# In vivo imaging and metabolism of hyperpolarized $^{13}$C diethyl succinate in mice

**N. Zacharias[1,2], N. Sailasuta[3], H. Chan[3], M. Wei[3], R. W. Grubbs[1], B. D. Ross[3], and P. Bhattacharya[3]**
[1]California Institute of Technology, Pasadena, CA, United States, [2]Enhanced Magnetic Resonance Laboratory, Huntington Medical Research Institutes, Pasadena, CA, United States, [3]Enhanced Megnetic Resonance Laboratory, Huntington Medical Research Institutes

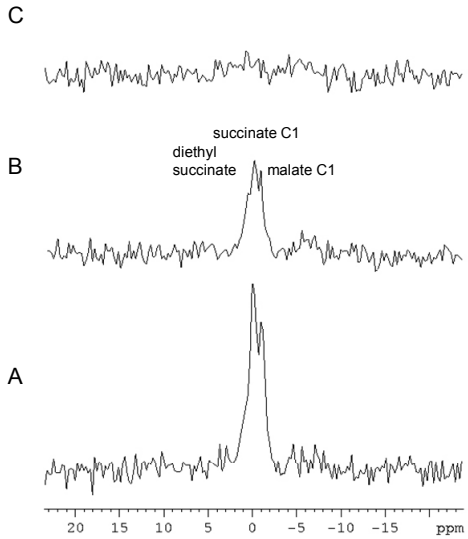
Purpose: The objective of this work is to illustrate the utility of hyperpolarized diethyl succinate for $^{13}$C MRI and MRS in normal and tumor bearing mice for *in vivo* real time metabolic imaging.

Background: Parahydrogen Induced Polarization (PHIP) can offer a 50,000 fold increase in signal strength under the right conditions. We have used PHIP hyperpolarized succinate, 2-hydroxy ethyl propionate, and 2,2,3,3-tetrafluoropropy-1-$^{13}$C propionate-$d_{2,2,3,3}$ (TFPP) for *in vivo* applications. However, all of these molecules have physiological barriers to being used in the clinic. For $^{13}$C succinate, the polarization transfer has to be done at acidic pH ≤ 3 or alkaline pH ≥ 9 for optimum hyperpolarization and there is limited transport of the charged succinate molecule across biological membranes and the blood brain barrier.[1,2] 2-hydroxy ethyl propionate is toxic and is not metabolized.[1] TFPP is not very water soluble and has to be injected in a 20% ethanol aqueous solution.[4] We have recently hyperpolarized diethyl succinate using PHIP. Diethyl succinate is water soluble, can be hyperpolarized at neutral pH, is known to be nontoxic, to cross biological membranes, and to be metabolized by cells using the TCA cycle.[5,6] We have preliminary data that illustrates the utility of diethyl succinate *in vivo* for real time metabolic imaging.

Methods & Results: Imaging and spectroscopy was done using 4.7T Bruker scanner and dual tuned volume coil for mice (Doty SC). Imaging protocol included ultrafast $^{13}$C FISP (fast imaging with steady state precession) 2D imaging followed by a single pulse (90$^o$ flip angle) $^{13}$C spectroscopy. In another protocol, single pulse (90$^o$ flip angle) $^{13}$C spectroscopy was performed and repeated thrice at an interval of 5 seconds. 12.5 mM aqueous solution of carbon-13 and deuterium labeled diethyl fumarate was hydrogenated to diethyl 3-$^{13}$C 4,5-$d_2$ succinate which was hyperpolarized by PHIP to P=3% before tail-vein injection into normal mice. $^{13}$C imaging or spectroscopy was then performed immediately after injection.

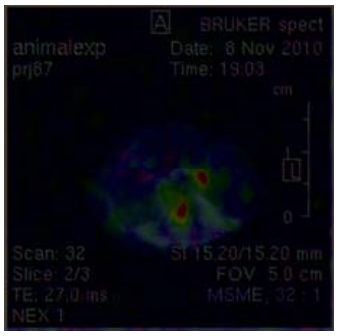
Our preliminary data reveals that diethyl succinate is taken up in normal mice and is metabolized rapidly (See Figure A). Based on the available data, diethyl succinate is being taken up by cells very quickly (<20s). The rapid uptake of diethyl succinate as compared to succinate is possibly due to the ester group which allows the diethyl succinate to pass through cell membranes more rapidly has described in literature.[7,8] Presently, we are determining the differences in imaging and metabolism of hyperpolarized diethyl succinate in normal mice compared to tumor bearing mice using low flip angle $^{13}$C MRS and CSI.

**A. *In vivo* metabolism of hyperpolarized diethyl succinate**




Three $^{13}$C spectra (single pulse, 90$^o$ pulse angle) from hyperpolarized diethyl succinate injected into a mouse. Spectra A, B, C taken 5 seconds after one another consectively. Resonances for diethyl succinate (large peak) and succinate are easily seen in scan A. Resonances for diethyl succinate, succinate, and malate C1 (TCA cycle metabolite) are seen in scan B

**B. $^{13}$C imaging with hyperpolarized diethyl succinate**




Superimposed $^{13}$C image of hyperpolarized diethyl succinate (red) and $^1$H image (light blue) of a mouse. $^{13}$C imaging was done with a FISP sequence and $^1$H imaging with MSME using identical FOV, central slice, voxel placement, slice thickness, and interslice distance.

Conclusions: Hyperpolarized diethyl succinate can be used for $^{13}$C imaging and metabolic studies. Diethyl succinate is quickly taken up by cells and metabolized, is water soluble, can be hyperpolarized at neutral pH, and is known to be nontoxic. It is a good candidate for future *in vivo* metabolic imaging in humans.

References:
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Acknowledgements: NZ would like to thank the James G. Boswell Foundation for their support.