G, Izquierdo JL, Jones P, Schaberg T, et al; MOSAIC Study Group.** Short-term and long-term outcomes of moxifloxacin compared to standard antibiotic treatment in acute exacerbations of chronic bronchitis. Chest. 2004;125:953-64. [PMID: 15006954]
29. **Suissa S.** Statistical treatment of exacerbations in therapeutic trials of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2006;173:842-6. [PMID: 16439716]
30. **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;37:238-43. [PMID: 20595151]
31. **Witham MD, Crighton LJ, Gillespie ND, Struthers AD, McMurdo ME.** The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure: a randomized controlled trial. Circ Heart Fail. 2010;3:195-201. [PMID: 20103775]
32. **Hurst JR, Vestbo J, Anzueto A, Locantore N, Müllerova H, Tal-Singer R, et al; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators.** Susceptibility to exacerbation in chronic obstructive pulmonary disease. N Engl J Med. 2010;363:1128-38. [PMID: 20843247]
33. **Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, et al; TORCH investigators.** Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med. 2007;356:775-89. [PMID: 17314337]
34. **Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al;**

UPLIFT Study Investigators. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. N Engl J Med. 2008;359:1543-54. [PMID: 18836213]
35. **Decramer M, Cooper CB.** Treatment of COPD: the sooner the better? Thorax. 2010;65:837-41. [PMID: 20805184]
36. **Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, et al.** Decreased histone deacetylase activity in chronic obstructive pulmonary disease. N Engl J Med. 2005;352:1967-76. [PMID: 15888697]
37. **Marik R, Fackler M, Gabrielson E, Zeiger MA, Sukumar S, Stearns V, et al.** DNA methylation-related vitamin D receptor insensitivity in breast cancer. Cancer Biol Ther. 2010;10:44-53. [PMID: 20431345]
38. **Essa S, Denzer N, Mahlknecht U, Klein R, Collnot EM, Tilgen W, et al.** VDR microRNA expression and epigenetic silencing of vitamin D signaling in melanoma cells. J Steroid Biochem Mol Biol. 2010;121:110-3. [PMID: 20153427]
39. **Bischoff-Ferrari HA, Willett WC, Wong JB, Stuck AE, Staehelin HB, Orav EJ, et al.** Prevention of nonvertebral fractures with oral vitamin D and dose dependency: a meta-analysis of randomized controlled trials. Arch Intern Med. 2009;169:551-61. [PMID: 19307517]

Current Author Addresses: Dr. Lehouck: Respiratory Medicine, University Hospitals Leuven, UZ Herestraat 49, Bus 7003, 3000 Leuven, Belgium.
Drs. Mathieu, Baeke, Decallonne, and Bouillon: Endocrinology and Experimental Medicine, University Hospitals Leuven, O&N I Herestraat 49, Bus 902, 3000 Leuven, Belgium.
Ms. Carremans and Dr. Janssens: Respiratory Medicine, University Hospitals Leuven, Herestraat 49, Bus 7003, 3000 Leuven, Belgium.
Drs. Verhaegen and Van Eldere: Laboratory of Bacteriology, University Hospitals Leuven, UZ Herestraat 49, Bus 7003, 3000 Leuven, Belgium.
Dr. Decramer: Respiratory Medicine, University Hospitals Leuven, UZ Herestraat 49, Bus 7003, 3000 Leuven, Belgium.

Author Contributions: Conception and design: C. Mathieu, C. Carremans, F. Baeke, J. Van Eldere, B. Decallonne, R. Bouillon, W. Janssens.
Analysis and interpretation of the data: A. Lehouck, C. Mathieu, F. Baeke, B. Decallonne, R. Bouillon, M. Decramer, W. Janssens.
Drafting of the article: A. Lehouck, C. Mathieu, F. Baeke, J. Verhaegen, W. Janssens.
Critical revision of the article for important intellectual content: C. Mathieu, F. Baeke, B. Decallonne, R. Bouillon, M. Decramer, W. Janssens.
Final approval of the article: C. Mathieu, F. Baeke, B. Decallonne, R. Bouillon, M. Decramer, W. Janssens.
Provision of study materials or patients: C. Mathieu, C. Carremans, J. Verhaegen, W. Janssens.
Statistical expertise: A. Lehouck, W. Janssens.
Obtaining of funding: C. Mathieu, M. Decramer, W. Janssens.
Administrative, technical, or logistic support: C. Carremans, F. Baeke, J. Van Eldere.
Collection and assembly of data: A. Lehouck, C. Mathieu, C. Carremans, F. Baeke, J. Verhaegen, J. Van Eldere, W. Janssens.

Appendix

Methods

Serum 25-(OH)D Levels

Total serum 25-(OH)D levels were measured in multiple batches by radioimmunoassay (DiaSorin, Brussels, Belgium) in all study participants according to the standard protocol. They are mean values of duplicate measures. Levels are expressed in nanograms per liter (conversion factor for nanomoles per liter, 2.5).

Plasma Cathelicidin Levels

Plasma cathelicidin levels were assayed by using an enzyme-linked immunosorbent assay (Hycult Biotechnology, Uden, the Netherlands), according to the standard protocol.

Quantitative Bacterial Cultures

Participants were asked to collect a spontaneous morning sputum on the day of a study visit. A cooling box to transport the sample was provided, and participants were instructed to blow their nose and rinse their mouth with water before collecting sputum. Sputum was homogenized by adding 0.1% dithiothreitol, and serial dilutions were prepared in phosphate-buffered saline at 1:100, 1:1000, 1:10 000, and 1:100 000. These dilutions were cultured on chocolate agar, blood agar, MacConkey agar, and mannitol salt agar plates. Undiluted but homogenized sputum samples were also cultured on chocolate agar and blood agar plates. Colonies of bacteria were examined and identified after 24-hour incubation at 37 °C. Colony-forming units were counted at their respective dilution in plates on which 1 to approximately 200 colonies could be differentiated.

Monocyte Phagocytosis Assay

The phagocytosis assay was performed by using the Phagotest Kit (ORPEGEN Pharma, Germany) containing fluorescein isothiocyanate (FITC)–labeled, opsonized *Escherichia coli*. Samples of 100 μL of peripheral blood with heparin were cooled on ice for 15 minutes, mixed with 2×10^7 FITC-labeled *E. coli*, and subsequently placed at 37 °C for 10 minutes. Control samples were kept on ice to inhibit phagocytosis. Next, 100 μL of brilliant blue (quenching solution) was added to delete fluorescence of nonphagocytosed bacteria sticking to the cellular membrane. After 2 washing steps, erythrocytes were lysed with lysis buffer for 20 minutes at room temperature. Three washing steps later, 50 μL of propidium iodide was added to stain leukocytes and intracellular bacterial DNA. The percentage of phagocytosis-positive cells in the monocyte gate was assessed by flow cytometry.

Results

Exacerbations and Serum 25-(OH)D Levels in the On-Treatment Population

Kaplan–Meier survival analysis showed no significant difference in the median time to first exacerbation between the vitamin D group and the placebo group (HR, 1.15 [CI, 0.83 to 1.60]; $P = 0.39$) (**Appendix Figure 1**, *top*). Supplementation with vitamin D resulted in a steep and significant increase in serum 25-(OH)D levels that remained stable during the study (mean, 52 ng/mL [SD, 16]), whereas baseline levels in the placebo group remained stable during the study (mean between-group difference, 30 ng/mL [CI, 27 to 33 ng/mL]; $P < 0.001$) (**Appendix Figure 1**, *middle*). No difference in the annual rate of exacerbations was observed ($P = 0.99$) (**Appendix Figure 1**, *bottom*).

Lung Function

Spirometric data from the study visits at baseline and at 4, 8, and 12 months were analyzed for trends over time in FEV_1. In 89% of the visits in the ITT population, appropriate FEV_1 measurements were obtained. Linear mixed-model analysis showed no significant differences in FEV_1 between the vitamin D and placebo groups, nor among study visits ($P = 0.87$) (**Appendix Figure 2** and **Appendix Table 1**).

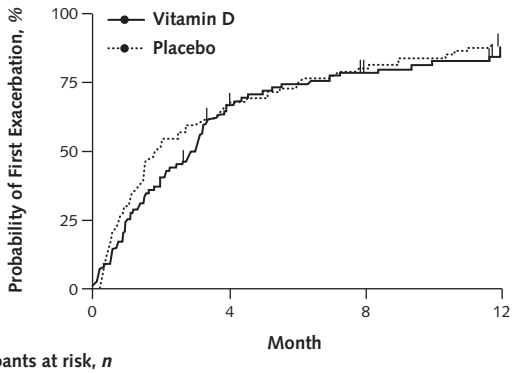
Quantitative Bacterial Cultures

The nature of spontaneous morning sputa cultures did not significantly differ for pathogenic strains or for the total number of colony-forming units when comparing both groups (**Appendix Table 4**).

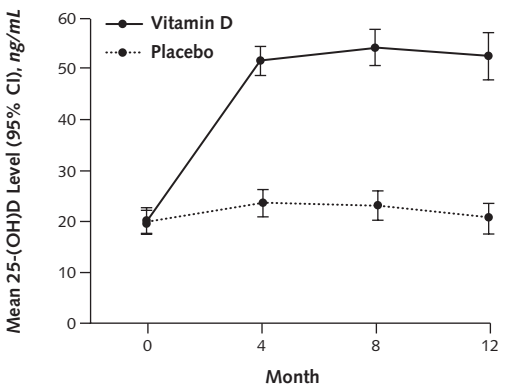
Monocyte Capacity for Phagocytosis

Monocyte function evaluated at the end of the study showed a significantly better capacity for phagocytosis in the vitamin D group than in the placebo group ($P = 0.002$) (**Appendix Figure 4**, *top*). The observed difference was more pronounced in the subgroup of participants with severe vitamin D deficiency at baseline ($P = 0.002$) (**Appendix Figure 4**, *bottom*).

Appendix Figure 1. **Mean serum 25-(OH)D levels and COPD exacerbation rates among the on-treatment population.**



Participants at risk, *n*				
Vitamin D	91	29	17	4
Placebo	91	29	18	9

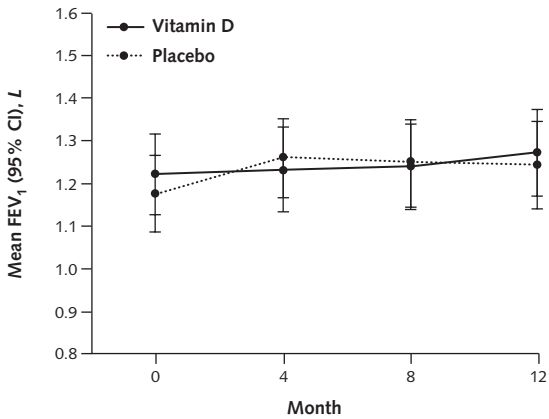


Participants, *n*				
Vitamin D	91	81	75	65
Placebo	91	83	73	65

Variable	Vitamin D (*n* = 91)	Placebo (*n* = 91)	Difference or RR (95% CI)	*P* Value
Mean 25-(OH)D level (SD), *ng/mL*				
At baseline	20 (12)	20 (11)	0.2 (–3 to 4)	0.90
During study	52 (16)	22 (13)	30 (27 to 33)	<0.001
COPD exacerbations per patient-year, *n*	2.8	2.8	0.99 (0.80 to 1.25)	0.99
Two-way ANOVA				<0.001

25-(OH)D = 25-hydroxyvitamin D; ANOVA = analysis of variance; COPD = chronic obstructive pulmonary disease; RR = rate ratio. **Top.** Kaplan–Meier plots of time to first exacerbation in the on-treatment population. **Middle.** Mean serum 25-(OH)D levels in the on-treatment population, excluding patients who started low-dose vitamin D supplementation during the trial. **Bottom.** COPD exacerbations per patient-year in the on-treatment population. Two-way ANOVA statistics for between-group differences in serum 25-(OH)D levels and interaction are given. For exacerbation rates, Poisson regression statistics are given. *P* values are unadjusted.

Appendix Figure 2. **FEV_1 from baseline to end of study in the intention-to-treat population.**



Participants, *n*				
Vitamin D	91	81	76	69
Placebo	91	84	81	77

Linear mixed-model analysis showed no significant differences between the groups and study visits ($P = 0.87$).

Appendix Table 1. **Serial Measurements of Lung Function and Quality of Life by Chronic Respiratory Questionnaire Scores Over 1 Year in the Intention-to-Treat Population***

Variable	Vitamin D Group (n = 91)	Placebo Group (n = 91)	Difference (95% CI)	Unadjusted P Value
FEV_1, *L*				
Baseline	1.2 (0.4)	1.2 (0.4)	0.05 (−0.08 to 0.17)	0.49
During study	1.2 (0.5)	1.2 (0.5)	−0.01 (−0.09 to 0.08)	0.95
P value†				0.88
Dyspnea score				
Baseline	4.7 (1.4)	4.7 (1.5)	−0.03 (−0.46 to 0.40)	0.90
During study	4.8 (1.5)	5.0 (1.5)	−0.24 (−0.49 to 0.14)	0.141
P value†				0.30
Emotional score				
Baseline	4.9 (1.3)	4.8 (1.3)	0.10 (−0.27 to 0.47)	0.60
During study	5.0 (1.3)	5.1 (1.3)	−0.07 (−0.31 to 0.16)	0.55
P value†				0.53
Fatigue score				
Baseline	4.1 (1.4)	3.9 (1.4)	0.19 (−0.22 to 0.59)	0.36
During study	4.3 (1.5)	4.1 (1.4)	0.15 (−0.12 to 0.42)	0.27
P value†				0.27
Mastery score				
Baseline	5.3 (1.3)	5.2 (1.3)	0.09 (−0.28 to 0.48)	0.63
During study	5.4 (1.3)	5.5 (1.3)	−0.02 (−0.26 to 0.22)	0.85
P value†				0.57

* Values are means (SDs).
† Two-way analysis-of-variance P value for interaction.

Appendix Table 2. **Serum 25-(OH)D Levels Over 1 Year in the ITT Population, Subgroup of Vitamin D–Naive Participants, and Subgroup of Vitamin D–Deficient Participants***

Variable†	Vitamin D Group	Placebo Group	Difference (95% CI)	Unadjusted P Value
ITT population				
Baseline (n = 91/91)	20 (12)	20 (11)	0.2 (–3 to 4)	0.90
4 mo (n = 83/86)	52 (13)	23 (13)	28 (25 to 32)	<0.001
8 mo (n = 79/81)	54 (15)	23 (12)	31 (27 to 35)	<0.001
12 mo (n = 70/74)	52 (19)	20 (13)	32 (26 to 37)	<0.001
During study	52 (16)	22 (13)	30 (27 to 33)	<0.001
P value‡				<0.001
Vitamin D–naive subgroup				
Baseline (n = 71/71)	17 (8)	16 (8)	0.8 (–2 to 3)	0.58
4 mo (n = 65/68)	51 (14)	20 (11)	31 (26 to 35)	<0.001
8 mo (n = 62/67)	52 (16)	20 (10)	32 (27 to 36)	<0.001
12 mo (n = 56/61)	50 (18)	18 (10)	33 (27 to 38)	<0.001
During study	51 (16)	19 (11)	32 (29 to 34)	<0.001
P value‡				<0.001
Vitamin D–deficient subgroup				
Baseline (n = 15/15)	8 (2)	7 (2)	0.6 (–1 to 2)	0.36
4 mo (n = 13/15)	49 (18)	15 (10)	34 (22 to 45)	<0.001
8 mo (n = 12/14)	51 (13)	12 (8)	39 (31 to 48)	<0.001
12 mo (n = 12/13)	50 (13)	8 (3)	43 (35 to 50)	<0.001
During study	50 (15)	12 (8)	38 (33 to 44)	<0.001
P value‡				<0.001

25-(OH)D = 25-hydroxyvitamin D; ITT = intention-to-treat.
* Values are means (SDs), reported in ng/mL. To convert values to nmol/L, multiply by 2.5.
† The numerator and denominator represent the numbers of participants in the vitamin D/placebo groups.
‡ Two-way analysis-of-variance P value for interaction.

Appendix Table 3. **Safety: Serial Serum Calcium and Phosphate Levels and Incidence of Hypercalcemia and Hypercalciuria Over 1 Year in Vitamin D and Placebo Groups in the Intention-to-Treat Population***

Variable†	Vitamin D Group	Placebo Group	Difference (95% CI)	*P* Value
Calcium level, *mg/dL*‡				
Baseline (*n* = 91/91)	9.4 (0.50)	9.4 (0.37)	0.04 (−0.09 to 0.16)	0.59
4 mo (*n* = 83/86)	9.4 (0.50)	9.3 (0.42)	0.07 (−0.07 to 0.21)	0.32
8 mo (*n* = 79/81)	9.3 (0.38)	9.3 (0.42)	0.05 (−0.07 to 0.18)	0.39
12 mo (*n* = 70/74)	9.3 (0.41)	9.2 (0.44)	0.11 (−0.04 to 0.26)	0.146
During study	9.4 (0.43)	9.3 (0.43)	0.08 (−0.01 to 0.16)	0.061
P value§				0.86
Phosphate level, *mg/dL*‖				
Baseline (*n* = 91/91)	2.8 (0.59)	2.8 (0.53)	−0.03 (−0.20 to 0.13)	0.70
4 mo (*n* = 83/86)	2.8 (0.61)	2.8 (0.60)	0.01 (−0.18 to 0.20)	0.93
8 mo (*n* = 79/81)	2.8 (0.57)	2.9 (0.59)	−0.06 (−0.25 to 0.12)	0.50
12 mo (*n* = 70/74)	2.9 (0.51)	2.9 (0.56)	−0.01 (−0.20 to 0.17)	0.90
During study	2.8 (0.57)	2.9 (0.58)	−0.02 (−0.13 to 0.09)	0.69
P value§				0.95
Hypercalcemia, *% (n)*				
Baseline (*n* = 91/91)	1 (2)	0	–	0.155
4 mo (*n* = 83/86)	2 (4)	0	–	0.043
8 mo (*n* = 79/81)	0	0	–	–
12 mo (*n* = 70/74)	0	0	–	–
Hypercalciuria, *% (n)*¶				
Baseline	–	–	–	–
4 mo	–	–	–	–
8 mo	–	–	–	–
12 mo (*n* = 39/40)	0	0	–	–

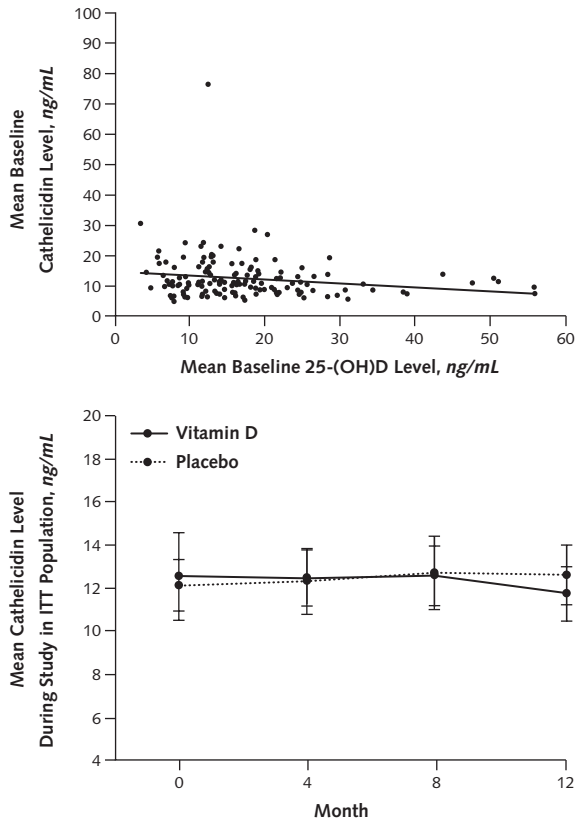
* Values are reported as means (SDs).
† The numerator and denominator represent the numbers of participants in the vitamin D/placebo groups.
‡ To convert calcium values to mmol/L, multiply by 0.25.
§ Two-way analysis-of-variance *P* value for interaction.
‖ To convert phosphate values to mmol/L, multiply by 0.323.
¶ The presence of hypercalciuria was examined only at the end of the study.

Appendix Table 4. **Sputum Samples and Bacterial Cultures**

Variable	Baseline	4 mo	8 mo	12 mo
Participants providing spontaneous sputum samples, *% (n)*				
Vitamin D group (*n* = 91)	54 (49)	44 (40)	33 (30)	20 (18)
Placebo group (*n* = 91)	49 (45)	47 (43)	44 (40)	30 (28)
P value*	0.55	0.66	0.128	0.088
Sputum samples with a positive culture, *% (n/n)*†				
Vitamin D group (*n* = 91)	26 (11/49)	28 (11/40)	40 (12/30)	28 (5/18)
Placebo group (*n* = 91)	52 (23/45)	47 (20/43)	45 (18/40)	32 (9/28)
P value*	0.011	0.074	0.68	0.75
Positive samples, *% (n)*				
Vitamin D group				
0 CFUs/mL (no potential pathogen)	74 (38)	–	–	72 (13)
10^5–10^6 CFUs/mL	7 (3)	–	–	0
10^6–10^7 CFUs/mL	3 (1)	–	–	0
10^7–10^8 CFUs/mL	7 (3)	–	–	11 (2)
$>10^8$ CFUs/mL‡	9 (4)	–	–	17 (3)
Placebo group				
0 CFUs/mL (no potential pathogen)	48 (22)	–	–	67 (19)
10^5–10^6 CFUs/mL	7 (3)	–	–	4 (1)
10^6–10^7 CFUs/mL	18 (8)	–	–	4 (1)
10^7–10^8 CFUs/mL	9 (4)	–	–	11 (3)
$>10^8$ CFUs/mL§	18 (8)	–	–	14 (4)

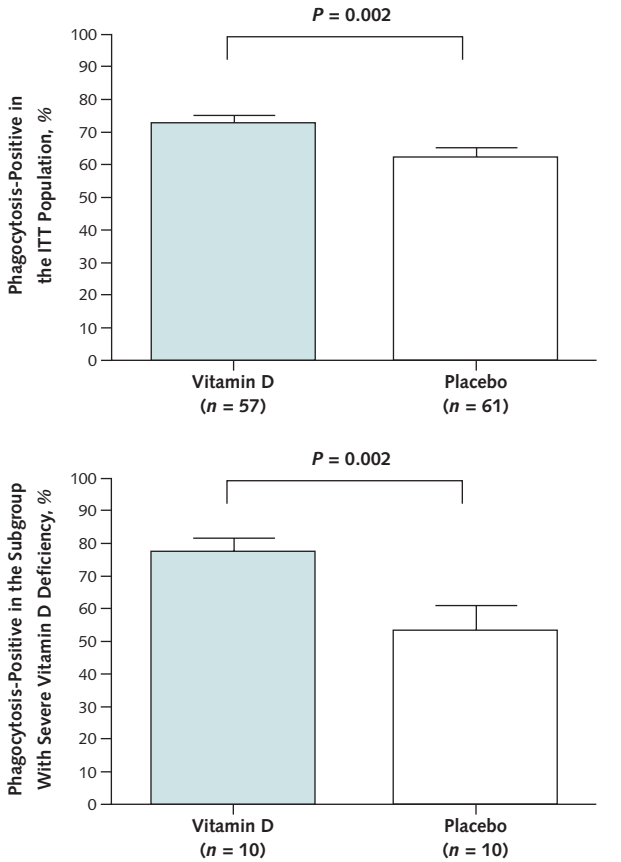
CFU = colony-forming unit.
* *P* values express differences between rows according to chi-square statistics.
† Bacterial growth of upper airway commensal flora was not taken into account.
‡ $P = 0.056$ between groups.
§ $P = 0.85$ between groups.

Appendix Figure 3. **Plasma cathelicidin levels.**



25-(OH)D = 25-hydroxyvitamin D; ITT = intention-to-treat. **Top.** Relationship between plasma cathelicidin and serum 25-(OH)D levels at baseline. Spearman $r = 0.027$; $P = 0.04$. **Bottom.** Plasma cathelicidin levels during the study in the ITT population. Linear mixed-model analysis showed no significant differences between the groups and study visits ($P = 0.85$).

Appendix Figure 4. **Monocyte phagocytosis assay results.**



ITT = intention-to-treat. **Top.** Percentage of monocytes positive for phagocytosis at the end of the study in the ITT population. **Bottom.** Percentage of monocytes positive for phagocytosis at the end of the study in the subgroup of participants with severe vitamin D deficiency (serum 25-hydroxyvitamin D levels <10 ng/mL).