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Dynamic Metabolic Imaging of Co-Polarized [2-¹³C]Pyruvate and [1,4-¹³C₂]Fumarate Using 3D-Spiral CSI with Alternate Spectral Band Excitation

Maninder Singh¹, Sonal Josan², Minjie Zhu¹, Aditya Jhahharia¹, Dirk Mayer¹

¹Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland, Baltimore, Maryland, USA

²Siemens Healthcare, Erlangen, Germany

Abstract

Purpose: Developing a method for simultaneous metabolic imaging of co-polarized [2-¹³C]Pyr and [1,4-¹³C₂]Fum without chemical shift displacement artifacts that also permits different excitation flip angles for substrates and their metabolic products.

Methods: The proposed pulse sequence consists of two frequency-selective RF pulses to alternately excite two spectral sub-bands each one followed by a fast 3D spiral chemical shift imaging (3D-spCSI) readout. Spectrally-selective RF pulses were designed to excite differential flip angles on substrates and products in each spectral sub-band. Number of signal averages analysis was utilized to determine a spectral width suitable to resolve the metabolites of interest in each of the sub-bands.

Results: Phantom experiments verified the co-polarization strategy and radiofrequency pulse design following differential flip angle used in our method. The signal behavior of the resonances in each sub-band was unaffected by the excitation of the respective alternate frequency band. Dynamic 3D ¹³C CSI data demonstrated the ability of the sequence to image metabolites like pyruvate-hydrate, lactate, alanine, fumarate and malate simultaneously and detect metabolic changes in the liver in a rat model of carbon tetrachloride-induced liver damage.

Conclusion: The presented method allows the dynamic CSI of a mixture of [2-¹³C]pyruvate and [1,4-¹³C₂]fumarate without chemical shift displacement artifacts while also permitting the use of different flip angles for substrate and product signals. The method is potentially useful for combined in-vivo imaging of inflammation and cell necrosis.

Keywords

hyperpolarized ¹³C; dynamic metabolic imaging; [2-¹³C]pyruvate; [1,4-¹³C₂]fumarate; liver

Introduction:

The health of an organ or tissue is characterized by its cellular metabolism involving various enzymatic biochemical reactions and alterations in these processes could indicate a disordered or diseased organ state. While in vivo magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) can noninvasively provide metabolic information, conventional ^1H and ^{13}C MRSI methods are inefficient owing to the low metabolite concentrations in cells or tissue generating very small signals.¹ To overcome this, dynamic metabolic imaging is used in conjugation with Hyperpolarization technique called dissolution-dynamic nuclear polarization (d-DNP) to enhance ^{13}C labelled signal of metabolites under study.² Pyruvate (Pyr) is an important probe for these kind of studies as it is the main bioenergetic component in cells. Additionally, conversion of hyperpolarized ^{13}C -labeled fumarate (Fum) to malate (Mal) has been proposed as a biomarker for cell necrosis.³ Metabolic imaging of hyperpolarized ^{13}C -labeled Pyr^{4–18} and Fum^{3,19–21} has shown great potential in characterizing multiple diseases and assessing response to treatment with Pyr already translated into the clinic.^{8–11} Thus, performing their measurements with a single injection of co-polarized substrates would be advantageous particularly for the clinical translation of this technology.

In a recent study on combined ^{18}F -2-fluoro-2-deoxy-D-glucose positron emission tomography and metabolic imaging of $[1-^{13}\text{C}]\text{Pyr}$ and $[1,4-^{13}\text{C}_2]\text{Fum}$ in a rat model of necrosis,¹⁹ A. Eldirdiri et al. reported a co-polarization scheme to polarize these substrates simultaneously. However, the small chemical shift (CS) dispersion of both substrates and metabolic products, e.g., the frequency difference between Fum and Ala is only 35 Hz at 3 Tesla (T), does not allow the use of signal-to-noise (SNR) efficient differential flip angles for substrate and products²² at clinical field strengths, potentially preventing the measurement of saturation kinetics that requires effective 90° excitation of the products.²³ The goal of this study is to investigate the feasibility of simultaneous metabolic imaging of co-polarized $[2-^{13}\text{C}]\text{Pyr}$ and $[1,4-^{13}\text{C}_2]\text{Fum}$ with different excitation flip angles on a clinical MR scanner taking advantage of the much larger CS dispersion of $[2-^{13}\text{C}]\text{Pyr}$ and its products $[2-^{13}\text{C}]\text{lactate}$ (Lac) and $[2-^{13}\text{C}]\text{alanine}$ (Ala) (>160 ppm).

Although, the large CS dispersion of resonances permits the desired differential flip angle scheme, it becomes more amenable to CS displacement artifacts for slice-selective acquisitions. Besides, this large frequency range is much beyond the practical spectral bandwidth limits of many fast MRSI methods and its encoding in interleaved manner significantly increases the imaging time, hence, severely limiting the temporal resolution in dynamic CSI. A solution is to excite smaller sub-bands of resonances with spectrally-selective RF pulses to avoid CS displacement artifacts due to the large CS dispersion.²⁴ Therefore, we applied number of signal averages (NSA) analysis used in least-squares estimation imaging approaches^{25,26} to investigate if a smaller spectral width (SW) corresponding to fewer spatial interleaves, would be sufficient to separate the metabolites of interest. The method presented here is based on 3D spiral chemical shift imaging (3D-spCSI) developed for metabolic imaging of $[2-^{13}\text{C}]\text{Pyr}$.²⁴ The sequence used frequency-selective RF pulses to alternately excite two spectral sub-bands each one followed by a fast 3D-spCSI readout. In our application the first frequency sub-band contains resonances

of [1,4- $^{13}\text{C}_2$]Fum (175.4 ppm) and [1,4- $^{13}\text{C}_2$]Mal (180.3 and 182.1 ppm) whereas the other sub-band consists of resonances of [2- ^{13}C]Lac (69.03 and 73.57 ppm), [2- ^{13}C]Ala (50.34 and 54.86 ppm) and [2- ^{13}C]pyruvate-hydrate (Pyh, 96.6 ppm). Pyh is in equilibrium with Pyr and itself metabolically inactive. Hence, it can serve as a surrogate marker for the injected substrate. To demonstrate the efficacy of the purposed imaging approach, time-resolved CSI data were acquired from rats before and after induce liver necrosis by administration of carbon tetrachloride (CCl_4).

Methods

The complete spectral dispersion of resonances of co-polarized mixture at 3 T is divided into two sub-bands: the first sub-band comprises the substrate [1,4- $^{13}\text{C}_2$]Fum and the doublet of its product [1,4- $^{13}\text{C}_2$]Mal (this frequency sub-band hereon is referred to as the *Fum* sub-band), whereas the other sub-band consist of [2- ^{13}C]Pyh and doublet of both [2- ^{13}C]Lac and [2- ^{13}C]Ala peaks (this frequency sub-band hereon referred to as the *Pyh* sub-band).

RF Pulse design:

To avoid CS displacement artifacts due to the large CS dispersion, two frequency-selective RF pulses (shown in Figure 1) were designed to alternately excite two spectral sub-bands each one followed by a fast 3D-spCSI readout. These pulses have different excitation bandwidths and exert differential flip angles for substrate and product resonances. For the *Fum* sub-band, a 14.08-ms spectrally-selective pulse with a passband of 50 Hz for the Mal doublet and 25 Hz for the Fum peak at 1% amplitude loss was used. For *Pyh* sub-band, a 2.22-ms spectrally-selective pulse with a passband of 1890 Hz at 1% amplitude loss was used. The width of these passbands was optimized by considering the maximum variation of B_0 of 13 Hz in the liver ROI from rat in-vivo data. The RF pulse waveforms, a combination of two (*Fum*-band) and three (*Pyh*-band) Hamming windowed-sinc functions with time bandwidth product of 2, together with their spectral profiles are shown in Figure 1. These spectral profiles were calculated by using the Shinnar-Le Roux algorithm.²⁷ For the design of the RF pulses it is important to sufficiently suppress excitation of [2- ^{13}C]Pyr in both bands and of [1,4- $^{13}\text{C}_2$]Fum during excitation of the *Pyh* band. The RF pulses were optimized for selective suppression of substrate peaks by iteratively changing the pulse parameters and calculating the frequency response.

The *Fum* sub-band pulse excites a differential flip angle on Fum and Mal peaks in the ratio of 1:4 (e.g., for 10° flip angle on Mal, it excites 2.5° on Fum). However, the *Pyh* sub-band pulse excites the Pyh, Lac, and Ala peaks with the same flip angle. The [2- ^{13}C]Pyr signal was not excited by any of these two pulses while excitation of Fum was suppressed in the *Pyh* sub-band (suppression factor of substrate peaks were: $\sim 1.6 \times 10^5$ for [2- ^{13}C]Pyr peak in the *Fum* sub-band and 3.5×10^5 for [2- ^{13}C]Pyr and 4.2×10^5 for [1,4- $^{13}\text{C}_2$]Fum peak in the *Pyh* sub-band) to limit the signal contamination from the injected substrates aliasing into the acquired frequency sub-bands and obscuring the low signal levels of the metabolic products. The RF pulses and transmitter frequency are shifted alternately during each subsequent sampling interval to acquire both sub-bands during same experiment.

Sample Preparation:

Both [2-¹³C]Pyr and [1,4-¹³C₂]Fum samples used in our experiments were prepared separately. The [2-¹³C]Pyr (14 M) sample is prepared by mixing AH111501 trityl radical (GE Healthcare, Oslo, Norway) in [2-¹³C]Pyruvic acid (Sigma Aldrich, USA) whereas the [1,4-¹³C₂]Fum (3.5 M) sample was prepared by dissolving [1,4-¹³C₂]Fumaric acid (Cambridge Isotope Laboratories, MA, USA) crystals in dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA) followed by the addition of AH111501 trityl radical. The co-polarized mixture consists of 50 µL of [2-¹³C]Pyr and 100 µL of [1,4-¹³C₂]Fum and the co-polarization was done using a SPINlab Hyperpolarizer (Research Circle Technology) operating at 5.0 T and 0.82 K. Following the procedure described by Eldirdiri¹⁹, the sample mixture was prepared by first adding the measured [2-¹³C]Pyr to the sample vial and freezing it. The [1,4-¹³C₂]Fum was then added on the top of it and the mixture is quickly frozen again to avoid mixing. The sample vial was quickly lowered into the polarizer in a single step to avoid the crystallization of fumaric acid and the sample was polarized for at least 3 hours. Upon dissolution, the polarized sample was neutralized with a solution of 80 mM NaOH mixed with 40 mM Tris buffer and 0.1 g/L of EDTA-Na₂, leading to a hyperpolarized solution of 40-mM Fum and 80-mM Pyr with a pH of 7.4 to 7.9. The liquid-state polarization of the substrates in the mixture was slightly lower compared to the case when polarizing compounds individually. The polarization at time of dissolution was measured in independent experiments as previously described:²⁸ 30 ± 2 % for Fum and 35 ± 3% for Pyr polarized as a mixture (mean ± standard deviation, n = 2) vs 38.5 ± 7% for Fum (n = 2) and 44 ± 2% Pyr (n = 2) polarized individually.

MR Experiments:

All experiments were performed on GE 750w 3.0 T clinical MR scanner (GE Healthcare, Waukesha, WI) with the maximum gradient strength of 33 mT/m and slew rate of 120 mT/m/ms. A single-loop ¹³C surface coil (40 mm diameter) for both signal excitation and reception was placed on top of the rat liver in the supine position. Proton MRI was performed using a small flex receive coil together with the scanner's body coil for anatomical localization and to confirm the location of the ¹³C surface coil on the organ of interest. An 8-M ¹³C-labeled urea phantom placed on the top of the rat liver was used for ¹³C RF calibration by nullifying/minimizing the signal for a reference 180° hard pulse. RF power was adjusted based on the maximum B₁ of the two sub-pulses to achieve a nominal 10° excitation for the respective passband. For phantom experiments assessing the performance of the RF pulses, a dual tuned (¹H/¹³C) volume transmit/receive RF coil (50 mm inner diameter, USA Instruments Inc., Aurora, OH) was used.

The 3D-spCSI method is based on a sequence developed by Josan *et. al.*²⁴ for frequency band-selective metabolic imaging of [2-¹³C]Pyr. Although implemented on a clinical MR scanner, that study used a high-performance gradient insert for small animal imaging²⁹ to achieve a SW high enough to sufficiently separate the multiple resonances of interest. Given the lower performance of the clinical gradients we explored NSA analysis to see if a smaller SW would be sufficient to separate each individual metabolite in both *Fum* and *Pyr* sub-bands without aliasing. Figure 2 shows the NSA calculated for 9 equally spaced echoes as a function of echo shift for the resonance frequencies at 3 T of the metabolites detected in the

two respective bands. The local maxima in the NSA plots correspond to the optimal sampling rate to differentiate the metabolites without overlap whereas the local minima represent echo shifts where aliasing leads to peak overlap. These results indicate an echo shift of 3.88 ms (corresponding to a SW of 258 Hz) is sufficient to separate metabolites excited in the two alternating sub-bands. Using a time optimal gradient waveform design algorithm³⁰ to generate spiral waveforms corresponding to a k-space trajectory for a field of view (FOV) of 50 mm with a matrix size of 10×10 , this SW can be achieved with only two spatial interleaves under the limitations of the clinical gradient system. Twenty echoes were used to encode the spectral information. With ten encoding steps in the phase encoding direction (FOV = 50 mm) the total acquisition time per volume was 2.5 s.

Hyperpolarized phantom experiments:

The performance of the RF pulses was evaluated for differential flip angle excitation and suppression of substrate peaks in both sub-bands through the hyperpolarized phantom experiments. For a syringe containing approximately 4 mL of a solution of co-polarized $[2\text{-}^{13}\text{C}]\text{Pyr}$ and $[1,4\text{-}^{13}\text{C}_2]\text{Fum}$, a simple pulse-and-acquire MRS sequence (TR = 3 s, SW=10 kHz, and 4096 points) was performed two times consecutively: first with alternate band excitation using the two spectrally-selective RF pulses (10° flip angle for the nominal product resonances, 10 excitations per band), and second with a 32- μs hard pulse (5.625° flip angle, 80 scans) starting 22 s after the end of the first scan. The signal dynamics of substrate $[2\text{-}^{13}\text{C}]\text{Pyr}$ and $[1,4\text{-}^{13}\text{C}_2]\text{Fum}$ peaks from both alternating RF and hard pulse MRS sequences were analyzed and compared for their suppression in both sub-bands.

In-vivo experiments:

Healthy male Wistar rats (n = 4, weight = 318 – 352 g) were used for in-vivo experiments. The animals were anesthetized using continuous isoflurane flow (1 – 3 %) with respiration and rectal temperature monitored throughout the experiments. Temperature was regulated using a warm water blanket. Hyperpolarized solutions at a dose of 10 mL/kg were injected approximately 30 s after dissolution via tail vein catheter at a rate of approximately 0.25 mL/s. Three animals received solution of co-polarized $[2\text{-}^{13}\text{C}]\text{Pyr}/[1,4\text{-}^{13}\text{C}_2]\text{Fum}$ and imaging was performed before and one day after intraperitoneal injection (i.p.) of CCl_4 at a dose of 2 mL/kg body weight (dissolved in olive oil in a ratio of 1:1 v/v) to induce liver necrosis. One animal also received a solution of $[2\text{-}^{13}\text{C}]\text{Pyr}$ only to assess potential excitation of other metabolic products of $[2\text{-}^{13}\text{C}]\text{Pyr}$ such as $[1\text{-}^{13}\text{C}]\text{acetylcarnitine}$ (Alcar) or $[5\text{-}^{13}\text{C}]\text{glutamate}$ (Glu) without interference from $[1,4\text{-}^{13}\text{C}_2]\text{Fum}$. All animal experiments were performed following approval from local Institutional Animal Care and Use Committee and were conducted following NIH guidelines for animal welfare.

^1H -T₂-weighted anatomical MR images for prescribing the ^{13}C CSI experiments were obtained using single-shot fast spin echo (FSE) sequence (FOV = $120 \times 72 \text{ mm}^2$, 2 mm slice thickness) in the axial, sagittal, and coronal planes. In addition, ^1H 3D spoiled gradient echo (3D-SPGR) images ($0.625 \times 0.625 \times 1.25 \text{ mm}^3$ resolution) were acquired, matching the ^{13}C CSI prescription for overlay of the metabolic images.

Post-dissolution and starting at the beginning of injection the polarized solution, the dynamic 3D ^{13}C CSI data were acquired during successive $\text{TR} = 2.5$ s interval for 30 timepoints. The RF pulse and transmit frequency was shifted alternately between successive timepoints to acquire 15 timepoints for each frequency sub-band corresponding to a temporal resolution of 5 s per each sub-band.

Data Reconstruction:

The FID data were reconstructed by applying a 15 Hz Gaussian filter along the spectral dimension and zero-filled by a factor of 2 followed by FFT. Metabolite signals were quantified by peak integration of the peak in absorption mode. The suppression factors for the substrate peaks were calculated as follows: The data from the hard-pulse sequence was used to calculate the T_1 s for both Fum and Pyr by fitting their time courses to a mono-exponential decay curve corrected for the 5.625° excitation. Using these T_1 s the longitudinal magnetization of Fum and Pyr was extrapolated to the times of the last two acquisitions of the first scan. Based on the measured signals the excitation flip angles for the two substrates were estimated and the corresponding suppression factors were calculated compared to a nominal 90° excitation.

The 3D ^{13}C CSI data were reconstructed using the same procedure as previously described.²⁴ Each frequency sub-band was reconstructed separately. The signal of each metabolic peak was integrated in absorption mode to obtain metabolic maps for each metabolite. The integration width of 40 Hz was used for both $[1,4-^{13}\text{C}_2]\text{Fum}$ and $[1,4-^{13}\text{C}_2]\text{Mal}$ peaks in the *Fum* sub-band whereas 30 Hz for $[2-^{13}\text{C}_2]\text{Pyh}$, $[2-^{13}\text{C}_2]\text{Lac}$ and $[2-^{13}\text{C}_2]\text{Ala}$ peaks in *Lac* sub-band. From an ROI encompassing liver for *in-vivo* rat experiments, the time courses for the detected metabolites were calculated. For display of individual spectra, a zero and first order phase correction was performed during spectral analysis to properly demodulate the phase. A paired t-test was performed to evaluate the change in product-to-substrate ratios in the liver after CCl_4 administration. Metabolite ratios are reported as mean values $\pm$ standard error.

Results:

Evaluation of RF pulse performance:

The performance of the RF pulses was evaluated on a sample of co-polarized Pyr and Fum where the pulses were inserted into a simple pulse-and-acquire MRS sequence that alternately excited the two sub-bands. The suppression factor for $[2-^{13}\text{C}]\text{Pyr}$ was 3.2×10^3 in the *Fum* sub-band and 1.9×10^3 in the *Pyh* sub-band. As the center frequency was misadjusted by 3 Hz relative to the Fum resonance, the corresponding theoretical values are 5.6×10^5 and 1.3×10^5 . Considering a B_0 inhomogeneity of 13 Hz the minimum theoretical suppression factor within this interval around $[2-^{13}\text{C}]\text{Pyr}$ is 4.6×10^4 in the *Fum* sub-band and 7.7×10^4 in the *Pyh* sub-band. The suppression factor for $[1,4-^{13}\text{C}_2]\text{Fum}$ in the *Pyh* sub-band was measured as 2.1×10^3 compared to the theoretical value of 4.5×10^5 at the adjusted frequency position and 8.9×10^4 within 13 Hz interval.

In-vivo data:

The *in-vivo* time averaged spectra for both sub-bands from a liver ROI ($20 \times 30 \text{ mm}^2$) in a representative animal after CCl_4 administration are shown in Figure 3. The spectra from the *Fum* sub-band shows the $[1,4\text{-}^{13}\text{C}_2]\text{Fum}$ peak as well as the doublet of its metabolic product $[1,4\text{-}^{13}\text{C}_2]\text{Mal}$ peaks indicating necrosis. The *Pyh* sub-band spectra show $[2\text{-}^{13}\text{C}]\text{Pyr-Hyd}$ peak and doublets of $[2\text{-}^{13}\text{C}]\text{Lac}$ and $[2\text{-}^{13}\text{C}]\text{Ala}$ peaks. In the *Fum* sub-band, the SW is sufficient to detect both Fum and Mal peaks without aliasing. However, for *Pyh* sub-band, relative to Lac peak in the middle of spectra, the other Lac peak was aliased once, Ala peaks were aliased -2 to -3 times and Pyh peak 3 times. This required separate reconstructions for the aliased signals to properly demodulate the phase.³¹ As illustrated by the three out of five separately reconstructed spectra shown in Figure 3B all the resonances of Pyh, Lac and Ala are well separated.

Figure 4(A) shows the representative ^{13}C metabolic maps of the detected metabolites from a slice through the liver superimposed onto the corresponding ^1H image. In addition to the liver, vasculature can also be seen in Fum and Pyh maps. The corresponding time courses of the metabolites from an ROI in the liver are plotted in Figure 4(B).

Product-to-substrate ratios averaged over ROIs in the liver from the time-averaged 3D spCSI data before and after administration of CCl_4 are shown in Figure 5. Whereas essentially no Mal was detected pre- CCl_4 ($\text{Mal/Fum} = 0.004 \pm 6 \times 10^{-4}$), the CCl_4 -induced necrosis lead to a statistically significant increase to 0.58 ± 0.045 ($p=0.0031$).

Discussion:

Our 3D-spCSI sequence represents an advancement of the of the previously described method²⁴ in a number of aspects. The normal Gaussian pulses were replaced by improved RF pulses permitting differential flip angle excitation capability. Furthermore, in the previous design the second sub-band only excited and detected the $[2\text{-}^{13}\text{C}]\text{Lac}$. With the new RF pulse and optimized SW the pulse sequence now permits the detection of $[2\text{-}^{13}\text{C}]\text{Lac}$, $[2\text{-}^{13}\text{C}]\text{Ala}$ and $[2\text{-}^{13}\text{C}]\text{Pyh}$ within the same acquisition.

Pyh was chosen as a surrogate marker for Pyr as it simplified the RF pulse design. Although the equilibrium between Pyr and Pyh is both temperature and pH dependent at physiological pH levels the ratio is almost constant.³² Knowledge of the exact ratio is not necessary when calculating the apparent rate constants for the conversion of Pyr to its metabolic products to differentiate healthy from diseased or damaged tissue. The equilibrium level as well as any differences in T_1 between Pyr and Pyh only correspond to a scaling factor. Note that any changes in the equilibrium, e.g., due to severe pH changes caused by a pathology, would also affect the detected Pyr level and, hence, the measurement of the apparent rate constants if Pyr is detected directly.

The previous use of a high-performance gradient insert allowed the spiral CSI acquisition with a SW of 607 Hz and a FOV of $80 \times 80 \times 60 \text{ mm}^3$ (5-mm isotropic resolution) with only two spatial interleaves. Achieving such a high SW with the clinical gradient system would require about 20 spatial interleaves for the same spatial parameters, hence, leading to

unacceptable scan times of 24 s per sub-band. However, using a SW of 258 Hz optimized by NSA analysis that can be achieved with two interleaves without high performance insert gradients permits the acquisition with the same spatial and temporal resolution per sub-band with only a small reduction in FOV.

The temporal resolution of each spectral sub-band acquired alternately was 5 s (two frequency sub-bands: 2.5 s acquisition per sub-band). Acquisition of more sub-bands would increase the sampling interval and decrease the temporal resolution that limits the dynamic metabolic imaging. Hence, depending on the specific application we have to trade-off between sampling interval and temporal resolution.

In addition to the [2-¹³C]Pyr signal in the phantom experiment, there are metabolic product resonances such as [1-¹³C]Alcar (175.2 ppm) and [5-¹³C]Glu (183.8 ppm) as well as the doublet of natural abundance [1-¹³C]Pyr (173.5 ppm) that could potentially affect the measurement. However, at least for rat liver imaging using the current hardware none of these resonances were detected as illustrated in Supporting Information Figure S1 of a time-averaged spectrum from a liver ROI of a healthy animal after injection of [2-¹³C]Pyr.

It has been shown that CCl₄-induced liver damage such as inflammation and increased alanine aminotransferase activity can be observed using metabolic imaging of hyperpolarized [1-¹³C]Pyr¹⁸. However, although both Lac/Pyh and Ala/Pyh slightly increased after CCl₄ (Lac/Pyh: from 0.64 ± 0.18 to 0.75 ± 0.12 , Ala/Pyh: from 0.49 ± 0.11 to 0.53 ± 0.06 in Figure 4) the change was not significant (Lac/Pyh: $p=0.14$, Ala/Pyh: $p=0.26$), most likely due to the small sample size.

A downside of using [2-¹³C]Pyr instead of [1-¹³C]Pyr is that for both [2-¹³C]Lac and [2-¹³C]Ala, the C2 carbon has a directly bonded proton. The stronger proton-carbon coupling not only leads to line splitting (2 loss in SNR for a doublet) but also to shorter T₁s compared to the case the product molecules are labelled in the C1 position. Using 90° excitations for the products, as is the goal when assessing the saturation kinetics, will mitigate the detrimental effect of the shorter T₁ as it reduces the time the magnetization is aligned the longitudinal axis.

As a surface coil was used for both signal excitation and reception in the ¹³C experiments, the RF excitation profile was inhomogeneous. This can be improved by using a volume transmit coil with greater B₁ homogeneity in combination with a surface coil for reception only.

While the proposed method was developed for a clinical field strength of 3 T it should also be applicable at higher field strengths such as 7 T. The larger dispersion of the chemical shift should be beneficial when designing the RF pulses with differential flip angles. However, in contrast to MRI of thermal equilibrium magnetization, higher field strengths do not necessarily improve SNR in hyperpolarized MRI as the SNR is determined by the magnetization at the time of injection. In fact, SNR could be reduced as the T₁s of many ¹³C-labeled compounds decrease at higher field strengths.

Conclusions:

The presented results demonstrate the feasibility of simultaneous metabolic imaging of [2-¹³C]pyruvate and [1,4-¹³C₂]fumarate using frequency band selective excitation without chemical shift displacement artifacts. The large spectral dispersion of resonances was divided into two frequency sub-bands and acquired in an alternate fashion to obtain dynamic time course information of various metabolites. The optimized spectral width of only 258 Hz was sufficient to separate each individual metabolite in the different sub-bands permitting a temporal resolution of 5 s using the clinical gradient system. The method was successfully applied to measure the different effects of CCl₄-induced liver damage in rats in-vivo. The ability to obtain these data in a single bolus injection should prove particularly important in the eventual translation to clinical studies of liver injury, e.g., nonalcoholic steatohepatitis, for which multiple bolus injections are likely infeasible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Golman K, Zandt R, Thaning M. Real-time metabolic imaging. *Proc Natl Acad Sci USA*. 2006;103(30):11270–11275. [PubMed: 16837573]
2. Ardenkjær-Larsen JH, Fridlund B, Gram A, et al. Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR. *Proc Natl Acad Sci USA*. 2003;100:10158–10163. [PubMed: 12930897]
3. Gallagher FA, Kettunen MI, Hu De-En, et al. Production of hyperpolarized [1,4-¹³C₂]malate from [1,4-¹³C₂]fumarate is a marker of cell necrosis and treatment response in tumors. *Proc Natl Acad Sci USA*. 2009;106(47):19801–19806. [PubMed: 19903889]
4. Guglielmetti C, Najac C, Didonna A, et al. Hyperpolarized ¹³C MR metabolic imaging can detect neuroinflammation in-vivo in a multiple sclerosis murine model. *Proc Natl Acad Sci USA*. 2017; 114(33):E6982–E6991. [PubMed: 28760957]
5. Xu HN, Kadlecsek S, Shaghghi H, et al. Differentiating inflamed and normal lungs by the apparent reaction rate constants of lactate dehydrogenase probed by hyperpolarized ¹³C labelled pyruvate. *Quant Imag Med Surg*. 2016;6(1):57–66.
6. Merritt ME, Harrison C, Sherry AD, Malloy CR, Burgess SC. Flux through hepatic pyruvate carboxylase and phosphoenolpyruvate carboxykinase detected by hyperpolarized ¹³C magnetic resonance. *Proc Natl Acad Sci USA*. 2011;108(47):19084–9. [PubMed: 22065779]
7. Park JM, Recht LD, Josan S, et al. Metabolic response of glioma to dichloroacetate measured in vivo by hyperpolarized ¹³C magnetic resonance spectroscopic imaging. *Neuro Oncol*. 2013;15(4):433–41. [PubMed: 23328814]
8. Chen H-Y, Larson PEZ, Bok RA, et al. Assessing Prostate Cancer Aggressiveness with Hyperpolarized Dual-Agent 3D Dynamic Imaging of Metabolism and Perfusion. *Can Res*. 2017;77(12):3207–3216.
9. Nelson SJ, Kurhanewicz J, Vigneron DB, et al. Metabolic imaging of patients with prostate cancer using hyperpolarized [1-¹³C]pyruvate. *Sci Transl Med*. 2013;14:198ra108.

10. Park Ilwoo, Larson Peder E. Z., Zierhut Matthew L., et al. Hyperpolarized ^{13}C magnetic resonance metabolic imaging: application to brain tumors. *Neu Onco.* 2010;12(2):133–144.
11. Aggarwal R, Vigneron DB, Kurhanewicz J. Hyperpolarized $[1-^{13}\text{C}]$ Pyruvate Magnetic Resonance Imaging Detects an Early Metabolic Response to Androgen Ablation Therapy in Prostate Cancer. *Eur Urol.* 2017;72(6):1028–1029. [PubMed: 28765011]
12. Cunningham CH, Lau JY, Chen AP, et al. Hyperpolarized ^{13}C metabolic MRI of the human heart: initial experience. *Circ Res.* 2016;119(11):1177–1182. [PubMed: 27635086]
13. Golman K, Petersson JS, Magnusson P, et al. Cardiac metabolism measured noninvasively by hyperpolarized ^{13}C MRI. *Magn Reson Med.* 2008;59:1005–1013. [PubMed: 18429038]
14. Schroeder MA, Cochlin LE, Heather LC, Clarke K, Radda GK, Tyler DJ. In vivo assessment of pyruvate dehydrogenase flux in the heart using hyperpolarized carbon-13 magnetic resonance. *Proc Natl Acad Sci USA.* 2008;105:12051–12056. [PubMed: 18689683]
15. Schroeder MA, Clarke K, Neubauer S, Tyler DJ. Hyperpolarized magnetic resonance A novel technique for the in-vivo assessment of cardiovascular disease. *Circ.* 2011;124:1580–1594.
16. Niles DJ, Gordan JW, Huang G, et al. Evaluation of renal metabolic response to partial ureteral obstruction with hyperpolarized ^{13}C MRI. *NMR in Biomed.* 2018;31:e3846.
17. DeVience SJ, Lu X, Proctor J, et al. Metabolic imaging of energy metabolism in traumatic brain injury using hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *Sci. Rep* 2017;7(1):1907. [PubMed: 28507314]
18. Josan S, Billingsley K, Orduna J, et al. Assessing inflammatory liver injury in an acute CCl_4 model using dynamic 3D metabolic imaging of hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *NMR in Biomed.* 2015;28:1671–1677.
19. Eldirdiri A, Clemmensen A, Bowen S, Kjær A, Ardenkjær-Larsen JH. Simultaneous imaging of hyperpolarized $[1,4-^{13}\text{C}_2]$ fumarate, $[1-^{13}\text{C}]$ pyruvate and ^{18}F -FDG in a rat model of necrosis in a clinical PET/MR scanner. *NMR in Biomed.* 2017:e3803.
20. Clatworthy MR, Kettunen MI, Hu DE, et al. Magnetic resonance imaging with hyperpolarized $[1,4-^{13}\text{C}_2]$ fumarate allows detection of early renal acute tubular necrosis. *Proc. Natl. Acad. Sci. USA* 2012;109(33):13374–13379. [PubMed: 22837393]
21. Zandt R, Karlsson M, Gisselsson A, Jensen PR, Hansson G, Lerche M. Acute Liver Failure Studied by Hyperpolarized $[1,4-^{13}\text{C}_2]$ Fumarate in CCl_4 Injured Rat Liver In Proceedings of the 17th Annual Meeting of ISMRM, Hawaii, USA, 2009 p. 4380.
22. Larson PEZ, Kerr AB, Chen AP, et al. Multiband excitation pulses for hyperpolarized ^{13}C dynamic chemical-shift imaging. *J Mag Reson.* 2008;194(1):121–127.
23. Xu T, Mayer D, Gu M, et al. Quantification of in vivo metabolic kinetics of hyperpolarized pyruvate in rat kidneys using dynamic ^{13}C MRSI. *NMR in Biomed.* 2011;24(8):997–1005.
24. Josan S, Hurd R, Park JM, et al. Dynamic Metabolic Imaging of Hyperpolarized $[2-^{13}\text{C}]$ Pyruvate using Spiral Chemical Shift Imaging with Alternate Spectral Band Excitation. *Mag Reson Med.* 2014;71(6):2051–2058.
25. Reeder SB, Wen Z, Yu H, et al. Multicoil Dixon chemical species separation with an iterative least-squares estimation method. *Mag Reson Med.* 2004;51(1):35–45.
26. Wiesinger F, Weidl E, Menzel MI, et al. IDEAL spiral CSI for dynamic metabolic MR imaging of hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *Mag Reson Med.* 2012;68:8–16.
27. Pauly J, Le Roux P, Nishimura D, Macovski A, Parameter relations for the Shinnar-Le Roux selective excitation pulse design algorithm [NMR imaging]. *IEEE Trans Med Imag.* 1991;10(1):53–65.
28. Mayer D, Yen Y, Josan S, Park JM, Pfefferbaum A, Hurd RE, Spielman DM. Application of hyperpolarized $[1-^{13}\text{C}]$ lactate for the *in vivo* investigation of cardiac metabolism. *NMR Biomed.* 2012;25: 1119–1124. [PubMed: 22278751]
29. Chronik B, Alejski A, Rutt BK. Design and fabrication of a three-axis multilayer gradient coil for magnetic resonance microscopy of mice. *Mag Reson Mat Phy Bio Med.* 2000;10:131–146.
30. Vaziri S, Lustig M. The Fastest Arbitrary k-space Trajectories In Proceedings of the 20th Annual Meeting of ISMRM, Melbourne, Australia, 2012 p. 2284.
31. Mayer D, Levin YS, Hurd RE, Glover GH, Spielman DM. Fast metabolic imaging of systems with sparse spectra: application for hyperpolarized ^{13}C imaging. *Mag Reson Med.* 2006;56(4):932–937.

32. Pocker Y, Meany JE, Nist BJ, Zadorojny C. Reversible hydration of pyruvic acid. I. Equilibrium studies. *J Chem Phys.* 1969;73(9):2879–2882.

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RF pulses and their spectral profiles

Figure 1:

Spectrally-selective multiband Hamming windowed-sinc RF *Fum*-band (A) and *Pyh*-band (C) pulses and their log-scale frequency profiles (B) and (D) with the passband in the *Fum* sub-band placed near the *Fum* peak and in the *Pyh* sub-band, centered near the down-field peak of the *Lac* doublet. The spectral profiles were calculated for the case of a 90° excitation on the respective metabolic products. The *Fum* sub-band pulse applies a differential flip angle at *Fum* and *Mal* peaks in the ratio 1:4 whereas the *Lac* sub-band pulse applies the same flip angle for *Pyh*, *Lac*, and *Ala* peaks. In both bands, the signal from the $[2-^{13}\text{C}]\text{Pyr}$ is suppressed.

Figure 2:

(A) Number of signal averages (NSA) with 9 equally spaced echoes for the resonances excited in the Fum sub-band (Fum and Mal doublet) and (B) Same as (A) but for the resonances excited in the Pyh sub-band (Pyh and the doublets of Lac and Ala). Based on these calculations, an echo spacing of 3.88 ms (~ 258 Hz) allows for resolving all resonances.

Figure 3:

In vivo 3D-spCSI data (FOV=5×5×5cm³, 10×10×10 matrix, 2.5 s per band) from a rat with CCl₄ - induced liver damage. (A) Time-averaged spectrum of the Fum sub-band from a liver ROI. Detection of the Mal doublet indicates necrosis of the liver. (B) Corresponding spectrum from the Lac sub-band. All resonances from Pyh, Lac, and Ala were well separated. Aliased signals required separate reconstructions to properly demodulate the phase caused by off-resonance. The number next to a peak corresponds to the number of times the resonance has been aliased with a negative sign indicating a smaller chemical shift.

Figure 4:

(A) Time-averaged metabolic maps from the data shown in Figure 3 of a slice through the liver superimposed onto corresponding ^1H MRI image. Whereas the metabolic products are only detected in the liver, the Fum and Pyh signals were also visible in the vasculature. (B) In vivo metabolite time courses after injection of co-polarized Pyr/Fum from a liver ROI in a rat one day after administration of CCl_4 .

Figure 5:

Product-to-substrate ratios averaged over ROIs in the liver from animals pre- and post-CCl₄. No Mal signal was detected before CCl₄ administration whereas a Mal/Fum ratio of about 0.6 after CCl₄ indicated liver necrosis. Lac/Pyh and Ala/Pyh ratios were slightly increased after CCl₄ but the change was not statistically significant.