Perioperative Plasma F₂-Isoprostane Levels Correlate With Markers of Impaired Ventilation in Infants With Single-Ventricle Physiology Undergoing Stage 2 Surgical Palliation on the Cardiopulmonary Bypass

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Abstract Cardiopulmonary bypass (CPB) produces inflammation and oxidative stress, which contribute to postoperative complications after cardiac surgery. F₂-Isoprostanes (F₂-IsoPs) are products of lipid oxidative injury and represent the most accurate markers of oxidative stress. In adults undergoing cardiac surgery, CPB is associated with elevated IsoPs. The relationship between F₂-IsoPs and perioperative end-organ function in infants with single-ventricle physiology, however, has not been well studied. This study prospectively enrolled 20 infants (ages 3–12 months) with univentricular physiology undergoing elective stage 2 palliation (bidirectional cavopulmonary anastomosis). Blood samples were collected before the surgical incision (T0), 30 min after initiation of CPB (T1), immediately after separation from CPB (T2), and 24 h postoperatively (T3). Plasma F₂-IsoP levels were measured at each time point and correlated with indices of pulmonary function and other relevant clinical variables. Plasma F₂-IsoPs increased significantly during surgery, with highest levels seen immediately after separation from CPB ($p < 0.001$). After separation from CPB, increased F₂-IsoP was associated with lower arterial pH ($q = -0.564; p = 0.012$), higher partial pressure of carbon dioxide ($PaCO_2; q = 0.633; p = 0.004$), and decreased lung compliance ($q = -0.783; p < 0.001$). After CPB, F₂-IsoPs did not correlate with duration of CPB, arterial lactate, or immediate postoperative outcomes. In infants with single-ventricle physiology, CPB produces oxidative stress, as quantified by elevated F₂-IsoP levels. Increased F₂-IsoP levels correlated with impaired ventilation in the postoperative period. The extent to which F₂-IsoPs and other...
bioactive products of lipid oxidative injury might predict or contribute to organ-specific stress warrants further investigation.

**Keywords** Cardiopulmonary bypass · Congenital heart disease · Isoprostanes · Oxidative stress

Cardiopulmonary bypass (CPB) produces inflammation and oxidative stress, which contribute to postoperative complications after cardiac surgery [28, 29]. Atrial fibrillation [4, 25], pulmonary dysfunction [2, 22], acute renal injury [13], and neurocognitive dysfunction [8, 24] all represent important end-organ injuries thought to be associated with perioperative oxidative stress. Postoperative pulmonary dysfunction in infants with single-ventricle physiology is particularly problematic due to their unique physiology, which requires low pulmonary resistance for passive pulmonary blood flow. The relationship between the extent of CPB-induced oxidative injury and the severity of postoperative pulmonary dysfunction in this population, however, has not been clearly demonstrated.

Isoprostanes (IsoPs) are prostaglandin-like molecules formed in vivo by free radical–mediated oxidation of arachidonic acid, which can be measured in all biologic fluids including plasma, urine, and cerebrospinal fluid [15, 18, 21]. The F2-IsoPs (named for their F-type prostane ring) are a particularly stable isoform, providing the most accurate measure of oxidative stress in vivo [12].

Cardiac surgery results in elevated F2-IsoP levels in adults [27], and studies comparing operations with and without CPB have found significantly higher plasma F2-IsoP levels in patients exposed to CPB [6], suggesting that this form of extracorporeal circulation is a unique contributor to oxidative stress.

Plasma levels of inflammatory cytokines, complement fragments, and other biomarkers of oxidative stress are known to be elevated in children and infants after cardiac surgery [5, 7, 16], but their association with end-organ dysfunction is less clear. Furthermore, there is no published data on the time course and extent of F2-IsoP elevation during cardiac surgery in pediatric patients, much less regarding the relationship between perioperative F2-IsoP levels and postoperative pulmonary function in this population.

This investigation aimed to determine the time course of perioperative oxidative injury as measured by plasma F2-IsoP levels and to test the hypothesis that oxidative injury correlates with postoperative pulmonary dysfunction in a population of children with single-ventricle physiology undergoing stage 2 bidirectional cavopulmonary anastomosis (BCPA).

**Methods**

**Patient Selection**

Infants 3 to 12 months of age with single-ventricle physiology undergoing elective stage 2 palliation (BCPA) at Vanderbilt Children’s Hospital were enrolled prospectively in the study. Patients were excluded if they had a history of premature birth (<36 weeks gestational age) or known genetic syndromes. Of the 34 infants who presented for stage 2 palliation between January 2009 and March 2010, 24 were eligible. Of these patients, 20 were enrolled and 19 completed the study. The study protocol was approved by the Vanderbilt University Institutional Review Board, and written informed consent was obtained from the parents of all the infants at the time of enrollment.

**Anesthesia and CPB**

The patients received preoperative sedation with oral midazolam (0.5–0.7 mg/kg). General endotracheal anesthesia was induced with a mixture of inhaled sevoflurane, nitrous oxide, and oxygen and maintained with isoflurane, fentanyl, and pancuronium. During mechanical ventilation, isoflurane was equilibrated to a 0.2–1% end-tidal concentration, and oxygen concentration was titrated to a systemic oxygen saturation goal of 75–90%. Continuous monitoring included standard 5-lead electrocardiography, pulse oximetry, end-expiratory CO₂, invasive arterial blood pressure, central venous pressure, rectal and nasal temperatures, and cerebral oximetry using near-infrared spectrophotometry (NIRS) (Somanetics, INVOS; Troy, MI, USA). Methylprednisolone (7 mg/kg) was administered intravenously to all the patients before the initiation of CPB.

All operations were performed on CPB using bicaval venous cannulation. The extracorporeal circuit included a roller pump (Terumo Cardiovascular Systems Corporation, Ann Arbor, MI, USA), tubing set, oxygenator with a hard-shell venous reservoir, hemoconcentrator, and continuous in-line monitoring (Terumo cardiovascular systems corporation). The circuit was primed with a mixture of albumin, packed red blood cells, fresh frozen plasma, and plasmalyte-A. After prebypass ultrafiltration of this mixture, mannitol, sodium bicarbonate, calcium chloride, and heparin were added to the washed circuit prime in doses adjusted for body surface area.

The perfusion flow rates during normothermia were 100–175 ml/kg/min, and the pump flow was reduced gradually during cooling per standard protocol. Moderate hypothermia (34–35°C) was achieved for cases not requiring aortic cross-clamping (n = 14), whereas lower core temperatures (31–32°C) were achieved during aortic cross-clamping (n = 5). In five cases requiring aortic cross-clamping, standard cold-blood cardioplegia solution...
(containing sodium bicarbonate, lidocaine, heparin, and potassium chloride) was used for myocardial protection. Modified ultrafiltration was performed for all the patients after termination of bypass with a pediatric hemofilter (Terumo Cardiovascular Systems Corporation).

Unfractionated heparin (400 U/kg) was administered intravenously, and an activated clotting time (ACT) of more than 400 s (International Technidyne Corporation, Edison, NJ, USA) was verified before the initiation of CPB. The ACT was monitored every 30 min and maintained more than 400 s during CPB. Heparin was neutralized with protamine in doses adjusted for the amount of circulating heparin and the patient’s ACT at the end of CPB.

Blood Sampling

Blood samples (5 ml) were collected after anesthesia induction and before initiation of surgery (baseline, T0), 30 min after the initiation of CPB (T1), after separation from bypass and protamine administration (T2), and 24 h after separation from CPB (T3). Blood was drawn from an indwelling arterial line (or from an indwelling central venous line in cases for which an arterial line was not available), transferred into tubes containing potassium ethylenediaminetetraacetic acid (EDTA), and placed immediately on ice. Samples were centrifuged at 3,200 g for 15 min within 1 h after collection, and the supernatant plasma was distributed in 0.5- to 1-ml aliquots, which then were stored at −70°C for later analysis.

Isoprostane Analysis

Plasma Sample Preparation

First, 1 ml of plasma was added to 1.0 ng of [2H_{4}]15-F_2-isoP ([2H_{4}]8-iso-PGF_{2α}; Cayman Chemical, Ann Arbor, MI, USA) internal standard. The solution then was processed by methods previously described such that derivatized F_2-isoP compounds were isolated, dried, and re-dissolved for GC/MS analysis [17, 19].

Mass Spectrometric Analysis of F_2-isoP

Gas Chromatography/Negative Ion Chemical Ionization-Mass Spectrometry (GC/NICI-MS) was performed with an Agilent 5973 Inert Mass Selective Detector coupled with an Agilent 6890n Network GC system (Agilent Labs, Torrance, CA, USA) using methods previously described. Levels of endogenous F_2-isoPs in a biologic sample are calculated from the ratio of intensities of ion m/z 569 (major ion generated by the F_2-isoP derivative) to ion m/z 573 (corresponding ion generated by the internal standard). The precision of this assay in biologic fluids is ±6%, and the accuracy is 94% [17, 19].

Clinical Data Collection

Clinical parameters including vital signs, cerebral oximetry, ventilatory parameters (including fraction of inspired oxygen [FiO_2], tidal volume [TV], peak inspiratory pressure [PIP], and positive end-expiratory pressure [PEEP]), and laboratory values including arterial blood gas analysis and hematoctrit were recorded at the time of each blood sample collection.

Lung compliance was determined using the following equation:

\[
\text{Dynamic lung compliance (Cd)} = \frac{TV}{(\text{PIP} - \text{PEEP})}
\]

Information regarding duration of CPB, temperature, inotropic support, ventilator time, length of intensive care unit (ICU) stay, length of hospital stay, and postoperative complications was collected from the patient medical record. Preoperative data including stage 1 operation, baseline hemodynamics (pulmonary artery pressures and pulmonary vascular resistance), and outpatient medications also were collected from the medical record and recorded.

Statistical Analysis

Data are presented as mean ± standard deviation unless otherwise noted. Levels of F_2-isoP were compared over time using repeated measures analysis of variance (ANOVA), and pair-wise comparisons were made using Tukey’s honestly significant difference (HSD) test to adjust for inflated type 1 error in the setting of multiple comparisons. Associations between F_2-isoP levels and continuous clinical parameters were analyzed using Spearman’s rank correlation. Tests were two-tailed, and a \( p \) value less than 0.05 was considered significant. Statistical analysis was performed using SPSS software v.19.0 (SPSS Inc., Chicago, IL, USA), R software 2.10.1 (R-project.org), and GraphPad Prism software v.5.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Patient Characteristics

Of 19 patients who underwent BCPA, 11 (58%) had hypoplastic left heart syndrome, 2 (11%) had another form of single right ventricle, and 6 (31%) had a single left ventricle. At least one concomitant operation was performed for 18 patients (95%), most often pulmonary artery augmentation at the site of Blalock-Taussig shunt take-down and BCPA construction (n = 12, 63%). Two patients (11%) had bilateral BCPA. No patients were left with antegrade pulmonary blood flow. The average CPB time was 89.1 ± 42.1 min. Five patients (26%) required aortic
cross-clamping, with mean duration of 17.4 ± 18.6 min (Table 1).

Clinical Outcomes

The median perioperative hospital length of stay was 11 days (range 4–54 days). The median intubation time was 1 day (range 1–25 days), and the median intensive care unit (ICU) time was 4 days (range 1–32 days). Four patients (21%) had persistent pleural effusions requiring chest tube placement for more than 5 days, with a median chest tube drainage time of 3 days (range 2–21 days).

Six patients (32%) required reoperation, including re-exploration, delayed chest closure, or wound debridement. Four patients (21%) with persistently elevated cavopulmonary pressures required inhaled nitric oxide (iNO) therapy in the immediate postoperative period, but only one of these patients was discharged receiving long-term pulmonary vasodilator therapy. The intubation time for these four patients was slightly longer than for the remainder of the cohort (median 2.5 days; range 2–7 days).

Other postoperative complications included infection in four cases (21%), new thrombosis in five cases (26%), and arrhythmia in one case (5%).

F2-Isoprostane Levels

Plasma F2-Isoprostane (F2-IsoP) changed significantly over time, with the highest levels seen immediately after separation from the bypass (p < 0.001). There was a significant increase in plasma F2-IsoP from baseline (T0, 0.062 ± 0.024 ng/ml) to after separation from bypass (T2, 0.084 ± 0.037 ng/ml) (95% confidence interval [CI] for the change, 0.01–0.04; p = 0.001), with a return to baseline levels or lower by 24 h postoperatively (T3, 0.047 ± 0.016 ng/ml) (Fig. 1). A positive correlation was observed between the baseline F2-IsoP level (T0) and the post-CPB F2-IsoP level (T2) (ρ = 0.678; p = 0.001).

F2-Isoprostane Correlations With Pulmonary Function and Clinical Variables

The change in plasma F2-IsoP levels from baseline (T0) to after separation from bypass (T2) was highly correlated with lower pH (ρ = −0.564; p = 0.012), higher partial pressure of carbon dioxide (PaCO2; ρ = 0.633; p = 0.004), and decreased lung compliance (ρ = −0.783; p ≤ 0.001) (Fig. 2a–c). Increased F2-IsoP levels did not correlate with duration of CPB, blood transfusion requirement, highest partial pressure of oxygen (PaO2) documented on the bypass, lactate, cerebral oxygenation, or transpulmonary gradient. Furthermore, F2-IsoP levels did not correlate with

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>4.7 ± 2.2</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (79)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (21)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>16 (84)</td>
</tr>
<tr>
<td>African American</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Primary diagnosis, n (%)</td>
<td>6.1 ± 1.0</td>
</tr>
<tr>
<td>HLHS</td>
<td>11 (58)</td>
</tr>
<tr>
<td>Other single RV</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Other single LV</td>
<td>6 (31)</td>
</tr>
<tr>
<td>Concomitant operations, n (%)</td>
<td></td>
</tr>
<tr>
<td>PA augmentation</td>
<td>12 (63)</td>
</tr>
<tr>
<td>Atrial septectomy</td>
<td>3 (16)</td>
</tr>
<tr>
<td>Removal RV–PA connection</td>
<td>3 (16)</td>
</tr>
<tr>
<td>DKS</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Bilateral BCPA</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Baseline SpO2 (%)</td>
<td>82.1 ± 7.4</td>
</tr>
<tr>
<td>Baseline Hgb (g/dl)</td>
<td>13.2 ± 2.0</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>89.1 ± 42.1</td>
</tr>
<tr>
<td>X-clamp time (min) (n = 5)</td>
<td>17.4 ± 18.6</td>
</tr>
</tbody>
</table>

n no. of patients; HLHS hypoplastic left heart syndrome; RV right ventricle; LV left ventricle; PA pulmonary artery; DKS Damus-Kaye Stansel procedure; BCPA bidirectional cavopulmonary anastomosis; SpO2 oxygen saturation by pulse oximetry; Hgb hemoglobin; CPB cardiopulmonary bypass; X-clamp, aortic cross-clamp

Fig. 1 F2-Isoprostane levels increase after cardiopulmonary bypass (CPB). Box-and-whisker plot demonstrates F2-Isoprostane (IsoP) levels at each time point: T0 (baseline), T1 (30 min on CPB), T2 (immediately after bypass), and T3 (24 h after bypass). The middle line represents the median value. The central box represents the values between the upper and lower quartiles. The vertical lines extend to 1.5 interquartile ranges from both ends of the box, excluding outliers (n = 19). *p < 0.05 (comparison of T0 and T2). **p < 0.05 (comparison of T2 and T3)
short-term postoperative outcomes such as ventilatory support requirement, duration of chest tube drainage, or length of hospital stay (Table 2).

Discussion

Our results show that plasma F$_2$-IsoP levels are elevated in children with univentricular physiology undergoing stage 2 surgical palliation (BCPA) on CPB. The temporal profile of oxidative stress surrounding CPB demonstrated in this study was similar to that observed in adults, although the highest levels in adults are during CPB [27], whereas our patients demonstrated the greatest elevation immediately after separation from bypass. This difference may be due to higher priming blood volumes required for infant CPB, causing dilutional changes in concentration and thus relatively lower measured F$_2$-Isops while the infant is on CPB.

We also observed an association between increased circulating F$_2$-IsoP levels and decreased pulmonary compliance in the immediate postoperative period. Because all patients received pressure-controlled ventilation after CPB, decreased pulmonary compliance resulted in lower pH and higher PaCO$_2$, which also were correlated with an increase in F$_2$-IsoP levels.

Pulmonary dysfunction is a known complication of cardiac surgery and CPB related to ischemia-reperfusion injury, blood transfusion, hypothermia, endothelial dysfunction, activation of inflammatory mediators, increased vascular permeability, and pulmonary edema [22, 26]. Despite improvements in perioperative management, including modified ultrafiltration to reduce total body water and pulmonary edema after CPB, pulmonary complications remain a major cause of morbidity after cardiac surgery [3, 10, 14].

Oxidative stress is known to contribute to lung injury after cardiac surgery, as well as during other pulmonary diseases in which F$_2$-IsoP levels are known to be elevated.

Table 2 | Spearman rank correlations between clinical variables and F$_2$-Isoprostane change from baseline (T0) to post-bypass (T2)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation coefficient ($\rho$)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPB time</td>
<td>0.070</td>
<td>0.776</td>
</tr>
<tr>
<td>Blood product transfusion requirement</td>
<td>-0.332</td>
<td>0.165</td>
</tr>
<tr>
<td>Peak PaO$_2$ on CPB</td>
<td>0.217</td>
<td>0.373</td>
</tr>
<tr>
<td>Highest perioperative lactate</td>
<td>-0.263</td>
<td>0.277</td>
</tr>
<tr>
<td>Lowest perioperative cerebral oxygen saturation (NIRS)</td>
<td>0.429</td>
<td>0.397</td>
</tr>
<tr>
<td>Transpulmonary gradient (T2)</td>
<td>-0.120</td>
<td>0.683</td>
</tr>
<tr>
<td>Indicators of perioperative respiratory mechanics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (T2)</td>
<td>-0.564</td>
<td>0.012*</td>
</tr>
<tr>
<td>PaCO$_2$ (T2)</td>
<td>0.633</td>
<td>0.004*</td>
</tr>
<tr>
<td>Dynamic Lung compliance (Cd) (T2)</td>
<td>-0.783</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Postoperative outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU days</td>
<td>0.046</td>
<td>0.855</td>
</tr>
<tr>
<td>Chest tube days</td>
<td>0.364</td>
<td>0.138</td>
</tr>
<tr>
<td>Total hospital days</td>
<td>0.203</td>
<td>0.420</td>
</tr>
</tbody>
</table>

CPB cardiopulmonary bypass; PaO$_2$ partial pressure of oxygen; NIRS near infrared spectrophotometry; PaCO$_2$ partial pressure of carbon dioxide; ICU intensive care unit

*p < 0.05
Although F₂-IsoPs are biologically inactive, other forms of isoprostanes produced in response to oxidative stress, specifically those with E-type prostane rings, exert biologic effects on nearly all cell types within the lung including airway smooth muscle, vascular smooth muscle and endothelium, and alveolar epithelial cells [11]. Because the biologically active isoprostane isoforms are relatively unstable, they are more difficult to analyze than the F₂-IsoP isoform. Elevated F₂-IsoPs, therefore, may represent not only a marker of overall oxidative stress after cardiac surgery but also ongoing cellular injury and oxidative stress due to the concomitant accumulation of biologically active isoprostanes. As our results suggest, this ongoing damage is evident within the lung tissue of infants undergoing CPB.

The extent and causes of postoperative pulmonary dysfunction are particularly relevant to patients with BCPA physiology. Decreased pulmonary compliance can lead to impaired pulmonary blood flow, cyanosis, and decreased cardiac output. Diastolic dysfunction and impaired ventricular filling, often seen in the immediate postoperative period as a result of CPB-induced myocardial ischemia-reperfusion injury, inflammation, and edema, can further worsen pulmonary blood flow in patients with this physiology, which relies on passive pulmonary blood flow and low filling pressures.

Furthermore, because pulmonary and cerebral circulations are now coupled in series, pulmonary vascular dysfunction can contribute to cerebral venous congestion and impaired cerebral perfusion. Because CO₂ has opposing effects on the pulmonary and cerebral circulation (with vasoconstricting effects in the lung and vasodilating effects in the brain), ventilatory management strategies after BCPA have been studied closely. Pulmonary vasodilators such as inhaled nitric oxide have been shown to improve pulmonary blood flow and cardiac output in patients struggling with cyanosis after BCPA [1].

Conversely, elevated PaCO₂, a potent pulmonary vasconstrictor, causes cerebral vasodilation and improved cerebral blood flow, which in BCPA physiology also translates to improved pulmonary blood flow and cardiac output during the postoperative period [9]. For this reason, many institutions use controlled hypoventilation as a strategy for maintaining a slightly higher PaCO₂ after BCPA. In our study, higher PaCO₂ after separation from bypass was associated with increased F₂-IsoP production, suggesting more overall oxidative injury and raising questions about how ventilatory manipulations may have an impact on perioperative oxidative stress and ongoing lung injury.

One notable observation in this study was that no clear correlation existed between the extent of F₂-IsoP elevation and intraoperative variables expected to contribute to oxidative stress such as duration of CPB, blood transfusion requirement, and highest PaO₂ on bypass. Furthermore, increased F₂-IsoPs were not associated with commonly used clinical monitoring parameters including arterial blood lactate, cerebral oxygenation, and transpulmonary gradient. Similarly, perioperative F₂-IsoP levels did not have a significant impact on short-term clinical outcomes including ventilatory support requirement, duration of chest tube drainage, or length of hospital stay. Additional intraoperative procedures, including aortic cross-clamping, also had no impact on F₂-IsoP levels or short-term clinical outcomes.

### Study Limitations

Studies in pediatric cardiac surgery are complicated by the heterogeneity of cardiac lesions and the variety of operations the children undergo, which limits statistical power and clinical applicability. Despite our attempts to minimize this problem by studying a relatively homogeneous group of patients, even this select group included a variety of diagnoses and surgical techniques, which certainly affected the power of our analysis. In addition, small sample size limited our ability to perform more in-depth statistical analysis, including multivariable linear regression and subgroup comparisons to identify predictors of increased F₂-IsoP production.

Finally, we recognize that children with a single-ventricle physiology represent a very complex group of patients whose F₂-IsoP production may differ significantly from that of other children requiring surgical intervention while on CPB. Future work should explore F₂-IsoP trends in a larger cohort of pediatric patients, ideally comparing perioperative F₂-IsoP production between children with cyanotic lesions and those with noncyanotic lesions. An additional focus on organ-specific assessment of lipid oxidation and long-term outcomes also should be considered.

### Conclusions

In infants with a single-ventricle physiology, CPB produces oxidative stress, as quantified by elevated F₂-IsoP levels. In this group, increased F₂-IsoP levels correlated with decreased pulmonary compliance and markers of impaired ventilation in the immediate postoperative period. The extent to which F₂-IsoPs and other bioactive products of lipid oxidative injury might contribute to perioperative organ-specific stress warrants further investigation. The utility of F₂-IsoPs as a predictor of long-term outcomes requires ongoing study.
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