Metabolic Phenotypes of asphyctic newborn with Hypoxic-Ischemic Encephalopathy

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INTRODUCTION

- **Birth asphyxia (BA)** is a leading cause of early neonatal death and long-term neurologic morbidity especially in low income countries.

- BA is characterized by periods of **hypoxia-ischemia** that lead to energy exhaustion especially in oxy-regulators tissues such as brain and myocardium.

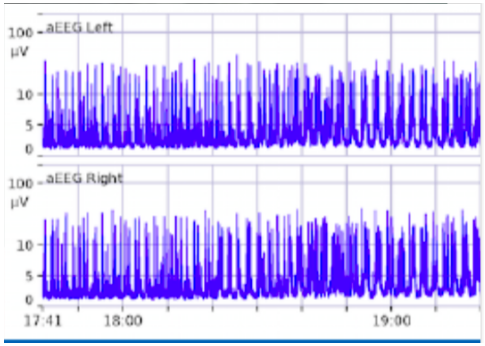
- **Hypoxic-ischemic encephalopathy (HIE)** is the neurological consequence of impaired blood flow and/or gas exchange during birth

- **Therapeutic Hypothermia (TH)** within 6 hours of birth has become the standard of care.

1. Clinical test
   - APGAR score
   - SARNAT score

Therapeutic hypothermia

- 6 h after birth
- Total 72 h

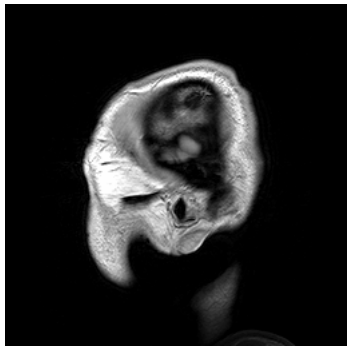


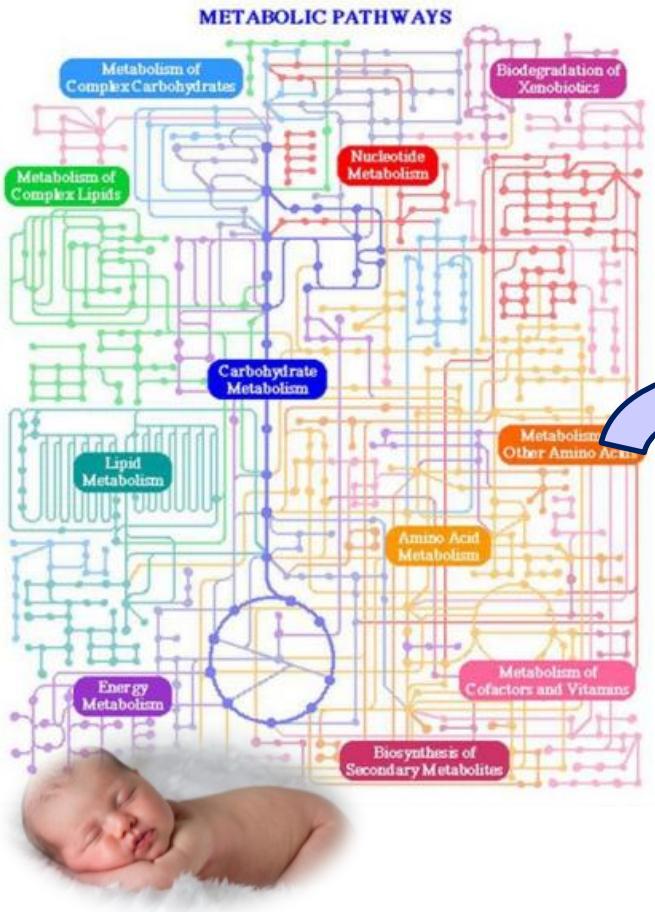
3. Electroencephalography (aEEG)

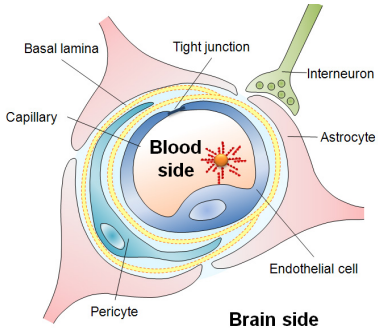
HIE

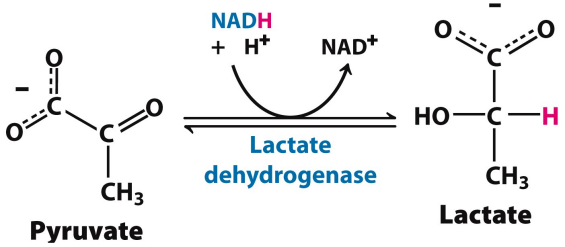
2. Biochemical parameters
   - Lactate
   - Cord gases
   - Metabolic acidosis

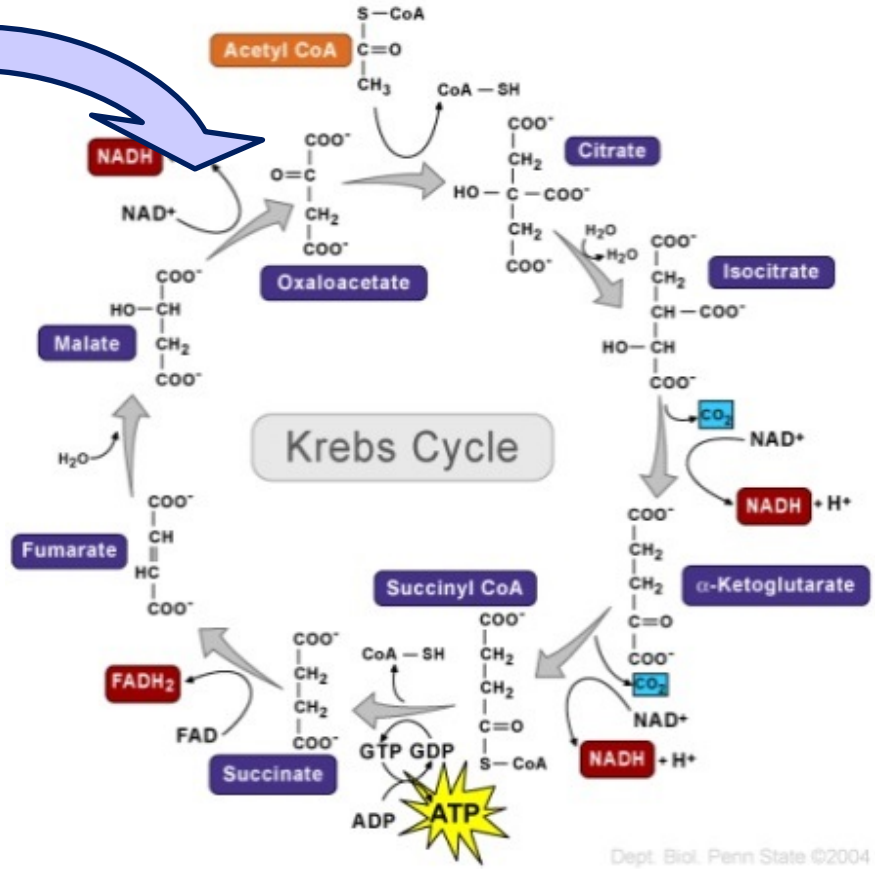
4. Magnetic Resonance Imaging (MRI)




METABOLIC PATHWAYS
Metabolism of Complex Carbohydrates
Biodegradation of Xenobiotics
Nucleotide Metabolism
Metabolism of Complex Lipids
Carbohydrate Metabolism
Lipid Metabolism
Amino Acid Metabolism
Energy Metabolism
Metabolism of Cofactors and Vitamins
Biosynthesis of Secondary Metabolites


Basal lamina
Tight junction
Interneuron
Capillary
Blood side
Astrocyte
Endothelial cell
Pericyte
Brain side

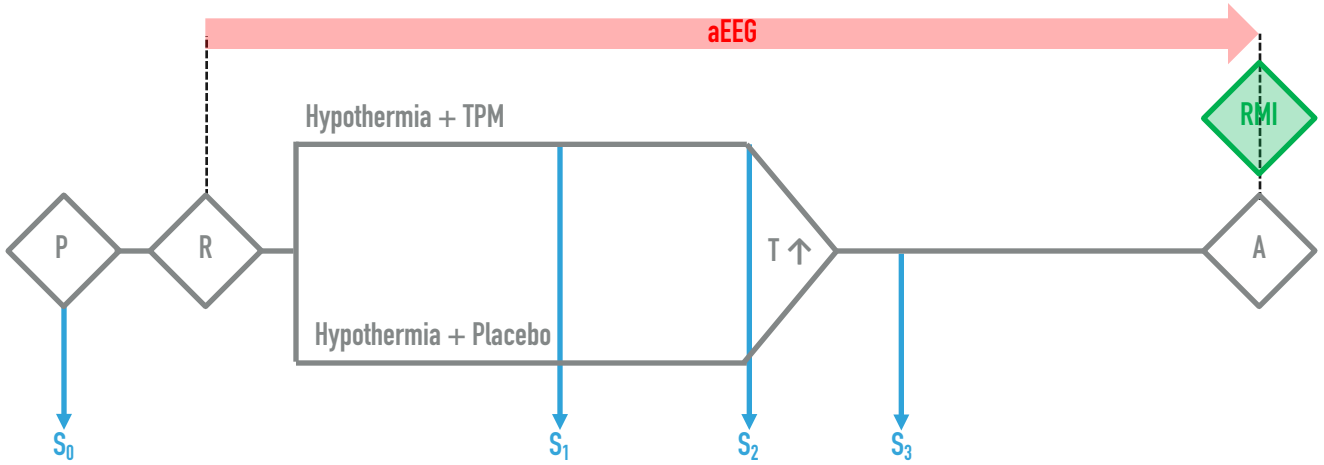

NADH + H+
NAD+
Lactate dehydrogenase
Pyruvate
Lactate


Acetyl CoA
CoA — SH
Citrate
NADH
NAD+
Oxaloacetate
Isocitrate
Malate
Krebs Cycle
Fumarate
Succinyl CoA
α-Ketoglutarate
FADH2
FAD
Succinate
GTP GDP
ADP
ATP
Dept. Biol. Penn State ©2004

OBJECTIVE

Assessment specific biomarkers in a cohort of newborn infatns evolving to moderate/severe HIE undergoing TH in which hypoxia-ischemia induced brain injury was assessed employing MRI.

POPULATION

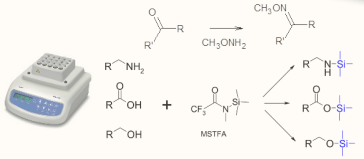


- Double-blinded, randomized, multicenter trial involving newborns with moderate-severe **hypoxic-ischemic encephalopathy** undergoing **hypothermia treatment.**
- **62 newborns** were enrolled.
- **MRI** between days 4 and 8 after birth.
- Grey matter, white matter and basal ganglia.
- Newborn were classified as "normal" (N=22) or "pathologic" (N=33).
- **Unstable neonates** may not be transported for MRI.

Gas-chromatography-mass spectrometry: Targeted

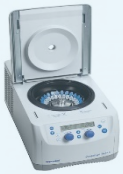
LACTATE
PYRUVATE

Protein precipitation and evaporation



Derivatization and analysis (GC-(EI)-Q-MS)

Sánchez-Ilana Á et al Sci Rep 2017

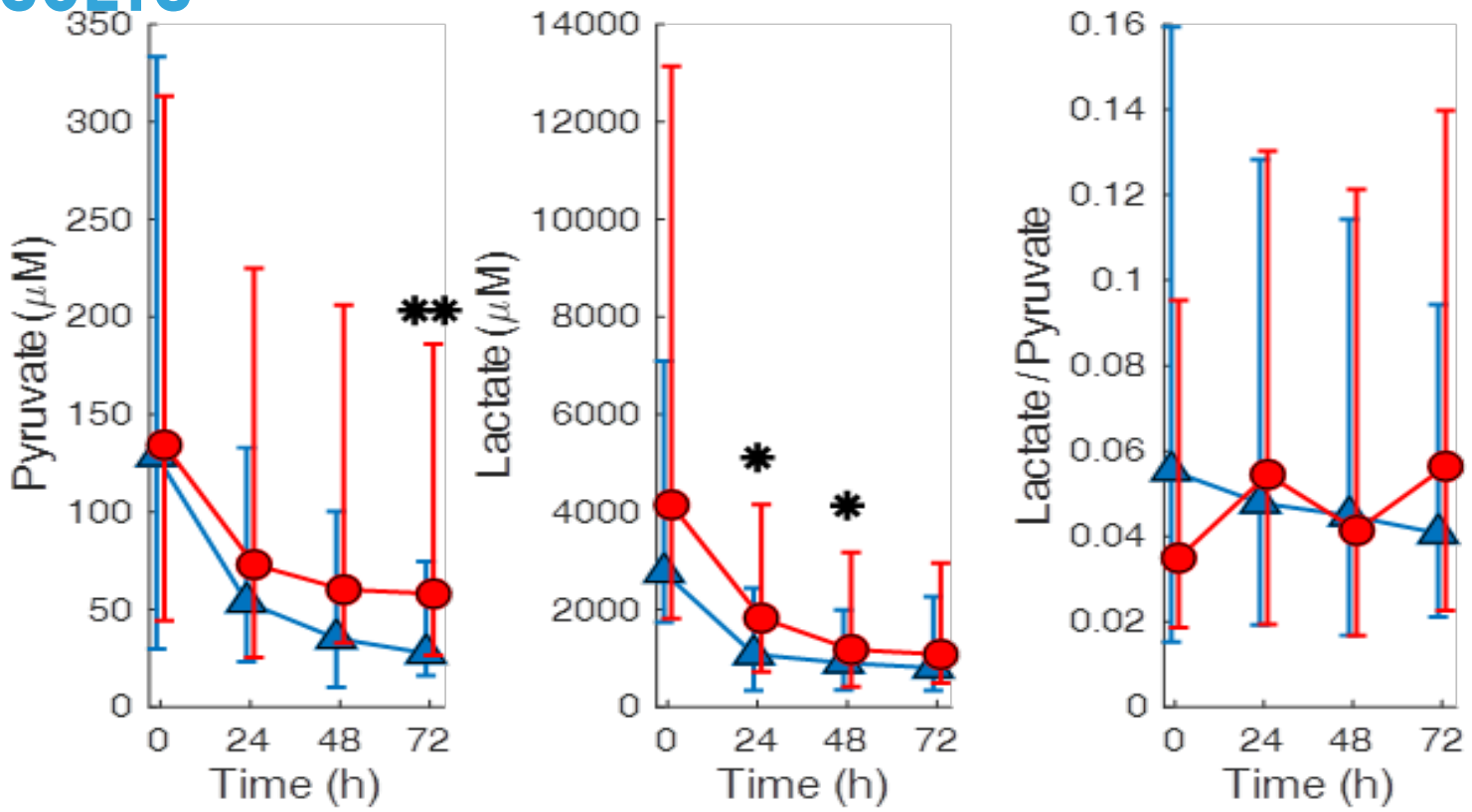


Protein precipitation and evaporation

Re-Dissolved with IS and analysis (UPLC-qTOF)

Liquid chromatography-time-of-flight-mass spectrometry: Untargeted

RESULTS

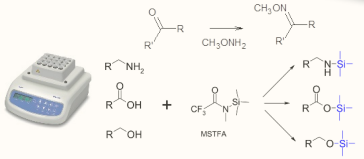


Evolution of lactate and pyruvate concentrations and pyruvate/lactate ratio in plasma samples from newborns with normal (blue) and pathologic (red) MRI outcomes

Gas-chromatography-mass spectrometry: Targeted

LACTATE
PYRUVATE

Protein precipitation and evaporation



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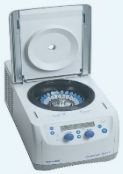
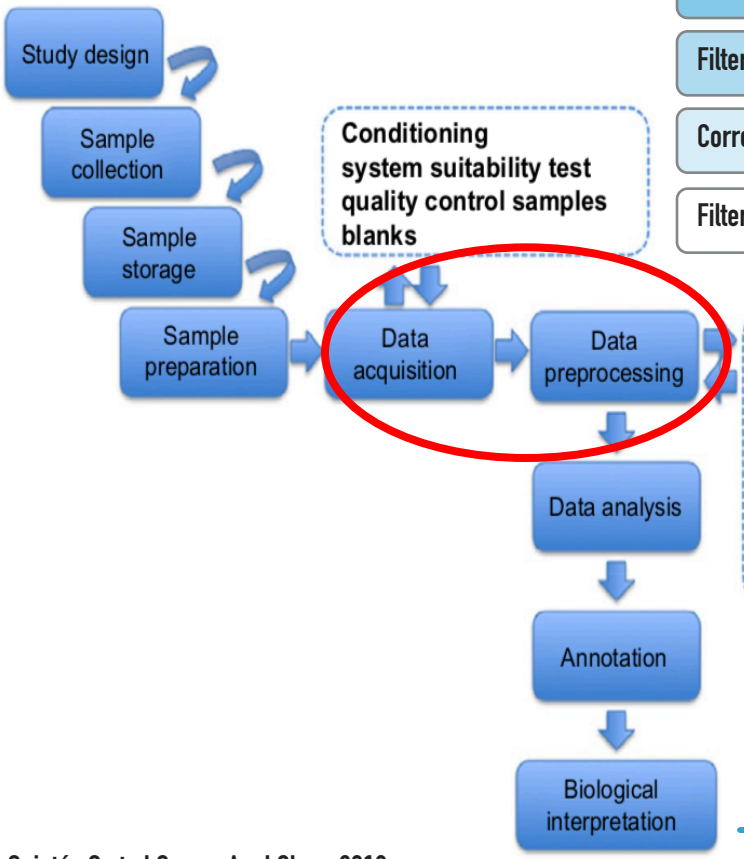


Protein precipitation and evaporation

Re-Dissolved with IS and analysis (UPLC-qTOF)

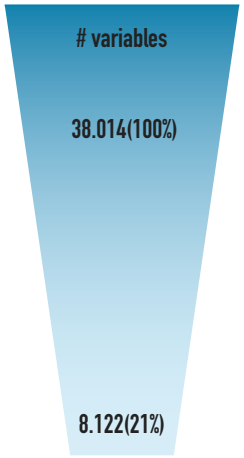
Liquid chromatography-time-of-flight-mass spectrometry: Untargeted

RESULTADOS



Peak Table extraction & data preprocessing

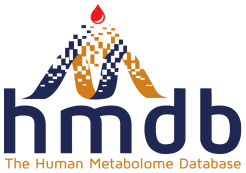
- Peak table extraction (XCMS online)
- Filtering: remove low intensity signals,
- Filtering: remove signals from blanks
- Correction of batch effect (QC-SVR)
- Filtering: Remove signals with RSD>15% in QC



Peak table generation
- Peak picking/integration
- Peak deconvolution
- Peak matching
- Peak alignment

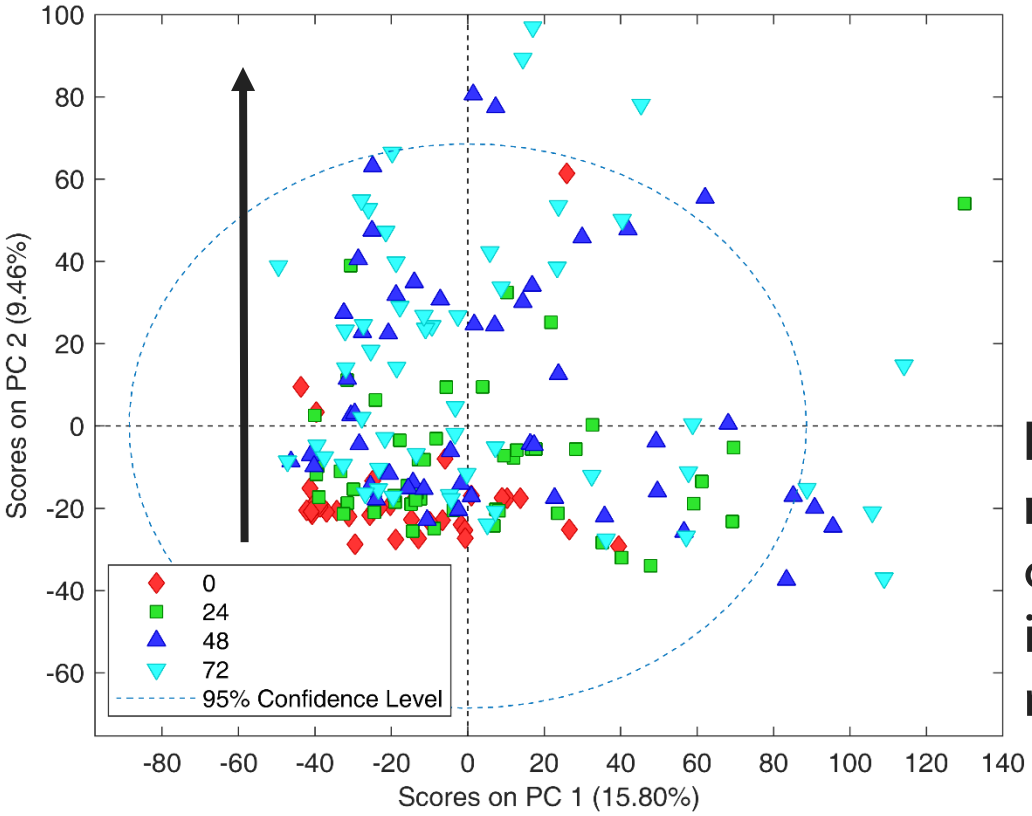
Batch effect
- Evaluation
- Correction

Data cleanup



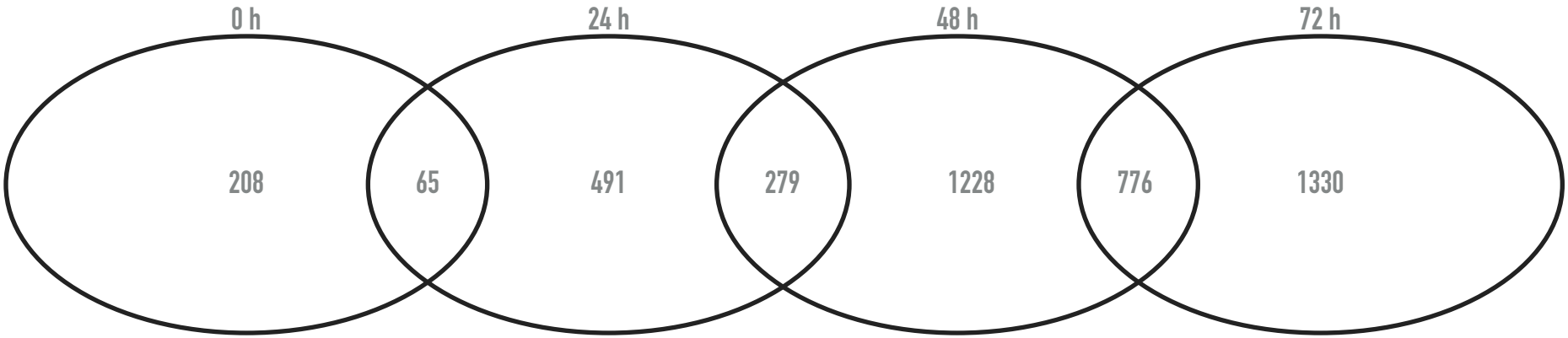
Quintás G et al Compr Anal Chem 2018

RESULTADOS



PCA scores of plasma metabolomic profiles. Direction of PC 2 shown the strong impact of time on the plasma metabolome.

RESULTADOS



Venn-diagram shown the longitudinal overlap among altered metabolomic features in plasma samples.

RESULTADOS

MetaboAnalyst 4.0

			T0		T24		T48		T72	
Pathway	Total # of metabolites	Hits (total)	Hits (sig)	Fisher´s P	Hits (sig)	Fisher´s P	Hits (sig)	Fisher´s P	Hits (sig)	Fisher´s
Alanine, aspartate and glutamate metabolism	24	19	6	0.14	5	0.9	13	0.4	16	0.04
Arginine and proline metabolism	77	50	10	0.5	21	0.4	41	0.002	41	0.003
Caffeine metabolism	21	2	2	0.04	2	0.2	2	0.4	2	0.4
D-Glutamine and D-Glutamate metabolism	11	7	1	0.8	3	0.6	7	0.03	7	0.04
Limonene and pinene degradation	59	7	2	0.4	1	1.0	4	0.7	7	0.04
Lysine biosynthesis	32	20	3	0.8	11	0.10	17	0.02	19	0.0013
Lysine degradation	47	32	7	0.4	17	0.07	21	0.4	28	0.002
Nitrogen metabolism	39	16	4	0.4	7	0.4	11	0.4	14	0.03
Phenylalanine metabolism	45	25	5	0.5	15	0.02	24	0.00009	21	0.02
Selenoamino acid metabolism	22	2	2	0.04	1	0.6	2	0.4	2	0.4
Steroid hormone biosynthesis	99	29	11	0.01	23	0.000009	25	0.004	25	0.006

CONCLUSIONS

- **The power of Sarnat** staging for an early prediction of normal vs. Pathologic brain imaging is poor, and a high proportion (i.e 50%) of infants staged as moderate HIE still developed brain injury at day 7.
- **The time-dependent evolution of pyruvate levels** in newborns with HIE undergoing TH. **Pyruvate and lactate** could potentially be used for discerning favorable/unfavorable outcomes at 72h.
- **We present the first metabolomic study** involving human subjects and serial sample collections in HIE for modelling brain injury as confirmed with MRI. Time-dependent perturbation of several metabolic pahtways were revealed. **The steroid hormones biosyntesis pathway** has been significantly altered in newborn with pathologic MRI outcomes.

THANK YOU FOR YOUR ATTENTION

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