Published in final edited form as:

NMR Biomed. 2017 February ; 30(2): . doi:10.1002/nbm.3670.

Doublet asymmetry for estimating polarization in hyperpolarized ^{13}C -pyruvate studies

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Abstract

Hyperpolarized ^{13}C MRS allows in vivo interrogation of key metabolic pathways, with pyruvate (Pyr) the substrate of choice for current clinical studies. Knowledge of the liquid-state polarization is needed for full quantitation, and asymmetry of the C_2 doublet, arising from 1% naturally abundant $[1,2-^{13}\text{C}]\text{Pyr}$ in any hyperpolarized $[1-^{13}\text{C}]\text{Pyr}$ sample, has been suggested as a direct measure of in-vivo C_1 polarization via the use of an in-vitro calibration curve. Here we show that different polarization levels can yield the same C_2 -doublet asymmetry, thus limiting the utility of this metric for quantitation. Furthermore, although the time evolution of doublet asymmetry is poorly modeled using the expected dominant relaxation mechanisms of carbon-proton dipolar coupling and chemical shift anisotropy (CSA), the inclusion of a C-C dipolar coupling term can explain the observed initial evolution of the C_2 doublet asymmetry beyond its expected thermal equilibrium value.

Graphical abstract

Asymmetry of C_2 doublet has been suggested as a direct measure of the instantaneous C_1 polarization, a parameter necessary for quantitation in in-vivo hyperpolarized ^{13}C Magnetic Resonance Spectroscopy of $[1-^{13}\text{C}]$ Pyruvate. Here we show that different polarization levels can yield the same C_2 -doublet asymmetry. A relaxation model including chemical shift anisotropy, carbon-proton and carbon-carbon dipolar coupling can explain the observed evolution of the C_2 doublet asymmetry.

Keywords

Hyperpolarized; carbon-13; magnetic resonance spectroscopy; liquid-state polarization; pyruvate; polarization measurement; doublet; asymmetry

Introduction

Measurements of energy regulation and mitochondrial function are critically important for understanding cell metabolism in normal and diseased states, and MRS of hyperpolarized ^{13}C -labeled substrates can provide unique non-invasive in vivo measurements of these processes (1). $[1-^{13}\text{C}]\text{Pyr}$ is currently the most commonly used substrate, being an endogenous compound playing a central role in catabolic and anabolic metabolism. In the cytosol, Pyr can be reduced to lactate as the end product of glycolysis or amidated to alanine. In mitochondria, Pyr can directly enter the TCA cycle via oxaloacetate or be converted to acetyl-CoA and CO_2 as the first step towards oxidative phosphorylation. Given its metabolic importance, low toxicity, and favorable NMR properties (in vivo T_1 relaxation rate $\sim 40\text{s}$), $[1-^{13}\text{C}]\text{Pyr}$ is the first hyperpolarized ^{13}C -compound to reach clinical trials (2), and is applicable to the study of cancer (2–4), cardiovascular pathologies (5–6), inflammation (7), and metabolic disorders (8).

Absolute quantitation can be important for fully interpreting in vivo hyperpolarized metabolic imaging data, and a key parameter required for such measurements is the liquid-state polarization of the injected substrate (9). Calculations using the thermal equilibrium values of the substrate signal can provide polarization values but are not practical for in-vivo applications due to the prohibitively long scan times required to achieve acceptable signal-to-noise ratio for the thermal equilibrium measurements. Doublet asymmetry of naturally abundant ^{15}N was used to determine the degree of polarization of the coupled carbon in a study of hyperpolarized ^{13}C labeled urea (10). This idea was extended by Hurd et al. to measure the polarization of $[1-^{13}\text{C}]\text{Pyruvate}$ using the asymmetry of the C_2 doublet from the 1% naturally abundant $[1,2-^{13}\text{C}]\text{Pyr}$ in any hyperpolarized $[1-^{13}\text{C}]\text{Pyr}$ sample, but it was concluded that the time spent in low field, as the sample is transferred from the polarizer to the scanner, confounds the measurement (11). Similar studies were conducted by Chen et al.

using selective excitation of [2-¹³C]Pyr resonance with the large error in polarization measurement (~20%) attributed to limited spectral resolution (12).

The relationship between the instantaneous polarization and the asymmetry of the C₂ doublet, as measured by the an asymmetry parameter $a_{C2} = (\text{inner peak} - \text{outer peak})/\text{sum of both peaks}$, of [1,2-¹³C]Pyr in whole pig blood was used to estimate in-vivo instantaneous C₁ polarization by Lau et al. (9). A paradoxical finding from this work was that the observed time evolution of a_{C2} did not appear to asymptotically approach the known thermal equilibrium value, and Lau et al. proposed determining instantaneous polarization from $a_{C2}(t)$ data based on a empirical calibration curve.

The validity of the Lau calibration curve was not tested for varying levels of initial polarization, and, in this work, we performed additional experiments to determine whether a single time-point measurement of the C₂ asymmetry is indeed sufficient to derive the instantaneous C₁ polarization. Although the origin of the initial C₂ doublet asymmetry due to the creation of two-spin order in a hyperpolarized homonuclear spin system sample is well known (13) and the final asymmetry can be calculated from residual strong coupling effects, the relaxation mechanisms responsible for the dynamic behavior and time evolution of the doublet asymmetry are less well understood. Based on in vitro data, we thus evaluated the contribution from different relaxation mechanisms to account for the observed asymmetry versus time curve. As predicted by theory, experimental data of the asymmetry curves derived from different initial polarizations demonstrate that a single time point measurement of asymmetry cannot unambiguously determine the instantaneous polarization.

Experimental Methods and Theory

Substrate preparation and polarization

Each sample consisted of 36μL of a mixture of 14M [1,2-¹³C] Pyruvic acid (Sigma-Aldrich Co., St. Louis, MO) and 15mM OX063 trityl radical (Oxford Instruments, Abingdon, UK). The samples were polarized using a dissolution dynamic nuclear polarization (DNP) system (SpinLab, GE Healthcare, Waukesha, WI) for different lengths of time to achieve approximately 17%, 22% & 40% liquid-state polarization at the time of dissolution, as calculated by comparison to the thermal equilibrium signal from each sample. The polarized sample was dissolved using 15g solution of 0.1g/L Na₂EDTA, 40mM Tris buffer, pH neutralized using a NaOH solution, and 4ml of the resulting 80mM solution of hyperpolarized pyruvate with a pH of 7 was used for dynamic MR measurements.

Signal acquisition

All experiments were performed on a clinical 3T MR scanner (40mT/m gradient amplitude, 150mT/m/ms slew rate; GE Healthcare, Waukesha, WI) using custom-built transmit/receive ¹³C and ¹H coils. A quadrature dual-tuned rat body coil (diameter=70 mm) was used for acquisition of the carbon spectrum. A non-selective 24-μs hard RF pulse-and-acquire sequence with an excitation flip angle of 5.6°, spectral width of 5 kHz, and 2048 points was used to acquire ¹³C spectra from the substrate every 3 seconds over a 4 minute period starting immediately after the pyruvate syringe was placed in the coil. The time from

dissolution to start of scan ranged from 38s to 52s for the various experiments as shown in Fig. 4. For the thermal equilibrium scan, the substrate was doped with 40 μ L of Gadolinium-diethylenetriaminepentacetate (Gd-DTPA, Magnevist, Bayer Schering Pharma, Germany), yielding 5mM in solution to reduce the T_1 of the slower relaxing C_1 carbon from 66s to ~1s, and signal was acquired using the same sequence with a 90° flip angle every 6s over a 40 minute period and averaged to obtain the thermal equilibrium spectrum.

Data processing

Custom developed scripts in MATLAB (MathWorks Inc., MA) were used for data processing and model fitting. The MR spectroscopic data were apodized with 10 Hz Gaussian line broadening and zero-filled by a factor of two. Zero-order and first-order phase correction was performed on the spectra, and the metabolite time courses were calculated by integrating the [1,2- 13 C] Pyr doublet peaks in absorption mode. Flip angle correction was applied to the integrated data and the time evolution of the asymmetry parameter for the C_1 & C_2 doublet was computed using the definition $a_C = (\text{inner peak} - \text{outer peak})/(\text{inner peak} + \text{outer peak})$ as given in (9). The center of the chemical shift between the two carbons is conventionally taken to be zero and the peaks closer to this center are considered “inner” peaks, whereas the peaks farther from the center are termed “outer”.

Model fitting

In order to model the observed a_{C2} versus time curves, we investigated the importance of several known relaxation mechanisms. With respect to the initial conditions, determining C_2 doublet asymmetry as the sample leaves the polarizer (defined as $t = 0$), is straightforward. Namely, the density operator at $t=0$, based on an exponential operator expansion without making the high temperature approximation, is given by (14):

$$\hat{\sigma}_0 \approx \frac{1}{4}\hat{E} + \frac{1}{2}P_0\hat{I}_z + \frac{1}{2}P_0\hat{S}_z + \frac{1}{2}P_0^2 2\hat{I}_z\hat{S}_z \quad [1]$$

where P_C is carbon polarization with initial value $P_0 = P_C(0)$, $I = C_1$ nucleus, and $S = C_2$ nucleus in [1,2- 13 C]Pyr. The last term, typically negligible under standard thermal equilibrium conditions but important for hyperpolarized studies where polarizations are often 10% – 50%, is a major contributor to C_1 and C_2 doublet asymmetry (please refer to supplemental material).

Following a non-selective RF excitation with flip angle ϕ , the $\hat{I}_z$ and $\hat{S}_z$ terms generate the expected in-phase doublets at $\Omega_I = \omega_0 - \omega_I$ and $\Omega_S = \omega_0 - \omega_S$ (rotating frame chemical shifts for the I and S spins respectively, where $\omega_0 = \gamma B_0$, and ω_I , ω_S are the Larmor frequencies of the I and S spins respectively), while the $2\hat{I}_z\hat{S}_z$ coherence, generates anti-phase doublets at both Ω_I and Ω_S . The net result is the generation of asymmetric doublets for both the C_1 and C_2 resonances as depicted in Fig. 1.

Defining a doublet asymmetry parameter $a_C = (\text{inner peak} - \text{outer peak})/(\text{inner peak} + \text{outer peak})$ based on the integral of the area under the doublet peaks (9), the C_2 doublet asymmetry is given by (see supplemental material):

$$a_{C_2} \approx P_0 \cos\phi + \sin\theta \quad [2]$$

with $\sin\theta = \frac{2\pi J}{\sqrt{(2\pi J)^2 + (\Omega_I - \Omega_S)^2}}$, the residual strong-coupling characteristic of the J-coupling interaction between the C_1 and C_2 carbons. For [1,2- ^{13}C]Pyruvate, using $J=64\text{Hz}$ and $(\Omega_I - \Omega_S)/2\pi = 1152\text{Hz}$ at 3T and 25°C, the asymmetry parameter at thermal equilibrium is $a_C^{eq} = \sin\theta = 0.056$ as reported earlier (9).

Given that the carbon polarization, $P_C(t)$, decays with a time constant of T_1 , a natural assumption is that a_C also decays towards a_C^{eq} with the same time constant. However, Eqn. [2] is strictly true only for $t=0$, the time at which the sample exits the polarizer. In an actual experiment, one needs to account for relaxation processes between $t=0$ and the time of the actual measurement. Lau et al. (9) and others (11,12) observed that, over the 2-min duration of a typical hyperpolarized ^{13}C study, $a_C(t)$ does not asymptotically approach a_C^{eq} but rather exhibits a decay curve seemingly independent of the ultimate thermal equilibrium value (see Figs. 3 and 4).

To investigate this unexpected observed behavior of $a_{C_2}(t)$, we started with the following analytical expression (see derivation in Supplemental material),

$$a_{C_2}(t) = \frac{\overline{\langle 2\hat{I}_z \hat{S}_z \rangle}(t)}{\overline{\langle \hat{S}_z \rangle}(t)} \cos\phi + \frac{1}{2} \frac{(\overline{\langle \hat{I}_z \rangle}(t) + \overline{\langle \hat{S}_z \rangle}(t))}{\overline{\langle \hat{S}_z \rangle}(t)} \sin\theta, \quad [3]$$

where $\overline{\langle \hat{A} \rangle}(t)$ denotes the time dependent ensemble average of the expectation value of operator $\hat{A}$. The relaxation behavior of the coherences of interest is then given by:

$$\frac{d}{dt} \begin{bmatrix} \overline{\langle \hat{I}_z \rangle}(t) \\ \overline{\langle \hat{S}_z \rangle}(t) \\ \overline{\langle 2\hat{I}_z \hat{S}_z \rangle}(t) \end{bmatrix} = - \begin{bmatrix} R_{11} & R_{12} & R_{13} \\ R_{21} & R_{22} & R_{23} \\ R_{31} & R_{32} & R_{33} \end{bmatrix} \begin{bmatrix} \overline{\langle \hat{I}_z \rangle}(t) - I_z^{eq} \\ \overline{\langle \hat{S}_z \rangle}(t) - S_z^{eq} \\ \overline{\langle 2\hat{I}_z \hat{S}_z \rangle}(t) - 2I_z S_z^{eq} \end{bmatrix} \quad [4]$$

where I_z^{eq} , S_z^{eq} and $2I_z S_z^{eq}$ are the thermal equilibrium values as given by the Boltzmann distribution for the expected values of the $\hat{I}_z$, $\hat{S}_z$, and $2\hat{I}_z \hat{S}_z$ respectively namely,

$$I_z^{eq} = S_z^{eq} = \frac{\hbar\gamma_C B_0}{2kT} \text{ and } 2I_z S_z^{eq} = \left(\frac{\hbar\gamma_C B_0}{2kT}\right)^2 \approx 0 \text{ for typical in vivo MRI conditions and } R_{ij} \text{ are the elements of the relaxation matrix } R.$$

We then derived analytical expressions for the elements of the relaxation matrix using Redfield theory considering the following dominant relaxation mechanisms for [1,2-¹³C]pyruvate: C-H dipolar coupling, the CSA of the carbon nuclei, and C-C dipolar coupling. Our analysis includes the dipolar coupling-CSA interference effect on the relaxation of scalar coupled nuclei (16), an effect well known in the NMR literature (17–18), but less so in the context of DNP.

The direct and cross relaxation terms of the relaxation matrix in Eqn [4] are given by (see supplemental material)

$$\begin{aligned}
 R_{11} &= \frac{1}{T_{1,CSA_1}} + \frac{1}{T_{1,HC_1}} + \frac{1}{T_{1,CC}} \\
 R_{22} &= \frac{1}{T_{1,CSA_2}} + \frac{1}{T_{1,HC_2}} + \frac{1}{T_{1,CC}} \\
 R_{33} &= \frac{1}{T_{1,CSA_1}} + \frac{1}{T_{1,CSA_2}} + \frac{1}{T_{1,HC_1}} + \frac{1}{T_{1,HC_2}} + \frac{1}{T_{2IzSz-2IzSz}} \approx \frac{1}{T_{1,CSA_1}} + \frac{1}{T_{1,CSA_2}} + \frac{1}{T_{1,HC_1}} + \frac{1}{T_{1,HC_2}} + \frac{3}{5T_{1,CC}} \\
 R_{12} &= R_{21} = \frac{1}{T_{Iz-Sz-cross}} \approx \frac{1}{2T_{1,CC}} \\
 R_{13} &= R_{31} = \frac{1}{T_{Iz-2IzSz-cross}} \approx \sqrt{\frac{6}{5}} \sqrt{\frac{1}{T_{1,CC}T_{1,CSA_1}}} \\
 R_{23} &= R_{32} = \frac{1}{T_{Sz-2IzSz-cross}} \approx \sqrt{\frac{6}{5}} \sqrt{\frac{1}{T_{1,CC}T_{1,CSA_2}}}
 \end{aligned}$$

[5]

where the subscripts denote relaxation due to proton-carbon dipolar couplings (HC), chemical shift anisotropy (CSA), carbon-carbon dipolar coupling (CC) and cross relaxation terms (cross), and the simplifying approximations are valid for the case of extreme narrowing.

Five independent parameters are thus required to fit this analytical model to hyperpolarized

data, namely $\frac{1}{T_{1,CSA_1}}$, $\frac{1}{T_{1,HC_1}}$, $\frac{1}{T_{1,CSA_2}}$, $\frac{1}{T_{1,HC_2}}$ and $\frac{1}{T_{1,CC}}$. We estimated these parameters using T_1 measurements from decay curves of 80mM hyperpolarized [1,2-¹³C]Pyr, [1-¹³C]Pyr and [2-¹³C]Pyr samples at 3T (using the same substrate preparation, polarization, signal acquisition and data processing methods described above for the asymmetry measurements), inversion recovery T_1 measurements of 1M thermally polarized samples taken at 7T (300MHz Varian Inova spectrometer), and by assuming CSA relaxivity scales with square of the main field (see supplemental material). The theoretical decay curves simulated using Eqn [4] were then compared to in vitro hyperpolarized [1,2-¹³C]Pyr data. For these calculations the initial values for the ensemble average of the coherences were computed using the first time point measurement of the individual C_1 and C_2 doublet peaks.

Results

The mean values of the pyruvate longitudinal relaxation times, as estimated from 3T and 7T experiments given in Table 1, were used to calculate the corresponding model parameters, namely $T_{1,HC_1} = 70s$, $T_{1,HC_2} = 55s$, $T_{1,CSA_1} = 1177s$, $T_{1,CSA_2} = 764s$, and $T_{1,CC} = 1079s$. In

principle, the longitudinal relaxation of the doublet C_1 and C_2 resonances is governed by the relaxation matrix R as defined in Eqns. [4] and [5], and therefore the decay of each peak is not mono-exponential. As an approximation, the integral of the area under the peak, and in the case of $[1,2-^{13}\text{C}]\text{Pyr}$, the sum of the areas under the doublet peaks, was used to estimate the longitudinal relaxation times of the C_1 and C_2 carbons. Experimental and theoretical a_{C_2} versus time curves are plotted in Fig. 2 from representative in vitro hyperpolarized $[1,2-^{13}\text{C}]\text{Pyr}$ dynamic data from 3T and corresponding model fits using 1) a relaxation model comprised of proton-carbon dipolar couplings & CSA of the carbon nuclei, and 2) a model that also included carbon-carbon dipolar coupling.

As shown by prior investigators (9,11), experimental doublet asymmetry parameter value appears to evolve beyond the thermal equilibrium value. Furthermore, a relaxation model assuming only proton-carbon dipolar couplings and CSA fails to account for this behavior. In contrast, the inclusion of carbon-carbon dipolar coupling, and its associated cross relaxation effects, yields a much better fit to the experimental data up to 250s after dissolution, the time interval during which asymmetry signal-to-noise ratio (SNR) is sufficient for measurement. The carbon spectra obtained at different time points are plotted in Fig. 3 along with the thermal equilibrium spectrum for comparison of the evolution of the doublet peaks. The initial evolution (e.g. at $t=38\text{s}$) is dominated by the faster relaxation of the two-spin order term $2\hat{I}_z\hat{S}_z$, that relaxes approximately at the rate of the sum of the $\hat{I}_z$ and $\hat{S}_z$ relaxation rates, whereas the later evolution beyond the thermal equilibrium value (e.g. at $t=110\text{s}$ and $t=254\text{s}$) is largely driven by the cross relaxation effects. Eventually, as the longitudinal coherences relax to their thermal equilibrium values, the asymmetry parameter converges to its thermal equilibrium value (Fig. 3 bottom right).

Time evolutions for the pyruvate C_1 and C_2 doublet asymmetries from in vitro hyperpolarized $[1,2-^{13}\text{C}]\text{Pyr}$ experiments at 3T and corresponding model fits are plotted in Fig. 4a for initial polarization values of $P_0 = 40\%$, 22% and 17% . Fig. 4b shows an alternative presentation of this data, mapping $a_{C_1}(t)$ and $a_{C_2}(t)$ to instantaneous polarization $P_C(t)$ as first presented by Lau et al (9). These plots clearly show that different initial polarization values result in different decay curves. Consequentially, a single measurement of a_{C_2} at a time $t = t_0$, can only be mapped to instantaneous polarization if the initial polarization value is known, i.e. the mapping of doublet asymmetry to instantaneous polarization is not 1:1 and thus non-invertible. For example, $a_{C_2} = 0.2$ corresponds to three different values of C_1 polarization of 0.14, 0.1 and 0.04 for $P_0 = 40\%$, 22% and 17% , respectively.

Discussion

Although the reported T_1 s of the C_1 carbon for both $[1-^{13}\text{C}]\text{Pyr}$ and $[1,2-^{13}\text{C}]\text{Pyr}$ are similar (11), from which one might expect C-C dipolar coupling to have minimal effects, these results demonstrate that by including C-C dipolar coupling and the associated dipolar coupling-CSA interference effect yields much improved fits and could explain the divergence of $a_{C_2}(t)$ from the expected asymptotic behavior during the first two minutes of a typical hyperpolarized experiment (Fig. 2). In particular, the data presented in Figs. 2 and 4 demonstrate that (1) instantaneous ^{13}C spin polarization can only be uniquely calculated if

the polarization at $t=0$ is known, and (2) proton-carbon dipolar couplings and CSA, in combination with carbon-carbon dipolar coupling, can model the observed time decay characteristics of the doublet asymmetries, with cross relaxation arising from the dipolar coupling-CSA interference effects being a significant contributing factor. This interference effect on the relaxation of scalar coupled nuclei is well known in the NMR literature (17) and causes the differential relaxation of the two doublet components, and therefore responsible for the different transverse relaxation times and linewidths (18). While it may appear from Fig. 3 that each of the doublet peaks have their individual apparent relaxation times, it is to be noted that the evolution of the $\hat{I}_z, \hat{S}_z$ and $2\hat{I}_z\hat{S}_z$ coherences are governed by the relaxation matrix R in Eqn. [4] and doublet peaks are the result of the evolution of these coherences after an RF pulse is applied (see supplemental material). However, the data and analysis presented here have some limitations.

The estimates of the five T_1 parameters needed to fully specify the model were only computed to limited accuracy. An alternative to the direct measurements of pyruvate C_1 and C_2 T_1 relaxation times at multiple field strengths would be least-squares fitting of the observed data to directly estimate the needed model parameters. This approach, however, may suffer from inaccuracies due to the large number of free parameters being fit.

Although the evolution of the asymmetry parameters a_{C1} and a_{C2} occurs over a much longer time scale than the direct T_1 relaxation rates, this is a differential effect. To calculate a_{C1} and a_{C2} , the areas under the doublet peaks need to be computed, and, unfortunately, the SNR of these measurements depends directly on the peak T_1 s. We thus think it doubtful that the effects investigated in this manuscript can be used to obviate any T_1 limits with current in vivo DNP studies.

While matching our in vitro data fairly well, the model presented here for the relaxation behavior of the hyperpolarized $[1,2-^{13}\text{C}]\text{Pyr}$ doublet asymmetries is incomplete, with in vivo factors such as compartmentalization, effects of paramagnetic ions, pH, chemical exchange, and relaxation due to multi-spin couplings and/or spin rotation ignored. However, the primary conclusion that instantaneous polarization can only be computed with knowledge of the polarization at $t=0$ likely remains valid.

For some cases, the polarization of the sample immediately after dissolution is indeed known, e.g. via the NMR measurement of an aliquot using a desktop polarimeter. However, decay occurring while the sample is moved from the polarizer to the scanner, during which time the sample experiences both low and high magnetic fields, is typically unknown and not modeled by the theory presented here as all the mechanisms responsible for relaxation at low fields are not considered. The best approach may be to perform an in vitro calibration run from which both hyperpolarized and thermal signals can be measured, with the time and path of sample transfers kept constant for subsequent in vivo experiments. Then, an extension of the approach proposed by Lau et al. (9) could be employed whereby a set of calibration curves for different initial polarizations are pre-computed and then used to determine the polarization at the time of measurement from the in vivo asymmetry measurements.

Conclusion

The liquid-state polarization is needed for full quantitation of hyperpolarized data, and asymmetry of the C₂ doublet, arising from 1% naturally abundant [1,2-¹³C]Pyr in any hyperpolarized [1-¹³C]Pyr sample, has been proposed as a direct measure of in-vivo C₁ polarization via the use of an in-vitro calibration curve. Here we showed that, due to direct and cross relaxation effects, different polarization levels can yield the same C₂-doublet asymmetry at the time of measurement, thus limiting the utility of this metric for quantitation. An extended calibration method utilizing a family of curves parameterized by the initial polarization may be an improved approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

NIH grants EB015891, EB019018, CA176836, S10 OD012283, Ruth L Kirschstein-NRSA F31 EB019821, Stanford-GE Healthcare Blue Sky grant program, Lucas Center for Imaging and the Stanford BioX Seed grant program. We would like to thank Dr. Stephen Russell Lynch for his help with the T₁ measurements at 7T.

Abbreviations & Acronyms

Pyr	pyruvate
Acetyl-CoA	acetyl coenzyme A
MRS	magnetic resonance spectroscopy
TCA	tri carboxylic acid
NMR	nuclear magnetic resonance
SNR	signal to noise ratio
RF	radio frequency
CSA	chemical shift anisotropy
FID	free induction decay
Gd-DTPA	gadolinium-diethylenetriaminepentacetate
DNP	dynamic nuclear polarization
EDTA	ethylenediaminetetraacetic acid

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Figure 1.

Schematic representation of the spectra resulting from [1,2-¹³C]Pyruvate with carbon polarization P_c , immediately following the application of an RF pulse with flip angle ϕ_I on the C₁ carbon and ϕ_S on the C₂ carbon. θ is the standard strong coupling parameter (see Supplemental Material). The sum of the in-phase doublets (left) resulting from the evolution of coherences $\hat{I}_y, \hat{S}_y, 2\hat{I}_y \hat{S}_z, 2\hat{I}_z \hat{S}_y$ yields the symmetric components of the pyruvate doublets, whereas, the sum of the anti-phase doublets (right) accounts for the observed the asymmetry.

Figure 2.

Representative model fitting of the time evolution for the asymmetry parameter, a_{c2} , of the C_2 pyruvate doublet. Black solid triangles = experimental data at 3T with $P_0=40\%$, red dashed line = model assuming relaxation due to proton-carbon dipolar couplings and chemical shift anisotropy, and blue dot-dashed line = model prediction including relaxation by carbon-carbon coupling. The thermal equilibrium value of a_{c2} (at 3T and 25°C) is indicated by the green dashed line. Experimental data was acquired every 3s starting 38s post dissolution and was stopped after 4 min due to low SNR. Model parameters used in the simulations as estimated from the T_1 measurements given in Table 1: $T_{1,HC1} = 70s$, $T_{1,HC2} = 55s$, $T_{1,CSA1} = 1177s$, $T_{1,CSA2} = 764s$, and $T_{1,CC} = 1079s$.

Figure 3.

In vitro carbon spectra of the hyperpolarized $[1,2-^{13}\text{C}]\text{Pyr}$ plotted at different time points highlight the temporal evolution of the asymmetries of the C_1 and C_2 doublets. Initial asymmetry (e.g. $t=38\text{s}$, top left) is dominated by the relaxation of the longitudinal two-spin order term $2\hat{I}_z\hat{S}_z$, whereas the evolution beyond thermal equilibrium is largely driven by interference effect between the C_1 - C_2 dipolar and CSA relaxations of the carbon nuclei (e.g. at $t=110\text{s}$ and $t=254\text{s}$) before eventually returning to the thermal equilibrium values (bottom right). The hyperpolarized spectra were acquired using 4ml of 125mM $[1,2-^{13}\text{C}]\text{Pyr}$ at 25°C with a flip angle of 5.6° , and the thermal equilibrium spectrum is the average of 400 transients, acquired after doping with 5mM Gd-DTPA, using 90° flip angle and $\text{TR}=6\text{s}$ with a total scan time of 40min. All spectra are normalized such that the tallest peaks have the same height with the scaling factors noted on the plots.

Figure 4.

Doublet asymmetry as a function of initial polarization P_0 ranging from 40% to 17%.

Relaxation model parameters used in the simulations are estimated from T_1 measurements given in Table 1: $T_{1,HC1} = 70s$, $T_{1,HC2} = 55s$, $T_{1,CSA1} = 1177s$, $T_{1,CSA2} = 764s$, and $T_{1,CC} = 1079s$. Top two rows (a) Measured and predicted time evolution of the asymmetry parameters, a_{C1} , and a_{C2} . The time delay between sample leaving polarizer and start of scan, are noted on the plots. Bottom row (b) Experimental and modeled C_2 and C_1 polarization versus C_1 and C_2 doublet asymmetry curves respectively (parameterized by measurement time). Black solid triangles = 3T experimental data, green dashed line = thermal equilibrium value, and blue dashed line = model assuming relaxation due to a combination of chemical shift anisotropy, proton-carbon dipolar couplings, and carbon-carbon dipolar coupling.

Table 1

T_1 measurements of C_1 and C_2 carbons in singly and doubly labeled pyruvate from decay curves of hyperpolarized samples at 3T and inversion recovery-based T_1 measurements of the ^{13}C nuclei from thermally polarized samples at 7 T. Using the mean values of these estimated T_1 values, the model parameters for Eqn [5] were calculated as: $T_{1,\text{HC1}} = 70\text{s}$, $T_{1,\text{HC2}} = 55\text{s}$, $T_{1,\text{CSA1}} = 1177\text{s}$, $T_{1,\text{CSA2}} = 764\text{s}$, and $T_{1,\text{CC}} = 1079\text{s}$.

T_1 Measurements (s) of C_1 , C_2 nuclei in Pyruvate				
	3 T		7 T	
	T_{1C1}	T_{1C2}	T_{1C1}	T_{1C2}
$[1,2-^{13}\text{C}]\text{Pyr}$	65.6 ± 2.6	47.4 ± 1.0	45.3 ± 0.9	36.0 ± 1.1
$[1-^{13}\text{C}]\text{Pyr}$	72.1 ± 1.3	–	49.5 ± 0.3	–
$[2-^{13}\text{C}]\text{Pyr}$	–	52.2 ± 1.1	–	40.1 ± 2.8