The Relationship Between Vitamin D Status and Adrenal Insufficiency in Critically Ill Children

J. Dayre McNally, Dermot R. Doherty, Margaret L. Lawson, Osama Y. Al-Dirbashi, Pranesh Chakraborty, Tim Ramsay, and Kusum Menon

Department of Pediatrics (J.D.M., M.L.L., P.C., K.M.), Faculty of Medicine, University of Ottawa, Children's Hospital of Eastern Ontario, Ottawa, Canada K1H 8L1; Research Institute (J.D.M., M.L.L., P.C., K.M.), Children's Hospital of Eastern Ontario, Ottawa, Canada K1H 8L1; Department of Anesthesia (D.R.D.), Faculty of Medicine, University of Ottawa and Children's Hospital of Eastern Ontario, Ottawa, Canada K1H 8L1; Department of Intensive Care (D.R.D.), Temple Street Children's University Hospital Dublin and University College Dublin 1, Ireland; Ontario Newborn Screening Laboratory (O.Y.A.-D., P.C.), Children's Hospital of Eastern Ontario, Ottawa, Canada K1H 8L1; Clinical Epidemiology Program (T.R.), Ottawa Hospital Research Institute, Ottawa, Ontario, Canada K1H 8L6; and Department of Epidemiology and Community Medicine (T.R.), Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8L6

Context: Recent studies in critically ill populations have suggested both adrenal insufficiency (AI) and vitamin D deficiency to be associated with worse clinical outcome. There are multiple mechanisms through which these pleiotropic hormones might synergistically influence critical illness.

Objective: The aim of the study was to investigate potential relationships between vitamin D status, adrenal status, and cardiovascular dysfunction in critically ill children.

Design: We conducted a secondary analysis of data from a prospective cohort study.

Setting and Patients: The study was conducted on 319 children admitted to 6 Canadian tertiary-care pediatric intensive care units.

Main Outcome Measures: Vitamin D status was determined through total 25-hydroxyvitamin D (25OHD) levels. AI was defined as a cortisol increment under 9 μg/dL after low-dose cosyntropin. Clinically significant cardiovascular dysfunction was defined as catecholamine requirement during pediatric intensive care unit admission.

Results: Using 3 different thresholds to define vitamin D deficiency, no association was found between vitamin D status and AI. Furthermore, linear regression failed to identify a relationship between 25OHD and baseline or post-cosyntropin cortisol. However, the association between AI and cardiovascular dysfunction was influenced by vitamin D status; compared to children with 25OHD above 30 nmol/L, AI in the vitamin D-deficient group was associated with significantly higher odds of catecholamine use (odds ratio, 5.29 vs 1.63; $P = .046$).

Conclusions: We did not find evidence of a direct association between vitamin D status and critical illness-related AI. However, our results do suggest that vitamin D deficiency exacerbates the effect of AI on cardiovascular stability in critically ill children. **(*J Clin Endocrinol Metab* 98: E877–E881, 2013)**

Recent epidemiological and biochemical studies have suggested vitamin D deficiency and adrenal insufficiency (AI) to be risk factors for illness severity and outcomes in critical illness (1–4). Although the mechanisms for these associations remain speculative, it is plausible that they are interrelated or partially dependent on each

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received January 14, 2013. Accepted March 15, 2013.
First Published Online April 1, 2013

Abbreviations: AI, adrenal insufficiency; CI, confidence interval; 1,25OH$_2$D, 1,25-dihydroxyvitamin D; 25OHD, 25-hydroxyvitamin D; OR, odds ratio; PICU, pediatric intensive care unit.

other. Vitamin D deficiency is thought to negatively impact the cardiovascular, respiratory, and immune systems directly through cellular vitamin D receptors (5). Similarly, critical illness-related AI is thought to contribute to cardiorespiratory instability through decreased myocardial contractility, impaired vascular tone, cytokine production, and amplified capillary leak (6, 7). Based on this overlap in clinical symptomatology, we hypothesized an interrelationship between vitamin D status, AI, and cardiovascular instability in critical illness.

Members of our research group recently completed the Adrenal Insufficiency in Pediatric Critical Illness Study (AIP) (6). Biological samples collected as part of this multicenter prospective observational study have been used to separately report on the epidemiology of AI and vitamin D deficiency in critically ill children (1, 6). The primary objective of this substudy was to investigate the potential association between AI and vitamin D status. Secondarily, we evaluated for mediation and effect modification between vitamin D status and AI in their previously described relationships with cardiovascular dysfunction.

Patients and Methods

This study represents a secondary analysis of data and samples collected as part of AIP (6). Patients were eligible if they were newborn to 17 years old, had arterial or central venous catheters, and could be enrolled within 24 hours of admission. AIP excluded patients who were born prematurely; had known or suspected adrenal, pituitary, or hypothalamic disease; received systemic steroids for more than 10 days in the previous month; received more than 1 systemic steroid dose within 24 hours of admission (except dexamethasone); expected to have care withdrawn; transferred from another pediatric intensive care unit (PICU); or whose primary clinician refused. Written informed consent was obtained for all AIP participants, and Research Ethics Board approval to measure 25-hydroxyvitamin D (25OHD) was obtained from 6 of the 7 centers (337 of 389 AIP participants).

All study participants had baseline blood collected within 24 hours of PICU admission. Immediately after baseline blood work, 1 μg cosyntropin (Cortrosyn; Amphastar Pharmaceuticals, Scarborough, Ontario, Canada) was given iv, and blood was resampled at 30 minutes. Plasma cortisol levels were measured using the Elecsys assay (Roche Diagnostics, Indianapolis, Indiana). Total 25OHD was measured using liquid chromatography–mass spectrometry (1).

For this study, AI was defined as a cortisol increment under 9 μg/dL after cosyntropin. Baseline cortisol under 5 μg/dL was also explored because it is commonly used in the clinical setting to determine adrenal status (8). Vitamin D status was evaluated using plasma 25OHD; multiple thresholds were used to define deficiency (30, 40, and 50 nmol/L). For descriptive statistics, continuous variables were provided as medians with interquartile ranges, and categorical variables frequencies were presented with 95% confidence intervals (CIs). Associations between categorical variables were investigated using χ^2 and Fisher exact tests. Binary logistic and multiple linear regression were further used to investigate relationships between vitamin D status, AI, and cardiovascular dysfunction. Infusions of catecholamine (dopamine, epinephrine, norepinephrine) identified clinically significant cardiovascular dysfunction. The Pediatric Risk of Mortality (PRISM) score was used to quantify illness severity (1, 6). Potential mediation of the previously described relationship between vitamin D status and cardiovascular dysfunction by AI was evaluated through multivariate regression. Effect modification was explored using interaction terms in logistic regression. A P value $< .05$ was considered statistically significant. SAS software (version 9.3; SAS Institute Inc, Cary, North Carolina) was used for statistical analysis, and Sigmaplot (version 12.3; Systat Software Inc, Chicago, Illinois) was used for graph preparation.

Results

Of the 337 potential study participants, 319 had concurrent cortisol and 25OHD measurements. Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) provides information on demographics, admission type, season, and illness severity measures (including catecholamine requirements). The cohort median 25OHD was 40.3 nmol/L (interquartile range, 29.1 to 54.1). Only 25 (7.8%) participants had 25OHD values above 75 nmol/L, and the highest were 93, 98, and 104 nmol/L. Of the 319 study participants, 90 (28.2%) met our AI definition, and 43 (13.5%) were determined to have a baseline cortisol under 5 μg/dL. Eighty-seven (27.3%) met the definition for cardiovascular instability, requiring at least 1 catecholamine infusion.

No significant difference was observed in median 25OHD levels for critically ill children with and without AI (39.5 vs 40.5 nmol/L; $P = .33$). Table 1 compares the rates of AI by vitamin D status using the three 25OHD thresholds. A nonsignificant trend toward higher rates of AI was observed for children with 25OHD under 30 nmol/L (35.4 vs 25.7%; $P = .10$). As shown in Supplemental Table 2, rates of baseline cortisol under 5 μg/dL did not coincide with higher vitamin D deficiency rates, regardless of definition. In fact, using the 50 nmol/L threshold, vitamin D-deficient children had a significantly lower odds ratio (OR, 0.42; 95% CI, 0.22 to 0.81) of baseline cortisol under 5 μg/dL. Visual examination for a linear relationship through scatter plots (Supplemental Figure 1) and regression analysis did not identify significant relationships between vitamin D and baseline or post-cosyntropin cortisol. For every 10 nmol/L decrease in 25OHD, we calculated: 1) decrease in cortisol increment by 0.4 μg/dL (95% CI, −0.4 to 1.3); 2) increase in baseline cortisol by 1.9 μg/dL (95% CI, −1.1 to 5.1); and 3) decrease

Table 1. Evaluation of AI Rates by Vitamin D Status

25OHD threshold	Vitamin D Deficient		Vitamin D Sufficient		OR (95% CI)
	AI	No AI	AI	No AI	
30 nmol/L	35.4% (29)	64.6% (53)	25.7% (61)	74.3% (176)	1.58 (0.92, 2.70)
40 nmol/L	29.5% (46)	70.5% (110)	27.0% (44)	73.0% (119)	1.13 (0.69, 1.84)
50 nmol/L	29.5% (65)	70.3% (154)	25.0% (25)	75.0% (75)	1.27 (0.74, 2.17)

AI is defined as a post-cosyntropin cortisol increment under 9 μg/dL. Data are expressed as percentages (counts). *P* values and ORs were calculated using χ^2 tests in SAS. For 25OHD, every 1 ng/mL is equivalent to 2.5 nmol/L.

in post-cosyntropin peak cortisol by −1.5 μg/dL (95% CI, −4.3 to 1.3).

Multivariate logistic regression was used to explore whether AI might be a mediator in the previously described relationship between vitamin D status and cardiovascular dysfunction. Supplemental Tables 3 and 4 show the OR change for different definitions of vitamin D status with the AI variable. The largest OR change was 6%, and it occurred with the addition of AI to the model using the 30 nmol/L threshold for vitamin D deficiency. Regardless, both vitamin D status (OR, 2.15; 95% CI, 1.25 to 3.72) and AI (OR, 2.38; 95% CI, 1.40 to 4.05) remained statistically associated with catecholamine administration.

The inclusion of a vitamin D and AI variable interaction term in logistic regression suggested that the association between AI and catecholamine use was stronger in the presence of vitamin D deficiency. This was observed for all 3 vitamin D deficiency thresholds, yet the difference only achieved significance using the 30 nmol/L cutoff (Table 2). Compared to children with 25OHD above 30 nmol/L, AI in the vitamin D-deficient group was associated with a significantly higher OR of catecholamine use (5.29 vs 1.63; $P = .046$).

Discussion

Through a secondary analysis of data from a large prospective cohort study, we evaluated the relationship between vitamin D status, AI, and cardiovascular dysfunction in critical illness and observed that vitamin D status modifies the strength of association between AI and cardiovascular dysfunction.

Our hypothesis of an association between vitamin D status, impaired adrenal-glucocorticoid function, and critical illness was based upon the overlap of clinical phenotype observed in these conditions. In patients with AI, disturbance of the hypothalamic-pituitary axis and inadequate cortisol production can lead to hemodynamic instability through decreased myocardial contractility, vasodilation, augmented cytokine production, and amplified capillary leak syndrome (6, 7). Likewise, cardiovascular and immune system dysfunction is suggested to be the primary noncalcemic mechanism for vitamin D deficiency-associated pathophysiology in critical illness (1, 5). Furthermore, other endocrine organs have hormone production influenced by vitamin D status (9, 10). For example, pancreatic cells have vitamin D receptors, and insulin secretion has been shown to vary with vitamin D status.

With this report, we provide evidence that the strength of relationship between AI and cardiovascular dysfunction is amplified by vitamin D deficiency, particularly 25OHD levels below 30 nmol/L. This finding is in agreement with the Institute of Medicine conclusion that 30 nmol/L is the appropriate threshold for defining increased risk of vitamin D deficiency (11). Biochemical studies have also demonstrated that boosted parathyroid and renal ac-

Table 2. Requirement for Catecholamine Infusion by Adrenal Status in Critically Ill Children With and Without Vitamin D Deficiency

	No AI	AI	OR (95% CI)	*P* value
Vitamin D sufficient				
Catecholamines, no	79.6% (140)	66.7% (43)		
Catecholamines, yes	20.4% (36)	33.3% (18)	1.6 (0.8, 3.2)	
Vitamin D deficient				
Catecholamines, no	73.6% (39)	34.5% (10)		
Catecholamines, yes	26.4% (14)	65.5% (19)	5.3 (2.0, 14.1)	.046

Vitamin D status is defined using a 25OHD threshold of 30 nmol/L. Data are expressed as percentages (counts). ORs represent risk associated with catecholamine use for adrenally insufficient patients in each vitamin D group. ORs are provided with 95% CIs. *P* values were calculated from change in likelihood ratio statistic after inclusion of an interaction term into the logistic regression model. *P* values and ORs were calculated using χ^2 tests in SAS. For 25OHD, every 1 ng/mL is equivalent to 2.5 nmol/L.

tivity cannot maintain 1,25-dihydroxyvitamin D ($1,25OH_2D$) production once 25OHD falls below 30 nmol/L (12). The plausibility of this previously unreported interaction is supported by limited literature describing vitamin D status modification of specific genetic polymorphisms and nutritional states on disease predisposition (13, 14). Furthermore, research also suggests that vitamin D may mediate positive and negative glucocorticoid effects on both cardiovascular and respiratory disease (15, 16).

This study also evaluated an association between AI and vitamin D status. Using multiple statistical techniques and different definitions, we were unable to find evidence of higher AI rates in vitamin D deficiency. The lack of change in strength of association between vitamin D status and catecholamine requirement with adjustment for AI suggests that the negative effects of vitamin D are not mediated through adrenal glucocorticoid dysfunction. These findings are consistent with the very limited literature in this area, including a clinical study reporting unchanged cortisol levels after $1,25OH_2D$ administration to healthy volunteers and laboratory research showing few vitamin D receptors in the adrenal cortex (17, 18). Surprisingly, application of the alternative measure of adrenal status identified significantly lower rates of baseline cortisol < 5 μg/dL among the vitamin D-deficient groups. The reason for this association is unclear, but it may reflect that random cortisol values do not correlate with worse clinical outcomes in critical illness (6, 19).

The major strength of this study is that the data were collected as part of a large prospective, multicenter study with assay and repeat sample validation. Importantly, exclusion of patients receiving significant steroids avoided confusion resulting from suppression of endogenous cortisol. The primary limitations relate to alternatives for measuring and defining both AI and vitamin D deficiency. For AI, we did not measure free cortisol, and our focus on cortisol precludes comment on hormones released by the vitamin D receptor-rich adrenal cortex (17, 20). Similarly, for vitamin D, one could speculate that plasma 25OHD is not a representative measure of vitamin D status in critical illness. First, significant dysfunction of the parathyroid or kidney organs can impair the endocrine production of active $1,25OH_2D$ hormone despite normal 25OHD concentrations. Second, tissue uptake of 25OHD and local tissue production of active hormone could be impaired by inflammation, hypoxia, toxin accumulation, or altered intracellular signaling. Finally, one must consider the existence of a relative vitamin D deficiency state, where accepted normal levels of 25OHD and $1,25OH_2D$ in health do not meet body requirements during critical illness.

In conclusion, despite similar clinical phenotypes and high prevalence among critically ill children, we were unable to find evidence of a relationship between vitamin D status and critical illness-related AI. However, we do provide hypothesis-generating evidence that vitamin D deficiency may augment the effect of AI on cardiovascular function. Because the described relationships with critical illness and interactions were derived from observational data they cannot definitively prove a thesis. Controlled trials will be required to test the hypothesis that optimization of vitamin D status before or after critical illness influences the positive and negative effects of AI (or glucocorticoid administration) during critical illness and improves outcome.

Acknowledgments

The authors thank Nathalie Earl, Larry Fisher, and Christine McRoberts for assistance with vitamin D determination and Roxanne Ward for help with the original AIP study design.

Address all correspondence and requests for reprints to: J. Dayre McNally, Children's Hospital of Eastern Ontario, 401 Smyth Road, Ottawa, Ontario, Canada K1S 3H2. E-mail: dmcnally@cheo.on.ca.

This work was supported by the Canadian Institutes of Health Research, Children's Hospital of Eastern Ontario Research Institute, and Children's Hospital of Eastern Ontario Popham Foundation.

Disclosure Summary: The authors have nothing to disclose.

References

1. **McNally JD, Menon K, Chakraborty P, et al.** The association of vitamin D status with pediatric critical illness. *Pediatrics*. 2012;130:429–436.
2. **Madden K, Feldman HH, Smith EE, et al.** Vitamin D deficiency in critically ill children. *Pediatrics*. 2012;130:421–428.
3. **Lee P, Eisman JA, Center JR.** Vitamin D deficiency in critically ill patients. *N Engl J Med*. 2009;360:1912–1914.
4. **Braun A, Gibbons F, Litonjua A, Giovannucci E, Christopher K.** Low serum 25-hydroxyvitamin D at critical care initiation is associated with increased mortality. *Crit Care Med*. 2012;40:63–72.
5. **Lee P.** Vitamin D metabolism and deficiency in critical illness. *Best Pract Res Clin Endocrinol Metab*. 2011;25:769–781.
6. **Menon K, Ward RE, Lawson ML, Gaboury I, Hutchison JS, Hébert PC.** A prospective multicenter study of adrenal function in critically ill children. *Am J Respir Crit Care Med*. 2010;182:246–251.
7. **Marik PE.** Critical illness-related corticosteroid insufficiency. *Chest*. 2009;135:181–193.
8. **Menon K, Lawson M.** Identification of adrenal insufficiency in pediatric critical illness. *Pediatr Crit Care Med*. 2007;8:276–278.
9. **Berger U, Wilson P, McClelland RA, et al.** Immunocytochemical detection of 1,25-dihydroxyvitamin D receptors in normal human tissues. *J Clin Endocrinol Metab*. 1988;67:607–613.
10. **Karnchanasorn R, Ou HY, Chiu KC.** Plasma 25-hydroxyvitamin D levels are favorably associated with β-cell function. *Pancreas*. 2012;41:863–868.

11. **Institute of Medicine.** *Dietary Reference Intakes for Calcium and Vitamin D*. In: Ross CA, Taylor CL, Yaktine AL, Bel Valle HB, eds. Washington, DC: The National Academies Press; 2011.
12. **Christensen MH, Lien EA, Hustad S, Almås B.** Seasonal and age-related differences in serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and parathyroid hormone in patients from Western Norway. *Scand J Clin Lab Invest*. 2010;70:281–286.
13. **Vimaleswaran KS, Cavadino A, Hyppönen E.** Evidence for a genetic interaction in allergy-related responsiveness to vitamin D deficiency. *Allergy*. 2012;67:1033–1040.
14. **Cheng TY, Neuhouser ML.** Serum 25-hydroxyvitamin D, vitamin A, and lung cancer mortality in the US population: a potential nutrient-nutrient interaction. *Cancer Causes Control*. 2012;23:1557–1565.
15. **Ahmed MA.** 2012 Impact of vitamin D(3) on cardiovascular responses to glucocorticoid excess [published online ahead of print September 19, 2012]. *J Physiol Biochem*. doi: 10.1007/s13105-012-0209-4
16. **Gupta A, Sjoukes A, Richards D, et al.** Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. *Am J Respir Crit Care Med*. 2011;184:1342–1349.
17. **Clark SA, Stumpf WE, Bishop CW, DeLuca HF, Park DH, Joh TH.** The adrenal: a new target organ of the calciotropic hormone 1,25-dihydroxyvitamin D3. *Cell Tissue Res*. 1986;243:299–302.
18. **Zofková I, Kancheva RL.** Lack of stimulatory effect of 1,25(OH)2 vitamin D3 on β-endorphin and cortisol secretion. *J Endocrinol Invest*. 1994;17:693–695.
19. **Briegel J, Schelling G, Haller M, Mraz W, Forst H, Peter K.** A comparison of the adrenocortical response during septic shock and after complete recovery. *Intensive Care Med*. 1996;22:894–899.
20. **Molenaar N, Johan Groeneveld AB, Dijstelbloem HM, et al.** Assessing adrenal insufficiency of corticosteroid secretion using free versus total cortisol levels in critical illness. *Intensive Care Med*. 2011;37:1986–1993.