Hyperpolarized singlet lifetimes of pyruvate in human blood and in mouse

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TARGET AUDIENCE: This work is aimed at basic scientists and radiologists who have an interest in the application of hyperpolarized ^{13}C-labelled cell substrates to investigate tissue metabolism *in vivo*.

PURPOSE: Hyperpolarized NMR is a promising technique for non-invasive imaging of tissue metabolism *in vivo*. However, the range of reactions that can be investigated is limited by the fast T_1-dependent decay of the nuclear spin order. In metabolites with coupled nuclear spin-1/2 pairs, polarization may be maintained for a longer time by exploiting the non-magnetic singlet (spin-0) state of the pair. This may allow preservation of hyperpolarization *in vivo* during transport to tissues of interest, such as tumours, and detection of slow metabolic processes. Also, a different peak pattern may be observed in the NMR spectrum depending on the magnetic field at which the metabolic reaction takes place, giving information on the metabolism that takes place before spectral acquisition. We show here that the ^{13}C singlet lifetime of [1,2-$^{13}C_2$]pyruvate is longer than T_1 in human blood and in a mouse *in vivo* at low field, albeit shorter than its T_1 in high magnetic field.

METHODS: Experiments in blood: [1,2-$^{13}C_2$]pyruvic acid or [1-^{13}C]pyruvic acid containing 15 mM trityl radical OX063 (GE Healthcare, Little Chalfont, UK) and 1.5 mM gadolinium chelate (Gadoteric acid, Dotarem®; Guerbet, Roissy, France) were hyperpolarized in a GE Healthcare DNP prototype hyperpolarizer working at 3.35 T and ~1.2 K and dissolved in 6 mL of a superheated D_2O buffer solution. After dissolution, pyruvate was diluted in either oxygenated or deoxygenated whole human blood to a final pyruvate concentration of 13.5 mM. For measurement of T_1 and T_S at low field, the blood samples were maintained in the NMR laboratory background field (~ 1 mT) at 37 °C and then inserted into the 9.4 T spectrometer magnet at intervals of ~ 20 s. ^{13}C spectra were acquired using a flip angle of 6° at 37 °C with a broadband probe in a 9.4 T vertical wide-bore magnet (100 MHz ^{13}C, Oxford Instruments, Oxford, UK) interfaced to a Varian ^{Unity}Inova console (Varian Inc., Palo Alto, CA). *In vivo* experiments: Hyperpolarization was carried out using a commercial Hypersense polarizer (Oxford BioTools, Oxford, UK), using the procedure described above. The levels of polarization for both types of experiment were typically ~ 20 % at the time of injection (within ~ 10 s after dissolution). The mouse was placed in a dual tuned ^{13}C/^{1}H volume coil (Rapid Biomedical, Germany), in a 7 T horizontal bore magnet (Agilent, Palo Alto, CA). Hyperpolarized [1,2-$^{13}C_2$]pyruvate was injected intravenously (0.2 mL, injected in 3-5 s) outside the magnet and each mouse was maintained at low field (~ 40 mT) for a delay time of 0, 3 or 7 s after the end of the injection before the mouse was rapidly inserted into the magnet for signal acquisition. Sixty ^{13}C spectra were acquired with a 2 s repetition time, using a non-slice-selective excitation pulse with a flip angle of 10º. Experiments were performed in compliance with the Animals (Scientific Procedures) Act of 1986 and were approved by local ethical review committees.

RESULTS AND DISCUSSION: The benefit of creating singlet order in [1,2-$^{13}C_2$]pyruvate is limited by the observation that the high field T_1 for both carbons is 1.6 – 2-fold longer than the T_S when in contact with blood. Furthermore, in D_2O buffer, although $T_S \sim 2T_1^{LF}$ (T_1 at low field) for [1,2-$^{13}C_2$]pyruvate, T_S of [1,2-$^{13}C_2$]pyruvate is comparable to T_1^{LF} of [1-^{13}C]pyruvate (~ 70 s in both cases [1]). Therefore, in the case of [1,2-$^{13}C_2$]pyruvate, preserving nuclear spin order in the long-lived singlet state is only beneficial for extending the lifetime of the substrate in D_2O buffer but does not provide any additional low-field metabolic information.

Our results also show that T_S of [1,2-$^{13}C_2$]pyruvate is longer than T_1^{LF} at ~ 40 mT in a live mouse. If hyperpolarized singlet order [1,2-$^{13}C_2$]pyruvate is injected into a subject maintained at low magnetic field, the ^{13}C nuclei of the product of any metabolic reaction will also be expected to be in the singlet order configuration, provided that the chemical bond between the two carbons is preserved and that the field is low enough to satisfy the near-equivalence condition. On the contrary, if the bond is broken (e.g. by decarboxylation of pyruvate to produce carbon dioxide), the product metabolite will be in the single-spin energy configuration, and since it will have originated from a zero-magnetization system no signal will be detected. Our observations show that singlet order was not preserved in the product [1,2-$^{13}C_2$]lactate produced at 40 mT from the injected [1,2-$^{13}C_2$]pyruvate. According to our predictions, the lactate signal we detect arises from the pyruvate metabolised at high field. This may be explained by the fact that at 40 mT the singlet-triplet states are not eigenstates of [1,2-$^{13}C_2$]lactate, accelerating the decay of the polarization. Consequently, if [1,2-$^{13}C_2$]pyruvate is injected at ~ 40 mT and the mouse is maintained at this field for a certain amount of time, metabolism will start occurring and polarization will leak through to the non-singlet lactate. Therefore, at the moment of detection at 7 T an apparent reduction in the amount of lactate will be observed in the NMR spectrum.

	T_1^{LF} (s) at 1 mT	T_S (s) at 1 mT	T_1^{HF} (s) at 9.4 T
[1,2-$^{13}C_2$]pyruvate	<5	19 ± 2	38.5 ± 0.4/30.0 ± 0.2 *(173 ppm/207 ppm)*
[1-^{13}C]pyruvate	8 ± 1	N.A.	45.0 ± 0.5

Table 1. ^{13}C relaxation time constants in whole human blood at 37 ºC. No differences were detected using oxygenated or deoxygenated blood.

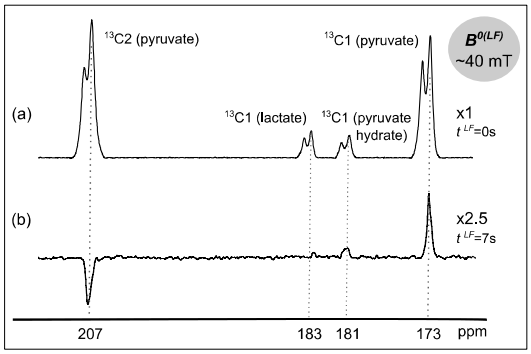

Figure 1. ^{13}C NMR spectra of a mouse tumor *in vivo* at 7.0 T after i.v. injection of hyperpolarized [1,2-$^{13}C_2$]pyruvate. (a) Acquired immediately following injection, (b) after maintaining the animal for ~ 7 s at ~ 40 mT after injection. Spectra were acquired with single scans.

CONCLUSION: Creating singlet order in doubly labelled molecules to extend polarization lifetime at low field would allow for instance, redistribution in the body and metabolism of the ^{13}C-labeled molecule in the singlet state while the patient is in low field and give more handling time in clinical applications. In the recent clinical trial of hyperpolarized [1-^{13}C]pyruvate in prostate cancer patients, the average time taken from dissolution to the completion of injection was 67.6 s [2] and presumably for most of this time the sample was in dissolution buffer at low field. However, the advantage of creating singlet order will only be fully realized if doubly labelled molecules can be identified for which T_S is longer than the T_1 of the singly labelled species, both at low and high magnetic field.

ACKNOWLEDGEMENTS: European Union Seventh Framework Programme (FP7/2007-2013) for support under the Marie Curie Initial Training Network *METAFLUX* (project number 264780).

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