Quantitative Biomarkers of Cancer from Metabolic Activity Decomposition using Stimulated-Echoes and Hyperpolarized Carbon-13 MR

Christine M Leon[1,2], Peder EZ Larson[1], Adam B Kerr[3], Robert Bok[1], John M Pauly[3], John Kurhanewicz[1], and Daniel B Vigneron[1]

[1]*Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, CA, United States,* [2]*UC Berkeley | UCSF Graduate Group in Bioengineering, University of California, Berkeley and University of California, San Francisco, San Francisco, CA, United States,* [3]*Department of Electrical Engineering, Stanford University, Stanford, CA, United States*

Introduction

Changes in enzyme expression precede tumorigenesis providing early biomarkers of disease[1]. Specifically, it is well known that the lactate dehydrogenase (LDH) enzyme is significantly upregulated in cancers[2]. It has been shown with NMR that HP ^{13}C substrates allow for observation of isotope flux through a single enzyme-catalyzed step[3]. *In vivo* the acquired signal is complicated by flow, perfusion, diffusion, membrane transport and pool size, in addition, to metabolism[4]. In this study, we applied the stimulated echo acquisition mode (STEAM) technique to suppress flow, perfusion and diffusion providing improved metabolic contrast without vascular effects[4]. Because the signal intensity of pyruvate and lactate in hyperpolarized (HP) Carbon-13 experiments is extremely sensitive the timing of acquisition, recent work has been directed toward acquisition of dynamic spectra[5]. We propose a new, robust method for quantification of dynamic data. Using Metabolic Activity Decomposition (MAD-STEAM)[4], we investigated real-time metabolic conversion parameters as kinetic biomarkers of cancer progression and treatment response.

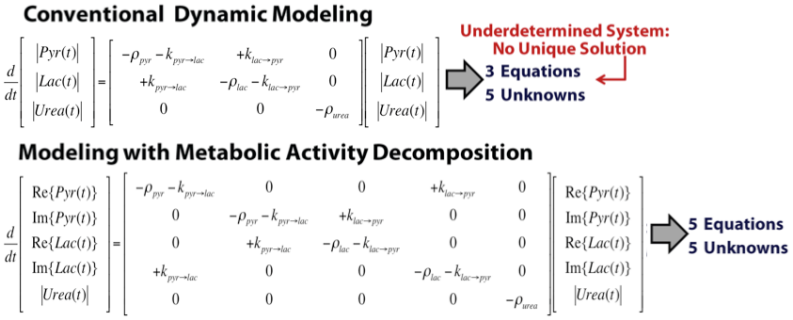

Figure 1: Comparision of conventional modeling versus Metabolic Acitivity Decomposition.

Methods and Modeling

STEAM in the presence of metabolic conversion creates a phase shift that depends on the resonance frequency and echo time (TE), $\Delta\varphi=2\pi\Delta f TE/2$[7]. By choosing $\Delta\varphi=\pi/2$, the spins generated during a mixing time (TM), can then be separated during reconstruction. ^{13}C-urea was used as a phase reference to correct shifts from homogeneous, bulk motion[4]. All data was acquired with TE=14ms, $\Delta\varphi_{Pyr\rightarrow Lac}=\pi/2$, TMs=1sec, 10x, slab 20 cm, progressive flip angle, adiabatic double spin echo. Dynamic spectra were obtained *ex vivo* from a phantom containing 6µL of HP [1-^{13}C]-pyruvate (2.84mM), LDH(2x excess), NADH (4x excess), and 15µL of HP ^{13}C-urea in PBS and in TRAMP tumor models (n=5) and normal (n=5) mice.

Conventional modeling using only the magnitude of the data yields four unknowns but only two equations; an underdetermined system of equations with no unique solution necessitating external measurements or assumptions such as $T_{1,Lac}=T_{1,Pyr}$ or $K_{Lac\rightarrow Pyr}=0$[6] (Fig 1). Using Metabolic Activity Decomposition, twice the amount of information can be obtained from a same acquisition providing a well-conditioned system of equations.

Conventional modeling underestimates the forward conversion rate $K_{Pyr\rightarrow Lac}$, compensating with inaccurate relaxation rates, which do not describe the actual kinetics of the system (Fig 2). However, Metabolic Activity Decomposition reconstruction of the same data provided a well-conditioned system of equations with single unique solutions yielding accurate rates of conversion.

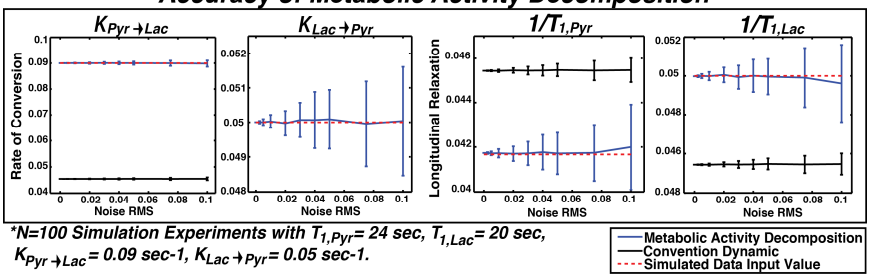

Figure 2: Comparision of stability of conventional modeling versus Metabolic Acitivity Decomposition modeling with increasing noise amplitudes.

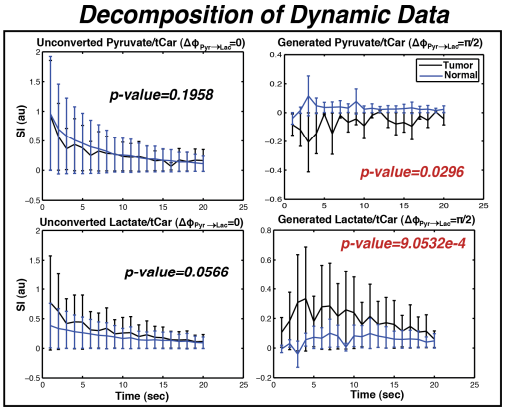

Figure 3: Dynamic curves from Metabolic Acitivty Decomposition showing increased lactate and decreased pyruvate generation normalized to total carbon in tumor versus normal tissue. P-values from Repeated Measures ANOVA (unpaired, n=5, $\alpha=0.05$).

Results and Discussion

In *ex vivo* LDH experiments, generation of phase shifted lactate signal demonstrated direct observation real-time conversion which can only be due to the LDH enzyme. Similar results were observed *in vivo* in a tumor, validating the method (Data not shown). Moreover, MAD-STEAM was able to differentiate generated lactate and pyruvate (Fig 3) from high rates of conversion of pyruvate to lactate in a tumor model, which is known to have high LDH activity. Simulations showed that Metabolic Activity Decomposition more accurately calculates relaxation and conversion rates (Fig 2). Moreover, fitting *in vivo* data with MAD-STEAM yielded $K_{Pyr\rightarrow Lac}$ values, which were better able to distinguish tumor versus normal, than conventional modeling (Fig 4).

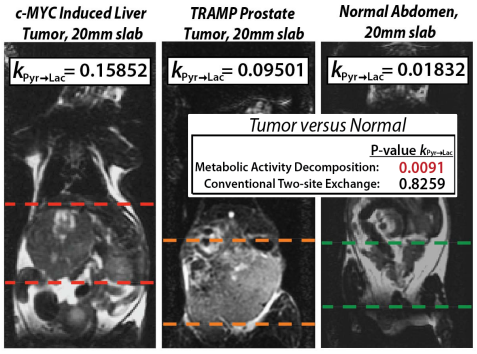

Figure 4: Rates of conversion from Metabolic Activity Decomposition and Conventional Exchange from dynamic spectra in tumor and normal tissue. P-values from two-sided unpaired t-test ($\alpha=0.05$, n=5).

Conclusion

MAD-STEAM improved real-time conversion and T_1 relaxation measurement and removes confounding factors for better observation of enzyme activity. The ability to probe enzyme activity directly and measure relaxation provides new quantitative biomarkers, which could improve assessments of early tumor formation and regression with increased specificity.

References: [1] Hu, et al. Cell Metab. 2011; 14:131-142. [2] Kroemer and Pouyssegur. Cancer Cell; 2008, 13(6): 472-382. [3] Merritt. P Natl Acad Sci USA 104, 19773-19777 (2007). [4] Larson et al. IEE Trans Med Imaging, 2011; *in press*. [5] Kurhanewicz, et al. Neoplasia. 2011; 13(2): 81-97. [6] Day. Nature Med 13, 1382-1387 (2007).

Acknowledgments: We would like to acknowledge Galen Reed, Simon Hu, Michael Ohliger, Cornelius Von Morze, Hikari Yoshihara, Peter Shin and Kristen Scott for technical assistance, as well as, funding from NIH P41-EB01359, NIGMS-IMSD R25-GM56847 and NIH R00-EB012064.