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First hyperpolarized [2-¹³C]pyruvate MR studies of human brain metabolism

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Abstract

We developed methods for the preparation of hyperpolarized (HP) sterile [2-¹³C]pyruvate to test its feasibility in first-ever human NMR studies following FDA-IND & IRB approval. Spectral results using this MR stable-isotope imaging approach demonstrated the feasibility of investigating human cerebral energy metabolism by measuring the dynamic conversion of HP [2-¹³C]pyruvate to [2-¹³C]lactate and [5-¹³C]glutamate in the brain of four healthy volunteers. Metabolite kinetics, signal-to-noise (SNR) and area-undercurve (AUC) ratios, and calculated [2-¹³C]pyruvate to [2-¹³C]lactate conversion rates (k_{PL}) were measured and showed similar but not identical inter-subject values. The k_{PL} measurements were equivalent with prior human HP [1-¹³C]pyruvate measurements.

Keywords

Hyperpolarized C13; Metabolic imaging; Brain metabolism

1. Introduction

Dissolution Dynamic Nuclear Polarization (dDNP) provides over 10,000 fold signal enhancement for hyperpolarized carbon-13 (HP-¹³C) MRI, enabling a novel stable-isotope

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

molecular imaging approach for preclinical and recently clinical research studies demonstrating both safety and translational potential for human HP- ^{13}C molecular imaging [1–4]. HP [1- ^{13}C]pyruvate MR metabolic imaging has been applied to identify tumor metabolism [5], assess aggressiveness [6], evaluate treatment response [7], and probe organ function [4,8].

MR detection of the conversion of HP [1- ^{13}C]pyruvate to [1- ^{13}C] lactate catalyzed by lactate dehydrogenase (LDH) has shown research value and clinical potential in Phase I trials of cancer patients reflecting the Warburg Effect [3] with greatly upregulated LDH activity [9,10]. In approaching the tricarboxylic acid (TCA) cycle, [1- ^{13}C]pyruvate is enzymatically metabolized via pyruvate dehydrogenase (PDH) and converted to $^{13}\text{CO}_2$, thereby preventing direct detection of downstream TCA cycle metabolites. Prior animal studies using HP pyruvate with the ^{13}C isotope enriched in the 2-position ([2- ^{13}C]pyruvate) have successfully shown direct detection as the HP ^{13}C labeled atoms are carried over into acetyl-CoA, a precursor to the TCA cycle, and on to [5- ^{13}C]glutamate, acetyl-carnitine and other metabolites as shown in Fig. 1 [11,12]. Therefore, HP [2- ^{13}C]pyruvate provides novel metabolic information different from HP [1- ^{13}C]pyruvate due to its unique positioning atop multiple anaplerotic and cataplerotic metabolic cascades in the TCA cycle with known fast conversions [13]. Prior preclinical studies have shown differences in [2- ^{13}C]pyruvate to [5- ^{13}C]glutamate metabolism with isocitrate dehydrogenase (IDH) mutations in brain tumor models that are not detected by HP [1- ^{13}C]pyruvate MR [14].

The goal of this study was to develop methods for the hyperpolarization and preparation of sterile [2- ^{13}C]pyruvate with FDA-IND and IRB approval for first-ever human studies. We sought to investigate HP [2- ^{13}C]pyruvate conversion to [2- ^{13}C]lactate and [5- ^{13}C] glutamate in the normal brain in four volunteers, demonstrating a significant first step for HP metabolic imaging to diagnose neurological disorders potentially at an early stage and monitor treatment response. Unlike animal studies, these human experiments were performed without anesthesia that significantly reduces brain pyruvate metabolism [15] and therefore are more relevant to future patient studies.

2. Methods

2.1. [2- ^{13}C]Pyruvate: FDA-IND, IRB, human volunteers

[2- ^{13}C]pyruvate was produced by MilliporeSigma Isotec Stable Isotopes (Miamisburg, OH) following Good Manufacturing Practices (GMP) for first-ever use in human HP MR studies. All human studies followed an IRB and FDA IND-approved protocol with informed consent. Proton T2-FLAIR anatomical reference imaging scans showed volunteers had no acute abnormalities.

2.2. Preclinical quality control: T1, polarization, purity, animal studies

T1 relaxation times and liquid-state polarization levels of [2- ^{13}C]pyruvate were measured with independent characterization experiments in solution and murine models. [2- ^{13}C]pyruvate T1 measurements of 47 sec and polarization levels of 15.61% reaffirmed literature values [16]. Pyruvic acid solution NMR testing was performed using a Varian

VNMRS 500 MHz (Varian Medical Systems, Palo Alto, CA) to confirm the absence of impurities.

In-vivo spectroscopic animal studies were performed on a 3 T GE MR scanner following IACUC approval, prior to human volunteer studies to test *in vivo* performance. Non-localized dynamic ^{13}C NMR spectra were acquired with hard-pulsed excitation (TR/TE = 3 sec/35 msec) in Sprague-Dawley rats for detection of $[5\text{-}^{13}\text{C}]\text{glutamate}$, $[2\text{-}^{13}\text{C}]\text{lactate}$ and other metabolite resonances such as acetylcarnitine and acetoacetate [17].

2.3. Clinical preparation: SPINlab hyperpolarization

A 1.46 g sample of 14 M 99% enriched $[2\text{-}^{13}\text{C}]$ -labeled pyruvic acid (Millipore-Sigma, Miamisburg, OH) mixed with 15 mM trityl radical (GE Healthcare, Oslo, Norway) was pre-filled in a single-use, pharma-kit polymer fluid pathway and polarized for over 2 h in a SPINlab polarizer (General Electric, Niskayuna, NY) operating at 5 Tesla and 0.77 Kelvin, with microwave irradiation frequency in the 94.0–94.1 GHz band. Following the protocol approved by the University of California San Francisco IRB and the FDA IND, and after dissolution and meeting all quality control specifications and pharmacist approval, 0.43 mL/kg of the hyperpolarized pyruvate solution (250 mM) was injected intravenously at a rate of 5 mL/sec using a power injector (Medrad Inc., Warrendale, PA) followed by 20 mL of sterile saline.

2.4. MR protocol

Volunteers were measured using a 3 T MR scanner (MR750, 50 mT/m gradient amplitude, 200 T/m/sec slew rate; GE Healthcare, Waukesha, WI) and scanned with a volume excitation and 32-channel receive ^{13}C array coil for brain studies [18]. A 400 μsec hard pulse excitation provided an approximately 2.5 kHz excitation bandwidth, with a nominal flip angle of 40° at the center frequency of 141 ppm calibrated using a built-in urea phantom. The $[2\text{-}^{13}\text{C}]\text{pyruvate}$, $[5\text{-}^{13}\text{C}]\text{glutamate}$, and $[2\text{-}^{13}\text{C}]\text{lactate}$ doublet resonances were excited with 7° , 30° , 5° and 2.1° flip angles respectively. The acquisition used temporal and spectral resolutions of 2 sec and 2.4 Hz across 30 timepoints for a total scan time of 2 min.

2.5. Data analysis

Dynamic spectroscopic data yielding kinetic rates and curves was reconstructed after zero-filling free induction decays. The 32-channel data was combined with a phase-sensitive summation followed by line broadening of 5 Hz [19].

For the pyruvate-to-lactate conversion (k_{PL}) kinetic model, the measured pyruvate magnetization functioned as the input for fitting the lactate magnetization. The MATLAB model was solved based on minimization of a constrained least-squares error computed across measured and estimated lactate using a trustregion-reflective algorithm. The input-less fitting was chosen over integral ratios due to improved accuracy by accounting for variability in delivery times [20]. The analytical tools used are available from the Hyperpolarized MRI Toolbox via the Hyperpolarized Technology Resource Center: <https://doi.org/10.5281/zenodo.1198915>.

Quantitative data processing and display were performed using MATLAB (The MathWorks Inc., Natick, MA) and MestReNova (Mestrelab, Santiago de Compostela, Spain). Zero- and first-order phase corrections were performed, and baseline was subtracted by fitting a spline to signal-free regions of the smoothed spectrum. Metabolites of interest were quantified following prior assignments by selecting and integrating across peak boundaries [17]. Single timepoint data 16 sec following injection was further analyzed and interpreted after performing singular value decomposition (SVD) signal enhancement techniques [20–22,24].

3. Results

3.1. Volunteer spectra

HP [2-¹³C]pyruvate, [2-¹³C]lactate, [5-¹³C]glutamate and other metabolites were successfully observed and quantitatively measured for the first time in four volunteers. Fig. 2 shows a representative summed spectra over the total 2 min scan time for a healthy volunteer using a pulse and acquire scheme with the RF profile shown in Fig. 3. Figs. 4 and 5 depict spectra and kinetics of measured metabolite resonances for each of the four volunteers.

3.2. SNR & metabolite ratios

Tables 1 and 2 summarize measured SNR from the single timepoint data and AUC metabolite ratios summed across all time-points for 4 volunteers. Measured values and calculated mean and standard error across volunteers were consistent within expected ranges [16]. The observed variations in SNR can be attributed to multiple factors including brain volumes, polarization values, and delivery times from the polarizer to the subject. These demonstrated however minimal effects on the ratios and kinetic values that showed tight agreement between volunteers. The third volunteer dataset showed the highest SNR with AUC ratios near median and was hence selected as the representative spectrum for peak identification in Fig. 2. The [2-¹³C]lactate (left and right peaks) correspond to the left and right resonances of the [2-¹³C] lactate doublet in the ¹³C MRS spectra. The left (downfield) resonance is about two-fold higher due to the excitation profile shown in Fig. 3.

3.3. [2-¹³C]Pyruvate k_{pL} Model:

Fig. 6 shows a MATLAB plot of a measured [2-¹³C]pyruvate k_{pL} value from the volunteer studies with calculated mean and standard error of $0.011 \pm 0.002 \text{ sec}^{-1}$. The values were consistent with prior [1-¹³C]pyruvate k values of 0.012 sec^{-1} acquired using a similar setup and non-selective pulse-acquire strategy [17,20]. Identical results of pyruvate to lactate kinetics across a previously processed [1-¹³C]pyruvate dataset and newly acquired [2-¹³C] pyruvate datasets from volunteers lends verification to the robust-ness and consistency of approach.

3.4. Initial volunteer EPI studies

Fig. 7 shows initial data and feasibility of HP ¹³C imaging of the [2-¹³C]pyruvate conversion to [5-¹³C]glutamate using a specialized ¹³C 32-channel head coil. As shown in Fig. 2 not only was the uptake of HP [2-¹³C]pyruvate in the human brain observed, but also its metabolic conversion to [2-¹³C]lactate, [5-¹³C]glutamate, and other metabolites, similar to prior animal study results [17].

4. Discussion and conclusion

In this study we worked with the ISOTEC Stable Isotope Division of MilliporeSigma, Merck KGaA to develop GMP grade 99% enriched [2-¹³C]pyruvate meeting the purity specifications established for [1-¹³C]pyruvate used in numerous human studies following FDA-IND and IRB approved protocols. Prior to human studies with HP [2-¹³C]pyruvate, we first tested the purity and polarization through *in vitro* NMR analysis and performed a process qualification for testing and demonstrating the sterility of the polarized solution. The NMR spectra in Fig. 4 and quantitative values in Tables 1 and 2 demonstrated excellent data repeatability affirming the consistency of the preparation and processing methods. Metabolite ratios and dynamic plots in these initial studies directly reflected the excitation profile of the RF pulse that was optimized to capture the bandwidth encompassing metabolic byproducts and provided normative values for future human brain HP [2-¹³C]pyruvate NMR studies. Lastly pyruvate to lactate kinetic modeling from these [2-¹³C]pyruvate studies yielded k_{PL} values that were consistent with results from a prior HP [1-¹³C]pyruvate dataset in healthy human brain.

4.1. Future directions

This study demonstrated feasibility and initial normative values for HP [2-¹³C]pyruvate NMR and thus serves as the groundwork for designing new studies of neurological disorders. These future studies would clearly benefit from an imaging approach to investigate HP [2-¹³C]pyruvate MRI variations associated with anatomy and pathology and examine differences using centrality metrics and connectomic analytical methods with HP [1-¹³C]pyruvate MRI [22]. HP metabolic information can also be linked with modalities such as functional and diffusion MRI to build increasingly comprehensive representations of neural function, structure and metabolism [23]. Centrality metrics processing higher-order descriptors of multi-valued metabolite kinetics with advances in machine learning may further elucidate new methods for detecting early stages of neurological disorders.

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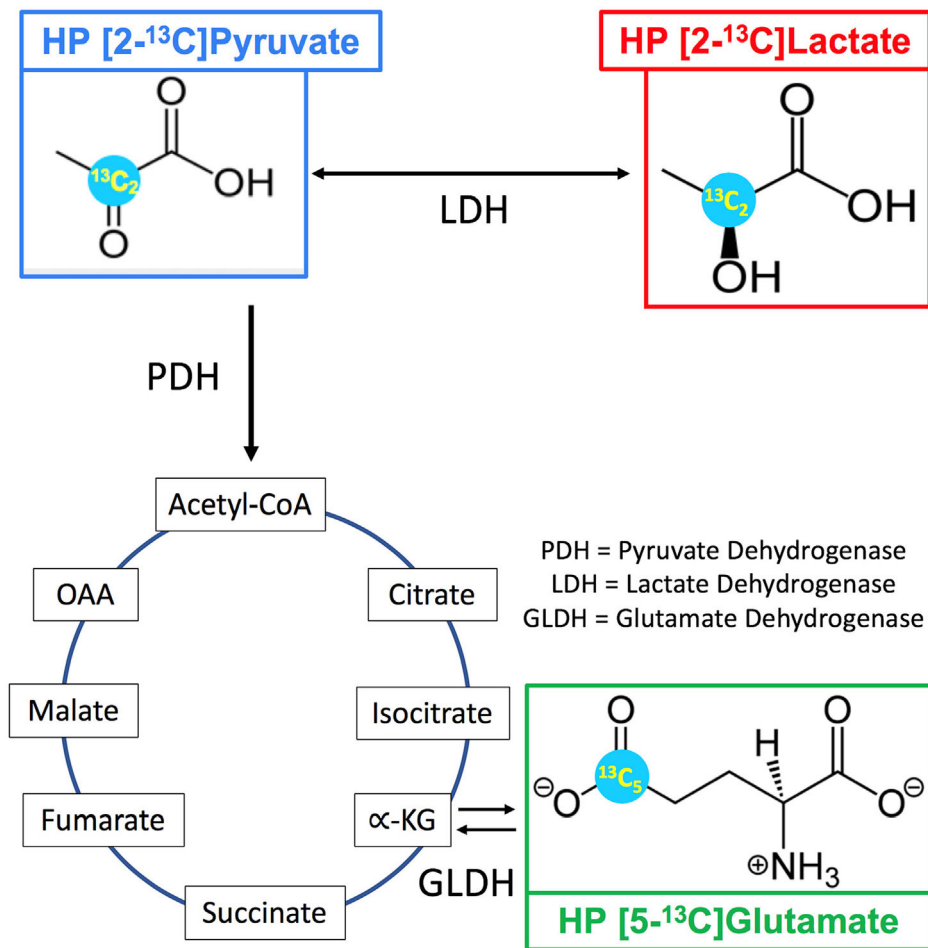


Fig. 1. Diagram showing [2-¹³C]pyruvate metabolism investigated in this hyperpolarized NMR spectroscopy study of the human brain.

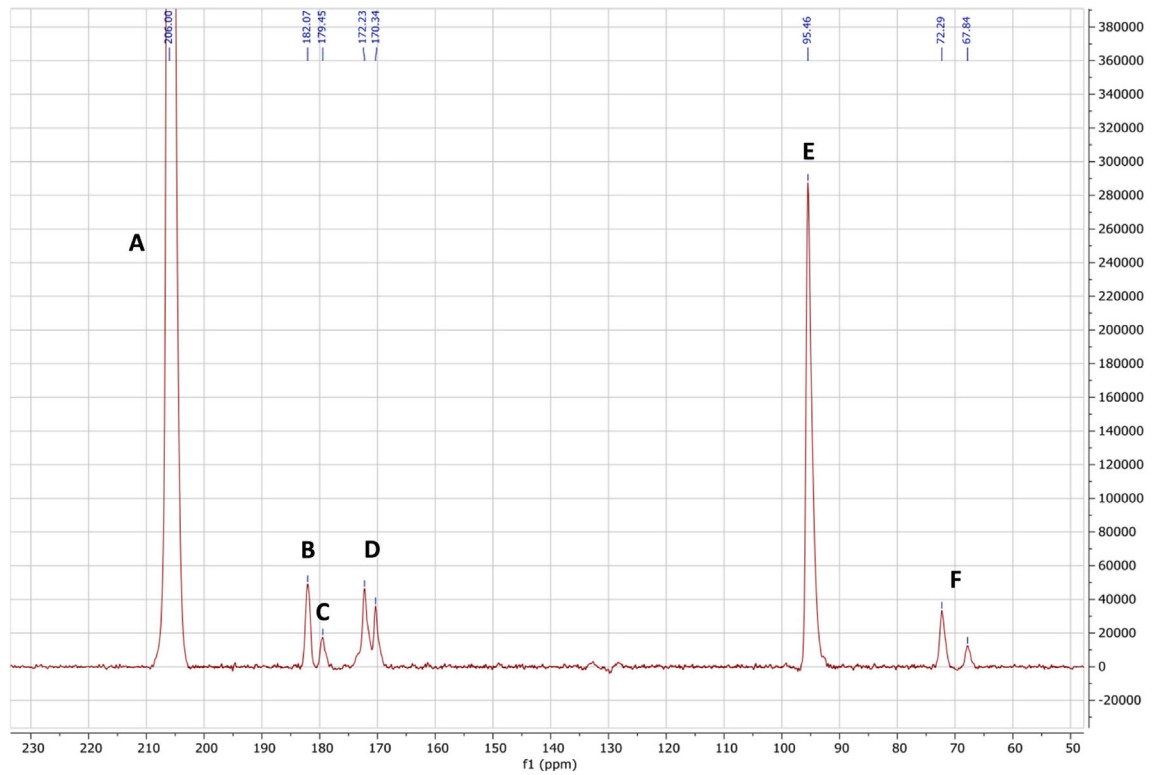


Fig. 2. Representative Carbon-13 NMR summed spectrum from the brain of a healthy volunteer acquired with a 32-channel head coil following an injection of 1.43 mL/kg of 250 mM [2-¹³C]pyruvate. Peak identification was assigned following those by Park et al. from studies of HP [2-¹³C]pyruvate in the murine brain: (A) [2-¹³C]pyruvate, (B) [5-¹³C]glutamate, (C) [1-¹³C]citrate and/or [5-¹³C]glutamine, (D) [1-¹³C]pyruvate (natural abundance doublet), (E) [2-¹³C]pyruvate-hydrate, (F) [2-¹³C]lactate doublet [17].

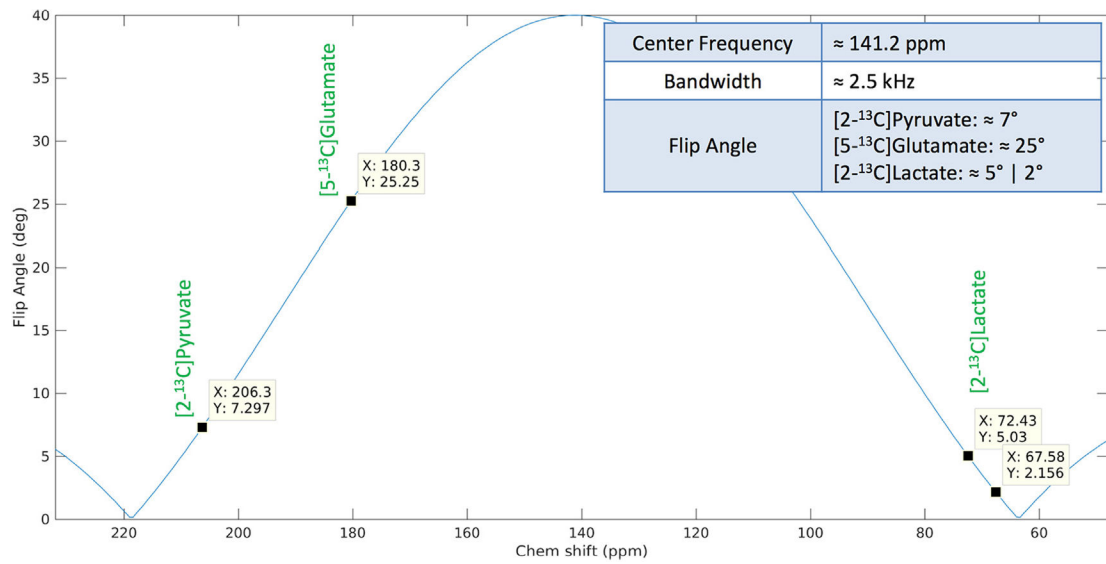


Fig. 3. Flip angle plot of the RF excitation pulse sequence with the parameters used for this study. Note the decreased excitation of the upfield [2-¹³C]lactate resonance versus the downfield by approximately one half.

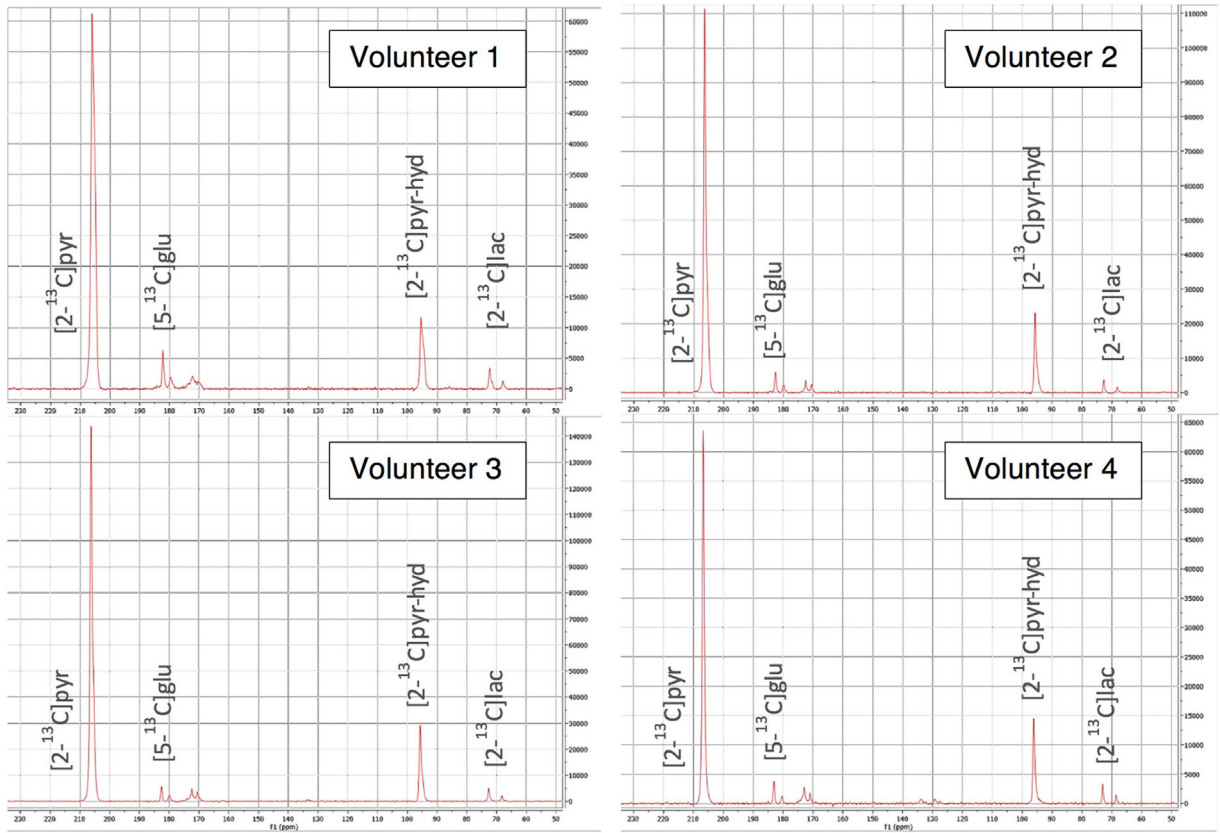


Fig. 4. Spectra for all four volunteers at a single timepoint 16 sec post-injection. Similar levels of $[5-^{13}\text{C}]$ glutamate and $[2-^{13}\text{C}]$ lactate reflect the underlying biochemistry of the healthy human brain of similar rates of conversion of $[2-^{13}\text{C}]$ pyruvate to $[2-^{13}\text{C}]$ lactate catalyzed by LDH as $[2-^{13}\text{C}]$ pyruvate to $[5-^{13}\text{C}]$ glutamate catalyzed by PDH.

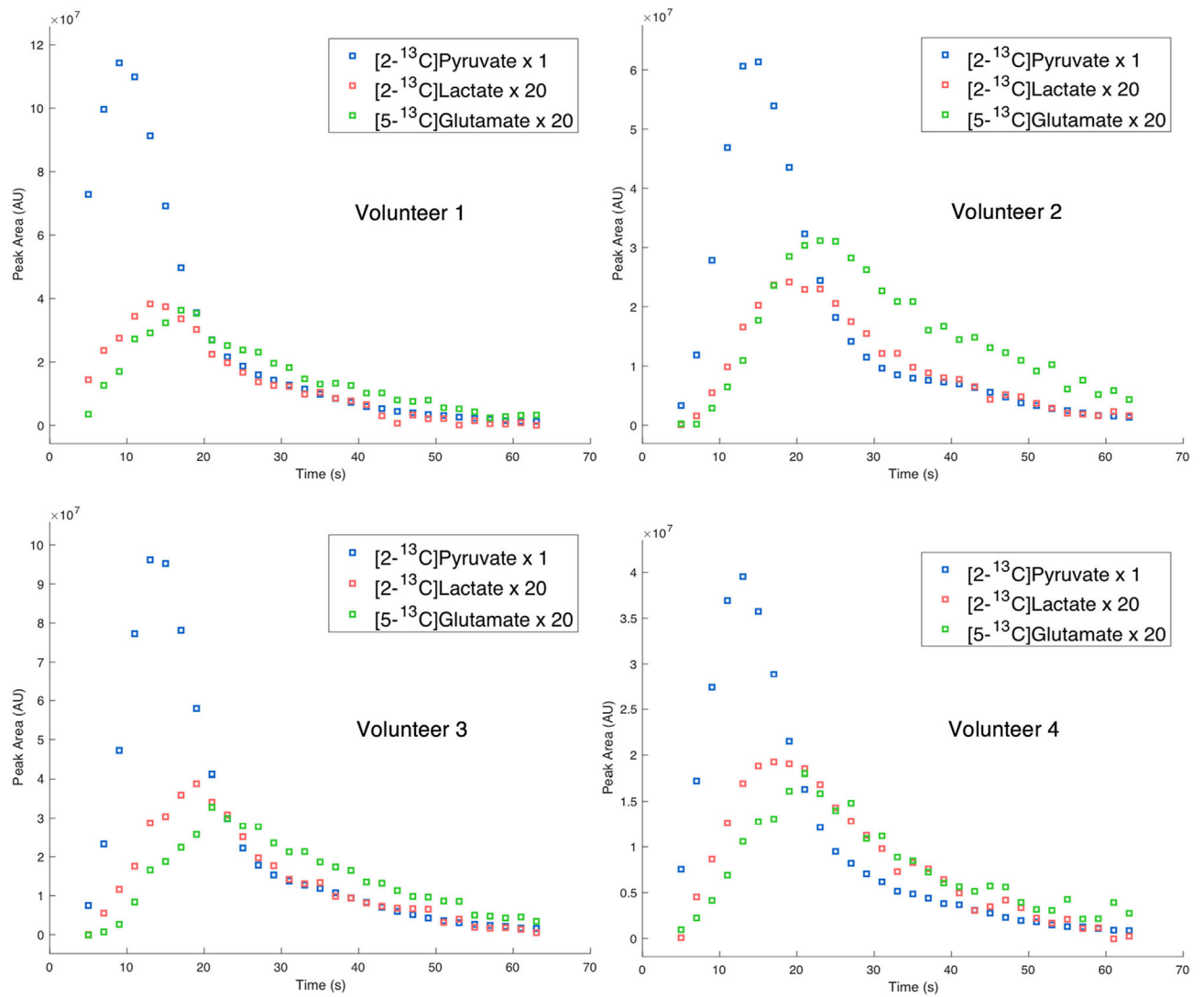
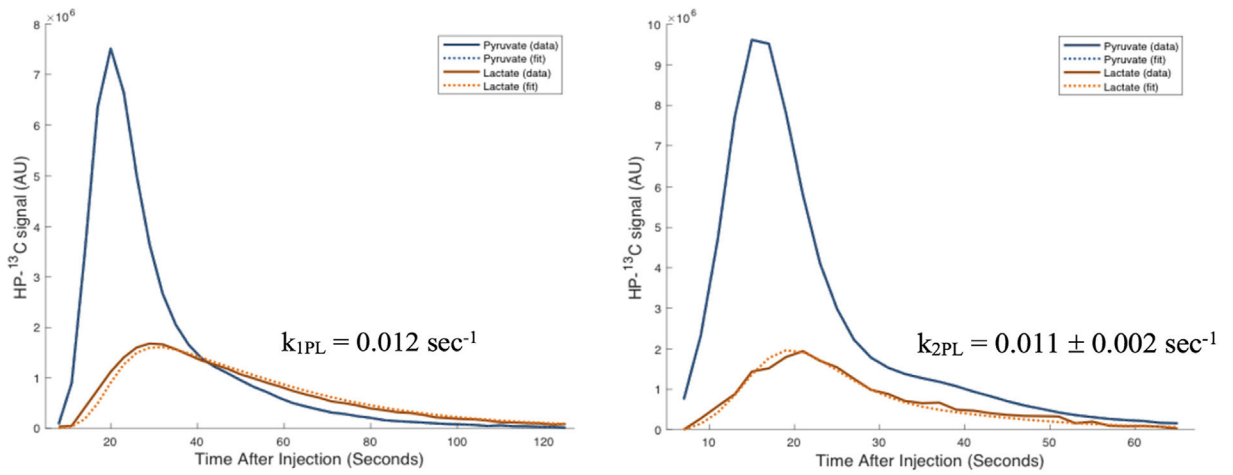


Fig. 5. Dynamic plots of metabolite kinetics for each of the four volunteers. Results were consistent noting minor differences in intensity scale. As shown in the corresponding Fig. 4, the rates of conversion of [2-¹³C]pyruvate to [2-¹³C]lactate and [5-¹³C]glutamate are similar in the normal human brain.

**Fig. 6.**

Plots showing k_{PL} analysis demonstrated similar results between a previously acquired [1-¹³C]pyruvate dataset from a volunteer (left) and [2-¹³C]pyruvate volunteer dataset acquired in this study with the mean \pm standard error for all 4 volunteers (right).

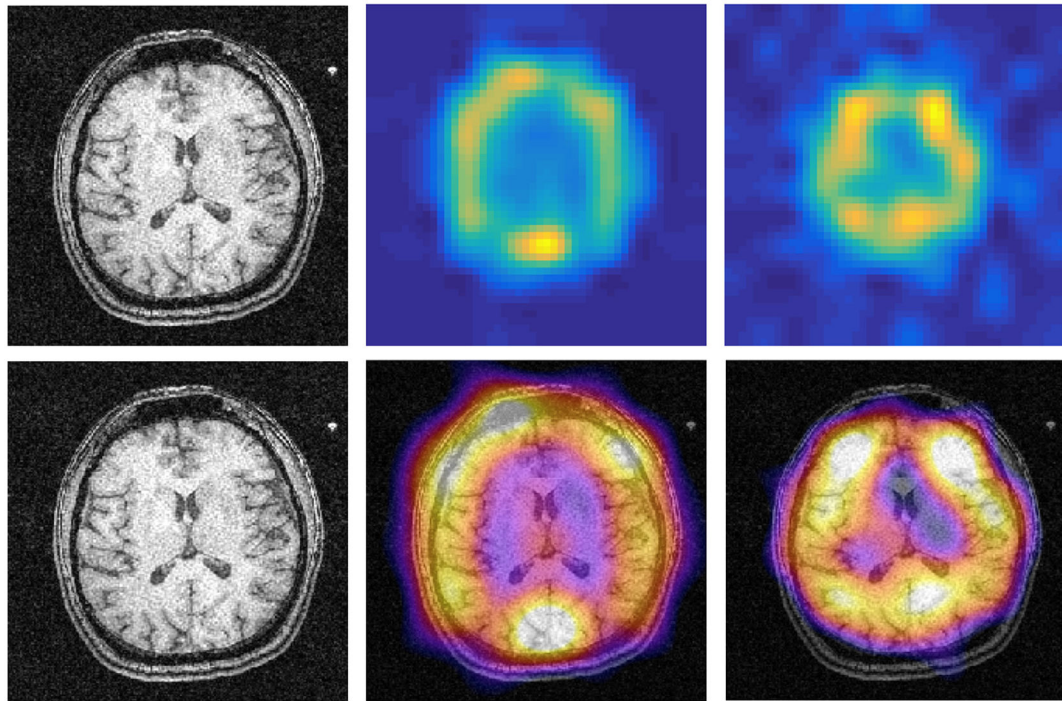


Fig. 7.

Images acquired using a metabolite-specific flip angle schedule and echo planar imaging (EPI) readout. From left to right ^1H proton reference image, overlaid $[2-^{13}\text{C}]$ pyruvate, and $[5-^{13}\text{C}]$ glutamate images of a volunteer's brain are shown. The single-shot HP ^{13}C EPI images were acquired with: in-plane resolution = $2.5 \times 2.5 \text{ cm}^2$, slice thickness = 5 cm, bandwidth = 8.06 kHz, TR = 100 msec, temporal resolution = 3 sec, TE = 19 msec, and flip angles $\theta_{\text{Pyr}} = 10^\circ$, $\theta_{\text{Glu}} = 60^\circ$. Average SNR of the pyruvate signal = 682 and glutamate signal = 31.1.

Table 1

SNR for each volunteer from a single timepoint 16 sec post-injection with calculated mean and standard error.

Volunteer	[2- ¹³ C]Pyruvate	[5- ¹³ C]Glutamate	[2- ¹³ C]Pyruvate-Hydrate	[2- ¹³ C]Lactate (Left Peak)	[2- ¹³ C]Lactate (Right Peak)
1	885.93	91.18	169.68	24.63	9.55
2	1278.34	68.36	265.33	42.58	18.55
3	2114.06	83.03	428.07	72.67	32.95
4	964.09	57.43	219.63	50.93	22.05
Mean	1310.61 ± 486.57	75.00 ± 13.03	270.68 ± 96.96	47.70 ± 17.27	20.77 ± 8.38

AUC metabolite ratios for each volunteer summed across all timepoints with calculated mean and standard error.

Table 2

Volunteer	Lac/Pyr	Glu/Pyr	Pyr-Hyd/Pyr	Glu/Lac
1	0.024	0.027	0.163	1.125
2	0.030	0.045	0.178	1.500
3	0.028	0.030	0.180	1.071
4	0.038	0.037	0.191	0.974
Mean	0.030 ± 0.005	0.035 ± 0.007	0.178 ± 0.010	1.168 ± 0.199