

# Combined 1H MRS and Near-Infrared Spectroscopy Measurements of Cerebral Blood Volume, Oxygenation, Cytochrome Oxidase, and Intracellular Metabolites During Perinatal Hypoxia-Ischaemia

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**Introduction:** Perinatal hypoxia-ischemia (HI) is associated with high mortality and morbidity rates worldwide. 1H magnetic resonance spectroscopy (MRS) can be acquired sequentially in our established piglet model during transient global HI. The thalamic lactate (Lac) / N-acetyl-aspartate (NAA) ratio is a highly sensitive and specific biomarker of long term neurodevelopmental outcome in infants with neonatal encephalopathy when acquired aged 5-14 d [1]. To investigate brain haemodynamic and metabolic changes during transient HI and recovery we integrated broadband near-infrared spectroscopy (NIRS) with MRS. This unique bimodality can characterise the time courses of brain oxygenation, haemodynamic and metabolic changes continuously and non-invasively offering insights into brain patho-physiology. Broadband NIRS can resolve changes in oxy- and deoxyhaemoglobin (HbO<sub>2</sub> & Hb) and the redox state of cytochrome-c-oxidase (ox-red CCO). CCO is the terminal electron acceptor of the mitochondrial electron transfer chain and catalyses over 95% of oxygen metabolism, thereby driving aerobic adenosine triphosphate (ATP) synthesis and playing a central role in the maintenance of mitochondrial function.

**Methods:** Experiments were performed under UK Home Office guidelines. A healthy piglet (aged < 24 hr) was anaesthetised and physiologically monitored with intensive life support. Transient cerebral HI was induced by reducing the inspired oxygen fraction to ~ 0.12 and inflating bilateral carotid artery occluders for 27.5 min after which the occluders were deflated. Inspired O<sub>2</sub> was increased step-wise to 0.19 during the last 10 min of transient HI and was returned to normal at the start of recovery. During, and up to 90 min after, transient HI whole-brain 1H MRS was acquired every minute using a 9.4 Tesla Varian spectrometer and a ~60 mm diameter MRS surface coil. 1H MRS used PRESS (repetition time 5 sec, echo time 288 ms, 12 averages, 2x2x2cm voxel centred entirely within the brain). Spectra were analysed using AMARES [2] as implemented in the jMRUI software [3] and the Lac/NAA peak area ratio was calculated. During the same time period, NIRS data were acquired continuously with 1 sec time resolution. The NIRS optodes were integrated with the surface coil and fixed on the top of the piglet's head. We used a broadband source and a multi-wavelength detection system to resolve the changes in the cerebral blood volume (CBV) (HbO<sub>2</sub>+Hb), cerebral oxygenation (HbO<sub>2</sub>-Hb) and ox-red CCO. This system allows for accurate measurements and has been used before in piglets [4 & 5] and human adults [6 & 7].

**Results:** Processed NIRS data are shown in Figure 1. At induction of transient HI there is a fast drop in brain oxygenation and a small fall in CBV. Upon reversal of the insult there is a hyperaemic phase followed by a slow recovery of CBV and brain oxygenation to baseline levels. Figure 2 shows ox-red CCO plotted with Lac/NAA on the same time axis. The ox-red CCO during transient HI becomes reduced when oxygenation dropped significantly indicating a reduction in oxidative metabolism. At the mid point of the insult as the inspired oxygen is titrated upwards, ox-red CCO starts to recover slowly. Upon reversal of the insult there is a period of reduced ox-red CCO and increased oxygenation. Lac/NAA begins to decline followed by an overshoot in ox-red CCO and a decline in oxygenation, indicating increased oxygen metabolism. Figure 3 shows the correlation between ox-red CCO and Lac/NAA during and following transient HI. During recovery this relationship appears to be approximately linear. During transient HI there is a clear reduction of ox-red CCO before Lac/NAA begins to increase.

**Discussion:** This is the first study combining broadband NIRS with 1H MRS during transient global HI in a neonatal model. Our measurements indicate that during the primary insult, haemoglobin oxygenation will fall and ox-red CCO will become reduced. In 1997 Cooper and Springett [4] used broadband NIRS and phosphorus MRS during HI in piglets and found a high linear correlation between the drop of ox-red CCO and [ATP]. In a later study of anoxic episodes, Springett et al. [5] correlated the drop of ox-red CCO with the decline in phosphocreatine concluding that at normoxia, oxidative phosphorylation and the oxidation state of the components of the electron transport chain are independent of cerebral oxygenation and that the reduction in the CCO signal occurs when oxygen tension limits the capacity of oxidative phosphorylation to maintain phosphorylation potential. During HI, Lac is produced by anaerobic glycolysis; in the early post-hypoxic state Lac is produced by astrocytes for recovery of synaptic function [8]. During the period of reduced oxidative metabolism during recovery, Lac is utilised and Lac/NAA declines. These data suggest that when Lac is sufficiently low, there is an overshoot in oxygen-based metabolism, indicating a switch-over in the physiological pathways. Although further studies are necessary to confirm these findings, a combination of 1H MRS and NIRS could allow study of the switch between anaerobic and aerobic metabolism in this model and be used to investigate potential therapies targeting oxidative stress at the beginning of recovery. NIRS provides complementary information to 1H MRS that may improve our understanding of the response of the newborn brain to HI.

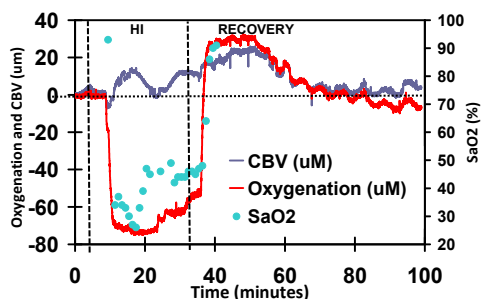


Figure 1: Processed NIRS data

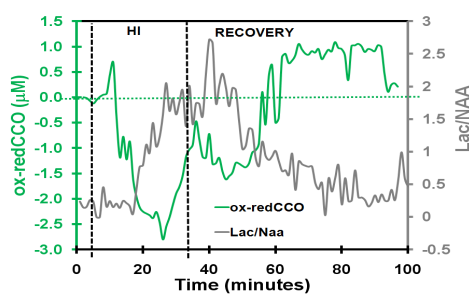


Figure 2: ox-red CCO plotted with Lac/NAA

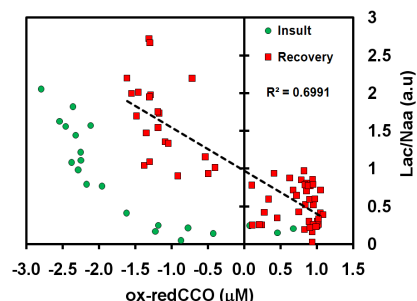


Figure 3: ox-red CCO plotted against Lac/NAA

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