# Effect of Albumin on Longitudinal Relaxation of [1-$^{13}C_1$]-Pyruvate

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## Introduction

The first trials of clinical $^{13}C$ imaging will likely monitor hyperpolarized [1-$^{13}C_1$]-pyruvate and its metabolic products. Albumin in blood binds numerous metabolites and drugs, and it is required for transport of long-chain fatty acids to the heart, liver and skeletal muscle. Albumin catalyzes the exchange of protons on the methyl group of pyruvate with solvent water, probably via formation of a Schiff base between pyruvate and lysine or arginine residues of albumin (1). Binding of pyruvate to albumin could reduce the $T_1$ of pyruvate and thereby reduce delivery of hyperpolarized pyruvate to the tissue of interest resulting in lower-quality images or the need to inject higher concentrations of pyruvate. The purpose of this study was to examine the effects of albumin on the hyperpolarized [1-$^{13}C_1$]-pyruvate signal and to confirm an earlier report of pyruvate binding to albumin (1).

## Methods

***Hyperpolarization Experiments.*** Rat hearts were perfused in the Langendorff mode with glucose-containing Krebs-Henseleit (KH) medium and 1) no BSA (bovine serum albumin), 2) 3.0 % BSA and 0.4 mM fatty acids or 3) 3.0 % fatty acid-free BSA. [1-$^{13}C_1$]-pyruvate was hyperpolarized using an Oxford Hypersense. HP [1-$^{13}C_1$]-pyruvate was injected into the heart and $^{13}C$ spectra were acquired using a 66 degree pulses with a 1 sec TR.

***Relaxation Experiments.*** [1–$^{13}C_1$]-Pyruvate (2.5 mM in buffered saline plus $D_2O$ for a lock) was added to 8 different concentrations of BSA (0 to 3%) with and without long-chain physiological fatty acids at a fixed ratio of 0.4 mM fatty acids : 3% BSA. $^{13}C$ $T_1$ relaxation times were measured at 150 MHz using standard inversion-recovery and saturation-recovery methods at 37 $^oC$.

***$^1H/^2H$ Exchange Experiments.*** Graded concentrations of [$^{12}C_3$]-pyruvate, 100 to 500 mM, were maintained at 37 $^oC$ with or without 3 % BSA and 0.4 mM fatty acids in > 95 % $D_2O$. Thirty-six $^1H$ NMR spectra were collected at intervals over 12 hours.

## Results

In the absence of albumin, hyperpolarized [1-$^{13}C_1$]-pyruvate at 2-3 mM typically produced signals from $CO_2$, $HCO_3$, lactate and alanine with a 1 s time resolution. In the presence of fatty acid-free BSA, essentially no signal was detected, but in the presence of BSA and fatty acids, [1-$^{13}C_1$]-lactate was detected (Figure 1a). At a higher concentration of hyperpolarized [1-$^{13}C_1$]-pyruvate (20 mM), [1-$^{13}C_1$]-lactate was observed even with fatty acid-free BSA. When fatty acids were not available to provide an alternative substrate for oxidation, $HCO_3^-$ was also observed. In the absence of BSA, the $T_1$ of [1-$^{13}C_1$]-pyruvate was 44 seconds and decreased to < 10 seconds above 2% fatty acid-free BSA. In the presence of fatty acids, $T_1$ was shortened at higher concentration of BSA but remained about 25 seconds at 3.0% BSA. A saturation-recovery experiment of [1-$^{13}C_1$]-pyruvate with 3 % fatted BSA resulted in a bi-exponential $T_1$ decay resulting in a very short component, 91 μs, and a longer component, 28 s. Slow exchange of solvent deuterons into pyruvate methyl protons, catalyzed by albumin, was confirmed (Figure 1b).

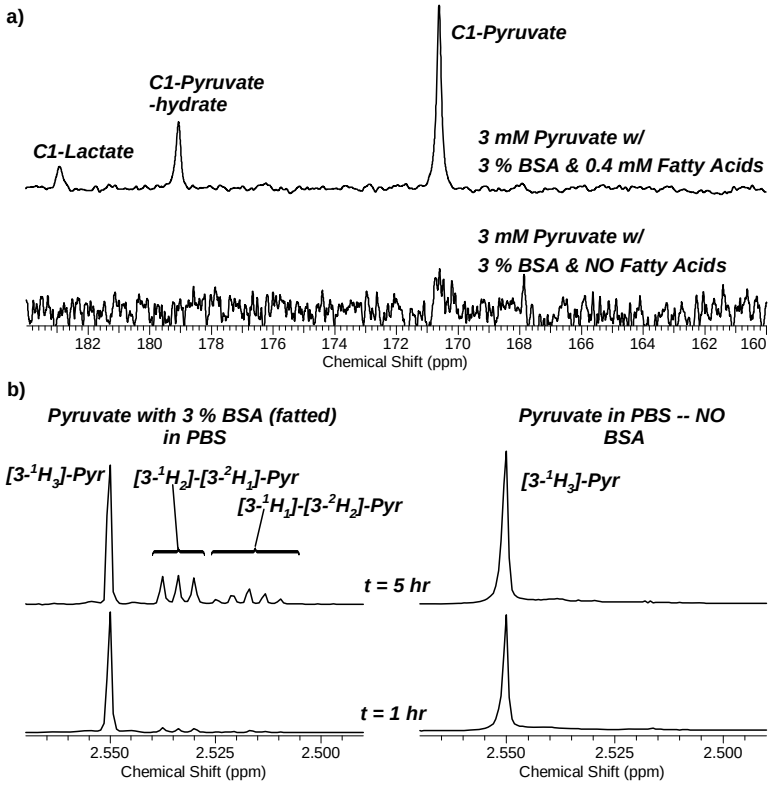


**Figure 1.** a) Effect of BSA on hyperpolarized [1-$^{13}C_1$]-pyruvate. b) $^1H/^2H$ exchange of pyruvate methyl protons in the presence BSA containing fatty acids (left) vs no BSA (right).

## Conclusions

The $T_1$ of [1-$^{13}C_1$]-pyruvate is reduced by interactions with albumin. The effect is most prominent in fatty-acid free albumin but is detected even in the presence of physiological concentrations of fatty acids. Transient covalent bonds between pyruvate and albumin may form spontaneously, although the role this reaction plays in the reduction of $T_1$ is unknown. These observations confirm earlier reports that the $T_1$ of pyruvate is reduced in vivo (2,3). Together, these data illustrate that an understanding of pyruvate binding to albumin is essential for quantitative interpretation of imaging data obtained with hyperpolarized [1-$^{13}C_1$]-pyruvate.

## References

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