# Use of Dichloroacetate to Aid the Investigation of Krebs Cycle Metabolism *In Vivo* in Normal Rat with Hyperpolarized [1-$^{13}$C]Pyruvate and [2-$^{13}$C]Pyruvate

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Introduction: Development of hyperpolarized technology utilizing dynamic nuclear polarization has enabled the measurement of $^{13}$C metabolism *in vivo* at very high SNR [1]. *In vivo* mitochondrial metabolism can, in principle, be monitored with pyruvate, which is catalyzed to acetyl-CoA via pyruvate dehydrogenase (PDH). The purpose of this work was to determine if the compound sodium dichloroacetate (DCA) could aid the study of mitochondrial metabolism with hyperpolarized pyruvate. DCA stimulates PDH by inhibiting its inhibitor, pyruvate dehydrogenase kinase (PDK). DCA's anti-glycolytic effect on a prostate cancer model was investigated in a prior hyperpolarized $^{13}$C study [2]. In this work, hyperpolarized [1-$^{13}$C]pyruvate and [2-$^{13}$C]pyruvate were used to probe mitochondrial metabolism in normal rats.

Methods: Normal male Sprague-Dawley rats were used (total n = 3 animals examined). All studies were performed on a GE 3T scanner with a custom $^{1}$H/$^{13}$C rat coil [3]. Polarizations were performed in a standard manner as described previously [3]. DCA was dissolved in saline (about 200 mg in 2 mL). Each rat received a baseline $^{13}$C study and then immediately thereafter was given a 150 mg/kg IP injection of DCA. 1.5 hours later, 150 mg/kg of DCA was delivered via IV injection, and then a follow-up $^{13}$C study was performed. 3 mL of 100 mM hyperpolarized pyruvate was injected over 12 sec. Non-localized dynamic data were collected (starting upon injection) with a double spin-echo sequence [4] with 5 degree hard pulse excitation, TE = 35ms, and TR = 3 sec. Data were processed with 10 Hz Gaussian apodization, and the first 32 time points were summed together.

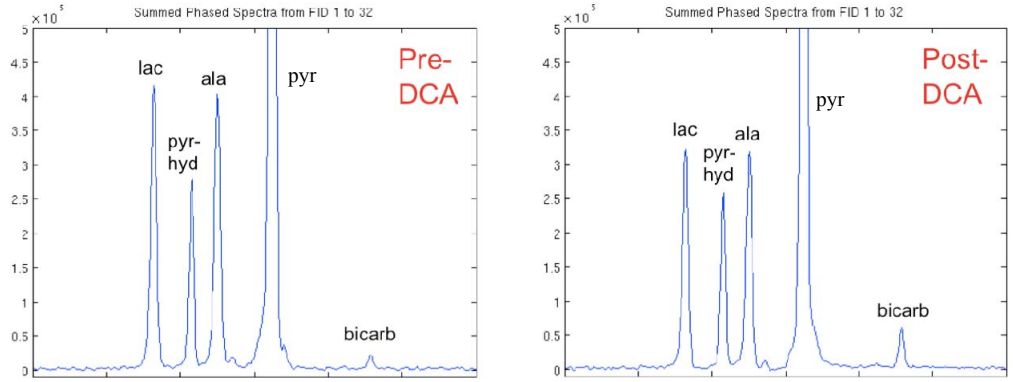


Figure 1: Non-localized rat spectra after injection of hyperpolarized [1-$^{13}$C]pyruvate pre and post DCA. No change in pyruvate or pyruvate-hydrate was observed. Decreased lactate and alanine and increased bicarbonate post DCA was consistent with reduced glycolytic flux and increased flux through PDH.

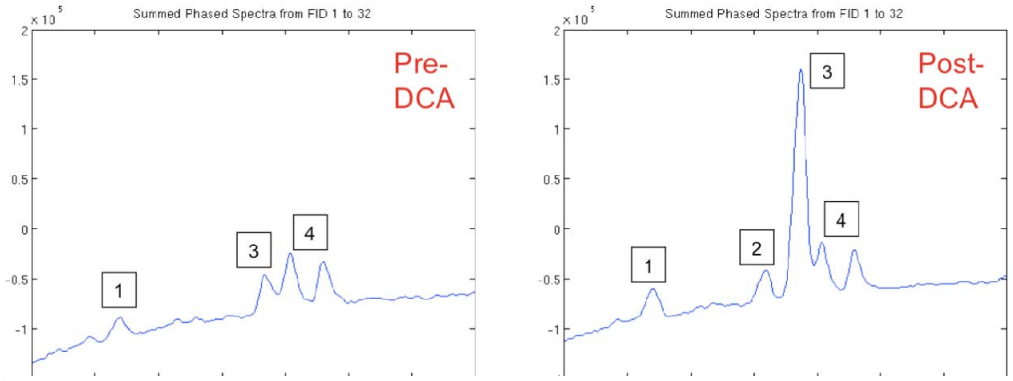


Figure 2: Non-localized rat spectra after injection of hyperpolarized [2-$^{13}$C]pyruvate pre and post DCA. The peaks and the measured ppm values in order from left to right are (1) [5-$^{13}$C]glutamate (183.9 ppm), (2) [2-$^{13}$C]acetoacetate (177.1 ppm), (3) [1-$^{13}$C]acetylcarnitine (175.0 ppm), and (4) a [1,2-$^{13}C_2$]pyruvate isotopomer doublet with minor lipid contamination (172.8 ppm). There was a dramatic increase in acetoacetate and acetylcarnitine post DCA. Glutamate might have increased slightly, and pyruvate remained constant.

Results: Figure 1 shows the pre and post DCA spectra when using hyperpolarized [1-$^{13}$C]pyruvate as the substrate. The injected pyruvate (and pyruvate-hydrate) levels did not change. Bicarbonate increased dramatically, and lactate and alanine decreased, suggesting that DCA was effective in stimulating PDH. Figure 2 shows the pre and post DCA spectra when using hyperpolarized [2-$^{13}$C]pyruvate as the substrate. The peak assignments and their ppm values are given in the Figure 2 legend. Peaks 1, 3, and 4 were assigned by comparing the ppm values with peaks assigned from a prior [2-$^{13}$C]pyruvate study [5], and peak 2 was assigned based on a standard metabolome database and consideration of plausible pathways. Once again, pyruvate did not increase, suggesting that injection volumes were consistent between pre and post acquisitions. Glutamate, increased perhaps slightly, and acetoacetate and acetylcarnitine increased dramatically.

Discussion: The increase in bicarbonate (in equilibrium with $CO_2$) demonstrates that DCA was effective in stimulating pyruvate flux through PDH. Acetyl-CoA derived from pyruvate entering the mitochondria has many metabolic fates. One of those is attachment to carnitine via carnitine acyltransferase I to form acetylcarnitine. As shown by Figure 2, most of the excess acetyl-CoA in a normal rat goes to form acetylcarnitine. In addition, acetyl-CoA can be converted to ketone bodies. Accordingly, Figure 2 shows production of the ketone body acetoacetate. Moreover, of course, excess acetyl-CoA can go through the TCA cycle, but in the case of the normal rat, there did not appear to be a marked increase in flux through the TCA cycle, as indicated by the glutamate peak. In summary, these results show that changes in carnitine and ketone body metabolism were detected in response to a physiological perturbation. These observations in the normal rat could serve as a basis for future disease studies. For example, carnitine deficiency occurs in diabetes, cirrhosis, and other metabolic disorders [6]. Furthermore, abnormal acetoacetate metabolism has been linked to cancer, with [$^{11}$C]acetate being investigated at a PET tracer [7].

References: [1] Ardenkjaer-Larsen et al. PNAS (2003) 100:10158 [2] Grant et al. 19th Proc. ISMRM (2011) p. 653 [3] Hu et al. MRI (2011) 29:1035 [4] Cunningham et al. JMR (2007) 187:357. [5] Schroeder et al. FASEB (2009) 23:2529 [6] Flanagan et al. Nutrition and Metabolism (2010) 7:30 [7] Leung. MICAD (2008). Pubmed ID: 20641818

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