Relaxation behaviour of hyperpolarized pyruvate in solution and in whole blood at 7 T

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Introduction: Dynamic nuclear polarization (DNP) offers up to four orders of magnitude in ^{13}C signal enhancement, enabling MR observation of biochemical processes *in vivo* (1). Monitoring of pyruvate and its downstream metabolites is of particular interest because of its altered metabolism in many types of solid tumours (2,3) and under some conditions such as ischemia (4). Many studies have focused on [1-^{13}C] pyruvate because its long T_1 allows several minutes of imaging time wherein lactate, bicarbonate, and alanine can be observed. However, the C_1 label is lost as $^{13}CO_2$ before entering the Krebs cycle as acetyl-CoA. With [2-^{13}C] pyruvate, Krebs cycle intermediates including glutamate, citrate, and acetylcarnitine have been observed *in vivo* (4). By using [1,2-$^{13}C_2$] pyruvate, pyruvate dehydrogenase flux, intracellular pH, and Krebs cycle metabolism can be monitored simultaneously with just a single dose, as demonstrated in pig hearts at 3 T (5). Figure 1 shows a 3 T ^{13}C spectrum, integrated over a whole pig heart, after infusion of [1,2-$^{13}C_2$] pyruvate in which a number of overlapping resonances from different metabolites can be seen. By increasing the field strength to 7 T, the larger chemical shift dispersion may improve resolution of these resonances and facilitate more accurate spectral quantification. However, longitudinal relaxation via chemical shift anisotropy (CSA) is enhanced at higher fields (6), which may shorten the effective imaging time of hyperpolarized compounds at 7 T. In this work, we report T_1 measured at 7 T of [1-^{13}C] pyruvate and [2-^{13}C] pyruvate in solution and characterize the relaxation behaviour of [1,2-$^{13}C_2$] pyruvate in solution and in whole blood to assess the values and challenges of transitioning from 3 T to 7 T.

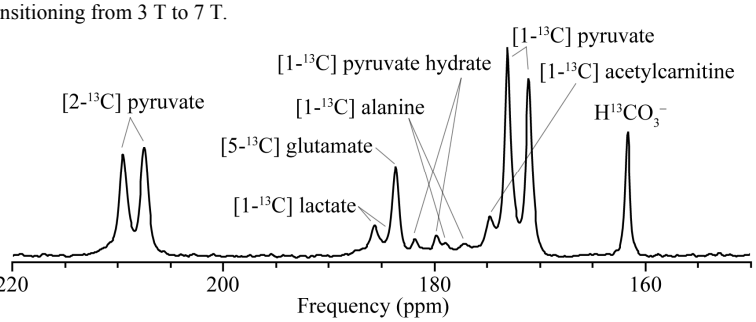

Figure 1. Representative ^{13}C spectrum of [1,2-$^{13}C_2$] pyruvate in whole pig heart at 3 T (10° flip angle, TR = 4 R-R, 4096 points, 10 kHz bandwidth) as described in (5).

Methods: Approximately 28 µL of ^{13}C-enriched pyruvic acid (99%, Isotec, Miamisburg OH) with 15 mM OX63 trityl radicals (Oxford Instruments, Abingdon, UK) and 1 mM Prohance (Bracco) was polarized to steady-state using a HyperSense DNP polarizer (Oxford Instruments) followed by rapid dissolution in 80 mM NaOH solution with 40 mM NaCl/tris buffer and 100 mg/L EDTA to fully neutralize the acid. Spectroscopy was performed on a Bruker BioSpec 70/30 USR 7 T small animal system (Bruker BioSpin, Germany) with a dual-tuned $^{1}H/^{13}C$ volume mouse coil (RAPID MR International, Columbus OH). The pulse sequence consisted of 125 µs hard pulses (5° for pre-polarized, 90° for thermal equilibrium experiments) and readouts of 4096 points (10 kHz bandwidth) with a 10 ms spoiler gradient at the end of each TR. For relaxation measurements in solution (N = 2), 2 mL of solution were drawn into a 5 mL syringe and transferred into the coil within 16 s, then 96 transients with TR = 3 s were acquired for [2-^{13}C] and [1,2-$^{13}C_2$] pyruvate, 128 transients for [1-^{13}C] pyruvate. Afterward, a spectrum at thermal equilibrium was collected with TR = 8 s and 384 transients following addition of 8 µL/mL Magnevist to shorten the T_1. For blood relaxation measurements (N = 2), whole blood was extracted, in accordance with institutional animal care protocols, from the femoral artery of 20 kg pigs into sodium heparin vacuum tubes (BD, Franklin Lakes NJ) and stored at 4°C until use. The blood was warmed to 37°C and 18 mL placed in a 60 mL syringe fastened to the coil. Approximately 2 mL of 80 mM [1,2-$^{13}C_2$] pyruvate solution was transported to the scanner within 15 s and injected into the blood syringe through a 92 cm microbore Luer lock-extension set (Codan US Corporation, Santa Ana CA) over 4 s. Immediately after injection, 96 transients were acquired with TR = 3 s. A spectrum at thermal equilibrium (20°C) was acquired with TR = 10 s and 384 transients after adding 200 µL of Magnevist to shorten the T_1. Spectral processing and analysis were performed using matNMR version 3.9.94 (7).

Results:
A representative equilibrium ^{13}C spectrum of [1,2-$^{13}C_2$] pyruvate in blood at 7 T is shown in Figure 2. Natural abundance macromolecules in red blood cells give rise to a broad resonance 5 ppm downfield of the C_1 doublet, which was not observed when fetal bovine serum was substituted for whole blood. Both C_1 and C_2 polarizations in blood were calculated using only the unaffected C_2 thermal equilibrium integral. Average initial polarizations of 17.9%, 13.3%, and 16.7% (C_1) / 14.9% (C_2) were achieved for [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] pyruvate respectively after adjusting for flip angle, number of transients, and temperature.

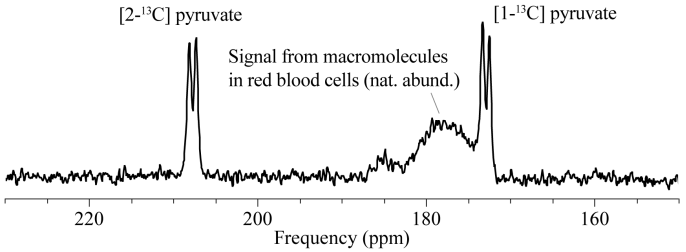

Figure 2. Thermal equilibrium spectrum of [1,2-$^{13}C_2$] pyruvate in blood at 7 T.

For [1,2-$^{13}C_2$] pyruvate, each doublet was fit to the sum of two Gaussian/Lorentzian curves to extract the integral of each resonance line. Mono-exponential fits were performed on the extracted peak integrals to obtain longitudinal relaxation time constants, presented in Table 1 with relaxation time constants in blood at 3 T included for comparison. Solution T_1 values at 7 T are compared to literature values at 3 T (3,5), 9.4 T (8), and 11.7 T (3) in Table 2.

Table 1: Longitudinal relaxation time constants (s) of [1,2-$^{13}C_2$] pyruvate spectral lines.

	C_1			C_2		
	Upfield	Downfield	Doublet	Upfield	Downfield	Doublet
Solution 7 T	48	62	54 ± 1	36	49	41 ± 1
Blood 7 T	35 ± 2	52 ± 1	44 ± 1	28 ± 1	44 ± 1	36 ± 1
Blood 3 T	35 ± 2	48 ± 3	42 ± 3	31 ± 3	43 ± 3	36 ± 3

Discussion:
In solution, single-labelled pyruvate exhibited 20% shorter T_1 at 7 T as compared to 3 T, whereas T_1 decreased only by 5% for dual-labelled pyruvate. These results suggest that auto correlated dipolar relaxation remains the dominant mechanism for [1,2-$^{13}C_2$] pyruvate at 7 T, but auto correlated CSA relaxation is likely dominant at 7 T for single-labelled pyruvate. Relaxation time constants in blood at 7 T were comparable to those measured at 3 T, suggesting that paramagnetic relaxation continues to be dominant in blood at 7 T.

Table 2: Solution T_1 (s) at 7 T and literature T_1 at different fields (3,5,8).

	[1-^{13}C] pyruvate	[2-^{13}C] pyruvate	[1,2-$^{13}C_2$] pyruvate	
	C_1	C_2	C_1	C_2
3 T	67.3 ± 2.5	58*	56	44
7 T	**58**	**43**	**54 ± 1**	**41 ± 1**
9.4 T	46 ± 6	37 ± 1		
11.7 T	48.3 ± 0.6			

*A.P. Chen, previously unpublished result

Conclusions:
We have demonstrated that T_1 of [1,2-$^{13}C_2$] pyruvate is not shortened significantly at 7 T in solution and in blood as compared to 3 T. Results from this work will lay the foundation for future quantitative investigations and modelling using [1,2-$^{13}C_2$] pyruvate at 7 T.

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