1H magnetic resonance spectroscopy of 2H-to-1H exchange quantifies the dynamics of cellular metabolism in vivo

Laurie J. Rich1,5, Puneet Bagga1,5, Neil E. Wilson1, Mitchell D. Schnall1, John A. Detre1,2, Mohammad Haris1,3,4 and Ravinder Reddy1,6*

Quantitative mapping of the in vivo dynamics of cellular metabolism via non-invasive imaging contributes to our understanding of the initiation and progression of diseases associated with dysregulated metabolic processes. Current methods for imaging cellular metabolism are limited by low sensitivities, costs, or the use of specialized hardware. Here, we introduce a method that captures the turnover of cellular metabolites by quantifying signal reductions in proton magnetic resonance spectroscopy (MRS) resulting from the replacement of 1H with 2H. The method, which we termed quantitative exchanged-label turnover MRS, only requires deuterium-labelled glucose and standard magnetic resonance imaging scanners, and with a single acquisition provides steady-state information and metabolic rates for several metabolites. We used the method to monitor glutamate, glutamine, γ-aminobutyric acid and lactate in the brains of unaffected and glioma-bearing rats following the administration of 2H2-labelled glucose and 2H3-labelled acetate. Quantitative exchanged-label turnover MRS should broaden the applications of routine 1H MRS.

Cellular metabolism is maintained by a network of biochemical reactions that are essential for normal tissue function1. These reactions form larger metabolic pathways that exist under tight regulatory control to help balance metabolic fluctuations experienced by the cell. Therefore, it is not surprising that abnormal metabolism is a hallmark of several pathologies, including neurodegeneration and cancer1,2. Probing of the kinetics of metabolic pathways in vivo plays a key role in studying disease mechanisms, identifying new treatment strategies and developing biomarkers of treatment response. To date, several non-invasive techniques have been established to monitor the relationship between cellular function and metabolism3.

Positron emission tomography (PET) using the glucose (Glc) analogue 2-18F-fluoro-2-deoxy-d-glucose is a widely utilized clinical tool that provides high-resolution maps of Glc uptake in cancer, neurodegeneration and cardiac diseases4–6. However, PET also requires the use of radioactive 2-18F-fluoro-2-deoxy-d-glucose and does not readily provide information on tissue metabolic activity beyond the initial step of glycolytic metabolism7. Conventional magnetic resonance spectroscopy (MRS) provides outstanding anatomical information without exposure to ionizing radiation, but only offers limited insight with regard to metabolism8. Chemical exchange saturation transfer MRI offers enhanced detection sensitivity for a variety of metabolites, but is limited in its ability to measure dynamic changes in metabolite turnover9,10. Proton magnetic resonance spectroscopy (1H MRS) is a gold standard for detecting and quantifying several endogenous tissue metabolites in a single acquisition, but it is not capable of tracking metabolic fluxes and pathways11. 13C MRS in combination with administration of 13C-labelled substrates has been used to study metabolic pathways in both preclinical and clinical settings12–14, but its low sensitivity has limited its routine use in human studies. The advent of dynamic nuclear polarization combined with 13C MRS has provided a strong boost to the sensitivity of this technique15,16. However, this approach is hindered by the short in vivo half-life of hyperpolarized 13C in addition to the requirement of onsite polarizers and 13C hardware.

Recently, 1H MRS—also referred to as deuterium MRS (DMRS)—has been evaluated for its potential to assess tissue metabolic kinetics following administration of deuterated substrates17. Preliminary studies have shown the utility of DMRS based deuterium metabolic imaging in the detection of human glioblastoma and hepatocellular carcinomas18. However, DMRS has low sensitivity relative to 1H MRS and still requires specialized hardware for use on clinical scanners. Deuterated substrates have also been utilized in combination with stimulated Raman scattering imaging for spectral tracing of deuterium (STRIDE) to measure the metabolic dynamics of newly synthesized cellular macromolecules including DNA, proteins, lipids and glycogen19. Although this approach provides high-resolution, biochemically informative images of Glc anabolic utilization, it is mostly restricted to superficial tissue measurements and requires prolonged exposure (~10 d) to deuterated substrates.

Here, we present a method—quantitative exchanged-label turnover MRS (QELT, or simply qMRS)—that increases the sensitivity of magnetic resonance-based metabolic mapping without the requirement for specialized hardware. Similar to DMRS, qMRS relies on the administration of deuterium-labelled substrates to track the production of downstream metabolites. However, instead of 1H-based detection of these metabolites, we performed 1H MRS. Since 1H is invisible on 1H MRS, replacement of 1H with 2H leads to an overall reduction in 1H MRS signal for the corresponding metabolites.

1Center for Magnetic Resonance and Optical Imaging, Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 2Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 3Research Branch, Sidra Medicine, Doha, Qatar. 4Laboratory Animal Research Center, Qatar University, Doha, Qatar. 5These authors contributed equally: Laurie J. Rich, Puneet Bagga.

*Correspondence: krr@pennmedicine.upenn.edu
This method takes advantage of the universal availability and ease of implementation of 1H MRS on all clinical and preclinical magnetic resonance scanners. The exceptional spectral resolution offered by 1H MRS enables tracking of label transfer to several important metabolites, including glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA) and lactate (Lac). Proof-of-principle qMRS studies were performed in the brains of unaffected and glioma-bearing rats following the administration of [6,6-2H2]Glc and [2,2,2-2H3]acetate. Together, our findings highlight qMRS as a straightforward and promising strategy for monitoring cellular metabolism in vivo with high potential for widespread clinical translation.

Results

Fundamentals of qMRS. The administration of labelled substrates such as Glc or acetate can provide information on metabolic flux through glycolysis and the citric acid cycle by tracking downstream labelling of metabolites including Glu, Gln, GABA and Lac (Fig. 1a)17–19. Building on the recent advances of DMRS, we developed qMRS, which relies on the indirect detection of a transferred deuterium label from deuterated substrates to intermediate metabolites. As a proof of concept, we performed qMRS in the brains of healthy rats before and after administration of [6,6-2H2]Glc. Figure 1b shows localized 1H MRS spectra from a rat brain before and after 60 min of [6,6-2H2]Glc infusion. Post-infusion, a reduction in the Glu-H4 amplitude at 2.35 ppm can be clearly observed. Similar observations were also made following infusion of [2,2,2-2H3]acetate (Supplementary Fig. 1). Subtraction of the post-infusion 1H MRS spectrum from the pre-infusion spectra revealed a marked increase in the 2.35 ppm Glu-H4 resonance, in addition to several Glu, Gln, GABA and aspartate (Asp) resonances, all of which were well above the background signal. A large reduction in the difference spectrum was also observed between 3.3 and 3.6 ppm, which was associated with non-deuterated proton resonances on the infused [6,6-2H2]Glc (Fig. 1b, right). In contrast, a considerable increase was observed between 3.6 and 4.0 ppm, corresponding to the deuterium-labelled Glc-H6 resonance (3.9 ppm) and Glu + Gln-H2 (Glx-H2; 3.8 ppm). A slight decrease was also observed at the 1.33 ppm Lac-H3 resonance, which was probably related to small amounts of unlabelled Lac produced by cerebral tissues due to Glc infusion19. This proof-of-principal study highlights the potential of qMRS to track 1H-based labelling of metabolites with high sensitivity, which can be performed simply with a traditional 1H MRS approach.

Comparative detection of neural metabolite turnover with qMRS and DMRS. Previous work has shown the ability of DMRS to track the production of Glx and Lac following [6,6-2H2]Glc infusion with high temporal resolution17. Therefore, we tested whether qMRS could also provide similar information following [6,6-2H2]Glc administration. To test this, we compared Glx measurements in healthy rat brains (n = 4) made with qMRS and DMRS using a combined 1H volume coil and 1H surface coil setup (Supplementary Fig. 2), with acquisition of qMRS and DMRS spectra alternating every 5 min following infusion. Figure 2 compares the time courses of qMRS and DMRS spectra pre- and post-infusion of [6,6-2H2]Glc. Infusion of [6,6-2H2]Glc revealed a visible reduction in the 2.35 ppm Glu-H4 peak after only 20 min of infusion, with the largest reduction observed at 60 min (Fig. 2a). The qMRS difference spectra confirmed this, with clear labelling of the Glu-H4 and other Glx resonances beginning after 20 min of infusion (Fig. 2b). Similarly, the formation of a Glx peak in DMRS was clearly observed within 20 min during infusion and continued to increase steadily over time (Fig. 2c). Surprisingly, a Lac peak was observed after 60 min of infusion using DMRS, which was not detected with qMRS. One explanation is that for DMRS studies, a surface coil with an unlocalized pulse-acquire sequence was utilized. Therefore, the signal measured with this approach could be from tissues other than the brain where labelled Lac is generated. In contrast, we performed localized spectroscopy for qMRS, where, as previously demonstrated15, we do not expect any Lac labelling in the healthy brain.

Following acquisition of combined qMRS and DMRS datasets, Glx concentrations were quantified using LCModel15 (qMRS) and AMARES23 (DMRS) for each time point. Comparisons of Glx measurements showed that they were in good agreement, with an initial linear increase that began to plateau after 45 min of infusion for both qMRS and DMRS (Fig. 3a). Furthermore, correlation analysis revealed a positive correlation (coefficient of determination, R² = 0.38) between these measurements, indicating that qMRS accurately reflects the dynamic labelling of Glx following deuterated Glc administration (Fig. 3b). This was confirmed using a Bland–Altman analysis, which revealed minimal bias between the Glx measurements obtained with qMRS and DMRS (Fig. 3c). In addition to Glx estimates, we compared signal-to-noise ratio (SNR) levels measured for the Glx peaks obtained with our qMRS and DMRS setups. We calculated the SNR to be ~41 for qMRS and ~8 for DMRS before subtraction, and ~8 and ~5 for the corresponding differences estimated for each spectrum. This represents an almost fivefold higher sensitivity for qMRS over DMRS, and 60% higher sensitivity for the corresponding difference spectra. Since DMRS was obtained with a surface coil with a pulse-acquire acquisition, while qMRS was obtained with a volume coil with point-resolved spectroscopy (PRESS) localization, the SNR advantage described for qMRS is still somewhat underestimated due to the coil sensitivity differences and longer echo times involved in the spatial localization.

Tracking of 1H labelling of neural metabolites with qMRS. While DMRS can provide temporal information on metabolite labelling, it is primarily restricted to Glx and Lac quantification due to the broad spectral peaks obtained through 1H detection. In contrast, since qMRS measures changes in the 1H MRS spectra, the high spectral resolution should enable detection of several key neural metabolites not obtainable with DMRS. For instance, instead of a single Glx peak, qMRS allows for individual detection of Glu and Gln, both of which are important components of neural metabolism and signalling. Therefore, we next set to measure labelling of these metabolites post-[6,6-2H2]Glc infusion. To quantify labelling, qMRS with [6,6-2H2]Glc infusion was performed in the brain of healthy rats (n = 6) over a 45-min period. All 1H MRS spectra were analysed with LCModel to obtain metabolite concentrations before and after infusion. To estimate the degree of labelling for each metabolite, post-infusion concentration measurements for each timepoint were subtracted from pre-infusion measurements, and divided by pre-infusion values to obtain the percentage of fractional enrichment. These values were adjusted by a correction factor of 1.33 to adjust for the theoretical probability that either a single or two 1H groups will be transferred from acetyl-CoA to downstream metabolites after [6,6-2H2]Glc infusion14.

Quantification of the Glx fractional enrichment (Fig. 4a) revealed a gradual increase and eventual plateau during infusion, with a 0.89 ± 0.23 mM increase (~9% enrichment) at 45 min post-infusion. Importantly, qMRS enabled individual quantification of changes in Glu (Fig. 4b) and Gln (Fig. 4c) levels, with a 0.68 ± 0.15 mM (~11% enrichment) increase in Glu and a 0.21 ± 0.12 mM (8% enrichment) increase in Gln observed 45 min post-[6,6-2H2]Glc infusion. It was also possible to estimate changes in the GABA (Fig. 4d) concentration, with a 0.25 ± 0.10 mM increase (10% enrichment) after infusion. Visually, the fractional enrichment of most metabolites followed an exponential pattern of increase over time. These labelling patterns are similar with regard to fractional enrichment of metabolites with published 13C MRS literature24,25. Changes in these metabolites were also compared with N-acetyl aspartate (NAA)—a highly abundant amino acid in the brain—as it should remain
unlabelled throughout the experiment. As expected, NAA levels (Fig. 4e) did not vary considerably throughout the experiment, suggesting it is a suitable reference for qMRS studies in the brain. In addition to [6,6′-2H2]Glc, infusion of unlabelled Glc was performed in a subset of animals (n = 4) to determine the stability of qMRS measurements over time. We did not observe significant changes in the metabolite concentrations following the infusion of unlabelled Glc (Supplementary Fig. 3). Quantification of Glu and Gln was also possible following infusion of [2,2,2′-2H3]acetate (Supplementary Fig. 4). These results clearly show the ability of qMRS to measure individual Glu and Gln metabolite labelling.

qMRS enables the detection of glycolytic metabolism in glioblastoma. It is well recognized that brain tumour development is associated with significant changes in cellular metabolism1. In particular, tumour cells are known to rely more heavily on glycolysis over oxidative phosphorylation (OXPHOS) to support cell growth, proliferation and survival2. A hallmark of this inefficient metabolic process is the increased uptake of Glc and subsequent conversion to Lac, with Lac production increasing significantly compared with normal physiological conditions. Given this, we hypothesized that qMRS should be capable of capturing this increased Lac turnover in brain malignancies. To address this, rats (n = 3) were orthotopically implanted with the F98 syngeneic rat glioma model and allowed to grow for 3 weeks, after which qMRS was performed. Figure 5 displays representative qMRS spectra from an F98 glioblastoma-bearing rat acquired before and 60 mins after [6,6′-2H2]Glc infusion. Before infusion, a large peak can be observed at 1.33 ppm, representing combined Lac and lipid peaks (Fig. 5a). After 60 min of [6,6′-2H2]Glc infusion, a marked reduction in the Lac-H3 peak at 1.33 ppm was observed, suggesting considerable Lac deuterium labelling (Fig. 5b). This was in contrast with non-tumour-bearing animals, in which no significant change in the Lac peak was observed. Subtraction of post-infusion 1H MRS spectra from the
pre-infusion spectra showed the time course of Lac labelling over the entire 60-min period, with a clear increase in labelling occurring only 10 min post-infusion (Fig. 5c). These results are consistent with previously published results using DMRS following the infusion of $[6,6'\text{H}_2]$Glc.

In vivo mapping of cerebral metabolism. Finally, to expand our observations beyond a basic single-voxel acquisition, we performed spectroscopic imaging to map deuterium labelling of metabolites occurring throughout the brain. To accomplish this, chemical shift imaging (CSI) was performed in a healthy rat brain before and 60 min after infusion of $[6,6'\text{H}_2]$Glc. A CSI volume of interest (VOI) was positioned over the centre of the brain (Fig. 6a, left). Metabolite maps were generated by calculating Glu and NAA concentrations for each voxel using LCModel, creating colour maps based on these estimates and overlaying these maps on an anatomical image. Before infusion, Glu and NAA maps revealed a range of metabolite levels across the imaging area (Fig. 6a, right).
After [6,6′-2H2]Glc infusion, a clear reduction in Glu levels was observed for the entire region, whereas NAA levels remained relatively constant. We then compared spectra obtained from a single CSI voxel within the VOI. Comparison of the spectra confirmed these findings, with a marked reduction in the Glu-H4 amplitude detected post-infusion and a clear peak formed at 2.35 ppm in the difference spectra (Fig. 6b). Spectra obtained for several of the acquired CSI voxels also confirmed these observations (Supplementary Fig. 5).

Together, these findings show the feasibility of performing 1H-based spectroscopic imaging in conjunction with deuterium labelling, and support the continued development of qMRS for human studies.

**Discussion**

We report a promising approach (qMRS) for measurement of the exchange of the deuterium label from metabolic substrates to metabolic intermediates in unaffected rat brain and rat glioblastoma using a common and easily implementable 1H MRS technique. qMRS not only provides a sensitivity advantage compared with DMRS, but it can also be carried out using standard clinical MRI hardware. Given ongoing efforts to establish robust acquisition and analysis methodologies for clinical 1H MRS, we expect that qMRS will be easily implemented on 3-T and 7-T clinical scanners, making it relatively straightforward to translate to clinical use. It is also possible to further improve qMRS by applying spectral denoising strategies (Supplementary Fig. 6). For instance, singular value decomposition noise reduction has recently been applied in 13C MRS analysis to enable differentiation of true from false peaks in noisy datasets (Supplementary Fig. 6). Integration of these approaches with qMRS could therefore enable measurement of tissue metabolic kinetics with high temporal resolution by reducing the number of averages necessary for spectral analysis.

**Fig. 4 | Kinetics of deuterium labelling of neural metabolites.** a–e, Fractional enrichment for Glx (a), Glu (b), Gln (c), GABA (d) and NAA (e) measured in normal rat brain (n = 6) during [6,6′-2H2]Glc infusion acquired over a 45-min period. As a visual aid, plots were fitted with the exponential plateau equation Y = Ym − (Ym − Yo) × exp(−k × x) (see Methods). Error bars represent s.e.m.

**Fig. 5 | Detection of glycolysis in rat glioblastoma.** a, Spectrum pre-[6,6′-2H2]Glc infusion (5 min acquisition; 128 averages), showing a large Lac/lipid peak observed at 1.33 ppm from a voxel placed within the tumour (inset). Scale bar, 5 mm. b, Spectrum acquired after 60 min of [6,6′-2H2]Glc infusion, showing a marked reduction in the 1.33 peak (dashed green line). c, qMRS difference spectra (pre – post) obtained every 10 min post-infusion, showing the increase in labelled Lac at 1.33 ppm. All studies were performed in rats (n = 3) bearing orthotopic F98 glioblastoma. MM, macromolecules.
Fig. 6 | Metabolic imaging of neural metabolism. a. Left: anatomical reference image showing the VOI (green box) sampled for CSI. Right: corresponding Glu and NAA metabolite maps acquired in a normal rat brain at 9.4 T before and after 60 min of [6,6-2H2]Glc infusion. Scale bar, 5 mm. b. Spectra representing a single CSI voxel within the VOI (yellow box in a) acquired before and after infusion. A clear reduction in the Glu-H4 resonance (Glu4) can be observed post-infusion (red arrow). A Glu4 peak was also observed in the corresponding difference spectrum.

Although only qMRS and DMRS were compared in this work, deuterated substrates have also been applied for stimulated Raman scattering-based imaging of cellular metabolism. Using the STRIDE approach, previous studies were able to produce high-resolution images of deuterium-labelled macromolecule metabolites (DNA, lipids and proteins) following the administration of deuterium-labelled Glc. While this technique was performed in a variety of tissues, including brain, intestine, liver and tumours, it is restricted to superficial imaging depths; therefore, its clinical potential is limited. Furthermore, in contrast with qMRS and DMRS, this approach requires prolonged administration of labelled Glc and has a 10-mM detection limit, which is considerably higher than qMRS (~0.5 mM). A major benefit of the qMRS approach compared with the recently developed DMRS is that it is feasible to measure deuterium labelling for individual metabolites such as Glu, Gln and Lac. Dynamic exchange of label on these metabolites as a function of time following the infusion of deuterium-labelled Glc (or acetate) can be used to derive rates of glycolysis, OXPHOS and Glu/GABA-Gln cycling in vivo in a spatially dependent manner. This information can be particularly useful when studying metabolic derangements in disease. For instance, Glu/GABA-Gln cycling exists in a careful balance within the healthy human brain to maintain normal neurotransmission. However, changes in this cycling are associated with severe neurological disorders such as Alzheimer’s and epilepsy. In addition, as observed in our rat glioblastoma studies, a hallmark of many cancers is an increase in Lac production due to a metabolic switch from OXPHOS to glycolysis to support tumour growth. Detection and quantification of the rate of Lac production may provide crucial information regarding tumour metabolism. Hence, qMRS is expected to open up new opportunities to probe changes in these metabolic rates in a variety of human diseases, including cancer and neurological disorders.

Although technically and experimentally challenging, over the past three decades, 13C MRS has become universally accepted as the only non-invasive method for providing information on the relationship between neuroenergetics and neuronal function. The time course of the incorporation of 13C label from 13C-labelled Glc into Glu-C4 and Gln-C4 resonances (fractional enrichment) is used in all studies to provide quantitative information regarding the rates of the glutamatergic citric acid cycle and neurotransmission. Similar to 13C studies, we observed the exponential pattern of 2H labelling of Glu and Gln resonances using qMRS in the rat brain. We have not quantified the metabolic fluxes by fitting the fractional enrichment data obtained via qMRS experiments in the present study. Nonetheless, we anticipate that these results would compare well with the values already reported in the literature.

qMRS offers several advantages over existing methods for measuring in vivo metabolism. Most notably, it only requires deuterium-labelled Glc, which can be given as an oral drink, along with standard 3-T MRI scanners (or 7-T MRI). There is no risk of exposure to ionizing radiation, no special equipment such as onsite hyperpolarizers for 13C and no multinuclear coils or cyclotrons for PET are needed. The deuterium label turnover is monitored directly using standard 1H MRS acquisition hardware and signal-processing strategies. Given this, the overall experimental simplicity and cost per scan makes qMRS an ideal choice for studying in vivo metabolic processes in the research and clinical setting. Another major advantage of qMRS is that it provides steady-state metabolic information of several metabolites along with the labelling kinetics of key metabolites in the same acquisition. Therefore, it is possible to obtain information on both labelled and unlabelled metabolites. While this method is demonstrated using Glc and acetate, it is also applicable to the measurement of label exchange from other deuterated substrates to their corresponding unlabelled metabolites. Moreover, this general approach is equally applicable to studying 19F-labelled substrates to probe metabolism and metabolite pool-size changes. Finally, we showed that it is possible to extend this approach to spectroscopic imaging modes, allowing labelling studies that can be performed across entire tissues with high sensitivity. Therefore, we anticipate that qMRS can also be implemented in other organ systems, including the liver, heart and breast.

Despite its advantages, this approach has some limitations. Since 1H MRS is sensitive to motion, repeated movements can lead to changes in peak linewidth, frequency shifts and even peak area. Therefore, subtraction of spectra acquired over time can be challenging. One approach to overcome motion-related frequency shifts is to perform block averaging using a small number of acquisitions (four or eight), and to perform a Fourier transform and frequency alignment using either NAA or creatine peaks as a chemical shift reference. This can also be combined with advanced quantification methods, such as LCModel, to improve the accuracy of metabolite turnover quantification. Furthermore, 1H MRS is also susceptible to background signals that will be augmented in cases where lipid signals are high (for example, tumours, breast, skeletal muscle and so on). However, strategies such as variable echo time and/or multi-quantum filtering could be applied to mitigate these issues. It is also important to note that while LCModel was used for quantification of metabolite labelling in this study, it is unclear how deuterium labelling patterns may affect the accuracy of these measurements. For instance, the average number of deuterons at the C4 position of Glu after [6,6-2H2]Glc infusion is always lower than two because of 2H label loss in the citric acid cycle at the conversion of acetyl-CoA to citrate. As described previously, the theoretical chance that both deuterons or only a single 2H atom is transferred from acetyl-CoA to Glx will be 1.33. Although we normalized the individual concentrations of metabolites obtained in the current study, further consideration is necessary to derive correction factors for all metabolites measured with qMRS. It is also likely that J-coupling patterns for specific metabolites may be altered following deuterium labelling, and that certain proton resonances will be labelled at greater frequencies than others. Ideally, individual basis sets should...
be created that mimic the expected labelling patterns in vivo, but this becomes increasingly difficult as these patterns change dynamically over time. Although this did not seem to negatively impact our analysis, optimizing these analysis parameters and/or determining the most suitable analysis method is a logical direction for future qMRS studies.

Outlook. In summary, we have demonstrated a technique that has the potential to measure glycolytic metabolism, OXPHOS and Glu–Gln cycling in normal brain and tumours via the utilization of deuterium-labelled substrates (Glc and acetate) and H MRS. Deuterated substrates can be safely and easily administered to humans at low doses. While the studies described here were preclinical in nature, we expect that this method can be easily extended to human studies at 3 T and 7 T, where CSI is routinely performed in research. As such, current efforts in our group are focused on developing qMRS for use in human subjects. This approach is expected to enable a wide range of studies probing metabolic derangements in vivo across medical disciplines.

Methods
Animal models and preparation. All of the animal experiments were performed under an approved Institutional Animal Care and Use Committee protocol by the University of Pennsylvania. For the qMRS metabolite tracking studies, seven 13- to 15-week-old male Fischer (CDF) rats (220–250 g; Charles River) were used. Four of the male CDF rats were then chosen for a second imaging session during which dual qMRS and DMRS were performed. For the brain tumour studies, three 6- to 8-week-old female F344/NCR rats (120–130 g; Charles River) were implanted with the rat glioblastoma cell line (F98), as described previously. Briefly, general anaesthesia was induced using 2% isoflurane mixed with air at 1 l min\(^{-1}\). Following an incision made on the skin covering the skull, a hole (6.5 mm diameter) was drilled by 1–2% isoflurane. The animal head was fixed on a stereotactic apparatus while temperature was monitored using a rectal temperature probe and maintained at 37 °C during the study. The maximum infusion volume over the imaging period (6.5-10 min) was 6.5–2.5 mm\(^3\) in both control (n = 6) and glioblastoma (4 × 4 × 4 mm\(^3\))-bearing (n = 3) rats using PRESS\(^{\text{\textregistered}}\) (TR/TE = 2,300/16 ms; spectral width = 4 kHz; 90° pulse bandwidth = 5,400 Hz; 180° pulse bandwidth = 2,401 Hz; number of points = 4,066; variable power radiofrequency pulses with optimized relaxation delays (VAPOR)\(^{\text{\textregistered}}\) water suppression; averages = 128). An additional qMRS sequence was used with the following parameters: repetition time/echo time = 300/16 ms; slice thickness = 3 mm; total field of view (FOV) = 35 × 35 mm\(^2\); excitation FOV = 10 × 6.5 mm\(^2\); matrix size = 12 × 12; number of points = 1,024; spectral width = 10,000 Hz; 90° pulse bandwidth = 8,000 Hz; 180° pulse bandwidth = 8,000 Hz; VAPOR water suppression; scan time = 20 min. In addition to the water-suppressed spectrum, another spectrum was acquired without water suppression to obtain the water reference signal for normalization.

In the remainder of the experiment. The maximum infusion volume over the imaging period (6.5-10 min) was 6.5–2.5 mm\(^3\) in both control (n = 6) and glioblastoma (4 × 4 × 4 mm\(^3\))-bearing (n = 3) rats using PRESS\(^{\text{\textregistered}}\) (TR/TE = 2,300/16 ms; spectral width = 4 kHz; 90° pulse bandwidth = 5,400 Hz; 180° pulse bandwidth = 2,401 Hz; number of points = 4,066; variable power radiofrequency pulses with optimized relaxation delays (VAPOR)\(^{\text{\textregistered}}\) water suppression; averages = 128). An additional qMRS sequence was used with the following parameters: repetition time/echo time = 300/16 ms; slice thickness = 3 mm; total field of view (FOV) = 35 × 35 mm\(^2\); excitation FOV = 10 × 6.5 mm\(^2\); matrix size = 12 × 12; number of points = 1,024; spectral width = 10,000 Hz; 90° pulse bandwidth = 8,000 Hz; 180° pulse bandwidth = 8,000 Hz; VAPOR water suppression; scan time = 20 min. In addition to the water-suppressed spectrum, another spectrum was acquired without water suppression to obtain the water reference signal for normalization.

Quantification. Metabolite concentrations measured by in vivo H MRS were quantified using LCMModel software (v.6.3)—a widely applied MRS analysis tool that employs a least-squares-based prior-knowledge fitting program. The concentration of metabolites was measured using the unsuppressed water peak as a concentration standard. LCMModel applied a 9.4-T spin echo (TE = 16 ms) basis set incorporating the following resonances: alanine (Ala), Asp, creatinine, glutamine, GABA, Glc, Glu, glycerophosphocholine, glutathione, myo-inositol, NAA, NAA + Glu, glycolate and taurine, with lipid resonances at 0.9, 1.3 and 2.0 ppm and macromolecule resonances at 0.9, 1.2, 1.4, 1.7 and 2.0 ppm. Once each metabolite concentration was estimated, calculation of fractional enrichment for each metabolite was performed by subtracting post-infusion levels from pre-infusion levels and dividing the change in metabolite levels by pre-infusion values. SNR estimates were calculated using Bruker topspin software by taking the ratio of the peak area divided by the standard deviation of the noise in the spectrum.

Analysis of CSI datasets was performed by calculating Glu and NAA concentrations for each voxel using LCMModel. LCMModel-based metabolite concentration estimates were loaded into MATLAB (R2018a) as a two-dimensional matrix and filtered using two-dimensional Gaussian smoothing. The 3 × 3 matrix was interpolated to a 9 × 9 grid and colourised using the jet colormap (Glu) and hot colormap (NAA). This 9 × 9 grid was then overlayed onto an anatomical grey-scale image for the entire FOV. For the CSI difference spectra, exponential line broadening (3 Hz) was applied to each individual spectrum, and the post-infusion spectrum was automatically shifted, phase corrected and Voigt line shape filtered to minimize differences between the pre- and post-infusion spectrum over the spectral ranges where no changes occurred.

Quantification of DMRS data was performed with the [MRUI software package (v.6.0) using a nonlinear least-squares quantitation algorithm named advanced method for accurate, robust and efficient spectral (AMARES) fitting by normalizing metabolite levels to the natural abundance deuterated water signal. As some deuterium labelling of water will occur following infusion of deuterated Glc, we normalized metabolite concentrations to the ratio of post-infusion water levels at each time point to pre-infusion levels. SNR estimates were calculated by taking the peak area and dividing it by the standard deviation of the noise level in the spectrum.

Data presentation. All statistical analyses and graphical displays of datasets were performed using GraphPad Prism (v.7.0) for MacOS (GraphPad Software). Minor smoothing was applied to difference spectra plots using a second-order polynomial averaging three neighbouring points on each side. All LCMModel-derived metabolite concentration plots report mean values with s.e.m. Fitted curves for concentration plots were generated to provide a visual aid of labelling using the following exponential plateau equation, \( Y = Y_0 - (Y_1 - Y_0) \times (e^{-k \times x}) \), where \( Y_0 \) is the starting population, \( Y_1 \) is the maximum population, \( k \) is the rate constant, Pearson's correlation analysis and Bland–Altman analysis was performed to compare Glx measurements made with qMRS and DMRS. P < 0.05 was considered significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The main data supporting the results of this study are available within the paper and its Supplementary Information. The raw and analysed datasets generated during the study are too big to be shared publicly, but they are available for research purposes from the corresponding author upon reasonable request.

Received: 25 June 2019; Accepted: 27 November 2019; Published online: 27 January 2020

References
1. DeBerardinis, R. J. & Thompson, C. B. Cellular metabolism and disease: what do metabolic outliers teach us? Cell 148, 1132–1144 (2012).
2. Pavlova, N. N. & Thompson, C. B. The emerging hallmarks of cancer metabolism. Cell Metab. 23, 27–47 (2016).
3. Fuss, T. L. & Cheng, L. L. Metabolic imaging in humans. Top. Magn. Reson. Imaging 25, 223–235 (2016).
4. Kelloff, G. J. et al. Progress and promise of FDG-PET imaging for cancer patient management and oncologic drug development. Clin. Cancer Res. 11, 2785–2808 (2005).

NATURE BIOMEDICAL ENGINEERING | VOL 4 | MARCH 2020 | 335–342 | www.nature.com/natbiomedeng
Articles
12. Beckmann, N., Turkalj, I., Seelig, J. & Keller, U. Carbon-13 NMR for the assessment of human brain glucose metabolism in vivo. *Biochemistry* **30**, 6362–6366 (1991).

13. Shulman, R. G. & Rothman, D. L. 13C NMR of intermediary metabolism: implications for systemic physiology. *Annu. Rev. Physiol.* **63**, 15–48 (2001).

14. De Graaf, R. A., Rothman, D. L. & Behar, K. L. State of the art direct 13C and indirect 1H-[13C] NMR spectroscopy in vivo. A practical guide. *NMR Biomed.* **24**, 958–972 (2011).

15. Mertz, M. E. et al. Hyperpolarized 13C allows a direct measure of flux through a single enzyme-catalyzed step by NMR. *Proc. Natl Acad. Sci. USA* **104**, 19773–19777 (2007).

16. Ross, B. D., Bhattacharya, P., Wagner, S., Tran, T. & Salsasuta, N. Hyperpolarized MR imaging: neurologic applications of hyperpolarized metabolism. *Am. J. Neuroradiol.* **31**, 24–33 (2010).

17. Lu, M., Zhu, X. H., Zhang, Y., Mateescu, G. & Chen, W. Quantitative assessment of brain glucose metabolic rates using in vivo deuterium magnetic resonance spectroscopy. *J. Cereb. Blood Flow. Metab.* **37**, 3518–3530 (2017).

18. De Feyter, H. M. et al. Deuterium metabolic imaging (DMI) for MRI-based 3D mapping of metabolism in vivo. *Sci. Adv.* **4**, eaat7314 (2018).

19. Zhang, I. et al. Spatial tracing of deuterium for imaging glucose metabolism. *NatuRe Biomedical Engineering* **3**, 402–413 (2019).

20. Brindle, K. M. Imaging metabolism with hyperpolarized 13C-labelled cell substrates. *J. Am. Chem. Soc.* **20**, 6418–6427 (2015).

21. De Graaf, R. A., Mason, G. F., Patel, A. B., Behar, K. L. & Rothman, D. L. In vivo 1H-[13C] NMR spectroscopy of cerebral metabolism. *NMR Biomed.* **16**, 339–357 (2003).

22. Provencher, S. W. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn. Reson. Med.* **30**, 672–679 (1993).

23. Naressi, A., Couturier, C., Castang, I., de Beer, R. & Graveron-Demilly, D. Java-based graphical user interface for MRUI, a software package for quantification of in vivo/magnetic resonance spectroscopy signals. *Comput. Biol. Med.* **31**, 269–286 (2001).

24. Van Eijden, P., Behar, K. L., Mason, G. F., Braun, K. P. & De Graaf, R. A. In vivo neurochemical profiling of rat brain by 1H-[13C] NMR spectroscopy: cerebral energetics and glutamatergic/GABAergic neurotransmission. *J. Neurochem.* **112**, 24–35 (2010).

25. Baslow, M. H. N-acetylaspartate in the vertebrate brain: metabolism and function. *Neurochem. Res.* **28**, 941–953 (2003).

26. Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029–1033 (2009).

27. Wilson, M. et al. Methodological consensus on clinical proton MRS of the brain: review and recommendations. *Magn. Reson. Med.* **82**, 527–550 (2019).

28. Donoho, D. L. De-noising by soft-thresholding. *IEEE T. Inform. Theory* **41**, 613–627 (1995).

29. Johnstone, I. M. & Silverman, B. W. Needles and straw in haystacks: empirical Bayes estimates of possibly sparse sequences. *Ann. Stat.* **32**, 1594–1649 (2004).

30. Brender, J. R. et al. Dynamic imaging of glucose and lactate metabolism by 13C-MRS without hyperpolarization. *Sci. Rep.* **9**, 3410 (2019).

31. Mason, G. F. et al. Simultaneous determination of the rates of the TCA cycle, glucose utilization, α-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *J. Cereb. Blood Flow. Metab.* **15**, 12–25 (1995).

32. Bak, L. K., Schousboe, A. & Waagepetersen, H. S. The glutamate/GABA-glutamate cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J. Neurochem.* **98**, 641–653 (2006).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒         |

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
All data were collected with the commercially available Bruker Paravision software (Version 6).

Data analysis
The generation of displayed MRS spectra was performed by using the Bruker Topspin plot editor software (Version 4.0.6). The quantification of 1H MRS datasets was conducted by using the commercially available LCModel (Version 6.1). The quantification of 2H MRS datasets was conducted via AMARES as a plugin for the open-source JMRUI program (Version 6.0). The processing of rat CSI datasets was performed by using LCModel and MATLAB (2018a).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The main data supporting the results in this study are available within the paper and its Supplementary Information. The raw and analysed datasets generated during the study are too big to be publicly shared, yet they are available for research purposes from the corresponding authors on reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: Sample sizes were determined on the basis of similar previously reported qualitative studies.
- **Data exclusions**: No data were excluded from the analysis.
- **Replication**: Proof-of-principle labelling studies were performed in six animals, with inter-animal reproducibility observed.
- **Randomization**: Because the work focused on technique development, randomization was not needed.
- **Blinding**: Because the work focused on technique development, blinding was not needed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                             | Involved in the study |
| ☑ Antibodies                   | ☑ ChIP-seq |
| ☑ Eukaryotic cell lines        | ☑ Flow cytometry |
| ☑ Palaeontology                | ☑ MRI-based neuroimaging |
| ☑ Animals and other organisms  |         |
| ☑ Human research participants  |         |
| ☑ Clinical data                |         |

Eukaryotic cell lines

Policy information about cell lines

- **Cell line source(s)**: F98 rat glioblastoma cell line (ATCC)
- **Authentication**: The cell line was not authenticated.
- **Mycoplasma contamination**: The cell line was tested to be negative for mycoplasma.
- **Commonly misidentified lines** (See ICLAC register): No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

- **Laboratory animals**: For qMRS metabolite tracking studies, sixteen 13–15-week-old male CDF rats (220–250 g, Charles River, Wilmington, MA) were used (seven for deuterated glucose, four for normal glucose, and five for deuterated acetate). For brain tumor studies, three 6–8-week-old female F344/NCR rats (120–130 g, Charles River, Wilmington, MA) were used.
- **Wild animals**: The study did not involve wild animals.
- **Field-collected samples**: The study did not involve samples collected from the field.
- **Ethics oversight**: All animal-imaging protocols were approved by the institutional animal care and use committee at the University of Pennsylvania.
### Magnetic resonance imaging

#### Experimental design

| Design type | Metabolite labelling in rats was performed by using deuterated substrates. |
|-------------|-------------------------------------------------------------------------|
| Design specifications | Imaging sessions consisted of pre-infusion scans and up to 70 minutes of post-infusion acquisitions. Acquisition time for 1H and 2H MRS datasets was ~5 mins. One 1H MRS dataset was acquired with 256 averages (10-min scan time). The acquisition time for the CSI datasets was ~20 min. |
| Behavioral performance measures | No behavioural performance measures were needed for this study. |

#### Acquisition

| Imaging type(s) | Magnetic resonance spectroscopy and spectroscopic imaging. |
|----------------|-----------------------------------------------------------|
| Field strength | 9.4T |
| Sequence & imaging parameters | For qMRS, data were acquired using a PRESS sequence (TR/TE=2,500/16 ms, spectral width = 4 kHz, 90 pulse bandwidth = 5,400 Hz, 180 pulse bandwidth = 2,400 Hz, number of points = 4,006, VAPOR water suppression, averages = 128). DMRS spectra were acquired using a non-localized single-pulse sequence acquired for the entire volume of the surface coil (TR = 300 ms, number of averages = 1,000). For rat CSI datasets, a PRESS based pulse sequence was used with the following parameters: TR/TE = 1,500/16 ms, total FOV = 35 x 35 mm, excited FOV = 10 x 6.5 mm, matrix size = 12 x 12, number of points = 1,024, spectral width = 10,000 Hz, 90 pulse bandwidth = 8,000 Hz, 180 pulse bandwidth = 8,000 Hz, VAPOR water suppression. |
| Area of acquisition | qMRS spectra were acquired from a voxel localized in the mid-brain (6.5 x 6.5 x 2.5 mm) in control animals and in a central portion of the tumor (4 x 4 x 4 mm) for glioblastoma-bearing rats. DMRS was acquired for an unlocalized area, mostly including the brain. |
| Diffusion MRI | Not used |

#### Preprocessing

| Preprocessing software | LCModel, jMRUI |
|------------------------|----------------|
| Normalization | Metabolite concentration was estimated by normalizing to the water signal in the same voxel. |
| Normalization template | N/A |
| Noise and artifact removal | N/A |
| Volume censoring | N/A |

#### Statistical modeling & inference

| Model type and settings | N/A |
| Effect(s) tested | N/A |
| Specify type of analysis | Whole brain, ROI-based, Both |
| Anatomical location(s) | Whole brain (DMRS) and mid-brain (qMRS). |
| Statistic type for inference | NA |
| Correction | NA |

#### Models & analysis

| n/a involved in the study | Functional and/or effective connectivity, Graph analysis, Multivariate modeling or predictive analysis |