# High-Dose Vitamin D₃ Administration Is Associated With Increases in Hemoglobin Concentrations in Mechanically Ventilated Critically Ill Adults: A Pilot Double-Blind, Randomized, Placebo-Controlled Trial

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## Abstract

*Background:* Anemia and vitamin D deficiency are highly prevalent in critical illness, and vitamin D status has been associated with hemoglobin concentrations in epidemiologic studies. We examined the effect of high-dose vitamin D therapy on hemoglobin and hepcidin concentrations in critically ill adults. *Materials and Methods:* Mechanically ventilated critically ill adults (N = 30) enrolled in a pilot double-blind, randomized, placebo-controlled trial of high-dose vitamin D₃ (D₃) were included in this analysis. Participants were randomized to receive placebo, 50,000 IU D₃, or 100,000 IU D₃ daily for 5 days (totaling 250,000 IU D₃ and 500,000 IU D₃, respectively). Blood was drawn weekly throughout hospitalization for up to 4 weeks. Linear mixed-effects models were used to assess change in hemoglobin and hepcidin concentrations by treatment group over time. *Results:* At enrollment, >75% of participants in all groups had plasma 25-hydroxyvitamin D (25(OH)D) concentrations <30 ng/mL, and >85% of participants across groups were anemic. In the 500,000-IU D₃ group, hemoglobin concentrations increased significantly over time ($P_{group \times time}$ = .01) compared with placebo but did not change in the 250,000-IU D₃ group ($P_{group \times time}$ = 0.59). Hepcidin concentrations decreased acutely in the 500,000-IU D₃ group relative to placebo after 1 week ($P$ = .007). Hepcidin did not change significantly in the 250,000-IU D₃ group. *Conclusion:* In these critically ill adults, treatment with 500,000 IU D₃ was associated with increased hemoglobin concentrations over time and acutely reduced serum hepcidin concentrations. These findings suggest that high-dose vitamin D may improve iron metabolism in critical illness and should be confirmed in larger studies. (*JPEN J Parenter Enteral Nutr.* 2018;42:87–94)

## Clinical Relevancy Statement

Anemia is highly prevalent in critical illness and associated with adverse patient outcomes. Vitamin D deficiency, also common in critical illness, has been identified as a risk factor for anemia. Our finding that hemoglobin concentrations increased following treatment with high-dose vitamin D is clinically relevant for clinicians and researchers contemplating therapeutic options for anemia in critically ill adults. Pending confirmation in larger studies, vitamin D repletion may have implications as a safe alternative or adjunct therapy to traditional therapies for anemia in critical illness, such as transfusions, which may carry risks for the patient.

## Introduction

Anemia is highly prevalent in critical illness. Nearly two-thirds of adults have anemia on admission to the intensive care unit (ICU), and an even greater proportion develops anemia within the first week of admission.[1,2] The presence of anemia is associated with increased risk of mortality, cardiovascular morbidity, and decreased oxygen-carrying capacity, potentially prolonging the requirement for mechanical ventilation.[3] This may be particularly concerning in critically ill patients with preexisting cardiopulmonary disease. Traditional therapies for anemia in critical illness include blood transfusions, erythropoiesis-stimulating agents, and iron repletion, but these are not without risks, and there is controversy regarding their efficacy in improving patient survival.[4-7] In light of the high burden of anemia and associated adverse outcomes, investigation into safe and efficacious alternative or complementary therapies to improve hemoglobin concentrations in critically ill adults is warranted.

Anemia in critical illness may develop due to repeated blood sampling, hemorrhage, renal disease, inflammation, and nutrient deficiencies, including iron, folate, and vitamin B₁₂.[3] Recently, vitamin D deficiency has also been

identified as a risk factor for anemia, particularly anemia of inflammation.[8,9] Where iron deficiency anemia occurs due to absolute iron deficiency—depletion of iron stores, most often measured via ferritin concentrations in the blood, and reduced circulating iron—anemia of inflammation is characterized by reduced circulating iron in the context of normal or elevated ferritin concentrations due to sequestration of iron within cells of the reticuloendothelial system.[10] In critical illness, inflammation may contribute to anemia due to elevations in proinflammatory cytokines and hepcidin, the major iron-regulatory hormone.[11] Increases in cytokines and hepcidin result in shortened red blood cell life span, decreased iron absorption, and iron sequestration within macrophages, limiting the amount of iron in circulation to support erythropoiesis and hemoglobin synthesis.[12] Vitamin D has been shown to reduce proinflammatory cytokines and suppress hepcidin transcription, thereby potentially improving iron egress from cells and increasing the amount of iron in circulation to support erythropoiesis. Furthermore, the hepcidin antimicrobial peptide gene (*HAMP*) has been found to contain a vitamin D response element, thus lending biological plausibility to the observed association between vitamin D deficiency and anemia.[13-15]

We have previously reported a high prevalence of vitamin D insufficiency and anemia in the ICUs at our centers,[16,17] and the VITdAL-ICU study found that serum hemoglobin concentrations were higher in the vitamin D–treated group than the placebo group 28 days after intervention.[18] However, few studies, particularly in the ICU population, have explored the therapeutic effect of vitamin D with hemoglobin as the primary outcome of interest. Therefore, we aimed to (1) examine the impact of high-dose vitamin $D_3$ ($D_3$) supplementation on hemoglobin concentrations in critically ill adults and (2) evaluate the effect of vitamin D on serum hepcidin concentrations to better understand the role of vitamin D in iron metabolism in this population. We hypothesized that treatment with high-dose vitamin $D_3$ would increase hemoglobin concentrations and reduce hepcidin concentrations in this population of critically ill adults.

## Methods

### Study Design and Participants

The study population for this analysis was derived from participants enrolled in a pilot (N = 30) double-blind, randomized, placebo-controlled trial of high-dose vitamin $D_3$ regimens in mechanically ventilated critically ill adults (NCT01372995).[19] The parent study was designed to test the efficacy of high-dose vitamin $D_3$ regimens in improving plasma 25-hydroxyvitamin D (25(OH)D) concentrations to levels ≥30 ng/mL. Briefly, upon enrollment, participants were stratified on Acute Physiology and Chronic Health Evaluation II (APACHE II) score (≤15 or >15) and randomized to receive placebo, a total enteral dose of 250,000 IU (6250 μg) of cholecalciferol ($D_3$), or a total enteral dose of 500,000 IU (12,500 μg) $D_3$. Participants were not included/excluded from the study on the basis of their 25(OH)D status at enrollment. The study drug was administered in 5 equal doses over 5 days (ie, the 500,000-IU group received a dose of 100,000 IU daily for 5 days after enrollment). Pills were dissolved in sterile water and administered through an enteral feeding tube. The cholecalciferol was manufactured from Tischon Corp (Westbury, NY) and BioTech Pharmacal (Fayetteville, AR). Inclusion criteria were age ≥18 years; receiving care in ICUs at Emory University Hospital, Emory University Hospital Midtown, or Grady Memorial Hospital; anticipated mechanical ventilation of ≥72 hours after study enrollment; anticipated survival and ICU stay of ≥96 hours; and enteral access and ability to tolerate enteral study drug administration. Potential participants were excluded due to pregnancy, shock, hypercalcemia, receipt of high-dose vitamin D therapy in the preceding 6 months, chronic renal dysfunction requiring

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dialysis, AIDS, cirrhosis, and receipt of any investigational drug in the 60 days prior to study entry. This study was approved by the Emory University Institutional Review Board, and written informed consent was obtained from the patient or legally authorized representative prior to enrollment.

## Data Collection

Data on demographics, medical history, and admitting diagnosis were collected on study entry.[19] Blood was drawn at study enrollment and weekly throughout the hospitalization for up to 4 weeks for assessment of plasma 25(OH)D and serum hepcidin concentrations. Plasma 25(OH)D concentrations were measured using an automated chemiluminescent technique (IDS-iSYS automated machine; Immunodiagnostic Systems, Fountain Hills, AZ) in a laboratory that participates in the Vitamin D External Quality Assessment Scheme (DEQAS, site 606) and the National Institute of Standards and Technology/National Institutes of Health (NIH) Vitamin D Metabolites Quality Assurance Program to ensure the accuracy of 25(OH)D measurements. Hepcidin concentrations were measured using an electrochemiluminescence immunoassay (Eli Lilly and Company, Indianapolis, IN) as previously described.[14,15,20,21] Hemoglobin measurements within 24 hours of the 25(OH)D measurement were abstracted from the electronic medical record, starting when the patient was enrolled in the study. Hemoglobin concentrations were determined via automated cell counting using standard hospital methods. Anemia was defined based on World Health Organization criteria as hemoglobin concentrations <13 g/dL in men and <12 g/dL in nonpregnant women.[22] Data on receipt and volume of red blood cell transfusion were extracted from participant electronic medical records. The transfusion trigger used at our sites is typically a hemoglobin concentration of 7 g/dL.

## Statistical Analysis

Descriptive statistics were performed for all variables and presented as mean ± standard deviation (SD) for normally distributed continuous variables, median (interquartile range [IQR]) for nonnormally distributed continuous variables, or percentages for categorical variables. Comparisons of characteristics between treatment groups at enrollment were performed using 1-way analysis of variance (ANOVA) for normally distributed continuous variables, Kruskal-Wallis tests for nonnormally distributed continuous variables, and Fisher's exact tests for categorical variables. Nonnormally distributed variables (hemoglobin and hepcidin) were transformed to the natural logarithmic scale for subsequent analyses. Such variables were then back-transformed so as to be expressed in their original unit of

measurement as geometric means (95% confidence interval [CI]).

Analysis of repeated measures was performed using linear mixed-effects models to ascertain mean differences in hemoglobin and hepcidin concentrations by time, treatment group, and time-by-treatment group interaction. Any differences between groups in outcomes over time are reflected in the time-by-treatment group term. $\beta$-Coefficients from linear mixed-effects models with log-transformed outcome variables (hemoglobin and hepcidin) were back-transformed and expressed as geometric mean ratios (GMRs) with their corresponding 95% CI. A GMR of 1 indicates no treatment effect. A 1-way ANOVA with Tukey's multiple testing correction was used to determine differences in treatment groups at specific time points, and paired $t$ tests were used to examine within-group changes in outcomes.

Sensitivity analyses were performed in which potentially confounding variables (age, sex, race, hemoglobin concentration at enrollment, hepcidin concentration at enrollment, transfusion volume, and admission ICU) were added to the model one at a time to determine the influence of each of these variables on the hemoglobin and hepcidin outcomes. Additional sensitivity analyses were performed with the linear mixed-effects models in which models were restricted to those with 25(OH)D concentrations <30 ng/mL at enrollment to determine if enrollment 25(OH)D status affected our outcomes. All analyses were 2-sided with an $\alpha$ of 0.05 and performed using SAS version 9.4 (SAS Institute, Cary, NC).

## Results

Demographic, biochemical, and health status characteristics at study enrollment were similar across treatment groups (Table 1). The median time from ICU admission to study enrollment was 4 days (interquartile range [IQR], 5) and did not differ between groups ($P = .53$). The study population was largely male, overweight, or obese, and approximately half were African American. Most participants had 25(OH)D concentrations <30 ng/mL at study enrollment. Nearly all of the participants were anemic at study enrollment, and the mean APACHE II score indicated a high degree of illness severity among the study population. Several participants had underlying comorbidities at enrollment, including diabetes and heart disease. In the 500,000-IU $D_3$ group, there was a higher proportion of a prior diagnosis of coronary artery disease compared with the other 2 groups. The groups did not differ in the proportion of participants who received blood transfusions or in the total volume of blood received throughout the course of the study.

As previously reported,[19] plasma 25(OH)D concentrations increased significantly after 1 week in the groups that

**Table 1.** Characteristics by Treatment Group at Time of Study Enrollment.[a]

| Characteristic | Placebo (n = 10) | 250,000 IU D₃ (n = 9) | 500,000 IU D₃ (n = 11) | P Value[b] |
|---|---|---|---|---|
| Age, mean ± SD, y | 64.8 ± 17.5 | 56.4 ± 15.4 | 68.1 ± 18.6 | .33 |
| Men | 6 (60.0) | 5 (55.6) | 8 (72.7) | .72 |
| Race | | | | .09 |
| African American | 4 (40.0) | 7 (77.8) | 3 (27.3) | |
| White | 5 (50.0) | 2 (22.2) | 8 (72.7) | |
| American Indian/Alaskan | 1 (10.0) | 0 (0.0) | 0 (0.0) | |
| BMI,[c] mean ± SD, kg/m² | 28.2 ± 9.9 | 33.4 ± 6.3 | 30.2 ± 6.1 | .36 |
| Plasma 25(OH)D, mean ± SD, ng/mL | 21.5 ± 12.2 | 23.2 ± 7.8 | 20.0 ± 7.3 | .75 |
| 25(OH)D <20 ng/mL | 5 (50.0) | 3 (33.3) | 5 (45.5) | .81 |
| 25(OH)D <30 ng/mL | 8 (80.0) | 7 (77.8) | 10 (90.9) | .70 |
| Hemoglobin, median (IQR), g/dL | 8.3 (2.0) | 9.4 (3.2) | 9.5 (1.8) | .22 |
| Anemia[d] | 10 (100.0) | 8 (88.9) | 10 (90.9) | .75 |
| Hepcidin, median (IQR), ng/mL | 82.1 (135.7) | 36.5 (67.7) | 15.7 (25.1) | .09 |
| APACHE II score, mean ± SD | 23.2 ± 8.8 | 20 ± 10.1 | 19.0 ± 7.5 | .53 |
| Admission ICU | | | | .16 |
| Medical | 7 (70.0) | 4 (44.4) | 3 (27.3) | |
| Surgical | 3 (30.0) | 5 (55.6) | 8 (72.7) | |
| Infection on admission | 6 (60.0) | 4 (44.4) | 3 (27.3) | .38 |
| Coronary artery disease | 1 (10) | 2 (22.2) | 7 (63.6) | .03 |
| Congestive heart failure | 1 (10) | 2 (22.2) | 5 (45.5) | .20 |
| COPD | 2 (20) | 1 (11.1) | 4 (36.4) | .49 |
| Asthma | 1 (10) | 1 (11.1) | 0 (0) | .52 |
| Diabetes | 4 (40.0) | 1 (11.1) | 2 (18.2) | .32 |
| Received blood transfusion[e] | 4 (40.0) | 2 (22.2) | 3 (27.3) | .78 |
| Transfusion volume,[f] mean ± SD, mL | 775 ± 505.8 | 700 ± 495.0 | 916.7 ± 700.6 | .91 |

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; COPD, chronic obstructive pulmonary disease; D₃, vitamin D₃; ICU, intensive care unit; IQR, interquartile range; IU, international unit; 25(OH)D, 25-hydroxyvitamin D.
[a]Values are presented as number (%) unless otherwise indicated.
[b]Analysis of variance for normally distributed continuous variables, Kruskal-Wallis test for nonnormally distributed continuous variables, Fisher's exact test for categorical variables.
[c]n = 8 for 250,000-IU D₃ group.
[d]Hemoglobin <13 g/dL for men, <12 g/dL for women.
[e]Receipt of any blood transfusion during the course of the study.
[f]Total volume of packed red blood cells received during the course of the study, among those who received a transfusion.

received 250,000 IU D₃ and 500,000 IU D₃, (to 45 ± 20 ng/mL and 55 ± 14 ng/mL, respectively) compared with no change in the placebo group ($P < .001$). These effects were sustained through week 4.

Hemoglobin concentrations increased significantly over time in the group that received 500,000 IU D₃ (Figure 1). Compared with the placebo group, those who received 500,000 IU D₃ demonstrated a significant 8% increase in hemoglobin concentrations per week (GMR, 1.08; 95% CI, 1.02–1.15; $P_{\text{group} \times \text{time}} = .01$). Hemoglobin concentrations in the 250,000-IU D₃ group did not change significantly over time relative to placebo (GMR, 0.99; 95% CI, 0.94–1.04; $P = .59$). By week 3, hemoglobin concentrations were significantly higher in the 500,000-IU D₃ group compared with placebo (11.30 g/dL [95% CI, 9.34–13.68] vs 8.19 g/dL [95% CI, 7.26–9.24]; $P = .03$). The prevalence of anemia remained high throughout the course of the study and did not differ significantly between groups at any time point ($P = 1.00$ at week 1 and $P = .72$ at

week 2; all remaining participants were anemic at weeks 3 and 4).

Hepcidin concentrations were only obtained for up to 3 weeks (Figure 2). During that period, there was not a statistically significant group-by-time interaction with hepcidin concentrations in either vitamin D–treated group relative to the placebo group ($P_{\text{group} \times \text{time}}$ [500,000 IU D₃ vs placebo] = .44; $P_{\text{group} \times \text{time}}$ [250,000 IU D₃ vs placebo] = .60). However, by 1 week, hepcidin concentrations did differ significantly between the 500,000-IU D₃ and placebo groups ($P = .007$). The 250,000-IU D₃ group did not differ from either the 500,000-IU D₃ group ($P = .22$) or the placebo group ($P = .38$) at 1 week. Within-group analyses showed that hepcidin concentrations decreased acutely by 66% after 1 week in the 500,000-IU D₃ group relative to study enrollment (GMR, 0.34; 95% CI, 0.20–0.59; $P = .002$). No significant changes were observed within the 250,000-IU D₃ or placebo groups from enrollment to 1 week ($P = .08$ and $P = .48$, respectively).
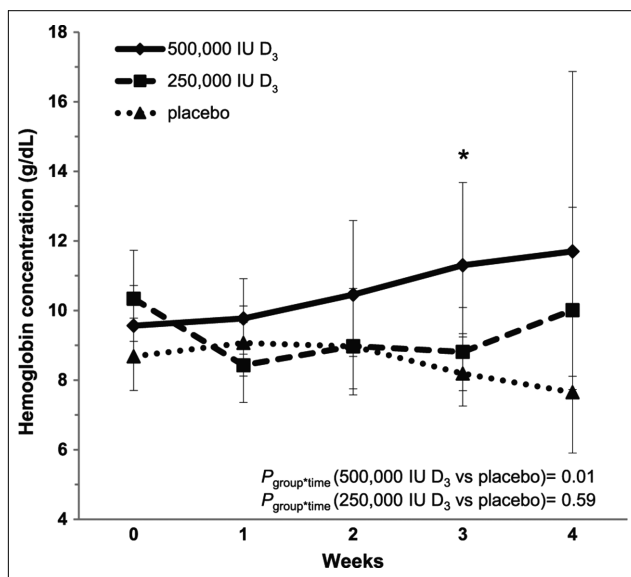
**Figure 1.** Geometric mean hemoglobin concentrations with corresponding 95% confidence intervals in critically ill adults. Hemoglobin concentrations are reported across time and by treatment group. Hemoglobin concentrations increased significantly over time in the group that received 500,000 IU $D_3$ compared with the placebo group; there was no significant change in the 250,000-IU $D_3$ group. By 3 weeks, hemoglobin concentrations in the 500,000-IU $D_3$ group differed significantly from the placebo group; there were no statistically significant differences between groups at other time points. *$P$ < .05; group*time, group-by-time interaction. Sample sizes in the placebo, 250,000-IU $D_3$, and 500,000-IU $D_3$ groups, respectively: enrollment, n = 10, 9, 11; 1 week, n = 9, 9, 9; 2 weeks, n = 8, 6, 5; 3 weeks, n = 5, 4, 2; 4 weeks, n = 2, 2, 1.
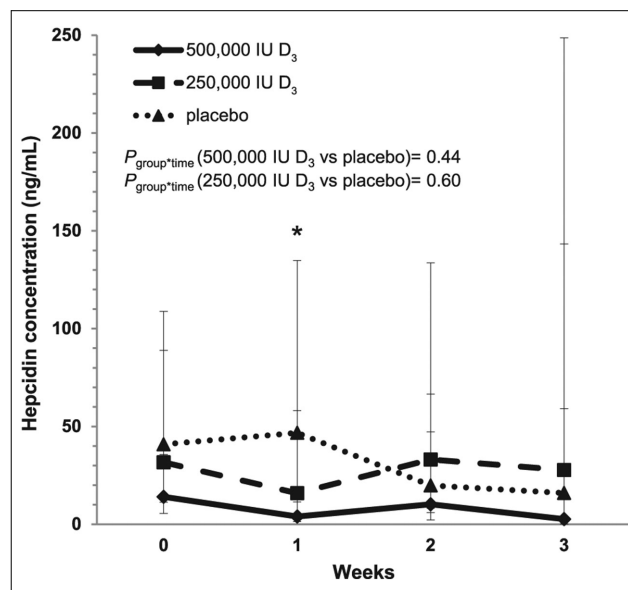


**Figure 2.** Geometric mean hepcidin concentrations with corresponding 95% confidence intervals in critically ill adults. Hepcidin concentrations are reported across time and by treatment group. There were no significant differences over time in hepcidin concentrations in either vitamin D–treated group relative to placebo. Hepcidin concentrations in the 500,000-IU $D_3$ group differed significantly from the placebo group 1 week after dosing; there were no statistically significant differences between groups at other time points. *$P$ < .05; group*time, group-by-time interaction. Sample sizes in the placebo, 250,000-IU $D_3$, and 500,000-IU $D_3$ groups, respectively: enrollment, n = 10, 9, 11; 1 week, n = 9, 6, 9; 2 weeks, n = 8, 6, 5; 3 weeks, n = 4, 4, 2.

## Sensitivity Analyses

Controlling separately for potentially confounding variables age, sex, race, hemoglobin concentration at enrollment, hepcidin concentration at enrollment, transfusion volume, and admission ICU did not affect the results of the linear mixed-effects models. The group-by-time interaction for hemoglobin remained significant in the 500,000-IU $D_3$ group ($P$ < .03 for all) and nonsignificant in the 250,000-IU $D_3$ group ($P$ > .45 for all). The group-by-time interaction for hepcidin remained nonsignificant for both the 500,000-IU $D_3$ ($P$ > .35 for all) and 250,000-IU $D_3$ ($P$ > .45 for all) groups relative to placebo with the addition of each control variable.

When linear mixed-effects models were restricted to those with 25(OH)D <30 ng/mL at the time of study enrollment, hemoglobin and hepcidin results were largely unchanged. Compared with the placebo group, those who received 500,000 IU $D_3$ demonstrated a significant 10% increase in hemoglobin concentrations per week (GMR, 1.10; 95% CI, 1.04–1.18; $P_{group \times time}$ = .003). Hemoglobin

concentrations in the 250,000-IU $D_3$ group remained unchanged relative to placebo (GMR, 0.99; 95% CI, 0.93–1.05; $P$ = .67). There was not a statistically significant group-by-time interaction with hepcidin concentrations in either vitamin D–treated group relative to the placebo group ($P_{group \times time}$ [500,000 IU $D_3$ vs placebo] = .34; $P_{group \times time}$ [250,000 IU $D_3$ vs placebo] = .47).

## Discussion

In this population of mechanically ventilated critically ill adults, we found that treatment with a total enteral dose of 500,000 IU $D_3$ was associated with a significant increase in hemoglobin concentrations over time. Although not sustained, there was an acute decrease in hepcidin concentrations by 1 week following treatment with 500,000 IU $D_3$ relative to the placebo group. No significant changes in hemoglobin or hepcidin concentrations were observed in the 250,000-IU $D_3$ group compared with the placebo group, suggesting a potential dose-related effect of vitamin $D_3$ on these outcomes.

Our findings are consistent with studies of patients with chronic kidney disease (CKD) in which treatment with vitamin D or its analogues increased hemoglobin concentrations.[23,24] However, studies in other patient populations have been mixed, but this is likely due to differences in the dose and form of vitamin D administered, as well as the prevalence of vitamin D deficiency and type of anemia in these studies.[25-27] Indeed, Sooragonda et al[26] found that among generally healthy adults with iron-deficiency anemia, treatment with 600,000 IU of cholecalciferol did not result in further increases in hemoglobin concentrations following correction of iron deficiency. This would suggest that the effects of vitamin D are likely specific to anemia of inflammation, which is consistent with the proposed mechanism of action outlined below.

The acute reduction in hepcidin that we observed is consistent with another study from our group in which healthy volunteers who received high-dose vitamin $D_3$ experienced a significant reduction in hepcidin concentrations after 1 week compared with the placebo group.[15] Given the physiology of iron recycling, the acute reduction in hepcidin observed in the 500,000-IU $D_3$ group likely potentiated the increase in hemoglobin, even though the change in hepcidin was not sustained over time.[28] During anemia of inflammation, which is a component of anemia in critical illness,[11] hepcidin is elevated, blocking iron egress from cells and sequestering iron within cells of the reticuloendothelial system.[12] This process limits the iron in circulation that can be used to support erythropoiesis and hemoglobin synthesis. Reductions in hepcidin may restore iron recycling by allowing iron to exit cells and be used in hemoglobin synthesis and erythropoiesis. Thus, increases in hemoglobin concentrations are likely to be preceded by reductions in hepcidin in the context of anemia of inflammation. Our findings of an acute reduction in hepcidin concentration with an increase over time in hemoglobin concentrations in the 500,000-IU $D_3$ group are in line with this process. Further, the gene coding for hepcidin has been demonstrated to contain a vitamin D response element,[13] lending strong biological plausibility to our findings. Other potential mechanisms underlying our findings may involve a role for vitamin D in supporting erythropoiesis through induction of erythroid progenitor cell proliferation,[29-31] but this was not evaluated in the present study.

Unexpectedly, we did not see significant changes in either hemoglobin or hepcidin concentrations in response to 250,000 IU $D_3$, despite significant increases in 25(OH)D concentrations in this group. It is possible that there may be differences in characteristics of the individuals randomized to these 2 groups that were not measured in this study or differences in unknown factors related to response to high-dose vitamin $D_3$. Another possibility is that a threshold level of 25(OH)D may need to be reached to provide adequate substrate for the local production of the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25[OH]$_2$D) by the macrophage to regulate hepcidin concentrations. While the groups were generally balanced at study enrollment, they differed in the prevalence of coronary artery disease, with the 500,000-IU $D_3$ group having a higher proportion with this condition. It is possible that this group was more responsive to vitamin D therapy in terms of hemoglobin and hepcidin concentrations. Indeed, in observational studies in patients scheduled for cardiac surgery and coronary angiography, higher 25(OH)D concentrations were associated with higher hemoglobin concentrations and protective against anemia.[32,33] Another potential explanation may be that even though 25(OH)D concentrations increased with both dosing regimens, it is possible that there was a differential between free 25(OH)D in these groups. Recent evidence has indicated that free 25(OH)D may be a better marker of vitamin D bioactivity than total 25(OH)D concentrations.[34] Thus, if the higher dose of vitamin D resulted in greater concentrations of free 25(OH)D, this may explain the observed changes in hemoglobin and hepcidin.

Our findings have potential clinical implications for vitamin D repletion as a safe and efficacious adjunct therapy for anemia in critical illness. Even though anemia was not completely resolved in our study population, hemoglobin concentrations did improve to a clinically meaningful extent in the group that received 500,000 IU $D_3$. The improvement in hemoglobin may reduce the frequency and necessity of blood transfusions. Transfusions are widely used in the ICU, but there is controversy regarding their use and threshold for initiation, as studies have found transfusions to be associated with increased length of hospital stay, hospital costs, and mortality.[1,2,35,36] Studies indicate that the hemoglobin threshold for initiation of transfusions typically falls between 7 and 9 g/dL and may vary by patient characteristics.[1,37,38] If vitamin D can improve hemoglobin concentrations to a level above which transfusions are typically initiated, as was demonstrated in our study, this may have positive effects on patient outcomes.

Strengths of our study included the rigorous clinical trial design, novel research question, and the well-characterized study population. However, there were important limitations. First, this trial was not specifically designed and powered with hemoglobin as the primary outcome, and the sample size of this pilot trial was relatively small. Nonetheless, the significant increase in hemoglobin that was observed, despite the small sample size, suggests that this was a fairly robust finding. We were also limited in that the randomization was unbalanced with respect to coronary artery disease. With only one person in the placebo group having the disease, we were unable to adjust for this variable in our analysis. We therefore cannot rule out the effect of this potential confounder on our outcomes, and our findings related to the 500,000-IU $D_3$ group may lack generalizability to populations with a different prevalence of underlying

comorbidities. Another potential limitation of this study was that the median time from ICU admission to study enrollment was 4 days. Therefore, the plasma 25(OH)D, serum hepcidin, and hemoglobin concentrations obtained at study enrollment may not reflect the participants' true baseline levels as concentrations of these biochemical markers may decrease after ICU admission due to hemodilution or other unknown factors. Finally, we were unable to fully evaluate the effect of other anemia treatments that may have been given as part of the patients' medical care. We did, however, find that the number of patients who received transfusions during their hospitalization did not differ by treatment group, nor did the volume of packed red blood cells received, suggesting that the increase in hemoglobin in the 500,000-IU $D_3$ group was unlikely due to differences in receipt of transfusions in this group.

In conclusion, we found that treatment with 500,000 IU of vitamin $D_3$ was associated with a significant increase in hemoglobin concentrations over time and an acute reduction in hepcidin concentrations in critically ill adults. Larger clinical trials of high-dose vitamin $D_3$ in critically ill adults with hemoglobin as the primary outcome are warranted to confirm these findings and further elucidate the therapeutic effect of vitamin D on anemia in critical illness.

## Statement of Authorship

E. M. Smith, G. S. Martin, T. R. Ziegler, and V. Tangpricha contributed to the conception and design of the work; E. M. Smith, J. L. Jones, J. E. Han, J. A. Alvarez, S. M. Zughaier, J. H. Sloan, and R. J. Konrad contributed to the acquisition and analysis of the data; E. M. Smith drafted the manuscript; and all authors contributed to the interpretation of the data and critically revised the manuscript. All authors read and approved the final manuscript and agree to be accountable for ensuring the accuracy and integrity of all aspects of the work.

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