Hyperpolarized [1-13C]acetylcarnitine as a tracer for cardiac metabolism

Jessica A. M. Bastiaansen[1], Tian Cheng[1,2], Rolf Gruetter[1,3], and Arnaud Comment[2]
[1]*Laboratory of Functional and Metabolic Imaging, EPFL, Lausanne, Switzerland,* [2]*Institute of Physics of Biological Systems, EPFL, Lausanne, Switzerland,*
[3]*Department of Radiology, University of Lausanne and Geneva, Lausanne and Geneva, Switzerland*

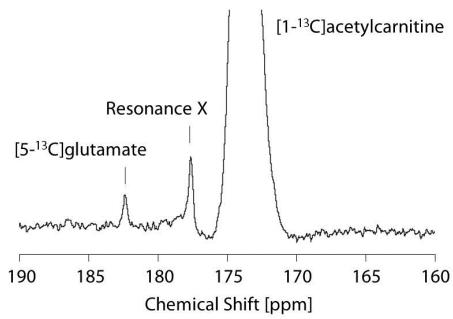
Introduction: Acetylcarnitine (ALCAR) is known to facilitate the influx and efflux of acetylgroups across the mitochondrial membrane and buffers an excess in acetylCoA in both cytosolic and mitochondrial compartments [1]. Acetylcarnitine is a necessary intermediate for mitochondrial acetate metabolism [2] and has been shown to easily cross the blood brain barrier and act as a neuroprotective agent [3] and used for Alzheimer's disease treatment in humans [4]. The advent of hyperpolarized MR allows for study of real-time metabolism and offers the possibility to determine the kinetics of specific biochemical transformations *in vivo* [5]. The conversion of hyperpolarized acetate into acetylcarnitine, acetylCoA [6] and citrate [7] has been probed *in vivo* in cardiac muscle and reflect several enzymatic activities. These downstream metabolites appear with a time delay and typically decay corresponding to their T_1. The aim of the present study was to explore the use of hyperpolarized acetylcarnitine as a tracer since it easily enters the mitochondrion and skips two cytosolic biochemical transformations needed for acetate to enter the mitochondrion. Secondly, the use of hyperpolarized acetylcarnitine could help elucidate the backflux through the reaction from acetylcarnitine to acetylCoA to describe the entire system of enzymatic reactions in more detail.

Materials and methods: Acetyl-1-^{13}C-L-carnitine hydrochloride (1.5M) solved in a 1:2 mixture of d_6-EtOD/D_2O containing 33mM TEMPO free radical were turned into frozen glassy beads and subsequently polarized in a custom-designed 5T DNP polarizer for 2 h [8]. After dissolution, the samples were transferred within 2 s into a home-built infusion pump located inside the magnet bore (9.4T) and connected to a catheter inserted into a D_2O filled phantom or the rat femoral vein (n=3). The 5 s long infusion of 1 mL hyperpolarized ALCAR solution was triggered 1 s later. The whole procedure was computer controlled in order to obtain an identical timing for all experiments. At the end of the *in vivo* acquisition, the residual solution in the infusion pump was extracted and measured in a high-resolution 400 MHz spectrometer (Bruker) to determine the acetate concentration. Series of single pulse acquisitions were recorded using 10° (*in vitro*) or 30° (*in vivo*) adiabatic RF pulses applied every 1 or 3 s starting at the end of the infusion with a homebuilt surface coil with ^{13}C in quadrature mode. The *in vivo* acquisitions were respiratory gated and cardiac triggered. Spectra were analysed in jMRUI and Matlab.

Results and discussion: The T_1 of [1-^{13}C]acetylcarnitine was found to be 15 s in blood at physiological temperature and around 16 s in D_2O (table 1). [1-^{13}C]acetylcarnitine was dynamically polarized with a buildup constant of 1390 s. After dissolution in a D_2O loaded phantom and data acquisition, the hyperpolarized time integrals were corrected for repeated adiabatic 10° excitations and the decay constant as a result of T_1 was 14.7 ± 1.0 s. *In vivo* experiments: A volume of 1 mL 100 mM [1-^{13}C]acetylcarnitine was infused in healthy rats and the time evolution of substrate and two metabolic products (figure 1) were measured in 3 animals. The linewidth of acetylcarnitine observed at 173.9 ppm was between 25 and 30 Hz. As expected given the relative short relaxation time the acetylcarnitine signal could be observed during 20 to 40 seconds exhibiting a mono-exponential decay of around 3 s regardless of the TR. The resonance of [5-^{13}C]glutamate appeared at 182.4 ppm, which cannot be observed using hyperpolarized acetate as a tracer because their resonances overlap (182.6 ppm vs. 182.4 ppm). Surprisingly [5-^{13}C]citrate, resonating at 179.7 ppm was not observed in these experiments but another, yet unknown metabolite resonance was observed at 177.7 ppm. High res NMR on the infused solution did not reveal any impurities. We have not been able to assign this resonance but a possible candidates are [5-^{13}C]glutamine or glutathione.

Conclusion: Despite its short T_1 it was possible to detect the formation of glutamate from hyperpolarized [1-^{13}C]ALCAR. An additional metabolite was detected which does not correspond to cardiac metabolites observed following a hyperpolarized [1-^{13}C]acetate injection. This study highlights the intricate relationship between reaction, transport and relaxation rates in the choice of hyperpolarized substrates.

References: [1] F. Stephens *et al*., J. Physiol. (2007) [2] P. Roberts *et al.*, Am J Physiol-Endoc (2005) [3] S. Scafidi *et al.*, J Neurochem (2010) [4] L. Parnetti *et al.*, Eur J Clin Pharmacol (1993) [5] J.H. Ardenkjaer-Larsen *et al.* , PNAS (2003) [6] P. Jensen *et al.*, *JBC* (2010) [7] J.Bastiaansen *et al.*, Proc ISMRM 5317 (2012) [8] A. Comment *et al.*, Concept Magn. Reson. (2007).
Acknowledgements: This work was supported by the Swiss National Science Foundation (grants 131087, #PP00P2_1335621 and 133562), the National Competence Center in Biomedical Imaging (NCCBI), the CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL, and the Leenaards and Jeantet Foundations.



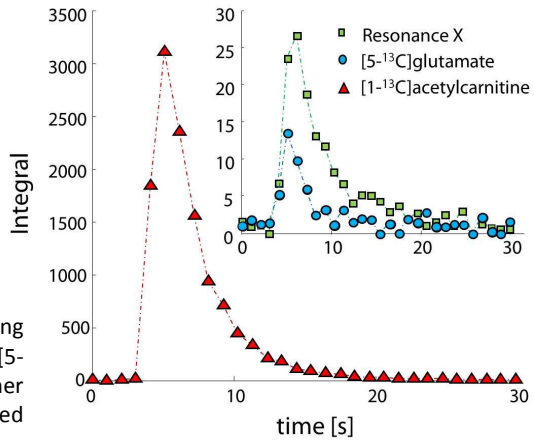


Fig. 1: *In vivo* cardiac ^{13}C NMR spectrum at t = 6 s following the infusion of hyperpolarized [1-^{13}C]ALCAR. [5-^{13}C]glutamate is observed at 182.4 ppm and another resonance at 177.7 ppm. The right panel shows a integrated spectral time course of an experiment with TR = 1 s

Solvent	T_1 in [s]
Plasma T = 310 K	14.9 ± 0.7
D_2O T = 295 K	10.3 ± 0.8
D_2O T = 305 K	14.3 ± 0.5
D_2O T = 310 K	15.5 ± 0.5

Table 1. Longitudinal relaxation times of [1-^{13}C]ALCAR in D_2O and plasma.