

Hyperpolarized pyruvate allows early detection of lactate in real-time metabolism of acute liver failure rats

Laia Chavarria^{1,2}, Jordi Romero-Giménez^{1,2}, Eva Monteagudo³, Juan Córdoba^{1,2}, and Sílvia Lope-Piedrafita^{3,4}

¹Liver Unit, Hospital Vall Hebron, Barcelona, Barcelona, Spain, ²Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Barcelona, Spain, ³Servei de RMN, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain, ⁴Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain

Introduction: Intracranial hypertension is a severe complication of acute liver failure (ALF) secondary to brain edema. The pathogenesis of cerebral edema in ALF is not clear but it is known that energy metabolism alterations are involved where a genesis of lactate seems to have an important role.¹

Aim: The aim of the study was to follow the dynamic synthesis of brain metabolites using hyperpolarized $1\text{-}^{13}\text{C}$ pyruvate in a rat model of ALF.

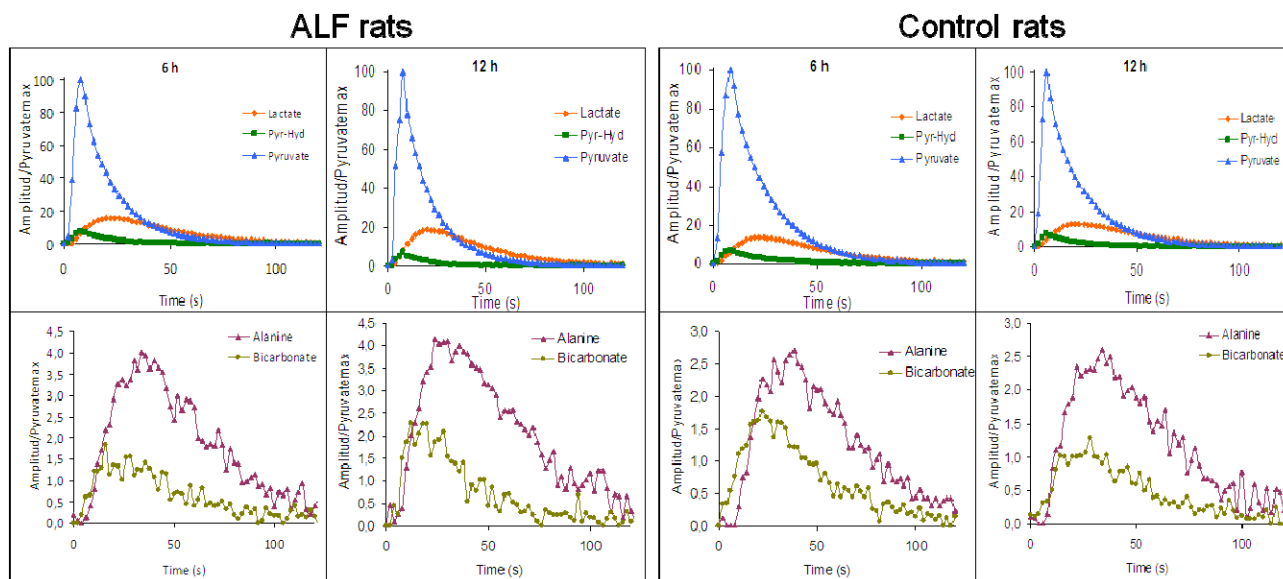
Methods:

Animal Model: Acute liver failure (ALF) was induced in Sprague-Dawley male rats (250-300g) by portocaval anastomosis (PCA) and hepatic artery ligation (HAL). Control rats underwent PCA and a sham surgery at the same timepoints as the ALF rats had the PCA and HAL surgeries. The ALF model is characterized by its highly predictable time course: the precoma stage is defined as a loss of the righting reflex at ~11h post HAL and coma stage as a loss of corneal reflex at ~14h post HAL. Control rats do not present any symptom of disease. Animals were anesthetized with isoflurane and body temperature was kept constant at $37\pm 0.5^\circ\text{C}$. The metabolism was assessed by hyperpolarized ^{13}C -pyruvate MRS. This study was performed on controls and ALF rats ($n=8/\text{group}$) repeatedly at 6 and 12 hours after sham or HAL surgery, respectively.

Magnetic resonance (MR): A sample of $[1\text{-}^{13}\text{C}]$ pyruvic acid, 15 mM OX63 trityl radical and 1.5 mM Dotarem was hyperpolarized using a HyperSense DNP polarizer for approximately 1 h ($\approx 94.1\text{ GHz}$, 100 mW). The sample was subsequently dissolved in a pressurized and heated alkaline buffer ($\approx 4\text{ mL}$) with a resulting polarization of $18\pm 2\%$ and physiological temperature and pH. 0.01 mL/g of the 80 mM hyperpolarized $[1\text{-}^{13}\text{C}]$ pyruvate solution was injected into the rat through the tail vein. ^{13}C -Magnetic resonance spectroscopy studies were performed in a 7T Bruker BioSpec using a $^1\text{H}/^{13}\text{C}$ surface coil placed on top of the rat head. ^{13}C -pulse-acquisition spectra were acquired for 3 min after injection (TR, 2s; excitation flip angle, 5° ; sweep width, 150 kHz; acquired points, 2048; frequency centered on the pyruvate resonance). Spectra were analyzed using AMARES algorithm as implemented in jMRUI software package.

Results:

Control and ALF rats did not show any change in injection time of pyruvate. ALF rats had a significant increase in the total ratio of lactate versus pyruvate in the average spectrum compared to Control rats (ALF at 6h: 0.475 ± 0.070 and at 12h: 0.606 ± 0.156 ; Control at 6h: 0.348 ± 0.052 and at 12h: 0.359 ± 0.068 ; $P=0.013$).



Additional MR parameters showed that the rate of lactate production was higher in ALF rats ($P<0.001$) with significant changes according to the severity of liver failure (6h: $1.41\pm 0.23\text{ s}^{-1}$ and 12h: $1.78\pm 0.44\text{ s}^{-1}$, $P=0.021$) in comparison to Control rats (6h: $1.06\pm 0.23\text{ s}^{-1}$ and $1.07\pm 0.17\text{ s}^{-1}$, $P=0.900$). There was also an increase in the rate of alanine production independent to the time course (ALF: $0.25\pm 0.10\text{ s}^{-1}$ and Control: $0.16\pm 0.05\text{ s}^{-1}$, $P=0.003$). The exponential signal decay term showed an accumulation of lactate in Control rats (ALF: $29\pm 3\text{ s}$ and Control: $32\pm 1\text{ s}$, $P<0.001$) and pyruvate (ALF: $18\pm 1\text{ s}$ and Control: $19\pm 1\text{ s}$, $P<0.001$).

Conclusion:

Magnetic resonance spectroscopy with hyperpolarized $[1\text{-}^{13}\text{C}]$ pyruvate detected early, before any symptom of disease, an increase in lactate in ALF rats, sign of anaerobic metabolism activation. This study shows for the first time the 'in vivo' brain metabolism of ALF rats at real-time. Hyperpolarization is a potential non-invasive technique to follow the *in vivo* metabolism involved in ALF.

Reference: [1] Bjerring PN, et al. Neurocrit Care 2008;9(1):3-7.