Radical-free mixture of co-polarized 13C-metabolites for probing separate biochemical pathways simultaneously in vivo by hyperpolarized 13C MR

Jessica AM Bastiaansen[1,2], Hikari AI Yoshihara[3,4], Andrea Capozzi[3], Juerg Schwitter[4], Matthew E Merritt[5], and Arnaud Comment[3]

[1]Department of Radiology, University Hospital (CHUV) and University of Lausanne (UNIL), Lausanne, Switzerland, [2]Center for Biomedical Imaging (CIBM), Lausanne, Switzerland, [3]Institute of Physics of Biological Systems, EPFL, Lausanne, Switzerland, [4]Division of Cardiology and Cardiac MR Center, University Hospital Lausanne (CHUV), Lausanne, Switzerland, [5]Advanced Imaging Research Center,Department of Radiology,Molecular Biophysics,Biomedical Engineering, University of Texas Southwestern Medical Center, Dallas, Texas, United States

INTRODUCTION: Advances in hyperpolarization enable the real time imaging of metabolism *in vivo*. The combination of hyperpolarized ^{13}C technology and co-administration of separate imaging agents allow simultaneous monitoring of both fatty acid and carbohydrate oxidation in the heart *in vivo* in a single experiment [1]. However, for the translation of hyperpolarized methods to the clinic, it is currently necessary to use ^{13}C preparations containing persistent radicals which require a time consuming filtration process before the injection. This, combined with additional pharmaceutical quality control tests on the solution, results in polarization losses, and loss of sensitivity of the experiment. Recent developments have shown that persistent-radical-free preparations of hyperpolarized pyruvic acid are possible, and applicable for metabolic studies *in vivo* [2]. In this study we aimed to extend the method to mixtures of ^{13}C-labeled endogenous metabolites, for the in vivo investigation of metabolic substrate competition.

METHODS: A solution of 50 uL [1-^{13}C]pyruvic acid and 50 uL of [1-^{13}C]butyric acid were mixed together and frozen into glassy beads in liquid nitrogen and then subjected to UV irradiation for 1 hr as described previously [2]. The beads were loaded in a 7T homebuilt polarizer, together with frozen NaOH for pH neutralization, and were dynamically hyperpolarized for 1 hr. After automatic dissolution and transfer to a separator infusion pump located inside the animal imager (9.4T, Varian/Magnet), 1.0 mL of a pH neutralized, hyperpolarized mixture of ^{13}C-labeled pyruvate and butyrate was injected into rats (male Sprague Dawley rats, n = 5). Cardiac triggered measurements were carried out on a 9.4 T imaging magnet (Varian/Magnex) using a ^{13}C surface coil, positioned over the chest, with 30° adiabatic RF pulses applied every 3 s with ^{1}H decoupling. NMR spectra were summed and fitted to Lorentzian lineshapes using Bayesian Analysis to obtain ratios of observed metabolites. Error bars indicate ± standard error of the mean.

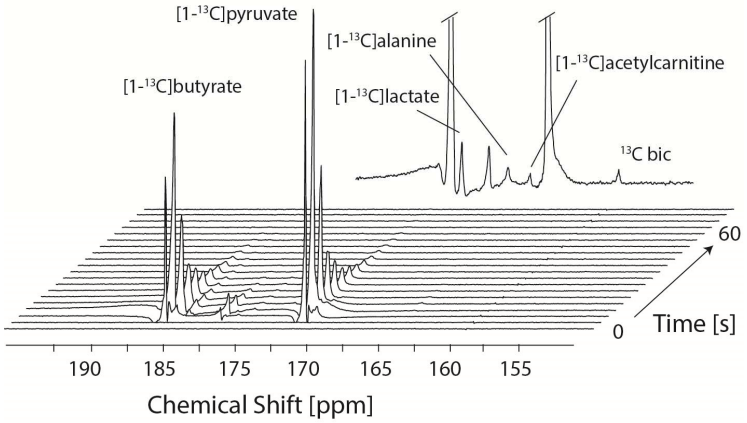

Fig 1. In vivo spectral time course of hyperpolarized 13C labeled pyruvate and butyrate in the heart. Polarization of both metabolites was achieved without the addition of a glassing agent or radicals. Downstream metabolites can be observed, using a substrate preparation free of radicals or glassy agent containing uniquely the 13C metabolite of interest. Different intensities of butyrate and pyruvate are substrate density related.

RESULTS AND DISCUSSION: Co-polarization of both ^{13}C labeled butyrate and pyruvate was achieved without the addition of radicals or glassing agents. In this case, the radicals created by UV irradiation of pyruvic acid act as polarizing agents not only for pyruvic acid itself, but also for the other admixed ^{13}C substrate, in the present case butyric acid. The metabolism of hyperpolarized pyruvate led to the detection of lactate, alanine, and ^{13}C bicarbonate. HP butyrate metabolism showed ^{13}C labeling in acetylcarnitine. The expected resonances of glutamate, β-hydroxybutyrate (BHB), citrate, acetoacetate, were absent in this study, most likely due to less than fully optimized conditions, and thus lower SNR. The relative signal ratios following the metabolism of this radical-free substrate preparation resulted in similar (bicarbonate) or higher values (alanine, lactate and acetylcarnitine) compared with previous reported data [1,3]

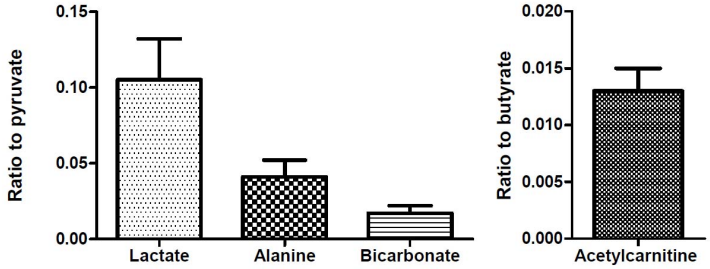

Fig 2. Relative ratios of observed metabolites in respect to the injected substrate signals. The left panel shows the relative signals of [1-^{13}C]lactate, [1-^{13}C]alanine and ^{13}C bicarbonate relative to pyruvate. The right panel shows the signal ratio of [1-^{13}C]acetylcarnitine relative to the injected substrate [1-^{13}C]butyrate.

CONCLUSION: Co-hyperpolarization of multiple ^{13}C-labeled metabolites is possible in UV-irradiated mixtures containing pyruvic acid. This enables noninvasive and simultaneous monitoring of separate metabolic pathways in a single experiment, without addition of persistent radicals or glassing solvents. This study illustrates a method for measuring substrate competition that could be generalized to humans. In addition, the removal of the need for filtering out free radicals could potentially shorten the time from dissolution to injection, increasing the signal-to-noise ratio in human studies.

References: [1] J. Bastiaansen *et al.,* Proc. Int. Soc. Mag. Res. Med. 22:987 (2014) [2] T. Eichorn *et al.*, PNAS 110: 18064-18069 (2014) [3] D. Ball *et al.,* MRM 71:1663-1669 (2014)