Total and free cortisol responses and their relation to outcomes after cardiopulmonary bypass in infants

Eric L. Wald, MD, MSCI,^a Carl L. Backer, MD,^b Joseph A. Dearani, MD,^c Zhuo Li, MS,^d William C. Oliver, MD,^e and Sheri S. Crow, MD^f

ABSTRACT

Background: Hypothalamic-pituitary-adrenal (HPA) axis dysfunction may be partially responsible for the hemodynamic instability experienced by infants after cardiopulmonary bypass (CPB). We report the full spectrum of the HPA response surrounding CPB for infant congenital cardiac surgery.

Methods: We enrolled 84 infants who received 1 mg/kg of dexamethasone before initiation of CPB. Total cortisol (TC), free cortisol (FC), adrenocorticotropic hormone (ACTH), and corticosteroid-binding globulin (CBG) were measured at 3 time points: immediately before CPB (TP1), on intensive care unit arrival (TP2), and at 24 hours after surgery (TP3). A 1- μ g ACTH stimulation test was performed at each time point to evaluate adrenal responsiveness.

Results: Sixty-eight infants completed all study procedures. Levels of TC, FC, CBG, and ACTH decreased significantly between the preoperative and 24-hour postoperative measurements. There were no significant associations between preoperative FC responses and clinical outcomes after adjusting for weight and Risk-Adjusted Scores for Congenital Heart Surgery. Infants with subnormal TC responses to ACTH stimulation ($<9 \mu\text{g/dL}$) at TP2 had greater fluid requirements ($P < .001$) and greater chest tube output ($P < .001$) during the first 24 hours, as well as longer length of stay (LOS) ($P = .007$). Except for LOS, these differences persisted for infants with subnormal stimulation tests at TP3.

Conclusions: We observed a significant decline in all aspects of the HPA axis throughout the first 24 hours after infant CPB. TC and FC levels were not associated with clinical outcomes. Subnormal ($\Delta < 9 \mu\text{g/dL}$) TC response to cosyntropin stimulation during the postoperative period was associated with increased fluid resuscitation and greater LOS. (J Thorac Cardiovasc Surg 2017;153:1155-63)

Suppression of perioperative free cortisol in 68 children undergoing CPB. * $P < .0001$ compared with TP1; $^{\wedge}P = .02$ compared with TP2.

Central Message

Total cortisol, but not free cortisol, response after the postoperative ACTH stimulation test may identify infants at risk for poor outcomes.

Perspective

Adrenal insufficiency is often invoked as a cause of hypocortisolemia after cardiopulmonary bypass. Previous studies have failed to measure free cortisol and have not tested adrenal insensitivity with ACTH stimulation. This study measures all components of the stress response, including free cortisol and response to low-dose ACTH, to truly evaluate the response of the infant hypothalamic-pituitary-adrenal axis to bypass.

See Editorial Commentary page 1164.

From the ^aDivisions of Critical Care and Cardiology, Department of Pediatrics and ^bDivision of Cardiovascular-Thoracic Surgery, Department of Surgery, Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, Ill; and Divisions of ^cCardiothoracic Surgery and ^dStatistics, ^eDepartment of Anesthesiology, and ^fDivision of Pediatric Critical Care, Department of Pediatrics and Adolescent Medicine, Mayo Clinic, Rochester, Minn.

This study was performed at Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine and the Mayo Clinic.

Received for publication June 9, 2016; revisions received Oct 7, 2016; accepted for publication Nov 4, 2016; available ahead of print Dec 22, 2016.

Address for reprints: Eric L. Wald, MD, MSCI, Division of Critical Care, Ann & Robert H. Lurie Children's Hospital of Chicago, Box 73, 225 E Chicago Ave, Chicago, IL 60611 (E-mail: Ewald@luriechildrens.org).

0022-5223/\$36.00

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<http://dx.doi.org/10.1016/j.jtcvs.2016.11.030>

Infants who undergo congenital heart surgery and experience hemodynamic instability following cardiopulmonary bypass (CPB) are at increased risk for morbidity and mortality.¹ CPB elicits a complex activation of cellular, humoral, cytokine, and complement cascades; endothelial changes; endotoxin release; and production of oxygen free radicals.² In response, the body releases endogenous

Scanning this QR code will take you to a video for the article.

Abbreviations and Acronyms

ACTH	= adrenocorticotrophic hormone
CBG	= corticosteroid-binding globulin
CPB	= cardiopulmonary bypass
CV	= coefficient of variation
FC	= free cortisol
HPA	= hypothalamic-pituitary-adrenal
IV	= intravenously
LCH	= Lurie Children's Hospital
LC-MS/MS	= liquid chromatography–tandem mass spectrometry
LOS	= length of stay
MC	= Mayo Clinic
RACHS	= Risk-Adjusted Scores for Congenital Heart Surgery
TC	= total cortisol
TP	= time point

glucocorticoids that modulate the inflammatory response by altering the proinflammatory and anti-inflammatory cytokine balance.³ Glucocorticoids influence hemodynamic stability through regulation of vascular tone and permeability, endothelial integrity, and enhanced vascular responsiveness to circulating catecholamines.^{4,5} Any perturbation of this innate corticosteroid response to stress, whether intrinsic or iatrogenic, has the potential to impact perioperative cardiovascular stability.

Early identification of adrenal dysfunction before a neonate or infant becomes clinically symptomatic could significantly impact morbidity and mortality. Children with an inadequate corticosteroid response to CPB-related stressors may benefit from exogenous steroid supplementation.⁶⁻⁹ Hypocortisolemia has been reported frequently after CPB in children,^{6,10-12} and although its etiology remains unclear, low cortisol levels often invoke a diagnosis of adrenal insufficiency. Nonetheless, it is standard practice in many heart centers to administer perioperative steroids to attenuate the inflammatory response of CPB, which may suppress native cortisol generation postoperatively. Alternatively, hypocortisolemia, and perhaps adrenal dysfunction, may be sequelae of CPB secondary to an overwhelming proinflammatory state.

Investigations reported to date have faced significant methodological challenges that include inconsistencies in the definition of a “normal” cortisol response and diverse approaches to both clinical and laboratory evaluation of the hormonal stress response. Previous studies have almost exclusively measured total cortisol (TC; bound) levels rather than biologically active free cortisol (FC) levels.^{10,13,14} CPB depletes corticosteroid-binding globulin (CBG),¹¹ and thus TC level can underestimate the actual

stress response. Recent studies have suggested that FC may provide a superior representation of the dynamic changes observed in the hypothalamic-pituitary-adrenal (HPA) axis during critical illness.^{15,16}

Our objective in the present study was to address these knowledge gaps and characterize the HPA response surrounding CPB for neonatal and infant congenital cardiac surgery. We measured TC, FC, CBG, and adrenocorticotrophic hormone (ACTH) levels and performed ACTH stimulation tests before, immediately after, and 24 hours after CPB. We hypothesized that children with lower TC and FC responses to ACTH stimulation after cardiac surgery would have poorer clinical outcomes in the first 24 postoperative hours and beyond. To our knowledge, this is the first study to simultaneously measure cosyntropin response and TC, FC, CBG, and ACTH levels in an attempt to comprehensively evaluate infants' HPA responses to CPB.

PATIENTS AND METHODS

This prospective observational study was approved by the Lurie Children's Hospital (LCH) and Mayo Clinic (MC) Institutional Review Boards. Written informed parental consent was obtained for all children.

Children were included if they were age <365 days and required CPB during cardiac surgery performed between 2011 and 2013. Children who received steroids within the 3 months before surgery, received etomidate for anesthesia induction, or weighed <3 kg were excluded.

CPB Protocol

Patients were treated with the institution's standard CPB protocol for size, age, and specific lesion. All patients at LCH received 1 mg/kg dexamethasone intravenously (IV) directly before CPB. Patients at MC received 0.5 mg/kg dexamethasone IV after anesthesia induction and 0.5 mg/kg IV directly before CPB, for a total dexamethasone dose of 1 mg/kg.

Anesthesia

All patients had arterial and central venous catheters in place, and received cefazolin for perioperative prophylaxis unless allergic to penicillin. At MC, anesthesia induction was achieved with fentanyl, midazolam, a muscle relaxant, and titration of a volatile anesthetic agent. At LCH, anesthesia induction generally involved a sevoflurane inhalation induction and/or IV or intramuscular ketamine. Heparin was given for anticoagulation before CPB. For heparin reversal, protamine was administered to all patients after CPB and completion of modified ultrafiltration.

Investigational Laboratory Tests

A 1- μ g cosyntropin stimulation test was performed, and levels of TC, FC, ACTH, and CBG were measured at 3 separate time points (TPs): TP1, before CPB (after anesthetic induction, before sternotomy and dexamethasone administration); TP2, on arrival at the intensive care unit; and TP3, at 24 hours after TP2.

Once the baseline blood sample was drawn, a 1- μ g dose of cosyntropin was administered IV. Thirty minutes later, blood was drawn to measure post-cosyntropin changes in the TC, FC, CBG, and ACTH levels. The reference ranges are 3-21 μ g/dL for total plasma cortisol, 19-45 mL/L for CBG, and 10-60 pg/mL for ACTH.¹⁷ A normal TC increase after cosyntropin administration was defined as >9 μ g/dL.¹⁸ Adequate FC in critical illness was defined as >2.0 μ g/dL.¹⁵ A normal FC after the cosyntropin test was defined as >3.1 μ g/dL.¹⁵

TC and FC

FC was measured in serum using a commercially available equilibrium dialysis plate (Thermo Fisher Scientific, Waltham, MA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Sample was added to the equilibrium dialysis plate in duplicate. Each well contained a vertical chamber of dialysate tubing (molecular weight cutoff 8000) surrounded by dialysate buffer. Once equilibrium was reached on LC-MS/MS, the resulting dialysate was combined with deuterated internal standard and analyzed using TurboFlow liquid chromatography (Cohesive Technologies, Franklin, MA) combined with atmospheric pressure chemical ionization, and tandem mass spectrometry (API 5000; AB SCIEX, Concord, Ontario, Canada). Cortisol and its internal standard (d3-cortisol) were monitored using multiple reaction monitoring scans at m/z 363.2/121.0 and 366.2/121.0, respectively.

TC was measured by LC-MS/MS. Serum was combined with deuterated internal standard and then underwent acetonitrile protein precipitation followed by liquid-liquid extraction with methylene chloride. The resulting extracts were dried, reconstituted, and injected onto a high-performance liquid chromatography system (Cohesive Technologies) combined with electrospray ionization and tandem mass spectrometry (API 4000; AB SCIEX). Cortisol and its internal standard (d4-cortisol) were monitored using multiple reaction monitoring scans at m/z 363.2/121.0 and 367.2/121.0, respectively. Dexamethasone was monitored and has a different m/z (393/147) and, in addition, was chromatographically resolved from cortisol; therefore, it did not contribute to the cortisol results.

ACTH was measured using a solid-phase sequential immunoassay with a Siemens Immulite 2000 automated immunoassay system (Siemens Healthcare Diagnostics, Deerfield, Ill). Intra-assay coefficients of variation (CVs) were 6.4% at 5.71 pg/mL, 2.7% at 27.7 pg/mL, and 3.2% at 401.9 pg/mL, and interassay CVs were 3.1% at 28.9 pg/mL and 4.6% at 429 pg/mL. The lower limit of the CRR was 5.0 pg/mL.

CBG was measured by competitive radioimmunoassay (DIAsource ImmunoAssays, Louvain-la-Neuve, Belgium). According to the manufacturer, the intra-assay CVs were 8.6% at 23 $\mu\text{g/mL}$ and 3.9% at 83 $\mu\text{g/mL}$, and interassay CVs were 10.8% at 25 $\mu\text{g/mL}$ and 4.8% at 114 $\mu\text{g/mL}$.

Clinical Variables

The following outcome variables were measured in all patients: vital signs in the first 24 hours, oxygenation indices, ventilator settings, lactate, fluid requirements in the first 24 hours, inotrope score, length of stay (LOS), use of supplemental corticosteroids, use of inhaled nitric oxide, and use of extracorporeal membrane oxygenation. There were no standard indications for administration of postoperative corticosteroids, and this decision was left to the discretion of the attending physician.

Inotrope score was calculated according to the following formula: dopamine $\times$ 1, plus dobutamine $\times$ 1, plus milrinone $\times$ 15, plus epinephrine $\times$ 100, plus norepinephrine $\times$ 100, plus phenylephrine $\times$ 100 = total inotrope score.¹⁹

Risk-Adjusted Scores for Congenital Heart Surgery (RACHS) were recorded for each procedure.²⁰

Statistical Methods

Descriptive statistics for categorical variables are reported as frequency and percentage, and continuous variables are reported as mean $\pm$ SD or median (range) as appropriate. Correlation between continuous variables was calculated using the Spearman correlation test. Mixed models were used to assess the differences in TC, FC, ACTH, and CBG between time points on a log scale, accounting for the longitudinal data format. Continuous variables were compared between 2 groups (determined by dichotomous cosyntropin stimulation test, adequate vs inadequate) using the 2-sample t test or Wilcoxon rank-sum test where appropriate. Multivariate linear regression models were used to see whether the differences in outcomes remained significant after adjusting for weight, CPB time, and RACHS score. Owing to the nonnormal distributions of outcome variables, we also used log transformation with modeling

to confirm the results. For easier interpretation, we present the results on the original scale. Boxplots were drawn to reflect the distribution of continuous variables at different time points. All statistical tests were 2-sided, with the α level set at 0.05 for statistical significance.

RESULTS

Eighty-four children, including 38 neonates (age <30 days) and 46 infants (age 30–364 days) were enrolled across 2 centers ($n = 56$ at LCH, $n = 28$ at MC). Sixteen patients were excluded because they received supplemental dexamethasone (for extubation) in addition to the preoperative dexamethasone dose within the first 24 hours after surgery. Sixty-eight patients completed blood draws and testing at all study time points. Patient characteristics are listed in Table 1. Four neonates underwent deep hypothermic circulatory arrest during their surgeries. Cardiac lesions and their surgical corrections are presented in Table 2. Three neonates received supplemental hydrocortisone in the first 48 hours after surgery owing to evidence of low cardiac output syndrome; these patients were not excluded from our analysis. There were no deaths within 28 days of the surgery, and no patients required postoperative extracorporeal membrane oxygenation.

HPA Variables

Median TC, FC, ACTH, and CBG levels decreased steadily from TP1 to TP3 (Figure 1). The median TC level fell from 14.2 $\mu\text{g/dL}$ (IQR, 7.3–20 $\mu\text{g/dL}$) at TP1 to a nadir of 3.0 $\mu\text{g/dL}$ (IQR, 0.84–15 $\mu\text{g/dL}$) at TP3 ($P < .001$). Similarly, the median FC level dropped from 1.7 $\mu\text{g/dL}$ (IQR, 0.93–2.5 $\mu\text{g/dL}$) at TP1 to 0.22 $\mu\text{g/dL}$ (IQR, 0.06–1.9 $\mu\text{g/dL}$) at TP3 ($P < .001$). A longitudinal analysis performed to evaluate the changes in both TC and FC using mixed linear models confirmed significant declines in TC, FC, and ACTH across the time periods ($P < .001$) (Tables 3–6). FC levels were highly correlated ($r = 0.94$ – 0.98 ; $P < .0001$) with TC levels at all

TABLE 1. Overall summary statistics (all patients, $n = 68$)

Characteristic	Value
Age, mo, median (range)	4 (0.43–5.8)
Weight, kg, median (range)	4.9 (3.6–6.1)
Total cardiopulmonary bypass time, min, mean $\pm$ SD	121 $\pm$ 49
Cross-clamp time, min, mean $\pm$ SD	63 $\pm$ 38
Length of stay, d, mean $\pm$ SD	14 $\pm$ 13
Inotrope score, mean $\pm$ SD	12.8 $\pm$ 5.9
Total intubation time, h, mean $\pm$ SD	66 $\pm$ 22
Total fluids in first 24 h, mL/kg, mean $\pm$ SD	184 $\pm$ 120
Chest tube output in first 24 h, mL, mean $\pm$ SD	106 $\pm$ 120
Highest postoperative lactate value, mean $\pm$ SD	2.1 $\pm$ 1.2
Highest mean arterial pressure in the first 24 h, mm Hg, mean $\pm$ SD	68 $\pm$ 35
Lowest mean arterial pressure first 24 h, mm Hg, mean $\pm$ SD	46 $\pm$ 10

SD, Standard deviation.

TABLE 2. Congenital heart lesions/surgeries in 68 pediatric patients undergoing CPB

Lesion or repair	
Tetralogy of Fallot	11 (3 palliated with BTS shunt)
AV septal defect	11 (2 partial)
VSD and ASD	8 (1 with pulmonary valve repair/transannular patch)
Transposition of the great arteries (ASO)	7
VSD (type II)	7 (1 with aortoplasty; 1 with subaortic membrane resection)
HLHS (Norwood or DKS procedure)	6
Cavopulmonary connection (Glenn)	5
Pulmonary stenosis	2 (1 with supralvalvular patch and arterioplasty, 1 with ASD repair)
Pulmonary atresia with IVS	2 (both with modified BT shunt)
Pulmonary atresia with VSD	1 (unifocalization, RV to PA conduit)
Coarctation of the aorta (ETEA)	2
TAPVR	2
Common arterial trunk	2
Pulmonary artery sling	2 (1 with ASD)

BTS, Blalock–Taussig shunt; *AV*, atrioventricular; *VSD*, ventricular septal defect; *ASD*, atrial septal defect; *ASO*, arterial switch operation; *HLHS*, hypoplastic left heart syndrome; *DKS*, Damus–Kaye–Stansel; *IVS*, intact ventricular septum; *RV*, right ventricle; *PA*, pulmonary artery; *ETEA*, end-to-end anastomosis; *TAPVR*, total anomalous pulmonary venous return.

time points. The decrement in median ACTH was profound, from 43 pg/mL (IQR, 17–101 pg/mL) at TP1 to 5.4 pg/mL (IQR, 2.5–9.8 pg/mL) at TP3 ($P < .001$). CBG concentrations fell from a median of 36 $\mu\text{g/mL}$ at TP1 to 21 $\mu\text{g/mL}$ at TP3 ($P < .001$). Absolute CBG levels and changes in CBG levels were not associated with outcomes at any time point.

Cosyntropin Stimulation Tests and Outcomes

“Adequate” TC responses were observed in 41% of patients ($n = 28$) preoperatively at TP1. This cohort was younger and smaller ($P = .001$), but neither baseline TC levels nor TC Δ values after stimulation at TP1 were associated with postoperative outcomes. Adequate FC responses were observed in 49% of patients at TP1, and similarly this group was younger, smaller, and underwent more complex surgeries ($P = .02$). Initially it appeared that the FC response after cosyntropin administration at TP1 may identify a cohort at risk for poorer outcomes, but after adjusting for age, weight, and RACHS score in a multivariate model, there was no association between FC levels (including baseline FC levels or values after cosyntropin stimulation) and clinical outcomes. Multivariable linear regression on the log-scaled outcomes showed similar results as the original scale (Table 7). In further exploratory analyses, we also analyzed the change in FC after cosyntropin as a continuous variable in an attempt to

explore possible associations with postoperative outcomes. When analyzed as a continuous variable, the change in FC after cosyntropin at TP1 was associated with increased fluid requirements in the first 24 hours ($P = .004$) as well as increased lactate ($P = .04$), even after adjusting for age, weight, and RACHS score (data not shown).

Postoperatively, adequate TC responses were seen in 59% of the infants ($n = 40$) at TP2, and 71% of the infants ($n = 48$) at TP3. Even after adjusting for age, weight, and RACHS score, infants with inadequate response to cosyntropin at TP2 had greater mean fluid requirements (282 mL/kg vs 135 mL/kg; $P < .001$) and mean chest tube output (32 mL/kg vs 13 mL/kg; $P < .001$) in the first 24 hours, and longer mean LOS (20 days vs 11 days; $P = .007$) (Figure 2). The differences in fluid requirements and chest tube output, but not for LOS, persisted in infants with inadequate stimulation test results at TP3 (Figure 3).

The normal FC response to cosyntropin in children is unknown. FC responses to cosyntropin increased significantly at each time point. FC levels more than doubled at TP1 (median, 3.7 $\mu\text{g/dL}$), increased by 5-fold at TP2 (median, 3.4 $\mu\text{g/dL}$) and by more than 15-fold at TP3 (median, 3.7 $\mu\text{g/dL}$). Limited adult data suggest a cutoff value of 3.1 $\mu\text{g/dL}$ to define a “normal” response after cosyntropin.¹⁵ In our series, however, there was no association between clinical outcomes and FC cosyntropin responses above or below the threshold of 3.1 $\mu\text{g/dL}$ at any time point.

DISCUSSION

Our findings show that all aspects of the HPA hormonal response (TC, FC, CBG, and ACTH levels) declined consistently throughout the first 24 hours after infant cardiac surgery when preoperative dexamethasone was used. Despite this profound postoperative hypocortisolemia, the postoperative cortisol response to cosyntropin stimulation was preserved in most of the children. There was no association between hormone levels and clinical outcomes, raising further questions regarding the clinical significance of isolated hypocortisolemia in the postoperative setting. We built on previous studies and performed cosyntropin stimulation testing in parallel with directly measured TC, FC, CBG, and ACTH levels at 3 successive time points surrounding pediatric cardiac surgery. Moreover, this is the first study to directly measure rather than calculate FC in the cardiac surgery setting. Measurement of FC failed to provide novel information about the stress response post-CPB, however, except for demonstrating that FC generation after cosyntropin stimulation remains consistent throughout the 24 hours surrounding surgery. Neither baseline FC levels nor FC increases after stimulation at various thresholds were associated with clinical outcomes. Although baseline TC levels were not consistently associated with clinical outcomes, a subnormal response to low-dose

FIGURE 1. Suppression of perioperative HPA axis variables in 68 children undergoing CPB. TP1, presternotomy; TP2, arrival at the intensive care unit; TP3, 24 hours after intensive care unit arrival; endpoint of upper whisker, third quartile plus 1.5 times the IQR or maximum; upper edge of box, third quartile; horizontal line inside box, median; diamond, mean; lower edge of box, first quartile; endpoint of lower whisker, first quartile minus 1.5 times the IQR or minimum; open circle, extreme value beyond endpoints of whiskers. **P* < .0001 compared with preoperative baseline (TP1); +*P* = .005 compared with TP1; ++*P* < .003 compared with TP1; ^*P* significant compared with TP2 (see Tables 3-6 for values). ACTH, Adrenocorticotrophic hormone; CBG, corticosteroid-binding globulin.

cosyntropin stimulation was associated with increased LOS and greater fluid requirements and chest tube output in the first 24 postoperative hours.

The study population displayed profound reductions in both central and peripheral HPA hormonal responses after surgery. Even with preoperative dexamethasone administration likely providing the corticosteroid systemic effects that

the patient’s own cortisol would be expected to accomplish, it was notable that postoperative stressors such as myocardial depression, blood pressure lability, pain, and agitation did not prompt ACTH and cortisol generation. Cortisol release is vital to maintaining cardiovascular and endothelial homeostasis, and yet TC and FC levels were quite low in our cohort. ACTH is released in response to

TABLE 3. TC (log scale): Longitudinal data analysis results using a mixed-effects model

Overall time effect		
Effect	F value	<i>P</i> value
Time point	20.68	<.0001

Difference between time points			
Effect	Estimate	SE	<i>P</i> value
TP2 vs TP1	−0.6225	0.1432	<.0001
TP3 vs TP1	−1.1988	0.1951	<.0001
TP2 vs TP3	0.5764	0.1862	.0028

SE, Standard error.

TABLE 4. FC (log scale): Longitudinal data analysis results using a mixed-effects model

Overall time effect			
Effect	F value	P value	
Time point	20.22	<.0001	
Difference between time points			
Effect	Estimate	SE	P value
TP2 vs TP1	−0.8523	0.2003	<.0001
TP3 vs TP1	−1.5429	0.2684	<.0001
TP2 vs TP3	0.6906	0.2851	.019

SE, Standard error.

TABLE 5. ACTH (log scale): Longitudinal data analysis results using a mixed-effects model

Overall time effect			
Effect	F value	P value	
Time point	60.34	<.0001	
Difference between time points			
Effect	Estimate	SE	P value
TP2 vs TP1	−1.45	0.1544	<.0001
TP3 vs TP1	−2.0469	0.1913	<.0001
TP2 vs TP3	0.5969	0.135	<.0001

SE, Standard error.

both biological stresses and low circulating cortisol levels. The low TC and FC levels measured postoperatively in our population should have triggered an increase in ACTH secretion. In contrast, the ACTH levels in our population declined steadily.

Exogenous corticosteroids can be potent inhibitors of the HPA axis through well-described negative feedback loops.⁴ Clearly, a postoperative random low cortisol level should not be equated with adrenal dysfunction in patients that receive preoperative steroids. Despite such suppression and resultant hypocortisolemia, there was no association with this phenomenon and clinical outcomes.

This study is the first to use mass spectrometry methods to accurately quantify FC levels in patients undergoing cardiac surgery. The current consensus is that FC is the biologically active mediator of the adrenal response.²¹ Traditionally reported TC levels measure only the amount of protein-bound cortisol, which is affected by circulating levels of CBG, the major carrier protein for cortisol that binds approximately 90% of plasma cortisol. An important regulator of free corticosteroid, CBG serves as a protein-bound steroid reserve to transport and supply hormone as needed. A greater percentage of circulating cortisol becomes biologically active (free) after the proinflammatory stimulus of CPB leads to dissociation of cortisol from CBG. As CBG levels decline in the postoperative

TABLE 6. CBG (log scale): Longitudinal data analysis results using a mixed-effects model

Overall time effect			
Effect	F value	P value	
Time point	6.1	.0042	
Difference between time points			
Effect	Estimate	SE	P value
TP2 vs TP1	−0.6052	0.2066	.0051
TP3 vs TP1	−0.7246	0.2318	.0029
TP2 vs TP3	0.1193	0.2184	.5871

SE, Standard error.

setting, FC is more abundant to act at the tissue level. Adult studies have demonstrated stronger associations between FC and illness severity compared with TC.^{16,22,23} Fluid shifts, capillary leak, and the use of modified ultrafiltration during surgery create additional challenges for the interpretation of traditionally measured TC levels. We hoped that direct measurement of FC would shed new light into the stress response surrounding CPB (Video 1).

Preoperative baseline median FC levels in our study population fell below the levels used to define adrenal insufficiency in the 2 previous pediatric studies that have measured FC levels during critical illness.^{24,25} Adult studies have defined adrenal insufficiency as FC <3.1 $\mu\text{g}/\text{dL}$ after cosyntropin stimulation.¹⁵ A significant fraction of children generated FC levels below that threshold at every time point, yet we were unable to find associations between FC levels and clinical outcomes. Interestingly, although FC concentrations decreased steadily over the first 24 hours, the median FC response to cosyntropin stimulation was similar at all 3 time points ($\sim 3.4\text{--}3.7 \mu\text{g}/\text{dL}$). FC levels strongly correlated with TC levels throughout the study period. However, plasma TC and FC are interrelated variables, and although correlation is expected, it does not imply that the tests can be used interchangeably. Previous investigations have demonstrated discordance in the diagnosis of adrenal insufficiency using TC and FC criteria in patients with liver disease and septic shock.^{22,23} Our findings represent a first step toward understanding the impact of the CPB-induced inflammatory response on the biologically active FC response and adds to the literature of what constitutes an adequate baseline FC level and response after cosyntropin stimulation during critical illness in children.

There is a lack of consensus regarding the evaluation of adrenal responsiveness in children. Even the appropriate cosyntropin dosage remains controversial, with previous studies using doses ranging from 1 μg to 250 μg .^{10,11,26} Advocates of the lower cosyntropin dose argue that the 250- μg dose may elicit supraphysiological ACTH concentrations that override adrenal resistance, and thus fail to diagnose patients with subtle secondary adrenal insufficiency.²⁷ These variances in testing hinder our ability to optimally identify and treat high-risk patients during critical illness. We chose a 1- μg cosyntropin dose in an attempt to provide the most sensitive indicator of adrenal responsiveness.^{28,29} In our cohort, there were subsets of children with a low TC response to cosyntropin stimulation at both TP2 and TP3. These findings were associated with increased fluid resuscitation in the first 24 hours, as well as longer LOS. These observations differed from our previous investigation, in which the majority of patients had an adequate TC response to cosyntropin stimulation.¹¹ The difference may stem from our decision to use the lower 1- μg cosyntropin dose in the

TABLE 7. Multivariate analysis of the difference between patients with FC >3.1 μg/dL versus <3.1 μg/dL at TP1, after adjusting for age, weight, and RACHS (1-3 vs 4-6)

Outcome*	Difference (95% CI)	P value	P value, log-transformation
Highest postoperative lactate value	0.41 (−0.29 to 1.1)	.252	.520
Inotrope score	2.23 (−1.11 to 5.57)	.192	.194
Duration of mechanical ventilation, h	99.46 (−74.96 to 273.88)	.264	.167
Intensive care unit length of stay	3.61 (−2.65 to 9.87)	.259	.224
Urine output, mL/kg	−16.48 (−33.67 to 0.72)	.0603	.072
Total fluids, mL/kg	−0.02 (−0.11 to 0.08)	.7421	.773

CI, Confidence interval. *Only outcomes that were significantly associated with FC in univariate analysis were analyzed in the multivariate model.

present study, thereby improving our ability to detect more subtle levels of adrenal dysfunction. Our observational study design prevents us from drawing any conclusions as to whether these poor responders represent a cohort with subtle adrenal dysfunction that may benefit from supplemental glucocorticoid therapy.

Our study has several limitations. First, there was no control group, making it impossible to fully ascertain the contribution of preoperative dexamethasone versus CPB-related alterations on the postoperative HPA response. Previous investigations have yielded diverse results in this setting, but do provide insight into the expected effect of CPB on the HPA axis in this population. Neonates undergoing both cardiac and noncardiac surgery have demonstrated intact preoperative adrenal reserve and adrenal responsiveness to both high-dose and low-dose cosyntropin dosing.^{10,11,30} In 2 previous studies that measured perioperative TC concentrations in both neonates and infants undergoing cardiac surgery who received steroids in the CPB prime, TC increased directly before CPB and peaked in the immediate postoperative period.^{31,32} In another study that evaluated neonates who did not receive steroids for CPB, TC and ACTH levels peaked immediately after CPB and then slowly decreased

to preoperative levels over the next 24 to 36 hours.¹³ In contrast, our study population showed a steady decline in postoperative TC levels. The explanation for these findings is likely multifactorial; however, dexamethasone suppression of the HPA axis is a plausible contributor to these observations and is the subject of ongoing investigation.

Another potential limitation of our study may have been the technical challenges of administering a 1-μg dose of cosyntropin. Less than one-half of our cohort (42%) generated an increase in TC level >9 μg/dL at TP1. This inevitably undercuts our findings in the cohort with decreased adrenal sensitivity at TP2 and TP3. Although the response to cosyntropin stimulation improved at TP2 and TP3, the percentage of children with low response was much higher than in our previous study.¹¹ The technical difficulty associated with the administration of tiny doses of cosyntropin has been observed in other studies and may have caused the variability in our results.³³ Alternatively, the anesthetic regimen can influence TC responses during surgery.³⁴ The TP1 stimulation test was administered following anesthesia induction, however. No significant differences in TC or FC levels at TP1 were seen between institutions despite differences in anesthetic approach.

FIGURE 2. Outcomes associated with Δ TC <9 μg/dL after ACTH stimulation at TP2. Endpoint of upper whisker, third quartile plus 1.5 times the IQR or maximum; upper edge of box, third quartile; horizontal line inside box, median; diamond, mean; lower edge of box, first quartile; endpoint of lower whisker, first quartile minus 1.5 times the IQR or minimum; open circle, extreme value beyond endpoints of whiskers. ICU, Intensive care unit.

FIGURE 3. Outcomes associated with Δ TC <9 $\mu\text{g/dL}$ after ACTH stimulation at TP3. Endpoint of upper whisker, third quartile plus 1.5 times the IQR or maximum; upper edge of box, third quartile; horizontal line inside box, median; diamond, mean; lower edge of box, first quartile; endpoint of lower whisker, first quartile minus 1.5 times the IQR or minimum; open circle, extreme value beyond endpoints of whiskers.

In summary, we observed a significant decline in all aspects of the HPA hormonal response (TC, FC, CBG, and ACTH levels) throughout the first 24 hours after infant CPB. Interestingly, this hypocortisolemia and down-regulation of the HPA axis was not associated with clinical outcomes in our cohort. Baseline TC and directly measured FC levels were not associated with clinical outcomes, and FC generation after cosyntropin remained consistent at all time points. Postoperatively a subnormal ($\Delta <9$ $\mu\text{g/dL}$) TC response to cosyntropin was associated with increased fluid resuscitation and longer LOS. The observed postoperative derangements in the HPA axis may reflect the hormonal impact of CPB, preoperative administration of dexamethasone, individual variations in dexamethasone metabolism, or a combination of these

and other factors. Further investigation of the HPA axis and the implications of preoperative dexamethasone administration is essential to evaluate the role of this treatment strategy in the modern CPB era.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Total and Free Cortisol Responses and Their Relation to Outcomes After Cardiopulmonary Bypass In Infants

Eric L. Wald MD,
Carl Backer MD,
Joseph Dearani MD,
Zhuo Li MS,
William Oliver MD,
Sheri S. Crow MD

VIDEO 1. Dr Eric Wald discussing the important implications of the article regarding the stress response to cardiopulmonary bypass in infants. Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(16\)31622-1/addons](http://www.jtcvsonline.org/article/S0022-5223(16)31622-1/addons).

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Key Words: cortisol, adrenal insufficiency, cardiopulmonary bypass, congenital heart surgery, clinical outcomes

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