Simultaneous multicompound hyperpolarization by DNP

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INTRODUCTION: Recent studies of hyperpolarized ^{13}C labeled compounds, specifically $^{13}C_1$-pyruvate, have been used to investigate metabolic processes associated with the Warburg effect [1,2]. These methods probe one specific pathway, the last step of glycolysis in which pyruvate is enzymatically converted to a number of products. Each of these products is indicative of the flux through the enzyme of choice, for example the production of lactate demonstrates LDH flux as a result. Though this information is relevant in itself, it is possible to investigate multiple pathways simultaneously by polarizing and injecting multiple hyperpolarized substrates at the same time. The purpose of this study is to demonstrate a methodology for hyperpolarizing multiple compounds simultaneously and its application to a bioreactor system showing the simultaneous metabolism of the hyperpolarized substrates.

METHODS: $^{13}C_1$-pyruvic acid (14.2M), acetic acid (10.2M), aspartic acid (2.6M), sodium bicarbonate (1.9M), glycine (4.0M), β-hdyroxybutyrate (4.0M) were prepared in various concentration of glycerol and DMSO. They were hyperpolarized separately and together using the Hypersense (Oxford Instruments) at a frequency of 94.080 Ghz as described previously [1] and 1mL of the multipolarization was injected into a custom designed 10mm NMR compatible bioreactor flow system or 2mL into a 10mm NMR tube for solution studies. For multipolarizations, each compound was prepared separately and then frozen together in the sample cup submerged in liquid N_2. 2 multipolarizations were conducted, first pyruvate was polarized with sodium bicarbonate (resulting in final concentrations of 2.5 mM and 20mM respectively), and then pyruvate, acetate, aspartate, beta-hydroxybutyrate and glycine were simultaneously polarized (at final concentrations of 1.3mM, 3.6mM, 23.1mM, 2.6mM, and 3.0mM respectively). The first multipolarization was done in the bioreactor, a completely contained 3D culture system with a continuous flow of 37°C media (containing RPMI, 10% FBS, and Penn/Strep) heated by water-jacketed inlet lines. Prior to entering the bioreactor, media is oxygenated using a Gas Exchange Module (GEM), filled with 95% Air/5% CO_2, to preserve physiologic conditions [3]. At the time of injection, the flow system was set to flow 4mL/min. The bioreactor contained either JM1 (rat hepatoma) cells electrostatically encapsulated in 500μm alignate beads, with a total cell concentration of 5 x 10^7 cells/mL. All ^{13}C NMR spectra were acquired in intervals of 3 sec using a 5° pulse for 300 secs on a narrow-bore 11.7T Varian INOVA (125MHz ^{13}C, Varian Instruments) equipped with a 10mm triple tune direct detect broadband probe. Prior to injection of the hyperpolarized compounds, a time course of ^{31}P spectra (202MHz ^{31}P) was acquired with a 90° pulse, nt=1024, and at=1s to assess the βNTP resonance as a function of time and infer cell health. pH measurements as a result of bicarbonate/CO_2 equilibrium were calculated using the Hendersen-Haselbach equation for a temperature of 37°C.

Fig. 1

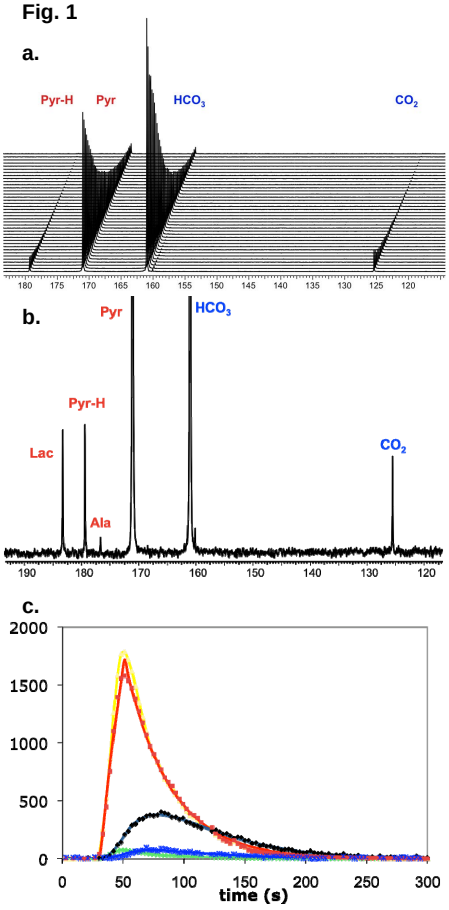

Figure 1. Copolarization of pyruvate and sodium bicarbonate injected into a 10mm NMR tube (a), 90s post injection into a bioreactor containing JM1 cells (b), and the dynamic measurement over time in the bioreactor (c). Yellow – bicarbonate, Red – pyruvate, Black – lactate x 10, blue - alanine x 10, and green – CO_2

RESULTS: The first series of studies included the copolarization of pyruvate and sodium bicarbonate. As shown in Figure 1a, the solution polarization of pyruvate and sodium bicarbonate yield highly resolved signals with good SNR. This solution was then injected into a bioreactor system containing JM1 cells in the log phase of growth. Figure 1b shows a scan at 90s post injection of the copolarization. The characteristic metabolism of pyruvate to lactate and alanine is shown as well as the resonances of bicarbonate and CO_2. Flux through LDH and ALT was not significantly different than injections of hyperpolarized pyruvate alone (12 nmols/s/10^8 cells and 2.5 nmols/s/10^8 cells respectively). The calculated pH was 7.54 using the integrals of the bicarbonate and CO_2 resonances, which was the same as the pH of the media. The second multipolarization explored was that of pyruvate, acetate, aspartate, glycine and beta-hydroxybutyrate. Figure 2 demonstrates the decay of the simultaneous polarization of all five compounds. The decay of signal is governed by the T_1 relaxation time of the different spin systems and is tabulated in Table 1 as well as the signal enhancements relative to equilibrium measurements. The T_1 of acetate and pyruvate appeared to be slightly longer, although the other metabolites were unchanged. The relative enhancements for most metabolites decreased relative to the polarization of the compounds alone, although acetic acid's polarization did not change significantly. Additionally, substantial enhancements were obtained for all of the hyperpolarized metabolic substrates (list the polarizations obtained for each compound of the mixture.)

Fig. 2

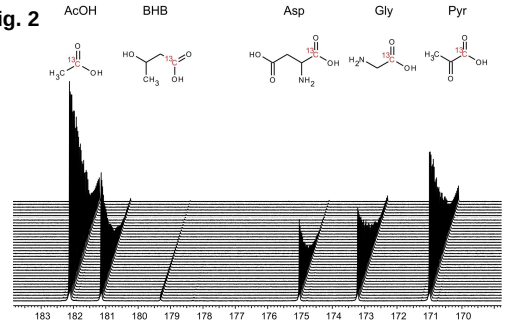

Table 1

	T1 (s) % Change	Enhancement (% Polarization)
Acetic Acid	57 (7%)	15892 (15.3%)
Aspartic Acid	30 (0%)	1043 (1.0%)
Glycine	48 (0%)	10921 (10.5%)
BHB	35 (0%)	9907 (9.5%)
Pyruvic Acid	60 (5%)	13669 (13.2)

DISCUSSION AND CONCLUSIONS: This study demonstrates, for the first time, the simulataneous hyperpolarization of multiple compounds as well as their injection into an NMR compatible 3D culture system. While there was some loss of hyperpolarization of individual compounds in the mixture relative to polarizing them alone, there remained substantial enhancements. Injections of combinations of compounds demonstrated several metabolic fluxes that were not different from that observed for the fluxes when the hyperpolarized substrates were injected individually. These data demonstrate the feasibility of probing multiple metabolic after one injection of multiple hyperpolarized metabolic substrates.

REFERENCES:

[1] Ardenkjaer-Larsen et al. PNAS 2003;100(18):10158-63 **[2]** Albers, MJ et al. Cancer Res.68(20):8607-15 **[3]** Keshari KR et al. (2008) ISMRM

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