# Measurement of apparent diffusion coefficients of hyperpolarized $^{13}$C-metabolites in-vivo

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## Introduction:

Hyperpolarized $^{13}$C labeled agents such as [1-$^{13}$C]pyruvate allow for studies of metabolism in real-time by in-vivo MR spectroscopy. Even low metabolite concentrations can be detected through the more than 10000-fold signal amplification of hyperpolarized spins by dissolution DNP (1). Monitoring the conversion of metabolites in tumors, e.g. pyruvate-to-lactate exchange, has the potential to detect early responses-to-treatment. However, the exchange rate depends on various factors like cellular uptake, enzyme activity, intracellular pool sizes, as well as perfusion, which cannot be separated in standard NMR measurements. Here, we suggest a new approach to characterize the metabolism by performing apparent diffusion coefficient (ADC) measurements of hyperpolarized metabolites in-vivo. The diffusion properties of the individual metabolites adds to the information obtained from exchange kinetics and the two methods can in principle be combined as demonstrated in vitro (2).

## Methods:

We designed a diffusion-weighted pulse sequence based on the pulsed gradient spin echo (PGSE) sequence using low flip angle ($\alpha = 20^\circ$) slice selective excitation (Fig. 1 A). A universal-rotation pulse optimized with optimal control theory (S-BURBOP-20%) (3) was implemented for the 180° refocusing pulse. The 9.1 ms long pulse is robust to 20 % $B_1$ imperfections and shows a stable refocusing and inversion profile over a bandwidth of 2 kHz. We used diffusion time $\Delta = 20$ ms, gradient time $\delta = 8$ ms, and rectangular gradient pulses with amplitudes up to g = 0.5 T/m in x-,y-, and z-direction. The sequence was repeated (TR = 200 ms) in a loop structure starting with a reference scan for b = 0 and then continuing with large b values in all three gradient directions (Fig. 1 B). The signal equation is given by the *Stejskal-Tanner* expression modified by an $T_{1,eff}$ decay, consisting of $T_1$ and loss of $M_z$ due to repeated excitations:

$$I_g = I_0 \times exp\left(\frac{-t}{T_{1,eff}}\right) \times exp(-bD) = I_0 \times exp\left(\frac{-t}{T_{1,eff}}\right) \times exp\left(-\left(\Delta - \frac{\delta}{3}\right)(\gamma\, g\delta)^2\, D\right)$$

An exemplary dataset from a diffusion experiment is shown in Fig. 1 C. For post-processing, a correction based on $T_{1,eff}$ was performed before data were fitted to the signal equation assuming a mono-exponential damping term (Fig. 2 A,B,C). Two rats were scanned using this protocol in a preclinical 4.7 T MR scanner (Agilent, USA), equipped with a 4-channel $^{13}$C receive array and a single volume transmit coil ($\gamma B_{1,max}$ = 714 Hz) (RAPID Biomedical, Germany). A 20 mm axial slice covering the animal's thigh muscle was chosen. Metabolite concentration maps were acquired with a FID-CSI sequence with spiral ordering (see Fig. 3, shown for coil element 1). [1-$^{13}$C]pyruvate was hyperpolarized using a HyperSense DNP polarizer (Oxford Instruments, UK) and 1.5 ml was injected into a tail vein over ~15 s followed by data acquisition 20 s later.

## Results and Discussion:

The new diffusion pulse sequence was tested in a reference phantom with two syringes, containing $^{13}$C-urea and $^{13}$C-glycine respectively. Shifting the transmitter offset to 500 Hz and 1000 Hz from resonance yielded equivalent ADC values (data not shown) proving the robustness of the sequence. The S-BURBOP pulse was shown to be useful because of its universal rotation property, broad bandwidth, frequency-selectivity, and $B_1$ robustness. In rats, we acquired $^{13}$C-metabolite ADCs in 3 spatial directions from lactate, alanine and pyruvate in muscle tissue at 4 different positions corresponding to the respective coil element. Quantitative ADC values are shown for all coil elements in Fig. 2. Lactate and alanine ADCs were lower compared to pyruvate ADC, indicating a more confined diffusion space consistent with the presence of the two metabolites in the intracellular compartment. In conclusion, we have demonstrated for the first time, that in-vivo measurement of $^{13}$C metabolite ADCs is possible using hyperpolarized nuclei. The analysis of diffusion properties of intracellularly produced hyperpolarized compounds might potentially be useful for characterizing pathological changes in tumor cells, e.g. if the cellular compartments and diffusion properties change due to breakdown of the plasma membrane.

**References:** (1) Ardenkjaer-Larsen, J. H. et al, PNAS 100:10158(2003)**;** (2) Schilling, F. et al. Proc. ISMRM 20(2012); (3) Janich, M. A. et al., J. Magn. Res. 213:126(2011)

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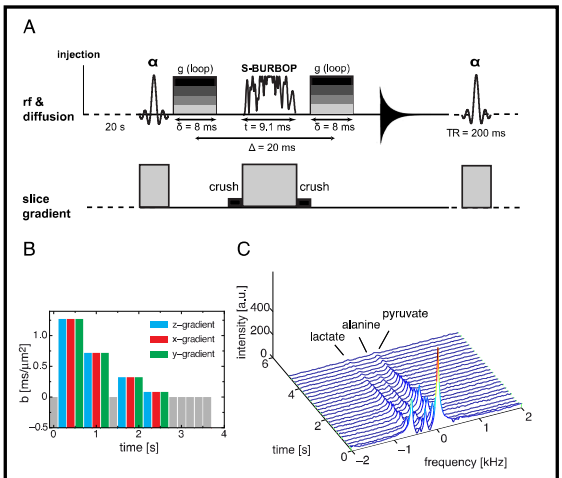


**Fig. 1**: (A) Pulse sequence for measurement of hyperpolarized $^{13}$C-metabolite ADCs in-vivo. (B) Gradient scheme for diffusion encoding in 3 orthogonal directions (C) Exemplary in-vivo diffusion dataset in muscle tissue acquired 20 s after injection of pyruvate.

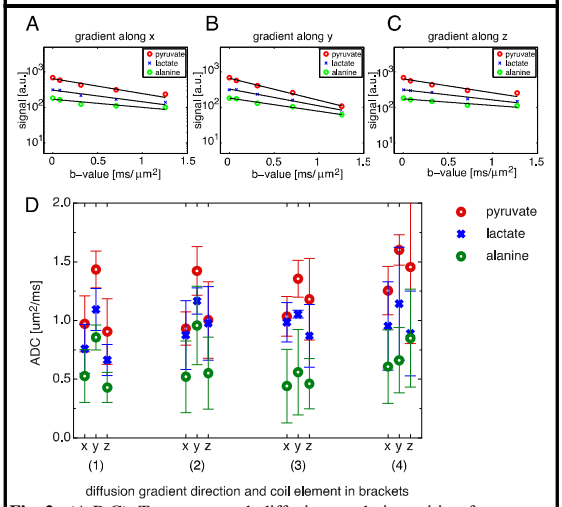


**Fig. 2**: (A,B,C) $T_{1,eff}$ corrected diffusion peak intensities for x-,y-,z-gradient direction and mono-exponential fits ($R^2$>0.95) for coil element 1. (D) Quantitative ADC values for all four coil elements.

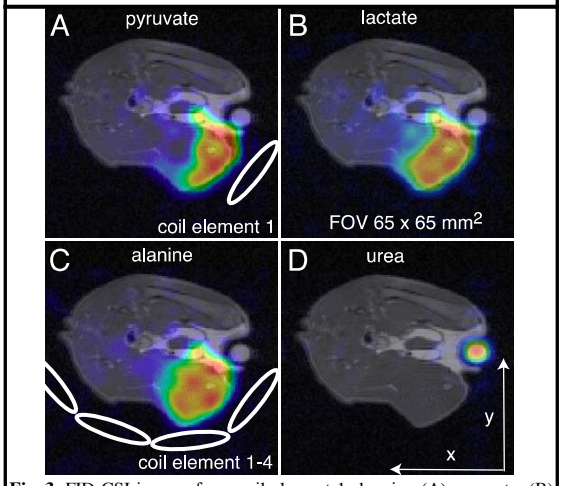


**Fig. 3**: FID-CSI images from coil element 1 showing (A) pyruvate, (B) lactate, (C) alanine distribution, and (D) a urea phantom. In (A) and (C) the coil positions are depicted. (D) shows the gradient directions.