USE OF HYPERPOLARIZED ^{13}C MR TO MONITOR CARDIAC METABOLISM *IN VIVO* IN TYPE 1 DIABETES

M. A. Schroeder[1], L. E. Cochlin[1], K. Clarke[1], G. K. Radda[1], and D. J. Tyler[1]
[1]Physiology, Anatomy and Genetics, University of Oxford, Oxford, Oxon, United Kingdom

Introduction

The application of ^{13}C MR spectroscopy for metabolic imaging has been limited by an intrinsically low sensitivity and the low natural abundance of ^{13}C. However, Ardenkjær-Larsen *et al* recently developed a method in which MR-active nuclei, hyperpolarized by Dynamic Nuclear Polarization (DNP), could be dissolved to obtain a solution polarized in excess of 20% [1]. When used in concert with MR spectroscopy, this method of hyperpolarization provides the MR signal necessary to detect low abundance molecules, enabling visualization of substrate uptake and *in vivo* metabolism in real time. This study used hyperpolarized 1-^{13}C-pyruvate as a metabolic tracer to detect changes in cardiac metabolism characteristic of type 1 diabetes. Specifically, the activity of pyruvate dehydrogenase (PDH), a tightly regulated mitochondrial enzyme fundamental to substrate selection in the myocardium, was monitored.

Methods

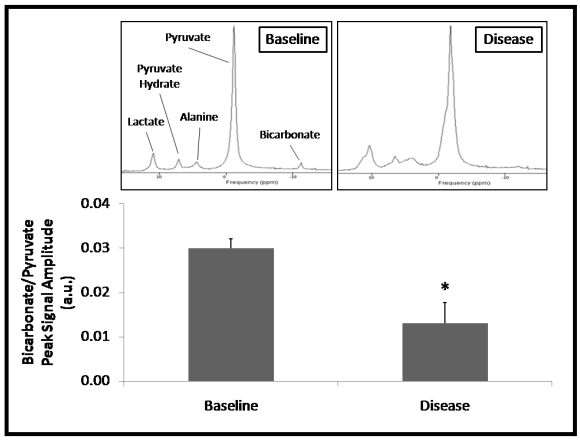
Six male Wistar rats were examined at baseline with hyperpolarized MR, as described below, such that each rat could serve as its own experimental control. Type 1 diabetes was subsequently induced with an intraperitoneal injection of freshly prepared streptozotocin, STZ, (50 mg/kg) in cold citrate buffer at pH 4.5. Five days after STZ-diabetes induction, rats were again examined by the hyperpolarized MR protocol. Rats were then recovered and sacrificed 1 hr later for tissue preparation.

MR-Protocol: 1-^{13}C-pyruvate was used for all experiments and was hyperpolarized and dissolved as previously described [2]. The resultant hyperpolarized tracer consisted of an 80 mM solution of hyperpolarized sodium 1-^{13}C-pyruvate at pH 7.2 – 7.9, with a polarization of ~30%. The tracer (1 ml) was injected over 10 s via a tail vein catheter into the anaesthetized rat which was positioned in the centre of a horizontal bore 7 T MR scanner. Immediately prior to injection, a ^{13}C-MR pulse-acquire spectroscopy sequence was initiated. Cardiac spectra were acquired for 1 min following injection with 1 s temporal resolution and a flip angle of approximately 5°. Spectra were localized to the heart by the use of a small butterfly surface coil placed over the chest.

Data Analysis: Conversion of pyruvate to lactate, alanine, and bicarbonate was monitored and spectra were quantified in jMRUI [3]. Maximum bicarbonate/pyruvate peak ratio was used as a marker of PDH activity. Variations in polarization were standardized between data sets by normalizing bicarbonate peak area to maximum pyruvate peak area. Plasma glucose, insulin, and fatty acid concentrations were measured, and heart tissue collected for subsequent analysis of PDH activity *in vitro*.

Results

All acquired spectra were of high SNR and peaks relating to the injected pyruvate, as well as the metabolic products, lactate, alanine and bicarbonate, were clearly visualized (Figure 1). The ratio of the maximal bicarbonate and maximal pyruvate peak areas showed a 57% reduction 5 days after the initiation of type 1 diabetes ($p<0.05$). This indicated a significant reduction in PDH activity in the type 1 diabetic heart. Further, bicarbonate peak area was shown to correlate negatively ($r=-0.90$) with blood glucose, an indicator of type 1 diabetes disease severity (Figure 2).



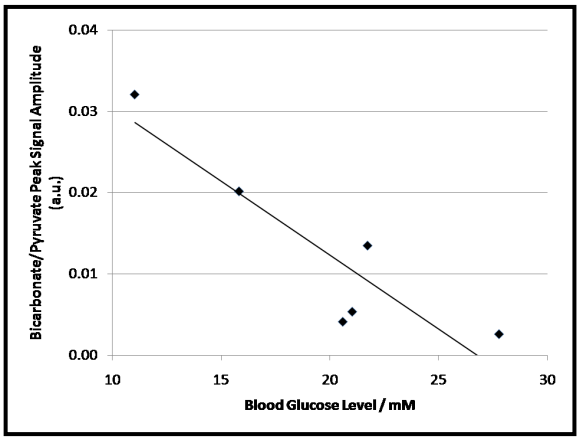


Figure 1, left *Ratio of bicarbonate/pyruvate peak areas at baseline and 5 days after induction of type 1 diabetes with STZ.Specta show a single acquisition with a 5° flip angle at t=10 s. *p<0.05 compared to baseline.* ***Figure 2, right*** *The relationship between bicarbonate peak signal area and blood glucose.*

Discussion

In this work we have demonstrated a reduction in PDH activity, indicated by a reduction in the level of bicarbonate production, in the type 1 diabetic heart. This reduction is caused by the increased expression of pyruvate dehydrogenase kinase (PDK), an inhibitor of PDH, in STZ-induced type 1 diabetes [4]. This work represents the first instance in which this pathological decrease in PDH activity has been detected *in vivo* and non-invasively. The ability to monitor PDH activity *in vivo* should provide a powerful tool for the study of disease progression and treatment in type 1 diabetes.

1. Ardenkjaer-Larsen, J.H., et al., *Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR.* Proc Natl Acad Sci U S A, 2003. **100**(18): p. 10158-63.
2. Golman, K., in't Zandt, R., Thaning, M., *Real-Time Metabolic Imaging.* Proc Natl Acad Sci U S A, 2006. **103**(30): p. 11270-11275.
3. Naressi, A., et al. , Computers in Biology and Medicine, 2001. **31**: p. 269-86.
4. Wu, P., et al., *Starvation and diabetes increase the amount of pyruvate dehydrogenase kinase isoenzyme 4 in rat heart.* Biochem J, 1998. **329 (Pt 1)**: p. 197-201.

Acknowledgements - This study was supported by the Medical Research Council, the British Heart Foundation and GE Healthcare.