# Metabolism of Hyperpolarized 1-$^{13}$C-Lactate in Living Breast Cancer Cell Cultures

**T. Harris[1], G. Eliyahu[2], L. Frydman[1], and H. Degani[2]**
[1]Chemical Physics, Weizmann Institute of Science, Rehovot, Israel, [2]Biological Regulation, Weizmann Institute of Science, Rehovot, Israel

## Introduction

The recent development of a technique to enhance $^{13}$C NMR polarizations by ≥10,000x may allow us to follow metabolic processes non-invasively with unprecedented sensitivity and temporal resolution [1]. The importance of such development for in vivo metabolic imaging is clear and many important works have been done showing the utility of hyperpolarized metabolic imaging both in cancer and in cardiac disease. However, in vivo metabolic studies may not sufficient in order to understand the underlying cellular processes that control the altered metabolism. For this purpose we have developed a bioreactor, allowing hyperpolarized metabolic measurements on living cell cultures maintained under well-controlled conditions.

We have previously applied this system to the study of pyruvate metabolism in T47D human breast cancer cells, and were able to characterize the kinetic parameters [2]. In this study we explore the feasibility of using hyperpolarized 1-$^{13}$C-Lactate as a biomarker to understand the glycolytic phenotype of cancer cells.

## Materials and Methods

Cell Culture: T47D (clone 11) human breast cancer cells were cultured in RPMI medium 1640 supplemented with 10% FCS (Biological Industries), 5 mL of L-glutamine, 0.4 mL of insulin, and 0.1% combined antibiotics (Bio-Lab). For the NMR perfusion studies, cells (>3 × 106) were seeded on 0.5 mL of Biosilon polystyrene beads (160–300 μm, Nunc) in silanized glass vials. Three hours later they were transferred to bacteriological dishes for cultivation, changing medium every second day, as well as on the day before the experiment. After 5–7 days of culture, cells on beads were transferred into a 10-mm NMR test tube and connected to the perfusion-injection system describe previously.[2]

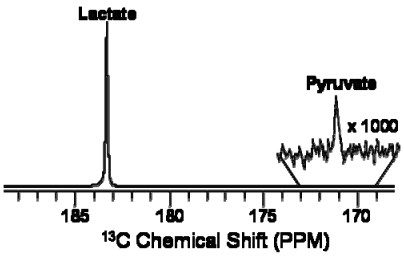
Hyperpolarization: A 0.84 M solution of $^{13}C_1$-Lactic Acid (Cambridge Isotopes) and 15 mM OX063 Trityl (GE Healthcare) was prepared, by mixing equal volume of $^{13}C_1$-Lactic Acid with $d_6$-DMSO. A 1.0 M solution of $^{13}C_1$-Sodium Pyruvate (Cambridge Isotopes) and 15 mM OX063 Trityl (GE Healthcare) was prepared, by mixing equal volume of $D_2O$ and $d_6$-DMSO. An aliquot of the metabolite solution was inserted into a Hypersense® 3.35-T low-temperature polarizer and irradiated at 1.5 K for executing dynamic nuclear hyperpolarization (DNP). After ≈90 min of microwave irradiation, the sample was dissolved in 4 mL of phosphate buffer solution containing 1 mg of EDTA; this solution was heated to 180 °C, pressurized to 10 bar, and flushed into the perfusion–injection system within 2–3 sec.

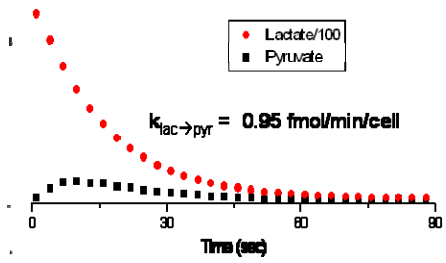
NMR Experiments: Spectra were recorded on an 11.7-T Varian spectrometer using a 10-mm broadband probe. These included $^{31}$P data recorded to monitor cell viability, and $^{13}$C spectra recorded immediately after injection of the hyperpolarized solution. 13C spectra were acquired

Data Analysis: The $^{13}C_1$-pyruvate and $^{13}C_1$-lactate signal intensity changes arising from these experiments were fit to a kinetic model using custom-written Matlab and Origin routines.

## Results and Discussion

The conversion of lactate to pyruvate could be observed in living breast cancer cells using a train of small angle pulses, as we have previously described for the pyruvate to lactate conversion [2,3] (Figure 1; left panel). The rate of pyruvate formation from lactate was much slower than the reverse reaction, and therefore to obtain kinetic measurements we employed selective pulses centered at the pyruvate peak. Analysis of the kinetic data, as well as normalization for cell number by using $^{31}$P spectra, yielded the rate constant for both directions of this reaction. At the same concentration of substrate (10mM) the rate of lactate→pyruvate was determined to be 0.95 fmol/min/cell, while the rate of pyruvate→lactate was previously shown to be 22.55 fmol/min/cell [2]. This approximately 20-fold difference agrees well with the difference between pyruvate and lactate uptake measured in tumor cells [4]. This result suggests that the metabolism of lactate to pyruvate is transport limited, as was previously shown for the pyruvate→lactate conversion. [2]




## Conclusion

This work shows the applicability of the perfusion-injection bioreactor in probing the kinetics of biomarkers such as pyruvate and lactate, despite their kinetics differing by an order of magnitude. By expanding the pool of biomarkers, greater understanding of the glycolytic phenotype of cancer cells can be advanced.

### References

[1] Ardenkjaer-Larsen, J.H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M. H., Servin, R., Thaning, M. & Golman, K. (2003) *Proc Natl Acad Sci USA* **100,** 10158-10163. [2] Harris, T., Eliyahu, G., Frydman, L., Degani, H. (2009) *Proc Natl Acad Sci USA* **106,** 18131-18136. [3] Day, S.E.,Kettunen, M.I.,Gallagher, F.A., Hu, D., Lerche, M., Wolbe, J., Golman, K., Ardenkjaer-Larsen, J.H. & Brindle, K.M.(2007) *Nature Medicine* **13,** 1382-1387. [4] Carpenter, L., Halestrap, A.P. (1994) *Biochem J* **351,** 751-760.

Financial support: Israel Science Foundation ISF ISF 235/08 and 447/2009; Helen Kimmel Award for Innovative Research.