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ORIGINAL RESEARCH

Perioperative Metabolites Are Associated With Adverse Neonatal Congenital Heart Disease Surgical Outcomes

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BACKGROUND: Clinical risk factors in neonatal cardiac surgery do not fully capture discrepancies in outcomes. Targeted metabolomic analysis of plasma from neonates undergoing heart surgery with cardiopulmonary bypass was performed to determine associations with clinical outcomes.

METHODS AND RESULT: Samples and clinical variables from 149 neonates enrolled in the Corticosteroid Therapy in Neonates Undergoing Cardiopulmonary Bypass trial with surgical treatment for congenital heart disease between 2012 and 2016 were included. Blood samples were collected before skin incision, immediately after cardiopulmonary bypass, and 12 hours after surgery. Outcomes include composite morbidity/mortality (death, extracorporeal membrane oxygenation, cardiac arrest, acute kidney injury, and/or hepatic injury) and a cardiac composite (extracorporeal membrane oxygenation, cardiac arrest, or increase in lactate level), hepatic injury, and acute kidney injury. Targeted metabolite levels were determined by high-resolution tandem liquid chromatography and mass spectrometry. Principal component and regression analyses were used to assess associations between metabolic profiles and outcomes, with 2 models created: a base clinical model and a base model+metabolites. Of the 193 metabolites examined, 40 were detected and quantified. The first principal component, principal component 1, was composed mostly of preoperative metabolites and was significantly associated with the composite morbidity/mortality, cardiac composite, and hepatic injury outcomes. In regression models, individual metabolites also improved model performance for the composite morbidity/mortality, cardiac composite, and hepatic injury outcomes. Significant disease pathways included myocardial injury (false discovery rate, 0.00091) and heart failure (false discovery rate, 0.041).

CONCLUSIONS: In neonatal cardiac surgery, perioperative metabolites were associated with postoperative outcomes and improved clinical model outcome associations. Preoperative metabolite levels alone may improve risk models and provide a basis for optimizing perioperative care.

Key Words: acute kidney injury ■ cardiac surgical procedures ■ cardiopulmonary bypass ■ extracorporeal membrane oxygenation ■ heart defects, congenital ■ infant, newborn ■ risk factors

Despite significant improvement in morbidity and mortality over the past 30 years, congenital heart surgery remains high risk. Neonates are among the most vulnerable of these patients, comprising mortality rates of 10%, compared with 3.0% and 1.7% in infants and adults, respectively.[1,2] Major and overall complication rates in neonates undergoing congenital heart surgery are high, at 19% and 67%, respectively,[3] leaving room for optimization of care. Current risk models incorporate clinical factors that are associated with increased morbidity and mortality, such as age, weight, preoperative status, including hospitalization before surgery, comorbidities, and surgical complexity. Perhaps the most widely recognized model, the

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CLINICAL PERSPECTIVE

What Is New?

- This study expands on perioperative metabolic changes, with a specific focus on neonates undergoing cardiopulmonary bypass. This cohort is larger than prior studies, is multicenter, and has a larger panel of metabolites that are included in the analysis.

What Are the Clinical Implications?

- A panel of metabolites that were primarily preoperative values was associated with postoperative outcomes, including death, renal and hepatic dysfunction, and cardiac arrest, which suggests that preoperative management could be more important than exposure to cardiopulmonary bypass.

Nonstandard Abbreviations and Acronyms

AIC	Akaike Information Criterion
AKI	acute kidney injury
CPB	cardiopulmonary bypass
FDR	false discovery rate; HPLC, high performance liquid chromotography; KEGG, Kyoto Encyopledia of Genes and Genomics
PC	principal component
PGE	prostaglandin E-1

Society of Thoracic Surgeons–European Association for Cardiothoracic Surgery category, combines age, surgical complexity, and preoperative length of stay to model mortality risk of surgery.[4] However clinical factors are static and mostly not modifiable and therefore not useful for operational improvements in care.

Metabolomics is a dynamic measure of the physiological state that can be leveraged in pediatric cardiology. Because metabolites are the result of the complex interplay between the genetic substrate and environmental influences, a patient's metabolic profile offers insight on the phenotype of the patient in the moment of sampling. Metabolic profiling has shown superior risk prediction for death or hospitalization in adult patients with heart failure compared with current standards, such as brain natriuretic peptide.[5] Published data are less prevalent in the pediatric population, although studies have examined metabolomic fingerprinting in children undergoing cardiac surgery, with associations to outcome measures, such as mortality and intensive care unit length of stay.[6,7]

The physiological disruptions experienced in neonates undergoing congenital heart surgery are why metabolomics could be enlightening for this vulnerable population. Disruptive factors include smaller body size, cyanosis, nutrition challenges, high likelihood of prostaglandin exposure preoperatively, and use of cardiopulmonary bypass (CPB). Although integral to cardiac surgery and thus life saving, CPB contributes to physiological derangements through exposure to nonphysiological pump circuit surfaces, organ ischemia/reperfusion, cooling, and rewarming.[8,9] The aims of this study are to: (1) describe perioperative changes in metabolic profiles in neonates requiring CPB, (2) associate metabolites with outcomes, and (3) identify potential metabolic pathways involved.

METHODS

Study Population

This study is a secondary analysis of the National Heart, Lung, and Blood Institute–funded, Corticosteroid Therapy in Neonates Undergoing Cardiopulmonary Bypass trial, a randomized controlled trial of intraoperative methylprednisolone or placebo (ClinicalTrials.gov Identifier: NCT01579513). Participants were enrolled between 2012 and 2016 from 2 enrolling sites. The trial primary end point was the incidence of a clinically derived composite morbidity-mortality outcome with additional secondary end points. The trial design and clinical outcomes have been previously reported.[10,11] Data supporting the findings of this study are available on reasonable request from the corresponding author.

Inclusion criteria for the parent study consisted of infants aged <1 month undergoing cardiac surgery with CPB. Exclusion criteria included prematurity, defined as <37 weeks postgestational age at the time of surgery, steroids within the 2 days before surgery, suspected infection, hypersensitivity that would be a contraindication to methylprednisolone, or use of mechanical circulatory support or active resuscitation at the time of proposed randomization. The protocol was approved by the institutional review board at the participating centers, and written informed consent was obtained from a parent/guardian before randomization. This secondary study was approved by the Johns Hopkins Institutional Review Board.

Outcomes

The parent study used a composite morbidity-mortality outcome, which has been previously validated in neonates undergoing cardiac surgery with CPB.[12] The composite outcome was met if any of the following occurred after surgery but before hospital discharge: death, cardiac arrest, extracorporeal membrane oxygenation, renal injury (defined as creatinine >2 times normal), hepatic injury (defined as aspartate

aminotransferase or alanine aminotransferase >2 times normal >36 hours postoperatively), or increasing lactate level (defined as >5 mmol/L). To focus on a more cardiac functional measure, we developed a cardiac composite outcome that only included death, extracorporeal membrane oxygenation, cardiac arrest, and increasing lactate level. Hepatic injury, using the same definition as in the composite outcome, and acute kidney injury (AKI), using the AKI Network's criteria, were further assessed individually from the composite morbidity/mortality outcome.[13] AKI was defined using the AKI Network's criteria for acute kidney injury as an increase in serum creatinine above the preoperative level by an absolute value of >0.3 mg/dL, a ≥50% increase, or urine output of <0.5 mL/kg per hour over a 6-hour period.[14]

Metabolite Identification and Quantification

Blood samples were collected from neonates at 3 time points: (1) before skin incision and steroid randomization, (2) immediately after CPB, and (3) 12 hours postoperatively. Samples were held on ice until the plasma was isolated, aliquoted, and then stored at −80 °C until assayed for metabolite concentrations using a targeted panel of 193 metabolites representing >366 metabolic pathways (Molecular Determinants Core at Johns Hopkins All Children's Hospital, St. Petersburg, FL). The full assay list is in Table S1.

Samples were initially filtered, with extraction blanks (water) versus extraction reference (human plasma) included on the filtration plate. Filtration was accomplished using Supelco 96-Well Protein Precipitation Filter Plate (Sigma Aldrich 55263-U) paired with PlatePrep 96-well Vacuum Manifold (Sigma Aldrich 57192-U).

Samples were then delivered to the mass spectrometry ionization source by high-pressure liquid chromatography (HPLC), using a Shimadzu HPLC, a SIL-30ACMP 6-MTP autosampler, and Nexera LC-30AD HPLC pumps. Targeted mass spectrometry assays used a Shimadzu 8060 triple-quadrupole instrument equipped with an electrospray ionization source used in both positive and negative modes.

Spectral Peak Data Processing

After review and verification of the accuracy of peak integrations, measured areas for each compound were normalized for within-batch drift by fitting a weighted scatterplot smoothing function generated from pooled quality control samples run between every 10th analytical sample.[15,16] Drift correction was performed using the R LOESS statistical package. To account for variation in extraction efficiency, samples were scaled using the response of a suite of isotopically labeled internal standards spiked into each sample at the beginning of the extraction procedure. Variations in overall instrument sensitivity between analytical batches were corrected by transforming raw data so that the mean within-batch areas for each compound were equivalent.

Statistical Analysis

Metabolomic data were corrected for sample osmolarity and then normalized, with the rare missing value replaced by half the minimum detectable level. No additional preprocessing of metabolomic data was performed. Using paired *t*-test analysis, metabolite concentrations at each time point were compared using a false discovery rate of 5% (Benjamini-Hochberg correction), which also established the *P*-value cutoff for significance. Metabolites that had significant differential expression between various time points were further divided into subcolumns based on increase or decrease in levels.

Additional features for association analysis were generated from metabolite concentrations, which included ratios between time points 1 and 2, 1 and 3, and 2 and 3, along with a cumulative value of all 3 time points. With the number of patients, metabolites measured at each time point, and the additional features derived between time points, this was a complex data set. Principal component (PC) analysis was performed to determine association between metabolites, generated features, and outcomes. PC analysis permitted simplification of the data set by reducing the dimensionality (ie, summarizing) the variations in the data points into smaller segments, which are the PCs.[17] To investigate the contribution of the different PCs to the various clinical outcomes, we used logistic regression models for binary outcomes.

To develop the base clinical model, clinical factors with known associations with surgical outcomes were evaluated for inclusion. Preliminary factors group assignment, steroid administration, cardiopulmonary bypass duration, and similar surgical factors ultimately included surgical center, preoperative prostaglandin E-1 (PGE) exposure, Society of Thoracic Surgeons category, preoperative lactate level, and gestational age at birth. PGE was included as a clinical variable because of the extensive hormonal,[18] renal,[19] hematologic,[20] and other systemic effects of PGE,[21] in addition to the associated high-risk physiologies that require PGE infusions for ductal patency. The following performance metrics were determined for the base clinical model (including only adjustment factors): Akaike Information Criterion (AIC) for binary and continuous outcomes and Wald X^2 and c-statistic for continuous outcomes. A second model was then created, by taking the base model followed by stepwise selection of the first 6 PC

loads (based on examination of the scree plot), with $P \leq 0.05$ to include and $P > 0.05$ to discard. Performance metrics were repeated for this second model (base model+PCs).

A final, third, model was created to determine associations between individual metabolites (or features derived from them) and outcomes. A multistep approach for model building was used, starting with narrowing the list of potential associated features for each outcome. Features with a $P < 0.10$ (indicating a potential association between the outcome and the features) were identified for each outcome, followed by a bootstrap resampling approach (500 samples, including between 20% and 80% of the total observations) to identify features with high reliability. Features with scores >50% were considered highly reliable. For the next step, we selected features with reliability scores >30%. Thereafter, the final regression models were built using backward elimination ($P < 0.05$ to remain) and included the clinical adjustment variables, the first 6 PC loads, and all metabolites or features thereof that met the above-mentioned reliability criteria. In some cases where 2 highly correlated features were both candidates for inclusion in the multivariable model, an intermediate step was added to compare the model with either feature, and the feature resulting in the best model fit (based on a lower model AIC) was selected.

Metabolite Enrichment Disease and Pathway-Associated Analysis

Final metabolites from the models or their features that improved an outcome were then evaluated using

Table 1. Cohort Characteristics and Clinical Status

Patient characteristics	Values, mean±SD or N (%)	Patient characteristics (cont.)	Values, mean±SD or N (%)
Total (N)	149	Surgical outcomes	
Site 2 (vs site 1)	38 (26)	Low cardiac output syndrome	69 (46)
Methylprednisolone arm	71 (48)	Mechanical cardiac support	3 (2)
		Increase inotropic support by 100%	50 (34)
Characteristics/clinical history		New inotropic agents	40 (27)
Age, d	8.7±5.3	Highest IS at 36 h	17.1±4.7
Gestational age at birth, wk	38.9±1.2	Highest VIS at 36 h	19.2±6.9
Sex (male)	89 (60)	Highest lactate level at 36 h (mmol/L)	4.0±2.4
Diagnosis		Ventilation time, d	7.7±15.1
Biventricular with arch hypoplasia	27 (18)	ICU length of stay, d	17.0±20.6
TGA	37 (25)	Hospital length of stay, d	30.4±39.1
All other biventricular disorders	40 (27)		
SV	45 (30)	Mortality/morbidity composite outcomes	55 (37)
		Death	7 (5)
Preoperative status		Cardiac arrest	9 (6)
Intubation before surgery	36 (24)	ECMO	7 (5)
PGE within 24 h of surgery	117 (79)	Renal	6 (4)
PGE ever before surgery	130 (87)	Hepatic	38 (26)
MOD ever before surgery	11 (7)	Lactate level	28 (19)
Highest creatinine (mg/dL)	0.8±0.5	Cardiac composite outcome	34 (23)
Highest lactate level (mmol/L)	3.5±2.7	AKI	66 (44)
Lactate level on day of surgery (mmol)	1.1±0.43		
Corrective procedure	90 (60)		
STAT category			
1–3	32 (21)		
4	77 (52)		
5	40 (27)		
CPB time, min	74.8±42.5		
DHCA, min	9.9±65.1		

AKI indicates acute kidney injury; CPB, cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IS, inotropic score; MOD, multiorgan dysfunction; PGE, prostaglandin E-1; STAT, Society of Thoracic Surgeons; SV, single ventricle; TGA, transposition of the great arteries; and VIS, vasopressor/inotropic score.

Metaboanalyst 5.0 for disease and metabolic pathways.[22] Findings were then compared for relevance to the outcome assessed and prior studies linking to diseases or pathways.

RESULTS

Subject Demographics

Samples from a total of 154 participants were available; of those, 5 subjects were excluded because of a missing sample for at least 1 of the time points. The final cohort included 149 patients who had metabolomic data available at all 3 time points. Demographics and baseline characteristics are shown in Table 1. The cohort consisted of 89 male patients (60%), and patients fit into following diagnostic categories: 27 (18%) biventricular physiology and arch hypoplasia, 37 (25%) transposition of the great arteries, 40 (27%) biventricular physiology not previously listed, and 45 (30%) with single-ventricle physiology. Most (79%) remained on PGE before surgery, and 87% had PGE exposure at some point before surgery. This is expected as most congenital heart disease that requires surgery in the neonatal period has a ductal-dependent physiology. Median age at surgery was 7 days (interquartile range, 5–10 days). A total of 55 patients (37%) experienced the composite morbidity/mortality trial outcome, 34 (23%) experienced the cardiac composite, 66 (44%) experienced AKI, 29 (19%) experienced elevated lactate level, and 38 (26%) experienced hepatic injury.

Differential Expression of Metabolites at Time Points

Of the 193 metabolites examined, 40 were detected and quantifiable in all 149 participants. The other 153 metabolites were not consistently identified or quantified. Results from paired *t*-test analysis comparing metabolite concentrations at various time points are shown in Table 2. A total of 34 metabolites changed significantly between all measured time points. The general pattern was either an immediate postoperative decrease or increase, with a trend back toward the preoperative baseline. There were many exceptions to this pattern, including methyladenosine, which showed a delayed increase at the 12-hour post-op; leucine and nicotinamide, which had the inverse finding of a delayed decrease at the 12-hour post-op time point; arginine, isoleucine, methionine, methionine sulfoxide, ornithine, serine, and glycocholic acid, all of which continued to decrease at the time points measured; and paraxanthine, indoxyl sulfate, and hippuric acid, which increased across all time points.

Association of Metabolites to Clinical Outcomes

The first 6 components of the PC analysis performed on metabolites and features individually had loads >3% and together explained 51% of the cumulative variance in metabolic compounds (detailed features of PCs in Table S2). In univariate analysis (Table 3), PC1 alone was significantly associated with the composite morbidity/mortality, cardiac composite, and hepatic injury outcomes. PC1 and PC3 were associated with acute kidney injury, and PC1 and PC6

Table 2. Metabolites With Statistically Significant Differences Between Different Time Points

Preoperative vs T2	T2 vs T3	Preoperative vs T3
Arginine*	Arginine*	Arginine*
Aspartic acid*	Isoleucine*	Asparagine
Carnitine*	Leucine*	Aspartic acid*
Creatinine*	Methionine*	Carnitine*
Glutamine*	Methionine sulfoxide*	Creatinine
Glutamic acid*	Nicotinamide*	Glutamine*
Histidine*	Ornithine*	Glutamic acid*
4-Hydroxyproline*	Proline*	Histidine*
Isoleucine*	Gluconic acid*	4-Hydroxyproline*
Lysine*	Galactitol*	Isoleucine*
Methionine*	4-Pyridoxic acid*	Leucine*
Methionine sulfoxide*	Glycocholic acid*	Lysine*
Ornithine*	Sn-glycero-3-phosphochocholine*	Methionine*
Phenylalanine*	1-Methyladenosine†	Methionine sulfoxide*
Proline*	N-acetylserine†	Nicotinamide*
Serine*	Histidine†	Ornithine*
Tryptophan*	Homoserine†	Phenylalanine*
Homoserine*	Carnitine†	Proline*
5-Oxoproline*	Creatinine†	Serine*
3-Methoxytyrosine*	Asparagine†	Tryptophan*
N-acetylserine*		5-Oxoproline*
Glycocholic acid*		3-Methoxytyrosine*
Gluconic acid†		Glycocholic acid*
N-acetyltryptophan†		Gluconic acid†
Paraxanthine†		Paraxanthine†
Galactitol†		Galactitol†
Indoxyl sulfate†		1-Methyladenosine†
Sn-glycero-3-phosphocholine†		Indoxyl sulfate†
Hippuric acid†		Sn-glycero-3-phosphocholine†
		Hippuric acid†
		N-acetylserine†

For metabolites with statistically significant differences, the fold change cutoff was 0.5/2.0 (*P*-value cutoff, 0.0125). * indicates significant decrease; †, significant increase. T2 indicates immediate postoperative; and T3, 12 hours postoperative.

Table 3. Association of PCs With Clinical Outcomes With Univariate Analysis

Outcome	PC*	OR (95% CI)	*P* value
Composite: mortality/morbidity	PC1	1.21 (1.10–1.33)	<0.001
Composite: cardiac†	PC1	1.14 (1.05–1.25)	0.003
Hepatic	PC1	1.27 (1.14–1.41)	<0.001
Lactate level	PC1	1.13 (1.03–1.24)	0.01
	PC6	1.25 (1.01–1.54)	0.04
AKI	PC1	1.06 (1.01–1.13)	0.03
	PC3	1.09 (1.01–1.16)	0.02

AKI indicates acute kidney injury; OR, odds ratio; and PC, principal component.

*Significant PC.

†Only includes death, extracorporeal membrane oxygenation, cardiac arrest, and lactate level outcomes.

were associated with increasing lactate level. Of the PC loads, PC1 was of special interest as 14 of the 20 metabolites within this group were preoperative (Table S2).

A base clinical model (adjusted for enrolling site, PGE use, Society of Thoracic Surgeons category, presurgery lactate level, and gestational age at birth) was significantly associated with composite morbidity/mortality, cardiac composite, and hepatic injury outcomes, but not AKI (Table 4). Of note, an additional analysis including steroid versus placebo administration was also run, without changes in results, so this factor was not included in further analysis. To determine if metabolite levels could improve the base clinical model performance, the 6 top PCs were incorporated into a regression analysis with the base clinical model (base model+metabolite PCs) and compared with the base clinical model using AIC, Wald X^2, and c-statistic as performance metrics (Table 4 and Table S3). For comparison, a lower AIC and higher c-statistic indicate improved model fit. Both the base clinical model and the base model+metabolite PCs were significant in their associations to the outcomes. However, the second model had improved model fit, as indicated in the difference in AIC and c-statistic. The addition of PC1 significantly improved the clinical model performance for the composite morbidity/mortality outcome, composite cardiac outcome, and hepatic injury (PC1 and PC3 for acute kidney injury and PC1 and PC6 for lactate level).

Association of Individual Metabolites With Outcomes

Given the significance of the association between clinical outcomes and metabolite PC loads, and improvement in base model+metabolite PCs compared with the clinical base model alone, we further investigated the association between individual metabolites (or

Table 4. Model Comparison of Associations Between PC Loads and Binary Outcomes With Multivariate Regression Analysis

Outcome	Base model*				Base model+PC					Base model+individual metabolites†				
	AIC	Wald χ^2	*P* value	C-statistic	AIC	Wald χ^2	*P* value	C-statistic	Significant PC	AIC	Wald χ^2	*P* value	C-statistic	Significant PC
Composite: mortality/morbidity	158.3	33.5	<0.001	0.828	137.3	38.2	<0.001	0.879	PC1	90.0	27.6	0.006	0.967	None
Composite: cardiac‡	140.2	24.7	<0.001	0.805	129.2	29.0	<0.001	0.850	PC1	113.0	30.4	<0.001	0.905	None
Hepatic	150.0	23.1	<0.001	0.788	121.7	32.5	<0.001	0.884	PC1	99.5	32.1	<0.001	0.936	None
Lactate level	123.8	32.2	<0.001	0.817	116.6	27.3	<0.001	0.845	PC1	93.5	27.1	0.001	0.930	None
AKI	210.9	5.3	0.38	0.607	204.6	13.6	0.06	0.672	PC1, PC3	182.0	29.6	0.003	0.811	PC6

AIC indicates Akaike Information Criterion; AKI, acute kidney injury; and PC, principal component.

*Adjusted for site, preoperative prostaglandin E-1, Society of Thoracic Surgeons category, preoperative lactate value, and gestational age at birth.

†Individual metabolites, as listed in Table S4.

‡Only includes death, extracorporeal membrane oxygenation, cardiac arrest, and lactate level outcomes.

features derived from them) and outcomes. As shown in Table 5, individual metabolites and features were significantly associated with outcomes. Individual performance metrics for the base model+individual metabolites are detailed in Table S4. Significant individual metabolites improved (lower AIC and higher c-statistic) the clinical model for the composite morbidity/mortality outcome, composite cardiac outcome, hepatic injury, and lactate level outcomes (Table 4). Association of individual metabolites with outcomes was not improved by including PC loads in the model for the composite morbidity/mortality outcome, composite cardiac outcome, hepatic injury, and lactate level outcomes.

Metabolite Enrichment Pathway and Disease-Associated Analysis

For the individual metabolites or features associated with clinical outcomes, we explored the metabolic pathways (Kyoto Encyclopedia of Genes and Genomes, (KEGG)) associated and if disease associated. Significant false discovery rate (FDR; <0.05) KEGG pathways represented included aminoacyl-tRNA biosynthesis, arginine biosynthesis, and metabolism of multiple amino acids. Using metabolite enrichment disease pathway analysis (Figure and Table 6), individual metabolites were significantly (FDR, <0.05) associated with disease pathways, including seizures (FDR, 2.67×10^{-6}), early markers of myocardial injury (FDR, 0.00091), and heart failure (FDR, 0.041).

DISCUSSION

Early identification of patients at risk for postoperative morbidity could improve outcomes. Clinical models alone using known, or suspected, risk factors, such as age, known comorbidities, weight, and complexity of surgery, have offered some insight into mortality risk, but may have no applicability to subtler organ-specific injury. The findings of this study show a significant association with metabolites and multiple clinical outcomes, and metabolite data improved on a clinical-only risk model. This study also suggests that metabolites may provide an actionable target to improve outcomes following congenital cardiac surgery. Prior studies comparing perioperative shifts in metabolic profiles and the relationship to postoperative outcomes have suggested the utility of incorporating metabolites into outcome prediction and/or risk stratification.[6,7] These pediatric studies have been limited by small patient cohort sizes from single centers and a broad age range. Our work expands on these studies using a multicenter and larger cohort, with a specific focus on neonates requiring cardiac surgery with CPB. This study also benefits from an expanded targeted assay panel of 193 metabolites. These measures allowed us to explore the potential metabolic pathways more specific to neonatal cardiac surgery and recovery.

In regard to the significant perioperative metabolite changes identified, these mostly represented aminoacyl-tRNA biosynthesis, arginine biosynthesis, and metabolism of multiple amino acids pathways. Enrichment analysis revealed that many of the individual metabolites were previously identified in studies of cardiac dysfunction and seizures, reflecting both cardiac dysfunction[23–25] and neurologic injury,[26] which are common postoperative morbidities. Although seizures were not recorded in the primary trial, we have shown previously that the brain injury biomarker GFAP (glial fibrillary acidic protein) was increased postoperatively in this cohort and was associated with a worse Bayley Scale of Infant Development III score at 12 months of age,[27] providing evidence of at least postoperative subclinical brain injury in this cohort and consistent with findings of seizure-associated metabolites. Three (L-isoleucine, L-leucine, and L-tyrosine) of the 8 amino acids associated with seizures were also associated with acute myocardial injury and heart failure, which could reflect a more global injury phenotype as opposed to specific organ injury.

Previously, dysregulation of glutathione and amino acid synthesis pathways was identified after congenital heart surgery and was confirmed in this study.[6,7] Shifts in glutathione metabolism are relevant to our study because glutathione has a role in antioxidant defense, nutrient metabolism, and regulation of protein synthesis, cell proliferation, and apoptosis.[28] We found

Table 5. Significant Individual Metabolites or Metabolite Features Associated With an Outcome

Composite: morbidity/mortality	Composite: cardiac	Composite: hepatic	Composite: lactate level	AKI
t1 Ornithine t1 Phenylalanine t1 Methionine t3 Cystine t3 4-Hydroxyproline t3 3-Methoxytyrosine r21 Homoserine	Cum proline t3 proline t3 leucine	t1 Carnitine t1 Aspartic acid t3 Tyrosine	t1 Methionine r31 Galactitol t3 Proline t3 Leucine	t1 Ornithine t1 Isoleucine t1 Aspartic acid t3 Alanine t3 Glutamic acid t3 4-Hydroxyproline t3 Serine

AKI indicates acute kidney injury; Cum, cumulative concentration from all time points; r21, ratio between time points 1 and 2; r31, ratio between time points 1 and 3; t1, time point 1 (preop); t2, time point 2 (immediate post-op); t3, time point 3 (12 hours post-op).

this compelling as neonates typically have limited glycogen and fat energy stores; therefore, they are more reliant on glucose-derived energy. Feeding preoperatively is typically restricted in neonates with congenital heart disease, with dependence on intravenous nutrition in the form of dextrose-containing fluids. Because of these feeding patterns, we postulate that our patients are at risk of even more poorly developed energy stores, which could explain glutathione dysregulation.

For most outcomes measured, high PC1 was universally associated with poor outcomes. Analyzing the individual components of this PC, we were surprised to find many of the metabolites were preoperative values, with the remaining components being cumulative values. This suggests that these patients were at risk because of their preoperative physiological state, and not because of surgical factors (CPB duration, circulatory arrest, and cross-clamp time). In the initial conceptualization of this study, we had hypothesized that the changes from surgery and CPB would be the most influential on patient outcomes. The recurring importance of PC1 strongly suggests that the patient's preoperative physiological state could be most important modification for improving morbidity and mortality.[23–25,29,30] As in the study by Davidson et al,[6] essential amino acids (leucine, methionine, and phenylalanine) were found to be associated with worse outcomes, in addition to conditionally

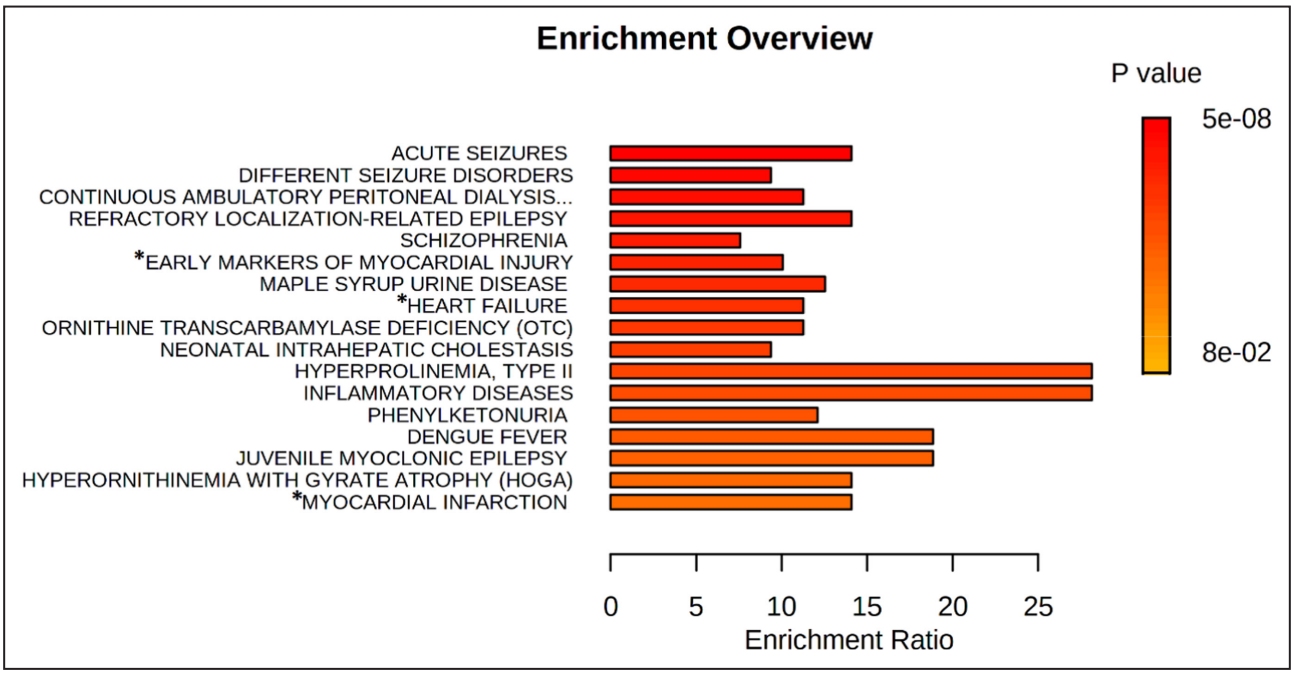

Figure. Metabolite enrichment disease pathway analysis.
Metabolite enrichment disease pathway analysis using individual metabolites that improved on the base model (listed in S4). Disease associations with metabolites are in the figure listed above. Asterisks (*) indicate attention to cardiac diseases that are of special interest to this patient population.

Table 6. Metabolite Disease Enrichment Analysis

Metabolite set enrichment analysis	Total	Hits	Raw *P* value	FDR	Perioperative metabolites associated with disease
Acute seizures	14	7	9.78E-09	2.67E-06	L-alanine, L-methionine, L-leucine, L-isoleucine, L-phenylalanine, L-histidine, L-tryptophan
Different seizure disorders	24	8	2.83E-08	3.87E-06	L-alanine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine
Refractory localization-related epilepsy	10	5	2.26E-06	0.000154	L-lysine, L-leucine, L-isoleucine, L-phenylalanine, L-tyrosine
Early markers of myocardial injury	14	5	1.69E-05	0.00091	L-alanine, L-isoleucine, L-leucine, L-proline, L-serine
Heart failure	10	3	0.00212	0.0414	L-isoleucine, L-leucine, L-tyrosine
Myocardial infarction	4	2	1.0	0.104	L-phenylalanine, L-tyrosine

FDR indicates false discovery rate.

essential (cystine and proline). The continued relevance of amino acids between our study and prior work supports the role that nutritional status plays in improving outcomes.

Despite increased cohort size compared with prior works, this study remains limited by a relatively small cohort produced from only 2 participating sites. Statistical analysis relied heavily on PC analysis, which allowed for interpretation of complex data in this relatively small cohort. A more straightforward approach using regression analysis for each metabolite against each outcome would likely have missed significant findings because of the smaller sample size. The drawback is that a general metabolic profile can be associated with an outcome (a PC), without individual metabolites being as clearly linked.

Other limitations include the primary trial enrolling criteria, and exclusion of younger neonates (aged <37 weeks) or older infants/children, which also left us unable to determine whether metabolite changes differ between these patient populations. These results may not be necessarily generalizable to nonneonates. Last, we used a targeted metabolomic analysis, which lacked a full range of metabolites, such as membrane and vasoactive lipids, which could potentially be informative.

The association between metabolites and outcomes in neonatal cardiac surgery that we have demonstrated herein suggests an additional tool beyond clinical data to help identify patients at risk for poor outcomes. What we found most promising was the association between preoperative metabolic profiles and outcomes of interest, suggesting improvement in outcomes begins preoperatively. Further research would include validation studies, and metabolomics in expanded age groups, in addition to targeted analysis of identified pathways. The proposed mechanism of preoperative nutritional status and effect on operational outcomes would bear further investigation also.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S4

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SUPPLEMENTAL MATERIAL

Table S1. Targeted panel of metabolites	
MDC ID	Compound name
0125_Ribulose 5-phosphate	Ribulose 5-phosphate sodium salt
0108_ADP / 0273_3,5-ADP	Adenosine diphosphate
0243_IDP	Inosine 5-diphosphate
0132_IMP	2-Inosine-5-monophosphate / Inosinic acid
0154_O-Phosphoethanolamine	O-Phosphoethanolamine
0179_Glucosamine 6-phosphate	D-GLUCOSAMINE 6-PHOSPHATE
0147_Gluconic acid	GLUCONATE
0246_Nicotinamide hypoxanthine dinucleotide	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE
0192_Ciliatine	(2-AMINOETHYL)PHOSPHONATE
0159_Tartaric acid	Tartaric acid
0367_Sn-Glycero-3-phosphocholine	SN-GLYCERO-3-PHOSPHOCHOLINE
0191_Glucuronolactone	D-GLUCURONOLACTONE
0200_Galactitol	GALACTITOL
0020_Citicoline	Cytidine 5-Diphosphocholine / Citicoline / Choline 5-cytidine diphosphate
0312_Glucosaminic acid	GLUCOSAMINATE
0341_Methionine sulfoximine	METHIONINE SULFOXIMINE
0048_4-Hydroxyproline	4-Hydroxy-L-Proline
0149_Dihydroorotic acid	Dihydroorotic acid
0028_Cystine	Cystine
0013_Aspartic acid	Aspartic acid
0223_N-Methylaspartic acid	N-METHYL-D-ASPARTIC ACID
0017_2,3-cyclic CMP	Cytidine 2,3-Cyclic Monophosphate / 2,3-Cyclic CMP
0168_Thiourea	Thiourea
0092_Orotic acid	Orotic acid
0012_Asparagine	L-Asparagine
0068_Serine	Serine
0038_Glutamine	Glutamine
0160_Nicotinamide mononucleotide	Nicotinamide mononucleotide
0234_Nalpha-Acetylasparagine	N-ALPHA-ACETYL-L-ASPARAGINE
0026_Cystathionine	Cysthathionine
0098_Homoserine	Homoserine
0057_Methionine Sulfoxide	Methionine sulfoxide
0070_Threonine / 0359_Allothreonine	Threonine/Allothreonine

0322_S-Carboxymethylcysteine	S-CARBOXYMETHYL-L-CYSTEINE
0183_Betaine	BETAINE
0226_N-methylglutamic Acid	N-METHYL-L-GLUTAMATE
0378_N-Acetylserine	N-ACETYL-DL-SERINE
0039_Glutamic acid	Glutamic acid
0073_TMP	Thymidine 5'-monophosphate / 5-Thymidylic acid
0021_Citrulline	Citrulline
0158_dCMP	2-Deoxycytidine 5-monophosphate
0064_Proline	Proline
0336_3-Ureidopropionic acid	3-UREIDOPROPIONATE
0008_Alanine	Alanine
0146_Sarcosine	Sarcosine
0302_C6_sugar_amine	C6 sugar amine
0076_Uracil	Uracil
0370_Trigonelline	TRIGONELLINE
0242_N-Acetylaspartic acid	N-AMIDINO-L-ASPARTATE
0369_2,3-Diaminopropionic acid	DL-2,3-Diaminopropionic acid monohydrochloride
0091_NAD	Nicotinamide adenine dinucleotide
0358_5-Hydroxylysine	DL-5-HYDROXYLYSINE
0321_Orthanilic acid	ANILINE-2-SULFONIC ACID
0143_Beta-Alanine	Beta-Alanine
0201_5-Oxoproline	5-OXO-D-PROLINE
0164_dAMP	Deoxyadenosine monophosphate
0023_Creatine	Creatine
0364_Citramalic acid	CITRAMALATE
0097_Xanthine	Xanthine
0061_Ornithine	Ornithine
0044_Histidine	Histidine
0371_3,4-Dihydroxyphenylglycol	3,4-DIHYDROXYPHENYL GLYCOL
0085_Fumaric acid	Fumaric acid
0055_Lysine	Lysine
0049_Hypoxanthine	Hypoxanthine / 6-Hydroxypurine
0325_N-Acetylglutamic acid	N-ACETYL-DL-GLUTAMIC ACID
0059_Nicotinic acid	Nicotinic acid
0077_Uridine	Uridine
0373_2-Hydroxybutyric acid	2-HYDROXYBUTYRIC ACID
0036_Gamma-aminobutyric acid	Gamma-aminobutyric acid / 4-aminobutanoic acid
0140_Pipecolic acid	Pipecolic acid

0018_3,5-cyclic GMP	Guanosine 3,5-Cyclic Monophosphate / 3,5-Cyclic GMP
0253_2,3-cyclic AMP	ADENOSINE 2,3-CYCLIC MONOPHOSPHATE
0030_Cytosine	Cytosine
0006_Adenylsuccinic acid	Adenylosuccinic acid / Adenylsuccinic acid
0204_Methylguanidine	METHYLGUANIDINE
0221_3-Hydroxymethylglutaric acid	3-HYDROXY-3-METHYLGLUTARATE
0346_4-Imidazoleacetic acid	4-IMIDAZOLEACETIC ACID
0047_Homocystine	Homocystine
0404_Maleic acid	MALEIC ACID
0438_2-Hydroxypyridine	2-HYDROXYPYRIDINE
0305_Thiamine monophosphate	THIAMINE MONOPHOSPHATE
0010_Arginine	Arginine
0170_3-Aminoisobutanoic acid	3-Aminoisobutanoic acid
0060_Ophthalmic acid	Ophthalmic acid
0211_4-Acetamidobutanoic acid	4-ACETAMIDOBUTANOATE
0014_3,5-cyclic AMP	Adenosine 3,5-Cyclic Monophosphate / 3,5-Cyclic AMP
0058_Nicotinamide	Nicotinamide
0461_Pterin	PTERIN
0051_Inosine	Inosine
0133_Purine	Purine
0056_Methionine	Methionine
0267_N-Alpha-acetyllysine	NALPHA-ACETYL-L-LYSINE
0024_Creatinine	Creatinine
0015_Carnitine	Carnitine
0189_O-Succinylhomoserine	O-SUCCINYL-L-HOMOSERINE
0327_6-Hydroxynicotinic acid	6-HYDROXYNICOTINATE
0491_Itaconic acid	ITACONATE
0086_Glutathione oxidized	Glutathione oxidized / Glutathione disulfide
0512_Vanillylmandelic acid	3-METHOXY-4-HYDROXYMANDELATE
0043_Guanosine	Guanosine
0259_5-Methylcytosine	5-METHYLCYTOSINE HYDROCLORIDE
0283_2-Deoxyguanosine	2-DEOXYGUANOSINE
0029_Cytidine	Cytidine
0071_Thymidine	Thymidine
0323_N-Acetylputrescine	N-ACETYLPUTRESCINE
0337_5-Aminopentanoic acid	5-AMINOPENTANOATE
0514_4-Quinolinecarboxylic acid	4-QUINOLINECARBOXYLIC ACID

0062_Pantothenic acid	Pantothenic acid
0202_4-Pyridoxic acid	4-PYRIDOXATE
0293_Indoxyl sulfate	INDOXYL SULFATE
0462_Ethylmalonic acid	ETHYLMALONIC ACID
0193_Selenomethionine	SELENOMETHIONINE
0278_Pyridoxamine	PYRIDOXAMINE
0351_Theobromine	THEOBROMINE
0426_Desmeninol	2-HYDROXY-4-(METHYLTHIO)BUTYRIC ACID
0178_2-Deoxycytidine	DEOXYCYTIDINE
0266_Octopamine	OCTOPAMINE
0004_Adenine	Adenine
0005_Adenosine	Adenosine
0205_1-Methyladenosine	1-METHYLADENOSINE
0442_Protocatechuic acid	3,4-DIHYDROXYBENZOATE
0194_Paraxanthine	PARAXANTHINE
0532_Hydroxyphenyllactic acid	3-(4-HYDROXYPHENYL)LACTATE
0075_Tyrosine	Tyrosine
0209_Urocanic acid	UROCANATE
0381_3-Hydroxykynurenine	3-HYDROXYKYNURENINE
0286_N-Acetylmethionine	N-ACETYL-DL-METHIONINE
0469_4-Aminobenzoic acid	4-AMINOBENZOATE
0531_3-Methyladenine	3-METHYLADENINE
0516_Pyridoxal	PYRIDOXAL
0034_Epinephrine	Epinephrine / Adrenaline
0536_Pyrrole-2-carboxylic acid	PYRROLE-2-CARBOXYLATE
0460_2-Quinolinecarboxylic acid	2-QUINOLINECARBOXYLIC ACID
0343_3-Methoxytyrosine	3-METHOXY-L-TYROSINE
0412_Pimelic acid	6-CARBOXYHEXANOATE
0533_Biotin	BIOTIN
0425_Thiopurine S-methylether	THIOPURINE S-METHYLETHER
0195_Caffeine	CAFFEINE
0421_Caffeic acid	CAFFEATE
0537_5-Hydroxyindoleacetic acid	5-HYDROXYINDOLEACETATE
0050_Isoleucine	Isoleucine
0459_Hippuric acid	HIPPURATE
0489_N-Acetylserotonin	N-ACETYLSEROTONIN
0481_Gentisic acid	2,5-DIHYDROXYBENZOATE

0525_3-Hydroxyphenylacetic acid	3-HYDROXYPHENYLACETATE
0349_5-Hydroxytryptophan	5-HYDROXY-L-TRYPTOPHAN
0229_Thyrotropin releasing hormone	THYROTROPIN RELEASING HORMONE
0163_Pyridoxine	Pyridoxine
0487_Dethiobiotin	DETHIOBIOTIN
0007_ADMA / 0067_SDMA Dimethylarginine	Dimethylarginine
0416_Mandelic acid	MANDELIC ACID
0053_Leucine	Leucine
0318_5-Deoxyadenosine	5-DEOXYADENOSINE
0295_Normetanephrine	DL-NORMETANEPHRINE
0503_N-Acetylleucine	N-ACETYL-L-LEUCINE
0338_Norleucine	NORLEUCINE
0493_Suberic acid	SUBERIC ACID
0430_2-Pyrocatechuic acid	2,3-DIHYDROXYBENZOATE
0410_Kynurenic acid	4-HYDROXY-2-QUINOLINECARBOXYLIC ACID
0033_Dopamine	Hydroxytyramine / Dopamine
0388_Xanthurenic acid	XANTHURENIC ACID
0473_2-Aminophenol	2-AMINOPHENOL
0450_3-Indoleacetamide	INDOLE-3-ACETAMIDE
0274_3-Nitrotyrosine	3-NITRO-L-TYROSINE
0506_Salicylamide	SALICYLAMIDE
0413_N-Acetylphenylalanine	N-ACETYL-L-PHENYLALANINE
0063_Phenylalanine	Phenylalanine
0466_Melilotic acid	3-(2-HYDROXYPHENYL)PROPANOATE
0479_Anthranilic acid	ANTHRANILATE
0433_Ferulic acid	FERULATE
0422_Lumichrome	LUMICHROME
0505_Salsolinol	1-METHYL-6,7-DIHYDROXY-1,2,3,4-TETRAHYDROISOQUINOLINE
0173_N-acetyltryptophan	N-acetyl-D-tryptophan
0052_Kynurenine	Kynurenine
0482_Azelaic acid	AZELAIC ACID
0420_Indoleacetic acid	N-ACETYL-L-ASPARTIC ACID
0395_Tyramine	TYRAMINE
0403_Melatonin	MELATONIN
0356_Guanidinosuccinic acid	INDOLE-3-ACETATE
0432_Indole-3-ethanol	INDOLE-3-ETHANOL
0436_10-Hydroxydecanoic acid C10:0(OH)	10-HYDROXYDECANOATE

0396_Cortisol	CORTISOL
0467_3-Methoxytyramine	3-METHOXYTYRAMINE
0069_Serotonin	Serotonin
0454_Lipoamide	LIPOAMIDE
0415_3,5-Diiodotyrosine	3,5-DIIODO-L-TYROSINE
0074_Tryptophan	Tryptophan
0434_Glycocholic acid	GLYCOCHOLATE
0423_Tryptophanamide	L-TRYPTOPHANAMIDE
0307_S-Hexylglutathione	S-HEXYL-GLUTATHIONE
0449_Hydrocortisone 21-acetate	CORTISOL 21-ACETATE
0463_3,5-Diiodothyronine	3,5-DIIODO-L-THYRONINE
MDC= Molecular Determinants Core	

Table S2. Top 20 metabolites (or features) contributing to the first 6 dimensions of PC analysis

PC1	PC2	PC3
t1 Phenylalanine	t2 Serine	t3 Leucine
t1 Proline	r32 4-Hydroxyproline	t3 Serine
cum Histidine	t2 Phenylalanine	t3 Phenylalanine
t1 Histidine	r32 Serine	t3 Lysine
t1 Isoleucine	r32 Histidine	t3 Histidine
t1 Serine	r32 Alanine	t3 Isoleucine
t1 Lysine	r32 Glutamine	t3 Proline
t1 Leucine	r32 Tyrosine	t3 Tyrosine
t1 Methionine Sulfoxide	r32 N-Acetylserine	t3 Methionine Sulfoxide
cum Methionine	t2 Histidine	t3 Glutamine
t1 Arginine	t2 Lysine	r31 Proline
cum Lysine	r32 Proline	t3 Alanine
t1 Alanine	r32 Cystine	t3 Methionine
t1 Methionine	t2 Tyrosine	r31 Alanine
cum Methionine Sulfoxide	r32_ Tryptophan	t3 4-Hydroxyproline
t1 5-Oxoproline	r32 Phenylalanine	r31 Phenylalanine
t1 Tyrosine	t2 Glutamine	t3 5-Oxoproline
t1 Tryptophan	t2 Alanine	r31 Isoleucine
cum Alanine	r32 Sn-Glycero-3-phosph	r31 Methionine

cum Phenylalanine	r32 Ornithine	r31 Glutamine
PC4	**PC5**	**PC6**
r21 Lysine	r32 Paraxanthine	Cum 3-Methoxytyrosine
r31 Lysine	r32 Isoleucine	t1 Asparagine
r31 Ornithine	r32 Leucine	t2 3-Methoxytyrosine
r21 5-Oxoproline	r32 Methionine	r31 Galactitol
r31 Histidine	r32 Phenylalanine	Cum Glutamic acid
r21 Histidine	r32 Tryptophan	Cum Cystine
r21 Ornithine	r32 2,3-Diaminopropioni	Cum Glutamine
r31 5-Oxoproline	r325-Oxoproline	Cum Asparagine
r31 Arginine	r32 Glutamic acid	t3 3-Methoxytyrosine
r21 Arginine	r32 Hippuric acid	t1 3-Methoxytyrosine
r31 Creatinine	r32 Sn-Glycero-3-phosph	r21 Gluconic acid
r21 Carnitine	r32 Proline	r21 Galactitol
r21 Creatinine	r32 Tyrosine	Cum 4-Hydroxyproline
t2 Caffeine	r32 Histidine	r31 Gluconic acid
t2 Paraxanthine	r32 Indoxyl sulfate	t2 Glutamic acid
Cum Paraxanthine	t3 Serine	t1 Ornithine
r31 Carnitine	t3 Glutamine	t1 4-Hydroxyproline
Cum Caffeine	t2 Sn-Glycero-3-phosphocho	t1 Cystine
t3 Caffeine	r32 Galactitol	t2 Cystine

t1 Paraxanthine	Cum Sn-Glycero-3-phosph	t1 Leucine

Abbreviations: t1 – time point one, immediate pre-op; t2 – time point 2, immediate post-op; t3 – time point 3, 12 hours post op; r21 – ratio of t2 to t1; r31 – ratio of t3 to t1; r32 – ratio of t3 to t2; cum – cumulative values from t1, t2, t3.

Table S3. Association between principal components loads and outcomes

Composite-mortality/morbidity						
Parameter	**Estimate**	**SE**	**OR**	**LCL**	**UCL**	**p-value**
Site = 2	2.5713	0.5524	13.0828	4.4186	38.7359	<.001
PGE ever = 0	-2.3215	0.6667	0.0981	0.0265	0.3637	0.001
STAT category	0.7071	0.2868	2.0281	1.1544	3.5632	0.01
Pre-surgery lactate	0.8811	0.5812	2.4136	0.7703	7.5622	0.13
Gestational age birth	-0.2656	0.209	0.7667	0.5085	1.1561	0.20
PC1	0.194	0.0489	1.2141	1.1029	1.3365	<.001
Composite - cardiac						
Parameter	**Estimate**	**SE**	**OR**	**LCL**	**UCL**	**p-value**
Site = 2	1.7387	0.5087	5.6899	2.0941	15.4606	0.001
PGE ever = 0	-1.1979	0.6765	0.3018	0.0799	1.1404	0.08
STAT category	0.7343	0.3098	2.084	1.1338	3.8307	0.02
Pre-surgery lactate	1.3524	0.5839	3.8667	1.2276	12.1796	0.02
Gestational age birth	-0.1182	0.2149	0.8885	0.5825	1.3554	0.58
PC1	0.1429	0.0448	1.1536	1.0564	1.2598	0.001
Hepatic						
Parameter	**Estimate**	**SE**	**OR**	**LCL**	**UCL**	**p-value**
Site = 2	1.7943	0.5538	6.0153	2.026	17.8592	0.001
PGE ever = 0	-2.5462	0.6967	0.0784	0.0199	0.3081	<.001
STAT category	0.9034	0.331	2.468	1.2879	4.7295	0.006
Pre-surgery lactate	-0.0927	0.6245	0.9115	0.2672	3.1094	0.88
Gestational age birth	-0.3044	0.2312	0.7376	0.4683	1.1617	0.19
PC1	0.2454	0.0556	1.2781	1.1458	1.4257	<.001
Lactate						

Parameter	Estimate	SE	OR	LCL	UCL	p-value
Site = 2	1.5581	0.5362	4.7498	1.6561	13.6226	0.004
PGE ever = 0	-1.2537	0.733	0.2854	0.0676	1.2052	0.09
STAT category	1.0129	0.3706	2.7536	1.3293	5.7038	0.006
Pre-surgery lactate	1.4959	0.6119	4.4634	1.3411	14.8542	0.01
Gestational age birth	-0.1564	0.238	0.8552	0.5358	1.3652	0.51
PC1	0.124	0.045	1.132	1.0362	1.2367	0.006

AKI

Parameter	Estimate	SE	OR	LCL	UCL	p-value
Site = 2	-0.0911	0.4223	0.9129	0.3982	2.0933	0.83
PGE ever = 0	0.3593	0.5544	1.4323	0.4819	4.2576	0.52
RACHS class	0.2391	0.2391	1.2701	0.794	2.0318	0.32
Pre-surgery lactate	-0.4263	0.4719	0.6529	0.2583	1.6503	0.37
Gestational age birth	0.1899	0.1599	1.2091	0.8831	1.6555	0.24
PC1	0.0655	0.0289	1.0677	1.0087	1.1301	0.02
PC3	0.0799	0.0358	1.0832	1.0096	1.1621	0.03

Abbreviations: AIC - Akaike Information Criterion, Estimate – regression parameter estimate, LCL – lower 95% confidence limit, OR – odds ratio, PC - principal component, PGE – prostaglandins, REL – Reliability score, SE – standard error, UCL – upper 95% confidence limit

Table S4. Final multivariable analysis between principal components loads, top metabolic hits and outcomes

Composite morbidity/mortality							
Parameter	**Rel**	**Estimate**	**SE**	**OR**	**LCL**	**UCL**	**p-value**
Site = 2	.	3.6594	0.9285	38.838	6.2645	240.7845	<.001
PGE ever = 0	.	-2.9055	1.0386	0.0547	0.0071	0.4212	0.005
STAT category	.	0.214	0.4158	1.2386	0.5471	2.804	0.61
Pre-surgery lactate	.	2.4624	0.9957	11.7329	1.6584	83.0091	0.01
Gestational age birth	.	-0.1315	0.3241	0.8768	0.4638	1.6576	0.68
T3 Cystine	100.0%	-0.0664	0.0216	0.9358	0.8969	0.9763	0.002
T3 4-Hydroxyproline	100.0%	0.0799	0.0213	1.0832	1.0388	1.1295	<.001
T3 3-Methoxytyrosine	95.6%	-3.7156	1.1672	0.0243	0.0025	0.2412	0.002
r21 Homoserine	93.4%	-1.3991	0.6561	0.2468	0.068	0.896	0.03
t1 Ornithine	98.2%	-0.0075	0.003	0.9925	0.9867	0.9984	0.01
t1 Phenylalanine	75.0%	0.0032	0.0012	1.0032	1.0009	1.0055	0.007
t1 Methionine	91.4%	0.0122	0.006	1.0123	1.0004	1.0243	0.04

Composite - cardiac							
Parameter	**Rel**	**Estimate**	**SE**	**OR**	**LCL**	**UCL**	**p-value**
Site = 2	.	2.1294	0.6018	8.4098	2.5776	27.4382	<.001
PGE ever = 0	.	-1.1273	0.7525	0.3239	0.0738	1.421	0.13
STAT category	.	0.7338	0.3276	2.083	1.0942	3.9651	0.03
Pre-surgery lactate	.	1.4609	0.6842	4.3098	1.1235	16.5329	0.03
Gestational age birth	.	-0.0094	0.252	0.9907	0.6038	1.6255	0.97
Cum Proline	90.8%	0.0014	0.0005	1.0014	1.0005	1.0023	0.003
T3 Proline	73.0%	0.0032	0.0013	1.0032	1.0006	1.0058	0.02
T3 Leucine	62.0%	-0.0202	0.0075	0.98	0.9657	0.9945	0.007

Hepatic

Parameter	Rel	Estimate	SE	OR	LCL	UCL	p-value
Site = 2	.	1.1931	0.6519	3.2973	0.9159	11.8708	0.07
PGE ever = 0	.	-2.898	0.8879	0.0551	0.0096	0.3156	0.001
STAT category	.	1.3183	0.444	3.7371	1.5618	8.942	0.003
Pre-surgery lactate	.	0.5358	0.7452	1.7088	0.3951	7.3899	0.47
Gestational age birth	.	-0.5332	0.3063	0.5867	0.3214	1.0711	0.08
t1 Carnitine	76.2%	0.0104	0.0025	1.0105	1.0054	1.0155	<.001
T3 Tyrosine	99.0%	0.0058	0.0018	1.0058	1.0022	1.0095	0.002
t1 Aspartic acid	93.2%	0.1012	0.0462	1.1065	1.0105	1.2116	0.03
r21 Caffeine	99.6%	-0.2577	0.107	0.7728	0.6263	0.9537	0.02

Lactate

Parameter	Rel	Estimate	SE	OR	LCL	UCL	p-value
Site = 2	.	0.6703	0.7341	1.9548	0.462	8.2714	0.36
PGE ever = 0	.	-0.8979	0.9172	0.4074	0.0672	2.4704	0.33
STAT category	.	0.9303	0.3016	2.5353	1.4017	4.5857	0.002
Pre-surgery lactate	.	1.3468	0.8898	3.8451	0.6692	22.0929	0.13
Gestational age birth	.	-0.2032	0.3036	0.8161	0.4494	1.482	0.50
R31 Galactitol	98.2%	0.0056	0.0021	1.0056	1.0016	1.0097	0.007
T3 Proline	98.8%	0.005	0.0016	1.005	1.0019	1.008	0.001
t1 Methionine	98.4%	0.007	0.0028	1.0071	1.0015	1.0126	0.01
T3 Leucine	74.6%	-0.0201	0.0086	0.9801	0.9637	0.9968	0.02

AKI

Parameter	Rel	Estimate	SE	OR	LCL	UCL	p-value
Site = 2	.	-0.3546	0.4885	0.7015	0.2686	1.8318	0.47
PGE ever = 0	.	0.3374	0.6385	1.4013	0.3996	4.9138	0.60

STAT category	.	0.1128	0.1818	1.1194	0.7831	1.6001	0.54
Pre-surgery lactate	.	-0.1401	0.5091	0.8693	0.3197	2.3638	0.78
Gestational age birth	.	0.2503	0.1766	1.2844	0.9078	1.8172	0.16
T3 Alanine	69.2%	0.0809	0.0439	1.0843	0.9947	1.1819	0.07
T3 Glutamic acid	76.4%	0.0086	0.0037	1.0087	1.0013	1.016	0.02
T3 4-Hydroxyproline	70.4%	0.0238	0.0093	1.0241	1.0055	1.043	0.01
T3 Serine	72.6%	-0.022	0.0082	0.9782	0.9627	0.9941	0.007
t1 Ornithine	80.6%	0.0024	0.0009	1.0024	1.0005	1.0042	0.01

Abbreviations: AIC - Akaike Information Criterion, Estimate – regression parameter estimate, LCL – lower 95% confidence limit, OR – odds ratio, PC - principal component, PGE – prostaglandins, REL – Reliability score, SE – standard error, UCL – upper 95% confidence limit