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## ARTICLE

# Pharmacometabolomics of Respiratory Phenotypic Response to Dexamethasone in Preterm Infants at Risk for Bronchopulmonary Dysplasia

Tamora Lewis<sup>1,\*</sup> , Prabhakar Chalise<sup>2</sup> , Cheri Gauldin<sup>1</sup> and William Truog<sup>1</sup>

A prospective cohort study was performed in preterm infants less than 32 weeks gestation at birth who were treated with dexamethasone for developing or established bronchopulmonary dysplasia (BPD). Respiratory phenotype (Respiratory Severity Score (RSS)), serum, and urine metabolomics were assessed before and after treatment. Ten infants provided nine matched serum and nine matched urine samples. There was a significant decrease in RSS with steroid treatment. Serum gluconic acid had the largest median fold change (140 times decreased,  $P = 0.008$ ). In metabolite set enrichment analysis, in both serum and urine, the urea cycle, ammonia recycling, and malate-aspartate shuttle pathways were most significantly enriched when comparing pretreatment and post-treatment ( $P$  value  $< 0.05$ ). In regression analyses, 6 serum and 28 urine metabolites were significantly associated with change in RSS. Urine gluconic acid lactone was the most significantly correlated with clinical response (correlational coefficient 0.915). Pharmacometabolomic discovery of drug response biomarkers in preterm infants may allow precision therapeutics in BPD treatment.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Bronchopulmonary dysplasia (BPD) is a common lung disease among preterm infants. Corticosteroids can improve symptoms, but there is large unexplained variability in clinical response. Pharmacometabolomics is an emerging tool to help understand variability in drug response.

### WHAT QUESTION DID THIS STUDY ADDRESS?

Are there serum and plasma metabolomic changes associated with dexamethasone therapy? Do certain metabolomic changes correlate with degree of drug response?

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This first pharmacometabolomic study in preterm infants with BPD shows that gluconic acid has the largest

fold change with steroid treatment and that the degree of change in urine gluconic acid correlates with drug response. Metabolic pathways are altered with dexamethasone therapy and certain metabolic signatures may be able to differentiate responders from nonresponders.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Identification of metabolomic biomarkers for likelihood of drug response, ones that could be measured early in treatment, may allow for precision therapeutics and early stopping of a high risk drug among infants unlikely to respond.

Bronchopulmonary dysplasia (BPD) is a lung disease that results from the combination of preterm birth, ventilator-associated lung injury, inflammation, and maladaptive lung growth. BPD is a common diagnosis in the neonatal intensive care unit (NICU), but there are no US Food and Drug Administration (FDA)- approved therapies for management. One class of drugs commonly used to prevent severe BPD, or to treat established BPD, are corticosteroids.<sup>1</sup> Use of this drug class in neonates and infants with BPD is challenging because of variability in clinical wanted effects

and an inability to target infants who are likely “responders.” Neonatologists lack an early biomarker of likely drug effect and cannot personalize therapy to improve outcomes.

Pharmacometabolomics is a novel and powerful tool to help understand variability in drug response. A patient metabolotype, or the full complement of circulating molecules in the plasma or urine, can be defined pretreatment and post-treatment with a drug. Either baseline metabolotype or change in metabolotype, in conjunction with close measurement of drug response, can inform treatment outcomes.

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Metabotype at baseline, and the discovery of signature metabotype changes with certain drug exposures, can elucidate the mechanisms of variation in drug response. For example, if a metabolic signature of responders vs. non-responders was available before treatment or early in the drug treatment course, then infants in the nonresponder group could receive alternate therapies empirically, or at least be spared the full 7–10 day course of steroids, which have been associated with adverse outcomes.<sup>2,3</sup>

Given the variability in steroid response observed in clinical practice, our goal is to perform a prospective cohort steroid pharmacometabolomic study nested in routine clinical care of preterm infants with evolving or established BPD. During this exploratory project, we aim to identify metabolomic changes that correlate with drug response and drug failure. Because neonatal metabolomics is an emerging field, our group aims to discover treatment response biomarkers and generate hypotheses for future research.

## METHODS

### Subjects and study design

This prospective pilot cohort study was reviewed and approved by the Children's Mercy Hospital institutional review board prior to patient enrollment. Parental consent was obtained in accordance with institutional review board regulations. Starting in October 2016, all preterm infants less than 32 weeks gestation at birth and treated with systemic dexamethasone per clinical care were eligible for enrollment. Demographic data and clinical data were abstracted from the clinical chart and by speaking with bedside clinicians in realtime. For this analysis, we only used data from the first course of systemic dexamethasone for each child.

In order to measure the clinical outcome, we measured short-term phenotypic response to systemic corticosteroids. The Respiratory Severity Score (RSS) was calculated before treatment (baseline) and on day 7 of treatment (drug response). In order to account for intraindividual variability, the average RSS for a 24-hour period was collected. The RSS is a quantitative description of the severity of lung disease while on mechanical

or noninvasive ventilation. Lower values of the RSS indicates better pulmonary function. RSS is calculated as the mean airway pressure  $\times$  fractionated of inspired oxygen ( $\text{FiO}_2$ ) (ranging from 21–100%). After starting treatment with dexamethasone, clinicians want the RSS to go down as pulmonary mechanics and gas exchange improve.

Blood and urine samples were collected two times, once in the 24 hours prior to starting systemic dexamethasone and once at days 3–6 after starting systemic dexamethasone. Both matrices were studied because the urine can be collected noninvasively with a cotton ball, whereas the serum may be a better reflection of pulmonary changes. The timing of post-treatment blood and urine sample was dictated by the infant having blood drawn for clinical laboratories within the target window. Samples were collected in the NICU and then either briefly refrigerated or immediately processed. Urine and serum were aliquoted and stored at  $-80^\circ\text{C}$  until metabolomic assay.

### Untargeted metabolomic assessment

Serum and urine samples were submitted for an untargeted metabolomic analysis through the National Institutes of Health (NIH)-funded West Coast Metabolomics Center in Davis, California. For analysis of serum and urine metabolites, metabolite levels were determined using an Agilent 7890A gas chromatograph coupled to a Leco Pegasus IV time-of-flight mass spectrometer, as previously described.<sup>4</sup> Acquired spectra were further processed using the 130 BinBase database,<sup>4,5</sup> including metabolite annotations by retention index and mass spectra matching. Data, reported as quantitative ion peak heights, were normalized by the sum intensity of all annotated metabolites across the entire study and used for further statistical analysis.

### Statistical analysis

The change in RSS, the clinical outcome, was assessed before and after treatment using the Wilcoxon signed rank test. In order to test for an association between baseline metabolite level and change in RSS, we performed regression

Table 1. Demographic and respiratory data

Infant	GA (weeks)	BW (kg)	Race	DOL steroid	Pre-RSS	Post-RSS	Change in RSS	Sample collection	Serum analysis	Urine analysis
1	25 1/7	0.480	WH	138	11.02	0.50 (nasal cannula)	-10.52	7.3	x	x
2	25 0/7	0.420	AA	36	4.65	2.63	-2.02	3.50	x	x
3	23 4/7	0.625	HIS	26	9.75	3.87	-5.88	5.33	x	x
4	23 4/7	0.595	HIS	27	8.03	2.90	-5.13	4.38	x	x
5	25 2/7	0.725	HIS	36	3.96	2.31	-1.65	5.63	x	x
6	24 6/7	0.700	WH	73	4.40	2.43	-1.97	6.58	x	x
7	28 6/7	1.530	WH	26	7.80	2.99	-4.81	4.42	x	—
8	24 4/7	0.370	AA	127	8.78	8.16	-0.6	5.54	x	x
9	25 5/7	0.850	WH	33	5.16	2.57	-2.59	6.50	—	x
10	26 4/7	0.770	WH	111	2.34	0.50	-1.84	3.50	x	x
Median	25 0/7	0.663	—	36	6.48	2.6	-2.3	5.44	—	—
IQR	24 2/7–26 0/7	0.465–0.790	—	27–115	4.29–9.02	1.86–3.21	-5.32 to -1.79	4.16–6.52	—	—

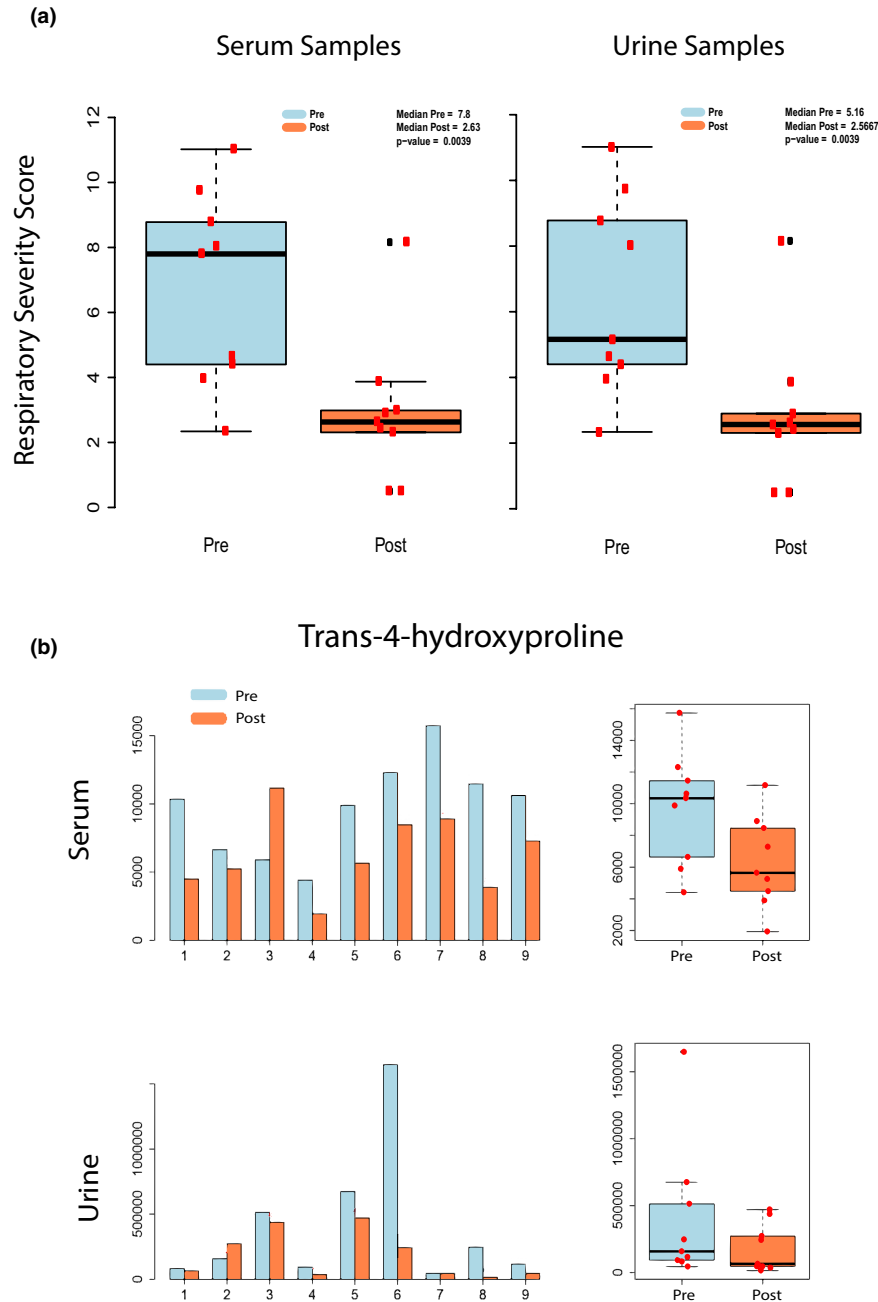
AA, African American; BW, birthweight; DOL steroid, day of life (age) when infant started steroids; GA, gestational age; HIS, Hispanic; IQR, interquartile range; RSS, Respiratory Severity Score; Sample collection, number of days from steroid start to post-treatment metabolomic sample collection; WH, white.

analysis. The differences in the metabolites assayed before and after treatment were assessed using the Wilcoxon signed rank test for each metabolite followed by multiple testing adjustments using Benjamini and Hochberg's false discovery rate method.

Each metabolite change was assessed from pretreatment to post-treatment using the Wilcoxon signed rank test and then they were sorted by the order of significance ( $P$  value). Then, Metabolite Set Enrichment Analysis (MSEA) was completed, as previously described<sup>6,7</sup> using MetaboAnalyst version 4.0. MSEA is a method of identifying biologically

meaningful patterns or the metabolic pathways that are significantly enriched in quantitative metabolomic data. The MSEA as implemented in the MetaboAnalyst version 4.0<sup>6</sup> uses the hypergeometric distribution based test followed by multiple test adjustments to evaluate whether a particular metabolite set is represented more than expected by chance within the given compound list.

Next, the association of change in metabolites with the change in the RSS scores before and after treatment was investigated using linear regression and correlation analyses. Prior to fitting the linear regression models, the association



**Figure 1.** Change in phenotype and example metabolite with dexamethasone therapy. Boxplots displaying (a) change in Respiratory Severity Score and (b) change in trans-4-hydroxyproline, an example metabolite that nearly universally decreased with steroid treatment.

of RSS with the birth weight, gender, race, mode of delivery, and antenatal steroids were examined. None of the variables were found to be associated with the RSS for both serum and urine, so they were not included in the regression model. Finally, for the exploratory analysis, the subjects were grouped into good, moderate, and poor responders' categories based on a decrease in RSS score. The top three subjects that had maximum decrease in RSS score were categorized as "good" responders, the three subjects having the least decrease in RSS were categorized as "poor" responders, and the remaining as "moderate" responders. The differences in the changes in metabolites among the three groups were assessed using the Kruskal–Wallis test. The tests were considered significant if the *P* value was <0.05.

The analyses were carried out separately for the serum and urine. All the analyses were performed using Statistical Software R (R Foundation for Statistical Computing, Vienna, Austria) and MetaboAnalyst version 4.0.

## RESULTS

Ten infants (median birthweight 663 g; median gestational age 25 0/7 weeks) provided nine matched pre-steroid and post-steroid treatment serum samples and nine matched pre-steroid and post-steroid treatment urine samples for analysis. Infant demographic and clinical phenotypic response data are provided in **Table 1**. All infants were treated with a protocolized dexamethasone wean over 7–10 days

**Table 2. Metabolite changes with dexamethasone therapy**

Metabolites	Median (pre)	Median (post)	Fold change	Direction	<i>P</i> value <sup>a</sup>
Serum					
Oxalic acid	10,808	15,403	1.425148	Up	0.0039
Lactic acid	41,371	81,823	1.977786	Up	0.0039
Malic acid	405	837	2.066667	Up	0.0078
Gluconic acid <sup>b</sup>	115,513	826	0.007151	Down	0.0078
Caprylic acid	902	4,800	5.321508	Up	0.0078
Alpha-ketoglutarate	669	958	1.431988	Up	0.0117
Saccharic acid <sup>b</sup>	8,474	868	0.102431	Down	0.0273
Cholesterol	1,581	2,221	1.404807	Up	0.0273
Arachidic acid	2,671	3,980	1.490079	Up	0.0273
Fumaric acid	524	1,049	2.001908	Up	0.038
Stearic acid	163,353	180,749	1.106493	Up	0.0391
<b>Xylitol</b>	<i>1,103</i>	<i>875</i>	<i>0.793291</i>	<i>Down</i>	<i>0.0547</i>
<i>Tryptophan</i>	<i>49,903</i>	<i>27,348</i>	<i>0.548023</i>	<i>Down</i>	<i>0.0547</i>
<i>Trans-4-hydroxyproline</i>	<i>10,342</i>	<i>5,637</i>	<i>0.545059</i>	<i>Down</i>	<i>0.0547</i>
<i>P-hydroxyphenyllactic acid</i>	<i>700</i>	<i>502</i>	<i>0.717143</i>	<i>Down</i>	<i>0.0547</i>
<i>Beta-gentiobiose</i>	<i>1,613</i>	<i>2,416</i>	<i>1.49783</i>	<i>Up</i>	<i>0.0547</i>
<i>Pantothenic acid</i>	<i>8,145</i>	<i>2,066</i>	<i>0.253653</i>	<i>Down</i>	<i>0.0587</i>
Urine					
Uracil	4,621	6,056	1.310539	Up	0.0039
Kynurenine	13,311	7,034	0.528435	Down	0.0039
Glucoheptulose	8,065	10,322	1.279851	Up	0.0039
1-Methylinosine	7,752	4,910	0.633385	Down	0.0039
Pseudo uridine	1,644,332	1,189,538	0.723417	Down	0.0078
Isohexonic acid	1,798,781	115,773	0.064362	Down	0.0078
Histidine	295,790	390,974	1.321796	Up	0.0078
Indole-3-lactate	21,523	7,731	0.359197	Down	0.0117
Sucrose	7232	15,092	2.086836	Up	0.0195
Nicotinamide	45,119	33,991	0.753363	Down	0.0195
7-Methylguanine	40,803	29,360	0.719555	Down	0.0195
5-Hydroxymethyl-2-furoic acid	53,570	28,530	0.532574	Down	0.0195
Pipecolic acid	4,543	2,124	0.467532	Down	0.0273
1,3,5-Trimethylcyanuric acid	64,839	50,777	0.783124	Down	0.0273
1,2-Cyclohexanedione	40,847	28,027	0.686146	Down	0.0391
<b>Uridine<sup>b</sup></b>	<i>1,028</i>	<i>878</i>	<i>0.854086</i>	<i>Down</i>	<i>0.0547</i>
<i>Metanephrine</i>	<i>48,104</i>	<i>40,710</i>	<i>0.846291</i>	<i>Down</i>	<i>0.0547</i>
<i>Ascorbic acid</i>	<i>35,228</i>	<i>107,869</i>	<i>3.062025</i>	<i>Up</i>	<i>0.0547</i>

**Bold:** Metabolite found significant in both pre–post comparison and regression analysis.

*Italics:* *P* value between 0.05 and 0.06, data included to encourage hypothesis generation.

<sup>a</sup>*P* value displayed in table is unadjusted for multiple comparisons. <sup>b</sup>Metabolite also found to differentiate three response groups.

(attending neonatologist made the decision about length of therapy). The dexamethasone was dosed as 0.15 mg/kg/day for 3 days, then 0.10 mg/kg/day for 2–3 days, then 0.05 mg/kg/day for 1–2 days, then 0.02 mg/kg/day for 1–2 days. The RSS was significantly less after steroid treatment than before for both serum and urine samples. For paired serum samples, median pre-RSS was 7.8, and median post-RSS was 2.63 ( $P$  value = 0.0039). For paired urine samples, the median pre-RSS was 5.16, and the median post-RSS was 2.57 ( $P$  value = 0.0039), **Figure 1**.

A total of 686 metabolites were assayed in both serum and urine samples, and 197 of these were known entities. Therefore, these 197 metabolites were included in the analysis. In serum, 11 metabolites were significantly different between presteroid and poststeroid treatments (**Table 2**). Serum gluconic acid displayed the largest median fold-change (139.8 times decreased with steroid therapy;  $P$  value = 0.008). Serum caprylic acid had the largest increase in median fold change (5.3 times;  $P$  value = 0.008). In urine, 15 metabolites were significantly different between pre-steroid and post-steroid therapy (**Table 2**). The largest decrease in the median fold change was with isohexanoic acid (fold change 15.5;  $P$  value = 0.008), and the largest increase was with sucrose (fold change = 2.09;  $P$  value = 0.0195). Regression analysis results for baseline metabolite and change in RSS are presented in **Table 3**. In serum, baseline xylitol levels are most associated with the degree of clinical steroid response, and in urine baseline saccharic acid is most associated.

Of the statistically significant metabolites in **Table 2** (change with steroid treatment), caprylic acid in serum and uridine in urine were also found to be significantly associated with degree of change in RSS in regression analyses. However, the changes in metabolite amount with dexamethasone treatment were not statistically significant after multiple testing adjustments.

The MSEA results for serum and urine are shown in **Figure 2**. The horizontal bars summarize the most significant metabolite sets identified to change with dexamethasone therapy, and the color intensities on the bars are based on their  $P$  values. For both serum and urine, urea cycle, ammonia recycling, and malate-aspartate shuttle pathways were the three most significantly enriched pathways ( $P$  values < 0.05) when comparing pre-steroid and post-steroid therapy. All of these pathways are implicated in energy homeostasis.

As expected, clinical improvement was variable with drug treatment (**Figure 4, top row**). Therefore, we performed a univariate regression analysis to investigate whether changes in individual metabolite levels were associated with change in RSS. The top three most correlated metabolites for serum and urine are displayed in **Figure 3**. Change in 6 serum metabolites and 28 urine metabolites were associated with change in RSS. Because of the small samples size, the majority of the associations (regression or correlation coefficients) did not retain their significance after adjusting for multiple comparisons. Urine metabolite gluconic acid lactone was the most significantly associated with a correlation coefficient of 0.915. Changes in the two metabolites threonic acid and isocitric acid were significantly associated with change in RSS for both serum and urine samples.

The most highly associated metabolites were capric acid in serum and gluconic acid lactone in urine.

In the final exploratory analysis, we compared changes in the metabolites among the three steroid response groups: good, moderate, and poor responders. In both serum and urine, none of the metabolites were significantly different among the three groups using a  $P$  value of <0.05. However, eight sera (uridine, saccharic acid, p-hydroxyphenyllactic acid, phosphoethanolamine, kynurenine, isohexanoic acid, gluconic acid, and erythrose) and eight urine metabolites (citrulline, uridine, trehalose, tagatose, mannitol, maltose, alpha-aminoadipic acid, and alloxanoic acid) were different between the three groups using a cutoff of <0.07 (**Supplemental Table S1**). The patient-specific change in RSS and select metabolites are displayed in **Figure 4**, with clinical response color-coded as green (good responders), yellow (moderate responders), and red (poor responders). Further research with bigger sample size is needed to investigate the association of these metabolites with degree of steroid response.

## DISCUSSION

Systemic steroid response is heterogeneous in preterm infants at risk for severe BPD, and the respiratory response data from this study confirm this. Although pharmacogenomics is beginning to shed light on this variability in drug response,<sup>8</sup> pharmacometabolomic research has the potential to unmask underlying physiology that contributes to drug response variability.<sup>9,10</sup> In this cohort study, we show that steroid treatment leads to changes in certain serum and urinary metabolites, and that certain metabolite changes are correlated with the degree of respiratory improvement after systemic dexamethasone therapy. Gluconic acid (lactone) is a metabolite that is significant in three of the analyses we performed: it is greatly decreased

**Table 3. Significant association between the baseline metabolite and change in RSS**

Metabolites	$\beta$	$P$ value	Correlation
Serum			
Xylitol	-0.00210	0.005	-0.83
N-Acetylmethionine	-0.00036	0.013	-0.78
Cystine	-0.00090	0.021	-0.75
Kynurenine acid	0.00242	0.035	0.70
Urine			
Saccharic acid <sup>a</sup>	0.00000	0.001	-0.89
N-Acetyl-D-tryptophan	-0.00002	0.003	-0.86
3,6-Anhydro-D-hexose <sup>a</sup>	-0.00052	0.004	-0.85
Uridine	-0.00066	0.015	-0.77
Gluconic acid lactone <sup>a</sup>	0.00000	0.017	-0.76
Hexaric acid	-0.00108	0.028	-0.72
Isoribose	-0.00021	0.037	-0.70
Maltotriitol	-0.00042	0.038	-0.69
Citrulline	0.00011	0.039	0.69
Beta-gentiobiose	-0.00003	0.043	-0.68

RSS, Respiratory Severity Score.

<sup>a</sup>Change in metabolite levels associated with change in RSS.

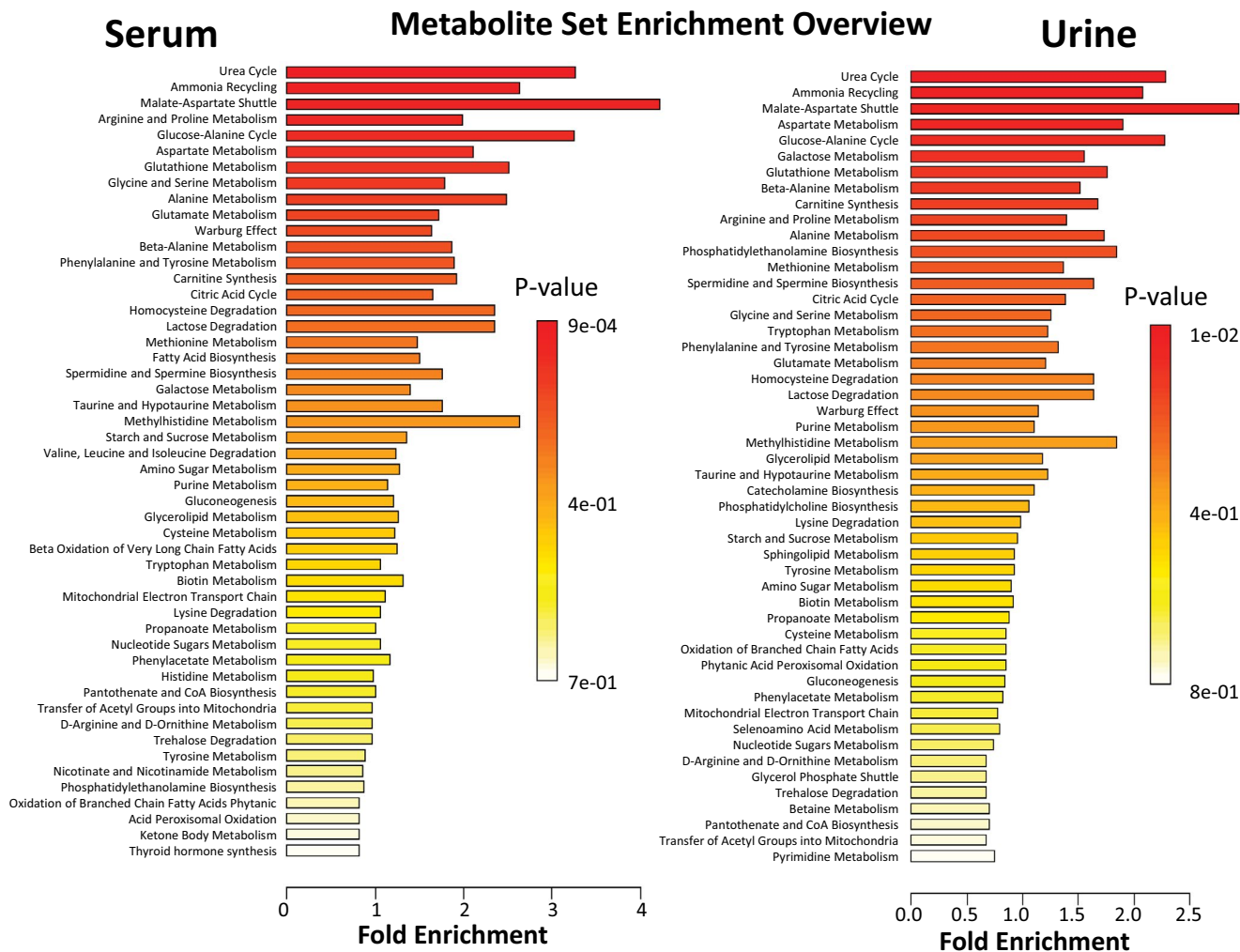


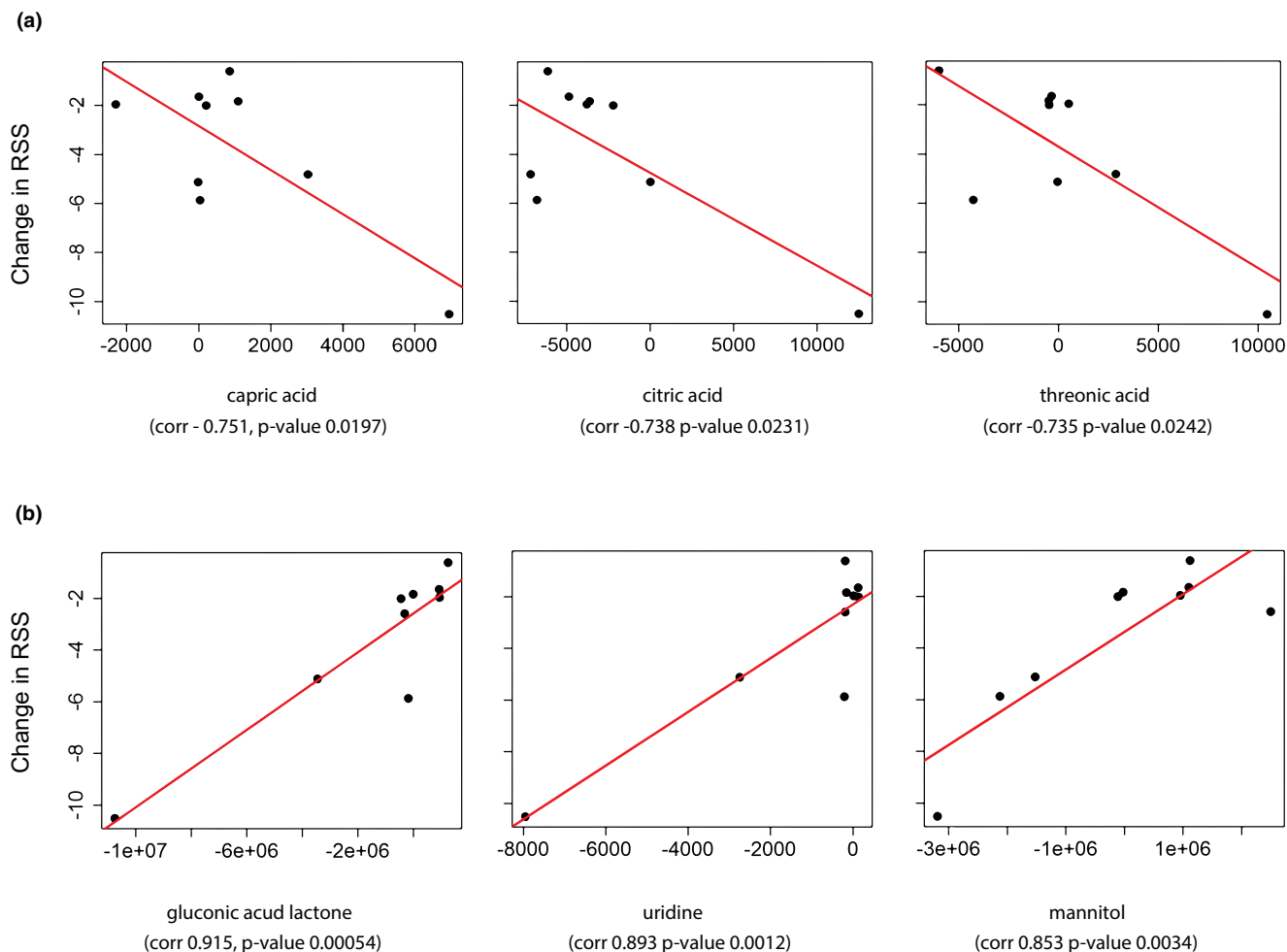
Figure 2. Metabolite set enrichment analysis.

with steroid therapy, the degree of decrease correlated with the degree of steroid response, and it is nearly significantly different among good, moderate, and poor responders.

Urine gluconic acid lactone, uridine, and mannitol were found to correlate with degree of steroid response. In all three, the more the metabolite decreased with treatment, the better the clinical response (more negative change in RSS). Gluconic acid (gluconate) is an oxidation product of glucose. The metabolism of gluconic acid is poorly understood in humans, but a recent study of human glucokinase shows that small changes in gluconate concentration lead to large shifts in the hexose monophosphate shunt.<sup>11</sup> The hexose monophosphate shunt produces nicotinamide adenine dinucleotide phosphate, a cofactor for glutathione reductase catalysis important in neutralizing oxidative stress. In addition, this shunt produces ribose-5-phosphate, a precursor molecule for nucleotide synthesis. The exact role of gluconic acid in human disease, let alone preterm infant disease, is not known, but multiple metabolomics studies have identified this molecule as a potential marker of inflammation and disease. Uridine is a nucleoside and has been shown in animal models to inhibit inflammation and fibrosis

in a model of lung fibrosis.<sup>12</sup> In an animal model of arthritis, direct uridine injection of the joint space prevented development of joint inflammation and inhibited local cytokine production.<sup>13</sup> In *ex vivo* cell adhesion models and a rat model of chemical-induced lung inflammation, uridine and/or 4-thiouridine decreases leukocyte adhesion, tissue edema, and tumor necrosis factor-alpha levels.<sup>14</sup> A decrease of this anti-inflammatory metabolite with steroid treatment in good responders may indicate the steroids are inhibiting inflammation in the lungs. The metabolite mannitol is thought to arise from the microbiome. Mannitol is produced by lactic acid bacteria,<sup>15</sup> pseudomonas species,<sup>16</sup> and streptococcal species.<sup>17</sup> Mannitol is a biomarker of congestive heart disease,<sup>17</sup> which is increasingly recognized as an inflammatory condition. In total, urine biomarkers associated with degree of steroid response in BPD seem implicated in the inflammatory cascade and oxidative stress, consistent with known disease physiology.

Fanos *et al.*<sup>18</sup> found that gluconates are one of five metabolites that, in urine samples collected shortly after birth, can distinguish between preterm infants who go on to develop BPD versus those who do not. A study on



**Figure 3.** Regression results of change in metabolite with change in Respiratory Severity Score (RSS). The three most strongly correlated metabolites in (a) serum and (b) urine are displayed.

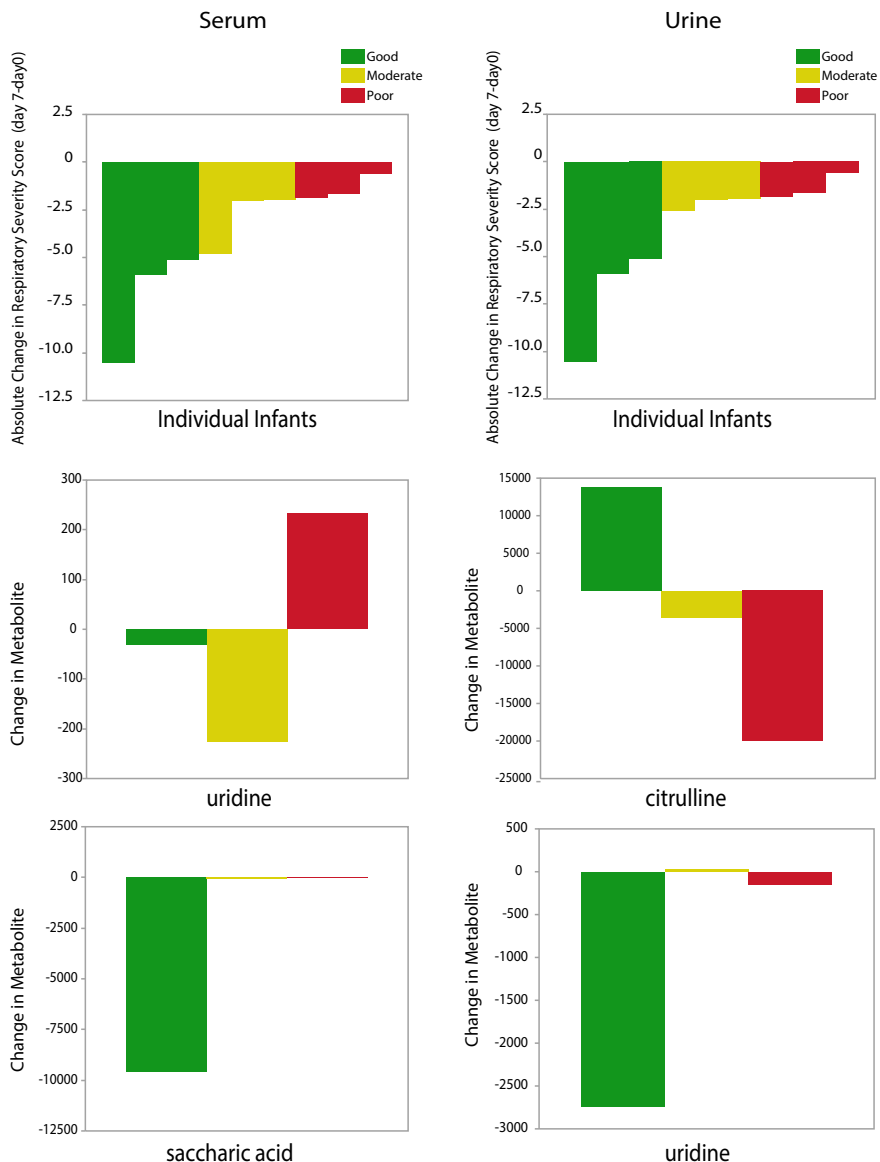
the metabolomics of necrotizing enterocolitis showed a marked increase in gluconic acid from urine samples of infants with necrotizing enterocolitis.<sup>19</sup> Gluconic acid is the only metabolite statistically significantly different in the cord blood of neonates with histologic chorioamnionitis vs. controls, furthering its implication in inflammation.<sup>20</sup> Thus, a decrease in gluconic acid with steroid treatment in preterm infants with lung disease is consistent with decreased inflammation. The greater the gluconic acid decrease, the better the clinical response to steroids, indicating that this metabolite may be a quantitative biomarker of drug response.

Trans-4-hydroxyproline is decreased in serum after steroid treatment. Trans-4-hydroxyproline is elevated in patients with idiopathic pulmonary fibrosis.<sup>21</sup> In 14 ventilator-dependent infants with BPD, high-dose dexamethasone treatment was associated with decreased urinary trans-4-hydroxyproline at days 3, 6, 9, and 12 of treatment, indicating suppressed collagen synthesis.<sup>22</sup> In rats, sepsis-associated lung injury is associated with increased pulmonary fibrosis and hydroxyproline content. In these animals, hydroxyproline was more elevated in

animals ventilated with a high tidal volume as opposed to a lower tidal volume, reflective of a more injurious ventilation strategy inducing more lung injury.<sup>23</sup> In sum, it seems that trans-4-hydroxyproline is a marker of lung injury and fibrosis, so a decrease with steroid treatment is biologically plausible.

Citric acid (citrate) and isocitric acid (isocitrate) were both associated with the degree of RSS improvement after steroid therapy. Both of these molecules are key intermediaries in the tricarboxylic acid cycle, which is an important metabolic pathway for the generation of ATP. If relatively higher levels of citrate and isocitrate are associated with improved lung function, this may imply that with steroid therapy the best responders exhibit quieting of their tricarboxylic acid cycle (and, thus, higher substrate availability), corresponding to less energy demand. Metabolic reprogramming occurs in human and animal models of chronic lung disease, implying that metabolic and energy perturbations may be implicated in BPD development and progression. In patients with BPD and growth failure, resting metabolic expenditure is elevated suggesting increased metabolic demand.<sup>24</sup> Gene expression profiles of newborn umbilical cord blood show that infants





**Figure 4.** Metabolite change by clinical response group. Left panel displays serum results and right panel displays urine results. For the absolute change in Respiratory Severity Score (RSS) in the top row, each bar represents an individual infant change in RSS with steroid treatment. For the change in metabolite, each bar represents the group mean.

who go on to develop BPD have lower levels of genes involved in oxidative phosphorylation and other bioenergetic pathways.<sup>25</sup>

Our study has strengths and weaknesses. A strength of our study is the ability to recruit a homogeneous group of preterm infants with severe lung disease and closely phenotype them before and after steroid treatment. Another strength is protocolized and standard dexamethasone dosing in our NICU, so dose variability does not confound our findings. Because extremely preterm infants treated with dexamethasone are rare at our level IV NICU, and because we limited our analysis to the first course of systemic dexamethasone, our sample size is small, and the statistical power is limited. We have not prospectively validated these findings. In addition, because the study was performed

during routine clinical care, the timing of the “post” steroid treatment sample was variable (collected when infants were getting blood drawn for clinical reasons). This variability in postdrug sample collection might confound the analysis, but we do not have sufficient sample size to control for this in the current cohort. Last, in this untargeted metabolomic study, we limited statistical analysis to only serum and urine metabolites with known identities. This could prevent our discovery of novel metabolites. Because there is so little known about pharmacometabolomics in neonates, our group felt it important to share this hypothesis-generating type of data while we continue to increase the size of our study cohort. In future research, we hope to secure the funding required for unknown metabolite identification and targeted quantification.

Metabolomics have been used in the neonatal population to identify disease states, such as histologic chorioamnionitis,<sup>20</sup> early and late-onset sepsis,<sup>26</sup> cytomegalovirus,<sup>27</sup> and intrauterine growth restriction.<sup>28</sup> In addition, metabolomic studies have investigated whether BPD severity and pulmonary hypertension can be predicted from umbilical cord blood metabolomics.<sup>29</sup> To our knowledge, this is the first study investigating pharmacometabolomics in preterm neonates. The power to understand how changes in biomarkers collected during routine clinical care correlate with likelihood of wanted clinical response to a drug is a potentially powerful tool for precision therapeutics. This study serves as an early investigation of pharmacometabolomics in preterm infants, and the research group is actively expanding the patient cohort to create a fuller understanding of the metabolomics of steroid response in preterm infants.

**Supporting Information.** Supplementary information accompanies this paper on the *Clinical and Translational Science* website ([www.cts-journal.com](http://www.cts-journal.com)).

#### Table S1.

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