Impact of Anesthesia and Surgery for Congenital Heart Disease on the Vitamin D Status of Infants and Children

A Prospective Longitudinal Study

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ABSTRACT

Background: Vitamin D is recognized as a pleiotropic hormone important for the functioning of organ systems, including those central to critical illness pathophysiology. Recent studies have reported associations between vitamin D status and outcome among critically ill adults and children. Preoperative vitamin D status, impact of operative techniques, and relationship between immediate postoperative vitamin D levels and clinical course have not been described in the pediatric congenital heart disease (CHD) population. The objective of this study was to describe the impact of CHD surgery on vitamin D status and relationship between postoperative levels and clinical course.

Methods: A prospective cohort study was conducted from 2009 to 2011 at a single tertiary care pediatric hospital. A total of 58 children with CHD were enrolled and blood collected preoperatively, intraoperatively, and postoperatively. Serum 25-hydroxyvitamin D (25OHD) was measured using liquid chromatography–mass spectrometry.

Results: The mean preoperative 25OHD was 58.0 nM (SD, 22.4), with 42% being deficient (<50 nM). Postoperatively, we identified a 40% decline in 25OHD to 34.2 nM (SD, 14.5) with 86% being deficient. Intraoperative measurements determined that initiation of cardiopulmonary bypass coincided with abrupt decline. CHD patients requiring catecholamines had lower postoperative 25OHD (38.2 *vs.* 26.5 nM, $P = 0.007$), findings confirmed through multivariate logistic regression. Lower postoperative 25OHD was associated with increased fluid requirements and intubation duration.

Conclusions: Most CHD patients are vitamin-D deficient postoperatively due to low preoperative levels and a significant intraoperative decline. Interventional studies will be required to determine whether prevention of postoperative vitamin D deficiency improves outcome.

What We Already Know about This Topic

- Vitamin D is a pleiotropic hormone, important not only for calcium homeostasis, but also for muscle strength, pathogen defense, immunomodulation, and myocardial health
- This study determined vitamin D status in children undergoing congenital heart disease surgery and evaluated its impact on outcome

What This Article Tells Us That Is New

- Most children are vitamin D deficient following congenital heart disease surgery, secondary to borderline preoperative levels and significant intraoperative decline
- Lower vitamin D levels were associated with worse clinical outcome

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Received from the Departments of Anesthesiology and Division of Pediatric Intensive Care Medicine, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada. Submitted for publication August 28, 2012. Accepted for publication February 5, 2013. The study was supported through grants from the Children's Hospital of Eastern Ontario Research Institute (Ottawa, Ontario, Canada) and Department of Anesthesia, University of Ottawa (Ottawa, Ontario, Canada). Research fellowship funding to J. Dayre McNally was provided by the Children's Hospital of Eastern Ontario Popham Foundation (Ottawa, Ontario, Canada). Paper submitted on behalf of the Canadian Critical Care Trials Group.

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 Anesthesiology 2013; 119:71-80

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CONGENITAL heart disease (CHD) is a common congenital anomaly affecting approximately 1% of newborns, resulting in approximately 15,000 procedures per year in North America.[1] For many children, anesthesia and cardiac surgery delineate a transition from a state of compensated heart disease to an acute multisystem critical illness state within a short period of time.[2,3] As a predictably ill pediatric cohort, benefits could be realized through the identification of new perioperative approaches that might modulate postoperative critical illness and decrease acute and chronic morbidity.

Vitamin D is recognized as a pleiotropic hormone important not only for calcium homeostasis, but also for muscle strength, pathogen defense, immunomodulation, and myocardial health.[4,5] As these organ systems are fundamental to pathophysiology following cardiac surgery, vitamin D deficiency could potentially represent a modifiable risk factor. Support for this hypothesis is available from observational studies on adult cardiovascular disease and critical illness which report associations between vitamin D and both morbidity and mortality.[6–12] The limited available pediatric research has also documented associations between vitamin D and severe asthma, acute respiratory infection, and cardiomyopathy.[13–17] A recently completed multicenter cross-sectional study in a large heterogeneous pediatric intensive care unit (PICU) population reported high rates of vitamin D deficiency that was associated with organ dysfunction and length of PICU stay.[18]

Despite having biological plausibility and associations with acute illness morbidities, a causal role for vitamin D in critical illness has yet to be widely accepted. The debate centers on the cross-sectional design of most available studies and an uncertainty whether lower reported blood vitamin D levels represent a marker of disease severity, or whether they alter the trajectory of critical illness.[4] For example, vitamin D levels may be altered in CHD patients due to large circulating fluid shifts associated with the cardiopulmonary bypass (CPB), blood loss, fluid administration, and interstitial leak of vitamin D binding proteins.[19–22]

Children undergoing CHD surgery may represent an ideal opportunity to study changes in vitamin D status associated with a complex surgical intervention and the subsequent systemic inflammatory response. Using a longitudinal study design, we sought to evaluate perioperative vitamin D status and the impact of CHD surgery. Secondary objectives were to test for associations between vitamin D and clinically important PICU outcomes. Finally, through a secondary analysis of a separate cohort of postoperative CHD patients who participated in a multi-center study addressing Adrenal Insufficiency in Pediatric Critical Illness (AIP), we sought to determine vitamin D deficiency rates and associations with clinical outcomes across a wider population.[23]

Materials and Methods

Prospective Study Design

We conducted a single-site, prospective observational study from August 2009 to June 2011. Inclusion criteria were children less than 18 yr of age undergoing elective or semielective surgery for CHD requiring admission to the PICU during the postoperative period. Patients who previously participated in this study, previous studies of vitamin D, and those scheduled for isolated pacemaker insertion or Blalock-Taussig shunts were excluded. A research nurse consecutively approached patients, parents, and guardians for consent during normal working hours after the patient was scheduled for operation by the cardiac surgical team. The Institutional Review Board at the Children's Hospital of Eastern Ontario approved the study in April 2009.

Surgical Protocol

Anesthetic and surgical management was left to the discretion of the anesthesiologist, perfusionists, and cardiovascular surgeon. In general, anesthesia was induced and maintained using a balanced narcotic (fentanyl or remifentanil) with sevoflurane technique. CHD patients under the age of 30 days received 30 mg/kg methylprednisolone preoperatively, which is standard in this institution. All cases received an arterial and central venous catheter. CPB and deep hypothermic cardiac arrest were performed as per our institutional protocol, with cooling to 24°C. After surgery, patients received modified ultrafiltration (MUF) as dictated by patient clinical state and residual volume in the circuit.

Postoperative Care

All patients received their postoperative care in the PICU under the direction of a pediatric intensivist. Patients were commenced on a standardized pathway which included a targeted sedation protocol; ventilation and weaning were undertaken by a respiratory therapist using an institutional algorithm incorporating a lung-protective strategy, standing orders for fluids which include baseline infusion at 75% maintenance and 5% albumin fluid boluses starting at 5 ml/kg to achieve patient-specific hemodynamic targets. In general, milrinone was commenced on all patients in the operating room, unless deemed by the operating room team to not be warranted. Similarly, inotropes were commenced in the operating room and PICU on a patient-specific basis. Finally, protocols are in place for the management of postoperative junctional ectopic tachycardia and hypertension.

Prospective Study Protocol

To limit discomfort and reduce infection risk, blood sampling was combined with clinically indicated tests using arterial or central venous catheters. The preoperative sample was collected after induction of anesthesia and prior to surgical incision from the arterial catheter. The postoperative samples were obtained at PICU admission, 4–8 h after admission, and with morning blood work on postoperative days 1 and 2. If arterial or central venous catheters had been discontinued, sample collection was attempted with clinically

indicated venipuncture. For the final 12 study participants, intraoperative specimens were collected to describe changes in vitamin D levels with the following intraoperative events: (1) blood immediately following CPB initiation, (2) blood on CPB prior to MUF (3) blood on CPB following MUF, and (4) 2 ml of the final MUF.

Vitamin D Status

Serum 25 hydroxyvitamin D (25OHD) is considered the best indicator of vitamin D status and was assayed in batches following discharge of patients from the PICU using a previously described liquid chromatography–mass spectrometry procedure.[24] The reported 25OHD concentrations represent both $25OHD_2$ and $25OHD_3$ metabolites. Vitamin D deficiency and severe deficiency were defined as 25OHD less than 50 and 25 nM, respectively.[25–27]

Clinical Data Collection

Relevant clinical data were prospectively collected and entered into a computerized database. Demographic variables included age, gender, weight, and ethnicity. Preoperative information on vitamin D intake was obtained from a food frequency survey. Details on the cardiac lesion including the risk-adjusted classification for congenital heart surgery scores were recorded.[28] Preoperative nutrition was assessed using both the Gomez (weight for age) and Waterlow (weight for length) approaches.[29] Intraoperative information gathered included the CPB duration, prime circuit volume, components in CPB prime, calcium administration, aortic cross-clamp time, and total surgery time. Fluid administered in the PICU, and duration of mechanical ventilation and PICU stay were also recorded. Postoperative administration of catecholamines was recorded and maximum inotrope score was calculated as previously published.[30] Albumin, phosphate, and pH-corrected ionized calcium concentrations were recorded from the beginning of anesthesia to postoperative day 3. Hypocalcemia was defined as an ionized value less than 1.1 mM.[31,32]

Retrospective AIP CHD Cohort

To confirm study findings, we completed a secondary analysis of clinical data and biological samples collected on a separate group of postoperative CHD patients who participated in a large multicenter study known as AIP.[23] AIP was a prospective cohort study conducted in seven tertiary care PICUs across Canada. There was no patient overlap between the prospective and retrospective cohorts. Blood samples were collected on study participants within 24 h of PICU admission. Institutional Review Board approval for this sub-study was obtained from six centers representing 128 of the original 149 AIP CHD patients.

Statistical Analysis

In addition to a description of the study cohort, the *a priori* statistical plan included an evaluation for changes in 25OHD levels over time, patient predictors of 25OHD levels, and associations between 25OHD levels and postoperative clinical course. A number of *post hoc* analyses were performed, including (1) a comparison of pre- and postoperative vitamin D status by CPB requirements, (2) comparison of vitamin D levels from intraoperative blood and MUF samples, (3) investigation of methylprednisolone and nutritional status as predictors of postoperative vitamin D status and their inclusion in the multivariate models. Presentation of vitamin D levels from CHD patients who participated in the AIP study, and exploration of potential associations with postoperative clinical course, also represented a *post hoc* secondary analysis. Subgroup and *post hoc* analysis replicate less well than other analyses.

Descriptive statistics are presented for the study populations with results for continuous variables provided as means with SDs or medians with interquartile range values. Results for categorical variables were provided as percentages with counts or 95% CI. A mixed linear model was used to evaluate for statistically significant differences in 25OHD levels longitudinally through the perioperative period; contrasts were used to assess differences at prespecified time points between groups. Univariate associations between 25OHD levels and participant characteristics were sought using chi-square or Fisher exact tests for categorical variables, and *t* tests, ANOVA, and Wilcoxon tests for continuous variables.

Linear regression and analysis of covariance were used to investigate potential predictors of pre- and postoperative 25OHD concentration. For clinical outcomes, the association with 25OHD levels was investigated using logistic regression for postoperative catecholamine administration, and linear regression for postoperative fluid requirements. Multivariate regression analysis was used to build a multipredictor model for postoperative 25OHD level and to control for potential confounders in assessing the association between 25OHD and clinical outcomes. A maximum of six and three variables were considered in linear and logistic regression models, respectively. The following measures were employed to avoid multivariate model over-fitting: (1) only variables with $P < 0.20$ in univariate analysis were considered, (2) only variables with P values less than 0.05 were retained, (3) due to anticipated high correlations between age and weight (spearman coefficient, 0.91), and CPB and aortic cross-clamp times (spearman coefficient, 0.88), only the most significant was retained for each regression analysis. A forward regression approach was used for evaluating the association between patient characteristics, 25OHD, and clinical outcomes. A sensitivity analysis performed by placing all the potentially predictive variables into the final multivariate models based on the significant results gave consistent results. Estimates and odds ratio for continuous variables were calculated per 10 nM increase in 25OHD; kilogram increase in preoperative weight; 10-min increase in total surgery, CPB and aortic cross-clamp times; 1 cc/kg increase in circuit prime volume; 1 ml/kg of total

operating room fluid; 0.1 unit increase in weight for age ratio; 1 unit increase in weight for length z score. Reference level for categorical variables were female, over 2 months of age, summer season, no genetic syndrome, both white parents, no methylprednisolone, risk-adjusted classification for congenital heart surgery score category 1 or 2, reported vitamin D intake less than 400 IU/day, no CPB during cardiac surgery, no platelets, no erythrocyte, and no fresh frozen plasma during the operation. For the logistic regression analysis, likelihood ratio statistic *P* values were used to evaluate variable contribution to the model. Cox regression was used to consider the association between vitamin D status and time to extubation and PICU discharge. For statistical analyses, a *P* value less than 0.05 was considered statistically significant, unless otherwise indicated. Specifically, as there were four contrasts in the evaluation of 25OHD levels over time, Bonferroni adjustment established the *P* value for statistical significance at 0.0125. Given the five time period contrasts in the comparison of 25OHD levels by catecholamine requirement, Bonferroni adjustment established a *P* value for significance of 0.01. Analyses were performed with the SAS software (Version 9.3, Copyright SAS Institute Inc., Cary, NC).

Results

Study Group Demographics

A total of 58 children with CHD were enrolled; a flow diagram showing screening, eligibility, and consent is provided in Supplemental Digital Content 1 (figure 1, http://links.lww.com/ALN/A918). Baseline characteristics for study participants are presented in table 1. The median age was 6 months, 57% were male, and 84% were identified as having two Caucasian parents. As shown in table 1, 76% of the CHD cohort had lesions corresponding to risk-adjusted classification for congenital heart surgery category 2 or 3. Information on individual patient lesions is available in Supplemental Digital Content 2 (table 1, http://links.lww.com/ALN/A919).

Preoperative 25OHD Concentrations

The mean total preoperative 25OHD level (D_2 + D_3) was 58.0 nM (SD, 22.4). The prevalence of moderate vitamin D deficiency was 42% (CI, 30–55) and 4% (CI, 1–12) for severe deficiency (table 2). The results of simple linear regression evaluating associations between preoperative 25OHD and 12 different patient characteristics can be found in Supplemental Digital Content 2 (table 2, http://links.lww.com/ALN/A919). Of these, only age less than 2 months (representing cutoff for maternal vitamin D levels) and preoperative administration of methylprednisolone were statistically associated with preoperative 25OHD. These two variables have significant correlation as only neonates undergoing CPB receive steroid at our institution (Spearman coefficient 0.64, *P* = 0.0001). After controlling for the most significant variable (age less than 2 months, *P* = 0.01),

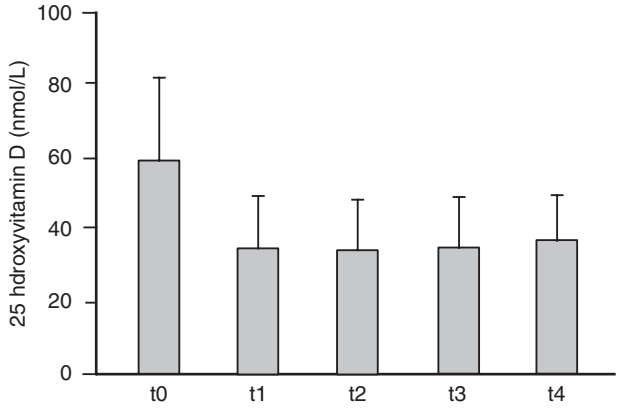

Fig. 1. Vitamin D levels before and after surgery. Graph shows group mean 25 hydroxyvitamin D levels with SDs (*error bars*) at five perioperative time points. Time points: t0, preoperative; t1, pediatric intensive care unit (PICU) admission; t2, 4–8 h after PICU admission; t3, morning postoperative day 1; t4, morning postoperative day 2.

Table 1. Demographic and Clinical Characteristics of Study Participants

Patient Characteristic	Result (n = 58)
Age, mo	8.4 (2.4–66.5)
Weight, kg	6.5 (4.3–12.8)
Male gender	56.9% (33)
Season of surgery	
Summer	27.5% (16)
Fall	24.1% (14)
Winter	31.0% (18)
Spring	17.2% (10)
RACHS	
Category 1	15.5% (9)
Category 2	41.3% (24)
Category 3	34.4% (20)
Category 4	8.6% (5)
Ethnicity*	
Any Caucasian	87.9% (51)
Both Caucasian	84.4% (49)
First Nations	6.9% (4)
African American	5.2% (3)
Other†	3.4% (2)
Genetic syndrome	
Trisomy 21	13.7% (8)
Digeorge/22q11	1.7% (1)
Emanuel syndrome	1.7% (1)
Unknown	1.7% (1)

Values are percentages with counts or medians with interquartile ranges.

* Percentages may add up to more than 100% as more than one ethnicity was recorded. † Other included Filipino (n = 1), South Asian (n = 1), and Iranian (n = 1).

RACHS = risk-adjusted classification for congenital heart surgery.

Table 2. Vitamin D Status and Deficiency Rates by Perioperative Time Point

Time Point	25OHD nM	% Deficiency*	% Severe Deficiency†
Preoperative (n = 57)	58.0 (22.4)	42 (29–55)	4 (0–8)
PICU admission (n =56)	34.2 (14.5)	86 (77–95)	27 (15–38)
Postoperative, 4–8 h (n = 50)	33.9 (13.5)	86 (76–96)	20 (9–31)
Postoperative, day 1 (n = 54)	34.2 (13.6)	87 (78–96)	24 (13–36)
Postoperative, day 2 (n = 47)	36.5 (12.2)	87 (78–97)	9 (1–17)

Values are means with SDs or percentages with 95% CIs.

* Deficiency defined as 25OHD level less than 50 nM. † Severe deficiency defined as 25OHD level less than 25 nM.

25OHD = 25 hydroxyvitamin D; PICU = pediatric intensive care unit.

neither methylprednisolone nor genetic syndrome improved the predictive ability of the model or was statistically significant (Supplemental Digital Content 2, table 2, http://links.lww.com/ALN/A919). Neither of the nutritional variables, weight for age or weight for length, was associated with preoperative vitamin D status.

Postoperative 25OHD Concentrations

The mean 25OHD level at PICU admission was 34.2 ± 14.5 nM, with 86% (CI, 77–95) meeting the criteria for moderate vitamin D deficiency and 27% (CI, 15–38) for severe deficiency (table 2). The 25OHD levels were determined to change significantly over the perioperative time course (fig. 1; $P < 0.001$). More specifically, the difference in pre- to immediate postoperative levels was significant (58.0 *vs.* 34.2 nM, $P < 0.001$) after Bonferroni adjustment.

Intraoperative 25OHD Decline, Timing, and Mechanism

The mean group decline in 25OHD was 25.0 ± 19.2 nM, representing a mean change of −39.6 ± 21.2% from the preoperative level. Initially, we explored the role of CPB in the intraoperative decline through a comparison of the percentile change in 25OHD between the four patients with coarctation of the aorta who did not undergo CPB (n = 4) and the remaining patients. As shown in figure 2A, the percent decline in 25OHD was statistically different between the CHD groups who did (41.8%, CI, 47.5–36.2) and did not require CPB (11.1%; CI, −9 to 31; $P = 0.01$). The difference in intraoperative decline remained significant when the comparison was restricted to neonates who did and did not require CPB (46.2 *vs.* 11.4%; $P = 0.01$). To explore the role of CPB in the intraoperative decline and determine whether the proposed CPB-induced change was acute or gradual, 25OHD levels were determined from blood sampled at three key points on CPB for the final 12 study participants (fig. 2B). A significant drop in 25OHD concentration was observed between the blood collected preoperatively and immediately following initiation of CPB (40.5%; CI, 28.5–52.5; $P = 0.0001$). No further decline in 25OHD was evident between the immediate post-CPB samples and any other intraoperative point. The 25OHD level in the MUF was 6.4 ± 3.5 nM.

Predictors of Postoperative 25OHD

The association between postoperative 25OHD and 18 demographic, preoperative, and intraoperative characteristics was initially considered through simple linear

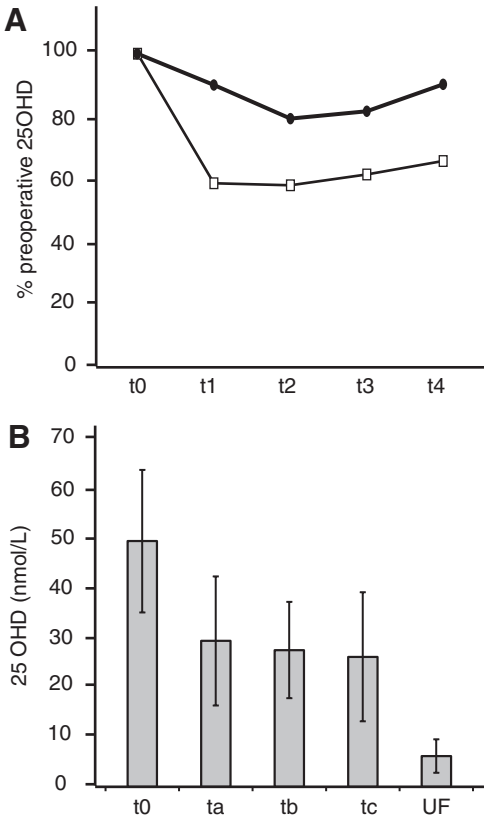

Fig. 2. Role of cardiopulmonary bypass (CPB) in the intraoperative decline in vitamin D levels. *A*, Compares 25 hydroxyvitamin D (25OHD) levels in study participants who did (*open squares*) and did not require CPB (*black circles*). Graph shows the group mean percent of the preoperative value at each perioperative time. Time points: t0, preoperative; t1, pediatric intensive care unit (PICU) admission; t2, 4–8 h after PICU admission; t3, morning postoperative day 1; t4, morning postoperative day 2. *B*, Graph shows group mean 25OHD levels with SDs (*error bars*) in preoperative and intraoperative samples for 12 study participants. Time points: t0, preoperative; ta, after CPB initiation; tb, on CPB before modified ultrafiltration (MUF); tc, following MUF; UF, final ultrafiltrate sample.

Table 3. Multivariate Linear Regression Model for Postoperative 25OHD Level

Characteristic	Adjusted Estimate	95% CI	*P* Value
Preoperative 25OHD	0.34	0.21 to 0.47	0.0001
Had CPB	–23.60	–11.2 to –47.5	0.002
Weight, kg	0.48	0.24 to 0.72	0.0002
Preop MP	–3.99	–11.54 to 4.52	0.38
Cross-clamp time	–0.18	–0.63 to 0.26	0.42
Prime volume per kg	–0.03	–0.26 to 0.21	0.83

Variables with *P* values > 0.2 in simple linear regression are provided in Supplemental Digital Content 2 (table 3, http://links.lww.com/ALN/A919). Estimates not calculated for age and CPB time due to high correlation with weight and cross-clamp time. *P* values for insignificant variables represent those calculated when tested individually in multivariate analysis with the significant predictors (*post hoc* sensitivity analysis).

25OHD = 25 hydroxyvitamin D; CPB = cardiopulmonary bypass; MP = methylprednisolone.

regression (see Supplemental Digital Content 2, table 3, http://links.lww.com/ALN/A919). As shown in table 3, of the potentially predictive variables from simple linear regression only three remained independently associated with postoperative 25OHD in multivariate analysis: lower preoperative 25OHD, need for CPB, and preoperative weight.

Postoperative Catecholamine Administration and 25OHD Levels

The relationship between 25OHD levels and postoperative cardiovascular dysfunction was evaluated using catecholamine requirements. Figure 3 compares pre- and postoperative 25OHD levels in the CHD groups who did (n = 18) and did not (n = 40) require catecholamines postoperatively. The difference in preoperative 25OHD did not achieve statistical significance (60 ± 21.7 *vs.* 54 ± 24 nM; $P = 0.32$). Conversely, there was a statistically significant difference between those participants who did and did not require catecholamines at all postoperative time points with Bonferroni adjustment (PICU admission, 26.5 ± 10 *vs.* 38.2 ± 15 nM, respectively; $P = 0.007$).

The association between postoperative catecholamines, 25OHD status, and 15 other demographic, preoperative, and intraoperative characteristics was considered through simple logistic regression (see Supplemental Digital Content 2, table 4, http://links.lww.com/ALN/A919). For vitamin D, the unadjusted analysis determined that every 10 nM increase in postoperative 25OHD decreased the odds of catecholamine administration by 57% (odds ratio, 0.43; CI, 0.22–0.84). As shown in table 4, multivariate regression identified that only three variables were independently associated with catecholamine administration. After controlling for aortic cross-clamp and preoperative weight, the odds ratio for postoperative 25OHD was unchanged (odds ratio, 0.50) and remained statistically significant ($P = 0.04$). No association between postoperative 25OHD and risk-adjusted classification for congenital heart surgery was observed in adjusted or unadjusted analysis ($P > 0.20$).

Clinical Outcome and Vitamin D Status

The association between severe vitamin D deficiency and other established markers of organ dysfunction and morbidity was investigated by grouping study participants based on severe deficiency status (table 5). Postoperative vitamin D status was determined from the first available postoperative sample, with severe deficiency defined as a level less than 25 nM. As shown in table 5, a statistically significant difference in postoperative fluid requirements (per kg) was determined for CHD patients with severe deficiency ($P = 0.001$). After multivariate linear regression analysis, including an evaluation of 16 other patient characteristics, 25OHD remained independently associated with postoperative fluid requirements (table 6). As shown in table 5, there was a significant association between severe vitamin D deficiency and median duration of intubation. Further evaluation using a time to extubation analysis calculated a hazard ratio of 1.3 (CI, 1.1–1.6; $P = 0.007$) for each 10 nM 25OHD increase. A statistical trend toward longer PICU length of stay determined a time to discharge hazard ratio of 1.2

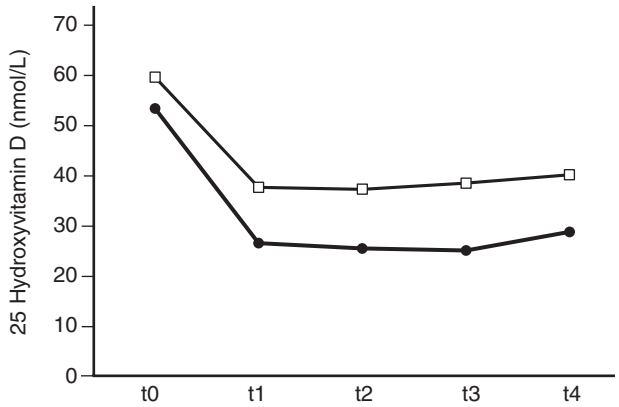

Fig. 3. Vitamin D levels by postoperative catecholamine requirements. Mean 25 hydroxyvitamin D levels are shown for study participant groups who did (*black circles*) and did not receive catecholamines (*white squares*). Time points: t0, preoperative; t1, pediatric intensive care unit (PICU) admission; t2, 4–8 h after PICU admission; t3, morning postoperative day 1; t4, morning postoperative day 2.

Table 4. Multivariate Logistic Regression Analysis of Postoperative Catecholamine Administration

Characteristic	Adjusted Odds Ratio	95% CI	LR *P* Value
Cross-clamp time	1.18	1.02–1.36	0.015
Postoperative 25OHD	0.50	0.23–1.08	0.04
Weight, kg	0.88	0.75–1.04	0.04
WFL z score	1.44	0.83–2.50	0.19
RACHS lesion three or four	1.73	0.43–7.18	0.40
Prime volume per kg	1.04	0.98–1.10	0.21
Total OR fluid per kg	1.02	0.99–1.06	0.15

P values calculated using likelihood ratio statistic. *P* values for insignificant variables represent those calculated when placed individually in multivariate regression with postoperative 25OHD and cross-clamp time in *post hoc* sensitivity analysis. Variables with *P* values > 0.2 in simple logistic regression are provided in Supplemental Digital Content 2 (table 4, http://links.lww.com/ALN/A919). Estimates not calculated for age and CPB time due to high correlation with weight and cross-clamp time.

25OHD = 25 hydroxyvitamin D; CPB = cardiopulmonary bypass; LR = likelihood ratio; OR = operating room; RACHS = risk-adjusted classification for congenital heart surgery; WFL = weight for length.

(CI, 1.0–1.4; $P = 0.06$) for each 10 nM rise in postoperative 25OHD. No relationship between vitamin D status and hypocalcemia or calcium administration was observed.

Retrospective Analysis of AIP Cohort

Serum was available for 122 of the 128 eligible AIP CHD study participants, with three sites contributing 120 samples. AIP CHD participants had a median age of 10 months and were 54% male. Information on cardiac lesion and surgery were used to compare 25OHD levels in patients with lesions who did and did not require CPB (39.9 nM ± 16.9 *vs.* 59.5 nM ± 18.3; $P < 0.001$). Mean 25OHD was not statistically different between the three main sites, ranging from 37.8 to 46.9 nM ($P = 0.11$). Of the AIP CHD study participants, 73% (CI, 65–80%) were vitamin D deficient immediately postoperatively. Statistically lower 25OHD were measured in participants requiring postoperative catecholamines (44.4 nM ± 19.1 *vs.* 36.6 nM ± 13.7; $P = 0.03$). Logistic regression determined that for every 10 nM rise in 25OHD, the odds of catecholamine use decreased by 25% (odds ratio, 0.75; CI, 0.58–0.98). For each 10 nM increase, Cox regression calculated a time to extubation hazard ratio of 1.1 (CI, 1.00–1.2; $P = 0.04$) and time to PICU discharge hazard ratio of 1.1 (CI, 1.02–1.22; $P = 0.02$).

Discussion

The results of this prospective study demonstrate that almost all CHD patients are vitamin D deficient following cardiac surgery, with lower levels independently associated with catecholamine requirements, fluid administration, and duration of intubation. The high prevalence of postoperative vitamin D deficiency was conferred by borderline normal or low preoperative levels and an acute intraoperative decline. Postoperative vitamin D status and association with clinical outcomes was confirmed in a secondary analysis of CHD patients who participated in the AIP study.[23]

Using accepted criteria for vitamin D status, many patients had borderline-normal or low vitamin D levels (mean 25OHD of 58 nM) prior to surgery, with 42% of the study cohort being deficient.[25–27] These levels are comparable, but slightly below, those reported on healthy Canadian children who have means closer to 75 nM.[33–35] Higher levels

Table 5. Comparison of Clinical Outcome Measures by Vitamin D Status

Outcome Measure	Vitamin D ≥25 nM (n = 40)	Vitamin D <25 nM (n = 18)	*P* Value
Received catecholamines	22% (9)	53% (8)	0.04
Inotrope score	11.25 (11.25–11.25)	12.25 (11.25–13.25)	0.02
Postop fluid boluses*	6 (3–9)	14 (8–17)	0.006
Total fluid intake, ml/kg*	276 (190–319)	427 (289–575)	0.001
Hypocalcemia†	29% (12)	33% (5)	0.76
No. of calcium boluses	1 (0–3)	1 (0–5)	0.33
Intubation duration, h	10 (2–119)	94 (22–250)	0.04
PICU length of stay, d	4 (3–8)	11 (4–15)	0.08

Values are percentages with counts or medians with interquartile ranges. *P* values calculated using Fisher exact test for percentages and Wilcoxon test for medians.

* Represents value over first three postoperative days. † Hypocalcemia defined as a pH corrected ionized value less than 1.1 mM.

PICU = pediatric intensive care unit.

Table 6. Multivariate Linear Regression Analysis of Postoperative Fluid Intake

Characteristic	Adjusted Estimate	95% CI	*P* Value
Postoperative 25OHD	–29.30	–52.74 to –5.87	0.015
Age, mo	–1.67	–2.43 to –0.91	0.0001
Preoperative MP	28.53	–72.84 to 129.91	0.57
Cross-clamp time	3.16	–3.21 to 9.522	0.32
Prime volume per kg	0.81	–2.37 to 4.00	0.61
Total OR fluid per kg	0.65	–0.30 to 1.60	0.17

P values for insignificant variables represent those calculated when placed individually in multivariate regression with the significant predictors in *post hoc* sensitivity analysis. Estimates not calculated for age less than 2 months and preoperative weight due to high correlation with preoperative methylprednisolone and age. Variables with *P* values > 0.2 in simple logistic regression are shown in Supplemental Digital Content 2 (table 5, http://links.lww.com/ALN/A919).

25OHD = 25 hydroxyvitamin D; MP = methylprednisolone; OR = operating room.

of preoperative 25OHD would have been expected given well-known recommendations for vitamin D supplementation and close supervision of CHD patients by a large group of health-care providers during the pre- and postnatal periods.[26,27,36] Inadequate preoperative vitamin D concentrations suggest either poor compliance with guidelines or that requirements for CHD patients differ from healthy children.

This study describes the novel observation of a CPB-associated 40% acute fall in serum 25OHD. Although we did not test for it specifically, it is highly unlikely that anesthetic technique factored into the decline in vitamin D. In fact, the results further indicate that the decline occurs immediately following initiation of CPB, suggesting a dilutional effect from the prime volume. Two recent adult studies have also described similar changes in vitamin D status associated with CPB.[19,37] Zitterman *et al.*[37] reported an approximate 25% drop following heart transplantation, but could not comment on the mechanism or timing as levels were measured on the sixth postoperative day. In a study of 19 adult patients, Krishnan *et al.*[19] demonstrated that CPB prime acutely reduces serum 25OHD, with levels returning to near normal following diuresis at 72 h. Other potential explanations for the intraoperative fall include absorption of 25OHD on the CPB tubing or oxygenator membrane. The absence of significant 25OHD in the ultrafiltrate indicates minimal loss due to MUF. While there may not be a clear explanation for why levels fall abruptly and remain depressed, the scale of the fall in levels and the strong association between low levels and clinically significant outcomes add weight to the body of evidence regarding the importance of this hormone in acute critical illness.

An important study finding is the temporal association between low postoperative 25OHD levels and postoperative catecholamine and fluid requirements, established measures of cardiovascular and immune dysfunction. Instinctively, vitamin D could negatively affect cardiovascular health through calcium homeostasis. Alternatively, low vitamin D concentrations could influence cardiac myocyte and endothelial function directly through cellular vitamin D receptors rapidly altering signal transduction, enzyme activity, and both gene and protein expression.[38,39] The described relationship between postoperative fluid requirements and vitamin D status would also be consistent with a greater systemic inflammatory response. Basic science research has shown that impaired immunomodulation and pathogen defense may be mediated directly through vitamin D receptors on immune cells, altering cell proliferation, cytokine release, and antimicrobial peptide production.[40–43] Finally, vitamin D deficiency could impair gas exchange and influence ventilator requirements through nerve dysfunction and muscle weakness.[44,45]

The major strength of this study is the prospective observational design, and the collection of intraoperative samples. The longitudinal design helps avoid the criticisms commonly applied to cross-sectional studies, and inability to determine when vitamin D deficiency arose. Importantly, we were able to demonstrate that postoperative vitamin D levels were determined well before CPB separation and are therefore unrelated to the pathophysiology, procedures, and interventions that occur postoperatively. This temporal order is an essential component when assessing for causation.

The biggest limitations of this study are patient heterogeneity and sample size. The total number of patients (n = 58) along with their heterogeneity in terms of age and disease severity limits the strength of conclusions drawn from multifactorial analysis. Such heterogeneity can also be perceived as beneficial as the study population is generalizable to many cardiac surgical programs. Yet, the primary outcome remained significant even after statistically allowing for a heterogenous study population. Furthermore, given the sample size, it is possible that demonstrated association between 25OHD and clinical outcomes represents a random or chance finding. We were able to partially overcome this limitation using postoperative blood collected on 122 CHD patients from the AIP study and confirm our single-center observations.[23] In addition, it remains possible that the demonstrated association between vitamin D and clinical outcome represents the results of an unmeasured association between vitamin D and another essential nutrient or nutritional status. As a descriptive study, the results cannot be used to prove that vitamin D supplementation and maintenance of vitamin D status following postcardiac surgery will

improve outcomes; this hypothesis will need to be tested in future randomized controlled studies. Finally, it is important to note that blood 25OHD may not accurately reflect vitamin D status in postoperative CHD patients if unmeasured dysfunction of the parathyroid and renal organs limits or reduces the conversion of 25OHD to active hormone (calcitriol). A study evaluating all components of the vitamin D axis could demonstrate a stronger relationship with clinical outcomes.

In conclusion, this report provides clear evidence that borderline normal vitamin D levels combined with an acute decline in vitamin D levels during cardiac surgery lead to high rates of vitamin D deficiency postoperatively. These findings suggest that extrapolation of supplementation recommendations for healthy children to the preoperative CHD patient is inadequate to maintain adequate vitamin D levels. Furthermore, our results indicate that lower postoperative levels are associated with heart dysfunction and other markers of organ dysfunction. This finding is supported by a recent interventional study on children with heart failure in a nonoperative setting.[46] Interventional studies will be required to determine if supplemental vitamin D in the CHD patient population will improve outcomes. Finally, the change in vitamin D levels that occurs during other complex pediatric surgeries and the association with anesthetic and operative procedures should be explored.

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