



## **High dose Vitamin D administration in ventilated intensive care unit patients: A pilot double blind randomized controlled trial**

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Abstract: Background: There is a high prevalence of vitamin D deficiency  
in the critically ill patient population. Several intensive care unit  
studies have demonstrated an association between vitamin D deficiency  
[25-hydroxyvitamin D (25(OH)D) < 20 ng/mL] and increased hospital length  
of stay (LOS), readmission rate, sepsis and mortality.

Material and Methods: Pilot, double blind randomized control trial  
conducted on mechanically ventilated adult ICU patients. Subjects were  
administered either placebo, 50,000 IU vitamin D3 or 100,000 IU vitamin  
D3 daily for 5 consecutive days enterally (total vitamin D3 dose =  
250,000 IU or 500,000 IU, respectively). The primary outcome was plasma  
25(OH)D concentration 7 days after oral administration of study drug.  
Secondary outcomes were plasma levels of antimicrobial peptide  
cathelicidin (LL37), hospital LOS, SOFA score, duration of mechanical  
ventilation, hospital mortality, mortality at 12 weeks, and hospital  
acquired infection.

Results: A total of 31 subjects were enrolled with 13 (43%) being vitamin  
D deficient at entry (25(OH)D levels < 20 ng/mL). The 250,000 IU and  
500,000 IU vitamin D3 regimens each resulted in a significant increase in  
mean plasma 25(OH)D concentrations from baseline to day 7; values rose to  
45.7±19.6 ng/mL and 55.2 ± 14.4 ng/mL, respectively, compared to  
essentially no change in the placebo group (21±11.2 ng/mL), p<0.001.  
There was a significant decrease in hospital length of stay over time in  
the 250,000 IU and the 500,000 IU vitamin D3 group, compared to the  
placebo group (25 ± 14 and 18 ± 11 days compared to 36 ± 19 days,  
respectively; p=0.03). There was no statically significant change in  
plasma LL-37 concentrations or other clinical outcomes by group over  
time.

Conclusions: In this pilot study, high-dose vitamin D3 safely increased  
plasma 25(OH)D concentrations into the sufficient range and was  
associated with decreased hospital length of stay without altering other  
clinical outcomes.



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Title: High Dose Vitamin D Administration in Ventilated Intensive Care Unit Patients: A Pilot  
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Dear Dr. Christian Koch,

We are grateful to you and the reviewers for taking the time and effort to improve our manuscript submission for publication to this esteemed journal. In regards to the specific reviewers concerns, we have addressed each issue raised with a response.

Thank you for your consideration of our work. Please address all correspondence concerning this manuscript to me at the address above and feel free to correspond with me by e-mail (jehan2@emory.edu).

Sincerely,

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Ms. Ref. No.: JCTE-D-16-00014

Title: High Dose Vitamin D Administration in Ventilated Intensive Care Unit Patients: A Pilot Double Blind Randomized Control Trial  
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“Reviewer #1: This reviewer has some minor comments:”

**1. In the abstract, it is important to indicate the secondary outcome of the study in the Material and Methods paragraph.**

-This has been inserted in the abstract under Material and Methods.

Page 2: “Secondary outcomes were plasma levels of the antimicrobial peptide cathelicidin (LL37), hospital LOS, SOFA score, duration of mechanical ventilation, hospital mortality, mortality at 12 weeks, and hospital acquired infection.”

**2. There are many abbreviations used in this manuscript, it would be desirable to add an abbreviation section as a footnote.**

1,25-dihydroxyvitamin D= 1,25(OH)<sub>2</sub>D<sub>3</sub>= calcitriol

25 (OH)D=25-hydroxyvitamin D

AMP= Antimicrobial peptides

APACHE II= Acute Physiology and Chronic Health Evaluation II

BALF= Bronchial alveolar lavage fluid

HAI=Hospital acquired infection

IU=International Units

LOS= length of stay

MAP= Mean arterial pressure

SOFA= Sequential organ failure assessment

-Abbreviation footnote added as page 16

**3. Page 4, second paragraph, line 4, replace 1 $\alpha$ -hydroxylase with CYP27b1.**

“In monocytes and macrophages, pathogens bind to cell surface toll-like receptors to CYP27b1 stimulate to convert 25(OH)D, the biomarker of vitamin D status, into the active form 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D<sub>3</sub>; calcitriol].”

-Revised as requested.

**4. Page 5, first paragraph, line 10, delete ref 24, and replace with a new ref by Leaf et al.(Am J Respir Crit Care Med Vol 190, Iss 5, pp 533-541, 2014).**

-This reference has been added to page 5, first paragraph.

“Leaf *et al* found that lower plasma 25(OH)D levels on admission to the ICU correlated with lower LL-37 plasma levels that were, in turn, associated with increased 90 day mortality and sepsis risk. [24, 25]”

**5. Page 7, last paragraph, lines 6 and 7, what is the vehicle used to dissolve vitamin D3 for enteral administration?**

-Dissolved in sterile water. Added to manuscript.

Page 7, second paragraph, 3<sup>rd</sup> sentence: "The medications were dissolved in sterile water and administered through an enteral feeding tube."

**6. Page 10, change "Measures" to "Measurements" of 25(OH)D. Same for LL-37.**

***Measurements of 25(OH)D***

***Measurements of LL-37***

-Revised as requested.

**7. Page 14, line 2, add ref. # after Nair et al.**

-Inserted as requested. Page 14, second sentence:

"Our results regarding LL-37 differ from Nair *et al* [40], who found an increase in serum 25(OH)D concentrations associated with increased serum LL-37 on day 1 and 3 in critically ill patients treated with cholecalciferol, raising the possibility that LL-37 regulation is time dependent within the first few days of vitamin D therapy and was not reflected in our measurements at day 7. [40]"

**8. Table 1, add unit (ng/ml) to baseline 25(OH)D.**

-Revised as requested.

“Reviewer #3: Several points need clarification:

Major:

**1. out of 658 screened only 31 subjects were randomized. This tells about very narrow internal validity of the results and should be more in-depth discussed in limitations section cautioning anyone from extrapolating the results to everyday practice.**

-Enrollment rates in critical care trials are historically lower than other specialty trials; however, we appreciate the reviewers comment and agree with this evaluation and have included in the limitations section on page 13 after the first sentence: “In addition, out of 658 patients screened we enrolled only 31 subjects and caution the generalizability of our results.”

**2. Intro - explain rationale why D3 replacement was chosen over D2. Ergocalciferol 50,000 units capsule is on the formulary of any hospital and if in future this trial results are confirmed in a larger study, then the cost of therapy will be much lower with Ergo replacement than asking special D3 preparation to be prescribed.**

We appreciate the reviewers concern that our study medication may not be widely available if we are able to confirm our results in future studies. The formulation of vitamin D<sub>3</sub> that was used in our study mirrors many large NIH funded trials including VITAL and VDAART study. Additionally, it is more readily available than the ergocalciferol 50,000 IU tablet. It can be purchased by hospitals/clinics/pharmacy or directly by a patient without a prescription. Furthermore, vitamin D<sub>3</sub> is the endogenously produced vitamin D as opposed to vitamin D<sub>2</sub> which must come as a supplement or in fungi/yeast.

In summary, we chose vitamin D<sub>3</sub> 50,000 IU because of its inexpensive cost (\$ 0.018 per pill), wide availability even without prescription, more widely used in other vitamin D trials, and it's the natural vitamin D ligand made endogenously.

**3. Steroid therapy was not listed as exclusion criterion. I understand patients with septic shock were excluded but what about the patients with COPD, ARDS or any other lung pathology who required steroid therapy. Steroids can affect metabolism of vitamin D and will have muscle effects and hence this concomitant therapy could affect the results and outcomes. What if vitamin D patients received less steroids and therefore by chance had less myopathy and had faster rehab process?**

-We appreciate this very important point that has been highlighted as a possible additional limitation. We did not analyze steroid use.

-We revised on page 13 in the limitations section: “Also, we did not analyze steroid use and may be an unknown confounding variable and limitation to this study and future studies will need to monitor corticosteroid dosing and duration as a potential confounder. “

**4. In the same venue, if the authors did not find inflammation modulatory effects of high dose vitamin D, then my best explanation would be some positive sub-acute**

**musculoskeletal effects from the vitamin D. this may have direct explanation why patients following vit. D replacement had earlier discharge from the hospital - they regained mobility after ICU stay faster so were deemed to be physically safe to be discharged to rehab, nursing home or home. Molecular mechanisms and musculoskeletal clinical implications needs to be discussed as so far the authors did not have an explanation why patients in the active group had earlier discharge from the hospital**

-We agree with this astute observation. We added to page 14 last line.

- "Therefore, there may be additional musculoskeletal effects that are unknown at this time, such as positive sub-acute effects that facilitated musculoskeletal recovery and thus expedited hospital discharge."

**5. The results section and Tables 2,3 and 4 will benefit from addition into calculations of combined two vitamin D groups into one because that may increase the power and unveil some other trends that may have not been seen due to low N in vitamin D groups separately.**

-One of the aims of the study was to see if there was a difference in dosing strategy on outcomes and safety. In addition, our pre-specified analysis plan was to analyze three treatment groups.

-If additional analysis were performed comparing placebo to both treatment groups, interpretation of the findings would be the results of exploratory post-hoc analysis with the potential for false positive results.

Minor:

**1. Please describe what LL37 is in the Abstract.**

-Revised as requested.

Page 2 abstract: "Secondary outcomes were plasma levels of the antimicrobial peptide cathelicidin (LL37), hospital LOS, SOFA score, duration of mechanical ventilation, hospital mortality, mortality at 12 weeks, and hospital acquired infection."

**2. 25-OH vitamin D was measured in the Emory lab. Was it Emory Hospital lab or a dedicated facility? Also did the assay determine both 25-OH vit. D2 and D3 to result in the final 25-OH vit. D level or authors have separate D2 and D3 isoforms measured to have more information on baseline and follow up levels following vit. D3 replacement**

The 25(OH)D was measured in the vitamin D laboratory at Emory University which is led by Dr. Vin Tangpricha. It is a research laboratory which is focused on the measurement of 25(OH)D and PTH. The 25(OH)D that is reported is the total of both the 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. In humans, very little 25(OH)D<sub>2</sub> is found in circulation unless a patient is prescribed ergocalciferol. This laboratory participates in the DEQAS ([www.deqas.org](http://www.deqas.org)) external quality control program for measurement of 25(OH)D.



## High Dose Vitamin D Administration in Ventilated Intensive Care Unit Patients: A Pilot Double Blind Randomized Control Trial

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**Figures:** 2

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**Conflicts of Interest:** The authors have no conflicts of interest to disclose.

## Abstract

**Background:** There is a high prevalence of vitamin D deficiency in the critically ill patient population. Several intensive care unit studies have demonstrated an association between vitamin D deficiency [25-hydroxyvitamin D (25(OH)D) < 20 ng/mL] and increased hospital length of stay (LOS), readmission rate, sepsis and mortality.

**Material and Methods:** Pilot, double blind randomized control trial conducted on mechanically ventilated adult ICU patients. Subjects were administered either placebo, 50,000 IU vitamin D<sub>3</sub> or 100,000 IU vitamin D<sub>3</sub> daily for 5 consecutive days enterally (total vitamin D<sub>3</sub> dose = 250,000 IU or 500,000 IU, respectively). The primary outcome was plasma 25(OH)D concentration 7 days after oral administration of study drug. Secondary outcomes were plasma levels of the antimicrobial peptide cathelicidin (LL37), hospital LOS, SOFA score, duration of mechanical ventilation, hospital mortality, mortality at 12 weeks, and hospital acquired infection.

**Results:** A total of 31 subjects were enrolled with 13 (43%) being vitamin D deficient at entry (25(OH)D levels < 20 ng/mL). The 250,000 IU and 500,000 IU vitamin D<sub>3</sub> regimens each resulted in a significant increase in mean plasma 25(OH)D concentrations from baseline to day 7; values rose to 45.7±19.6 ng/mL and 55.2 ± 14.4 ng/mL, respectively, compared to essentially no change in the placebo group (21±11.2 ng/mL), p<0.001. There was a significant decrease in hospital length of stay over time in the 250,000 IU and the 500,000 IU vitamin D<sub>3</sub> group, compared to the placebo group (25 ± 14 and 18 ± 11 days compared to 36 ± 19 days, respectively; p=0.03). There was no statically significant change in plasma LL-37 concentrations or other clinical outcomes by group over time.

**Conclusions:** In this pilot study, high-dose vitamin D3 safely increased plasma 25(OH)D concentrations into the sufficient range and was associated with decreased hospital length of stay without altering other clinical outcomes.

**Clinical Trial Registration:** [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT01372995)

**Primary Source of Funding:** This project was supported, in part, by National Institutes of Health grants: NIH R21 HL110044 (GSM, TRZ), K24 DK096574 (TRZ), UL1 TR000454 (JEH, GSM, TRZ, VT), T32 AA013528 (JEH) and T32 DK007298 (JLJ).

Word Count: 297

**Key Words:** vitamin D, lung failure, critical care, LL-37, antimicrobial peptides

## Introduction

There is a high prevalence of vitamin D deficiency in the critically ill patient population, with approximately 60% of patients found to be vitamin D deficient, (25(OH)D concentrations <20 ng/mL), and an additional 30% of patients being vitamin D insufficient, (25(OH)D= 20-30 ng/mL). [1-10] Several intensive care unit (ICU) studies have demonstrated an association between vitamin D deficiency and important clinical outcomes: increased hospital length of stay (LOS), readmission rates, sepsis and mortality. [3-9, 11, 12] Vitamin D deficiency is also associated with increased risk of acute respiratory failure in critically ill patients. [13, 14] Furthermore, Dancer *et al* found that vitamin D deficiency is nearly universal in development of the acute respiratory distress syndrome and mechanistically related to lung inflammation and alveolar epithelial cell injury. [15]

Vitamin D has pleiotropic effects on the host immune pathway and may be uniquely involved with lung immune function and alveolar capillary barrier function. In monocytes and macrophages, pathogens bind to cell surface toll-like receptors to CYP27b1 stimulate to convert 25(OH)D, the biomarker of vitamin D status, into the active form 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D<sub>3</sub>; calcitriol]. 1,25(OH)<sub>2</sub>D<sub>3</sub> in turn upregulates mRNA expression of human cationic antimicrobial protein (hCAP-18), which is cleaved to produce LL-37, a major anti-microbial peptide (AMP) with activity against gram-positive/gram-negative bacteria, fungi and viruses. [16-18] Although data are inconsistent, existing evidence suggests that supplementation with vitamin D<sub>3</sub> may decrease susceptibility or enhance recovery to infections such as influenza, recurrent pneumonia and tuberculosis.[19-21]

An observational study in patients with serum 25(OH)D < 20 ng/ml found that improved vitamin D status before hospital admission decreased the odds of all cause-mortality.[22] A recent large randomized study by Amrein *et al* demonstrated decreased mortality in a subgroup of subjects with severe vitamin D deficiency (<12 ng/ml) given a one-time bolus dose of 540,000 IU of enteral cholecalciferol.[23] Leaf *et al* found that lower plasma 25(OH)D levels on admission to the ICU correlated with lower LL-37 plasma levels that were, in turn, associated with increased 90 day mortality and sepsis risk. [24, 25] In addition, a single intravenous dose of 2 µg calcitriol in adult ICU patients with severe sepsis or shock significantly upregulated leukocyte mRNA for hCAP-18 and the anti-inflammatory cytokine interleukin-10 24 hours after dosing. [24, 26] Therefore AMPs may be important modifiers of the immune response in critically ill patients in response to vitamin D status or exogenous administration.

We designed this pilot study to evaluate the safety and efficacy of two doses of vitamin D<sub>3</sub>, 250,000 or 500,000 IU, given in divided doses over 5 consecutive days to increase plasma 25(OH)D concentrations to the sufficient range (> 30 ng/mL) and to increase plasma LL-37 in adult ventilated patients requiring intensive care.

## **Methods**

### ***Trial Design***

The study was approved by the Emory University Institutional Review Board and written informed consent was obtained from the patient or legal surrogate prior to study enrollment. The enrollment goal of this pilot study was 36 patients (12 in each group) from two Atlanta, Georgia hospitals; Emory University Hospital (EUH) and Emory University Midtown (EUH-M). The study was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov)

(NCT01372995) and the protocol was amended to include bronchoscopy at both baseline and study day 7. A Data Safety Monitoring Board reviewed progress and adverse event reports every 6 months during the duration of the trial. The entire study duration was 84 days; however, blood samples were only taken every 7 days while the patient was hospitalized.

### ***Participant Selection***

Enrollment started in July 2011 and was completed in March 2014. Inclusion criteria were: 1) receiving care in an ICU; 2) age greater than 18 years; 3) expected to require mechanical ventilation for at least 72 hours after study entry; 4) expected to survive and remain in the ICU for at least 96 hours after study entry; 5) enteral access in place to enable delivery of vitamin D<sub>3</sub> or placebo and are deemed to be able to tolerate enteral drug administration. Exclusion criteria were: 1) inability to obtain or declined informed consent from the subject and/or legally authorized representative; 2) current pregnancy; 3) ongoing shock, [defined as unstable blood pressure despite vasopressor support and mean arterial pressure (MAP) < 60 mm Hg on at least 3 consecutive readings within a 3-hour period prior to study entry]; 4) current hypercalcemia (albumin-corrected serum calcium > 10.8 mg/dL or ionized calcium > 5.2 mg/dL); 5) history of therapy with high-dose vitamin D<sub>3</sub> (greater than or equal to 50,000 IU a week) to treat vitamin D deficiency, within previous 6 months; 6) history of disorders associated with hypercalcemia (history of cancer with history of hypercalcemia within the past 1 year, hyperparathyroidism, sarcoidosis, nephrolithiasis); 7) chronic dialysis; 8) known history of cirrhosis; 9) known HIV; 10) received any investigational drug within 60 days prior to study entry. The initial protocol included bronchoscopy on day 7 of intubation, but the

protocol was amended to include a baseline bronchoscopy and then a repeat on day 5-7 if the patient remained intubated, in order to increase our sample size. The protocol was amended in January, 2012 to permit enrollment of any adult critically ill mechanically ventilated patients.

***Intervention:***

Following informed consent, subjects' Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated. [27] Treatment assignments were stratified according to clinical center and dichotomized APACHE II score  $\leq$  or  $>15$ . Subjects were randomly assigned in a 1:1:1 ratio to placebo or a total of 250,000 IU vitamin D<sub>3</sub> or 500,000 IU vitamin D<sub>3</sub> in divided equal doses over 5 days.

Treatment groups were assigned by a blinded block randomization schedule overseen by biostatisticians of the Atlanta Clinical and Translational Science Institute (ACTSI) biostatistics core. The placebo arm received two inactive medication tablets daily for 5 days; treatment arm 1 received one 50,000 international units (IU) of vitamin D<sub>3</sub> and 1 placebo pill daily for 5 days (250,000 IU total) and treatment arm 2 received 2 pills of 50,000 IU of vitamin D<sub>3</sub> daily for 5 days (500,000 IU total). The medications were dissolved in sterile water and administered through an enteral feeding tube.

Cholecalciferol 50,000 IU tablets were manufactured from Tischon (Westbury, NY) and Biotech (Fayetteville, AR) and bioavailability testing conducted during the trial showed the capsules to be within 10% of the stated dose. EUH and EUH-M Investigational Drug Service pharmacists maintained the code and delivered all study drugs to the respective study subject's primary nurses for administration per research protocol. With the exception of the pharmacists, all study staff were blinded to the group allocation.

### ***Clinical and demographic characteristics***

Clinical and demographic data were collected at baseline. Sequential organ failure assessment (SOFA) [28] scores, laboratory values and other clinical data were collected daily while the subjects were hospitalized. Hospital-acquired infections (HAI) were measured as a composite of all infectious complications that occurred during hospitalization according to the 2009 Center Disease Control definitions.[29] Measured safety parameters included serial serum creatinine, calcium, and phosphorous concentrations, and adverse events.

### ***Biospecimen collection***

Twenty mL of venous blood was collected at baseline and again at 7 and 14 days. The bronchoalveolar lavage procedure was performed by serial instillation of 30 mL aliquots of normal saline into a pulmonary subsegment and BALF was collected by suction. BALF was centrifuged (1000 rpm; 15 min) and supernatant frozen at -80°C. BALF was concentrated 5- to 10-fold for 25(OH)D determinations. Plasma 25(OH)D was measured using a chemiluminescent-based automated machine (IDS- iSYS; Immunodiagnostic Systems, Scottsdale, AZ). The Emory Vitamin D laboratory participates in the Vitamin D External Quality Assessment Scheme and NIST/NIH Vitamin D Metabolites Quality Assurance Program. The 25(OH)D assay also included internal control samples with known 25(OH)D concentrations as an additional quality measure. Plasma LL-37 was determined by ELISA (Hycult Biotech Inc., Plymouth Meeting, PA) and corrected according to BALF urea concentrations.

### ***Sample Size and Statistical Methods***



The primary outcome was the change in plasma 25(OH)D from baseline to 7 days. Using our preliminary data [1], 12 subjects in each group were required to test the hypothesis that high-dose vitamin D<sub>3</sub> would achieve plasma 25(OH)D levels  $\geq$  30 ng/mL with power of 0.94 and  $\alpha$  of 0.05. The primary analysis was according to intent-to-treat and global tests based on a generalized linear models, to estimate the effect of time and treatment on the outcomes. Multiple pairwise comparisons were used to test the difference between groups. In addition, the proportion of patients in each group achieving 25(OH)D levels of  $>$  30 ng/mL was compared using an uncorrected chi-squared statistic. For outcomes that were not normally distributed, such as ICU LOS and hospital LOS, log-transformation was performed. Correlations were calculated using Spearman's rho ( $\rho$ ). P values  $<$  0.05 are considered significant. Based upon our previous data on plasma LL-37 levels in adult ICU patients, enrolling 12 subjects per group provided 99% power to detect an increase in LL-37 to levels in normal healthy subjects (27.2 ng/mL).[1] Planned secondary outcomes were hospital LOS, development and/or persistence of organ dysfunction quantified using the SOFA score, duration of mechanical ventilation, hospital mortality, mortality at 12 weeks, and HAI.

## Results

A total of 31 subjects were enrolled before project funding was ended. Study enrollment, randomization and follow-up are shown in the CONSORT diagram (**Figure 1**). Of 658 patients screened for inclusions/exclusion criteria, a total of 31 subjects were eligible and randomized, and data from 30 subjects were analyzed. Per spousal request, one patient withdrew from the study after completion of study medication. All subjects received all study medication except one subject who received the first day of

study drug before a decision was made for comfort care; this individual was included in the assigned intention-to-treat group. No subjects were lost to follow-up. Baseline demographic and clinical characteristics were comparable across all three groups (**Table 1**). At baseline, comorbidities, including history of heart failure, chronic obstructive pulmonary disease, chronic kidney disease, diabetes, asthma and diagnosis of acute infection did not differ between groups. A history of coronary artery disease was different between groups at baseline; 10.0% (1/10) in the placebo group, 22.2% (2/9) in the 250,000 IU vitamin D<sub>3</sub> group, and 45.5% (7/10) in the 500,000 IU vitamin D<sub>3</sub> group, (p = 0.03).

### ***Measurements of 25(OH)D***

On the day of enrollment, 43% of subjects were vitamin D deficient with plasma 25(OH)D levels < 20 ng/mL and 40% were vitamin D insufficient, defined as plasma 25(OH)D levels 20-30 ng/mL (both similar between groups). Baseline 25(OH)D in BALF did not differ between treatment groups (**Table 1**). Plasma concentrations of 25(OH)D significantly increased from baseline in both vitamin D<sub>3</sub> treatment groups at study day 7 (to 45±20 ng/mL in the 250,000 IU and 55±14 ng/mL in the 500,000 IU vitamin D<sub>3</sub> treatment groups, respectively; each p< 0.001) (**Figure 2**). The mean change from baseline to day 7 in 25(OH)D levels within groups over time was 0.4±7 ng/mL in the placebo group versus 26±13 ng/mL and 33±16 ng/mL in the 250,000 IU and 500,000 IU vitamin D<sub>3</sub> treatment groups, respectively, (p<0.001).

### ***Measurements of LL-37***

Plasma LL-37 levels on day 7 for placebo, 250,000 IU and 500,000 IU were 62.04 ng/mL (IQR 48.56,103.02), 63.16 ng/mL (IQR 47.26,72.14) and 112.3 ng/mL (IQR

63.88,229.22),  $p=0.42$  respectively. There was no statistically significant change in plasma levels of LL-37 from baseline by group over time (days 7 and 14 after entry) and no correlation with plasma 25(OH)D levels in plasma (**Table 2**). There was no significant correlation between plasma 25(OH)D, BALF 25(OH)D, plasma LL-37 and BALF LL-37, except for a negative correlation between plasma 25(OH)D concentrations and BALF 25(OH)D concentrations across all groups over time Spearman  $\rho = -0.59$ , ( $p=0.001$ ) (**Table 3**).

### ***Clinical Outcomes***

There was a difference in hospital LOS between groups; placebo  $36\pm 19$  days compared to 250,000 IU group  $25\pm 14$  days compared to 500,000 IU group  $18\pm 11$  days, ( $p=0.03$ ) with no statistical differences in ICU LOS or duration of mechanical ventilation (**Table 4**). Changes in daily SOFA score, frequency of hospital-acquired infections and hospital mortality were similar between groups (**Table 4**).

### ***Safety and Adverse Events***

Mean serum calcium, creatinine and phosphorus concentrations determined during each subject's hospitalization stay were similar between groups at all time points. There was no significant rise in calcium, creatinine and phosphorus per patient during the entire study period (**Appendix 1**). There was no difference in adverse events between groups. Two subjects were re-hospitalized within 30 days of drug discontinuation and those events were deemed to not be related to study drug.

### **Discussion**

In critically ill patients with respiratory failure, this randomized, controlled, double blind, pilot study demonstrated that either of two high-dose vitamin D<sub>3</sub> treatment regimens, given orally in divided doses over a 5-day period, safely increased plasma 25(OH)D to >30 ng/mL by day 7, which was sustainable at least through day 28. These pilot data suggest that high-dose vitamin D<sub>3</sub> supplementation favorably influenced hospital LOS, complementing the recent findings of Quraishi *et al*, that serum 25(OH)D levels were inversely associated with hospital LOS in surgical ICU patients.[6] From a safety perspective, high-dose vitamin D supplementation was well tolerated and without significant adverse events in these critically ill and mechanically ventilated patients.

This pilot study adds to the currently limited literature of high dose vitamin D therapy in critically ill patients, particularly by focusing on critically ill and mechanically ventilated patients without other major comorbidities, as an important test population within the larger population of patients with respiratory failure. In comparison, the VITdAL-ICU clinical trial administered 540,000 IU of vitamin D<sub>3</sub> as a one-time enteral dose followed by a monthly oral dose of 90,000 IU over 5 months to mixed medical and surgical ICU patients.[30] Clinical outcomes were unaffected except for the subgroup of patients with the lowest 25(OH)D levels (<12 ng/mL) in which hospital mortality was significantly decreased.[30] Our study differed from this previous important study in that VITdAL-ICU targeted hospitalized subjects who had documented 25(OH)D levels less than 20 ng/mL, and only 65% of the VITdAL-ICU patients were mechanically ventilated, in contrast to our study which focused on mechanically ventilated patients without vitamin D status eligibility requirements. Another similar study is that of Quraishi *et al*, who administered 200,000 IU or 400,000 IU of vitamin D<sub>3</sub> in critically ill patients with

sepsis and also found no correlation between 25(OH)D and LL-37.[31] In addition, baseline plasma 25(OH)D were similar in both studies, although Quraishi's post-treatment plasma 25(OH)D and LL-37 concentrations were lower than in the current study.

Despite a rapidly growing body of scientific literature, the mechanisms underlying the potential benefit of high-dose vitamin D therapy remain uncertain. Several epidemiologic studies have shown an association between vitamin D deficiency and increased risk of respiratory infections [32, 33] as well as a direct relationship between vitamin D and decreased lung function in patients with chronic obstructive pulmonary disease and interstitial lung disease.[34-37] Therapeutically, administration of 25(OH)D was associated with an increase in lung function for adult patients with asthma [38] and with improved one year survival in patients with cystic fibrosis. [39] Given that vitamin D may uniquely impact lung function, it is possible that therapeutic benefits of high dose vitamin D<sub>3</sub> may be particularly evident in critically ill patients with respiratory failure, but this would need to be tested in a rigorous clinical trial.

Limitations of this randomized clinical trial were the small sample size, imbalances in chronic conditions between treatment groups at study entry and the relatively low rate of infection in the cohort. In addition, out of 658 patients screened we enrolled only 31 subjects and caution the generalizability of our results. Also, we did not analyze steroid use and may be an unknown confounding variable and limitation to this study and future studies will need to monitor corticosteroid dosing and duration as a potential confounder. Our study is the first in critically ill patients to administer high-dose vitamin D in divided doses over a period of several days instead of a one-time dose, to

ensure adequate medication effect to effectively test our hypotheses, as critically ill patients often have gastrointestinal intolerance and variable drug absorption. The randomized nature of the study design, with control for disease severity in the randomization scheme, strengthens the results, although we interpret the clinically important improvements in hospital LOS with caution due to the risk of type I error in a pilot study. Our results regarding LL-37 differ from Nair *et al* [40], who found an increase in serum 25(OH)D concentrations associated with increased serum LL-37 on day 1 and 3 in critically ill patients treated with cholecalciferol, raising the possibility that LL-37 regulation is time dependent within the first few days of vitamin D therapy and was not reflected in our measurements at day 7. [40]

Currently, clinical practice guidelines do not exist regarding therapeutic dosing of vitamin D in critically ill patients. The growing body of literature suggests potentially important clinical benefits, such as for hospital LOS and survival, particularly for those critically ill patients with the lowest serum 25(OH)D and with infections or respiratory failure. High-dose vitamin D may have multifactorial effects that could contribute directly or indirectly to hospital LOS, including salutary effects, via improved 25(OH)D levels on respiratory or other skeletal muscle function, by modulation of the pro-inflammatory milieu, and by regulation of immune functions, among other contributors.[41] Therefore, there may be additional musculoskeletal effects that are unknown at this time, such as positive sub-acute effects that facilitated musculoskeletal recovery and thus expedited hospital discharge. Additional studies are needed to define the putative mechanisms underlying such benefits, and to clearly determine the clinical outcome benefits of high-dose vitamin D<sub>3</sub> therapy.

**Figure 1. CONSORT diagram of study subject enrollment, allocation and follow-up.**

**Figure 2. Plasma 25-hydroxyvitamin D concentrations by group over time.** At study entry the mean plasma 25(OH)D was similar across all groups. By day 7, mean plasma 25(OH)D concentrations significantly increased in the vitamin D treatment groups compared to placebo, with sustained effects up to day 28. There was no statistical difference in the mean plasma 25(OH)D concentration between the 250,000 IU vitamin D<sub>3</sub> group and the 500,000 IU vitamin D<sub>3</sub> group.

**Abbreviations:**

1,25-dihydroxyvitamin D= 1,25(OH)<sub>2</sub>D<sub>3</sub>= calcitriol

25 (OH)D=25-hydroxyvitamin D

AMP= Antimicrobial peptides

APACHE II= Acute Physiology and Chronic Health Evaluation II

BALF= Bronchial alveolar lavage fluid

HAI=Hospital acquired infection

IU=International Units

LOS= length of stay

MAP= Mean arterial pressure

SOFA= Sequential organ failure assessment

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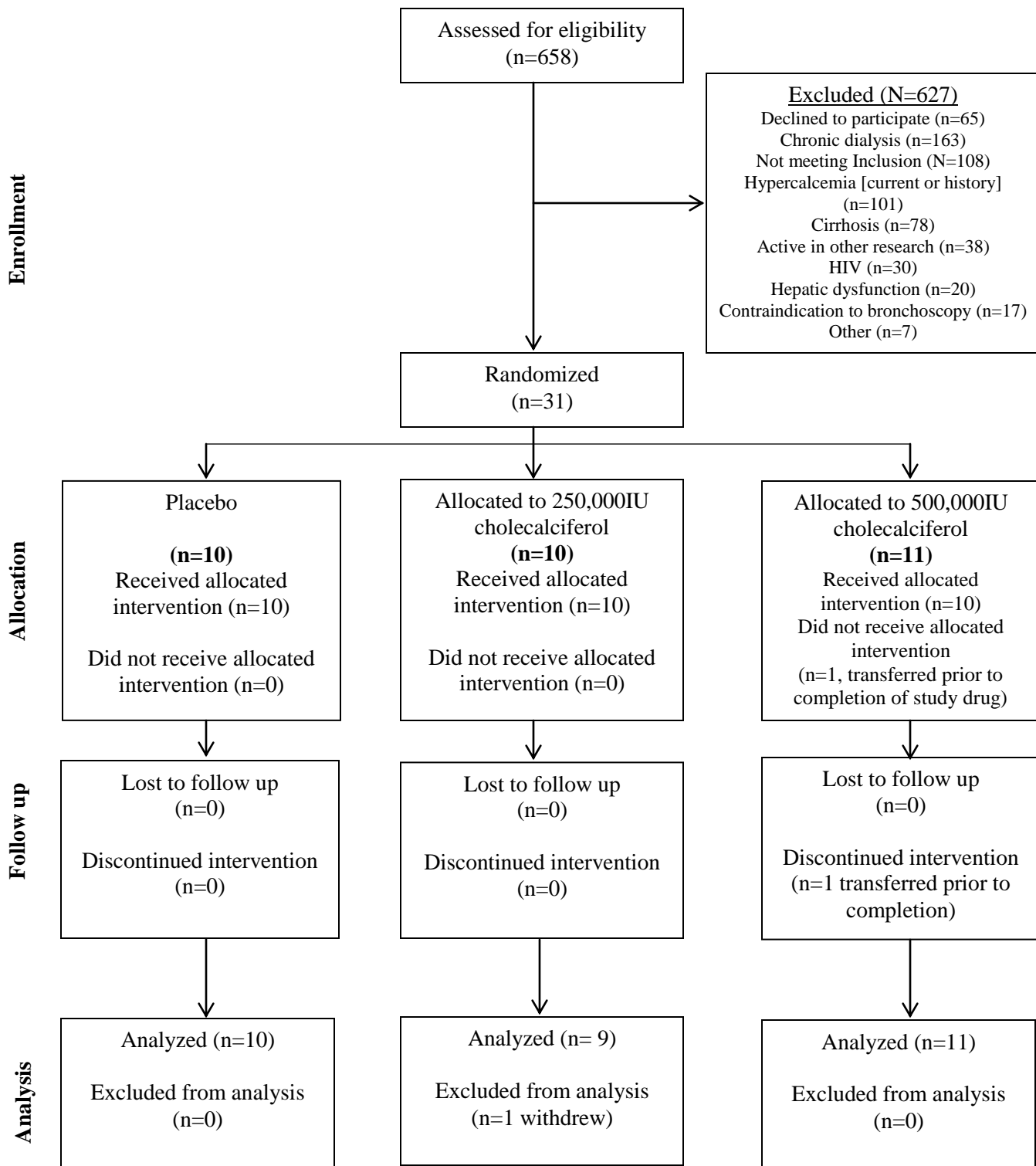
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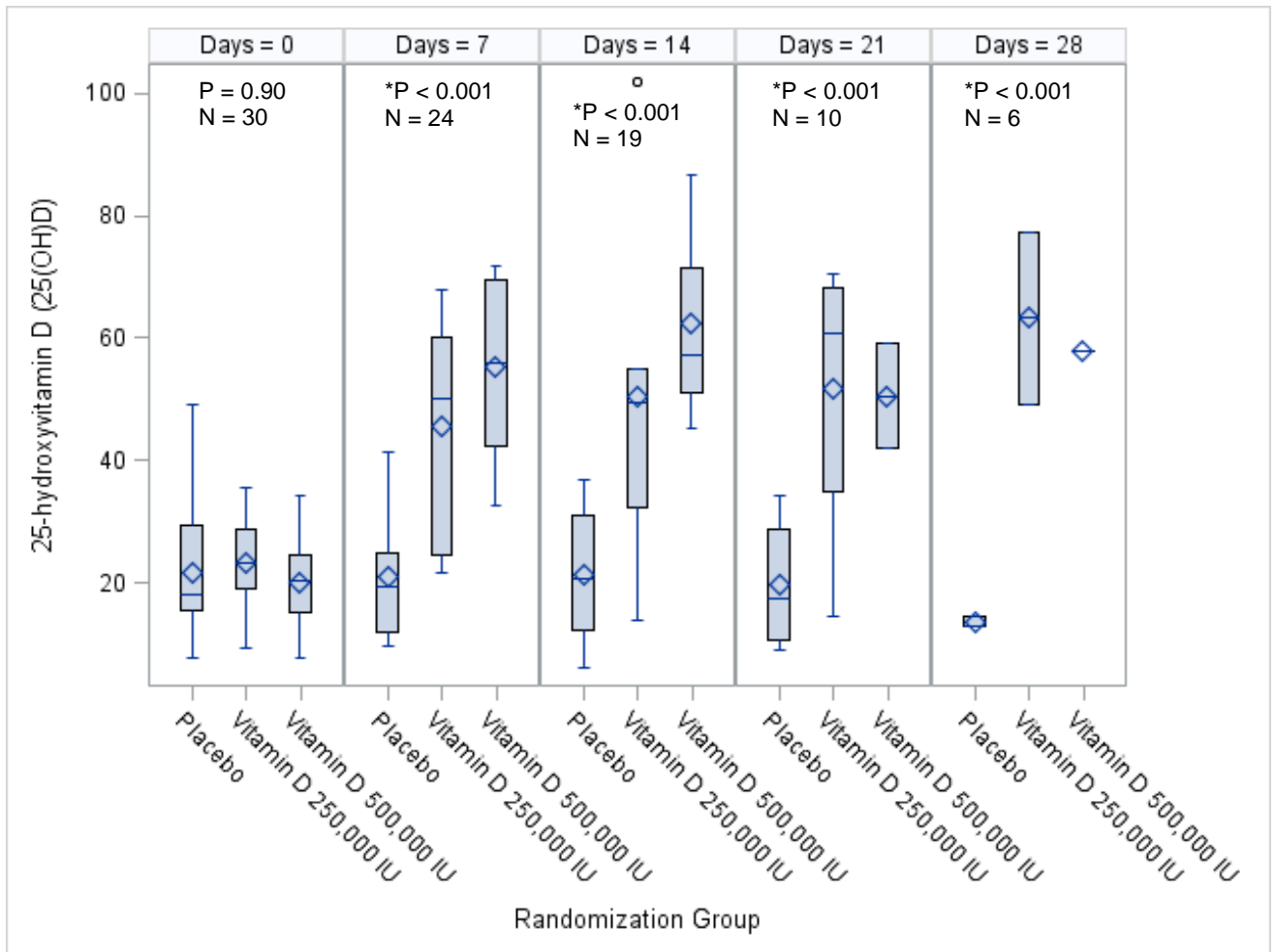
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Figure 1. CONSORT Diagram





\* P values of <0.05 are considered statistically significant

Table 1. Baseline demographic characteristics

Variables	Placebo	Vitamin D <sub>3</sub> 250,000 IU	Vitamin D <sub>3</sub> 500,000 IU	P-Value
	N=10	N=9	N=11	
Female N (%)	4 (40.0)	4 (44.4)	3 (27.3)	0.72
Race				
African American	4 (40.0)	7 (77.8)	3 (27.3)	0.09
Caucasian	5 (50.0)	2 (22.2)	8 (72.7)	
American Indian/Alaskan	1 (10.0)	0 (0)	0 (0)	
Age, mean (SD)	64.8 (17.5)	56.4 (15.4)	68.1 (18.6)	0.22
BMI, mean (SD)	28.2 (9.9)	33.4 (6.3)	30.2 (6.1)	0.62
APACHE II, mean (SD)	23.2 (8.8)	20.0 (10.1)	19.0 (7.5)	0.55
SOFA Day 0, mean (SD)	8.6 (4.3)	8.9 (3.6)	9.1 (3.1)	0.47
Infection on Admission	6 (60.0)	4 (44.4)	3 (27.3)	0.38
Admission ICU				
Surgical	3 (30.0)	5 (55.6)	8 (72.7)	0.16
Medical	7 (70.0)	4 (44.4)	3 (27.3)	
Baseline 25(OH)D (ng/mL), mean (SD)	21.5 (12.2)	23.2 (7.8)	20.0 (7.3)	0.75
Baseline 25(OH)D Category				
Deficient (<20ng/mL)	5 (50.0)	3 (33.3)	5 (45.5)	0.87
Insufficient (20-30ng/mL)	3 (30.0)	4 (44.4)	5 (45.5)	
Sufficient (>30 ng/mL)	2 (20.0)	2 (22.2)	1 (9.1)	
Baseline 25(OH)D BALF, mean (SD)	14.6(9)	13(7)	10.4(2)	0.63¥
Baseline plasma LL-37ng/mL, median (25%-75%IQR)	58.4 (37.2,97.3)	45.5 (41.2,76.6)	57.9 (37.1,284.2)	0.42
Baseline LL-37 BALF (ng/mL) mean (SD)	1(1.0)	4.9	0.4(0.4)	0.17£
Coronary Artery Disease	1 (10.0)	2 (22.0)	7 (64.0)	0.03*
Congestive Heart Failure	1 (10.0)	2 (22.0)	5 (45.5)	0.20
Diabetes	4 (40.0)	1 (11.0)	2 (18.0)	0.32
COPD	2 (20.0)	1 (11.0)	4 (36.0)	0.49
Asthma	1 (10.0)	1 (11.0)	0 (0)	0.52

25(OH)D = 25 hydroxyvitamin D, BALF=Bronchial Alveolar Lavage Fluid, IQR=interquartile range,  
COPD=chronic obstructive pulmonary disease

\*p<0.05 statistically significant

¥, placebo N=7; 250,000 N=5, 500,000 N=7

£, placebo N=4; 250,000 N=1, 500,000 N=1

Table 2. Change of Plasma LL-37 Overtime by Groups

	Placebo (ng/mL)	Vitamin D <sub>3</sub> 250,000 IU (ng/mL)	Vitamin D <sub>3</sub> 500,000 IU (ng/mL)	P value
Total N of Observations	N = 9	N = 7	N = 9	
Change of Plasma LL-37 day 7 compared to baseline, median (IQR)	-3.8 (-18.9, 26.9)	6.0 (-13.4, 39.2)	-13.5 (-48.4, 37.4)	0.42
Total N of Observations	N = 8	N = 6	N = 5	
Change of Plasma LL-37 day 14 compared to baseline, median (IQR)	1.1 (-24.6, 10.8)	-12.3 (-36.1, 8.1)	-6.0 (-27.3, 2.0)	0.29

Table 3. Spearman Correlation of 25-hydroxyvitamin D between Plasma and Bronchoalveolar Lavage Fluid by Treatment Group and All Subjects

	Placebo	Vitamin D <sub>3</sub> 250,000 IU	Vitamin D <sub>3</sub> 500,000 IU	All
Spearman Coefficient	-0.26	-0.87	-0.76	-0.59
P value	0.47	0.05	<b>0.03*</b>	<b>&lt;0.001*</b>

\* P values of <0.05 are considered statistically significant



Table 4. Clinical outcomes

	Placebo	Vitamin D <sub>3</sub> 250,000 IU	Vitamin D <sub>3</sub> 500,000 IU	P- value Group by Day
Hospital length of stay days, mean (SD)	36(19)	25(14)	18(11)	<b>0.03*</b>
ICU length of stay days, mean (SD)	23(14)	17(14)	15(10)	0.30
Ventilator days, mean (SD)	20(15)	12(10)	14(10)	0.29
Change in SOFA score from baseline, mean (SD)	-2(3)	-3(3)	-2(3)	0.72
Hospital acquired infection, N (%)	3(30)	3(33)	2(18)	0.77
Hospital mortality, N (%)	1 (10)	0	1(10)	0.76
Day 84 mortality, N (%)	2(20)	1(11)	4(36)	0.33

N=30, \*p<0.05, p-value interaction between group and time, length of stay log transformed

## Highlights (for review)

- First double blind RCT of vitamin D therapy in mechanically ventilated patients
- Treatment with placebo, 250000IU or 500000IU enteral vitamin D3 was well tolerated
- Significant increase in plasma 25(OH)D from baseline to day 7
- Significant decrease in hospital length of stay for vitamin D3 treated subjects
- No change in plasma LL-37 according to treatment group

Appendix 1. Average Blood Calcium, Creatinine, and Phosphorus for all patients by group

	Group			P-Value
	Placebo	Vitamin D3 250,000 IU	Vitamin D3 500,000 IU	
Mean Calcium mg/dL mean (SD)	8.33 (0.75)	8.40 (0.52)	8.41 (0.63)	0.95
Mean Creatinine mg/dL mean (SD)	2.15 (1.41)	2.21 (3.13)	1.51 (0.83)	0.67
Mean Phosphorus mg/dL mean (SD)	3.26 (1.40)	4.22 (1.80)	4.03 (0.88)	0.28