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***In vivo* hyperpolarized ^{13}C MR spectroscopic imaging with ^1H decoupling**

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Abstract

Application of ^{13}C MRS *in vivo* on whole body MR system has been limited due to the low static field (and consequent low signal to noise ratio—SNR) of these scanners; thus there have been few reports of ^1H decoupled ^{13}C MRS *in vivo* using a clinical MR platform. The recent development of techniques to retain highly polarized spins in solution following DNP in a solid matrix has provided a mechanism to use endogenous pre-polarized ^{13}C labeled substrates to study real time cellular metabolism *in vivo* with high SNR. In a recent *in vivo* hyperpolarized metabolic imaging study using ^{13}C pyruvate, it has been demonstrated that the line shape (signal decay) of the resonances observed are greatly affected by J_{CH} coupling in addition to inhomogeneous broadening. This study demonstrates the feasibility of improving hyperpolarized ^{13}C metabolic imaging *in vivo* by incorporating ^1H decoupling on a clinical whole body 3 T MR scanner. No reduction of T_1 of a pre-polarized ^{13}C substrate ([1- ^{13}C] lactate) in solution was observed when ^1H decoupling was applied with WALTZ16 sequence. Narrower linewidth for the [1- ^{13}C] lactate resonance was observed in hyperpolarized ^{13}C MRSI data *in vivo* with ^1H decoupling.

Keywords

DNP; Hyperpolarized ^{13}C MR metabolic imaging; ^1H decoupling; T_1 ; Clinical MR system

1. Introduction

^1H decoupling for ^{13}C MRS experiments is performed routinely on analytical NMR systems for both simplification of the NMR spectrum as well as enhancement of peak amplitude signal to noise. It has also been utilized in *in vivo* MRS experiments with dedicated high field animal MR systems [1]. Application of ^{13}C MRS *in vivo* on whole body MR systems has been limited due to the low static field of these scanners (hence lower sensitivity). Thus there have been few reports of ^1H decoupled ^{13}C MRS *in vivo* using a clinical MR platform [2,3].

The recent development of techniques to retain highly polarized spins in solution from DNP [4] has provided a mechanism to use endogenous pre-polarized substrates to study real time cellular metabolism *in vivo* [5]. It has been shown following injection of pre-polarized [1- ^{13}C] pyruvate, that its metabolic products [1- ^{13}C] lactate, [1- ^{13}C] alanine and [1- ^{13}C]

bicarbonate can be observed *in vivo* [5,6]. In a recent *in vivo* hyperpolarized metabolic imaging study using ^{13}C pyruvate, it was demonstrated that the line shape (signal decay) of the resonances observed were greatly affected by J_{CH} coupling in addition to inhomogeneous broadening ($T2^*$) [6]. Since the ^{13}C spectrum in this particular application is fairly sparse, the broadened lines do not affect the ability to identify and quantify each of the metabolite resonances individually. However, as the pre-polarized substrate and DNP applications move beyond $[1-^{13}\text{C}]$ pyruvate, stronger proton-carbon coupling and smaller chemical shift difference between metabolites may hinder interpretation and quantification of the data.

One important consideration for ^{13}C MRS or MRSI experiments is the longitudinal relaxation time of the pre-polarized substrate as well as its metabolite products. A previous study reported a reduction of $T1$ s of ^{13}C spins in non-hyperpolarized substrates when conventional ^1H decoupling scheme (such as WALTZ16 [7]) was applied [8]. In the present study, the effect of ^1H decoupling on the $T1$ of a pre-polarized ^{13}C substrate was investigated at both low and high magnetic fields. This study also examined the feasibility of improving hyperpolarized ^{13}C metabolic imaging *in vivo* by incorporating ^1H decoupling on a clinical whole body 3 T MR scanner.

2. Methods

2.1. MR hardware

All *in vivo* and some solution studies of the pre-polarized ^{13}C metabolite $T1$ measurement in solution were performed using a 3 T GE SignaTM scanner (GE Healthcare, Waukesha, WI) equipped with the MNS (multinuclear spectroscopy) hardware package. This system was also equipped with a separate ^1H RF channel for decoupling. The ^1H decoupling unit was programmed with WALTZ16 pulse sequence and allowed bi-level irradiation. The RF coil used in the 3 T experiments was a custom build dual-tuned ^1H - ^{13}C birdcage coil with a quadrature ^{13}C channel and linear ^1H channel constructed based on an earlier design [9]. The inner coil diameter was 8 cm and the length of the coil was 9 cm. A Varian INOVA 14.1 T spectrometer (Varian, Palo Alto, CA) equipped with an 8 mm $^{31}\text{P}/^{13}\text{C}$ dual-tuned probe with ^1H decoupling outer coil was also used for pre-polarized ^{13}C metabolite $T1$ measurements in solution.

2.2. Polarizer and compound

A HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK) was used in this study. Two different preparations of ^{13}C samples were polarized and dissolved in this study: first was a mixture $[1-^{13}\text{C}]$ lactate (Isotec, Miamisburg, OH), water, DMSO and OX063 trityl radical (Oxford Instruments, Abingdon, UK). The mixture contained 38.5% $[1-^{13}\text{C}]$ -lactate and 30% DMSO. The trityl radical concentration was 15 mM. The second preparation, used in the *in vivo* experiments was a mixture of neat (99% purity) $[1-^{13}\text{C}]$ pyruvic acid (Isotec) and 15 mM of OX063 trityl radical. Both mixtures were polarized in a field of 3.35 T at approximately 1.4 K by irradiation of microwaves (94.116 GHz for lactate mixture, and 94.113 GHz for pyruvic acid mixture) similar to what was described previously [4]. Each ^{13}C lactate sample ($n = 9$) was polarized for ~100 min prior to dissolution with a 100 mM phosphate buffer (50 μl of lactate mixture/5 ml of buffer). Each $[1-^{13}\text{C}]$ pyruvic acid sample (two for each animal, four total) was polarized for ~60 min prior to dissolution with 40 mM TRIS buffered NaOH solution (32 μl of pyruvic acid mixture/~5 ml of buffer) that gave a nominal final pH of 7.4 [6].

2.3. Simulation

Simulated $[1-^{13}\text{C}]$ lactate data with and without J_{CH} coupling were created in SAGE software (GE Healthcare, Waukesha, WI). One dataset was created with $T2^*$ of 200 ms (1.6 Hz linewidth) without J_{CH} coupling and the other dataset was created with $T2^*$ of 32 ms (10 Hz

linewidth) without J_{CH} coupling. Area under the $T2^*$ decay curve were calculated for both datasets without and with the presence of J_{CH} coupling to estimated the SNR benefit of $[1-^{13}C]$ lactate data acquired with 1H decoupling vs. without 1H decoupling (3.33 Hz for methine-carbonyl coupling, and 4.1 Hz for methyl-carbonyl coupling, empirically measured at 9.4 T, data not shown).

2.4. $T1$ measurement of pre-polarized ^{13}C metabolite in solution

Experiments to estimate the $T1$ of pre-polarized $[1-^{13}C]$ lactate with and without decoupling were conducted at both 14.1 and 3 T. A small tip angle (five degree in all experiments) pulse-acquire pulse sequence was used on both platforms. For measurements at 3 T, 64 spectra were acquired at interval of 3 s ($TR = 3$ s) and sampling duration was 1.638 s (5000 Hz spectral bandwidth, 8192 pts). Four $T1$ measurements of pre-polarized $[1-^{13}C]$ lactate were performed at 3 T: without decoupling ($n = 2$) and with WALTZ 16 1H decoupling ($n = 2$). A syringe filled with 1.77 M $[1-^{13}C]$ lactate solution was tested prior to the experiments with the pre-polarized $[1-^{13}C]$ lactate to determine the decoupling parameters, The WALTZ-16 sequence was used with a 90 pulse-width of 1.25 ms and power of 30 W during acquisition. Using the bi-level irradiation feature of this 1H RF channel, decoupling power was attenuated by 10 dB during non-sampling portion of the pulse sequence. The center frequency used for decoupling was 127.76315 MHz (between the methine and the methyl protons of lactate). For measurements at 14.1 T, 64 spectra were acquired at intervals of 3.5 s ($TR = 3.5$ s) and sampling duration was 2 s (16,000 Hz spectral bandwidth, 32,000 pts). Five $T1$ measurements of pre-polarized $[1-^{13}C]$ lactate were performed at 14.1 T: without decoupling ($n = 2$), WALTZ16 1H decoupling during sampling only ($n = 1$), and WALTZ16 1H decoupling continuously ($n = 2$) during the entire experiment. The WALTZ16 sequence had a 90° pulse duration of 29.2 μs for a pre-calibrated power setting at 14.1 T. To estimate the longitudinal relaxation time, ^{13}C lactate peak integrals were plotted as a function of time and the signal decay curve was fitted to the equation: $I_{(i)} = I_0 \cos^{(i-1)} \theta e^{-(i-1)TR/T1}$, where $I_{(i)}$ is the signal measured at each time point, I_0 is the signal of the first point, θ is the RF tip angle [10]. The fitting was performed using Marquardt's non-linear least square routine as implemented in MATLAB software (The MathWorks Inc., Natick, MA). For the 3 T measurements, the entire sample was within the detection volume of the RF coil, for the 14.1 T measurements, most of the sample was within the detection volume of the coil. Shimming was performed manually in all *in vitro* experiments using a syringe (3 T) or an nmr tube (14.1 T) filled with non-hyperpolarized $[1-^{13}C]$ lactate solution.

2.5. In vivo ^{13}C MRSI experiments

All animal experiments followed a protocol approved by the UCSF institutional animal research committee. Two hyperpolarized ^{13}C MRSI experiments were performed on each of the two normal male Sprague–Dawley rats included in the study. For each animal, the MRSI experiments were performed following two separate (1 h apart) tail vein injections of 2.5 ml/100 mM of pre-polarized $[1-^{13}C]$ pyruvate, with 1H decoupling performed in one of the two experiments. Care was taken to ensure the body temperature of the animal was constant throughout the imaging procedures by maintaining a flow of heated water through the pad supporting the rat. Both the oxygen saturation and pulse rate of the animal was continuously monitored to ensure that the animals were under similar apparent physiological state for the two injections. Shim current values were kept the same throughout the experiment for a given animal. Shim current values were set by the standard auto-shimming routine on the MRI system performed during the 1H anatomical scans, as endogenous natural abundant ^{13}C spins in a normal rat was inadequate for shimming. A double spin–echo pulse sequence with a flyback echo-planar readout trajectory was used in the experiments with $TE = 35$ ms and $TR = 160$ ms (Fig. 1) [11]. Spectral resolution was 9.85 Hz/pts (101.5 ms sampling time, 581 Hz spectral bandwidth, 59 pts). The spatial encoding matrix was $12 \times 8 \times 16$, and FOV was 72 mm \times 48

mm × 96 mm, resulted in (6 mm)³ spatial resolution. The acquisition started 35 s after the beginning of the injection (injection duration: 12 s) [6,12]. The total acquisition time was ~15 s. The WALTZ16 parameters used in the *in vivo* experiments were the same as what was described above in the *in vitro* experiments at 3 T. Peak amplitude and linewidth (FWHM) of both ¹³C lactate and ¹³C pyruvate resonances were measured (from all voxels within the rat body) and compared between experiments with and without ¹H decoupling. Liquid state polarizations were presumed to be the same for the two experiments in each animal at the time of injection. The paired *T*-test was used to examine if the changes in peak amplitude and linewidth were significant for the experiments with ¹H decoupling as compared to those without.

3. Results

The simulated spectrum of [1-¹³C] lactate with 200 ms *T*₂^{*} and *J*_{CH} coupling is shown in Fig. 2 (upper left). The corresponding *T*₂^{*} decay curves with and without the *J*_{CH} coupling (Fig. 2, upper right) demonstrated an SNR benefit of ¹H decoupling even when a short sampling duration was used. More specifically, when 100 ms of the FID was sampled, the integral under the *T*₂^{*} curve is was 78% higher with ¹H decoupling as compared to without decoupling; when only 50 ms of the FID was sampled, the integral under the curve with ¹H decoupling was still 24% higher. [1-¹³C] lactate was also simulated as a peak with 10 Hz Gaussian line shape (32 ms *T*₂^{*}, Fig. 2, lower left). In this case, when 50 ms of the FID was sampled, a 19% gain with ¹H decoupling was attained when ¹H decoupling was used as compared to without ¹H decoupling (Fig. 2, lower right).

Representative data from the *in vitro* *T*₁ measurement of pre-polarized [1-¹³C] lactate at 3 T are summarized in Fig. 3. The effect of ¹H decoupling was demonstrated in the representative free induction decay and spectra from both experiments (Fig. 3, upper and middle plots). Average lactate linewidth (FWHM) for the experiments at 3 T with ¹H decoupling was 2.4 Hz and that for the experiments without ¹H decoupling was 12.5 Hz. The *T*₁ estimated from the experiment with WALTZ16 decoupling was approximately 50.5 s (50.6 and 50.4 s in repeated experiments) and it was approximately 48.3 s (48.1 and 48.4 s) for the experiment without ¹H decoupling (Fig. 3, lower plot). Data from *T*₁ measurement of pre-polarized [1-¹³C] lactate at 14.1 T are summarized in Fig. 4. A shortening of *T*₁ was observed at higher field. *T*₁s of pre-polarized lactate were approximately 33.0 (average of 33.4 and 32.7 s), 34.8 and 35.1 s (average of 35.0 and 35.2 s), respectively, for experiments without ¹H decoupling, with WALTZ16 decoupling RF irradiation during sampling only and decoupling RF irradiation continuously. The lactate carbonyl linewidth for the experiments at 14.1 T with ¹H decoupling was 2.3 Hz and that for the experiments without ¹H decoupling was 13.0 Hz. The average *R*² values of the signal decay curve fit was >0.99.

Representative data from ¹³C MRSI acquired in one normal rat are shown in Fig. 5. In some voxels within the volume imaged, narrower linewidth for [1-¹³C] lactate was clearly observed in the experiment with ¹H decoupling as compare to experiments without decoupling. On average for both animals (average over all voxels), ¹³C lactate linewidth was 7% narrower (10.9 Hz with decoupling, 11.8 Hz without decoupling) and ¹³C pyruvate was 4% narrower (12.0 Hz with decoupling, 12.5 Hz without decoupling) for the experiments with decoupling as compared to the experiment without decoupling. The standard deviation of the lactate linewidth was 4.9 Hz and the standard deviation of the pyruvate linewidth was 5.8 Hz over all the voxels in the animals; this high variability was presumably due to the large variation of local *B*₀ within the rat body. A 16% higher peak amplitude SNR (2.06 vs. 1.77) for ¹³C lactate resonance was also observed for the ¹H decoupled experiment, while peak amplitude SNR for ¹³C pyruvate was 2% lower (3.91 vs. 4.01). For both of the animals in the study, the change in lactate linewidth was statistically significant (*p* = 0.04), while the change in pyruvate

linewidth was not ($p = 0.11$). And the change in SNR was not significant for either the lactate ($p = 0.19$) or the pyruvate resonances (0.42).

4. Discussion

Proton decoupling for ^{13}C MRS/MRSI experiments can provide higher peak amplitude SNR and help resolve overlapping resonances in some applications. It has been reported, however, that spin-lattice relaxation times of carbonyl carbons measured with fast small tip angle experiments can be substantially reduced by the application of the WALTZ ^1H decoupling scheme [8]; and the effect is particularly pronounced when the decoupling sequence irradiation was applied continuously during the experiment. If this phenomenon is also true for pre-polarized carbonyl ^{13}C spins, it would greatly affect one's approach to ^{13}C DNP MRS/MRSI experiments when ^1H decoupling is desired.

In this study, the spin-lattice relaxation time of the carbonyl carbon on pre-polarized ^{13}C lactate was estimated by the same fast small tip angle approach mentioned above, with and without the application of WALTZ16 ^1H decoupling during the experiment. The aim of these measurements was to investigate if proton decoupling negatively affected the T_1 relaxation of the pre-polarized spins *in vivo* (such as $[1-^{13}\text{C}]$ lactate). In data acquired at both 14.1 and 3 T, no reduction of T_1 was observed when ^1H decoupling was applied, although a shortening of the $[1-^{13}\text{C}]$ lactate T_1 was observed at the higher field as compared to lower field, presumably due to increased influence of chemical shift anisotropy [13]. In addition, the T_1 for $[1-^{13}\text{C}]$ lactate was unchanged when ^1H decoupling RF was applied continuously or only during sampling.

This absence of any change in T_1 is rationalized as follows. Proton decoupling *can* alter the apparent relaxation rate, by driving the populations of proton sub-levels from their equilibrium values, provided that dipole-mediated cross-relaxation is strong enough to give rapid cross-relaxation between the carbon and proton magnetizations. In the instance presented in this study, however, the large proton-carbon inter-nuclear distance precludes rapid cross-relaxation (and, in consequence, any large Overhauser enhancement). The comparative isolation which results between proton and carbon magnetizations also ensures that the dipolar cross correlation effects, which are predicted [14] to survive decoupling, will be likewise be of small importance.

Then, given that the carbonyl carbon of lactate interacts but weakly with protons and relaxes slowly, compared to a methyl or methylene carbon, its T_1 is expected to be long, and also relatively immune to decoupling effects. The long T_1 is in fact essential to the successful use of hyperpolarized substrates *in vivo*, and is correlated, not incidentally, with the weak J_{CH} scalar coupling, given that both types of coupling—through bond, and through space—grow larger as the inter-nuclear distance decreases. It is expected that the T_1 of a methyl carbon should be more sensitive to decoupling than that of the lactate carbonyl, simply because the proximity of the carbon and hydrogen in the methyl group, and the resulting increase in cross-relaxation.

Although the effect of ^1H decoupling on $[1-^{13}\text{C}]$ pyruvate was not measured, but since the J_{CH} coupling for the $[1-^{13}\text{C}]$ pyruvate carbonyl is even less than that of $[1-^{13}\text{C}]$ lactate, it is likely that the T_1 of $[1-^{13}\text{C}]$ pyruvate will not be shortened by ^1H decoupling either. It is also not clear what the role of NOE plays in the presence of large non-equilibrium polarization of ^{13}C nuclei, theoretical modeling and more experimental investigation may be necessary to completely understand our observation [15].

This study also demonstrated the feasibility of applying ^1H decoupling for hyperpolarized ^{13}C MRSI on a standard clinical 3 T MR system. The spectral resolution of the

experiment was limited due to the speed of acquisition required for *in vivo* hyperpolarized MRSI experiments; this limited the accuracy of the linewidth measurements in these *in vivo* studies. Both the limited spectral resolution and the various tissue type within the imaging volume (B_0 variation) contributed to the large standard deviation of the linewidth measurements. But as demonstrated by the simulation (Fig. 2), one would expect a SNR benefit with ^1H decoupling for resonances having linewidths similar to the *in vivo* lactate carbonyl (10 Hz), even when using short data acquisition times (50 ms). In addition, clear linewidth changes were observed in some of the voxels (Fig. 5), and the overall average linewidth was also narrower for the ^1H decoupled experiment. In some voxels, ^1H decoupling did not demonstrate an observable effect; again a large slab through the rat body contains various tissue types, the inhomogeneous broadening ($T2^*$) may be the dominant decay mechanism in some voxels while J_{CH} coupling may be making more contribution in other voxels that have higher degree of local B_0 homogeneity, hence the differential response to decoupling. In future application involving larger animals and humans, where the regions of interest are confined to an organ or a specific tissue type (brain, liver or prostate), the overall benefit of ^1H decoupling should be more clearly observed. Furthermore, improved B_1 homogeneity of the ^1H coil used and a more appropriate phantom (similar loading compare to the subject) for optimizing decoupling may improve the efficacy of ^1H decoupling throughout the region of interest. Adiabatic decoupling pulse sequences were not available on the 3 T MR scanner used in this study, but utilizing such sequences could provide more uniform decoupling B_1 over the region of interest and potentially allow ^1H decoupling to be performed at lower power (lower SAR).

No significant increase in SNR was observed for either the $[1-^{13}\text{C}]$ pyruvate or the $[1-^{13}\text{C}]$ lactate resonances in the *in vivo* MRSI data. However, the change in lactate linewidth was significant. This is consistent with the larger ~ 3 Hz couplings from methine and methyl protons in lactate compared to the ~ 1.5 Hz couplings from methyl protons in the $[1-^{13}\text{C}]$ pyruvate case. The observed difference in SNR (although not significant) for $[1-^{13}\text{C}]$ lactate also was consistent with the simulation (Fig. 2, lower plots) for the linewidth and the readout duration used in these experiments. To better quantify the potential gain in SNR *in vivo* with ^1H decoupling, more studies (larger animals at 3 T or small animals using dedicated animal imager with high performance gradients) and accurate liquid state polarization measurements will need to be performed.

5. Conclusions

The potential and feasibility of performing ^1H decoupling for hyperpolarized ^{13}C MRSI experiment *in vivo* was demonstrated in this study in rats using a clinical 3 T MR system. With application of ^1H decoupling using the WALTZ16 sequence, we found no decrease of spin-lattice relaxation time of the pre-polarized substrate, and some improvement in the quality of ^{13}C MRSI data. ^1H decoupling for future *in vivo* applications with pre-polarized ^{13}C substrates in larger animals and humans may be an important consideration for optimized ^{13}C MRSI studies.

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References

1. Gruetter R, Adriany G, Choi I, Henry P, Lei H, Oz G. Localized *in vivo* ^{13}C NMR spectroscopy of the brain. *NMR Biomed* 2003;16:313–338. [PubMed: 14679498]
2. Ross B, Lin AP, Harris K. Clinical experience with ^{13}C MRS *in vivo*. *NMR Biomed* 2003;16:358–369. [PubMed: 14679500]

3. Saito M, Matsuda T, Tropp J, Inubushi T, Nakai T. Assessment of the specific absorption rate and calibration of decoupling parameters for proton decoupled carbon-13 MR spectroscopy at 3.0 T. *EJRAD* 2005;55:289–293.
4. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of >10, 000 times in liquid-state NMR. *Proc. Natl. Acad. Sci. USA* 2003;100(18):10158–10163. [PubMed: 12930897]
5. Golman K, Zandt R, Thaning M. Real-time metabolic imaging. *PNAS* 2006;103(30):11270–11275. [PubMed: 16837573]
6. Kohler SJ, Yen Y, Wolber J, Chen AP, Albers MJ, Bok R, Zhang V, Tropp J, Nelson S, Vigneron DB, Kurhanewicz J, Hurd RE. In vivo 13 carbon metabolic imaging at 3 T with hyperpolarized ¹³C-1-pyruvate. *Magn. Res. Med* 2007;58(1):65–69.
7. Shaka AJ, Keeler J, Frenkiel T, Freeman R. An improved sequence for broadband decoupling: WALTZ-16. *J. Magn. Reson* 1983;53(2):335–338.
8. Day IJ, Mitchell JC, Snowden MJ, Davis AL. Applications of DNP-NMR for the measurement of heteronuclear T1 relaxation times. *J. Magn. Reson* 2007;187:216–224. [PubMed: 17521933]
9. Derby K, Tropp J, Hawryszko C. Design and evaluation of a novel dual-tuned resonator for spectroscopic imaging. *J. Magn. Reson* 1990;86:645–651.
10. Patyal BR, Gao J, Williams RF, Roby J, Saam B, Rockwell BJ, Thomas RJ, Stolarski DJ, Fox PT. Longitudinal relaxation and diffusion measurements using magnetic resonance signals from laser-hyperpolarized 129Xe nuclei. *J. Magn. Reson* 1997;126:58–65. [PubMed: 9177796]
11. Cunningham CH, Chen AP, Albers MJ, Kurhanewicz J, Hurd RE, Yen Y, Nelson SJ, Vigneron DB. Double spin-echo sequence for rapid spectroscopic imaging of hyperpolarized 13C. *J. Magn. Reson* 2007;287(2):357–362. [PubMed: 17562376]
12. Chen AP, Albers P, Cunningham CH, Kohler SJ, Yen Y, Hurd RE, Tropp J, Bok R, Nelson SJ, Kurhanewicz J, Vigneron DB. Hyperpolarized C-13 spectroscopic imaging of the TRAMP mouse at 3 T-initial experience. *Magn. Res. Med* 2007;58(6):1099–1106.
13. Levy GC, Edlund U. Carbon-13 chemical shift anisotropy relaxation in organic compounds. *J. Am. Chem. Soc* 1975;97(17):5031–5032.
14. Werbelow LG, Grant DM. Proton-decoupled carbon-13 relaxation in ¹³CH₂ and ¹³CH₃ spin systems. *J. Chem. Phys* 1975;63(11):4742–4748.
15. Merritt ME, Harrison C, Mander W, Malloy CR, Sherry AD. Dipolar cross-relaxation modulates signal amplitudes in the ¹H NMR spectrum of hyperpolarized [¹³C]formate. *J. Magn. Reson* 2007;189(2):280–285. [PubMed: 17945520]

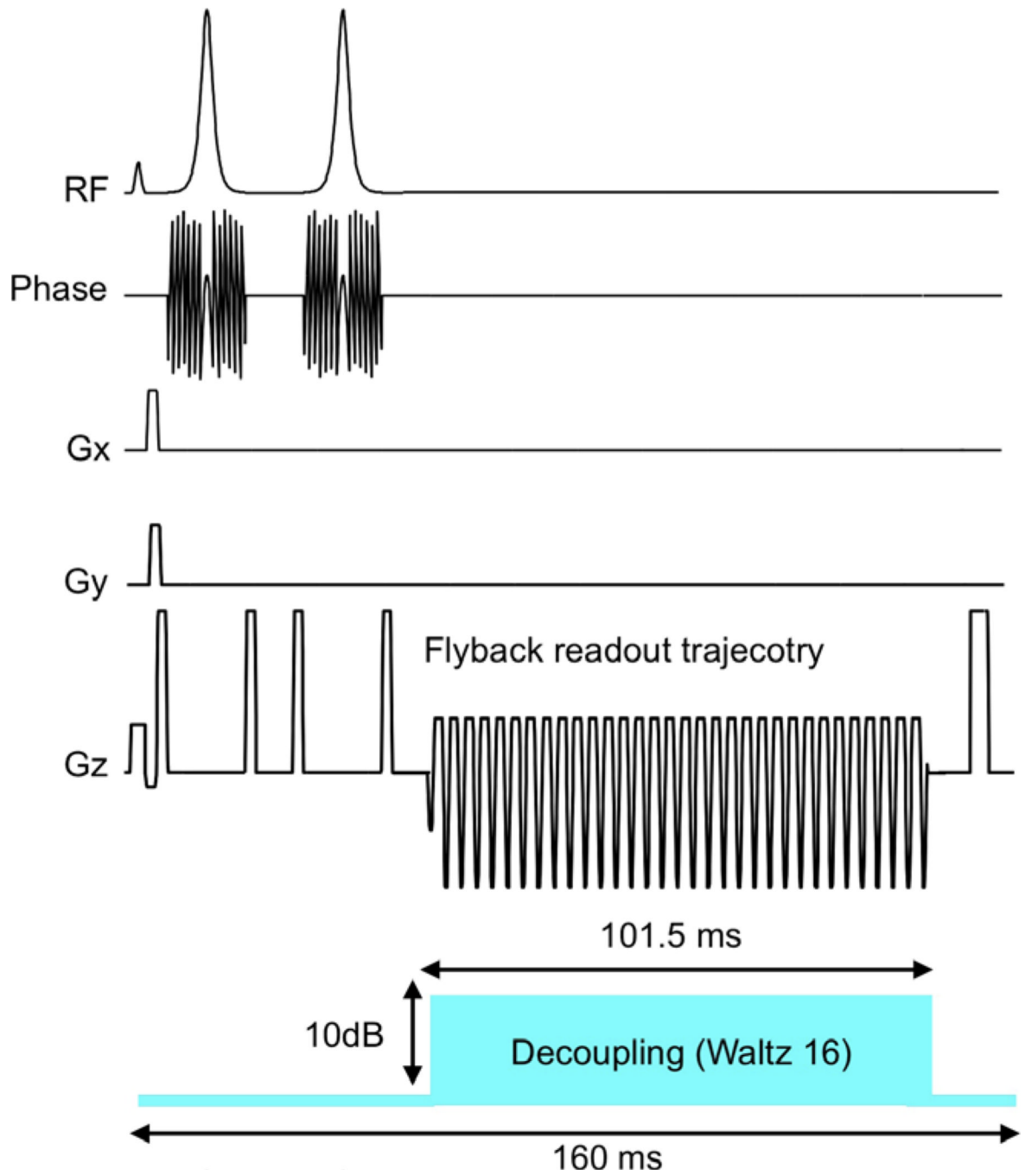


Fig. 1. ^{13}C pulse sequence (with ^1H decoupling) diagram for *in vivo* 3D MRSI experiment.

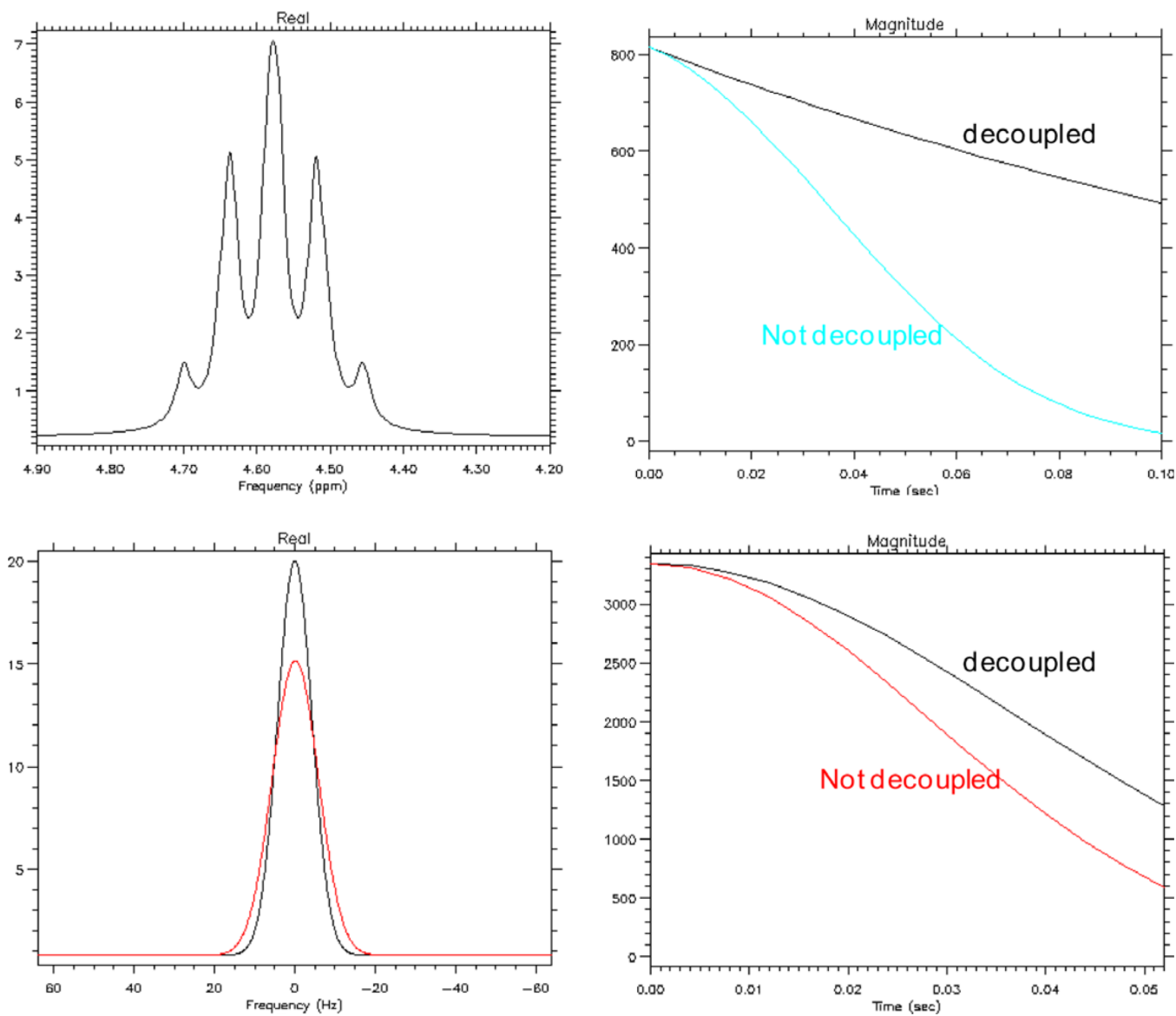


Fig. 2. Simulated $[1-^{13}\text{C}]$ lactate data with 200 ms T_2^* (spectrum: upper left, T_2^* decay curves: upper right) and 32 ms T_2^* (spectrum: lower left, T_2^* decay curves: lower right) are shown. When sampling 50 ms of the FID, in data with 200 ms T_2^* , ^1H decoupling improve the area under the curve by 24%; in data with 32 ms T_2^* , ^1H decoupling improve the area by 18%.

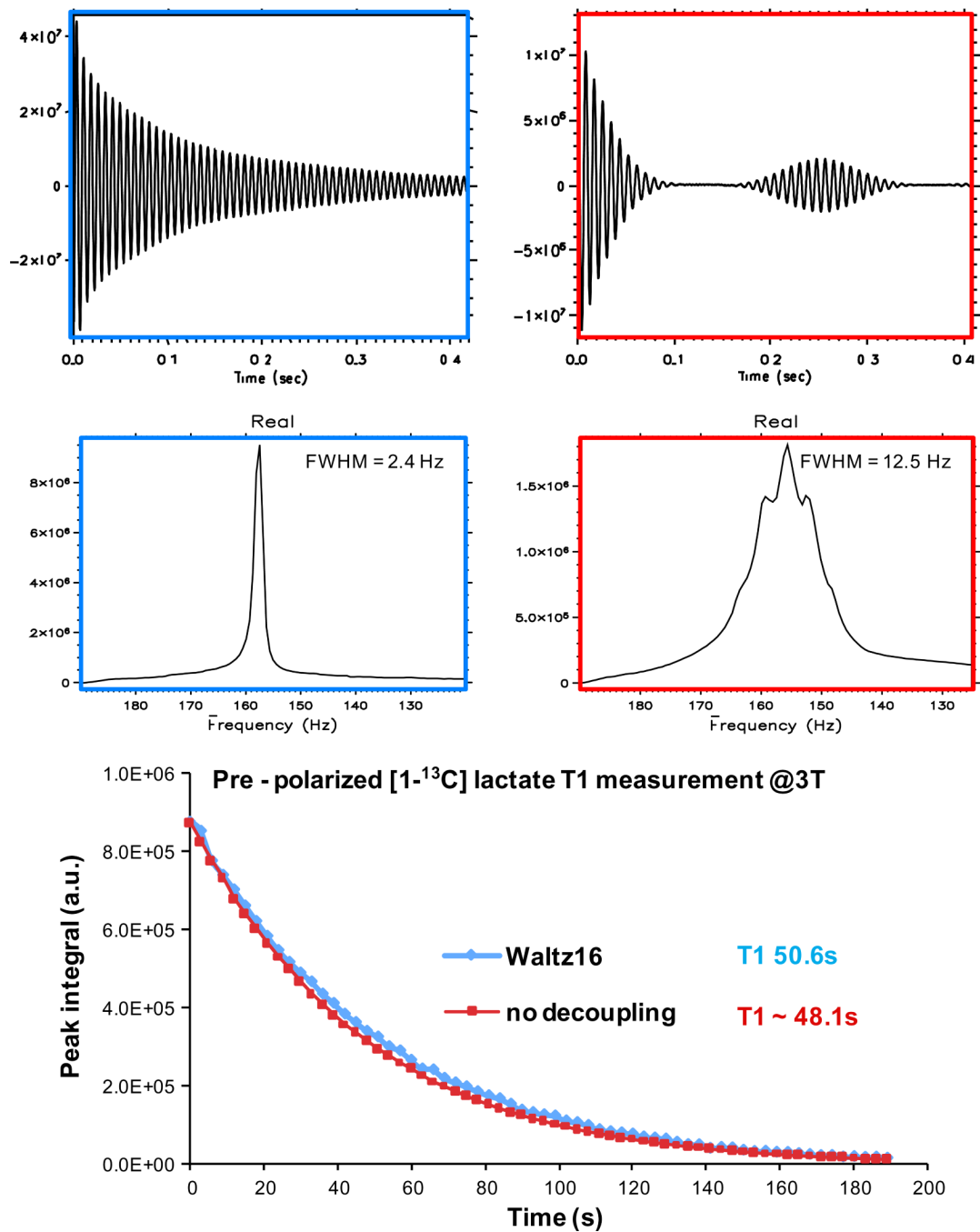


Fig. 3. *In vitro* T₁ measurement of pre-polarized [1-¹³C] lactate at 3 T. Representative FIDs and spectra from both the ¹H decoupled experiments (upper and middle left) and the experiments without decoupling are shown (upper and middle right). Signal decay curve from the representative data are plotted (lower plot), the two curves are normalized by the first time point of each experiment.

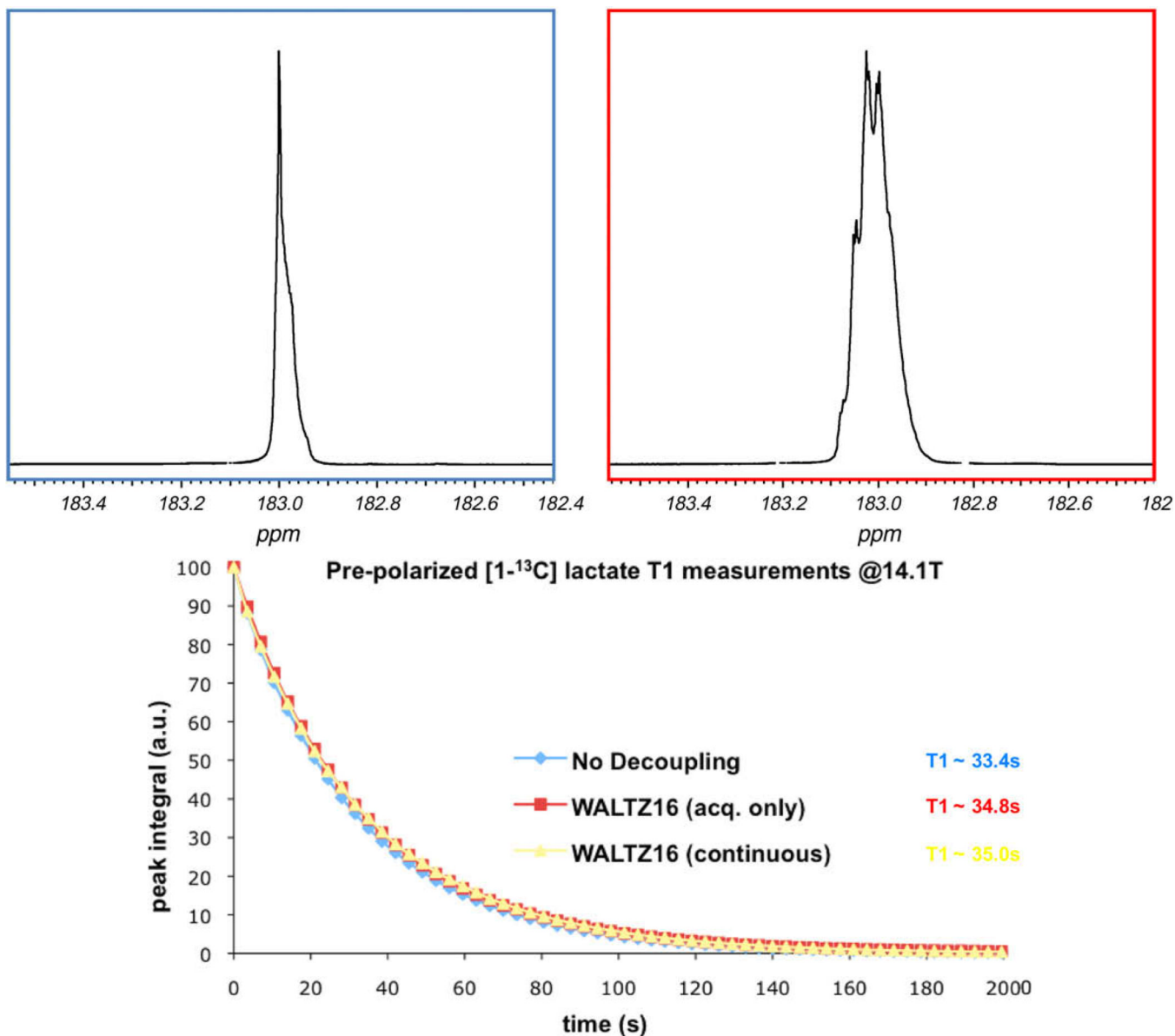


Fig. 4. Data from *in vitro* T1 measurement of pre-polarized [1-¹³C] lactate at 14.1 T. Representative spectra with ¹H decoupling (upper left) and without ¹H decoupling (continuous decoupling, upper right) are shown. Signal decay curves from the data were plotted (lower plot), the curves were normalized by the first time point of each experiment.

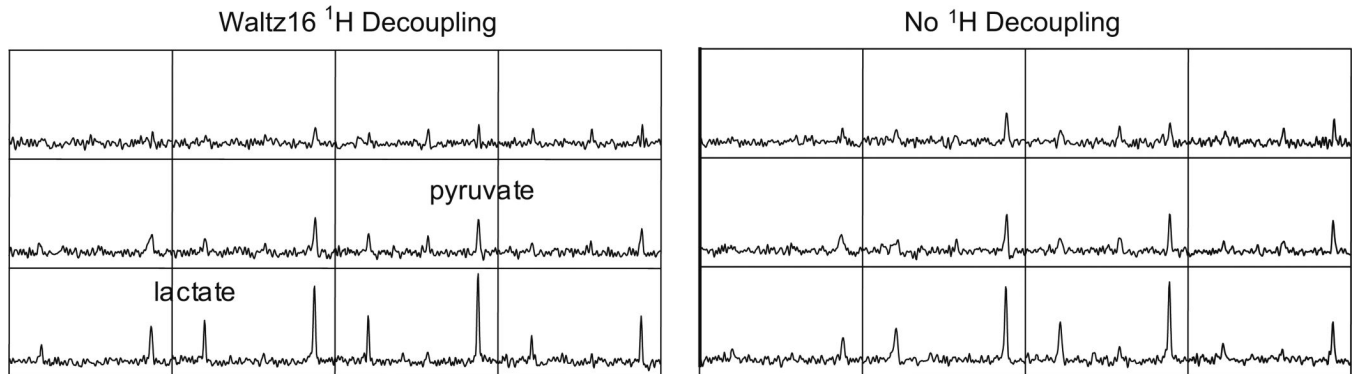
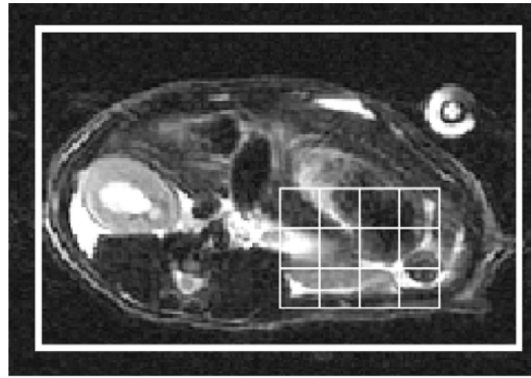


Fig. 5. Representative ^{13}C spectra obtained from a normal rate with and without ^1H decoupling following separate boluses of pre-polarized ^{13}C pyruvate. Change in linewidth of the ^{13}C lactate and pyruvate resonances were observed in some voxels with ^1H decoupling.