In vivo ^{13}C MRS investigation of alterations in cerebral oxidative metabolism in a chronic liver disease rat model

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Introduction:

In vivo dynamic ^{13}C MRS together with the administration of [1,6-^{13}C]-glucose and an appropriate mathematical model of neuronal-glial metabolism is a powerful and unique technique to non-invasively investigate compartmentalized cerebral energy metabolism [1]. In the diseased liver (chronic or acute liver failure), ammonia passes directly into the blood circulation, leading to hepatic encephalopathy (HE) [2-4]. Ammonia detoxification occurs mainly in the astrocytes through the glutamine synthetase enzyme, leading to an increase of brain glutamine (Gln), cell swelling and consequently making the astrocytes the principal target of ammonia neurotoxicity. The clinical manifestations of HE extends from mild cognitive and motor impairment to coma and death [2-4]. The molecular mechanisms that characterize HE are complex and sometimes controversial. In the last decades several hypothesis were elaborated: the increase of Gln creates an osmotic imbalance leading to brain edema; or that other mechanisms (i.e. energy dysfunction) may lead to edema [5]. To date, no *in vivo* dynamic ^{13}C MRS studies were performed in animal models with chronic liver disease (CLD) to assess energy metabolism. Few *ex vivo* studies were performed on animal models with acute liver failure [6]. In CLD it has been previously shown that the osmotic imbalance created by the increase of Gln can be compensated at early stages by a decrease of other osmolytes, but with minimal brain edema at latest stages of the disease [7]. In this context, the aim of the present study was to identify if at latest stages the increase of Gln impacts brain energy metabolism leading to edema.

Methods:

Localized ^{13}C spectra were measured on 3 control rats and 3 CLD rats (Wistar, 300±30g, VOI=5x8x8mm^3) under isoflurane anesthesia. The CLD model was created by bile duct ligating (BDL) the animals 8 weeks before the ^{13}C aquisition, while the controls were sham-operated. On the day of the experiment, the femoral arteries and veins were catheterized for monitoring blood gases, blood pressure, glucose concentration, and for infusion of glucose. An exponentially decaying bolus of 99%-enriched [1,6-$^{13}C_2$] glucose was administrated over 5 min, followed by a continuous infusion of 70%-enriched glucose for 6h. Glucose was infused at a rate adjustable to the concomitantly measured plasma glucose concentrations to maintain the desired glycemia level (around 300 mg/dl). All data were acquired on a 9.4T system (Varian/Magnex Scientific) using a home-built 10mm (^{13}C)/13mm (^{1}H quad) surface coil as RF transceiver and the semi-adiabatic DEPT polarization transfer sequence (TR=2.5s, interpulse delay 3.8ms (J_{CH}=130Hz), 45° for last ^{1}H pulse to simultaneously measure signals from CH, CH_2, CH_3 groups) [8]. ^{1}H spectra were acquired in the same VOI before the glucose injection using an ultra-short-TE localized SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 128 scans), to measure glutamate (Glu) and Gln pool sizes.

Results and Discussion:

Total Glu was 6.3±0.8 µmol/g and total Gln 5.8±0.6 µmol/g in BDL rats versus 7.4±0.3 µmol/g and 4.2±0.1 µmol/g in control rats, respectively. High quality *in vivo* ^{13}C spectra could be acquired both in the controls and BDL rats (fig. 1A). The uptake curves of the carbon positions C4, C3 and C2 of Glu and Gln were successfully fitted by the two-compartmental neuroglial model [1], both for the BDL (fig. 1B) and the control rats groups (not shown). The fluxes and their precision (as determined by Monte Carlo simulation) obtained on the two rat groups are the following:

Fluxes in µmol/g/min	Vtca^g	Vpc	Vnt	Vtca^n	Vx
BDL rats	0.10 ± 0.02	0.028 ± 0.003	0.07 ± 0.01	0.54 ± 0.03	0.52 ± 0.19
Control rats	0.15 ± 0.04	0.021 ± 0.003	0.08 ± 0.01	0.55 ± 0.03	0.52 ± 0.13

No major flux changes were observed between the two groups, except for the pyruvate carboxylase flux (Vpc), which was significantly higher ($p < 0.05$) in the BDL rats group than in the controls. This anaplerotic pathways is directly involved in the de novo synthesis of glutamine in the glial compartment, which is known to be upregulated in the hyperammonemic conditions generated by CLD [5,9]. A tendency to a decrease in the glial TCA cycle activity was observed, without reaching statistical significance.

In conclusion, the present ^{13}C MRS study of HE induced by CLD characterized for the first time brain oxidative metabolism *in vivo* in a CLD model. As compared with controls, a significant increase of pyruvate carboxylase activity was measured in the BDL rats. However, although significant changes in amino acids and osmolytes concentrations were measured at this stage of the disease development in previous studies [7], no significant effect on oxidative metabolism and apparent neurotransmission was observed, suggesting that mild brain edema in CLD-induced HE is primarily caused by other effects of hyperammonemia, such as osmotic imbalance and resulting cell swelling.

References [1] R Gruetter R et al., Am J Physiol Endocrinol Metab 2001; [2] Norenberg MD et al, Metab Brain Dis 2009; [3] Caudle SE et al, J Pediatr. 2010; [4] Braissant O, Mol Genet Metab. 2010; [5] Rama Rao KV et al, Neurochem Int 2011; [6] Zwingmann C, J Neurosci Res 2007; [7] Cudalbu C et al, Proc Intl Soc Mag Reson Med 2012; [8] Henry PG et al, Magn Reson Med 2003; [9] Braissant O et al, J Inherit Metab Dis 2012 **Acknowledgements**. Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, the Leenaards and Jeantet Foundations; SNF grant 131087.

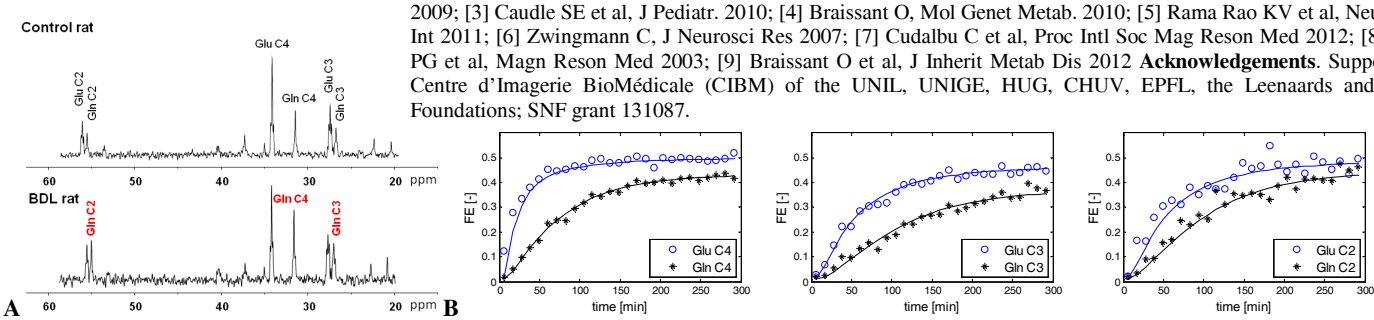

Fig. 1:A) *In vivo* ^{13}C brain spectra acquired in a rat model of CLD and a control at 9.4T at the end of the [1,6-^{13}C]glc infusion. The ^{13}C experiment was undertaken 8 weeks after BDL or sham ligation; B) C4, C3 and C2 uptake curves of glutamate and glutamine averaged over the BDL rat group and fitted with two-compartmental neuroglial metabolic modeling (solid lines).