Real-time *in vivo* monitoring of pyruvate C_1 polarization using C_2 integral ratios

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Introduction:
Dynamic nuclear polarization (DNP) allows *in vivo* observation of metabolic substrates by affording up to four orders of magnitude in ^{13}C signal enhancement (1). However, the signal enhancement decreases with time as the spin system returns toward thermal equilibrium. For quantitative analysis, it is highly desirable to monitor the instantaneous polarization throughout the experiment. With appropriate receiver calibration, metabolite concentrations can be obtained after accounting for the instantaneous polarization. Determination of ^{13}C polarization using the doublet asymmetry of a neighbouring *J*-coupled nucleus was first demonstrated in [^{13}C]-urea with natural abundance ^{15}N (1). Initial C_1 polarization of [1,2-$^{13}C_2$]-pyruvate was determined *in vitro* (2) and *in vivo* (3) using the C_2 asymmetry, but the accuracy of instantaneous polarization measurement, as the polarization decays, using this method is complicated by the multiplet effect, resulting in differential relaxation of spectral lines for homonuclear AB systems (4). We present here an empirical relation between the instantaneous polarization and the C_2 spectral line ratio of [1,2-$^{13}C_2$]-pyruvate in whole pig blood. We used this model to obtain retrospective estimates of instantaneous polarization during *in vivo* spectroscopic experiments in pig heart. Furthermore, we were able to estimate real-time *in vivo* C_1 polarization decay curves.

Methods:
Experiments were performed in accordance with institutional animal care protocols. Whole blood was extracted from either the femoral or carotid artery of 20 kg pigs into sodium heparin vacuum tubes (BD, Franklin Lakes NJ) and stored at 4°C until use. Before the scan, 18 mL of blood was warmed to 37°C and placed into a 60 mL syringe. The syringe was fastened with the bolus centred in a dual-tuned $^{1}H/^{13}C$ rat coil (Magvale, San Francisco CA) and in a GE MR750 3T scanner (GE Healthcare, Waukesha WI). Approximately 25 µL of [1,2-$^{13}C_2$]-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). A heated 80 mM NaOH solution with 40 mM NaCl/tris buffer and 100 mg/L EDTA was injected in sufficient volume to rapidly dissolve and neutralize the frozen acid. The polarized pyruvate solution was transported to the 3T scanner and 2 mL injected through a 92 cm microbore Luer lock-extension set (Codan US Corporation, Santa Ana CA) into the blood syringe within 20 s. A pulse-acquire sequence with 10° flip angle (TR = 3 s, 4096 points/10 kHz readout, 96 transients) was started immediately after injection. A 20°C spectrum at thermal polarization was acquired with 90° flip angle and 384 transients after adding 200 µL Gd-DTPA (Magnevist) to shorten T_1 sufficiently to allow full magnetization recovery within TR = 10 s. *In vivo* ^{13}C spectra from pig hearts (integrated over the entire heart) were acquired using a ^{13}C transmit/receive surface coil and a cardiac-gated sequence with nominal 10° hard pulses every 4 R-R beginning simultaneously with intravenous infusion of 15 mL of 83 mM [1,2-$^{13}C_2$]-pyruvate over approximately 15 s, as described in (5).

Results:
Longitudinal relaxation time constants of each spectral line in whole blood are compared to values in solution (6) in Table 1. Instantaneous polarizations were calculated by dividing the doublet integral in each hyperpolarized spectrum by the corresponding doublet integral from the thermal spectrum after adjusting for flip angle, number of transients, and population difference at thermal equilibrium (2.48 ppm at 37°C, 2.63 ppm at 20°C). As a measure of C_2 doublet asymmetry, we examined the ratio of the upfield partial integral to the downfield partial integral. The relationship between this C_2 ratio and the C_1 instantaneous polarization can be approximated with a linear model, forming a calibration curve such as that in Figure 1. Due to noise, only points with polarizations above 1% were considered in determining the slope and intercept of this line.

The average calibration slope and intercept were used to estimate the instantaneous polarizations during three sets of *in vivo* pig heart experiments. Reasonable initial polarizations of 17%, 20%, and 23% were obtained. More interestingly, we were able to estimate *in vivo* polarization decay as a function of time using recorded heart rates, such as the curve shown in Figure 2. This was feasible even with the large variation in signal amplitude as the bolus washed through the chambers.

Table 1: Longitudinal relaxation time constants (sec) for each spectral line and each full doublet.

	C_1			C_2		
Pyruvate Concentration	Upfield	Downfield	Doublet	Upfield	Downfield	Doublet
8 mM in blood (37°C)	35 ± 2	48 ± 3	42 ± 3	31 ± 3	43 ± 3	36 ± 3
8 mM in blood (20°C)	30 ± 3	37 ± 3	34 ± 2	27 ± 2	35 ± 2	31 ± 2
80 mM solution (20°C)	55 ± 5	66 ± 4	62 ± 5	43 ± 3	53 ± 2	48 ± 2

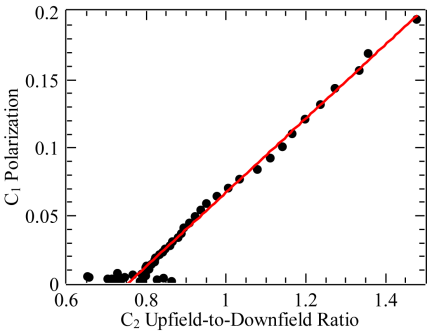

Figure 1. Calibration curve relating C_2 ratio, *z*, to C_1 instantaneous polarization, *p*. From regression, $p = 0.272z - 0.205$, $r^2 = 0.997$.

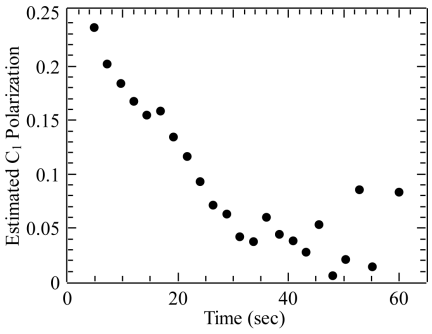

Figure 2. Estimated *in vivo* C_1 polarization during whole pig heart ^{13}C spectroscopic measurement with recorded heart rate of 100 beats per minute.

Discussion:
In blood, body temperature longitudinal relaxation time constants were longer than those at room temperature. Due to field-dependent relaxation at low fields (7) that the solution may experience during transport from polarizer to scanner, it is important to obtain calibration data with transfer times and handling procedures comparable to those used during *in vivo* studies. Estimated *in vivo* C_1 polarizations appear to follow a mono-exponential decay only within the first 10 s, although it continues to decrease monotonically as expected. As the pyruvate-blood bolus enters the right atrium, its relaxation behaviour is expected to resemble that observed in our blood phantom experiments. Over time, the pyruvate becomes distributed among the four compartments of the heart. These separated pyruvate pools may experience different relaxation conditions, resulting in multiple contributions in the spectral intensities to which our calibration data may not apply.

Conclusions:
We have demonstrated an empirical model that allows *in vivo* C_1 polarization monitoring in real-time. Polarization estimates obtained from this method are effectively independent of signal amplitude variations resulting from perfusion and metabolic activity. With accurate *in vivo* instantaneous polarization measurements, it would be possible to determine the number of moles of each metabolite within a voxel after accounting for DNP enhancement, coil sensitivity, and normalization to an external ^{13}C reference standard. This information could allow the determination of enzymatic conversion and exchange rates in physical units, enabling quantitative comparisons between studies and potentially providing further insight into the biochemistry of disease onset, progression, and response to treatment.

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References: (1) J.H. Ardenkjaer-Larsen *et al*. *Proc. Natl. Acad. Sci. USA* **100**: 10158 (2003). (2) R.E. Hurd *et al*. 50th ENC, p438. (3) A.P. Chen *et al*. *Proc. Intl. Soc. Mag. Reson. Med.* **18**: 3261 (2010). (4) S. Schäublin *et al*. *J. Magn. Reson.* **13**: 196-216 (1974). (5) A.P. Chen *et al*. *NMR Biomed.* doi: 10.1002/nbm.1749 (2011). (6) J.Y. Lau *et al*. *Proc. Intl. Soc. Mag. Reson. Med.* **19**: 1513 (2011). (7) F.M. Martinez-Santiesteban *et al*. *Proc. Intl. Soc. Mag. Reson. Med.* **19**: 656 (2011).