Magnetic resonance imaging of tumor glycolysis using hyperpolarized $^{13}$C-labeled glucose

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In this study, we monitored glycolysis in mouse lymphoma and lung tumors by measuring the conversion of hyperpolarized [U-$^{2}$H, U-$^{13}$C]glucose to lactate using $^{13}$C magnetic resonance spectroscopy and spectroscopic imaging. We observed labeled lactate only in tumors and not in surrounding normal tissue or other tissues in the body and found that it was markedly decreased at 24 h after treatment with a chemotherapeutic drug. We also detected an increase in a resonance assigned to 6-phosphogluconolactone in the pentose phosphate pathway. This technique could provide a new way of detecting early evidence of tumor treatment response in the clinic and of monitoring tumor pentose phosphate pathway activity.

Tumor cells frequently display high rates of aerobic glycolysis. Although the hypoxic tumor microenvironment might select for cells that are glycolytic, even under aerobic conditions, and thus can generate ATP in the absence of oxygen, it is clear that this and the other metabolic changes observed in tumor cells are driven by oncogene activation and loss of tumor suppressor gene function. Moreover, although these metabolic changes are important for generating ATP under anaerobic conditions, they also have other important functions, such as the generation of metabolic intermediates for biosynthetic pathways. For example, glycolytic flux is diverted into the pentose phosphate pathway (PPP) to generate NADPH for lipid biosynthesis and to combat the increased oxidative load experienced by many tumors.

The aberrant metabolism displayed by tumor cells provides opportunities for tumor detection and treatment response monitoring using metabolic imaging. Positron emission tomography (PET) measurements of the uptake and trapping of $^{18}$fluorodeoxyglucose ($^{18}$FDG) have been used to detect tumors and their metastases, and decreases in FDG uptake have also been used to detect treatment response in some tumor types.

$^{13}$C magnetic resonance spectroscopy (MRS), which can detect signals from multiple cellular metabolites following administration of a $^{13}$C-labeled substrate, including $^{13}$C-labeled glucose, has been widely used to follow metabolic processes in vivo. However, its relatively low sensitivity is a major limitation. The recent development of dynamic nuclear polarization (DNP), which dramatically increases the sensitivity of the $^{13}$C MRS experiment (>10,000 times), has allowed real-time imaging of several substrates and the metabolites formed from them in vivo. The most widely used substrate to date has been hyperpolarized [1-$^{13}$C]pyruvate, and decreased label exchange between labeled pyruvate and endogenous lactate in tumors has been shown to be a marker of treatment response.

The major drawback of the technique is the short half-life of the hyperpolarization, which for [1-$^{13}$C]pyruvate is ~30 s in vivo. This means that subsequent metabolism must be relatively fast in order to detect it and that imaging should be accomplished within 2–3 min of injection of the polarized material. Previous studies with hyperpolarized [U-$^{2}$H, U-$^{13}$C]glucose have shown that hyperpolarized $^{13}$C-labeled lactate can be detected in Escherichia coli cells, yeast and tumor cells in vitro. Hyperpolarized $^{13}$C-labeled glucose has been imaged in rats in vivo, although detection of glucose metabolism was not demonstrated. We show here that injection of hyperpolarized [U-$^{2}$H, U-$^{13}$C]glucose allows real-time imaging of glycolytic flux in two mouse tumor models in vivo and that this flux is decreased in a lymphoma model 24 h after treatment with the chemotherapeutic drug etoposide. We also show that a resonance assigned to 6-phosphogluconolactone (6PG), generated by PPP activity, can be detected. Given that hyperpolarized [1-$^{13}$C]pyruvate imaging has already been used in the clinic in a study in prostate cancer, imaging of hyperpolarized glucose and its metabolic product lactate may offer a more sensitive approach for imaging tumor treatment response in the clinic.

RESULTS
Measurements in vivo

Intravenous (i.v.) injection of hyperpolarized [U-$^{2}$H, U-$^{13}$C]glucose (100 mM, 0.35 mL) into mice bearing tumors derived from T cell lymphoma (EL4) cells (EL4 tumors) ($n = 6$) resulted in tumor signals from all six $^{13}$C nuclei in both anemic forms (60–100 p.p.m.) (Fig. 1a,b). The polarization decayed with an apparent $T_1$ for the combined resonances of 8.9 ± 0.6 s ($n = 12$, average from all animals before and after treatment) (Fig. 1b). We also observed signal from the labeled C1 carbon of lactate in the tumor spectra at 15 s after injection. Lactate detection was not specific to EL4 tumors, as similar spectra were obtained from Lewis lung carcinoma (LL2) tumors (Fig. 2a). We did...

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not observe labeled lactate in brain, heart and liver or kidney when the surface-coil receiver was placed over these tissues (Fig. 2a), indicating that the lactate signal arises from within the tumor itself rather than from other tissues via the circulation. A typical $^{13}$C chemical-shift image acquired at ~$15$ s after injection of hyperpolarized $[\text{U-2H}, \text{U-13C}]$glucose from an untreated EL4 tumor–bearing mouse (Fig. 2b) showed that the hyperpolarized lactate signal was predominantly within the tumor, consistent with the findings from the localized spectra (Fig. 2b). The distribution of lactate in the chemical-shift image was similar to that observed after injection of hyperpolarized $[\text{U-2H}, \text{U-13C}]$glucose. For clarity, only every other time point is shown. The top spectrum in the stacked plot is the sum of the first $20$ s of data acquisition. AU, arbitrary units.

**Flux of $^{13}$C label from hyperpolarized $[\text{U-2H}, \text{U-13C}]$glucose to lactate was decreased $24$ h after treatment of EL4 tumor–bearing animals with etoposide.** The lactate/glucose signal ratio was decreased by $62\%$ at $24$ h after treatment with etoposide ($1.82 \pm 0.42\%$ in untreated tumors versus $0.69 \pm 0.11\%$ in treated tumors, $P = 0.026, n = 6$).

There was no evidence of appreciable pyruvate oxidation in the tricarboxylic acid cycle in these tumors. In animals injected with hyperpolarized $[\text{U-13C}]$pyruvate ($0.2$ mL, $75$ mM i.v.) the HCO$_3^-$ signal was $0.01 \pm 0.006\%$ of the $[\text{U-13C}]$lactate signal ($n = 2$) in slice-selective spectra and $0.80 \pm 0.14\%$ ($n = 2$) in non–slice-selective spectra, where there is some contribution from underlying tissue. Dichloroacetate (150 mg per kg body weight i.v.) injected $3$ min before the pyruvate had no measurable effect on this ratio.

**High-resolution MRS measurements on tumor extracts**

The effects of etoposide treatment on the concentrations of unlabeled and $^{13}$C-labeled glucose and lactate were determined from high-resolution

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**Figure 1 $[\text{U-2H}, \text{U-13C}]$glucose signals are detectable in vivo.** (a) $^{13}$C NMR spectrum of $[\text{U-1H}, \text{U-13C}]$glucose in vitro. The resonance at $-63.5$ p.p.m. is from glucose C6α (63.4) and C6β (63.6); the resonances between 70 and $80$ p.p.m. are from glucose C2α (74.3), C2β (77.0), C3α (75.6), C3β (78.6), C4α (72.4), C4β (72.4) and C5α (74.2), C5β (78.6) and the resonances at 94.8 and 98.7 p.p.m. are from glucose C1α and C1β, respectively. All the resonances are split into multiplets due to $J$-coupling between $^{13}$C-$^{13}$C and $^{13}$C-$^2$H. All the resonances are split into multiplets due to $J$-coupling between $^{13}$C-$^{13}$C and $^{13}$C-$^2$H.

(b) Representative $^{13}$C tumor spectra acquired between $16$ s and $36$ s after the i.v. injection of $0.35$ mL of $100$ mM hyperpolarized $[\text{U-2H}, \text{U-13C}]$glucose. For clarity, only every other time point is shown. The top spectrum in the stacked plot is the sum of the first $20$ s of data acquisition.

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**Figure 2 $^{13}$C spectroscopic imaging showing the spatial distribution of labeled glucose and lactate.** (a) Representative $^{13}$C MR spectra acquired from subcutaneous EL4 and LL2 tumors, brain, heart and liver and kidneys $15$ s after the injection of $0.35$ mL of $100$ mM hyperpolarized $[\text{U-2H}, \text{U-13C}]$glucose. The lactate spectra are the sum of $4$ transients collected over a period of $1$ s, whereas a single transient was acquired for the glucose spectra. Flux of hyperpolarized $^{13}$C label was only observed between $[\text{U-2H}, \text{U-13C}]$glucose (63–99 p.p.m.) and lactate C1 (doublet at $-185$ p.p.m.) in EL4 and LL2 tumors. (b) Representative chemical-shift–selective images obtained ~$15$ s after i.v. injection of $0.4$ mL $200$ mM hyperpolarized $[\text{U-2H}, \text{U-13C}]$glucose into an EL4 tumor–bearing mouse. The spatial distributions of glucose, urea and lactate are displayed as voxel intensities relative to their respective maxima. The $^1$H MR images, shown in grayscale, were used to define the anatomical location of the tumor (outlined in white). A urea phantom was included to serve as a reference. The color scales represent arbitrary linearly distributed intensities for the hyperpolarized images.
13C nuclear magnetic resonance (NMR) spectra (Supplementary Fig. 2) and 1H NMR spectra of EL4 tumor, liver and blood extracts taken from animals at 20 and 150 s after injection of [U-13C]glucose, which was injected at the same concentration as the hyperpolarized [U-2H, U-13C]glucose. Labeled material was distinguished from unlabelled material, in which 13C was only present at natural abundance (1.1%), by the presence of 13C–13C spin coupling in the labeled material. The concentrations of unlabeled glucose and lactate determined from the natural-abundance 13C signal showed good agreement with the concentrations determined from the 1H NMR spectra (data not shown). There was no detectable labeled lactate in the blood, confirming that the hyperpolarized 13C-labeled lactate observed in the tumor in vivo is unlikely to have been washed in from other tissues.

Following drug treatment and at 150 s after injection of labeled glucose, there was a 50% decrease in the labeled lactate concentration, which was comparable to the 39% decrease in the steady-state unlabeled lactate concentration (Table 1 and Supplementary Fig. 2). The ratio of the lactate C3 singlet intensity (from [3-13C]lactate) (corrected for the contribution from background natural abundance signal) to that of the C3 doublet (from [2,3-13C]lactate) was \( 7 \pm 1\% \) \((n = 5)\), indicating that flux through the PPP was \(~7\%\) of the glycolytic flux, assuming that the singlet arising from lactate C3 is the product of the oxidative branch of the PPP.

Table 1 Glucose and lactate concentrations measured in tissue extracts

| Glucose and lactate concentrations measured in tissue extracts |
|---------------------------------------------------------------|
| Glucose | Lactate | Glucose | Lactate |
|----------------|----------|----------|----------|
| Natural abundance signals | | | | |
| Glucose | Lactate | Glucose | Lactate |
|----------------|----------|----------|----------|
| 20 s after injection | | | | |
| EL4 (µmol g\(^{-1}\) WT) | Untreated | 0.42 ± 0.06 | 20.7 ± 3.0 | 0.13 ± 0.02 | 0.49 ± 0.10 |
| | Treated | 0.46 ± 0.13 | 12.7 ± 1.9* | 0.16 ± 0.04 | 0.50 ± 0.13 |
| Blood (µmol mL\(^{-1}\)) | Untreated | 3.3 ± 0.1 | 3.3 ± 0.4 | 1.8 ± 0.1 | n.d. |
| | Treated | 3.2 ± 1.0 | 3.3 ± 0.6 | 3.8 ± 0.9 | n.d. |
| Liver (µmol g\(^{-1}\) WT) | Untreated | 11.4 ± 1.1 | 7.2 ± 1.3 | 1.7 ± 0.4 | 0.20 ± 0.06 |
| | Treated | 10.6 ± 1.5 | 5.4 ± 0.6 | 1.4 ± 0.3 | 0.06 ± 0.02* |
| 150 s after injection | | | | |
| EL4 (µmol g\(^{-1}\) WT) | Untreated | 1.4 ± 0.3 | 20.0 ± 1.0 | 0.40 ± 0.07 | 1.9 ± 0.4 |
| | Treated | 1.5 ± 0.3 | 12.0 ± 0.5* | 0.49 ± 0.10 | 1.2 ± 0.2 |
| Liver (µmol g\(^{-1}\) WT) | Untreated | 9.2 ± 3.2 | 6.0 ± 0.3 | 1.1 ± 0.1 | 0.31 ± 0.01 |
| | Treated | 9.8 ± 3.0 | 5.3 ± 0.7 | 1.3 ± 0.2 | 0.29 ± 0.04 |

Tumors were freeze-clamped and extracted 20 s or 150 s after i.v. injection of 0.35 µmol of 100 mM [U-13C]glucose. \( n = 5 \) for EL4 and liver samples after 20 s, \( n = 4 \) for EL4 and liver samples after 150 s and \( n = 3 \) for blood plasma samples. Data are expressed as mean ± s.e.m.

* \( P < 0.05 \), n.d., not detected. µmol g\(^{-1}\) WT, µmol per gram body weight.

There was no difference between untreated and treated tumors, and in liver the corresponding value was 38 ± 9% \((n = 5)\).

**DISCUSSION**

Previous studies have demonstrated that treatment response can be detected in murine tumor models from the decrease in tumor 13C-labeled lactate concentration in animals injected with 13C-labeled glucose\(^{22,23}\). However, in these studies with non-hyperpolarized glucose, much higher glucose concentrations were used\(^{22,23}\), the data were acquired for longer periods of time (80–120 min) and there was insufficient signal for imaging. We have shown here that hyperpolarized [U-2H, U-13C]glucose has a sufficiently long \( T_1 \) and degree of polarization to allow detection and imaging of glycolytic flux in mouse tumors \( in vivo \). Furthermore, the experiment detects tumor treatment response, with a 62% decrease in the lactate/glucose signal ratio at 24 h after treatment of lymphoma tumors with etoposide. This decrease was larger than the 39% decrease in steady-state lactate concentration and the 50% decrease in labeled lactate concentration determined from measurements on tumor extracts, which may be due, in part, to the slightly higher blood glucose concentration in the drug-treated animals.

Only tumor tissue showed detectable levels of labeled lactate; no signal was detected in brain, heart and liver or kidney, and no labeled lactate was detected in the blood. Our measurements of labeled lactate concentrations in tumor extracts gave a lactate production rate of \( \sim 0.8 \) µmol min\(^{-1}\) g\(^{-1}\), similar to rates reported for other tumor cell types\(^1\) and comparable with the glucose consumption rate measured in rat brain (0.75 µmol min\(^{-1}\) per g tissue)\(^{24}\) and heart muscle (\( \sim 1 \) µmol min\(^{-1}\) per g tissue)\(^{25}\).

Presumably, these tissues showed no signal because their steady-state lactate concentrations were lower than those in tumors\(^{24,25}\). The sensitivity of MR detection of hyperpolarized [U-2H, U-13C]glucose and the lactate produced from it is much lower than PET detection of [\(^{13}\)C]FDG and also lower than MR detection of hyperpolarized [\(^{13}\)C]label exchange between [\(^{1}\)C]pyruvate and endogenous lactate. The signal-to-noise ratio for the lactate signal produced from hyperpolarized [U-2H, U-13C]glucose was \( \sim 10 \) when the glucose was injected at 0.3 g per kg body weight (g per kg) and \( \sim 60 \) when 0.7 g per kg...
was injected and an optimized signal acquisition protocol was used, as compared to ~400 for the lactate produced from hyperpolarized [1-13C]pyruvate (injected at ~0.07 g per kg)\textsuperscript{11}. Nevertheless, hyperpolarized [U-2H, U-13C]glucose has some potential advantages for detecting tumor treatment response. First, it does not use ionizing radiation. Second, detection of 13C-labeled lactate could be advantageous in detecting response in those tumors where 18FDG-PET shows poor contrast, such as those in the brain and prostate. Lactate, on the other hand, is much higher in concentration in brain tumors than in the surrounding brain tissue, and the absence of labeled lactate in the kidney in this study indicates that detection of treatment response in prostate cancer could also be possible, as there will be little or no labeled lactate in the adjacent bladder.

The polarized pyruvate and 18FDG-PET experiments investigate only a few steps in glucose metabolism: glucose transport and hexokinase activity in the case of 18FDG-PET and monocarboxylate transporter and LDH activities in the case of polarized pyruvate. In contrast, in principle, measurements of hyperpolarized 13C label flux between glucose and lactate can be used to assess flux through the entire glycolytic pathway. This depends on two assumptions. First, we assume that there is unidirectional flux of label from glucose to pyruvate. As there are three effectively irreversible steps in the glycolytic pathway between glucose and pyruvate, the glycolytic intermediate concentrations are relatively low and there is no measurable glucogenic flux; because we have never detected labeled glucose in this tumor model in experiments with hyperpolarized [1-13C]pyruvate or [1-13C]lactate\textsuperscript{11,26}, this assumption appears justified. Second, we assume that most of the hyperpolarized 13C label that reaches pyruvate exchanges into the much larger lactate pool, which has been demonstrated\textsuperscript{27}, and that little of the pyruvate is oxidized in the mitochondria. Experiments with hyperpolarized [1-13C]pyruvate showed that pyruvate oxidation in the tumor was minimal compared with exchange of 13C label with endogenous lactate, with only 0.01 ± 0.006% of the hyperpolarized label in lactate appearing in H\textsubscript{13}CO\textsubscript{3}-. In non–slice-selective spectra, which include some contribution from underlying tissue, this figure increased to 0.80 ± 0.14%. Dichloroacetate, which has been shown to increase pyruvate oxidation\textsuperscript{28}, had no effect on this ratio. In a rat brain glioma model, the signal from hyperpolarized H\textsubscript{13}CO\textsubscript{3} was 4% of that in [1-13C]lactate versus 16% in normal brain\textsuperscript{28}. Therefore, we conclude that measurements of hyperpolarized 13C label flux between glucose and lactate in those tissues that have low rates of pyruvate oxidation, such as tumors, can be used to assess net flux through the glycolytic pathway. This measured flux should be sensitive to drugs that inhibit any step in the pathway or which divert flux into other pathways. In principle, the polarized pyruvate experiment gives similar information about glycolytic flux because the flux is sensitive to changes in lactate concentration; increases in lactate concentration result in increased lactate exchange\textsuperscript{11}. However, this need not be the case. In human breast cancer cells (MCF7) treated with an inhibitor of mitogen-activated protein kinase, there was decreased lactate labeling despite an increase in lactate concentration. The increased lactate concentration was attributed to an increase in glycolytic flux, and the decreased labeling to inhibition of the monocarboxylate transporter MCT-1 (ref. 29). Another advantage of the polarized glucose experiment is that glucose can be used at physiological concentrations, whereas pyruvate is used at supraphysiological concentrations. In the first clinical trial of hyperpolarized [1-13C]pyruvate, pyruvate was injected at 0.43 mL per kg body weight of a 250 mM solution (NCT01229618), which equates to a whole-blood concentration of ~1.5 mM (physiological concentration, ~0.060 mM). In clinical i.v. glucose tolerance tests, glucose is injected at up to 0.5 g per kg body weight, which equates to a whole-blood concentration of ~40 mM.

The production of 13CO\textsubscript{2} in the irreversible oxidative decarboxylation catalyzed by 6PG dehydrogenase potentially provides a measure of net flux into the PPP. Measurements with [1,2-13C]glucose showed that ~7% of labeled lactate was produced via the PPP, which is comparable with a measured hyperpolarized H\textsubscript{13}CO\textsubscript{3}+/6PG 13C\textsubscript{1} ratio of ~10%. However, this measurement may be compromised if there is appreciable 13CO\textsubscript{2} production resulting from pyruvate decarboxylation in the reaction catalyzed by pyruvate dehydrogenase. In non–slice-selective spectra in animals injected with hyperpolarized [1-13C]pyruvate, the hyperpolarized H\textsubscript{13}CO\textsubscript{3}− signal was 0.8% of the [1-13C]lactate signal, as compared with ~2% in animals injected with hyperpolarized [U-2H, U-13C]glucose. Because flux into the pathway is controlled by glucose 6-phosphate dehydrogenase activity\textsuperscript{30}, labeling of 6PG may provide a more reliable measure of PPP flux.

The major limitation of using hyperpolarized [U-2H, U-13C]glucose is its short polarization lifetime. Reducing the degree of 13C substitution in the molecule, and thus homonuclear dipolar relaxation, will extend the lifetime, although the effect is relatively small. The T\textsubscript{s} of the resonances from natural abundance C4 carbon in [U-2H]glucose were ~30% longer than those from the C4 carbons in [U-2H, U-13C]glucose. Using [U-2H, 3,4-13C]glucose or [U-2H, 3-13C]glucose would have the added advantage of producing [1-13C]lactate (if they are metabolized via the glycolytic pathway), which would improve detection, as the lactate resonances would be a singlet. This method could also provide another assessment of PPP activity, as [U-2H, 3,4-13C]glucose or [U-2H, 3-13C]glucose should also produce [1,2-13C]lactate if metabolized via the PPP. Sensitivity could also be improved by increasing the level of polarization (from the ~15% achieved here) and by injecting higher glucose concentrations, although this may only be possible in preclinical studies.

### METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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### AUTHORS CONTRIBUTIONS

T.B.R. and M.I.K. designed the research; T.B.R., E.M.S., B.W.C.K., D.-E.H. and M.I.K. performed the research; T.B.R. and M.I.K. analyzed data; and T.B.R., M.I.K. and K.M.B. wrote the paper.
COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Cell culture. Mouse T cell lymphoma (EL4) and Lewis lung carcinoma (LL2) cells were obtained from the American Type Culture Collection (ATCC). EL4 cells were grown in RPMI 1640 medium (Invitrogen) supplemented with 2 mM L-glutamine and 10% FCS (PAA Laboratories). LL2 cells were grown in DMEM (Invitrogen) supplemented with 4.5 mg mL⁻¹ glucose, 2 mM L-glutamine and 10% FBS (PAA laboratories).

Animal preparation. Experiments were conducted in compliance with project and personal licenses issued under the Animals (Scientific Procedures) Act of 1986 and were designed according to the UK Co-ordinating Committee on Cancer Research guidelines for the welfare of animals in experimental neoplasia. The Cancer Research UK Cambridge Institute Animal Welfare and Ethical Review Body approved the work.

EL4 cells (5 × 10⁶) were resuspended in ice-cold PBS and implanted into female C57BL/6 mice (n = 40, 6–8 weeks of age; Charles River) by subcutaneous injection in the lower flank. LL2 cells were implanted (6 × 10⁶) in a single animal. At this location, there was no detectable respiratory motion in magnetic resonance images. MRS was performed when the tumors had grown to a size of ~2 cm³, which for EL4 tumors was typically 10 d following implantation. Animals were imaged before and 24 h after treatment with 67 mg per kg body weight etoposide (Eposin, 20 mg mL⁻¹; PCH Pharmachemie) and were anesthetized by inhalation of 1–2% isoflurane (Isoflo; Abbotts Laboratories) in 75% air, 25% O₂ (2 L per min) and body temperature maintained by blowing warm air through the magnet bore. Breathing rate (~80 b.p.m.) and body temperature was monitored during the experiment using an infrared thermometer (Biotrak). Hyperpolarized agents were injected i.v. via a tail-vein catheter.

Perchloric acid extracts from untreated (n = 15) and etoposide-treated (n = 14) EL4 tumor-bearing mice were prepared following injection of 0.35 mL 100 mM [U-1³C]glucose. Protonated glucose was used to avoid the complex multiplets arising from 1³C–H coupling. The mice were killed by cervical dislocation after either 20 s (n = 5, treated; n = 5, untreated) or 150 s (n = 4, treated; n = 4, untreated), and the tumors and livers were rapidly freeze-clamped in liquid nitrogen–cooled tongs. Another cohort was similarly prepared (n = 3, untreated; n = 3, etoposide-treated), and blood was obtained by cardiac puncture after 20 s. A third cohort was killed by cervical dislocation 150 s after injection with 0.35 mL of 100 mM [1,2,1³C₂]glucose (n = 3, treated; n = 2, untreated) and the tumors and livers rapidly freeze-clamped. Perchloric acid extracts were prepared with 7% perchloric acid (1:8 w/v), which were then neutralized with KOH, lyophilized, and dissolved in 99.9% <air, 25% O₂ (2 L per min) and body temperature maintained by blowing warm air through the magnet bore. Breathing rate (~80 b.p.m.) and body temperature was monitored during the experiment using an infrared thermometer (Biotrak). Hyperpolarized agents were injected i.v. via a tail-vein catheter.

Hyperpolarization of [U-²H, U-¹³C]glucose. For therapy response studies in EL4 tumors and of ¹³C MR spectra from LL2 tumors, brain, heart and liver and kidneys, trityl radical (25.8 mM, OX063; GE Healthcare), gadolinium chelate (2.6 mM, Dotarem; Guerbet) and [U-²H, U-¹³C]glucose (3.55 M; Cambridge Isotopes) were dissolved in 50 µl deuterium oxide.

Magnetic resonance imaging and spectroscopy in vivo. Experiments were performed in a 7.0-T horizontal bore magnet (Varian) using an actively decoupled dual-tuned ¹³C/¹H volume transmit coil (Rapid Biomedical) and a 20-mm ¹³C receiver surface coil (Rapid Biomedical) placed over the tissue of interest. Hyperpolarized [U-²H, U-¹³C]glucose (0.35 mL 100 mM, or 0.4 mL 200 mM; the dead volume of injection line was ~50 µl) was injected i.v. over a period of 3 s and the animal placed inside the magnet. Data acquisition was started 15 s after the start of injection, with a total time between dissolution and data acquisition of ~30 s. A series of frequency-selective ¹³C spectra were collected, with four spectra collected from the lactate region (1 ms sinc pulse with flip angle 20°) followed by one spectrum collected from the glucose region (flip angle 10°) and the sequence repeated over a period of 40 s. The spectral width was 4 kHz collected into 768 complex points, the repetition time was 0.2 s and echo time 0.8 ms. In two animals, 9 lactate spectra were acquired followed by 1 glucose spectrum with a flip angle of 10° (0.75 ms sinc pulse), a repetition time of 0.1 s, echo time of 0.45 ms and a spectral width of 6 kHz. In three animals, two ¹³C chemical-shift–selective images were collected (field of view 32 × 32 mm, repetition time 30 ms, echo time 0.8 ms, spectral width 6 kHz, data matrix 16 × 16, flip angle 5°), the first from the glucose resonance (15 s after injection) and the second from the lactate resonance (25 s after injection). A urea phantom was included for reference. An identical chemical-shift–selective image was acquired in the same animals 25 s after injection of hyperpolarized pyruvate. Data were overlaid on ¹H spin-echo reference images (field of view 32 × 32 mm, data matrix 128 × 128, repetition time 1.8 s, echo time 20 ms, slice thickness 2 mm).

Lactate and glucose spectra were summed separately and phase- and baseline-corrected using Matlab (MathWorks). Spectra were referenced to the glucose C1B carbon at 98.7 p.p.m. Ratios of the lactate (183–187 p.p.m.) and glucose (60–100 p.p.m.) peak integrals, summed over the whole time course, were calculated. The signals were not corrected for the different number of ¹³C nuclei in glucose (6) and lactate (1) or for differences in flip angle. For comparison the summed first second of data acquisition (four lactate spectra, one glucose spectrum) were also analyzed and similar results were obtained. For determination of the apparent glucose T₁, signal integrals were fitted to a mono-exponential decay function. In order to account for possible polarization variations at the time of glucose injection, spectra were normalized using the initial glucose signal intensity.

For experiments with [1-¹³C]pyruvate 0.2 mL of the hyperpolarized solution (75 mM) was injected i.v. and data acquisition started at 15 s after injection. A series of alternating slice-selective and non–slice-selective spectra were collected with 100 ms between spectra, using the same acquisition conditions as used for the glucose experiments. The imaging slice was selected through tumor. For some experiments dichloroacetate (150 mg per kg body weight) was injected i.v. 3 min after the hyperpolarized pyruvate.

High-resolution ¹³C and ¹H NMR spectroscopy. High-resolution ¹H and ¹³C-decoupled ¹³C NMR spectra of tumor, liver and whole blood extracts were obtained at 14.1 T (25 °C, pH 7.2) using a Bruker 600 MHz NMR spectrometer (Bruker) using a 5-mm probe. The acquisition conditions were: ¹H, 90° pulses; 7.3 kHz spectral width; 0.9 s acquisition time; 32,000 data points; 64 transients; and 12.5 s recycling time; ¹³C, 30° pulses; 36.0 kHz spectral width; 0.9 s acquisition time; 32,000 data points; 2048 transients; and 14 s recycling time. Chemical shifts were referenced to 3-(trimethylsilyl)-2,2,3,3-tetadeuteropropionic acid (TSP, 0.0 p.p.m.). Spectral deconvolution and multiplet structures were analyzed using the PC-based (Intel Centrino Platform) NMR program, ACDSpecManager (ACD/Labs). Data were zero-filled twice and multiplied by an exponential function before Fourier transformation. All NMR resonance areas were normalized relative to the 5 mM TSP resonance integral. For reference purposes, a high-resolution ¹³C NMR spectrum was acquired from a [U-²H, U-¹³C]glucose solution, using the same acquisition parameters as described above (Fig. 1a).

Statistical analyses. Results are expressed as mean ± s.e.m. unless stated otherwise. Statistical significance was tested using Excel (Microsoft) with a two-tailed Student’s t-test.