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Effect of Cholecalciferol Supplementation on Vitamin D Status and Cathelicidin Levels in Sepsis: A Randomized, Placebo-Controlled Trial

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Objectives: To compare changes in vitamin D status and cathelicidin (LL-37) levels in septic ICU patients treated with placebo versus cholecalciferol.

Design: Randomized, placebo-controlled, trial.

Setting: Medical and surgical ICUs of a single teaching hospital in Boston, MA.

Patients: Thirty adult ICU patients.

Interventions: Placebo ($n = 10$) versus 200,000 IU cholecalciferol ($n = 10$) versus 400,000 IU cholecalciferol ($n = 10$), within 24 hours of new-onset severe sepsis or septic shock.

Measurements and Main Results: Blood samples were obtained at baseline (day 1) and on days 3, 5, and 7, to assess total 25-hydroxyvitamin D, as well as vitamin D-binding protein and

albumin to calculate bioavailable 25-hydroxyvitamin D. Plasma LL-37 and high-sensitivity C-reactive protein levels were also measured. At baseline, median (interquartile range) plasma 25-hydroxyvitamin D was 17 ng/mL (13–22 ng/mL) and peaked by day 5 in both intervention groups. Groups were compared using Kruskal-Wallis tests. Relative to baseline, on day 5, median change in biomarkers for placebo, 200,000 IU cholecalciferol, and 400,000 IU cholecalciferol groups, respectively, were as follows: 1) total 25-hydroxyvitamin D, 3% (–3% to 8%), 49% (30–82%), and 69% (55–106%) ($p < 0.001$); 2) bioavailable 25-hydroxyvitamin D, 4% (–8% to 7%), 45% (40–70%), and 96% (58–136%) ($p < 0.01$); and 3) LL-37: –17% (–9% to –23%), 4% (–10% to 14%), and 30% (23–48%) ($p = 0.04$). Change in high-sensitivity C-reactive protein levels did not differ between groups. A positive correlation was observed between bioavailable 25-hydroxyvitamin D and LL-37 (Spearman $\rho = 0.44$; $p = 0.03$) but not for total 25-hydroxyvitamin D and LL-37.

Conclusions: High-dose cholecalciferol supplementation rapidly and safely improves 25-hydroxyvitamin D and bioavailable 25-hydroxyvitamin D levels in patients with severe sepsis or septic shock. Changes in bioavailable 25-hydroxyvitamin D are associated with concomitant increases in circulating LL-37 levels. Larger trials are needed to verify these findings and to assess whether optimizing vitamin D status improves sepsis-related clinical outcomes. (*Crit Care Med* 2015; XX:00–00)

Key Words: 25-hydroxyvitamin; cathelicidin; cholecalciferol; intensive care unit; LL-37; vitamin D

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the last two decades (4–7), in general, the mortality associated with each of these is estimated to be 16%, 20%, 46%, and more than 80%, respectively (8–10). The annual incidence of sepsis exceeds 1.6 million cases in the United States alone and results in costs exceeding \$24 billion every year (10, 11).

Current theories about the onset and progression of sepsis focus on dysregulation of inflammatory responses, including the possibility that a massive and uncontrolled release of proinflammatory mediators initiates a chain of events that lead to widespread tissue injury (12). The degree of immune dysfunction is thought to correlate with the severity of sepsis (13). Recently, cells of the innate and adaptive immune system were shown to express the vitamin D receptor (VDR) (14). In contrast to its inhibitory role in adaptive immunity, vitamin D is a potent activator of the innate immune system (15) and may be integral for natural defense mechanisms against microbial invasion (16).

In humans, circulating 25-hydroxyvitamin D (25OHD) is the most abundant vitamin D metabolite (17). As such, it is often used as a proxy for total body vitamin D status (18). Growing evidence suggests that a significant proportion (50–90%) of critically ill patients have low 25OHD levels during ICU admission (19–23). Suboptimal 25OHD levels, in turn, appear to be associated with a higher risk of mortality in critically ill patients (21–23). Although it is known that patients with sepsis typically have low 25OHD levels (24, 25) and that vitamin D status is inversely associated with the severity of sepsis (26, 27), little is known regarding the effects of vitamin D supplementation in this patient cohort. Therefore, our primary goal was to determine whether high-dose cholecalciferol (vitamin D₃) supplementation in patients with severe sepsis or septic shock can 1) rapidly influence vitamin D status and 2) affect systemic expression of cathelicidin (LL-37), a vitamin D–dependent, endogenous, antimicrobial peptide (28). Our secondary goal was to determine whether high-dose cholecalciferol supplementation modifies systemic cytokine levels in patients with severe sepsis or septic shock.

MATERIALS AND METHODS

Study Cohort

Patients were recruited from a single medical ICU and two surgical ICUs at the Massachusetts General Hospital (MGH) in Boston, MA. These three, 18-bed ICUs receive admissions from all medical and surgical services, except for Cardiology and Cardiac Surgery. MGH is a large teaching hospital and a level-1 trauma center, which serves a diverse population in eastern Massachusetts. The Partners Human Research Committee (Institutional Review Board) approved the study protocol, and the trial was registered on ClinicalTrials.gov (NCT01896544). All subjects were enrolled between January 1, 2014, and March 1, 2014.

Inclusion and Exclusion Criteria

All adult men and women, at least 18 years old, admitted to the medical or surgical ICUs at MGH, and within 24 hours of new-onset sepsis, were potentially eligible to participate.

During the initial screen, for patients transferred from within the hospital (but not the emergency department) or another healthcare facility, we performed a detailed review of the electronic medical records to determine an approximate time of when the patients met sepsis criteria according to the 2012 Surviving Sepsis Campaign (29). For patients who presented from home and were admitted to the ICU through the emergency department, we reviewed the initial documentation on arrival to MGH, and for those who met criteria for sepsis, we considered time of arrival to the emergency department as the time of new-onset sepsis. At the time of enrollment, only those patients who met criteria for severe sepsis or septic shock (according to the consensus definition) (29) were approached for study consideration. Informed consent was obtained from subjects if they were oriented to person, place, and time in addition to testing negative on the Confusion Assessment Method for the ICU test (30); surrogate consent was obtained when subjects did not meet these criteria. Subjects were only included in the study if the baseline blood sample and subsequent cholecalciferol supplementation could be completed within 24 hours of new-onset sepsis.

Exclusion criteria included a known history of renal stones, diagnosis of hypercalcemia within the past year, baseline serum total calcium more than 10 mg/dL, established diagnosis associated with increased risk of hypercalcemia (e.g., metastatic cancer, sarcoidosis, multiple myeloma, and primary hyperparathyroidism), and current vitamin D supplementation. Due to the need for repeated blood samples, we also excluded patients with a known history of anemia at the time of ICU admission (defined as hematocrit < 25%) and any pregnant or immediately postpartum women. We also excluded any patients with high gastrointestinal output requiring continuous decompression as cholecalciferol administration through the nasogastric/orogastric tube would not be possible. Finally, patients were also excluded if they did not have a suitable healthcare proxy (when they were not able to provide consent), if they had a high likelihood of dying within the first 48 hours of ICU admission (as determined by the treating ICU team), if they had transitioned to a “comfort care only” status, or if they refused to participate.

Blood Sample Processing and Biomarker Assays

Following informed consent, fresh blood from an indwelling arterial or central venous catheter was collected directly into an EDTA-containing tube (this was designated as day 1 of the study). The sample was immediately stored on ice and then centrifuged within 30 minutes to separate out plasma. All samples were centrifuged at 2,300 rpm for 15 minutes at a temperature of 4°C. The separated plasma was immediately transferred to polypropylene tubes and stored at –80°C until biomarker testing was ready to be initiated. Follow-up blood samples were collected and processed in a similar manner on days 3, 5, and 7.

Assays were performed at the Harvard Medical School Clinical and Translational Science Award core laboratory at MGH. 25OHD (combined D₂ and D₃) and vitamin D–binding protein (DBP) were measured by enzyme-linked immunosorbent assay (ELISA), using commercially available kits

(Abbott Laboratories, Abbott Park, IL, and Hycult Biotech, Plymouth Meeting, PA, respectively). Intra- and interassay coefficients of variation (CV) were both less than 8% for 25OHD and less than 12% for DBP, respectively. LL-37 was also measured by ELISA, using a commercially available kit (Hycult Biotech), with intra- and interassay CVs of less than 10% for both, respectively. The MGH core laboratory is a Clinical Laboratory Improvement Amendments–certified facility, which uses rigorous methods and continuously updated reference standards for the assessment of albumin and high-sensitivity C-reactive protein (hsCRP). Bioavailable 25OHD was calculated using previously described formulas using total 25OHD, DBP, and albumin levels (31). Plasma cytokine levels of interleukin (IL)-1 β , IL-4, IL-6, IL-10, tumor necrosis factor (TNF)- α , and interferon (INF)- γ were measured using a commercially available kit (Cytokine Human Magnetic 10-Plex Panel; Life Technologies, Grand Island, NY) using a Luminex FLEXMAP 3D (Luminex, Austin, TX) multiplex assay analyzer.

Vitamin D Supplementation

Cholecalciferol and matched placebo were both supplied in a clear liquid form by a commercial vendor (Ddrops Company, Toronto, ON, Canada) directly to the MGH Research Pharmacy. The Research Pharmacy was responsible for establishing the study code using a computer-generated block randomization algorithm, preparing syringes containing the study drug (each syringe contained 7 mL of liquid), and dispensing the coded syringes directly to a member of the research team after verifying subject enrollment in the study. Once dispensed by the research pharmacy, a member of the research team witnessed the administration of the study drug as a single bolus to all enrolled subjects. The study drug was administered either orally (when clinically appropriate) or via an indwelling nasogastric/orogastric tube by a registered nurse responsible for the care of each patient. The standard MGH protocol involves flushing all gastric tubes with 20 mL of saline before and after drug administration. Suctioning through the gastric tube was held for at least 1 hour after study drug administration.

Clinical Data Collection

Partners Healthcare electronic medical records system was used to obtain baseline information related to age, sex, race, body mass index (BMI), Acute Physiology and Chronic Health Evaluation (APACHE) II score, and type of patient (medical vs surgical ICU). Microbiology results were also abstracted from the electronic medical records system. Total body fluid balance (TBB) from admission to the time of study-related blood sample acquisition was calculated from the bedside ICU flow sheet for each subject. The 30-day mortality rate for the analytic cohort was confirmed by reviewing individual medical records and cross-referencing each case using the Social Security Death Index Master File up to 90 days following hospital discharge.

Statistical Analysis

Descriptive statistics were tabulated in aggregate for the groups who received placebo versus 200,000 IU cholecalciferol versus 400,000 IU cholecalciferol. Continuous data were reported as

medians with interquartile ranges (IQRs) and compared using Kruskal-Wallis tests. Categorical values were expressed as proportions and compared using chi-square tests. Length-of-stay (LOS) data were compared using Log-rank tests. We performed Spearman correlation testing to assess the relationship between the change in 25OHD and bioavailable 25OHD with change in LL-37 levels.

The study was powered to investigate our primary goal. Based on previous studies, we assumed that the mean 25OHD level in severely septic or septic shock patients at MGH would be 17 ng/mL (31). We also assumed that by day 7, 25OHD levels in the placebo group would decrease by 2 ng/mL, while a single dose of 400,000 IU cholecalciferol would raise levels by 15 ng/mL (32). Assuming a common SD of 10 ng/mL and $\alpha = 0.05$, a sample size of six patients in each group (placebo vs intervention) would allow us to detect this difference with a power of 80%. Similarly, based on preliminary data, we assumed that the baseline LL-37 levels in ICU patients would be 50 ng/mL. We also assumed that by day 7, LL-37 levels in the placebo group would decrease by 10 ng/mL, while supplementation with 400,000 IU cholecalciferol would raise levels to 100 ng/mL. Assuming a common SD of 40 ng/mL and $\alpha = 0.05$, a sample size of seven patients in each group (placebo vs intervention) would allow us to detect this difference with a power of 80%. To compensate for potential study withdrawal, drug intolerance, or death before completing the study, we enrolled 10 patients in each arm. All analyses were performed using STATA 12.0 (StataCorp LP, College Station, TX). A two-tailed *p* value of less than 0.05 was considered to statistically significant.

RESULTS

A total of 87 patients were screened for study inclusion. Fifty-one patients did not meet criteria for severe sepsis or septic shock according to the 2012 Surviving Sepsis Campaign guidelines (29) and were therefore excluded. Furthermore, four patients were expected to die within 48 hours of ICU admission (transitioned to “comfort care only” measures), while two patients died within 24 hours of new-onset sepsis and therefore could not be approached for study participation. This left 30 patients in the analytic cohort. Their baseline characteristics and clinically relevant outcomes are shown in **Table 1**.

The median age of study participants was 64 years (IQR, 58–68 yr) and median baseline 25OHD level was 17 ng/mL (IQR, 13–22 ng/mL). Overall, subjects were predominantly men (60%) and white (93%). There was no difference between groups at baseline in terms of age, sex (ratio of women vs men), BMI, APACHE II score, type of patient (medical vs surgical ICU), and site(s) of positive microbial culture.

Peak change in 25OHD levels was achieved by day 5 in both intervention groups (**Fig. 1**). To account for variations in the individual responses to acute stress and to cholecalciferol supplementation, changes in 25OHD, bioavailable 25OHD, LL-37, hsCRP, and cytokines were expressed as a percentage of baseline and compared between days 1 and 5 (**Figs. 2–4**). No adverse events related to cholecalciferol supplementation were observed in either intervention group.

TABLE 1. Demographic Factors, Baseline Clinical Information, and Major Clinical Outcomes Among 30 Patients With Severe Sepsis or Septic Shock, According to Randomly Assigned Treatment Group

Variable	Treatment Group			p
	Placebo (n = 10)	200,000 IU (n = 10)	400,000 IU (n = 10)	
Age (yr)	65 (58–70)	64 (55–66)	62 (59–67)	0.91
Sex (%)				1.00
Female	40	40	40	
Male	60	60	60	
Body mass index (kg/m ²)	28 (26–35)	28 (27–33)	30 (28–37)	0.40
Acute Physiology and Chronic Health Evaluation II	22 (16–28)	21 (19–32)	23 (16–25)	0.62
ICU type (%)				0.26
Medical	60	50	50	
Surgical	40	50	50	
Positive culture (%) ^a				0.74
Pulmonary	40	50	40	
Bloodstream	60	60	70	
Urinary	10	10	10	
Day 1: 25OHD (ng/mL)	19 (13–22)	15 (12–20)	17 (13–25)	0.76
Day 5: 25OHD (ng/mL)	19 (11–23)	22 (16–25)	29 (23–41)	0.04
Day 1: B25OHD (ng/mL)	1.4 (0.8–2.1)	1.9 (1.2–2.5)	2.5 (1.7–2.9)	0.16
Day 5: B25OHD (ng/mL)	1.6 (1.2–2.1)	1.8 (1.6–3.7)	4.0 (3.2–4.5)	0.04
Day 1: LL-37 (ng/mL)	53 (43–62)	52 (48–59)	51 (41–52)	0.52
Day 5: LL-37 (ng/mL)	50 (35–54)	54 (40–64)	67 (59–68)	0.02
Day 1: hsCRP (mg/L)	106 (60–267)	208 (90–359)	119 (85–247)	0.62
Day 5: hsCRP (mg/L)	53 (38–72)	98 (12–130)	31 (26–160)	0.99
Day 1: TBB (mL)	1,592 (1,086–2,117)	1,730 (925–3,075)	1,518 (696–2,088)	0.55
Day 5: TBB (mL)	2,996 (1,980–4,081)	3,241 (2,228–6,586)	3,060 (1,355–3,803)	0.63
ICU LOS (d)	12 (7–15)	4 (3–11)	3 (2–11)	0.83
Hospital LOS (d)	21 (18–31)	13 (12–16)	14 (8–21)	0.03
30-d readmission (%)				< 0.001
No	80	100	100	
Yes	20	–	–	
30-d mortality (%)				0.18
Dead	30	30	20	
Alive	70	70	80	

25OHD = 25-hydroxyvitamin D, B25OHD = bioavailable 25-hydroxyvitamin D, LL-37 = cathelicidin, hsCRP = high-sensitivity C-reactive protein, TBB = total body fluid balance, LOS = length of stay.

^aTotals do not equal 100% since some patients had more than one location where a positive culture was obtained.

Data presented as either median (interquartile range) or proportions and compared using Kruskal-Wallis tests or chi-square tests, respectively. Length-of-stay data are compared using log-rank tests for trend. Boldface values represent statistically significant results. Dashes denote there are no patients in the respective category.

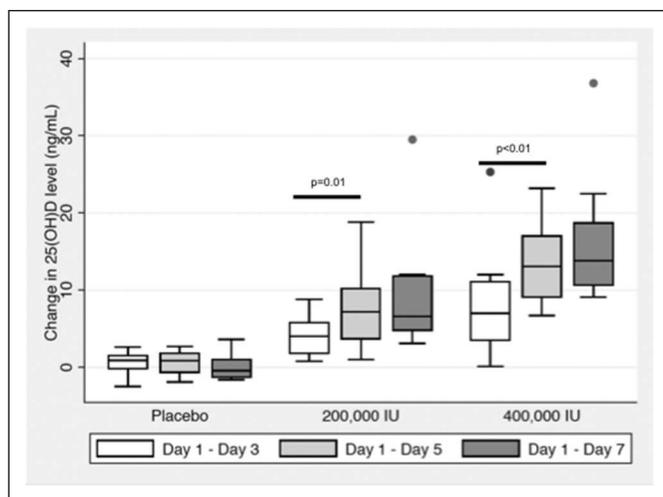


Figure 1. Median (with interquartile ranges) change in 25-hydroxyvitamin D (25(OH)D) levels on days 3, 5, and 7 relative to baseline (day 1): Peak plasma levels are achieved by day 5 in both the 200,000 IU cholecalciferol and 400,000 IU cholecalciferol groups.

Between days 1 and 5, median change in plasma total 25OHD level was 3% (IQR, -3% to 8%) for the placebo group, 49% (IQR, 30–82%) for the 200,000 IU cholecalciferol group, and 69% (IQR, 55–106%) for the 400,000 IU cholecalciferol group ($p < 0.001$). The calculated median change in bioavailable 25OHD level between days 1 and 5 was 4% (IQR, -8% to 7%), 45% (IQR, 40–70%), and 96% (IQR, 58–136%), respectively ($p < 0.01$). Relative to baseline, on day 5 median change in plasma LL-37 level was -17% (IQR, -9% to -23%), 4% (IQR, -10% to 14%), and 30% (IQR, 23–48%), respectively ($p = 0.04$). Change in hsCRP levels did not differ between groups: -39% (IQR, -36% to 64%), -58% (IQR, -40% to -77%), and -41% (IQR, -8% to -74%), respectively ($p = 0.59$). Correlation testing demonstrated a significant relationship between the change in bioavailable 25OHD and change in LL-37 levels (Spearman $\rho = 0.44$; $p = 0.03$) but not between the change in total 25OHD and change in LL-37 levels (Spearman $\rho = 0.23$; $p = 0.23$).

Between days 1 and 5, median change in plasma IL-1 β was 4% (IQR, -36% to 16%) for the placebo group, -32% (IQR, -66% to 5%) for the 200,000 IU cholecalciferol group, and -53% (IQR, -36% to -71%) for the 400,000 IU cholecalciferol group ($p = 0.02$). The median change in IL-4 between day 1 and day 5 was -16% (IQR, -40% to 5%), -15% (IQR, -36% to 5%), and -25% (IQR, -9% to -41%), respectively ($p = 0.57$). The median change in IL-6 between day 1 and day 5 was -29% (IQR, -14% to -50%), -48% (IQR, -12% to -78%), and -78% (IQR, -53% to -93%), respectively ($p = 0.02$). The median change in IL-10 between day 1 and day 5 was -3% (IQR, -18% to 3%), 2% (IQR, -7% to 31%), and 16% (IQR, -15% to 42%), respectively ($p = 0.07$). The median change in TNF- α between day 1 and day 5 was 5% (IQR, -38% to 68%), -20% (IQR, -65% to 48%), and -22% (IQR, -46% to 61%), respectively ($p = 0.61$). And, relative to baseline, on day 5, median change in INF- γ was -40% (IQR, -6% to -50%), -22% (IQR, -26% to 2%), and -5% (IQR, -14% to 5%), respectively ($p = 0.09$).

With respect to key clinical outcomes, there was no difference between groups regarding ICU LOS: 12 days (IQR, 7–15 d) for the placebo group, 4 days (IQR, 3–11 d) for the 200,000 IU cholecalciferol group, and 3 days (IQR, 2–11 d) for the 400,000 IU cholecalciferol group (test for trend, $p = 0.83$). However, there was a difference between groups regarding hospital LOS: 21 days (IQR, 18–31 d), 13 days (IQR, 12–16 d), and 14 days (IQR, 8–21 d), respectively (test for trend, $p = 0.03$). Although there was a significant difference in the 30-day ICU readmission rate between groups (both cases were for respiratory failure and sepsis): 20%, 0%, and 0%, respectively ($p < 0.001$), there was no difference between groups regarding 30-day all-cause mortality (30%, 30%, and 20%, respectively; $p = 0.18$). When the two cholecalciferol groups were combined into a single intervention arm and compared with the placebo group, there was no difference in ICU LOS: 12 days (IQR, 7–15 d) for the placebo group versus 3 days (IQR, 3–12 d) for the cholecalciferol group ($p = 0.77$). However, hospital LOS was still significantly longer in the placebo group compared with the cholecalciferol group: 21 days (18–31 d) versus 13 days (10–18 d) ($p = 0.01$), respectively. And although 30-day ICU readmission rate was still higher in the placebo group compared with the cholecalciferol group (20% vs 0%; $p < 0.001$, respectively), there was no difference between them in terms of 30-day all-cause mortality (30% vs 25%; $p = 0.43$, respectively).

DISCUSSION

In this randomized controlled trial of critically ill patients, we compared the effects of placebo versus 200,000 IU cholecalciferol versus 400,000 IU cholecalciferol on vitamin D status and expression of an endogenous, vitamin D-dependent, antimicrobial peptide. We demonstrated that a single bolus dose of 400,000 IU cholecalciferol is safe and effective for rapidly improving circulating 25OHD levels, bioavailable 25OHD, and expression of LL-37 in patients with severe sepsis or septic shock. We also observed that cholecalciferol supplementation can modulate cytokine expression, resulting in a significant reduction in systemic levels of IL-1 β and IL-6. Although the present study was underpowered to reliably detect differences in clinical outcomes, our results provide important mechanistic evidence to explain why rapidly improving vitamin D status in patients with severe sepsis or septic shock may improve clinical outcomes and support the design and implementation of larger, multicenter trials.

The primary results of our trial are consistent with evidence that cells of the innate and adaptive immune system express the VDR (14). Vitamin D is an important link between Toll-like receptor (TLR) activation and antibacterial responses (33). In vitro studies have shown that in low vitamin D states, stimulation of human macrophages by TLR induces a series of important responses, which include 1) increased expression of VDR (34); 2) conversion of 25OHD to its most biologically active form of 1,25-dihydroxyvitamin D (1,25(OH)₂D) (35); and 3) enhanced production of LL-37 (28, 36). Our study builds on these findings and demonstrates in vivo that improving bioavailable 25OHD in humans with overwhelming infections

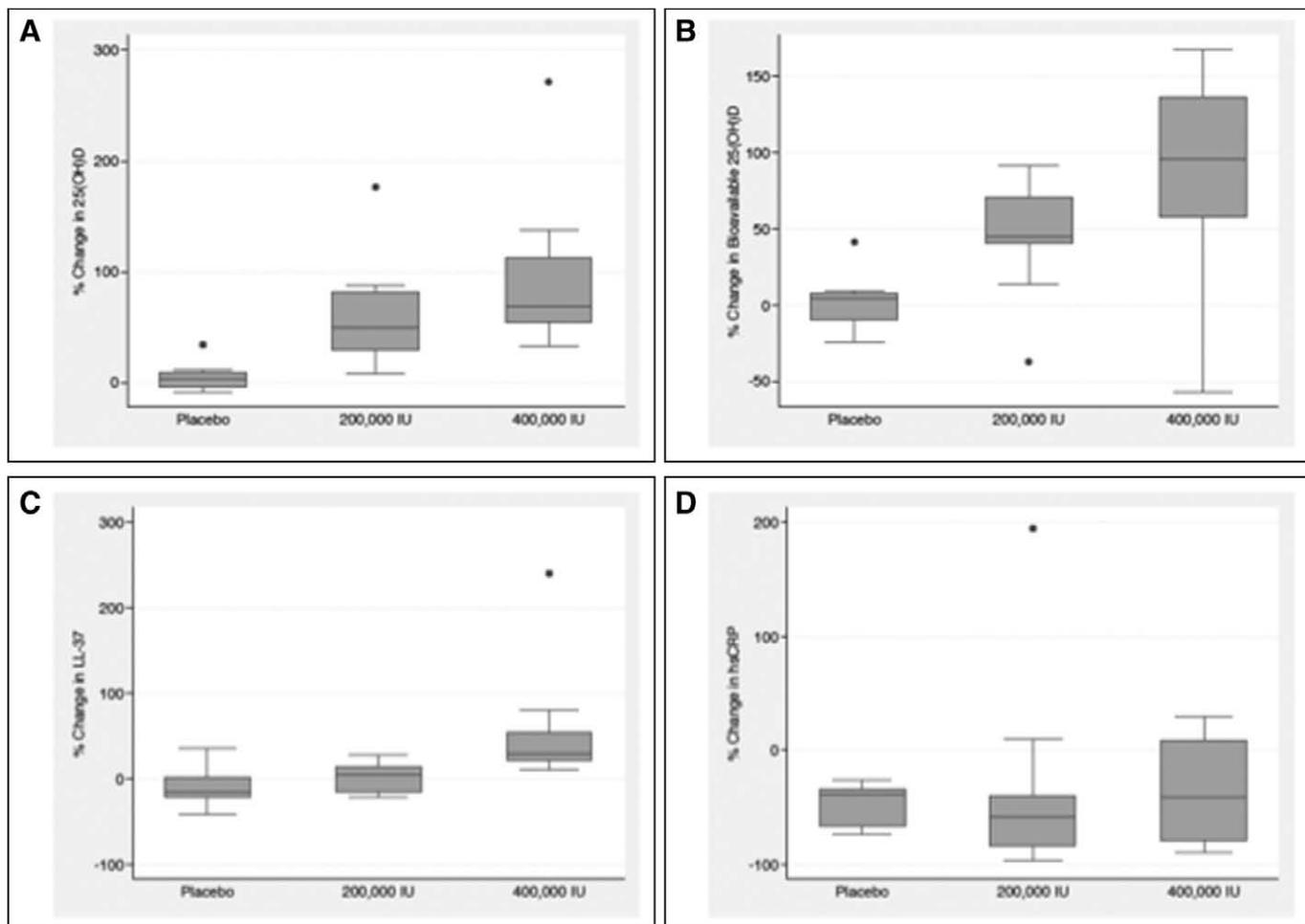


Figure 2. Change in biomarker levels on day 5 expressed as a percentage of baseline levels (day 1). Significant differences in 25-hydroxyvitamin D (25OHD) (**A**), bioavailable 25-hydroxyvitamin D (**B**), and cathelicidin (LL-37) (**C**) levels were observed between groups. No difference was observed in high-sensitivity C-reactive protein (hsCRP) (**D**) levels.

is associated with elevated systemic LL-37 levels. However, to fully appreciate the significance of these findings, it is important to discuss two fundamental concepts: total 25OHD versus bioavailable 25OHD and the role of cathelicidins in innate immune responses.

In humans, cholecalciferol is either obtained through the diet or synthesized by skin upon exposure to ultraviolet B radiation (17). Cholecalciferol is converted to 25OHD in the liver and then to 1,25OH₂D by the kidneys and by cells of the immune system (among others) for paracrine use (37). Circulating 25OHD levels are, however, approximately 500–1,000 times higher than 1,25OH₂D levels, and both 25OHD and 1,25OH₂D are predominantly protein bound in circulation (38). Only 0.03% of 25OHD is free (24), with close to 88% bound to DBP and the remainder to albumin (38). Although free 25OHD is most readily available for conversion into 1,25OH₂D (37), the albumin-bound component of total 25OHD can be easily mobilized in times of need and provides a larger pool of 25OHD to maintain optimal vitamin D-mediated autocrine and paracrine activity (39). By contrast, the 25OHD-DBP complex is very stable, with an affinity of binding several orders of magnitude greater than

that of 25OHD to albumin (37). 25OHD bound to DBP is typically involved in the regulation of gene expression, requiring intracellular enzymatic cleavage of the 25OHD, and thus, it is thought to have limited biological activity during acute stress (39, 40). As such, the free and albumin-bound 25OHD are collectively referred to as “bioavailable” 25OHD, and it has been hypothesized that assessment of the bioavailable forms of vitamin D may have greater predictive value for important ICU-related outcomes when compared with total serum 25OHD (41, 42).

Cathelicidins are a family of antimicrobial and endotoxin-binding proteins largely found in peroxidase-negative granules of mammalian neutrophils (43). The cathelicidins are synthesized as pre-pro-proteins, and after removal of the signal peptide, they are stored in granules as inactive proforms (43). The active biologic domains of the cathelicidins generally reside in the C-terminus (44). The C-terminal antibacterial peptides are activated when cleaved from the proforms of the cathelicidins by serine proteases from azurophilic granules (44). Although most mammals express several types of cathelicidins, hCAP-18 is the only human cathelicidin (45). Although hCAP-18 is a major protein in specific granules of neutrophils (46), it is also

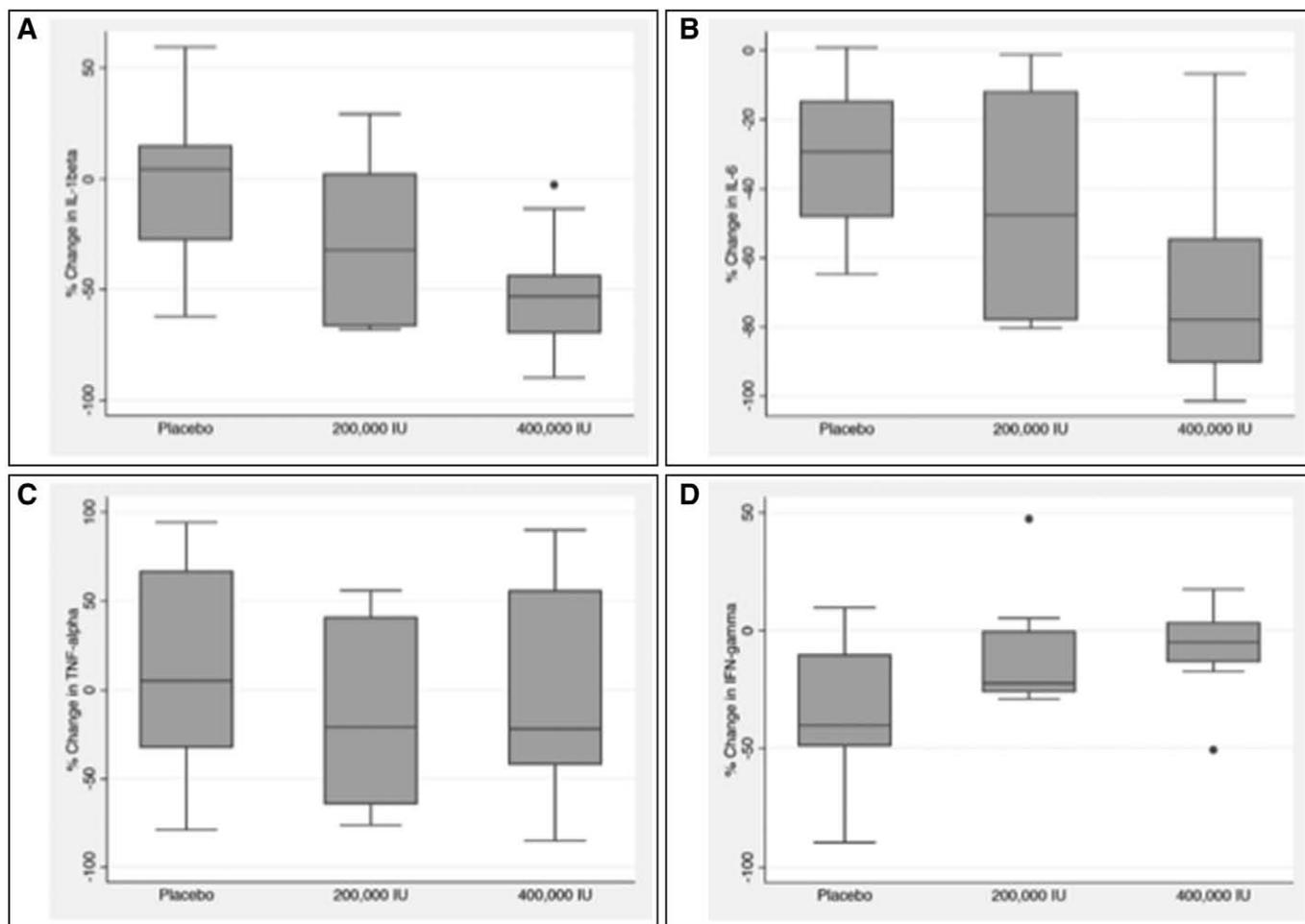


Figure 3. Change in proinflammatory cytokines on day 5 expressed as a percentage of baseline level (day 1). Significant differences in interleukin (IL)-1 β (A) and IL-6 (B) levels were observed between groups. A trend toward significant difference in interferon- γ (D) was also observed between groups. No difference was observed in tumor necrosis factor (TNF)- α (C) levels between groups.

present in subpopulations of lymphocytes as well as monocytes (47) and in squamous epithelia at barrier sites, such as skin, lungs, gut, and the genitourinary tract (48–50). Plasma also contains a significant concentration of hCAP-18 bound to lipoproteins (51). The antibacterial C-terminus of hCAP-18, LL-37, exhibits potent activity against a broad spectrum of infectious agents such as both Gram-positive and Gram-negative bacteria, viruses, fungi, and mycobacteria (52). LL-37 also has synergistic antibacterial effects with the defensins (another major class of endogenous, antimicrobial peptides) (53) and is a chemotactic agent for neutrophils, monocytes, and T cells (54). As such, LL-37 expression by neutrophils and epithelial cells may represent an important first line of defense for the innate immune system (55).

In addition to up-regulation of antimicrobial peptide production, our study demonstrates that high-dose cholecalciferol supplementation results in a significant reduction in IL-1 β and IL-6 but not TNF- α levels. Although TNF- α is often regarded as the “master regulator” of proinflammatory cytokines (56), both IL-1 β and IL-6 play important roles in the early inflammatory response that characterizes sepsis. IL-1 β is a major mediator of inflammation-induced coagulation (57), lipid mediators, and

reactive oxygen and nitrogen species (58). IL-1 β also amplifies the inflammatory cascade in an autocrine and paracrine manner by activating macrophages to secrete other proinflammatory cytokines (e.g., IL-6) (59). All these actions have been shown to be critical in the development of sepsis-induced organ dysfunction (60). On the other hand, a key function of IL-6 is the induction of fever (61) and the mediation of the acute phase response (62, 63). IL-6 has also recently been shown to have an important role in sepsis-induced myocardial dysfunction (64) and therefore may play a critical role in the progression of sepsis to multiple organ failure (60). It is worth noting that though not statistically significant, we observed a trend toward less suppression of INF- γ and higher IL-10 levels in patients who received high-dose cholecalciferol. INF- γ production is tightly regulated and stimulated by macrophage-derived cytokines (especially TNF- α) (65). It is normally not detectable in the plasma of healthy humans, but levels can be elevated in patients with sepsis (66). Recent evidence suggests that INF- γ may play an important role in the reversal of sepsis-induced immunoparalysis (67, 68). In contrast, expression of IL-10 is largely associated with anti-inflammatory functions, such as suppression of proinflammatory mediators (e.g., TNF- α , IL-1 β , and IL-6) (69, 70). Furthermore, data

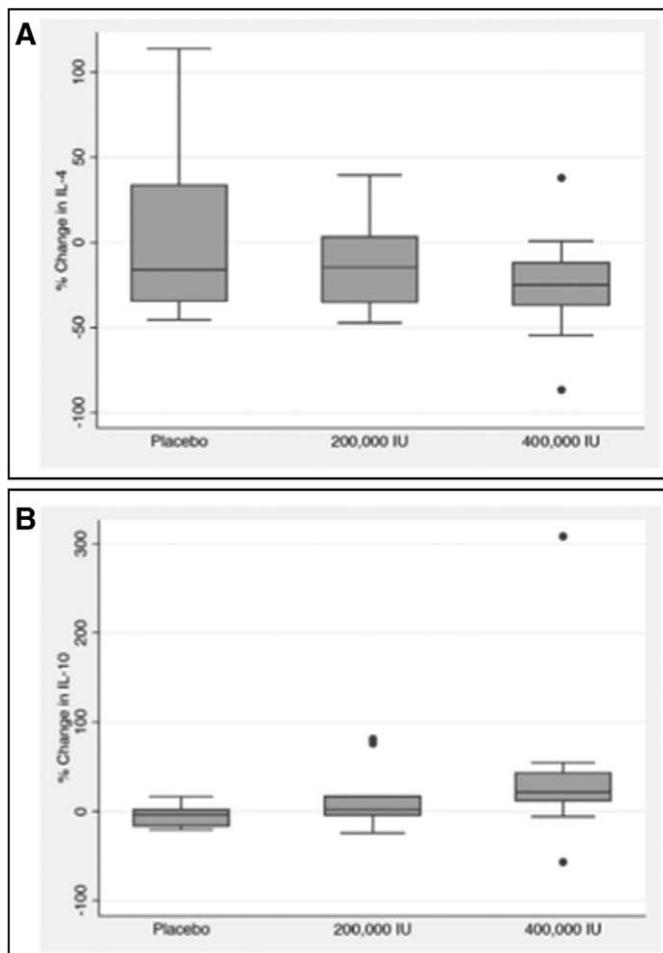


Figure 4. Change in anti-inflammatory cytokines on day 5 expressed as a percentage of baseline level (day 1). A trend toward significant difference in interleukin (IL)-10 (**B**) levels was observed between groups. No difference was observed in IL-4 (**A**) levels between groups.

suggest that IL-10 may be a critical regulator of the transition from early reversible sepsis to late irreversible septic shock and multiple organ failure (71, 72). Although IL-4 is a cytokine with many immunoregulatory functions in addition to regulation of cell proliferation, differentiation, and apoptosis, its role in sepsis remains unclear (60). Our findings do not support a relationship between cholecalciferol supplementation and systemic IL-4 expression in patients with sepsis. Taken all together, our results suggest that early administration of high-dose cholecalciferol in patients with severe sepsis or septic shock may significantly influence cytokine expression compared with patients who received placebo.

Our study findings build upon two recently published randomized, placebo-controlled, clinical trials. Leaf et al (73) reported that 1,25OH₂D administration did not result in higher plasma LL-37 levels in patients with sepsis. However, it is important to recognize that the intervention group in this study received a single dose of 1,25OH₂D, which has a half-life of only a few hours (37). As such, 1,25OH₂D resulted in a significant increase in leukocyte messenger RNA expression of LL-37 but did not significantly elevate systemic levels of the antimicrobial peptide. The use of cholecalciferol in our study

likely provided substrate for continuous paracrine and/or autocrine production of 1,25OH₂D and was therefore likely more effective in boosting systemic levels of LL-37. Additionally, Amrein et al (74) reported that high-dose cholecalciferol supplementation in critically ill patients with 25OHD levels up to 12 ng/mL resulted in a significant reduction of in-hospital mortality. Although the mechanisms underlying such an outcome remain unclear, the results of our study suggest that antimicrobial and immunomodulatory effects of vitamin D may play an important role during critical illness.

Although our findings are novel and intriguing, we acknowledge the potential limitations of our trial. First, our a priori sample size calculation was only aimed at adequately powering the trial to assess whether two different doses of cholecalciferol could rapidly improve vitamin D status and enhance circulating LL-37 levels in patients with severe sepsis or septic shock. Although we did find a significant difference in cytokine levels and the 30-day readmission rate between groups, our current study was not specifically powered to assess these or other important clinical outcomes, such as ICU LOS, hospital LOS, and mortality. Second, we only measured a number of the cytokines most commonly associated with sepsis (60). A deeper exploration of cytokine expression and immunophenotyping are likely needed to enhance our understanding of the immunomodulatory effects of vitamin D in acute illness. Third, we enrolled patients from both medical and surgical ICUs. Differences in patient-specific factors such as underlying comorbidities, baseline vitamin D status, and variable absorption through the alimentary tract may have influenced the observed outcomes. Fourth, fluid loading, renal loss of albumin, and inflammatory changes, which are typical of the sepsis syndrome, are known to affect circulating 25OHD levels. We attempted to control for these factors by calculating the bioavailable 25OHD (which takes into consideration alterations in albumin and DBP levels) in addition to measuring TBB and hsCRP. However, our calculations for bioavailable 25OHD are based on formulas validated in noncritically ill patients. Although a number of commercial tests for free 25OHD levels have recently become available (75), their role is not yet clear. Furthermore, methods for direct measurement of bioavailable 25OHD are not readily available. These issues merit consideration in the design of future trials that aim to confirm and expand on our novel findings.

CONCLUSION

Administration of 400,000 IU cholecalciferol as a single bolus at the outset of severe sepsis or septic shock is a safe and effective intervention to rapidly improve 25OHD levels and bioavailable 25OHD in patients with low vitamin D status. In turn, the changes in bioavailable 25OHD level are associated with concomitant increases in circulating LL-37 levels. Cholecalciferol supplementation also significantly influenced cytokine expression in patients with severe sepsis or septic shock. Larger trials are needed to verify these findings and to assess whether optimizing vitamin D status may improve sepsis-related clinical outcomes.

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