Table 1: Summary of metabolomic studies in animal models of hypoxia/asphyxia and/or resuscitation protocols.

Author/year	Experimental model	Aim	Biological fluid	Analytical platform	Key findings
van Cappellen van Walsum et al/2001[41]	Fetal lambs	Investigate if mild hypoxia induces changes in cerebral metabolism vs. severe hypoxia	Cerebrospinal fluid	^{1}H NMR	• Increased choline in severe hypoxia • After 2 hours of mild hypoxia and in severe hypoxia, levels of lactic acid, alanine, phenylalanine, tyrosine, lysine, branched chain amino acids, and hypoxanthine were found increased
Atzori et al/2010[49]	Newborn piglets	Characterize the metabolic profiles of newborn undergoing hypoxia-reoxygenation	Urine	^{1}H NMR	• Metabolic variations were observed in the urine of piglets treated with different oxygen concentrations. Discriminant metabolites: urea, creatinine, malonate, methylguanidine and hydroxyisobutyric acid
Solberg et al/ 2010[19]	Newborn piglets	Detection of markers of hypoxia	Plasma	Flow injection analysis MS/MS and LC-MS/MS	• Ratios of alanine to branched chained amino acids and of glycine to BCAA were highly correlated with the duration of hypoxia • Reoxygenation with 100% oxygen delayed cellular metabolic recovery • Metabolites of the Krebs cycle (alpha keto-glutarate, succinate, fumarate) were significantly reduced at different rates depending on the resuscitation, showing a delay in recovery in the 100% reoxygenation groups. • Oxysterols and acylcarnitines showed different responses to reoxygenation
Beckstrom et al/2011[22]	Newborn non-human primate	Identify significant metabolites affected by birth asphyxia	Blood	GC×GC-TOFMS	• 10 metabolites increased after asphyxia • Lactate, creatinine, succinic acid, malate and arachidonic acid could help as potential biomarkers
Liu et al/ 2011[18]	Neonatal rats	Distinguish different insults, treatments and recovery stages after applying hypothermia	Brain slice	^{1}H/^{31}P NMR	• Metabolites differed in treatment and outcome groups, especially phosphocreatine, ATP and ADP • ATP levels severely decreased at normothermia, and restored equally by immediate and delayed hypothermia • Cell death was decreased by immediate hypothermia, but was equally substantially greater with normothermia and delayed hypothermia
Skappak et al/2013 [23]	Newborn piglets	Identify hypoxia using urinary metabolomic profiling	Urine	NMR	• 13 urinary metabolites differentiated hypoxic versus nonhypoxic animals (1-methylnicotinamide, 2-oxoglutarate, alanine, asparagine, betaine, citrate, creatine, fumarate, hippurate, lactate, N-acetylglycine, N-carbamoyl-β-alanine, and valine). • Using metabolomic profile, it was able to blindly identify hypoxic animals correctly 84% of the time compared to nonhypoxic controls • Metabolomic profiling of urine has potential for identifying neonates that have undergone episodes of hypoxia
Liu et al/ 2013[25]	Neonatal rats	Distinguish metabolic differences in glia and neurons	Brain slices	^{13}C NMR	• [2-C]Glutamine increased in the hypothermia group compared to delayed hypothermia and normothermia group • [3,4-C]glutamate, [2-C]taurine and phosphocreatine were mostly associated with adenosine triphosphate preservation
Liu et al/ 2013[51]	Neonatal mice	Identify biomarkers and distinguish differences applying hypothermia	Brain extracts	^{1}H NMR	• Hypothermia group was separated from non-hypothermia and controls
Fanos et al/2014[20]	Piglet model	Investigate metabolomic profiles according to oxygen concentration (18%, 21%, 40%, and 100%) administered at resuscitation	Urine	^{1}H NMR	• 21% of oxygen is the most “physiological” and appropriate concentration to be used for resuscitation
Takenouchi et al/2015[29]	Neonatal rats	Decipher the mechanisms through which hypothermia regulates metabolic dynamics in different brain regions	Brain tissue	MS/MS	• 107 metabolites were investigated • Hypothermia diminished the carbon biomass related to acetyl moieties, such as pyruvate and acetyl-CoA, and increased deacetylated metabolites (carnitine and choline) • Hypothermia diminished the acetylcholine contents in hippocampus and amygdala, where carnitine was increased

Chun et al/2015[39]	Non-human primate model	Identify indicators of brain injury, repair and prediction of neurodevelopmental outcome	Plasma	GC×GC-TOFMS	• 63 metabolites identified as potential biomarkers • 8 metabolites (arachidonic acid, butanoic acid, citric acid, fumaric acid, lactate, malate, propanoic acid, and succinic acid) correlated with early and/or long-term neurodevelopmental outcomes • Citric acid, fumaric acid, lactate and propanoic acid correlated with combined outcomes of death or cerebral palsy • Circulating metabolome has the potential to predict neurodevelopmental outcome
Solberg et al/2016[33]	Newborn piglets	Identify early brain hypoxia biomarkers	Plasma	LC-TOFMS	• Increased plasma metabolites at the end of hypoxia, reflecting a metabolic adaptation to prolonged anaerobiosis • Metabolite levels returned to base line after resuscitation
Sachse et al/2016[34]	Newborn pigs	Identify biomarkers for subject characterization, intervention effects and possibly Prognosis	Plasma/Urine	NMR	• Plasma and urine metabolites showed severe alterations consistent with hypoxia and acidosis 2 and 4 hours after return of spontaneous circulation • Baseline plasma hypoxanthine and lipoprotein concentrations were inversely correlated to the duration of hypoxia sustained before asystole occurred • No evidence for a differential metabolic response to the different resuscitation protocols or in terms of survival
Blaise et al / 2017[47]	Newborn mice	Investigate the effects of excitotoxicity in metabolome	• Brain tissue • Plasma	MS	• No difference in plasma metabolic profile • The amino acids glutamine, proline, serine, threonine, tryptophan, valine, and the sphingolipid SM C26:1 were increased in the brain. Glycerophospholipids were decreased • Metabolomics could identify excitotoxic effects
Brown et al /2017[35]	Newborn mice	Investigate if intrauterine inflammation alters the metabolome of the amniotic fluid, fetal and neonatal brain, and if sex makes difference	• Amniotic fluid • Brain	LC-MS	• Intrauterine inflammation enhances amino acids and purine metabolites • Hypoxanthine pathway metabolites were increased in amniotic fluid. They can be potential biomarkers. • Fatty acids pattern differed in neonatal brain in a sex-specific manner

NMR: nuclear magnetic resonance (spectroscopy), MS: mass spectrometry, LC-MS: Liquid Chromatography - Mass Spectrometry, GC×GC-TOFMS: 2-dimensional gas chromatography-time-of-flight-mass spectrometry, LC-TOFMS: Liquid chromatography-time of flight mass spectrometry.