# Imaging TCA Cycle Metabolism by PHIP Hyperpolarization of $^{13}$C Succinate *In Vivo*

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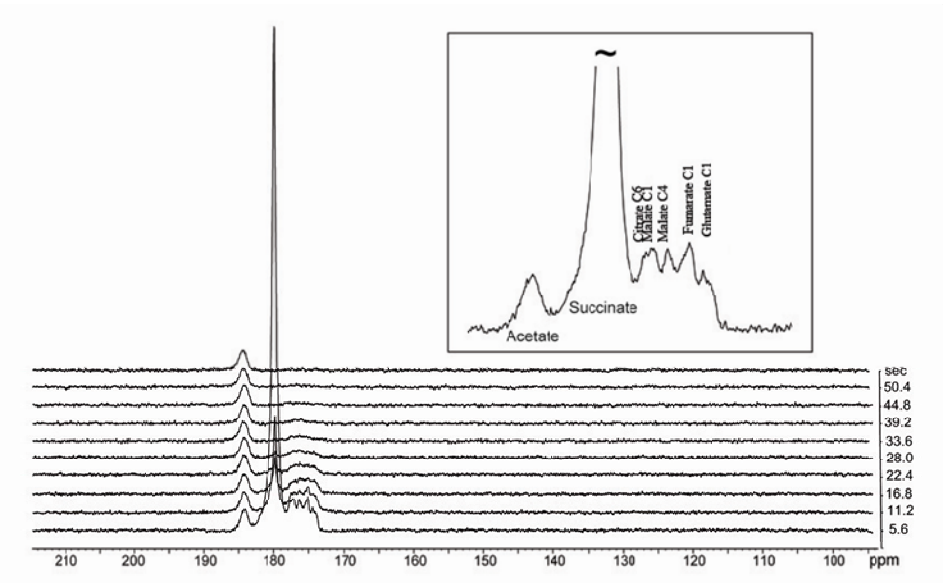
Purpose: The objective of this work was to overcome inherent insensitivity of *in vivo* MR by demonstrating metabolic imaging of reactions of the Krebs Tricarboxylic Acid Cycle *in vivo* using PHIP (Parahydrogen Induced Imaging) method of hyperpolarization [1,2]. While DNP with pyruvate demonstrated early on the potential for visualizing several sequential steps of metabolism with retention of hyperpolarized spins, to the best of our knowledge, because no 'metabolizable' molecule was available, TCA cycle metabolic imaging has never been observed with the alternative technology of PHIP.

Methods: $^{13}$C deuterated fumarate was hydrogenated by parahydrogen and rhodium-catalysis to 1-$^{13}$C succinate and hyperpolarized to 8±2% before tail-vein injection into tumor-bearing mice. $^{13}$C hyperpolarized signals were recorded at 3 second intervals over 120 seconds. A second injection was performed to permit high speed $^{13}$C *in vivo* imaging of the anatomic distribution of all hyperpolarized signal with reference to the underlying implanted tumor. 30 studies were performed in each of two (N=15) distinct tumor models, the renal cancer (RENCA) and a Lymphoma A20 with the goal of enhancing the likelihood of detecting onward hyperpolarized metabolism of succinate.

A. RENCA: renal cancer




B. Lymphoma A20

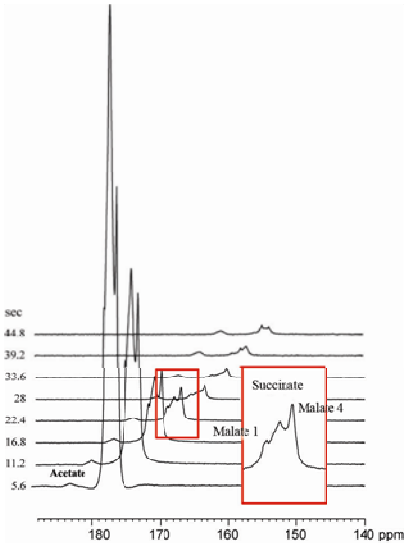



**Figure A**: Metabolic products of hyperpolarized $^{13}$C-succinate in RENCA tumor in a living mouse using MRS. 3M non-polarized $^{13}$C-acetate phantom was used as a chemical shift reference. **B:** Metabolic products from hyperpolarized 1-$^{13}$C succinate in Lymphoma A20 tumor bearing mice.

Results: After injection of hyperpolarized 1-$^{13}$C succinate, the substrate was observed in all spectra acquired during the putative $^{13}$C $T_1$ of hyperpolarized succinate. Whereas in prior studies with PHIP no hyperpolarized metabolites were observed, hyperpolarized metabolic products were detected with 20,000 fold increased sensitivity in all 30 of the *in vivo* trials reported here, with secondary hyperpolarized metabolites observed over 3 – 5 minutes (Figure). $^{13}$C images produced during the $T_1$ of hyperpolarized succinate and/or its metabolites showed restricted distribution of intravenously delivered succinate within the implanted tumors, and occasionally beyond their anatomic boundaries as defined by synchronous $^{1}$H MRI. The metabolic fate of hyperpolarized $^{13}$C succinate differed in the two tumor populations (N=15): in RENCA renal carcinoma metabolic products malate C1 and C4, fumarate C1, glutamate C1 and citrate C6 were defined; and in Lymphoma A20 the hyperpolarized metabolic products were limited to $^{13}$C malate C1 and C4 (Figure). The differences in metabolic profile were tentatively assigned to the presence of hypoxia inducing factor HIF1α in Lymphoma A20 which is absent from RENCA. Finally, since hyperpolarized $^{13}$C succinate chemical shift is pH sensitive, some function of intra-tumor pH may be recovered from these data.

Conclusion: Hyperpolarized MR effectively images $^{13}$C intermediates of the Krebs TCA cycle using PHIP with potential for preclinical and clinical application in cancer molecular imaging. In a prior report from this Laboratory, metabolites were observed in *in vivo* brain tumor – but only after they had returned to Boltzmann equilibrium [3].

References:
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3. Chekmenev EY et al. PASADENA Hyperpolarization of Succinic Acid for MRI and NMR. J Am Chem Soc 2008; 130:212-4213.