Metabolic response of glioma to dichloroacetate measured in vivo by hyperpolarized $^{13}$C magnetic resonance spectroscopic imaging

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**Background.** The metabolic phenotype that derives disproportionate energy via glycolysis in solid tumors, including glioma, leads to elevated lactate labeling in metabolic imaging using hyperpolarized [1-$^{13}$C]pyruvate. Although the pyruvate dehydrogenase (PDH)–mediated flux from pyruvate to acetyl coenzyme A can be indirectly measured through the detection of carbon-13 ($^{13}$C)-labeled bicarbonate, it has proven difficult to visualize $^{13}$C-bicarbonate at high enough levels from injected [1-$^{13}$C]pyruvate for quantitative analysis in brain. The aim of this study is to improve the detection of $^{13}$C-labeled metabolites, in particular bicarbonate, in glioma and normal brain in vivo and to measure the metabolic response to dichloroacetate, which upregulates PDH activity.

**Methods.** An optimized protocol for chemical shift imaging and high concentration of hyperpolarized [1-$^{13}$C]pyruvate were used to improve measurements of lactate and bicarbonate in C6 glioma-transplanted rat brains. Hyperpolarized [1-$^{13}$C]pyruvate was injected before and 45 min after dichloroacetate infusion. Metabolite ratios of lactate to bicarbonate were calculated to provide improved metrics for characterizing tumor metabolism.

**Results.** Glioma and normal brain were well differentiated by lactate-to-bicarbonate ratio ($P = .002, n = 5$) as well as bicarbonate ($P = .0002$) and lactate ($P = .001$), and a stronger response to dichloroacetate was observed in glioma than in normal brain.

**Conclusion.** Our results clearly demonstrate for the first time the feasibility of quantitatively detecting $^{13}$C-bicarbonate in tumor-bearing rat brain in vivo, permitting the measurement of dichloroacetate-modulated changes in PDH flux. The simultaneous detection of lactate and bicarbonate provides a tool for a more comprehensive analysis of glioma metabolism and the assessment of metabolic agents as anti-brain cancer drugs.

**Keywords:** bicarbonate, dichloroacetate, glioma, hyperpolarized $^{13}$C, pyruvate.

**Glioblastoma multiforme** is one of the most aggressive cancers, with less than half of patients surviving beyond 1.5 years even with optimal treatment. New treatments, such as targeted molecular therapies and metabolic modulation, with improved effectiveness have been actively developed in the last decade, but there is still a need for methods that can be used to gauge the effects of therapy acutely after administration.

Unlike normal tissues, which derive the bulk of energy needs via oxidative phosphorylation (OXPHOS) of multiple energy substrates, solid tumors, including glioma, derive a disproportionate amount of energy via glycolysis (GLY), even when oxygen tension levels are high, also known as the Warburg effect. Contrary to Warburg’s original idea, however, mitochondrial dysfunction is not the cause of this metabolic change, raising several other proposed possibilities, including the capacity to derive energy from glucose more quickly if not more efficiently through GLY, the need to create biomass and to support proliferation, and the development of a more favorable (for the cancer cell) acidic environment. Interest
in the Warburg effect, whatever its underlying function, has been increasing recently due in large part to the hope that a better understanding will lead to novel therapeutic anticancer strategies.

Until recently, a major difficulty in studying cancer metabolism has been that limited tools exist with which to study it repeatedly in the intact organism. Using 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) PET scanning, the diagnostic effectiveness of which is based on the Warburg effect (due to increased glucose utilization by cancer tissue), provides no information as to how glucose is processed within cells because FDG-PET cannot differentiate metabolic products from the injected substrate.9,10 Elevated lactate (Lac) concentration in brain tumors due to upregulated glycolytic rate can be detected using proton (1H) and carbon-13 (13C) magnetic resonance spectroscopy (MRS).11,12 In 1H-MRS, Lac quantification is hampered by signal overlap from lipids that can arise within tumors13 as well as from fat outside the brain containing signal the spectra due to non-ideal spatial localization. Although spectral editing techniques are available to help differentiate Lac from lipids,14,15 these methods also reduce the signal-to-noise ratio (SNR).16 can be prone to patient motion during long acquisition, and are not routinely used in the clinic.13C-MRS provides better signal separation due to a wider dispersion of the chemical shift, but because of its low sensitivity, it requires long acquisition times that severely hinder real-time in vivo investigations.17,18

Hyperpolarized 13C-MRS, using dynamic nuclear polarization (DNP) in combination with a rapid dissolution process to retain high levels of nuclear spin polarization in the liquid state, enables the real-time investigation of in vivo metabolism with more than 10,000-fold signal increase over conventional 13C-MRS methods.19,20 Using [1-13C]pyruvate (Pyr), a substrate occupying a key nodal point in the glucose metabolic pathway, allows one to quantitatively follow the in vivo conversion of Pyr to Lac, alanine (Ala), or acetyl coenzyme A (CoA).

Pyr has shown great potential for differentiating cancer from normal tissue and detecting metabolic response to treatment.24–26 Recently, hyperpolarized [1-13C]Pyr has been applied to rat brain implanted with human gliomas,27 and the early changes in tumor metabolism after exposure to temozolomide treatment,28 therapy targeted to the phosphatidylinositol 3′-kinase (PI3K) pathway,29,30 and radiotherapy31 have been reported. Up to now, however, cancer studies utilizing 13C-MRS of hyperpolarized Pyr have focused primarily on Lac production, in large part because of the poor SNR of Bic, thus representing a serious hurdle in being able to use this methodology to assess the balance between GLY and OXPHOS. Whereas Bic has been consistently detected in normal rat brain,32,33 detection in glioma has not been reported yet. Grant et al.34 recently demonstrated that the reduction of Lac in a transgenic mouse model for prostate cancer after a week of daily administration of dichloroacetate (DCA) could be consistently observed, but Bic was detected only post-DCA.

In this study we improve detection of Bic in brain of tumor-bearing rats by using single time-point spiral chemical shifting imaging (CSI)35 to increase SNR and by injecting [1-13C]Pyr with higher concentration. Furthermore, we measure the metabolic response of glioma and normal-appearing brain tissue to administration of DCA. DCA promotes mitochondrial function by inhibiting pyruvate dehydrogenase kinase (PDK), thus upregulating pyruvate dehydrogenase (PDH), which catalyzes the production of acetyl CoA from Pyr.36–41 DCA has been shown to reduce tumor growth both in vitro and in vivo42,43 and is currently in clinical trials (for details, see clinicaltrials.gov). Due to its small molecular size, DCA can readily transverse the blood–brain barrier, and the efficacy of DCA has been demonstrated for patients with malignant gliomas.44

**Materials and Methods**

**MR Hardware and Radiofrequency Coils**

All experiments were performed on a 3T Signa MR scanner (GE Healthcare) equipped with a high-performance insert gradient coil operating at a maximum amplitude of 500 mT/m with a slew rate of 1865 mT/m/ms.45 A custom-built dual-tuned 13C-1H quadrature volume radiofrequency (RF) coil (Ø = 50 mm)46 operating at 32.1 MHz and 127.7 MHz, respectively, was used for both RF excitation and signal reception, respectively.

**Animal Preparation and Tumor Model**

Implantation of 1 × 106 C6 glioma cells (derived from an N-methyl-N-nitrosourea–induced tumor47 as previously described48,49) was performed in the right striatum of 5 male Wistar rats (body weight = 235 ± 10 g) 10.5 days before 13C-MRS imaging. The estimated tumor size, measured from hyperintense regions of contrast-enhanced T1-weighted proton images, was 95.3 ± 17.9 mm3. Three healthy male Wistar rats (body weight = 262 ± 7 g) were also examined, using the same imaging protocol, to compare the metabolic responses with DCA of normal brain of healthy rats and normal-appearing brain of tumor-bearing rats.

Animals were anesthetized with 1.5%–3.0% isoflurane in oxygen (−1.5 L/min) followed by tail vein cannulation for intravenous administration of hyperpolarized [1-13C]Pyr and DCA. Body temperature was monitored by a rectal probe and maintained at 37°C using a warm water blanket underneath the animal. Vital signs including respiration, temperature, and oxygen saturation were monitored throughout the MR experiments.
All procedures were approved by the local Institutional Animal Care and Use Committee.

**Polarization Procedure**

A mixture of 14-M [1-13C]-labeled pyruvic acid and 15 mM trityl radical was polarized using a HyperSense DNP system (Oxford Instruments Molecular Biotechs). The solvent solution consisted of 40 mM tris-hydroxy-methylaminomethane, 125 mM NaOH, 100 mg/L ethylene diamine tetraacetic acid, and 50 mM NaCl and was used to produce a 125-mM solution of [1-13C]Pyr. Using a high concentration exploits the unsaturatable component of Pyr transport into the brain. Liquid-state polarization at dissolution was calculated to be ~25% based on solid-state polarization and independent calibration experiments. A volume of 2.6–3.4 mL of the solution with a pH of ~7.5 was injected through the tail vein catheter followed by a flush of 0.5 mL of saline with 1% of heparin to clear the catheter line. Injection rate was maintained at 0.25 mL/s. The amount of injected solution was adjusted to maintain a dose of 1 mmol/kg body weight.

**MR Protocol**

Single-shot fast spin echo (FSE) images with pulse repetition time (TR)/echo time (TE) of 1492/38.6 ms, slice thickness of 2 mm, and in-plane resolution of 0.47 mm were acquired for anatomical reference from up to 43 slices in axial, coronal, and sagittal planes. A dual-echo T2-weighted FSE sequence (TR/TE1/TE2 = 5000/11.3/56.7 ms, slice thickness = 1 mm, in-plane resolution = 0.25 mm, matrix size = 256 × 192, echo train length = 8) in the axial plane was used to determine the location of the tumor. The homogeneity of the B0 field over the region of the brain that included the tumor was manually optimized with a point-resolved spectroscopy sequence by minimizing the line width of the unsuppressed water signal using the linear shim currents.

Spiral CSI with 4 spatial interleaves (spectral bandwidth = 932.8 Hz) was used for metabolic imaging of hyperpolarized [1-13C]Pyr and its metabolic products, [1-13C]Lac, [1-13C]Ala, and 13C-Bic. The 13C transmit RF power was calibrated over the targeted slice using a reference phantom (3.4-M solution of [1-13C]Lac) placed on top of the animal’s head. Data were acquired using a variable flip angle scheme leading up to 90 degrees from a 6- to 8-mm slice with a nominal in-plane resolution of 2.7 mm × 2.7 mm (field of view [FOV] = 43.5 mm, matrix size = 16 × 16, TR = 125 ms, number of echoes = 96, acquisition time = 0.5 s). Further details of the pulse sequence are described by Mayer et al. Single time-point images were acquired 30 s after the start of the injection. The time delay was chosen to maximize Bic based on prior dynamic metabolic imaging experiments. Following the last 13C spiral CSI acquisition, each animal was scanned with T1-weighted proton spin-echo sequence (TE/TR = 12/700 ms, 29 slices, slice thickness = 1 mm, number of excitations [NEX] = 6, FOV = 87 mm, matrix size = 256 × 256, acquisition time = 9:02 min) both prior to and after injection of 0.9 mL of a 1:2 mixture of gadolinium–diethylene triamine pentaacetate (Magnevist, Bayer Schering Pharma) and saline to confirm tumor location and size.

**Dichloroacetate Infusion and Histology**

13C-MRS of each animal was obtained following a bolus injection of hyperpolarized [1-13C]Pyr solution before and after DCA administration. Beginning 45 min before the second scan, 200 mg/kg body weight of sodium DCA (Sigma-Aldrich) dissolved in saline (30 mg/mL) was injected through the tail vein catheter. About 0.5 mL of the DCA solution was injected as a bolus, with the rest infused at a rate of 0.1 mL/3 min.

**Data Processing and Analysis**

All 13C data sets were first corrected for differences in polarization at time of injection estimated from the solid-state polarization level and dissolution-to-injection delay using a T1 of 60 s and then processed using MatLab (Mathworks) as described. Briefly, the k-space data were apodized by a 10-Hz Gaussian filter and zero-filled by a factor of 4 in the spectral dimension and by a factor of 2 in both spatial dimensions. After a fast Fourier transform in the time domain, the chemical shift artifact along the readout direction was removed followed by gridding onto a Cartesian grid and a 2-dimensional fast Fourier transform in the 2 spatial frequency domains. Metabolic maps were calculated by integrating the corresponding metabolite peaks in absorption mode.

For analysis, the glioma region of interest (ROI) was selected based on the hyperintense region of the contrast-enhanced T1-weighted proton images. An ROI of normal-appearing brain was chosen in the contralateral hemisphere of the brain. In healthy rats, the ROIs were selected in similar locations to the ROIs of normal-appearing brain in tumor-bearing rats. Metabolic data for each ROI were evaluated as ratios to total carbon (tC), which was calculated as the sum of the detected Pyr, Lac, pyruvate hydrate (PyrH), Ala, and Bic signals. Additionally, the analysis was performed for the corresponding Lac-to-Bic ratios. For the display of individual spectra, a first-order phase correction was applied, and the baseline was subtracted by fitting a spline to signal-free regions of each spectrum.

Values are reported as mean ± standard errors. Differences of the Lac/tC, Bic/tC, and Lac/Bic ratios from normal brain and glioma were assessed for statistical significance using a paired Student’s t-test unless explicitly stated. Likewise, the metabolic responses of glioma and normal-appearing brain to DCA were also evaluated using a paired t-test.
Results

Metabolites in Glioma and Normal-Appearing Brain Before DCA Infusion

Figure 1A shows the spectra of glioma (red line) and normal-appearing brain (blue line) from a representative rat (#G1) before DCA infusion. Each spectrum was averaged over the voxels within the respective ROIs in the imaged 8-mm axial slice and normalized to the [1-13C]Pyr peak at 173 ppm. [1-13C]Lac and 13C-Bic peaks, produced from Pyr via Lac dehydrogenase (LDH) and PDH, were detected at 185 and 163 ppm, respectively. PyrH at 181 ppm was in dynamic equilibrium with Pyr and itself not metabolically active. The spectra illustrate the high SNR afforded by hyperpolarization. For example, SNR was 231 for Pyr, 85 for Lac, and 19 for Bic in the spectrum from normal-appearing brain. Due to the low Ala aminotransferase concentration in brain, Ala, which has a chemical shift of 179 ppm, was undetectable. Increased Lac and decreased Bic of glioma relative to normal-appearing brain are clearly evident in the spectra.

Figure 1B–D shows the corresponding single time-point metabolic images of Pyr, Lac, and Bic. The positions of the ROIs for glioma and normal-appearing brain are indicated in Fig. 1E. The protruding region at the skin above the glioma ROI was edema caused by the tumor cell implantation procedure. Averaged Lac contrast. DCA increased Bic production, measured by Bic/tC, in normal-appearing brain from 0.049 ± 0.004 to 0.1 ± 0.009 (P = .002, n = 5), whereas Lac/tC was unchanged (0.31 ± 0.02 for pre-DCA, 0.28 ± 0.02 for post-DCA, P = .1). Lac/Bic, which gave the highest contrast between glioma and normal-appearing brain, decreased from 6.4 ± 0.5 to 2.9 ± 0.2 (P = .0006). Metabolic change in glioma tissue due to the injected DCA was greater than in normal-appearing tissue. Post-DCA Lac/tC in glioma rose from 0.018 ± 0.002 to 0.056 ± 0.005 (P = .0008), and post-DCA Lac/tC was reduced from 0.44 ± 0.03 to 0.39 ± 0.04 (P = .05). Post-DCA Lac/Bic in glioma recovered to the pre-DCA level of normal brain by decreasing from 26.5 ± 3.6 to 7.4 ± 1.1 (P = .002). Accordingly, the post-DCA difference between glioma versus normal-appearing brain became less significant compared with pre-DCA in all the ratio metrics (P = .003 for Bic/tC, .008 for Lac/tC, and .005 for Lac/Bic).

Glioma was better differentiated from normal-appearing brain using metabolite ratios. Lac/tC in glioma was 0.44 ± 0.03, whereas it was 0.31 ± 0.02 in normal-appearing brain (P = .001). On the other hand, Bic/tC was 0.018 ± 0.002 in glioma versus 0.049 ± 0.004 in normal-appearing brain (P = .0002). Lac/Bic was 26.5 ± 3.6 in glioma and 6.4 ± 0.5 in normal-appearing brain (P = .002). Lac/tC, Bic/tC, and Lac/Bic in control rats were comparable to those values in normal-appearing brain in tumor-bearing rats (equal-variance t-test, Ps = .5 for Lac/tC, .6 for Pyr/tC, and .2 for Lac/Bic).

Response to DCA

For both pre- and post-DCA infusion, metabolic maps of Lac/tC, Bic/tC, and Lac/Bic from the same animal are shown in Fig. 2. The ratio images were edited to include only the brain to provide adequate image contrast. DCA increased Bic production, measured by Bic/tC, in normal-appearing brain from 0.049 ± 0.004 to 0.1 ± 0.009 (P = .002, n = 5), whereas Lac/tC was unchanged (0.31 ± 0.02 for pre-DCA, 0.28 ± 0.02 for post-DCA, P = .1). Lac/Bic, which gave the highest contrast between glioma and normal-appearing brain, decreased from 6.4 ± 0.5 to 2.9 ± 0.2 (P = .0006). Metabolic change in glioma tissue due to the injected DCA was greater than in normal-appearing tissue. Post-DCA Lac/tC in glioma rose from 0.018 ± 0.002 to 0.056 ± 0.005 (P = .0008), and post-DCA Lac/tC was reduced from 0.44 ± 0.03 to 0.39 ± 0.04 (P = .05). Post-DCA Lac/Bic in glioma recovered to the pre-DCA level of normal brain by decreasing from 26.5 ± 3.6 to 7.4 ± 1.1 (P = .002). Accordingly, the post-DCA difference between glioma versus normal-appearing brain became less significant compared with pre-DCA in all the ratio metrics (P = .003 for Bic/tC, .008 for Lac/tC, and .005 for Lac/Bic).

Fig. 1. (A) Representative 13C spectra (rat ID: G1) from the ROIs of glioma (red line) and normal-appearing brain (blue line) before DCA infusion. Each spectrum was normalized by Pyr peak. Metabolic images of (B) Pyr, (C) Lac, and (D) Bic from the rat acquired before DCA infusion. Higher Lac and lower Bic signals were detected in the glioma region as compared with normal-appearing brain. (E) ROIs of glioma and normal-appearing brain are shown on the contrast-enhanced T1-weighted proton image.
The metabolite pattern for the control group was similar to the changes in normal-appearing brain in the glioma animals: Bic/tC increased from 0.055 ± 0.002 to 0.11 ± 0.003 (P = .002), Lac/tC increased from 0.27 ± 0.3 to 0.29 ± 0.01 (P = .1), and Lac/Bic decreased from 4.8 ± 0.3 to 2.6 ± 0.08 (P = .009).

Detailed results of individual subjects including controls are presented in Fig. 3A–C. The statistical distributions of Lac/tC, Bic/tC, and Lac/Bic are shown in Fig. 3D–F, with the vertical lines indicating the 95% confidence interval.

The change of total carbon signal due to DCA infusion was not significant in either glioma (P = .05) or normal-appearing brain (P = .3).

**Discussion**

Lac/Pyr, as measured by hyperpolarized $^{13}$C-MRS, has served as a common metric to detect GLY-dependent tumor energy metabolism by correlating LDH and Lac labeling. However, relative Lac labeling cannot be directly used to assess mitochondrial function in tumor cells. On the other hand, Bic production can be used to monitor PDH activity, which measures Pyr conversion to acetyl CoA, so as to potentially serve as a surrogate marker for cellular mitochondrial metabolism. Both Lac and Bic labeling using hyperpolarized [1-$^{13}$C]Pyr in combination with drug-induced changes in observed peaks has not been demonstrated previously in any in vivo tumor model. However, the primary purpose of this work was not to discover a new mechanism by which DCA alters tumor metabolism. Rather, the importance of our work is to demonstrate an in vivo imaging technique by which changes in energy metabolism from GLY toward OXPHOS can be detected and quantified. Our results demonstrate for the first time that in addition to Lac, Bic can be reliably measured both in normal brain and in glioma tissue using an optimized MR acquisition protocol and high substrate concentration. The effect of DCA on Pyr metabolism was also clearly identified by comparing the measured Lac/tC and Bic/tC from normal brain and glioma tissues. By contrast, there was no significant change of detected total carbon after DCA infusion in either glioma or normal brain tissues, suggesting that treatment response to DCA in balancing metabolic pathways cannot be detected by a simple Pyr uptake measurement alone. However, further comparison experiments are necessary to validate this hypothesis.

Although both Bic/tC and Lac/tC are useful to differentiate tumor from normal brain tissue and to assess DCA effects, Lac/Bic was the parameter that was most sensitive to distinguish glioma from normal brain. More studies of Lac/Bic, or alternatively Bic/Lac, as a metric to evaluate therapeutic responses of other cancers are needed to verify its utility as a biomarker for cancer metabolism.

Due to its capacity to increase PDH flux, DCA has also proven efficacious as a Lac-lowering drug in various tissues, including blood, cerebrospinal fluid, and within a cell. Scapulio et al.\(^{41}\) reported that a single administration of DCA could decrease Lac by up to 60%, and the effect was maximized about 10 h after DCA administration for all tested doses. However, as shown in Fig. 3A and D, Lac labeling did not change significantly in glioma after DCA infusion, which might be due to the high dose of infused Pyr that saturated LDH activity even after PDH activity was elevated or due to the short time scales associated with the experiments, saturating the targeted enzymatic pathways is also an advantage in that only under such conditions are the products limited by enzyme activities (the primary quantities being targeted in these experiments) rather than substrate concentrations. In addition, no adverse hemodynamic event up to 10 mM of plasma Pyr concentration has been reported.\(^{54}\) Using $^{13}$C-labeled glucose as a substrate instead of Pyr is a potential alternative method to observe metabolic reaction to DCA under more physiological conditions. However, the short $T_1$ of $^{13}$C-glucose (1–2 s) is highly problematic. Recently, metabolic kinetics of hyperpolarized...
[U-2H, U-13C]glucose and products were demonstrated in *Escherichia coli*, but no in vivo metabolic products have been detected thus far in animal models. Because of the relatively coarse spatial resolution compared with the size of brain tumor, partial volume averaging effects were unavoidable in this study, likely...

![Fig. 3. Summary of metabolic changes in (A) Lac/TC, (B) Bic/TC, and (C) Lac/Bic of all 5 individual animals’ glioma (solid line) and normal-appearing brain (dashed line), along with data from the control rats (n = 3) shown by the dotted line. Average values of corresponding parameters from ROIs within the tumor-bearing animals, along with 95% confidence intervals, are presented in (D), (E), and (F). The differences between normal-appearing brain and tumor for all 3 parameters were significant both before and after administration of DCA (baseline: \( P = .001 \), and 0.0002, 0.002; post-DCA: \( P = .008 \), 0.003, and 0.005 for Lac/TC, Bic/TC, and Lac/Bic, respectively). With respect to DCA-induced changes, Bic/TC increased in both tumor and normal tissue (\( P = .0008 \) for glioma, \( P = .002 \) for normal-appearing brain) as the Lac/Bic ratio (\( P = .002 \) for glioma, \( P = .0006 \) for normal-appearing brain) did. In contrast, DCA-induced changes in Lac/TC ratios were not statistically significant.](https://academic.oup.com/neuro-oncology/article-abstract/15/4/433/1055623)
resulting in overestimating Bic and underestimating Lac, especially in rats with small tumors. Therefore, higher SNR would be necessary to reduce the voxel size while still being able to reliably detect Bic in tumors. This can be achieved either by using surface coils or improved pulse sequences or by polarizing at a lower temperature and/or high magnetic field to increase the polarization.\textsuperscript{38,39} Alternatively, the higher SNR can be leveraged to perform dynamic metabolic imaging permitting the calculation of apparent conversion rate constants that are less sensitive to experimental parameters such as injection time or shape of Pyr bolus than are metabolite ratios.\textsuperscript{60}

Although Bic/tC is a measure of PDH activity, it is not a direct measure of OXPHOS. Specifically, increased Bic may not necessarily mean increased OXPHOS, as not all acetyl CoA necessarily enters the TCA cycle. For example, acetyl CoA produced from Pyr might not enter the TCA cycle but instead could be directed toward other functions, such as fatty acid metabolism. Conversely, low Bic does not necessarily mean low OXPHOS, as metabolic pathways into the TCA cycle other than oxidative decarboxylation of Pyr via PDH can contribute relevant molecules.\textsuperscript{61} One potential strategy to assess TCA cycle more directly is to use hyperpolarized Pyr with the label in the C2 position, since the label is then retained in the conversion to acetyl CoA. However, to date, TCA-cycle intermediates have been detected in the heart when only hyperpolarized [2-\textsuperscript{13}C]Pyr is used in vivo.\textsuperscript{62} Moreover, the differential DCA effect between normal-appearing brain and glioma tissues could be affected by the increased perfusion of DCA into the tumor because of the disruption in the blood–brain barrier. Nonetheless, MRS measures of Lac/tC, Bic/tC, and Lac/Bic clearly relate to GLY and OXPHOS and are likely still useful for measuring in vivo tumor metabolism in both animal models and—with increasing availability of polarizers capable of human studies—the clinic.

We believe the ability to robustly measure such metabolic shifts in vivo could have major significance in assessing the efficacy of multiple antitumor therapies currently under development that target reversing the Warburg effect as a means of controlling tumor growth. For example, the failure of drugs such as DCA in some studies could be due to dosing regimes that fail to maintain the desired metabolic shift for a sufficient duration of time. Observing only Lac, as has been shown in numerous studies of both cancer patients and animal tumor models, may be inadequate in that changes in Lac labeling are not necessarily informative with respect to OXPHOS and the TCA cycle. Future work will be aimed at assessing the glioma treatment effect of DCA in longitudinal studies and estimating the appropriate combination of DCA dosage and frequency of the treatment. Combining PDH upregulation with downregulation of LDH activity, which can be achieved by glucose deprivation and ketogenic diets, might also represent an effective treatment for brain tumors, which could be monitored using the hyperpolarized \textsuperscript{13}C MRS imaging techniques presented in this study.

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