

Untargeted ¹H-NMR Urine Metabolomic Analysis of Preterm Infants with Neonatal Sepsis

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Supplementary materials

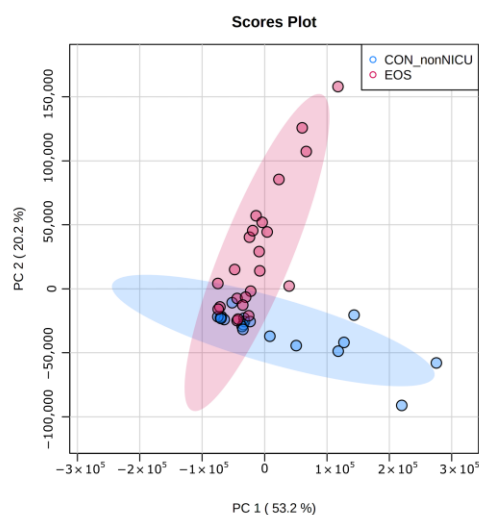
Table S1. ¹ H NMR Chemical Shifts of Metabolites detected in urine samples of neonates and their main metabolic pathway.

	Metabolites	δ _H (ppm) and multiplicity	Pathway
1.	1-Methylnicotinamide	4.46 (s), 8.17 (m), 8.88 (d), 8.95 (d), 9.26 (s)	Nicotinate and Nicotinamide metabolism
2.	2-Hydroxybutyrate	0.89 (t), 1.66 (m)	Propanoate metabolism
3.	2-Hydroxyisobutyrate	1.35 (s)	Amino acid degradation and ketogenesis
4.	2-Oxoglutarate	2.99 (t), 2.43 (t)	Amino acids metabolism
5.	4-Hydroxybenzoate	6.96 (d), 7.75 (d)	Phenylalanine metabolism
6.	Acetate	1.92 (s)	Pyruvate metabolism
7.	Acetone	2.22 (s)	2-propanol and Ketone body metabolism
8.	Alanine	1.46 (d)	Urea, Glucose-Alanine cycle
9.	Ascorbate	3.72 (m), 3.75 (m), 4.02 (m), 4.53 (d)	Vitamin digestion and absorption, Biosynthesis of cofactors, Tyrosine metabolism
10.	Betaine	3.25 (s), 3.88 (s)	Glycine, serine and threonine metabolism
11.	Choline	3.19 (s), 3.50 (m), 4.05 (m)	Cell membrane synthesis-one carbon metabolism, Glycine, serine and threonine metabolism
12.	Citrate	2.56 (d), 2.68 (d)	Citrate cycle
13.	Creatine	3.025 (s), 3.91 (s)	Glycine, serine and threonine metabolism
14.	Creatinine	3.04 (s), 4.06 (s)	Creatine catabolism
15.	Cysteine	3.19 (dd), 3.39 (dd)	Cysteine and methionine metabolism
16.	Dimethylamine	2.71 (s)	Choline metabolism

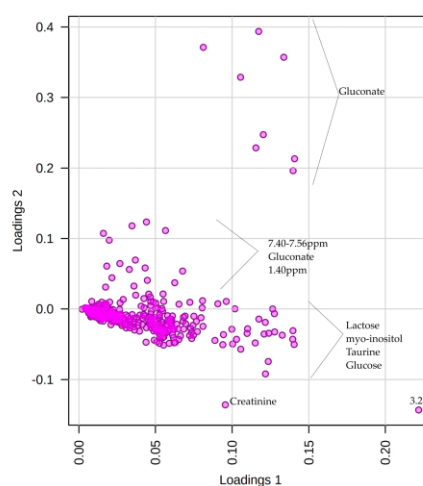
17.	Ethanol	1.17 (t)	Ethanol degradation
18.	Ethanolamine	3.13 (t), 3.13 (t)	Glycerophospholipid metabolism
19.	Formate	8.44 (s)	Glyoxylate and dicarboxylate metabolism
20.	Fumarate	6.52 (s)	Arginine biosynthesis, Citrate cycle
21.	Gluconate	3.65 (m), 3.77 (m), 3.82(m), 4.02 (m), 4.12 (d)	Pentose Phosphate Pathway
22.	Glucose	3.40 (m), 3.45 (m), 3.48 (m), 3.52 (dd), 3.70 (m), 3.83 (m), 3.89 (dd), 4.63 (d), 5.22 (d)	Glycolysis / Gluconeogenesis
23.	Glycine	3.55 (s)	Nitrogen metabolism
24.	Hippurate	7.53 (m) , 7.62 (m), 7.82 (d), 4.97 (d)	Phenylalanine/ Phenolics metabolism
25.	Hypoxanthine	8.18 (s), 8.20 (s)	Purine metabolism
26.	Isobutyrate	1.08(s)	Biphenyl degradation
27.	Lactate	1.31 (d)	Glycolysis / Gluconeogenesis, Pyruvate metabolism
28.	Lactose	4.44 (d), 4.67(d), 5.24 (d)	Lactose synthesis, Galactose metabolism
29.	Levulinate	2.22 (s), 2.43 (t), 2.74 (t)	Secondary metabolism
30.	Lysine	1.71 (m), 1.90 (m), 3.02 (t), 3.76 (t)	Protein digestion and absorption & Biotin metabolism
31.	Mannose	5.17 (d), 4.89 (d)	Galactose metabolism, Fructose & mannose metabolism
32.	<i>myo</i> -inositol	3.26 (t), 3.52 (dd), 3.62(t), 4.05 (t)	Galactose and Inositol phosphate metabolism, ABC transporters, Phosphatidylinositol signaling system, Ascorbate/ alderate metabolism
33.	<i>N, N</i> Dimethylglycine	2.91 (s)	Glycine, serine and threonine metabolism
34.	Oxypurinol	8.31 (s)	Purine metabolism
35.	Propylene glycol	1.13 (d), 3.44 (dd), 3.54 (dd)	Pyruvate metabolism
36.	Pyruvate	2.36 (s)	Urea cycle, Pyruvate metabolism
37.	Succinate	2.47 (s)	Citric Acid Cycle
38.	Taurine	3.25 (t), 3.42 (t)	Taurine and Hypotaurine Metabolism, Primary bile acid biosynthesis
39.	Threonine	1.31 (d), 3.58 (d), 4.25 (m)	Protein digestion and absorption, Biosynthesis of

			amino acids and Glycine, serine and threonine metabolism
40.	Trigonelline	8.07(m), 8.92 (m), 9.02 (m), 9.11 (s, br)	Nicotinate and nicotinamide metabolism
41.	Trimethylamine	2.86 (s)	Oxide, choline and carnitine metabolism
42.	Tyrosine	6.88 (m), 7.17 (m)	Phenylalanine, tyrosine and tryptophan biosynthesis
43.	Urea	5.8 (s, br)	Urea cycle, Arginine and proline metabolism
44.	Valine	0.97 (d), 1.03 (d), 2.23 (m), 3.59 (d)	Protein digestion and absorption, Valine, leucine and isoleucine degradation
45.	Xanthine	7.97 (s)	Purine metabolism

* s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad.



(a)



(b)

Figure S1. Scores and loadings plot of PCA for NMR data belonging to neonates diagnosed with EOS (pink circles) and healthy neonates without need for NICU hospitalization (blue circles). (a) PCA scores plot of the EOS and healthy non-NICU preterms . (b) Loadings plot of PC1 and PC2.

STOCSY 1D

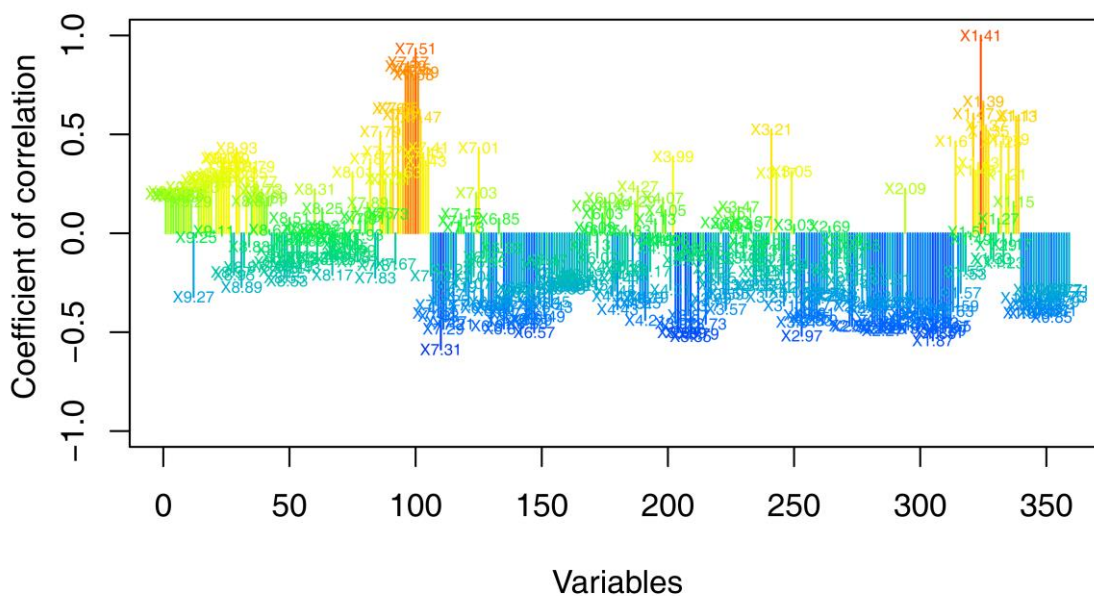


Figure S2. 1D-STOCY pseudo-NMR spectrum of correlation coefficients to the other signals in the median urine NMR spectrum and maximum intensity correlation of peaks are color encoded and projected into statistical difference spectra: “driver peak” was set the one at 1.41 ppm.

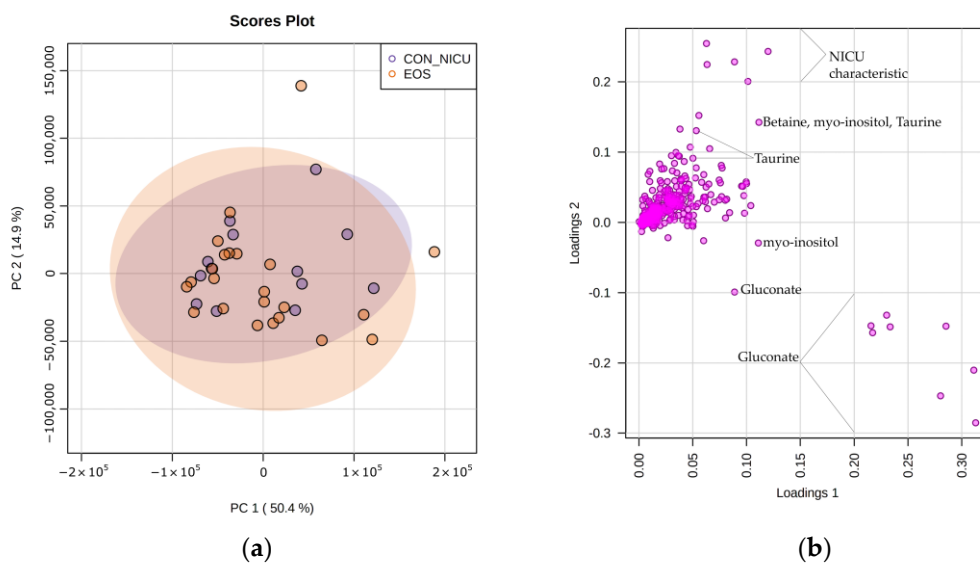


Figure S3. Scores and loadings plot of PCA for NMR data belonging to neonates diagnosed with EOS (orange circles) and neonates hospitalized in NICU without EOS (purple circles). (a) PCA scores plot of the EOS and control group. (b) Loadings plot of PC1 and PC2.

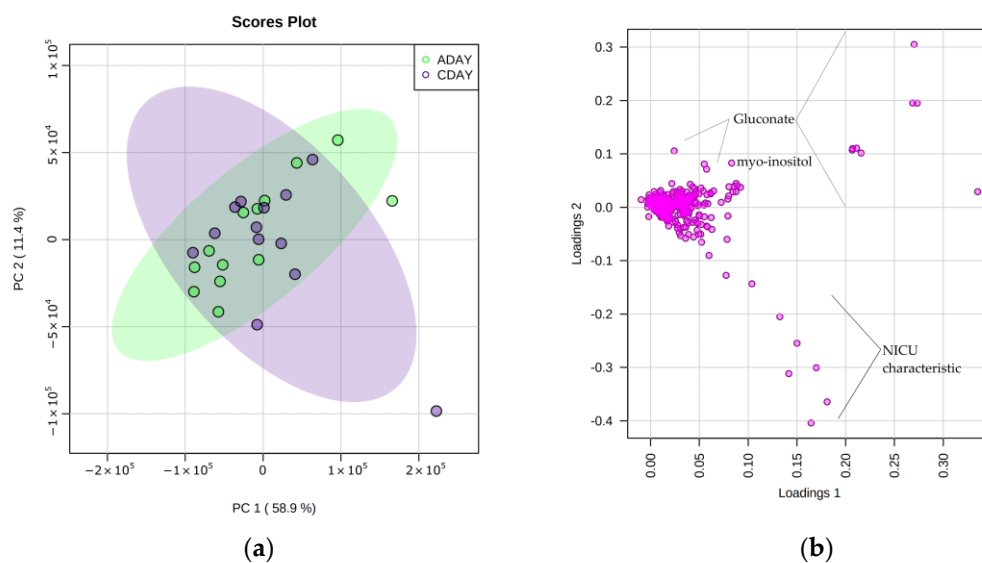


Figure S4. Scores and loadings plot of PCA for NMR data belonging to urine samples of neonates diagnosed with EOS the first (green circles) and the third day (blue circles) of their life. (a) PCA scores plot of the first- and third-day's samples. (b) Loadings plot of PC1 and PC2.

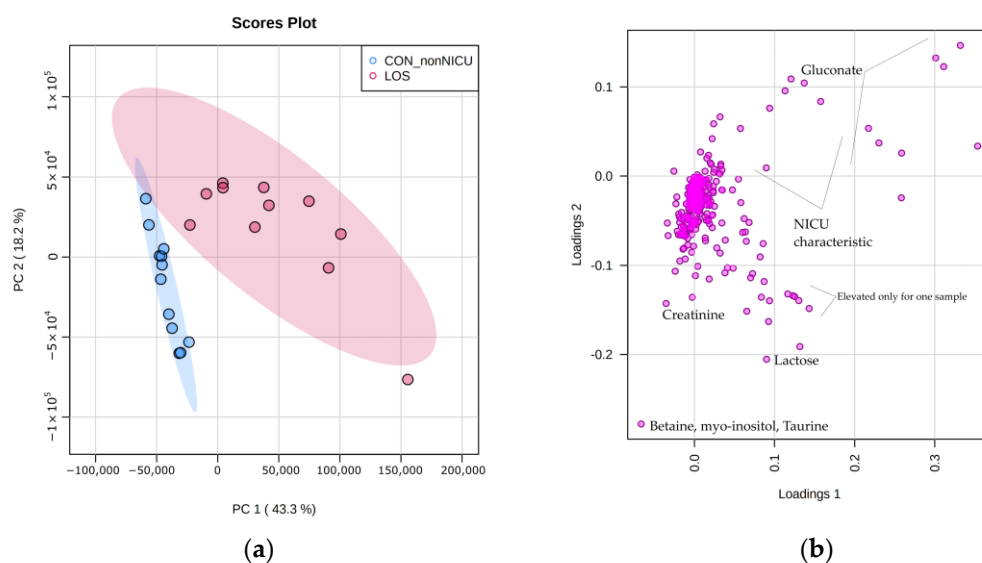


Figure S5. Scores and loadings plot of PCA for NMR data belonging to neonates diagnosed with LOS (pink circles) and control neonates without need for hospitalization (blue circles). (a) PCA scores plot of the LOS and healthy non-NICU preterms without need for hospitalization. (b) Loadings plot of PC1 and PC2.

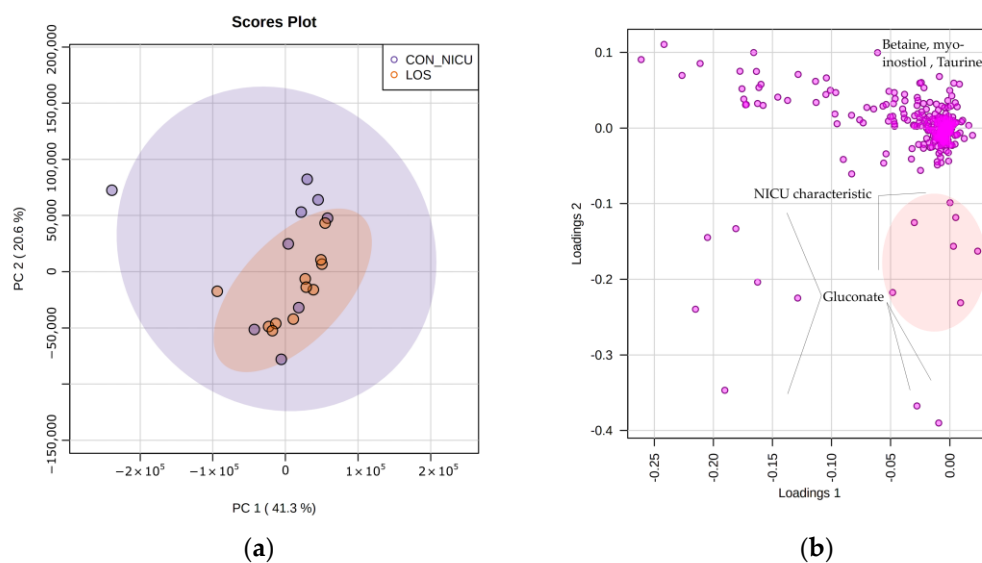


Figure S6. Scores and loadings plot of PCA for NMR data belonging to neonates diagnosed with LOS (orange circles) and control neonates of NICU (purple circles). (a) PCA scores plot of the LOS and control group. (b) Loadings plot of PC1 and PC2.

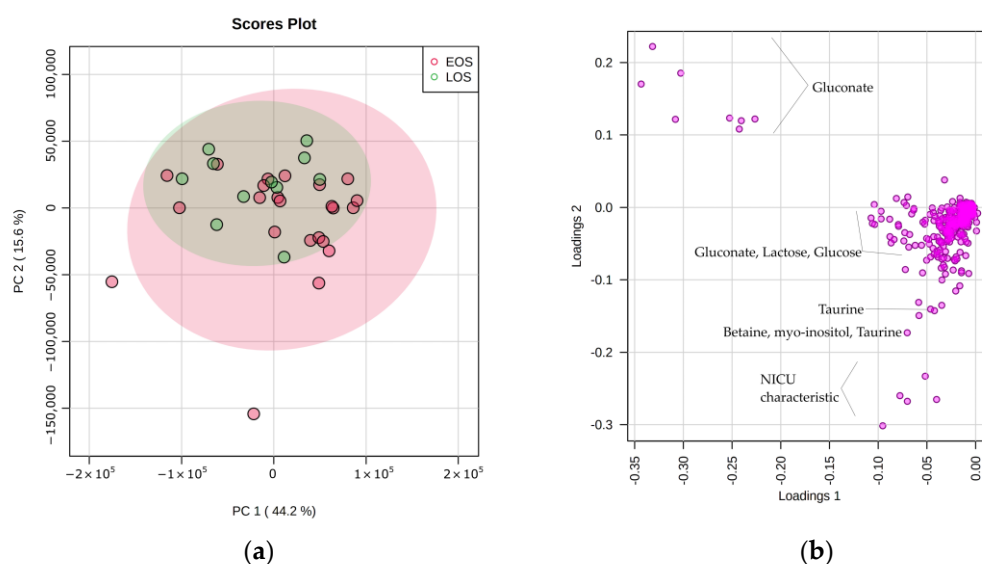


Figure S7. Scores and loadings plot of PCA for NMR data belonging to urine samples of neonates diagnosed with EOS (red circles) and neonates with LOS (green circles). (a) PCA scores plot of EOS and LOS group. (b) Loadings plot of PC1 and PC2.

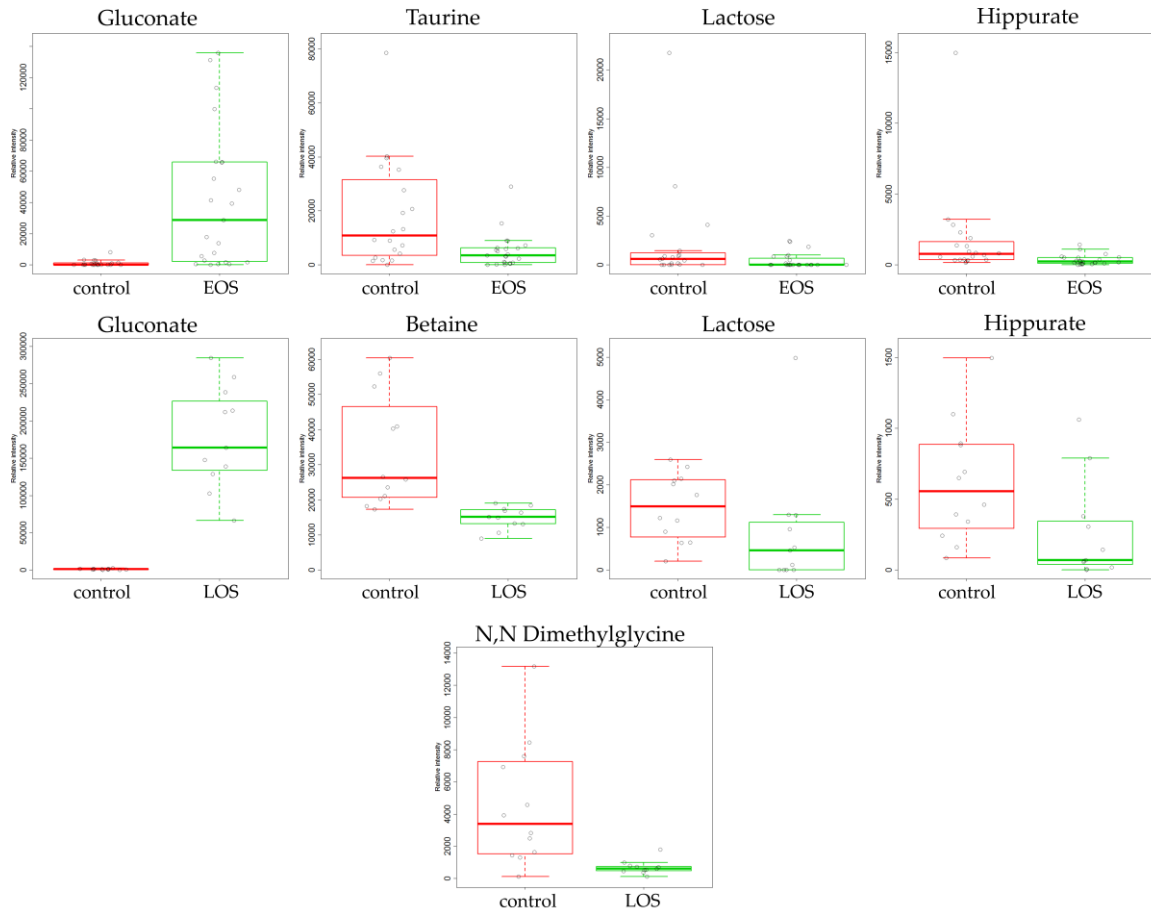


Figure S8. Box plots of the statistically significant metabolites highlighted from univariate analysis with p -value <0.05 , between healthy control non-NICU neonates, LOS and EOS groups.

Table S2. Detailed results from the pathway analysis of the EOS group's significant metabolites.

Pathway name	Metabolites	p-value	Pathway impact
Ascorbate/ alderate metabolism	myo-inositol	0.040642	0.0
Taurine/ Hypotaurine metabolism	Taurine	0.040642	0.42857
Phenylalanine Metabolism	Hippurate	0.050574	0.0
Pentose Phosphate Pathway	Gluconate	0.1083	0.04712
Glycolysis/Gluconeogenesis	Glucose	0.12684	2.1E-4
Galactose metabolism	myo-inositol	0.13143	0.0
Phosphatidylinositol signaling system	myo-inositol	0.13599	0.03736
Inositol phosphate metabolism	myo-inositol	0.14505	0.12939
Glycine/ serine and threonine metabolism	Betaine	0.15849	0.05034
Primary bile acid biosynthesis	Taurine	0.2146	0.00758

Table S3. Detailed results from the pathway analysis of the LOS group's significant metabolites.

Pathway name	Metabolites	p-value	Pathway impact
Glycine/ serine and threonine metabolism	Betaine, N,N Dimethylglycine	0.011364	0.12294
Ascorbate/ alderate metabolism	myo-inositol	0.040642	0.0
Taurine/ Hypotaurine metabolism	Taurine	0.040642	0.42857
Phenylalanine Metabolism	Hippurate	0.050574	0.0
Pentose Phosphate Pathway	Gluconate	0.1083	0.04712
Galactose metabolism	myo-inositol	0.13143	0.0
Phosphatidylinositol signaling system	myo-inositol	0.13599	0.03736
Inositol phosphate metabolism	myo-inositol	0.14505	0.12939
Primary bile acid biosynthesis	Taurine	0.2146	0.00758