Differentiation of Flux and Isotopic Exchange using Co-administration of Hyperpolarized [2-13C]Pyr and [1-13C]Lac

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Introduction: Hyperpolarized ^{13}C magnetic resonance spectroscopy (MRS), using Dynamic Nuclear Polarization (DNP) in combination with a rapid dissolution process to retain high levels of nuclear spin polarization in the liquid state, enables the real-time investigation of *in vivo* metabolism [1]. Using [1-^{13}C]pyruvate (Pyr), a substrate occupying a key nodal point in the glucose metabolic pathway, allows one to quantitatively follow its *in vivo* conversion to lactate (Lac), alanine (Ala), or acetyl CoA. Pyr to Lac conversion, in particular, has been widely investigated due to its correlation with disease models, such as cancer [2]. However, because the conversion of Pyr to Lac is reversible, it is difficult to differentiate metabolic flux of Pyr to Lac from isotopic exchange. Moreover, it has been shown that increasing the pool size of unlabeled Lac increases the isotopic exchange and, thus, improves [1-^{13}C]Lac detection [3]. Recently, Wilson et al. demonstrated a co-polarization method using frozen layers of ^{13}C-bicarbonate (Bic) and [1-^{13}C]Pyr in the same sample cup [4]. The objective of this work is to differentiate flux and isotopic exchange by injecting co-polarized [1-^{13}C]Lac and [2-^{13}C]Pyr. We also verified the effect of ethanol (EtOH) in a rat liver, which is known to increase the flux from Pyr to Lac by increasing NADH [5], using the co-administration of hyperpolarized Pyr and Lac with different labels.

Method: All measurements were performed on a clinical 3-T GE MR scanner. A custom-built ^{13}C surface coil ($Ø_{inner}$ = 28 mm) operating at 32.12 MHz was used for both RF excitation and signal reception. A proton birdcage coil (Ø = 70mm) was used for anatomical reference. Two male Wistar rats (316-318 g) were anesthetized with 1-3% isoflurane in oxygen (~1.5L/min). Compounds were polarized using HyperSense DNP and administered through the tail vein For rat 1, 3.5 mL of 80-mM [2-^{13}C]Pyr was first injected for a baseline measurement. To evaluate the effect of increased Lac pool size and increased NADH from EtOH oxidation, the 2nd injection consisted of 80-mM [2-^{13}C]Pyr with 40-mM unlabeled (^{12}C) Lac in the dissolution buffer. The 3rd injection consisting again of 80-mM [2-^{13}C]Pyr with 40-mM unlabeled (^{12}C) Lac in the buffer was given 45 min after infusion of EtOH. (0.1 g of EtOH per kg body weight diluted with saline to be 20 %). Rat 2 received two injections of a mixture of co-polarized 80-mM [2-^{13}C]Pyr and 40-mM [1-^{13}C]Lac before and 45 min after EtOH infusion. A sample with layered substrates of Lac (bottom layer) and Pyr (top layer) was used for co-polarization. Dynamic MRS data were acquired following the injection of the hyperpolarized compounds using the dynamic free induction decay sequence with a 5.625^{o} hard pulse, and the surface coil positioned over the liver (spectral bandwidth = 10,000 Hz, 4096 spectral points, scan time = 2 min, temporal resolution = 3s). The obtained time-course data were fit using the two-site exchange model [6]. Assuming net flux is from Pyr to Lac, it can be estimated that **Flux** = $[Pyr]\cdot k_{Pyr\rightarrow Lac} - [Lac]\cdot k_{Lac\rightarrow Pyr}$, **Exchange** = $[Pyr]\cdot k_{Pyr\rightarrow Lac} - \text{Flux} = [Lac]\cdot k_{Lac\rightarrow Pyr}$, where [Pyr] and [Lac] include labeled and unlabeled substrates.

Result: Time-averaged spectra from rat 1 normalized by [2-^{13}C]Pyr peak are shown in Fig. 1(a). The detected [2-^{13}C]Lac doublet increased in comparison to the baseline (blue line) when unlabeled 40-mM Lac was added to the buffer (red) with a further increase after EtOH infusion (green). As shown in Fig. 1(b), [2-^{13}C]Lac acquired after co-administration of labeled Pyr and Lac (rat 2) increased by a similar amount after EtOH infusion as for rat 1. Time-curves of labeled Pyr and Lac acquired from rat 2 after EtOH is shown in Fig. 1(c) as an example of obtained time-course. The apparent conversion rate constants from Pyr to Lac ($k_{Pyr\rightarrow Lac}$) were estimated from detected $^{13}C_2$-labeled Pyr and Lac. As Fig. 1(d) shows, $k_{Pyr\rightarrow Lac}$ increased from 0.014 s^{-1} to 0.019 s^{-1} when Lac was injected with Pyr, and further increased to 0.022 s^{-1} after EtOH infusion in both rats. Similarly, the apparent conversion rate constants from Lac to Pyr ($k_{Lac\rightarrow Pyr}$) were estimated from $^{13}C_1$-labeled Lac and Pyr. Unlike $k_{Pyr\rightarrow Lac}$, $k_{Lac\rightarrow Pyr}$ did not show significant change after EtOH from rat 2. $k_{Lac\rightarrow Pyr}$ could not be estimated from the data of rat 1 since injected Lac was unlabeled. The increased flux from Pyr to Lac due to increase of NADH is illustrated in Fig. 1(e). Whereas the injected $^{13}C_2$ Pyr concentration is dominant over the intrinsic unlabeled Pyr ([Pyr]≈[$^{13}C_2$Pyr]), unlabeled Lac concentration might not be negligible depending on the [$^{13}C_1$Lac].

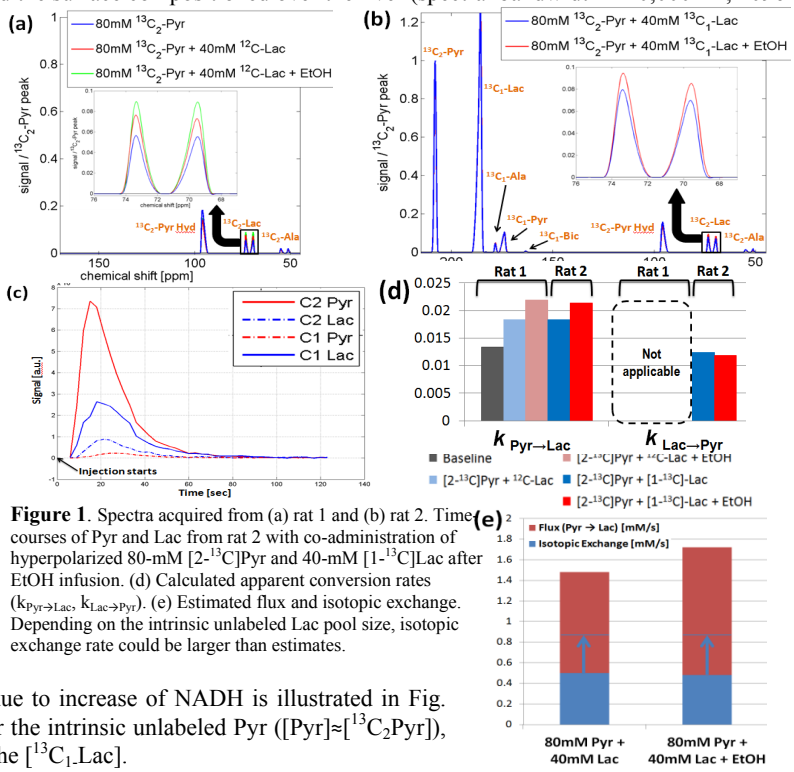

Figure 1. Spectra acquired from (a) rat 1 and (b) rat 2. Time courses of Pyr and Lac from rat 2 with co-administration of hyperpolarized 80-mM [2-^{13}C]Pyr and 40-mM [1-^{13}C]Lac after EtOH infusion. (d) Calculated apparent conversion rates ($k_{Pyr\rightarrow Lac}$, $k_{Lac\rightarrow Pyr}$). (e) Estimated flux and isotopic exchange. Depending on the intrinsic unlabeled Lac pool size, isotopic exchange rate could be larger than estimates.

Discussion: Bi-directional metabolic conversion can be estimated by simultaneously injecting both co-polarized substrates. The amount of flux and isotopic exchange can be estimated by the difference in flow of each substrate. However, the co-administration leads to changes in pool sizes of both substrates, resulting in potentially unphysiological condition, such as a highly enlarged Lac pool. On the other hand, low concentration of injected [1-^{13}C]Lac might result in underestimated exchange because of the contribution of intrinsic unlabeled Lac pool ([Lac]>[$^{13}C_1$ Lac]). In the case of non-negligible intrinsic Lac pool, isotopic exchange in Fig. 1(e) should be increased by [Lac]/[$^{13}C_1$ Lac]-fold, and flux should be reduced accordingly, maintaining total amount of conversion. Moreover, since we may not have saturated exchange with the 40-mM Lac in the bolus, some of the rate increase from EtOH could be exchange due to the increased steady state Lac pool generated during the 45 min when EtOH was in the system.

Conclusion: We demonstrate the differentiation of flux and isotopic exchange between Pyr and Lac by injecting polarized [2-^{13}C]Pyr and [1-^{13}C]Lac together, and observed increased flux from Pyr to Lac after EtOH infusion. The effect of Lac pool size on flux and isotopic exchange, and determination of intrinsic Lac pool in body need further investigation by varying the concentrations of [1-^{13}C]Lac and [2-^{13}C]Pyr.

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Acknowledgements: NIH: RR09784, AA 05965, AA018681, AA13521-INIA, and EB009070, DOD: PC100427, The Lucas Foundation, and GE Healthcare