

# Metabolic spectroscopy and imaging of lung inflammation using hyperpolarized $1\text{-}^{13}\text{C}$ pyruvate

Sarmad Siddiqui<sup>1</sup>, Hooria Shaghaghi<sup>1</sup>, Stephen Kadlecek<sup>1</sup>, Harrilla Profka<sup>1</sup>, and Rahim Rizzi<sup>1</sup>  
<sup>1</sup>Department of Radiology, University of Pennsylvania, Philadelphia, PA, United States

## Introduction

It is generally accepted that inflammatory cell activity, particularly that of neutrophils, may contribute to, or even exacerbate existing lung injury [1]. Release of inflammatory factors from these cells is known to contribute to injury in ventilator-induced lung injury (VILI), acute respiratory distress syndrome (ARDS), and other similar injuries. As such, techniques for reducing neutrophil infiltration may lead to better clinical outcomes. Directly visualizing the progression of lung inflammation would be very useful. The conventional techniques for measuring inflammatory cell activity, biopsies and lavage, are not very effective in sampling regional differences. Radiological methods are not well-suited either as they are highly dependent on alterations of tissue density. However, it has been shown that the metabolic state of inflammatory cells, particularly glycolytic activity, is much greater than that of normal lung tissue [2]. As such, in this study, the uptake of hyperpolarized  $[1\text{-}^{13}\text{C}]$  pyruvate is investigated as a potential marker for inflammation in lung tissue.

## Methods

Two groups of Sprague-Dawley rats (5 healthy and 5 Bleomycin-exposed inflammatory model) were used in this study. Lungs of male rats (250-350g) were excised and placed in a 20mm NMR tube (9.4T vertical bore magnet) while perfused with a modified Krebs-Henseleit buffer containing 3% (w/v) fatty-acid free BSA. The perfusate was passed through an oxygenating column under a constant flow of 1 atm 95:5  $\text{O}_2/\text{CO}_2$ , and warmed to  $36.5^\circ\text{C}$  via passage through a water-jacketed tubing. The perfusate pH was adjusted with 1N HCl or NaOH to maintain a physiological value of  $7.4 \pm 0.1$  during perfusion. The temperature of the perfusate in the NMR tube was continuously monitored and maintained at  $36.5 \pm 1^\circ\text{C}$ . The health of the tissue was monitored using  $^{31}\text{P}$  spectroscopy. 28.5mg  $[1\text{-}^{13}\text{C}]$  pyruvic acid was polarized with a HyperSense DNP system (Oxford Instruments). 4 mL Tris-buffered saline with 100 mg/L EDTA was heated to  $190^\circ\text{C}$  at 10 bar and was used to rapidly dissolve the frozen sample. This sample was further diluted in different volumes of oxygenated Krebs-Henseleit buffer (without BSA) yielding a neutral, isotonic solution of 32 mM  $[1\text{-}^{13}\text{C}]$  pyruvate. This solution was then injected at 10mL/min, and low flip-angle ( $\alpha=15^\circ$ ) carbon spectra were acquired for the duration of the hyperpolarized signal. The spectra were fitted and analyzed using custom MATLAB routines.

## Results

Figure 1 shows the (a) superimposed  $^{31}\text{P}$  spectra of control and inflammatory rat models. Eight distinct peaks can be identified in each spectrum. There is a significant difference in the glycerophosphocholine and glycerophosphoethanolamine concentrations of each model as seen in (b).

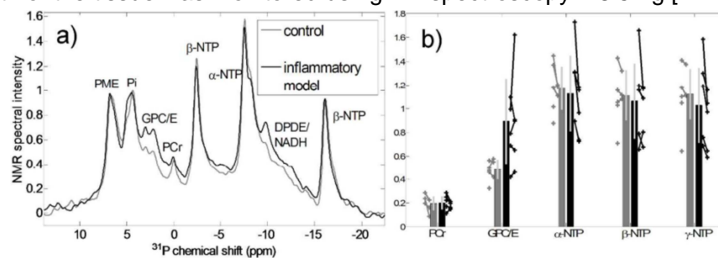
Figure 2(a) shows a stacked time-series of non-selective spectra from an inflammatory model subject; Figure 2(b) the infusion of hyperpolarized  $1\text{-}^{13}\text{C}$  pyruvate followed by lactate and alanine metabolic products. The inflammatory model rats show an approximately 4-fold increase in lactate production compared to control rats. This increase is observed across the entire acquisition period (2b, bottom).

Figure 3 shows the chemical shift imaging of injected pyruvate (middle) and lactate (right) reveals a localized region of rapid lactate production when superimposed on a  $^1\text{H}$  image of perfused lungs (left).

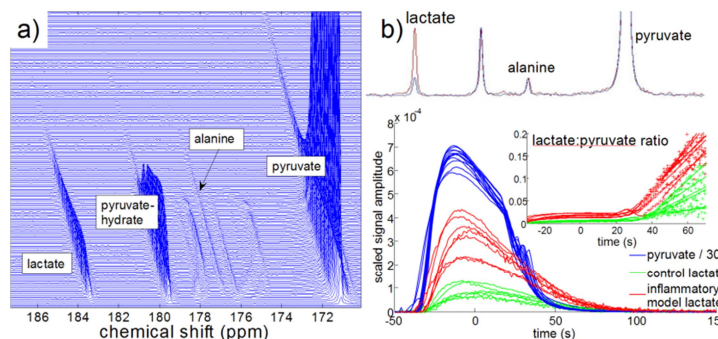
## Conclusions

Our study demonstrates that hyperpolarized carbon-13 spectroscopy and imaging can be used to detect and localize pulmonary inflammation. Because the baseline metabolic activity of the lung is relatively low, this technique allows us to visualize a multiple-fold increase in signal contrast from inflammatory cells. The added advantage of this technique is that the measurement is from a direct metabolic pathway, the glycolytic pathway, of the infiltrating inflammatory cells. Future studies will focus on probing the response of the cells to a variety of other insults, both *ex vivo* and *in vivo*.

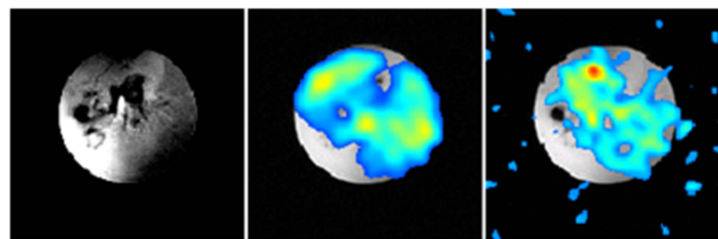
**References** [1] Ross SD, et al. Ann Thorac Surg 67(5):1428-1433 (1999). [2] Borregaard N, Herlin T.J Clin Invest 70:550-554 (1982)



**Fig. 1:** (a) spectra and (b) quantification of metabolites in control and inflamed rat models.



**Fig. 2:** (a) time series of an inflamed rat model (b) and comparison of lactate metabolism in healthy and inflamed rat models



**Fig. 3:** Chemical Shift Imaging of pyruvate and lactate superimposed on the proton spectra of a perfused lung