Published in final edited form as:

Magn Reson Imaging. 2011 October ; 29(8): 1035–1040. doi:10.1016/j.mri.2011.07.001.

In Vivo Measurement of Normal Rat Intracellular Pyruvate and Lactate Levels after Injection of Hyperpolarized [1-¹³C]Alanine

Simon Hu¹, Minhua Zhu¹, Hikari A.I. Yoshihara¹, David M. Wilson¹, Kayvan R. Keshari¹, Peter Shin¹, Galen Reed¹, Cornelius von Morze¹, Robert Bok¹, Peder E.Z. Larson¹, John Kurhanewicz¹, and Daniel B. Vigneron¹

¹Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA

Abstract

Hyperpolarized technology utilizing dynamic nuclear polarization has enabled rapid and high sensitivity measurements of ¹³C metabolism *in vivo*. The most commonly used *in vivo* agent for hyperpolarized ¹³C metabolic imaging thus far has been [1-¹³C]pyruvate. In preclinical studies, not only is its uptake detected, but also, its intracellular enzymatic conversion to metabolic products including [1-¹³C]lactate and [1-¹³C]alanine. However, the ratio of ¹³C-lactate/¹³C-pyruvate measured in this data does not accurately reflect cellular values since much of the [1-¹³C]pyruvate is extracellular depending on timing, vascular properties, and extracellular space and monocarboxylate transporter activity. In order to measure the relative levels of intracellular pyruvate and lactate, in this project we hyperpolarized [1-¹³C]alanine and monitored the *in vivo* conversion to [1-¹³C]pyruvate and then the subsequent conversion to [1-¹³C]lactate. The intracellular lactate/pyruvate ratio of normal rat tissue measured with hyperpolarized [1-¹³C]alanine was 4.89 ± 0.61 (mean $\pm$ standard error) as opposed to a ratio of 0.41 ± 0.03 when hyperpolarized [1-¹³C]pyruvate was injected.

Keywords

Hyperpolarized carbon-13; Dynamic nuclear polarization (DNP); Monocarboxylate transporter (MCT); Magnetic resonance spectroscopy / spectroscopic imaging

Introduction

Hyperpolarized MR imaging technology utilizing dissolution dynamic nuclear polarization (DNP) has made feasible the rapid and high sensitivity detection of *in vivo* metabolism of prepolarized ¹³C compounds (1). The most widely used DNP agent for both *in vitro* and *in vivo* applications thus far has been [1-¹³C]pyruvate. After injection, [1-¹³C]pyruvate is actively transported into cells by monocarboxylate transporters (MCTs) and acts as a metabolic precursor whose flux to [1-¹³C]lactate and [1-¹³C]alanine is catalyzed by the enzymes lactate dehydrogenase (LDH) and alanine transaminase (ALT) respectively. The levels of hyperpolarized metabolites have been shown to inform on the disease state of

© 2011 Elsevier Inc. All rights reserved.

Correspondence to: Dr. Daniel B. Vigneron, Dept. of Radiology and Biomedical Imaging, Box 2512, University of California, San Francisco, 1700 4th St. QB3 Building, Suite 102, San Francisco CA, 94158-2512, Phone: (415) 476-3343, Fax: (415) 514-4451, dan.vigneron@radiology.ucsf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

tissue in many preclinical studies. In particular, in cancer animal models, dramatic increases in $[1-^{13}\text{C}]\text{lactate}$ have been observed (2,3,4). Typically, $[1-^{13}\text{C}]\text{lactate}$ is measured relative to other hyperpolarized metabolites, for example $[1-^{13}\text{C}]\text{pyruvate}$ or $[1-^{13}\text{C}]\text{total carbon}$ (the sum of hyperpolarized pyruvate, alanine, and lactate). However, the ratio of $^{13}\text{C}\text{-lactate}/^{13}\text{C}\text{-pyruvate}$ measured typically does not accurately reflect cellular values since much of the $[1-^{13}\text{C}]\text{pyruvate}$ is extracellular depending on timing, vascular properties, and extracellular space and MCT activity. In order to measure the relative levels of intracellular pyruvate and lactate, we hyperpolarized $[1-^{13}\text{C}]\text{alanine}$ and monitored the *in vivo* conversion to $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$. With the assumption that normal cells exhibit little or no leakage of ALT, then hyperpolarized alanine must be transported into cells in order for conversion to hyperpolarized pyruvate and then lactate to take place, and thus, the detected levels of pyruvate and lactate would reflect intracellular ratios.

Methods

Polarizer and Preparations

All hyperpolarizations were performed using a HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK). The alanine preparation was created by mixing together 350 mg $[1-^{13}\text{C}]\text{L-alanine}$ (Cambridge Isotope Laboratories, Andover, MA, USA), 250 μL 18.94M NaOH, and 100 μL DMSO. The resulting alanine and NaOH concentrations were ~ 6.2 M and ~ 7.5 M respectively. Also added were 15 mM OX063 trityl radical and 0.3 mM Dotarem® gadolinium (Guerbet). A 100 μL sample of the final mixture was polarized at a field of 3.35T at ~ 1.3 K. The microwave irradiation frequency for the polarization was determined through a microwave frequency sweep procedure and the power was set to 25 mW. The preparation was polarized for 2 hours and then dissolved in a phosphate buffer containing 0.3 mM Na_2EDTA (ethylenediaminetetraacetic acid disodium salt) with HCl added to neutralize the NaOH. The final dissolved alanine had a concentration of 100 mM and a pH ~ 7.3 . For each hyperpolarized experiment, a small aliquot (~ 0.5 mL) of the dissolved alanine was used to measure liquid state polarization, which was 12.6% (standard deviation = 1.1%, $n = 4$, calculated back to time of dissolution using an experimental *in vitro* T_1 of 41.5 sec [standard deviation = 2.2 sec, $n = 6$]). For the experiments with pyruvic acid, a mixture consisting of 35 μL (approximately 45 mg) of $[1-^{13}\text{C}]\text{pyruvic acid}$ (Cambridge Isotope Laboratories, Andover, MA, USA) with 15 mM OX063 trityl radical and 1.5 mM Dotarem® gadolinium was used. The preparation was polarized for 1 hour and then dissolved in an aqueous solution with 40 mM Tris, 100 mM NaOH, and 0.3 mM Na_2EDTA . The final dissolved pyruvic acid had a concentration of 100mM and a pH of ~ 7.5 , and the liquid state polarization was typically $>30\%$ at the time of dissolution.

Animal Handling

All animal studies were carried out under a protocol approved by the UCSF Institutional Animal Care and Use Committee. Normal male Sprague-Dawley rats were used. For each experiment, a rat was anesthetized with an initial dose of isoflurane (2–3%), placed on a heated pad, and had a tail vein catheter inserted while under continuous anesthesia (1–2% isoflurane). An extension tube was attached to the main catheter to facilitate the eventual administration of the hyperpolarized substrate. The rat was then transferred to a heated water pad (with continuous circulation at 37 °C) in the radiofrequency (RF) coil in the MR scanner, strapped in place, kept under anesthesia with continuous delivery of isoflurane (1–2%) through a nose cone at an oxygen flow of 1 L/min, and periodically monitored for respiratory rate and general tissue perfusion.

MR Studies

All experiments were performed on a 3T GE MR scanner (GE Healthcare, Waukesha, WI, USA) equipped with multinuclear spectroscopy capability. A custom dual-tuned $^1\text{H}/^{13}\text{C}$ rat coil was used for both transmit and receive. Axial T2-weighted anatomical images were acquired with a standard fast spin-echo sequence. The hyperpolarized ^{13}C data were acquired using a double spin-echo sequence (5) with TE = 35 ms, TR = 3 sec, flip = 30 degrees, spectral bandwidth = 5000 Hz, and spectral resolution = 2.44 Hz. The dynamic acquisition was started 35 seconds after the start of injection of the hyperpolarized substrate. In each experiment, a total of 3 mL of 100 mM hyperpolarized material was delivered to the animal.

Data Processing

For each dynamic time point, a 10 Hz Gaussian apodization filter was used, a 1D FFT was performed, and a zero-order phase correction was applied. Due to the substantial subcutaneous fat in these animals, a significant, natural abundance ^{13}C lipid resonance ~1.4 ppm from pyruvate was detected in varying intensities in the ^{13}C MR spectra. To correct for this, a non-hyperpolarized baseline scan was acquired following the hyperpolarized scan to quantitate the fat signal contribution, which was then subtracted from the hyperpolarized ^{13}C MRS data. For metabolite quantification, two metrics—SNR and peak area ratios—were used. For all quantification, the first 15 dynamic time points were first summed together. To calculate SNR for the ^{13}C resonances of hyperpolarized pyruvate, lactate, and alanine, the maximal peak height (with the mean of the baseline of the adjacent non-peak region subtracted) was divided by the standard deviation of the noise. (Note: the pyruvate values used in this paper refer to pyruvate + pyruvate-hydrate.) Peak areas were calculated by integration over the full baseline of each peak with subtraction of the noise contribution.

Results

Figure 1 shows a comparison of hyperpolarized spectra obtained from injecting $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ alanine. As illustrated by Figure 1a, in the hyperpolarized $[1-^{13}\text{C}]$ pyruvate injection experiment, hyperpolarized $[1-^{13}\text{C}]$ lactate and hyperpolarized $[1-^{13}\text{C}]$ alanine are produced by LDH and ALT catalyzed reactions respectively. Note that for clarity, the MCT step and the transport of pyruvate into the mitochondria/ decarboxylation to acetyl-CoA and $^{13}\text{CO}_2$ are not shown. In contrast, when hyperpolarized $[1-^{13}\text{C}]$ alanine is injected, hyperpolarized $[1-^{13}\text{C}]$ lactate only appears after passage through hyperpolarized $[1-^{13}\text{C}]$ pyruvate as an intermediate. Therefore, detection of lactate also indicates initial conversion to pyruvate. Figure 1b shows some of the anatomical regions (experiments were non-localized), including liver, kidneys, stomach, intestines, and muscle, from which spectral data (Figure 1c and 1d) were obtained. Figure 1c shows a typical spectrum (sum of first 15 FIDs) after injection of 3 mL of 100 mM hyperpolarized $[1-^{13}\text{C}]$ pyruvate. Pyruvate (and some pyruvate-hydrate), alanine, and lactate were the primary resonances detected. Resonances from ^{13}C -bicarbonate, from the carbonic anhydrase mediated equilibrium with $^{13}\text{CO}_2$ after the pyruvate decarboxylation step, were also detected. The pyruvate, alanine, and lactate SNRs were 8310, 3140, and 3070 respectively (total SNR = 14520), and the lactate to pyruvate ratio of SNRs was 0.37. The lactate to pyruvate ratio calculated using peak areas was 0.39. Figure 1d shows a corresponding typical spectrum after injection of 3 mL of 100 mM hyperpolarized $[1-^{13}\text{C}]$ alanine. The inset of Figure 1d, which has the same vertical scale as Figure 1c, shows that most of signal is from the injected alanine. With a 92-fold zoom, the lactate and pyruvate peaks become apparent (lipid subtracted). The pyruvate, alanine, and lactate SNRs

in this spectrum were 16.2, 7150, and 70.0 respectively (total SNR = 7240). The lactate to pyruvate ratios using SNR and peak areas were 4.32 and 4.14 respectively.

Figure 2 shows the identification of pyruvate in the alanine injection experiments and highlights the benefit of fat subtraction. Figure 2a shows FIDs 16 through 64 of a spectrum following a hyperpolarized [$1\text{-}^{13}\text{C}$]pyruvate injection. The earlier FIDs, which contain overwhelming pyruvate signal, were omitted to make the lipid resonance visible. As shown in Figure 2a, the distance from lactate to pyruvate was 391 Hz, and the distance between the fat peak and pyruvate was 46 Hz. The lipid resonance, due to its proximity in the spectrum to pyruvate, most likely originates from a fatty acyl chain in pure adipose tissue (6). As expected, the pyruvate peak increased only when earlier dynamic time points were included, and the fat peak increased even when later time points after the hyperpolarized signal had decayed away were averaged. When hyperpolarized [$1\text{-}^{13}\text{C}$]alanine was injected (Figure 2b, first 15 FIDs), the same resonances with the same peak separations were detected. The distance between the outermost peaks, lactate and pyruvate, was 393 Hz, and the distance from pyruvate to fat was 44 Hz, which was in close agreement with Figure 2a considering the spectral resolution of the acquisitions was 2.44 Hz. Figure 2c shows the pyruvate peak after lipid subtraction. The top part of Figure 2c (same data as Figure 2b) shows the original unsubtracted spectrum with pyruvate as a shoulder on the lipid resonance. The middle of Figure 2c shows a non-hyperpolarized baseline scan immediately following the hyperpolarized alanine injection scan in which there was no shoulder on the lipid resonance. The bottom of Figure 2c is the resulting difference spectrum, showing the lipid completely removed and only the pyruvate present. These data show that pyruvate, albeit very little, was detected after injection of hyperpolarized alanine. Lactate, on the other hand was abundantly detected compared with pyruvate.

Tables 1 and 2 summarize all the hyperpolarized SNR and peak area data. As shown in Table 1, the total SNR when injecting alanine was only a little more than a factor of 2 lower than when injecting pyruvate, which is consistent with the polarization values measured (reported in the methods section). However, following hyperpolarized ^{13}C alanine injections, the majority of the SNR came from the pre-polarized alanine, and the SNRs of pyruvate and lactate were much lower. The same trend can be seen in Table 2, which reports integrated peak areas (normalized to total carbon area) instead of SNR. Both Table 1 and Table 2 show the measured lactate to pyruvate ratio was an order of magnitude higher when alanine was injected instead of pyruvate. The lactate to pyruvate ratios were comparable between the two tables, thus corroborating each other and showing that the ratios were insensitive to the quantification method.

As Table 1 demonstrates, non-localized SNRs of pyruvate and lactate were relatively low for the alanine injection experiments. Sensitivity enhancements would be needed to enable finely localized studies. However, as Figure 3 shows, slice-localized data with adequate lactate SNR may be feasible with current techniques. The data in Figure 3 were acquired with the same parameters described in the methods section except RF excitation was limited to a slice localized to the rat liver (Figure 3a). The lactate SNR in the alanine injection spectrum in Figure 3b was 11.2, and the pyruvate SNR was too low to measure. If the lowest detectable SNR is ~ 3 , then the lactate to pyruvate ratio would be greater than at least 3.5. The lactate to pyruvate SNR ratio for this same slice when pyruvate was injected (Figure 3c) was 0.55. Thus, the difference in ^{13}C -lactate to ^{13}C -pyruvate ratio between the alanine injection and pyruvate injection acquisitions in this initial liver-localized experiment is in agreement with what was detected in the non-localized studies.

Discussion

Hyperpolarized ^{13}C metabolic imaging, with its many advantages—including high sensitivity, virtually no background signal, and no ionizing radiation—has the potential to become an important research and ultimately clinical tool (7). Use of hyperpolarized ^{13}C technology to monitor metabolism could have a significant impact on the diagnosis/staging of disease and measurement of its response to treatment (7). Substantial effort has already been devoted to the evaluation of this technology in preclinical cancer models, and it has been demonstrated that perturbation of hyperpolarized ^{13}C lactate levels is a reliable and ubiquitous trait (2, 3, 4). However, when $[1-^{13}\text{C}]\text{lactate}$ is visualized with $[1-^{13}\text{C}]\text{pyruvate}$, care must be taken when interpreting the results. The injected pyruvate that enters the cell would depend on perfusion and uptake through MCTs, with the MCT transport step indicated to be the rate-limiting step in hyperpolarized pyruvate to lactate flux (8). In the scenario that pyruvate uptake is incomplete and persists in the extracellular space, this complicates the modeling of pyruvate and lactate kinetics. The use of hyperpolarized $[1-^{13}\text{C}]\text{alanine}$ in this study provides insight into intracellular steady-state lactate to pyruvate ratios since alanine must be transported into cells in order for pyruvate and lactate to be produced, and thus the observed ^{13}C -pyruvate is intracellular as is its conversion to ^{13}C -lactate. This approach provides a means to quantify pyruvate levels that are 1) intracellular and 2) are not the injected substrate. In normal, non-localized rat tissue, the lactate to pyruvate ratio was more than 10 times higher when injecting hyperpolarized alanine instead of pyruvate, showing that during hyperpolarized pyruvate injections, the majority of pyruvate originates from outside the cell. *In vivo* investigation of steady state levels of low concentration metabolites such as pyruvate would be severely limited without the sensitivity enhancement from hyperpolarization.

The lactate to pyruvate ratio in this study was quantified using both SNR and peak area metrics, and the mean and variation were similar for both methods. The variability in the measurement when using hyperpolarized alanine was most likely due to the low pyruvate levels, and thus SNR, observed. Despite the low steady state pyruvate levels, alanine $\rightarrow$ pyruvate flux must have been at least equal to pyruvate $\rightarrow$ lactate flux because, as shown by Figure 1a, alanine must pass through pyruvate to become lactate, which was consistently measurable. This is quantitatively shown in the hyperpolarized alanine injection row of Table 1, which shows relatively low SNR for lactate and very low SNR for pyruvate. The distribution and kinetics of amino acid transporters in the body may be an important factor in hyperpolarized alanine flux (9). The lower lactate and pyruvate SNRs could be explained by slower uptake of alanine. Due to these low SNRs, a limitation of this study was that the entire rat body, including liver, kidneys, stomach, intestines, and muscle was used to derive the intracellular lactate/pyruvate ratios in most of the experiments (adequate lactate SNR was detected in an initial liver-localized experiment). Future developments for enhanced sensitivity may enable localized hyperpolarized spectroscopic imaging.

Hyperpolarized alanine may have applications that extend beyond probing intracellular lactate and pyruvate levels. Alanine is the primary amino acid released by muscle tissue to be transported to the liver for gluconeogenesis during postabsorptive and fasted states (10), and it is plausible that during conditions such as these, alanine uptake and thus metabolic flux would be more pronounced. Hyperpolarized experiments have already shown a change in alanine metabolism in the liver when $[1-^{13}\text{C}]\text{pyruvate}$ was used as the injected substrate in fasted rats (11). In that experiment, lower alanine levels were detected, perhaps pointing to flux in the direction of alanine to pyruvate for gluconeogenesis. Finally, hyperpolarized alanine may also have utility in disease studies as alanine levels have been known to be perturbed in many metabolic diseases (10). In recent hyperpolarized pyruvate experiments, alanine levels were found to increase in diseased versus healthy states in a transgenic mouse

model of liver cancer (12, 13). The continued investigation of [1-¹³C]pyruvate and other agents in animal models will lead to a better understanding of hyperpolarized biomarkers and assist in the refinement of a technology that may ultimately benefit individualized treatment of disease.

Conclusions

Uptake of hyperpolarized [1-¹³C]alanine and its flux to [1-¹³C]pyruvate and [1-¹³C]lactate were detected *in vivo*, with the enhancement from hyperpolarized technology enabling the reliable detection of low steady state levels of pyruvate. The lactate to pyruvate ratios, which reflected intracellular values, were substantially higher than those measured when [1-¹³C]pyruvate was injected, suggesting that the majority of pyruvate detected in [1-¹³C]pyruvate studies is of extracellular origin.

Acknowledgments

The authors thank Dr. James Tropp for designing and building the coil used in this study and Kristen Scott for animal handling assistance. This study was supported by NIH grant R01 EB007588 and UC Discovery grant ITL-BIO04-10148 in conjunction with GE Healthcare.

References

1. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. *Proc Natl Acad Sci.* 2003; 100:10158–10163. [PubMed: 12930897]
2. Golman K, Zandt RI, Lerche M, Pehrson R, Ardenkjaer-Larsen JH. Metabolic imaging by hyperpolarized ¹³C magnetic resonance imaging for *in vivo* tumor diagnosis. *Cancer Res.* 2006; 66:10855–10860. [PubMed: 17108122]
3. Day SE, Kettunen MI, Gallagher FA, Hu DE, Lerche M, Wolber J, Golman K, Ardenkjaer-Larsen JH, Brindle KM. Detecting tumor response to treatment using hyperpolarized ¹³C magnetic resonance imaging and spectroscopy. *Nat Med.* 2007; 13:1382–1387. [PubMed: 17965722]
4. Albers MJ, Bok R, Chen AP, Cunningham CH, Zierhut ML, Zhang V, Kohler SJ, Tropp J, Hurd RE, Yen Y, Nelson SJ, Vigneron DB, Kurhanewicz J. Hyperpolarized ¹³C lactate, pyruvate, and alanine: noninvasive biomarkers for prostate cancer detection and grading. *Cancer Res.* 2008; 68:8607–8615. [PubMed: 18922937]
5. Cunningham CH, Chen AP, Albers MJ, Kurhanewicz J, Hurd RE, Yen Y, Pauly JM, Nelson SJ, Vigneron DB. Double spin-echo sequence for rapid spectroscopic imaging of hyperpolarized ¹³C. *J Magn Reson.* 2007; 187:357–362. [PubMed: 17562376]
6. Canoni P, Alger JR, Shulman RG. Natural abundance carbon-13 nuclear magnetic resonance spectroscopy of liver and adipose tissue of the living rat. *Biochem.* 1983; 22:4974–4980. [PubMed: 6639939]
7. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, Deberardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR. Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research. *Neoplasia.* 2011; 13:81–97. [PubMed: 21403835]
8. Harris T, Eliyahu G, Frydman L, Degani H. Kinetics of hyperpolarized ¹³C₁-pyruvate transport and metabolism in living human breast cancer cells. *Proc Natl Acad Sci.* 2009; 106:18131–18136. [PubMed: 19826085]
9. Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev.* 1990; 70:43–77. [PubMed: 2404290]
10. Felig P. The glucose-alanine cycle. *Metabolism.* 1973; 22:179–207. [PubMed: 4567003]
11. Hu S, Chen AP, Zierhut ML, Bok R, Yen Y, Schroeder MA, Hurd RE, Nelson SJ, Kurhanewicz J, Vigneron DB. *In vivo* carbon-13 dynamic MRS and MRSI of normal and fasted rat liver with hyperpolarized ¹³C-pyruvate. *Mol Imaging Biol.* 2009; 11:399–407. [PubMed: 19424761]

12. Hu S, Lustig M, Balakrishnan A, Larson PEZ, Bok R, Kurhanewicz J, Nelson SJ, Goga A, Pauly JM, Vigneron DB. 3D compressed sensing for highly accelerated hyperpolarized ^{13}C MRSI with *in vivo* applications to transgenic mouse models of cancer. *Magn Reson Med*. 2010; 63:312–321. [PubMed: 20017160]
13. Hu S, Balakrishnan A, Bok RA, Anderton B, Larson PEZ, Nelson SJ, Kurhanewicz J, Vigneron DB, Goga A. ^{13}C -Pyruvate imaging reveals alterations in glycolysis that precede tumor formation and regression. *Cell Metab*. 2011; 14:131–142. [PubMed: 21723511]

Figure 1.

A) Schematic of the interconversion between pyruvate, alanine, and lactate and the redistribution of the ^{13}C label. (Note: for clarity, the entry of pyruvate into the mitochondria and its subsequent metabolism is not shown.) Of particular relevance in this diagram is that the ^{13}C label on injected alanine must transfer to a pyruvate intermediate before going to lactate. B) T2-weighted coronal image of the central portion of the rat body from which hyperpolarized data were obtained. The ^{13}C experiments were nonlocalized. C) A hyperpolarized ^{13}C non-localized summed spectrum after injection of $[1-^{13}\text{C}]$ pyruvate. Very high pyruvate, lactate, and alanine signals were detected. A modest amount of bicarbonate was also observed. D) A typical lipid-subtracted hyperpolarized ^{13}C non-localized summed spectrum after injection of $[1-^{13}\text{C}]$ alanine. As shown in the upper right inset, in contrast to C), the vast majority of the signal came from the injected substrate. The other metabolites are visualized with a 92X zoom, showing a lactate to pyruvate ratio reflective of intracellular concentrations and much higher than in C).

Figure 2.

A) Hyperpolarized ^{13}C non-localized data after injection of $[1-^{13}\text{C}]$ pyruvate with the earlier time points in the summed spectrum omitted (shown are time points 16 through 64) to allow for visualization of a lipid resonance ~ 1.4 ppm to the left of pyruvate. The earlier time points contain pyruvate signal that overwhelms the fat peak. An example of a spectrum with the earlier time points is given in Figure 1C). B) Hyperpolarized ^{13}C non-localized data after injection of $[1-^{13}\text{C}]$ alanine showing the same lipid resonance as in A). The distances between major peaks and pyruvate were the same in A) and B), showing that pyruvate was indeed present in the $[1-^{13}\text{C}]$ alanine data set. C) Fat subtraction. The pyruvate peak in the original $[1-^{13}\text{C}]$ alanine data set (top), though noticeable, was made more conspicuous (bottom) after subtraction of the lipid peak estimated from a non-hyperpolarized reference scan (middle).

Figure 3.

A) T2-weighted coronal image with a box depicting the 20 mm slice localized to the rat liver from which the hyperpolarized data were acquired. B) A hyperpolarized ^{13}C liver-localized summed spectrum after injection of $[1-^{13}\text{C}]$ alanine. A clear lactate signal was detected, indicating that transport of alanine and passage through pyruvate to lactate occurred in the liver. C) A corresponding spectrum in the same slice after injection of $[1-^{13}\text{C}]$ pyruvate. Pyruvate was higher than lactate in this spectrum, in contrast to the spectrum following $[1-^{13}\text{C}]$ alanine injection (B) and thus is consistent with the non-localized results in Figure 2.

Summary of pyruvate injection (n = 4) and alanine injection (n = 4) hyperpolarized SNR data. SNR was taken to be maximal metabolite peak height in a phased spectrum divided by noise standard deviation measured from a non-peak region. Quantification was performed after lipid subtraction.

Table 1

	Pyr SNR	Ala SNR	Lac SNR	Total SNR	Lac/Pyr
Pyr Injection	8200 ± 430	3210 ± 410	3370 ± 280	14800 ± 980	0.41 ± 0.03
Ala Injection	11.9 ± 2.0	6640 ± 710	55.1 ± 6.1	6700 ± 710	4.89 ± 0.61

Note: ± are given as standard error of the mean.

Table 2

Summary pyruvate injection (n = 4) and alanine injection (n = 4) hyperpolarized peak area data. The peak areas were calculated by integrating over the full width of metabolite peaks in a phased spectrum, with the noise contribution subtracted out. Quantification was performed after lipid subtraction.

	Pyr Area	Ala area	Lac Area	Lac/Pyr
Pyr Injection	0.558 ± 0.015	0.214 ± 0.012	0.229 ± 0.006	0.41 ± 0.02
Ala Injection	2.14e-3 ± 2.82e-4	9.89e-1 ± 7.34e-4	9.09e-3 ± 4.84e-4	4.96 ± 0.94

Note: the values have been normalized to total carbon area, and ± are given as standard error of the mean.