Hyperpolarized [2-^{13}C]-D-fructose Uptake and Metabolism in Brain Tissue

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Introduction: Classical neuroenergetic models suggested that glucose is the exclusive fuel for the human brain. However, there is growing evidence that other carbon sources such as fructose may play a role in neuroenergetics as well [1]. Depending on tissue, D-fructose is metabolized through Fructose-1-phosphate, or Fructose-6-phosphate (F-6-P) metabolic pathway (Fig.1). However, Bergbauer et al. reported that in brain the F-6-P metabolic pathway is used exclusively [5]. Despite good evidence that fructose can cross the blood brain barrier, there is still much to be learned about this physiological process and the roles that fructose may play in the brain. The aim of the study was to find out if hyperpolarized [2-^{13}C]-D-fructose is able to pass across the blood brain barrier fast enough to enable the ^{13}CMMR measurement. Another goal was to observe [2-^{13}C]-D-fructose metabolism in vivo, and to find out if the method allows distinguishing the metabolites in D-fructose metabolic pathway.

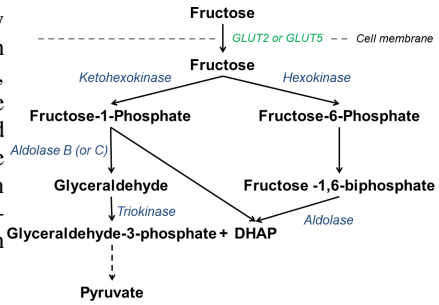

***Fig.1**: D-Fructose metabolism (enzymes are in blue and membrane transporters in green).*

Methods: [2-^{13}C]-D-fructose (EurIso-Top, Germany) was prepared as described by Keshari et al. [2]. The mixture was hyperpolarized using HyperSense DNP polarizer (Oxford Instruments, UK). The hyperpolarized probe was injected intravenously (injected dose 5 mL/kg) to healthy, fed Lewis rats. We performed two injection/measurements on each rat using GE Signa 3.0 T MR Scanner (GE Healthcare, USA), equipped with dual-tuned ^{1}H/^{13}C birdcage coil. First, a ^{13}C spectrum was acquired, and second chemical shift imaging using a spiral sequence with IDEAL encoding [3] was performed to measure the fructose distribution in brain, heart, liver, and kidney tissues. ^{1}H anatomical images (axial orientation) were obtained using a gradient echo sequence after the ^{13}C-hyperpolarization experiments. Data analysis and image reconstruction were done using MATLAB 2011b (Mathworks, USA) and jMRUI.

Results: We were able to hyperpolarize [2-^{13}C]-D-fructose up to a polarization level of 8.9±1.2% with T_1 in solution of 18.7±0.9 s, and observe a very clear signal in all of the tissues. This implies that D-fructose was able to cross blood brain barrier in sufficient amount and fast enough enabling the in-vivo measurement. In Fig. 3 we present the signal spectrum observed in slice containing the brain area. All three isomers of D-frucose have been observed: β-fructopyranose (100.8 ppm), β-fructofuranose (104.2 ppm), and α-fructofuranose (107.0 ppm); The ratio of the D-fructose isomers in brain slice was 13.8:5.2:1.

***Fig.2**: Localized signal of β-fructopyranose in rats brain 8, 12, 16, 31 and 35 s after injection.*

Discussion: As expected, the majority of the signal was detected in liver. However, even 35 s after the injection sufficient fructose signal was observed in the brain. Keshari et al. (2009) suggested that higher amount of β-fructopyranose (100.8 ppm) compared to β-fructofuranose (104.2 ppm) can be connected to fructose metabolism and/or higher uptake of exclusive β-fructopyranose isomer. If we consider this fact, there are areas in brain with more intensive signal of β-fructopyranose compared β-fructofuranose (Fig. 4C). We demonstrated successfully the polarization of [2-^{13}C]-D-fructose and in-vivo imaging. In conclusion we can say that D-fructose is a good candidate for functional imaging studies, and due to its non-toxicity to human it may be later applied in human subject examinations.

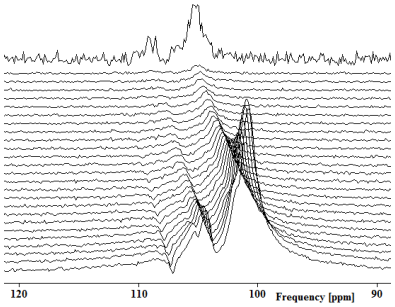

***Fig.3**: Spectrum in brain slice: β-fructo-pyranose (100.8 ppm), β-fructofuranose (104.2 ppm), and α-fructofuranose (107.0 ppm). Time gap 1.5 s. Last line represents the sum of the next 39 spectra.*

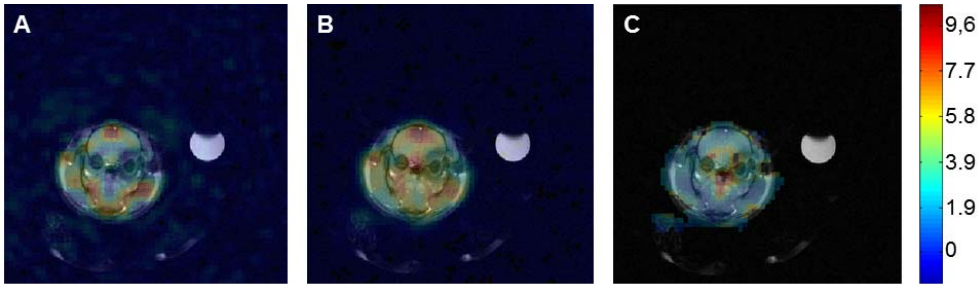

***Fig.4**: Spatial concentration of β-fructofuranose (A) to β-fructopyranose (B), and ratio β-fructopyranose: β-fructo-furanose in brain 8 s after injection (C).*

References: [1] Funari VA et al, Cerebellum. 2007;6(2):130-40. [2] Keshari KR, et al., J Am Chem Soc. 2009;131(48):17591-17596. [3] Wiesinger F, et al., Magn Reson Med. 2012;68(1):8-16. [4] Meier S, et al., Mol. BioSyst. 2011;7(10):2834-2836. [5] Bergbauer K, et al., Dev Neurosci. 1996;18:371–9.

Acknowledgements: Funded by BMBF13EZ114, and the European Social Fund. Project PO KL „Information technologies: Research and their interdisciplinary applications").