Vitamin D Supplementation in Sepsis and Critical Illness: Where Are We Now?

There is growing recognition that vitamin D deficiency is very common in critically ill adults and children, with or without sepsis (1–10). Low serum concentrations of the biomarker of vitamin D status, 25-hydroxyvitamin D (25[OH]D), when documented at intensive care unit (ICU) admission, remain unchanged or significantly decrease during the subsequent 7- to 10-day period without adequate vitamin D supplementation (2–5). Several studies have variously demonstrated a significant association between vitamin D deficiency (blood 25[OH]D levels $<$ 15–20 ng/ml] in the ICU and adverse clinical outcomes (e.g., length of hospital stay, readmission rates, and mortality) (4–10). For example, in a retrospective observational study in 1,325 patients, Braun and colleagues found that serum 25(OH)D levels $\leqslant$ 15 ng/ml obtained within 7 days before or after initiation of critical care were associated with higher 30-, 60-, and 365-day mortality, even after multivariate adjustment (6). Large retrospective cohort studies indicate that low serum levels of 25(OH)D obtained at time points within 1 year prior to ICU admission are associated with higher rates of sepsis, bloodstream infection, and mortality (11, 12). The reasons for the high rate of vitamin D deficiency in the ICU is likely multifactorial. Decreased exposure to sunlight and/or dietary intake of vitamin D prior to ICU admission may occur, and inflammation, decreased synthesis of vitamin D–binding protein (VDBP; which may result in urinary loss of 25[OH]D), interstitial extravasation, and hemodilution due to fluid loads may play a role after admission (1, 13).

Despite these data, vitamin D concentrations are not routinely determined in patients admitted to the ICU, and no clinical practice guidelines for monitoring vitamin D status and/or preventing or treating vitamin D deficiency in the ICU are published.

In addition, a rapidly growing body of literature demonstrates a link between vitamin D and immune functions (14–18). Vitamin D_3, primarily derived from skin, undergoes hepatic hydroxylation to the major circulating metabolite, 25(OH)D (15). Circulating 25(OH)D is converted by 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) to the biologically active hormone vitamin 1,25-dihydroxyvitamin D ($1,25[OH]_2D$; also termed calcitriol), classically in the kidney (15). $1,25(OH)_2D$ interacts with the nuclear vitamin D receptor (VDR) to mediate vitamin D effects (15, 18). However, numerous other cells and tissues express both VDR and CYP27B1, including immune cells (14, 15, 18). Vitamin D deficiency results in reduced available 25(OH)D substrate for $1,25(OH)_2D$ synthesis and action (14, 15).

Recent studies show that $1,25(OH)_2D$ induction of antibacterial activity in mononuclear macrophages is mediated, in part, by up-regulation of the antimicrobial peptide (AMP) LL-37 (also termed cathelicidin) (16, 18). LL-37 is the C-terminal peptide fragment of the pre-proprotein hCAP18 derived from the human cathelicidin antimicrobial peptide gene (*CAMP)* and induces a variety of antimicrobial effects mediated via chemotaxis, cytokine production, apoptosis, and other functions (18). $1,25(OH)_2D$ also enhances innate immunity by several AMP-independent mechanisms and regulates adaptive immunity through a variety of effects on T cells, including IFN-γ release and induction of autophagy (17, 18).

Given these data, there is a growing interest in evaluating the clinical and immunological impact of vitamin D supplementation in critical illness and sepsis. Unfortunately, surprisingly few studies on the impact of vitamin D supplementation in ICU patients are published (2, 3), although several trials of high-dose cholecalciferol (vitamin D_3) administration are in progress (e.g., ClinicalTrials.gov identifiers NCT01372995 and NCT01896544).

In this issue of the *Journal*, Leaf and colleagues (pp. 533–541) report the results of a double-blind, randomized, placebo-controlled trial on the immune/physiologic and clinical effects of a single intravenous dose of 2 μg calcitriol ($1,25[OH]_2D$) in 67 adult ICU patients with severe sepsis or shock, of whom 36 patients were randomized to calcitriol (19). Blood and urine indexes were obtained at baseline and at 6, 24, and 48 hours after study drug administration. The primary endpoint was plasma levels of cathelicidin (LL-37) protein (change from baseline) 24 hours after study drug administration; secondary endpoints included whole-blood leukocyte cathelicidin messenger RNA (mRNA) expression, urinary kidney injury markers (neutrophil gelatinase-associated lipocalin and kidney injury molecule-1), protein and leukocyte mRNA pro- and antiinflammatory cytokines (IL-1β, IL-2, IL-6, IL-10, and tumor necrosis factor-α), and clinical outcome measures (organ function indexes, change in illness severity scores, length of stay, and mortality) (19). The dose of calcitriol was chosen based on prior safety/efficacy studies in non-ICU patients with chronic kidney disease and on the single prior ICU study of calcitriol administration, in which an intravenous dose of 2 μg calcitriol was given on each of Days 0, 2, 4, and 6 to 11 adult ICU patients and found to be safe (2).

The two groups of study subjects were well matched for baseline clinical and demographic characteristics and the primary site of infection responsible for sepsis/shock (19). The dose of calcitriol was without adverse effects, and resulted in a fivefold increase in plasma $1,25(OH)_2D$ concentrations 6 hours after administration; no change in plasma levels of 25(OH)D or other vitamin D metabolites occurred. Calcitriol did not alter plasma cathelicidin protein levels at 24 hours (or at other time points) compared with placebo, but significantly increased total leukocyte cathelicidin and antiinflammatory IL-10 mRNA expression at 24 hours. No

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differences in other cytokines, urinary injury markers, or clinical outcomes were noted between groups (19).

Strengths of the report by Leaf and colleagues include the randomized, double-blind design and that this is the first report to evaluate exogenous vitamin D administration on immune functions in ICU/septic patients. This study is unique in that the authors administered the active vitamin D hormone $1,25(OH)_2D$. In the only other such study in ICU patients, Mata-Granados gave four identical calcitriol doses (2 μg) every other day for 6 days in a small cohort of ICU patients, but immune, physiologic, and clinical outcomes were not measured (2). Serum blood levels obtained 24–48 hours after dosing did not reveal changes in either 25(OH)D or $1,25(OH)_2D$ concentrations in that study; thus, a weakness of the current study is that only a single dose of calcitriol was given and dose-finding and time course data are unavailable. In addition, the timing of calcitriol dosing in relation to the diagnosis of sepsis is unclear. Nonetheless, this single $1,25(OH)_2D$ dose did increase cathelicidin mRNA in leukocytes 24 hours later, suggesting that the rapid increase in $1,25(OH)_2D$ blood levels may have immunomodulatory effects in adults with sepsis. In addition, leukocyte mRNA expression of the antiinflammatory cytokine IL-10 also increased (19). Thus, these data can inform subsequent dose and time-course studies on immune functions of this agent in patients with sepsis. However, without more detailed studies in specific cell types (including *ex vivo* studies), the potential salutary immunological impact of changes in cathelicidin and IL-10 gene expression at 24 hours only are difficult to assess. A substantial proportion (56%) of the calcitriol-treated subjects of Leaf and colleagues had a respiratory source of sepsis (19); given that LL-37/cathelicidin is expressed in lung and is detectable in bronchoalveolar lavage fluid (BAL) (20), studies of the impact of various vitamin D regimens on AMP and cytokine expression in BAL fluid of ICU/septic patient will be of interest. The bioavailability of 25(OH)D for conversion to $1,25(OH)_2D$ is dependent on VDBP levels; in a previous study, we found that VDBP levels in blood were significantly lower in septic compared with adult ICU patients without sepsis (1). Therefore, subsequent studies on the metabolic, immune and clinical impact of vitamin D regimens in ICU settings should probably include VDBP levels and calculated indexes of vitamin D bioavailability in data interpretation.

In the only other published study of vitamin D treatment in ICU patients, Amrein and colleagues gave a single high dose of 540,000 IU (13.5 mg) of cholecalciferol (vitamin D_3) to 12 adult ICU patients versus placebo (n = 13) (3). Subjects randomized to vitamin D_3 had no adverse clinical or metabolic effects and demonstrated a substantial increase in serum concentrations of both 25(OH)D and $1,25(OH)_2D$ over 7 days. Some data suggest that the intracrine conversion of 25(OH)D to $1,25(OH)_2D$ within immune cells themselves may be a key signal for immune cell responses to vitamin D (18). This is supported by a recent study in individuals with HIV/AIDS given high-dose vitamin D_3 (7,000 IU/d for 12 wk) (21). This regimen substantially increased serum levels of both 25(OH)D and $1,25(OH)_2D$; however, *ex vivo* monocyte *CAMP* mRNA expression over time was only positively related to 25(OH)D and not to $1,25(OH)_2D$ concentrations (21). Thus, administration of cholecalciferol may induce a more robust immune response relative to calcitriol, but comparative efficacy data on this question in ICU patients are needed.

Many questions remain with regard to the clinical, metabolic, and immune efficacy of vitamin D administration in ICU and septic patients, including optimal dosing, timing of administration, and form of vitamin D that is most efficacious. Nonetheless, given that vitamin D deficiency is widespread in ICUs and is associated with adverse outcomes, coupled with the potentially beneficial immunomodulatory effects of exogenous vitamin D suggested in the report by Leaf and colleagues and others, additional studies of this nutrient in catabolic, septic, and critically ill patients are warranted. ■

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Particulate Air Pollution and Lung Function

In this issue of the *Journal*, Lepeule and colleagues (pp. 542–549) present remarkable additional evidence that exposure to air pollution adversely affects lung function and pulmonary health (1). Lung function measures (FVC and FEV_1) from 858 elderly men who lived in the Boston area and were enrolled in the Normative Aging Study were evaluated. Concentrations of black carbon, a surrogate of exposure to traffic-related air pollution, were estimated for the geocoded participants' addresses using data from 148 monitoring stations and a spatiotemporal land use regression model. Elevated pollution exposures were associated with significant reductions in baseline lung function levels as well as accelerated rates of lung function decline. The statistically robust results reported are remarkable, even surprising, for several reasons: air pollution levels in the Boston area are quite low (well within U.S. air quality standards), the study is not exceptionally large, and the statistical approach is quite ambitious by estimating joint effects on baseline lung function as well as rate of decline. Nevertheless, given the widespread nature of exposure, these results suggest measurable adverse respiratory health effects from traffic-source air pollution and have important public health and policy implications.

This latest work augments an already substantial literature that demonstrates that breathing contaminants, especially combustion-related particles, affects pulmonary function. It has long been known that cigarette smoking contributes to accelerated lung function decline in aging adults (2). There is also evidence that exposures to combustion-source particulate air pollution may have similar but smaller effects (3, 4). Daily time-series panel studies, mostly of children, provide evidence that short-term (one to a few days) elevated exposure to fine particulate matter is associated with small transient declines in lung function (5). A recent analysis of data from the Framingham Heart Study observed similar air pollution–related declines in lung function in adults (6). Lepeule and colleagues, also using the data from the Normative Aging Study, observed reduced lung function effects associated with 4 to 28 days of exposure to fine particulate matter and other measures of traffic-source air pollution (7). Results from the Southern California Children's Health study indicate that chronic exposures to fine particulate and related air pollutants over a period of years were associated with deficits in rate of lung function growth and that exposures to traffic-related pollution as well as pollution from other sources have adverse effects on children's lung function (8).

Lepeule and colleagues correctly suggest that understanding how long-term air pollution exposures impact lung function in the elderly is important in understanding possible pathways for the mortality and morbidity associated with air pollution (1). This study provides important evidence that air pollution has adverse effects on lung function. Other studies indicate that air pollution, including traffic-related fine particulate pollution, contributes to chronic obstructive pulmonary disease and mortality (9). However, the simple mechanistic concept that air pollution induces accelerated decline in lung function, resulting in loss of reserve capacity, disability, and eventual death due to pulmonary disease, is clearly incomplete. Most of the particulate air pollution effects on mortality seem to be due to cardiovascular mortality (10), and a broad evaluation of the literature suggests that exposures to particulate air pollution are strongly associated with cardiovascular disease (11). Cardiovascular and pulmonary diseases have substantial common comorbidities, and deficit in lung function is a strong predictor of cardiovascular disease and mortality (12). Inflammation associated with pulmonary disease appears to contribute to cardiovascular risk (13). Lepeule and colleagues have also used data from the Normative Aging Study to explore epigenetic influences on inflammation, air pollution, and lung function (7, 14).

The findings that traffic-related pollution is associated with deficits in lung function and accelerated rates of lung function decline are intriguing but leave important unanswered questions: How do deficits in lung function fit into a more comprehensive understanding of the biological pathways that link air pollution exposure to cardiopulmonary disease and death? What specific constituents or characteristics of the traffic-source pollution are really responsible for the adverse effects on lung function? Are the adverse health effects due to pollutants from gasoline combustion, diesel combustion, brake and/or tire wear, something else, or a combination of all? Are the effects due primarily to particulate