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Metabolic Profiling of Children Undergoing Surgery for Congenital Heart Disease

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Objective: Inflammation and metabolism are closely interlinked. Both undergo significant dysregulation following surgery for congenital heart disease, contributing to organ failure and morbidity. In this study, we combined cytokine and metabolic profiling to examine the effect of postoperative tight glycemic control compared with conventional blood glucose management on metabolic and inflammatory outcomes in children undergoing congenital heart surgery. The aim was to evaluate changes in key metabolites following congenital heart surgery and to examine the potential of metabolic profiling for stratifying patients in terms of expected clinical outcomes.

Design: Laboratory and clinical study.

Setting: University Hospital and Laboratory.

Patients: Of 28 children undergoing surgery for congenital heart disease, 15 underwent tight glycemic control postoperatively and 13 were treated conventionally.

Interventions: Metabolic profiling of blood plasma was undertaken using proton nuclear magnetic resonance spectroscopy. A panel of metabolites was measured using a curve-fitting algorithm. Inflammatory cytokines were measured by enzyme-linked immunosorbent assay. The data were assessed with respect to clinical markers of disease severity (Risk Adjusted Congenital heart surgery score-1, Pediatric Logistic Organ Dysfunction, inotrope score, duration of ventilation and pediatric ICU-free days).

Measurements and Main Results: Changes in metabolic and inflammatory profiles were seen over the time course from surgery to recovery, compared with the preoperative state. Tight glycemic control did not significantly alter the response profile. We identified eight metabolites (3-D-hydroxybutyrate, acetone, acetoacetate, citrate, lactate, creatine, creatinine, and alanine) associated with surgical and disease severity. The strength of proinflammatory response, particularly interleukin-8 and interleukin-6 concentrations, inversely correlated with PICU-free days at 28 days. The interleukin-6/interleukin-10 ratio directly correlated with plasma lactate.

Conclusions: This is the first report on the metabolic response to cardiac surgery in children. Using nuclear magnetic resonance to monitor the patient journey, we identified metabolites whose concentrations and trajectory appeared to be associated with clinical outcome. Metabolic profiling could be useful for patient stratification and directing investigations of clinical interventions. (*Crit Care Med* 2015; 43:1467–1476)

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Key Words: bioinformatics; biomarkers; congenital heart disease; critical illness; metabolic profiling

Congenital heart disease is relatively common, affecting between 4 and 14 individuals in every 1,000 live births (1). Around one third of affected children require surgery during early childhood. Interventions to correct or palliate congenital heart disease present their own challenges. Young age, abnormal circulatory physiology, altered blood flow and hypothermia during cardiopulmonary bypass, and the trauma of surgery may cause splanchnic hypoperfusion and tissue ischemia-reperfusion. The resulting dysregulation of homeostatic pathways regulating inflammation, metabolism, and the endocrine system has important clinical consequences including the severity of organ failure and secondary morbidity (2–5).

A better understanding of the interaction between homeostatic derangement and host organ failure in severe illness could improve diagnostic accuracy and patient stratification through the use of clinically relevant biomarkers. These are chemical entities that can be objectively measured and evaluated as an indicator of biological processes. Many biomarker studies have focused on genomic and transcriptomic methods, alongside protein identification in tissues and body fluids. New developments in metabolic profiling techniques such as nuclear magnetic resonance, spectroscopy, and mass spectrometry have opened up the possibility to build a picture of the metabolic environment in cells, tissues, or biofluids and identify key metabolic biomarkers of disease risk, severity and outcome. This approach of assessing metabolites within a biological sample has been used as a tool for biomarker identification and evaluation in a range of disease states, including surgical oncology, sepsis, obesity, and malnutrition (6–12).

We sought to evaluate the metabolic profiling approach and explore the effect of a tight versus conventional glycemic control regime on the metabolic response to surgery in a group of children with congenital heart disease. We used this pilot study to test the following hypotheses: 1) tight glycemic control post surgery reduces the inflammatory and metabolic dysregulation following surgery; 2) metabolic profiling has a role in augmenting stratification of and clinical management of children undergoing congenital heart surgery.

METHODS

Patients

The study was approved by the Royal Brompton Hospital Research Ethics Committee (REC Ref 10/H0801/8). Children undergoing elective surgery for congenital heart disease were recruited preoperatively, with informed parental/patient consent.

The children in this study were also enrolled into the Control of Hyperglycemia in Pediatric Critical Illness trial, with a treatment arm of using insulin to target blood glucose to a range of 72 to 126 mg/dL (4.0–7.0 mmol/L), and control arm of conventional glycemic control, with a target level below 216 mg/dL (12.0 mmol/L) (13).

Children were eligible to participate in the study if they were between 36 weeks of corrected gestational age and 16 years of age, if they had been admitted to a PICU, and if they had an arterial catheter in place and were receiving mechanical ventilation and vasoactive drugs, with an anticipated duration of treatment of at least 12 hours. Children were excluded if they had diabetes mellitus, a confirmed or suspected inborn error of metabolism, if withdrawal of treatment was being considered, or if they had been in a PICU for more than 5 days.

Patient Biological Samples

Peripheral blood was obtained from intra-vascular lines preoperatively (at induction of anesthesia), and at serial timepoints postoperatively following admission to the PICU. Specifically, samples were taken on admission (0 hr), and then at 6, 24, and 48 hr post surgery for as long as an indwelling vascular catheter was in situ. Blood was collected into endotoxin-free sodium heparin (168 IU) Vacutainer tubes (Beckton Dickinson, Oxford, United Kingdom). Plasma was extracted by centrifugation of blood at 1,200*g* for 10 minutes and the supernatant was stored at –80°C until analysis.

Cytokine Assays

Cytokine concentrations in plasma samples were determined in duplicate using MSD MULTI-SPOT immunoassay kits for interleukins (IL) 10, 6, 8 and IL-1 receptor antagonist (IL-1ra) (Mesoscale Discovery, Gaithersburg, MD), according to the manufacturer's protocol. Analysis was undertaken using MSD Workbench, version 3.0.18, on the Sector Imager 2400 (MSD)

Statistical Analysis of Demographic Variables

Values are shown as median (interquartile range) except where indicated.

^{1}H NMR Spectroscopic Analysis

The protocols for sample preparation and NMR acquisition are based on published protocols by Dona et al (14). A Bruker AVANCE III spectrometer (Bruker Biospin, Billerica, MA) with a broadband inverse 600 MHz 5 mm Z gradient probe with automatic tuning and matching was used for all the ^{1}H NMR spectroscopy experiments. Further details are provided in the **supplementary methods** (Supplemental Digital Content 1, http://links.lww.com/CCM/B267).

Data Reduction and Multivariate Analysis

The acquired ^{1}H NMR spectra of plasma samples were zero filled to 132 K points, Fourier transformed, phase and baseline corrected using Bruker Topspin 3.1 (Bruker Biospin) Using both in-house developed scripts (Drs T. Ebbels, O. Cloarec, and M. Rantalainen) for MATLAB (version 2012b; The MathWorks, Inc, Natick, MA) and python 2.7.4 (15–17). Further details on the analysis and model fitting (18–23) are provided in the supplementary methods (Supplemental Digital Content 1, http://links.lww.com/CCM/B267).

Peak Fitting

For deconvolution and relative metabolite quantification in the spectra the R (22) package BATMAN (Bayesian AuTomated Metabolite Analyzer for NMR spectra) was used (23). Relative concentrations for the metabolites were used to obtain Pearson correlation matrices and plot heatmaps in python (15–17) using the plotting library matplotlib (24). Fifteen metabolites were relatively quantified in this way: lactate, creatine, creatinine, glucose, citrate, formate, 3-hydroxybutyrate, acetone, acetate, acetoacetate, alanine, valine, isoleucine, leucine, and threonine. Further details are provided in the supplementary methods (Supplemental Digital Content 1, http://links.lww.com/CCM/B267).

RESULTS

Patients

There were 28 patients with a median age of 6.6 months (4.0–18.9 mo), and median weight of 6.2 kg (4.0–8.52). The median number of PICU-free days at 28 days was 24 (20.25–26). Children in this cohort were relatively undernourished, with a median weight age *z* score of –2.03 (–2.94 to –1.49). As a marker of organ failure, we used the Pediatric Logistic Organ Dysfunction (PELOD) score. During the first 24 hours, children enrolled in the study had a median PELOD score of 11 (11–20.75). Further clinical details on the patient clinical variables are shown in **Table 1**. The natures of the congenital heart lesions of the children enrolled in the study are shown in **Table 2**.

Fifteen children had tight glycemic control postoperatively, and the remaining 13 had conventional blood glucose management. Median glucose (±interquartile range) for tight and conventional glycemic control groups was 6.1 mmol/L (5.8–6.5) and 6.7 mmol/L (6–8.02), respectively ($p = 0.046$). There was no difference in age, weight, weight age Z score, PELOD score, Inotrope Score, or duration of ventilation and PICU-free days between the two groups (**Table 3**).

TABLE 1. Clinical Characteristics of the Patients Enrolled in the Study

Clinical Variable	Median (IQR)
Age (mo)	6.605 (4.49 to 15.56)
Weight (kg)	6.25 (4.00 to 8.35)
Weight age *z* score	−2.03 (−2.85 to −1.69)
Risk Adjusted Congenital heart Surgery Score -1 (25)	2 (2 to 3)
Pediatric Logistic Organ Dysfunction score (26) on PICU admission	11 (11 to 20.5)
Maximum inotrope score (27)	7.65 (5 to 11.5)
Highest lactate on blood gas	1.95 (1.4 to 2.75)
Highest base excess on blood gas	−5.35 (−8.3 to −4.35)
Highest arteriovenous oxygen saturation difference on blood gas	33.2 (29.05 to 40.1)
Days free of PICU at 28 d	24 (20.5 to 26)

IQR = interquartile range.

TABLE 2. Congenital Heart Defects of Patients Enrolled to the Study

Congenital Heart Defect	*n*	% of Total
Tetralogy of fallot	8	28.57
Ventricular septal defect	9	32.14
Hypoplastic left heart	2	7.14
Atrioventricular septal defect	1	3.57
Truncus arteriosus	2	7.14
Transposition of the great arteries	3	10.71
Mitral valve stenosis	1	3.57
Total anomalous pulmonary venous connection	1	3.57
Pulmonary atresia	1	3.57
Total	28	

Metabolic Profiles Showed No Difference Between Glycemic Control and Conventional Glucose Management Groups

^{1}H NMR spectra of plasma profiles showed no difference in postoperative samples from children with glycemic control intervention compared with the control group. The prediction metrics for the PLS-DA model of the global ^{1}H NMR profile are R^2Y 0.75, Q^2Y –0.44, and R^2Y 0.46, Q^2Y –0.78 for the model generated using a subset of 15 relatively quantified metabolites.

The model validation metric Q^2Y is obtained through cross-validation, and is therefore a more robust estimate against model overfitting. Because in both cases the obtained Q^2Y values are negative, our data do not support the hypothesis of a difference in metabolic profiles caused by a tighter glycemic control compared with the conventional regime.

Metabolic Profiles Showed a Temporal Evolution That Returned Toward the Presurgical Profile With Clinical Recovery

Substantial differences between pre- and postoperative samples were found. This is summarized in the principal components analysis scores plot including all pre- and postoperative timepoints showing that samples are generally well clustered according to the time at which they were taken (**Fig. 1**). As expected from a fairly heterogeneous population, there was a considerable amount of patient variability that became particularly visible postoperatively. The preoperative samples formed a tight cluster in the top left corner of the plot inferring relatively low variation in the biochemical composition of plasma, whereas samples obtained postoperatively were dispersed across the scores plot indicative of

TABLE 3. Comparison of Clinical Variables Between Those Children Undergoing Conventional Management and Those With Tight Glycemic Control

	Conventional Management ($n = 13$)	Tight Glycemic Control ($n = 15$)
Mean glucose during PICU admission[a]	6.7 (6.0 to 8.2)	6.1 (5.8 to 6.5)
Mean dose of insulin[b]	0 (0 to 0)	0.06 (0.01 to 0.08)
Age (wk)	8.25 (2.54 to 18.0)	6.3 (4.6 to 25.84)
Weight (kg)	6.5 (4.4 to 9.15)	6 (3.9 to 7.5)
WAZ	−1.96 (−2.61 to −1.7)	−2.13 (−3.34 to −0.82)
RACHS score	2 (2 to 3.5)	2 (2 to 3)
PELOD score	11 (11 to 16)	11 (11 to 21)
Duration of ventilation (hr)	48 (35.5 to 108)	72 (48 to 168)
PICU-free days at 28 d	24 (23 to 26)	21 (20 to 26)

[a]$p = 0.046$.
[b]$p = 0.002$.

variation in the individual response to surgery or ultrafiltration. In general, there was a time trend post surgery with the samples immediately postoperatively (orange circles) migrating in the first principal component (PC) with the 6-hour (yellow) and 24-hour (green) samples differentiating from the preoperative samples in the second PC. By 48 hours postoperatively (purple circles), some of the patients were comapping with the preoperative samples, indicating a recovered metabolic profile.

Changes in Metabolic Profile Were Related to Pre- and Postsurgical Factors

The strength of the metabolic response to surgery was influenced by surgical and/or disease severity. This is demonstrated by trajectories for two patients that are highlighted in Figure 1. Patient 19 underwent major surgery and had a more prolonged postoperative recovery (RACHS-1 score 4, invasive ventilation for 241 hr and 7 d free of PICU at 28 d). The pattern of change

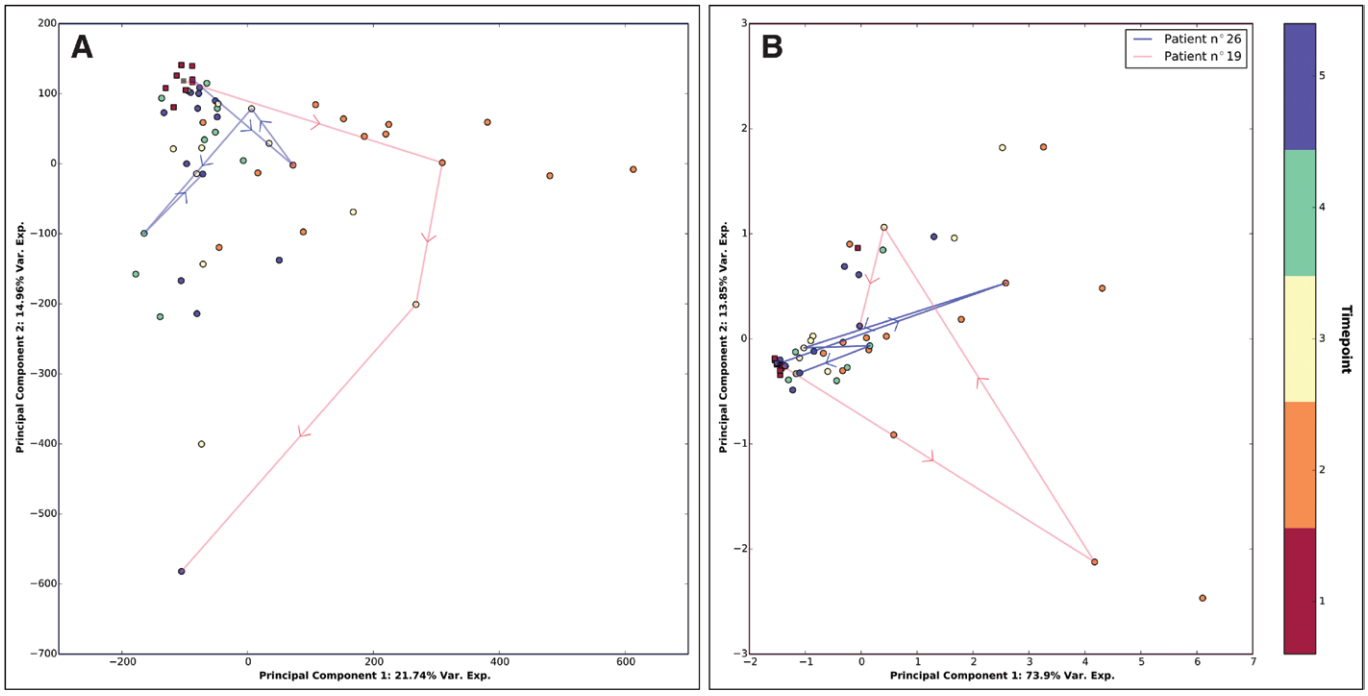

Figure 1. Principal component analysis score plots for the full nuclear magnetic resonance profile (**A**) and cytokine measurements (**B**). Coordinates are colored according to time of sampling and show systematic changes in both metabolite and cytokine profiles over time. Timepoint 1 (*red*): preop; timepoint 2 (*orange*): 0 hr postop; timepoint 3 (*yellow*): 6 hr postop; timepoint 4 (*green*): 24 hr postop; timepoint 5 (*purple*): 48 hr postop. We draw attention to this for two patients with different clinical severities by connecting the coordinates of their plasma profile in chronological order. The patient with mild disease (*blue* line) has a smaller trajectory than the patient with severe disease (*red*), who had a more deranged trajectory that did not recover to baseline by the end of the sampling period (48 hr post surgery).

in metabolic profile over time demonstrates that this patient had a correspondingly strong metabolic response. In contrast, a tighter trajectory was seen in samples from patient 26 whose surgery was less severe and recovery quicker (RACHS-1 score 2, invasive ventilation 21 hr and 25 d free of PICU at 28 d).

Comparison of the preoperative samples with samples collected immediately postoperatively showed clear differentiation in the first PC (PC1), with coclustering of preoperative samples (**Fig. 2*A***).

We analyzed the loading plots for the PCA model (**Fig. 3, *A*** and ***B***) to identify metabolites that had significantly higher concentrations in the postoperative samples. Those identified included acetate, acetoacetate, acetone, alanine, citrate, formate, glucose, 3-hydroxybutyrate, isoleucine, leucine, *N*-acetylated glycoprotein, threonine, and valine.

Analysis of Key Metabolites in Predictive Models of Clinical Outcome

The loadings plot highlighted the influence of therapeutically administered drug and nutrition resonances (Fig. 3, *B*). This made it crucial to develop a sound analytical approach to identify relevant compounds that might be hidden by the peaks of some of these agents.

We recorded all the administered drugs and feeds and undertook analysis of their metabolic profiles. We then incorporated these data into our patient sample analysis. We then examined those integrals from fitted peaks that showed a relationship with clinical factors, but which were independent of the influence of any exogenous agents.

We selected the most significant metabolites identified in the multivariate analyses, then deconvolved and relatively quantified them. In addition to the metabolites identified through this method, we included known markers of organ dysfunction in critical illness. These included lactate (a well-established marker of tissue perfusion), creatine and creatinine (markers of muscle metabolism and renal function, respectively).

PCA scores plots based solely on the quantified metabolites gave similar results to those models using the global spectral profile (**Fig. 2, *B***). However, samples collected preoperatively were not so tightly clustered and one individual, denoted by an asterisk, appeared as an outlier postoperatively. The profile from this individual was distinguished from the rest mainly by high levels of lactate.

To more specifically identify the spectral features associated with the observed time course, OPLS regression was performed against time. Analysis of the regression coefficients revealed that the predominant variables largely match the highest loadings of PC1 on the pre- versus postoperative whole profile PCA plot.

Next, we used orthogonal partial least squares discriminant (OPLS) analysis and OPLS-DA to screen for patterns in the full plasma profiles across individual timepoints.

Using the 15 fitted metabolites, the OPLS models on samples taken immediately postoperation indicated moderate predictive capacity of the fitted metabolites for clinical outcome. These included RACHS-1 score (R^2Y = 0.70, Q^2Y = 0.26), PELOD (R^2Y = 0.66, Q^2Y = 0.17), and PICU-free days (R^2Y = 0.71, Q^2Y = 0.11).

By 6 hours post operation, the ability of the metabolite panel to predict clinical outcome was stronger: RACHS (R^2Y = 0.77, Q^2Y = 0.70), PELOD (R^2Y = 0.73, Q^2Y = 0.70), and PICU-free days (R^2Y = 0.70, Q^2Y = 0.69). In addition, some predictive capacity of the metabolite panels for inotrope score (R^2Y = 0.64, Q^2Y = 0.59) and mechanical ventilation hours (R^2Y = 0.68, Q^2Y = 0.37) was evident.

Cytokine Profiles Had a Similar Postoperative Time Trajectory

PCA scores plots of the cytokine measurements (Figs. 1, *B* and 2, *C*) displayed a similar scattering pattern to the corresponding

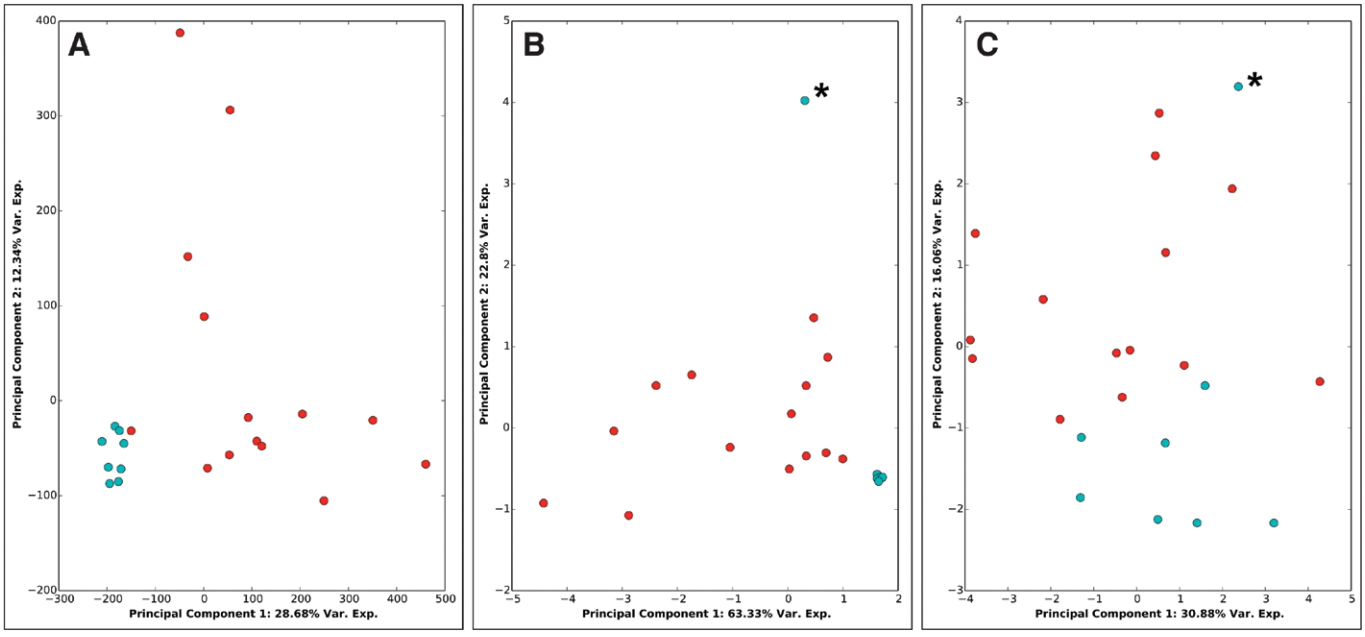

Figure 2. Principal component analysis score plots for preoperative (*cyan*) and postoperative (*red*) detailing the variability in the full nuclear magnetic resonance profile (**A**), cytokine concentrations (**B**), and quantified metabolites (**C**).

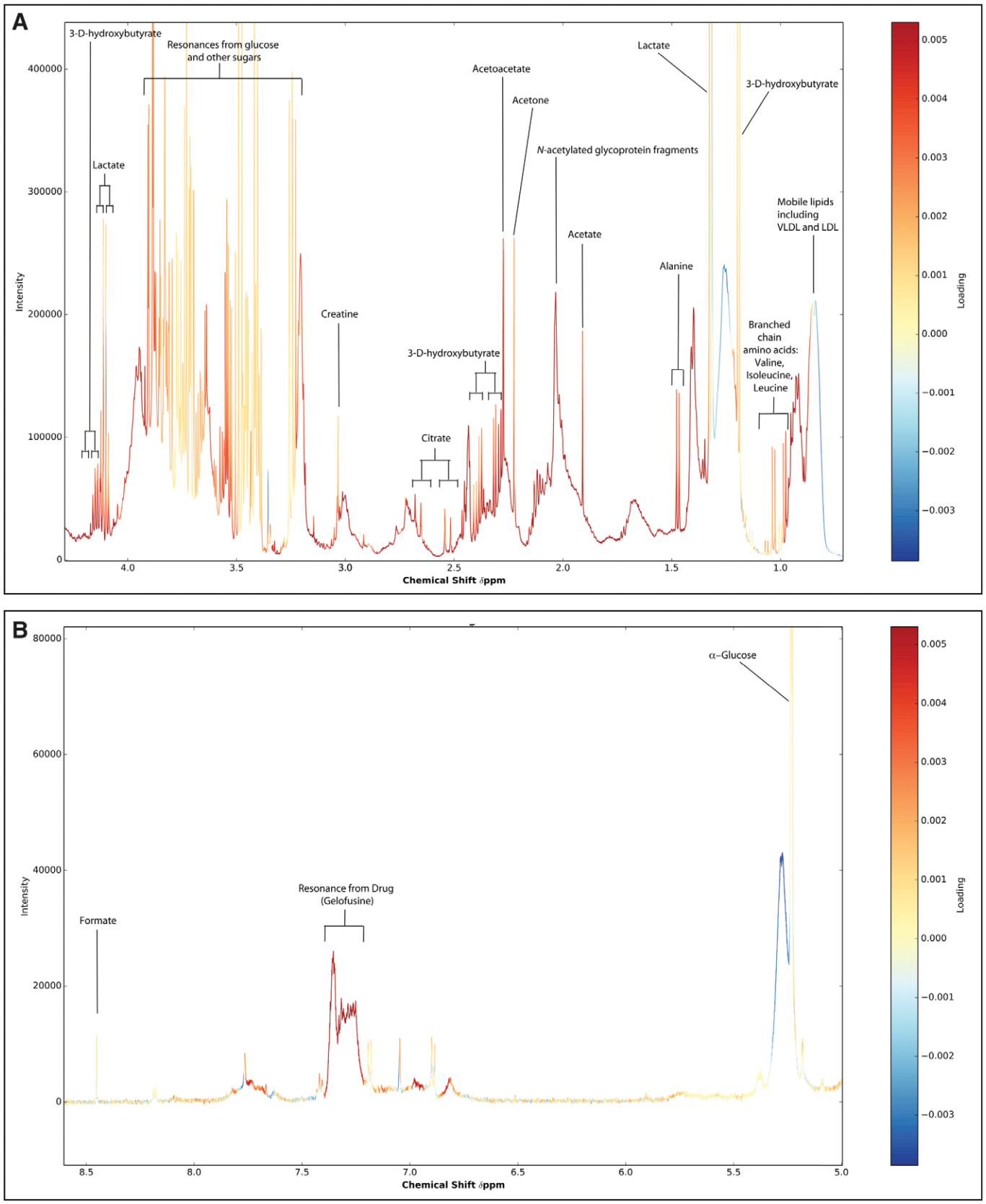

Figure 3. Loadings for the first principal component, projected onto the median nuclear magnetic resonance spectra for both pre- and postsurgery samples: δ 4.3–0.6 region (**A**) and δ 8.1–5.1 region (**B**).

metabolite scores plots, with preoperative samples clustering tightly and the samples from subsequent timepoints demonstrating a trajectory through time. However, in contrast to the metabolic profiles, cytokine levels regressed to preoperative values as early as 6 hours postoperatively for some patients. The key cytokines influencing the deviation in PC1 (corresponding to the immediate response to surgery) were an increase in the IL-6/IL-10 ratio and IL-1ra concentrations, which increased rapidly post surgery and then smoothly decayed back to the baseline. The other cytokines followed a similar but less defined pattern.

At 6 hours post surgery, concentrations of some cytokines displayed inverse significant correlations with the subsequent number of days free of PICU at 28 days (IL-1ra – r: –0.62, $p = 0.017$; IL-6 – *r*: –0.56, $p = 0.034$; IL-8 – *r*: –0.64, $p = 0.013$; IL-10 – *r*: –0.71, $p = 0.0041$). For IL-6 and IL-8, concentrations measured at 24 hours continued to have association with PICU-free days at 28 days (IL-6 – *r*: –0.73, $p = 0.026$; IL-8 – *r*: –0.76, $p = 0.017$).

As with the metabolic profile data, no significant difference was noted in cytokine concentrations between the postoperative glycemic control groups.

Assessment of the Integrated Response of Metabolites and Cytokines to Congenital Heart Surgery

To examine relationships of the key metabolic and inflammatory markers, we prepared a correlation map, which showed associations between coherent groups of variables. We observed a significant association between IL-6 and IL-8, the ketone bodies (acetone, acetoacetate and 3-D-hydroxybutyrate), and the branched chain amino acids (valine, leucine, and isoleucine) across all time points (**Fig. 4, *A–C***).

Some relationships varied across time. For example, preoperatively, alanine was inversely correlated with ketone body levels. Lactate and creatine were strongly correlated with IL-6, IL-8, and the IL-6/IL-10 ratio (Fig. 4, *A*).

Postoperatively, we observed a change in association pattern. Immediately postoperatively 3-D-hydroxybutyrate, acetoacetate, and to some extent acetone were positively correlated with the branch chain amino acids, particularly leucine (Fig. 4, *B*). Another significant correlation immediately postoperatively was an inverse association between the ketone bodies and cytokines IL-6 and IL-8. Neither of these correlations persisted through to later timepoints (Fig. 4, *C*).

Other correlations of interest included citrate and acetate, evident only in samples obtained immediately post operation, and between alanine and the branched chain amino acids, which showed a strong positive correlation postoperatively.

We also investigated the correlation of the quantified lactate with the IL-6/IL-10 ratio and found a strong correlation on preoperative samples ($r = 0.98$; $p = 3.15^{-5}$). This correlation becomes nonsignificant immediately post operation ($r = –0.38$; $p = 0.169$), but reverts to the preoperative status afterward ($r = 0.76$; $p = 1.75^{-6}$).

DISCUSSION

Metabonomic technology has been applied to several studies in critical care and neonatal medicine (28). However, previous studies have largely ignored confounders such as drugs (in this case, particularly antibiotics, sedatives, and anesthetics) and feed type. The spectral signatures from critically ill patients are known to be heavily "contaminated" by signals from exogenous resonances from treatments such as drugs, intravenous feeds, and organ perfusion solutions (29). The raw data from plasma spectra were confounded by metabolites derived from administered feeds and drugs and displayed very complex and dynamic biological composition. We were concerned that this would obfuscate large regions of the spectra. Therefore, we developed a method of peak fitting and relative signal quantification in plasma spectra, which was shown to be robust by demonstrating association with known metabolic biomarkers of outcome (such as lactate) and some of the important clinical outcomes, despite being a small patient cohort.

No Effect of Glycemic Control Was Found on Clinical Outcome or on Metabolite or Cytokine Responses to Surgery

Our patient cohort was a subset of patients recruited to a larger clinical trial of glycemic control in 1,300 critically ill children (13). We examined the effect of tight glycemic control on the global metabolic profile and a cytokine panel in response to congenital heart surgery when compared with the standard glycemic control procedures. While glucose concentrations differed between groups, the magnitude was small, but statistically significant (0.6 mmol/L or 10 mg/dL). In the wider clinical trial, a similar small but significant difference in mean blood glucose concentrations between the two groups of 0.42 mmol/L or 7.6 mg/dL was reported (13).

We did not observe any difference in inflammatory or metabolic parameters, or in clinical outcome. It was not possible to obtain any reliable OPLS-DA model discriminating between the two glycemic control regimes at any of the timepoints after insulin administration started, using full plasma metabolic profile, relatively quantified metabolites, or cytokine measurements.

Our data are consistent with results from the wider clinical trial, which concluded that tight glycemic control in critically ill children had no significant effect on major clinical outcomes (13).

Metabolic Panels Reflect the Effects of Surgery and Clinical Outcome

Our analytical approach enabled us to uncover profound changes in plasma composition following heart surgery in critically ill infants and children. Each patient's metabolic response took a trajectory that demonstrated distinct phases relating to the pre-/postoperative stage. Each individual demonstrated a unique response to the surgery and this could be mapped as a patient journey, whereby both the metabolic and the cytokine panels could be followed and modeled temporally.

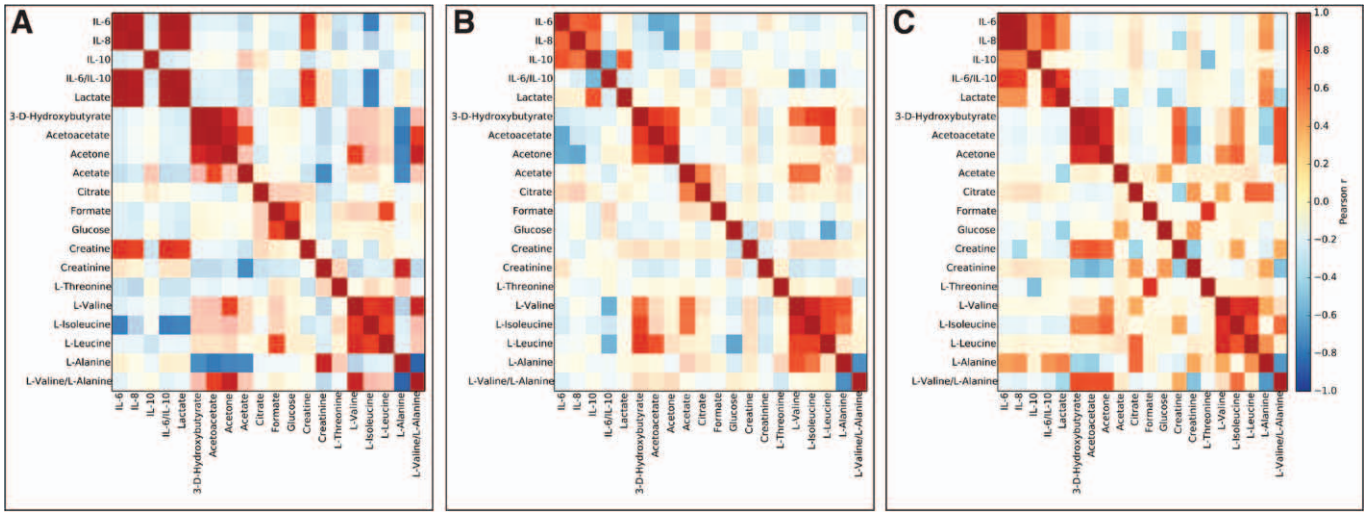

Figure 4. Pearson correlation heat maps for the presurgery (**A**), immediately postsurgery (**B**), and combined 6–48 hr postsurgery samples (**C**). Cells with *opaque* coloring specify correlations with a value of $p < 0.05$ (Student *t* test). *Red* cells indicate positive correlation and blue cells negative correlation.

We noted, even in this small patient cohort, that severity of surgery and critical illness affected the nature of the postoperative metabolic trajectory and its return to baseline. It would be interesting to explore this in a larger cohort and uncover important preoperative and postoperative factors including cyanosis, heart failure, splanchnic ischemia, and the effects of any intra- or postoperative complications.

Our population was undernourished, as measured by the weight age *z* score. Children with congenital heart disease are known to exhibit early and progressive falls in their growth trajectory compared with healthy children, with reductions in WAZ score, head circumference, and length for age *z* score (30, 31). It is also known that surgery and bypass, and the burden of cardiac failure and chronic disease, result in significant metabolic and nutritional stress. Furthermore, inadequate nutritional intake in the postoperative period results in further challenges to restoring normal growth parameters (32, 33). Our patient cohort received standard nutritional care pre- and postoperatively. No additional dietetic interventions are currently in place in our institution. Our analysis has demonstrated that changes in feed have a rapid and strong effect on metabolic profile, and it would be interesting to compare the profile with that in well-nourished children. The reduction in plasma very low-density lipoprotein concentrations postoperatively and increase in ketone bodies is consistent with fasting of these children. Thus, despite the confounding variables, it was possible to observe some interesting relationships that warrant further investigation.

Lactate (34–36), inflammatory cytokines, and IL-6/IL-10 ratio (5, 37) have been shown to be markers of outcome severity in critically ill patients. The former has been shown to correlate well with classical scoring systems of critical care, such as Acute Physiology and Chronic Health Evaluation II (37, 38) Our data demonstrate an association between inflammatory cytokine balance (IL-6/IL-10 ratio) and lactate, suggesting a close relationship between inflammation and metabolic derangement in the days after surgery for congenital heart disease. The increase in *N*-acetylated glycoprotein fragments immediately post-surgery is consistent with inflammation, which also reflects the general spike in plasma cytokines.

Using the relatively quantified metabolites, it was possible to obtain reliable OPLS models for the PELOD and RACHS-1 clinical scores, particularly in the samples taken in the early postoperative period (on PICU admission and at 6 hr post surgery). Analysis of the OPLS model coefficients showed that both the RACHS-1 and the PELOD scores were mainly associated with lactate, citrate, and to a lesser extent creatinine and alanine. Ketone bodies, particularly acetoacetate and creatine, displayed the inverse association with these scores. In all analyses, creatine showed the inverse trend of creatinine (more creatinine and less creatine associated with higher scores and greater severity). All these metabolic parameters were also associated with PICU-free days but with their trends reversed, as expected. The main metabolites positively associated with inotropic score were alanine, citrate, and lactate, with creatine inversely correlated. In general, ketone bodies seem to correlate with better surgical outcomes, whereas citrate, lactate, alanine, and higher creatinine-to-creatine ratio with the inverse.

Our data are similar to previously published work regarding metabolism in adults with critical illness (39). Increased catecholamine, cortisol, and glucagon secretion in critical illness are known to promote lipolysis and ketone body generation (39, 40). Triglycerides are metabolized to fatty acids and glycerol, a gluconeogenic substrate. High glucagon and low insulin concentrations also promote oxidation of free fatty acids to acyl CoA. The latter is hepatically converted to ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone), as a water-soluble fuel source.

To our knowledge, this work is the first metabonomic study in children undergoing surgery for congenital heart disease. The study demonstrates that nuclear magnetic resonance can be applied to biological samples from children with congenital heart disease, and that changes occur in metabolic profile during the response to the surgical insult. Because the

postoperative metabolic spectra are heavily confounded by resonances deriving from feeds and drugs, the use of the whole ^{1}H NMR plasma or urine profile can be quite limited, other than to identify gross outliers. Despite these shortcomings, we show that through an improvement in the prep-processing procedures, it is possible to reliably measure the evolution of various markers of interest.

We accept that our study group was small, and for this reason we could not keep separate training and test datasets for a more stringent assessment of the multivariate models. To further validate the PLS models in the setting of a small sample size, a permutation test was performed on the most relevant clinical parameters (such as surgical and disease severity scores and outcomes). In this test, the models are refitted and cross-validated multiple times, after permuting class labels or dependent variable values. The *p* values for the tests to assess the metabolite panel were highly significant (< 0.01) for RACHS score, PELOD score, and PICU-free days in models using data from samples at 6 hours post operation. Nevertheless, any putative marker identified in this study has to be properly validated in a larger cohort, as it is likely that the full extent of variability in this type of critical care population has been under sampled.

Because ketone bodies and lipid metabolism were indicated as predictive of clinical outcomes and displayed alterations in response to surgical insult, additional studies should include evaluation of liquid chromatography-mass spectrometry to profile the lipidomic response to critical illness. Liquid chromatography-mass spectrometry methods generate 2D datasets (chromatographic retention time and mass-to-charge ratio) and have less form overlap between exogenous and endogenous molecules. Our data on inflammatory links to metabolic shifts and clinical outcome post surgery suggest that targeted mass spectrometry methods could be of interest to identify other biological markers of acute inflammation.

In summary, our study suggests that metabolic profiling could be a clinically relevant tool for stratifying patient responses to congenital heart surgery. Furthermore, we show evidence that tight glycemic control does not impact the post-surgical metabolic profiles, supporting the clinical trial from which this study was drawn that failed to show improvement in early clinical outcomes with tight glycemic control. With improved data analysis workflows and targeted metabolite quantification, detailed and informative metabolic data could inform patient stratification and critical care management.

In summary, we have identified a panel of metabolites, which is predictive of clinical outcome. This metabolite panel will require validation in a larger cohort of children.

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