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Investigation of Tumor Hyperpolarized [1-¹³C]-Pyruvate Dynamics Using Time-Resolved Multiband RF Excitation Echo-Planar MRSI

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Abstract

Hyperpolarized [1-¹³C]-pyruvate is an exciting new agent for the in vivo study of cellular metabolism and a potential cancer biomarker. The nature of the hyperpolarized signal poses unique challenges because of its short duration and the loss of magnetization with every excitation. In this study, we applied a novel and efficient time-resolved MR spectroscopic imaging (MRSI) method to investigate in a prostate cancer model the localized temporal dynamics of the uptake of [1-¹³C]-pyruvate and its conversion to metabolic products, specifically [1-¹³C]-lactate. This hyperpolarized ¹³C method used multiband excitation pulses for efficient use of the magnetization. This study demonstrated that regions of tumor were differentially characterized from normal tissue by the lactate dynamics, where tumors showed later lactate detection and longer lactate duration that was statistically significant ($P < 0.001$). Compared to late-pathologic-stage tumors, early- to intermediate-stage tumors demonstrated significantly ($P < 0.01$) lower lactate total signal-to-noise ratio (SNR), with similar temporal dynamic parameters. Hyperpolarized pyruvate dynamics provided uptake, perfusion, and vascularization information on tumors and normal tissue. Large, heterogeneous tumors demonstrated spatially variable uptake of pyruvate and metabolic conversion that was consistent with cellularity and necrosis identified by histology. The results of this study demonstrated the potential of this new hyperpolarized MR dynamic method for improved cancer detection and characterization.

Keywords

hyperpolarized C-13 pyruvate; dynamic MRSI; multi-band RF pulses; prostate cancer; metabolic imaging; TRAMP

While the current commercially available MRI/¹H MR spectroscopic imaging (MRSI) examination has shown promise for improving the detection and characterization of prostate cancer in individual patients, there is clinical consensus that a need exists for more sensitive and specific imaging (1). In the study of this disease, the transgenic adenocarcinoma of mouse prostate (TRAMP) mouse has been valuable as an established and well-studied murine model of prostate cancer (2,3). This model mimics human disease progression with a neoplastic evolution from early to late stages and has a very similar metabolic profile. Metastases are also

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common in this model, especially in the lymph nodes, but also to the lungs, kidneys, adrenal glands, liver, and bone (3).

Carbon-13 MR spectroscopy has shown the ability to noninvasively probe the metabolic profile and distinguish between malignant and normal tissue (4). Recently, hyperpolarization techniques have been developed to retain dynamic nuclear polarization (DNP) signal enhancements of ^{13}C compounds through a dissolution process that provides solutions with greater than 40,000-fold signal increases compared to thermal equilibrium (5–7). These hyperpolarized compounds have been used recently to investigate a variety of biological and cellular processes (5,6,8–28). This has allowed for the in vivo study in animals of ^{13}C metabolism with rapid scan times, good signal-to-noise ratio (SNR), and no background signal. In particular, hyperpolarized $[1-^{13}\text{C}]$ -pyruvate has the potential to observe cellular bioenergetics, such as glycolysis, the citric acid cycle, and fatty acid synthesis. Its conversion to $[1-^{13}\text{C}]$ -lactate and $[1-^{13}\text{C}]$ -alanine has been observed extensively in vivo in animal models (5,6,8–19).

Previous studies have begun to investigate the use of in vivo MRSI with hyperpolarized $[1-^{13}\text{C}]$ -pyruvate for the study of prostate cancer in the TRAMP mouse (11,18). In Chen et al. (11), differential conversion to $[1-^{13}\text{C}]$ -lactate and $[1-^{13}\text{C}]$ -alanine was detected using a fast three-dimensional MRSI acquired in a 14-sec interval starting 35 sec after injection. The tumors and metastases were characterized by increased $[1-^{13}\text{C}]$ -lactate and decreased $[1-^{13}\text{C}]$ -alanine. No studies have examined localized dynamics of $[1-^{13}\text{C}]$ -pyruvate and its metabolic products, but the study of the TRAMP mouse and potentially other animal models would benefit from rapid and serial observation of the metabolic signal evolution, thereby providing information of uptake, perfusion, vascularization, and tissue retention.

Hyperpolarized MRSI poses several unique pulse sequence design challenges, particularly for time-resolved acquisitions. The duration of the hyperpolarized signal and thus the imaging window is determined by the T_1 relaxation of the labeled molecules. For $[1-^{13}\text{C}]$ -pyruvate, this window is only a few minutes. Furthermore, each excitation pulse will use up some of the magnetization, which is unrecoverable. In dynamic imaging, particular care must be taken in selecting excitation parameters to preserve magnetization for later images. One method that addresses these challenges is the dynamic lactate-specific imaging (29). Only lactate is excited, which allows for fast temporally resolved imaging, as well as preserving pyruvate for conversion to lactate at later times. This method, however, provides no information about the pyruvate substrate or its conversion to alanine, thus limiting its value for monitoring dynamic metabolism.

We have recently introduced a new dynamic spectroscopic imaging method for hyperpolarized agents that efficiently uses the magnetization through tailored multiband excitation pulses (30). Pyruvate is excited with a small flip angle to preserve its magnetization, while lactate and alanine are excited with larger flip angles to improve their SNR. In this paper, we improved and applied this new method to studies of prostate cancer in vivo using the TRAMP model. The goal of this project was to provide new unique dynamic metabolic information, including the perfusion of pyruvate, differential conversion to lactate and alanine, and the duration and detection times of the metabolites, creating a more detailed metabolic profile of tumors and normal tissue than previously reported (11,18).

MATERIALS AND METHODS

Hyperpolarized $[1-^{13}\text{C}]$ -Pyruvate

The $[1-^{13}\text{C}]$ -pyruvate was hyperpolarized using recently introduced dynamic nuclear polarization methods (5) to yield more than 40,000-fold signal enhancement of ^{13}C compounds

in solution. The compound polarized was a mixture of [$1\text{-}^{13}\text{C}$]-pyruvic acid and the trityl radical (tris[8-carboxyl-2,2,6,6-tetra[2-(1-hydroxyethyl)]-benzo(1, 2-d:4,5-d')bis(1,3)dithiole-4-yl] methyl sodium salt) obtained from GE Healthcare (Oslo, Norway). The purity of the pyruvic acid (Isotec, Miamisburg, OH) was >98% and the degree of labeling was >99%. A tris (hydroxymethyl)aminomethane (TRIS)/NaOH/ethylenediaminetetraacetic acid (EDTA) dissolution medium was used to bring the final dissolved sample to the desired concentration of 80 mM and an acceptable pH level. An aliquot of the pyruvate solution was taken and injected into a polarimeter to measure the percentage of polarization (which had a range of 13.9 to 27.5% at the time of injection for these experiments). This was also used to measure the pH of the final solution (range of 7.0 to 7.7). The compound was polarized for at least 45 min prior to dissolution by a HyperSense (Oxford Instruments, Abingdon, UK) at 3.35 T and a temperature of 1.4 K. The complete details of the polarization and dissolution methods are described in Ardenkjaer-Larsen et al. (5).

Radio Frequency (RF) Pulse and Pulse Sequence

This new dynamic MRSI method has a much improved imaging efficiency by using tailored multiband RF pulses that preserve pyruvate magnetization (30). A majority of the pyruvate magnetization is retained for subsequent conversion into lactate and alanine, making them more readily detected in later images. With this pulse, the metabolite flip angles could be individually chosen such that the hyperpolarized substrate—pyruvate—was excited much less than its products of lactate and alanine. This reduces the loss of hyperpolarized pyruvate magnetization that would occur if it were excited with the larger flip angles required to observe lactate and alanine, which have a much lower concentration. Another advantage of this flip angle scheme is that it provides better detection of the conversion kinetics. This is because the primary conversion direction of pyruvate is to lactate via lactate dehydrogenase and to alanine via alanine aminotransferase. Thus, the lactate and alanine signal will be replenished during the experiment, provided there is still polarized pyruvate, but pyruvate will not be substantially replenished. Overall, this results in a more efficient use of the magnetization and thus improves the SNR for dynamic imaging. The pulse design is described in more detail elsewhere (30, 31) and is summarized here.

The spectral-spatial pulse used for all experiments is shown in Fig. 1a–c. The flip angles have been chosen empirically to provide enough lactate and alanine SNR, as well as a long window in which to sufficiently observe their dynamics. Pyruvate is excited with a 3.3° flip angle (θ_{pyr}), while lactate and alanine are excited with a 20° flip angle ($\theta_{\text{lac,ala}}$), a ratio of 1:6. In several initial experiments, this pulse was scaled down to $\theta_{\text{pyr}} = 2.5^\circ$ and $\theta_{\text{lac,ala}} = 15^\circ$, but the flip angles of $\theta_{\text{pyr}}: \theta_{\text{lac,ala}} = 3.3^\circ:20^\circ$ were found to provide a better balance of SNR and temporal window. The pulse was designed to exclude pyruvate-hydrate excitation because its kinetics follow that of pyruvate, and any pyruvate-hydrate excitation would result in additional pyruvate saturation due to their exchange. A complex optimization spectral filter design with spectral factorization is used to achieve the desired response, shown by the dashed lines in Fig. 1b, while also minimizing the RF power (32). Furthermore, these pulses were corrected for chemical-shift misregistration, as shown by the flat top and bottom of the spectral-spatial profile in Fig. 1c. The design is very flexible, and several other excitation pulse designs are shown in Larson et al. (30). The pulse in Fig. 1 used for these experiments has a 5 mm minimum slice thickness, with a maximum gradient amplitude of 4 G/cm, 0.0453 G peak RF amplitude, and a 18.8-ms duration. Each metabolite had an excitation bandwidth of 0.8 parts per million (ppm), which was found in previous experiments (30) to sufficiently cover the inhomogeneity across a mouse.

The pulse sequence used is shown in Fig. 1d. The multi-band excitation pulse described previously was followed by two hyperbolic secant adiabatic refocusing pulses. This pair of

adiabatic pulses ensured that no magnetization was lost due to flip-angle inaccuracies while forming a spin echo (33). During the readout, an echo-planar spectroscopic imaging (EPSI) gradient was used to encode both spectral and spatial information (34). A full echo acquisition was used for improved SNR and allowed for magnitude spectral reconstructions (35). Several initial studies used a fly-back EPSI gradient and later studies used a symmetric EPSI readout because it was more SNR efficient (36). The entire fly-back gradient waveform was 101.48 ms, with a spectral resolution of 9.83 Hz, 581-Hz spectral bandwidth, 16 spatial points, and a minimum resolution of 5.4 mm. Only data from the plateaus of the gradient were used in the reconstruction. The symmetric gradient was 102.08 ms, with a spectral resolution of 9.79 Hz, 581-Hz spectral bandwidth, 18 spatial points, and a minimum resolution of 4.45 mm. The positive and negative lobes were reconstructed separately, and then their magnitudes were summed to form the final spectra. The data acquired on the ramps were gridded onto uniformly spaced k -space locations prior to the Fourier transform, which allowed for improved SNR and smaller voxel sizes.

This sequence was repeated for eight phase-encode steps, resulting in a 16×8 (fly-back EPSI) or 18×8 (symmetric EPSI) effective matrix. All images were acquired with echo time = 160 ms, pulse repetition time = 250 ms, 2 sec per image, temporal resolution of 5 sec, and a 5 mm slice thickness. With the fly-back EPSI gradient, the in-plane resolution was 5×5.4 mm, resulting in a 0.135-cc voxel size. The symmetric EPSI acquisitions had an in-plane resolution of 5×5 mm and 0.125 cc voxels. With eight phase-encode steps and the metabolite flip angles applied, we expect, per image, a pyruvate magnetization loss of either 0.76% ($\theta_{\text{pyr}} = 2.5^\circ$) or 1.32% ($\theta_{\text{pyr}} = 3.3^\circ$) and a lactate/alanine magnetization loss of 14.2% ($\theta_{\text{lac,ala}} = 15^\circ$) or 29.2% ($\theta_{\text{lac,ala}} = 20^\circ$). Unless specified otherwise, experiments used $\theta_{\text{pyr}}: \theta_{\text{lac,ala}} = 3.3^\circ:20^\circ$ and the symmetric EPSI readout with 0.125-cc voxels.

Animal Experiments

All transgenic prostate cancer (TRAMP) mouse studies were performed under a protocol approved by the UCSF Institutional Animal Care and Utilization Committee. For all the mice included in this study, a 28-gauge port that extended from the dorsal surface of their neck was surgically implanted into one of their jugular veins at least 1 day before the hyperpolarized MR study. At the time of the MR experiment, the mice were anesthetized with 1–1.5% isoflurane, and a 90-cm-long, 24-gauge extension tube was connected to the jugular vein port, using a hard plastic connector (Strategic Applications, Libertyville, IL). This special extension tubing made it possible to quickly inject the hyperpolarized agent inside the human MR scanner while maintaining a low dead volume in the tubing. Next, the mice were placed on a water-filled, temperature-controlled pad that was heated to approximately 37°C by circulating water and positioned inside a custom-built, dual-tuned mouse birdcage coil. This coil, used for RF transmission and signal reception, was based on a design from Derby et al. (37).

A total of 11 TRAMP mice with tumors were studied in 15 experiments, and three TRAMP wild-type mice were studied twice each to provide six control acquisitions. In each of the mice studied, 0.35 mL of the final pyruvate solution was injected into the mouse over a 12-sec period, followed by a 0.15-mL normal saline flush. The injection volume was approximately 10% of the mouse blood volume, which demonstrated no adverse effects. The pyruvate injection of 80 mM leads to higher blood concentrations than normal physiological levels, especially during the initial bolus (38). Experiments were performed on a GE Excite 3-T clinical MRI system (GE Healthcare, Waukesha, WI) with 40 mT/m, 150 mT/m/ms gradients, and a broadband RF amplifier. For the hyperpolarized experiments, the RF transmitter gain was determined based on previous phantom and animal experiments and was estimated to be within $\pm 10\%$ of the appropriate value. The center frequency was calibrated using a syringe filled with 1.77-M

[1-¹³C]-lactate inserted next to the animal in the coil. T_2 -weighted fast spin-echo (FSE) images were acquired in axial, coronal, and sagittal orientations as anatomical references.

At the end of the study, mice were sacrificed by asphyxiation with carbon dioxide. Their genitourinary tracts were dissected promptly, and individual prostate lobes or primary tumor pieces were isolated and snap frozen in liquid nitrogen subjected to formalin fixation/paraffin-embedding for subsequent pathologic and immunohistochemical (Ki-67 and caspase-3) analysis. The percentage of well-differentiated, moderately differentiated, and poorly differentiated prostate cancer tissue, shown in Table 1, was estimated from this pathology. Mice with less than 25% poorly differentiated and invasive cancerous tissue were classified as early-intermediate stage, and those with >25% were classified as late stage.

Analysis

Data analysis was performed in MATLAB (The Mathworks Inc., Natick, MA), using the detected peak amplitudes for pyruvate, lactate, and alanine. A normalized SNR accounting for the [1-¹³C]-pyruvate polarization was calculated as:

$$nSNR = \frac{S}{N} \cdot \frac{25\%}{P}, \quad [1]$$

where nSNR is the normalized SNR, S is the peak amplitude, N is the noise standard deviation, and P is the measured polarization, which is normalized relative to 25%. From these peak amplitudes, we extracted several parameters. The total metabolite signal (tSNR) was calculated by summing the normalized SNR over time and then normalizing to the total acquisition time. Total hyperpolarized carbon (tHC) was calculated by summing the tSNR of lactate, alanine, and pyruvate. In some studies, the first three to four images were not used in these calculations because of spreading artifacts from the pyruvate bolus, and the signal was normalized appropriately. A “mean time” (MT) was calculated as the first moment of the peak amplitudes, $S[i]$, versus time, $t[i]$:

$$MT = \frac{\sum_{i=1}^N S[i]t[i]}{\sum_{i=1}^N S[i]}. \quad [2]$$

This is identical to the centroid of the dynamic curves. The full width at half maximum (FWHM) of the curves was calculated as the time the peak amplitude was above half of the maximum peak amplitude in each voxel. Peak amplitudes were linearly interpolated to provide finer FWHM time resolution. Both the MT and FWHM were only calculated in voxels with at least two images where the peak amplitude was greater than 2.5 noise standard deviations.

Since we can expect normally distributed data, t tests were used for all statistical analysis. A two-sample (independent) t test, which tests the hypothesis that the two sample groups characteristics have the same mean, was used to highlight overall characteristic differences. For data within a given animal, a paired-sample t test was applied, testing the hypothesis that, within animals, the two sample group means are the same. This highlighted differences within animals and controls for both inter animal variations (respiration, vascularization, bolus size and delivery) and interexperiment variability (pyruvate polarization, coil loading, flip angle calibration).

RESULTS

Typical data with this new method in TRAMP mice are shown in Fig. 2. The tumor in Fig. 2a–e had a high lactate signal, high lactate to pyruvate ratio, and no alanine, all of which are typical for TRAMP tumors, as was demonstrated previously (11). The representative image shown included the liver, which had some of the highest detected $[1-^{13}\text{C}]$ -alanine. The hyperpolarized ^{13}C MRSI data were collected from throughout the mouse abdomen, and various metabolite amounts and dynamics were observed. The effect and timing of the injection were also observable in the detected pyruvate (Fig. 2c). The large pyruvate signal in the neck was from the jugular catheter used for the injection. In the pyruvate curves, a second peak was seen 20 sec after injection that corresponds to the pyruvate in the catheter that is displaced by saline flushed between 12 and 18 sec after injection, creating a second bolus. This was done to clear the catheter line of hyperpolarized pyruvate and also resulted in modulations of the lactate and alanine in some voxels. Representative metabolic dynamics in a normal prostate, early-stage tumor, and late-stage tumor are shown in Fig. 2f. In the normal prostate, low amounts of hyperpolarized pyruvate, lactate, and alanine were detected, while high lactate was observed in the tumors. The largest lactate was seen in the late-stage tumor, which is consistent with previous nondynamic studies (11,18). The low metabolite signal in the normal prostate makes it difficult to compare the dynamic characteristics between normal and cancerous prostates.

Images of the lactate FWHM and MT, as well as metabolite tSNR, pyruvate FWHM, and pyruvate MT, are shown in a large, late-stage tumor in Fig. 3. The coronal orientation allowed for comparison between the tumor and other organs, such as the liver. While the highest lactate tSNR was within the tumor, there were voxels in the gut with lactate on the level of many tumor voxels. The data analysis showed that the lactate dynamics were distinct within the prostate tumors, with the highest lactate FWHM and lactate MT seen in the tumor, as is shown in Fig. 3d,e. Other tissues with high hyperpolarized lactate levels, such as the gut, did not share these lactate dynamic characteristics. The pyruvate dynamics also provided new information. In particular, the pyruvate MT (Fig. 3e) showed areas of more rapid uptake in the right, superior portion of the tumor (pyr MT = 23.69 sec), which also had the highest pyruvate tSNR (12.80). This region had the highest lactate tSNR (44.08) and an earlier lactate MT (34.63 sec), suggesting it was a better-perfused and vascularized region of the tumor, in addition to being highly metabolically active. The rest of the tumor had relatively high lactate tSNR (25.03) but a later pyruvate MT (25.72 sec) and lactate MT (36.93 sec), suggesting that perfusion was delayed in these regions. The regional differences between pyruvate and lactate in both their tSNR and MT maps demonstrate the unique heterogeneity information provided by dynamic imaging. Alanine was seen in the liver and gut but not in the tumor.

Figure 4 shows a comparison of the pyruvate and lactate characteristics between tumor and liver voxels in eight TRAMP mice, as well as the liver voxel characteristics in three wild-type normal mice, showing differences that were strongly statistically significant for many parameters. Only animals with primary prostate tumors and no evidence of metastases were included in these comparisons, and voxels used were required to have a minimum SNR to ensure accurate and consistent dynamic parameterizations. The liver was chosen as the healthy reference tissue because it was more metabolically consistent than the gut, where activity probably varies with digestion and peristalsis, and some portion of the liver was always present and viable in the coronal orientation. The pyruvate and lactate characteristics in normal mice livers (x's) agree with this hypothesis of the liver as a healthy reference and also show the variance of these parameters resulting from physiologic (cardiac and liver function, flow), experimental (injection, bolus, RF calibration), and/or other sources. In agreement with the visual observations shown in Fig. 3, there were significant statistical differences in the lactate dynamics (FWHM and MT), and both were strictly increasing from liver to tumor within each

animal. This resulted in the strongest significance when the paired-sample *t* test was applied, which controls for interanimal and interexperiment variations. Both the tSNR and fraction of alanine (not shown) were statistically significant, as expected because of the normally high alanine in the liver and little to no alanine in the prostate. The pyruvate to tHC and lactate to tHC ratios were significantly different between tumor and liver, but this was not true for the lactate tSNR or pyruvate tSNR (not shown). The tHC ratio differences are most likely due to alanine, which is included in tHC and detected in the liver. The lack of statistically significant tSNR differences is because the liver often had substantial lactate, especially relative to early-stage tumors. The pyruvate FWHM and MT (not shown) showed no statistically significant differences, suggesting similar perfusion and flow in the tumor and liver. These data suggest the lactate FWHM is the most sensitive dynamic parameter for differentiating tumor and normal tissue.

Figure 5 compares hyperpolarized ^{13}C dynamic data acquired in two TRAMP tumors with different pathologic grades and rates of cellular proliferation based on Ki-67 staining. The TRAMP in Fig. 5a–c had a relatively sparse and ill-defined tumor consisting of 75% moderately differentiated and 25% poorly differentiated tissue. There was no clearly delineated tumor nodule or mass in the FSE image, and Ki-67 staining showed a low density of proliferating tumor cells. On the other hand, the TRAMP in Fig. 5d–f had a well-defined tumor on the FSE image and a high density of proliferating tumor cells with 100% poorly differentiated tissue. The lactate was substantially larger in the more aggressive tumor, with a lactate tSNR of 18.88 as compared to 7.07 for the less aggressive tumor. The pyruvate was similar, with an average pyruvate tSNR of 2.39 and 1.41 for the less and more aggressive tumors, respectively. The dynamic data analysis yielded lactate MTs of 38.37 and 34.43 sec, indicating an earlier arrival and likely better vascularization in the more advanced, aggressive tumor. The lactate FWHM values were 35.00 and 30.62 sec, showing more persistent lactate in the less aggressive tumor, which may be due to poorer vascularization. Heterogeneity was observed across the more aggressive tumor, which demonstrated better pyruvate perfusion and more lactate in the top-left voxels corresponding to the more homogenous region in the FSE image.

Table 1 summarizes the tSNR, lactate MT, and lactate FWHM from 10 hyperpolarized $[1-^{13}\text{C}]$ -pyruvate studies in six of the TRAMP tumors analyzed with histology ($N = 3$ had early- to intermediate-stage tumors, $N = 3$ had late-stage tumors, as determined by histological analysis). There was a significant difference between the late- and early-/intermediate-stage tumors in lactate tSNR, which is consistent with previous results (11,18). On average, the dynamic parameters (MT and FWHM) for lactate and pyruvate showed no statistically significant differences between early- to intermediate- and late-stage tumors. One possible explanation is that the majority of lactate and pyruvate detected was from actively proliferating cells, which could have a specific dynamic profile (MT and FWHM), where the amount of lactate corresponds to the density of these cells while the dynamic profile is stable. The pyruvate tSNR was highly variable, indicating a wide range of tissue perfusion. Also, a metabolic difference from normal tissues was that all tumors demonstrated little to no alanine signal, which is high in the normal liver and muscle tissues.

Dynamic imaging data and pathology from the corresponding tissue in a heterogeneous tumor are shown in Fig. 6. Overall, the pyruvate and lactate signal variations were consistent with the variations in cellular density. In particular, the left anterior portion of this tumor (green arrows) had less lactate (lac tSNR = 12.34), which corresponded to a region of reduced cellular density (lighter and pinker hematoxylin and eosin staining) and reduced tissue density (tissue integrity compromised). As shown in the histology, the tissue separated during processing, indicating weakness or lack of structure that was most likely due to the presence of necrosis. There was also lighter staining along the anterior and right sides of the tumor, which had relatively less lactate and pyruvate (lac tSNR = 12.55, pyr tSNR = 4.21). The central posterior

region had the greatest tumor cell density and the highest lactate and pyruvate (lac tSNR = 30.30, pyr tSNR = 10.42). The pyruvate and lactate had a similar distribution across the tumor, but the contrast was greater in the lactate signal.

The perfusion information provided by the dynamic imaging is shown in the lactate and pyruvate MT (Fig. 6f). Within the tumor, the shortest MT for both pyruvate and lactate was near the central posterior region (pyr MT = 23.42 sec, lac MT = 36.70 sec), nearest to the major vessels, and the MT increases in the peripheral regions (pyr MT = 24.40 sec, lac MT = 38.64 sec). In the probable-necrosis region (green arrows), the lactate MT is particularly high (39.54 sec), most likely because it has the worst vascularization and perfusion. The lactate FWHM was relatively similar across the entire tumor (average $\pm$ standard deviation = 31.29 ± 1.53 sec), as compared to nontumor tissue (data shown in Figs. 3e and 4).

A lymph node metastasis in this slice (red arrow) was easily identified by its high lactate signal (lac tSNR = 27.41). The lymph node had low pyruvate (pyr tSNR = 5.37) and a later pyruvate MT (24.90), both similar to the peripheral, lower-density regions of the primary tumor. This is related to its naturally slower lymphatic fluid supply and/or relatively poorer vascularization. However, the lactate tSNR, MT (35.31 sec), and FWHM (29.07 sec) were similar to the central primary tumor region, suggesting high active cellular density in the lymph node metastasis.

DISCUSSION

Time-resolved, multiband RF excitation, echo-planar MRSI provided high spatial (0.125–0.135 cc) and temporal (every 5 sec) resolution [$1\text{-}^{13}\text{C}$]-pyruvate metabolic data from throughout the abdomens of TRAMP mice. By observing the pyruvate uptake dynamics, it was possible to assess tissue perfusion, indicative of vascularization, in the primary tumor, metastatic disease and in surrounding healthy tissues. The resulting lactate and alanine time course provided detailed information on the metabolic profile not only in terms of overall metabolite conversion but also the rate and timing of this conversion. Specifically, prostate tumors showed later lactate detection and a longer duration of lactate conversion when compared to normal tissue that was statistically significant ($P < 0.001$). The high-spatial-resolution dynamic MRSI data obtained in this study allowed for an assessment of the pathologic heterogeneity of tumors, as reflected by differences in metabolic dynamics in different regions of the tumor having different cellularity, perfusion, and viability. The clinical management of prostate cancer patients is complicated by the pathologic and biologic heterogeneity of the human prostate and prostate cancer, and dynamic hyperpolarized ^{13}C MRSI may be able to provide critical information concerning this heterogeneity.

The wild-type normal mice studied with this method did not provide substantial additional information about the prostate pyruvate metabolism dynamics. This is because the normal mouse prostate had relatively low pyruvate uptake and lactate conversion (Fig. 2f), as shown previously (11,18), and was also smaller than our voxel sizes. The result was that there was insufficient SNR in the normal prostate to reliably quantify the metabolite MT and FWHM parameters.

The longer duration of lactate conversion and its later mean detection time in tumors relative to healthy tissue could have several explanations. One explanation is that lactate may not wash out of the tumor as quickly as other healthy tissues. Another explanation is that there is an intracellular uptake of pyruvate in tumor tissue that would result in a longer duration of lactate conversion. A longer T_1 of lactate in tumors, due to its surrounding molecular environment, would also cause the lengthened detection of hyperpolarized lactate. While the lactate and pyruvate tSNR was highly variable across and within individual tumors, the lactate FWHM and MTs were relatively consistent across the tumors. One possible explanation is that

primarily active tumor tissue is contributing to the hyperpolarized lactate signal in these voxels, resulting in dynamics characteristic of the tumor tissue. In this case, the lactate tSNR would vary with the density of active tumor cells, while the lactate FWHM and MT would indicate a cancerous region regardless of density. This hypothesis is supported by the histological comparisons shown in Figs. 5 and 6.

We found a significant difference in the lactate tSNR between tumor stages, which is consistent with previous studies (11,18). Interestingly, in this initial study no significant difference in the dynamic metabolic parameters was noted between late- and early- to intermediate-stage tumors. However, this finding needs to be proven in a larger number of TRAMP mice. Even if the dynamic metabolic data do not provide pathologic-grade information, they provide important information concerning cellularity and necrosis (Fig. 6), as well as differences in perfusion and substrate delivery (Figs. 3 and 6).

The primary benefit of dynamic imaging over single-time-point imaging is that it provides measures of the perfusion and substrate delivery timing. There may be additional benefits to dynamic imaging in human prostate imaging. We have observed that the delivery of pyruvate to different tissues is relatively similar between mice, but in humans this will likely not be the case, especially for older patients who may have compromised cardiac function. In single-time-point imaging, the metabolite SNR and ratios, such as the lac:pyr and lac:tHC, have been proposed as biomarkers of tumor presence and pathologic grade (11,18) and are dependent on the timing of the imaging acquisition, as is shown in the presented dynamic data. Therefore, results from single-time-point imaging would be confounded by the varying rates of substrate delivery between human subjects, while dynamic imaging would be robust to these variations. Another potential benefit is the observed consistency of the lactate MT and FWHM in cancerous tissue, which could confirm the presence of disease. This would likely be more valuable in other tumors and metastatic disease, particularly within tissues with high lactate signal, where lactate tSNR or peak lactate may not be significantly increased compared to normal tissue but the lactate dynamics could provide a marker of early-stage disease.

Our initial five experiments were performed with slightly smaller flip angles of while the rest of our experiments used $\theta_{\text{pyr}}: \theta_{\text{lac,ala}} = 2.5^\circ:15^\circ$ as this improved the SNR while maintaining a long enough temporal window. While this difference will result in perturbations of the observed dynamics, we found that this did not substantially alter the dynamic parameters. This is demonstrated by TRAMP #1 in Table 1, for whom two experiments were performed on the same day identically except with the different flip angles, and the analysis parameters vary only a fraction of their standard deviations. All experiments used for the comparison of tumor to liver tissue (Fig. 4), were acquired with $\theta_{\text{pyr}}: \theta_{\text{lac,ala}} = 3.3^\circ:20^\circ$.

CONCLUSIONS

We have developed a dynamic MRSI method that provided localized, time-resolved metabolic information following the injection of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate. This method uses novel multiband excitation pulses to preserve hyperpolarized magnetization for improved dynamic imaging and allowed the imaging of metabolites for 60 sec following injection consistently. Applying this method to the TRAMP mouse model of prostate cancer has provided new dynamic metabolic information for improved tumor characterization. The dynamic data are indicative not only of the metabolic profile but also the tissue perfusion, metabolic rates, and retention of metabolites in tissue. These TRAMP tumors were characterized by high $[1-^{13}\text{C}]$ -lactate, low $[1-^{13}\text{C}]$ -alanine, late detection of $[1-^{13}\text{C}]$ -lactate, and a long $[1-^{13}\text{C}]$ -lactate duration. The tumor lactate dynamics, described by the MT and FWHM, had statistically significant differences from normal tissue. With this new method, we have also observed tumor heterogeneity in the pyruvate and lactate dynamics that was

consistent with cell density as determined by histology. Distinction of tissue heterogeneity may be even more valuable for human prostate studies because of the large amount of surrounding normal tissue and the need to assess tumor aggressiveness.

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FIG. 1.

Multiband spectral-spatial excitation pulse with an echo-planar gradient and a 5 mm minimum slice thickness. **a:** RF pulse—real (solid) and imaginary (dashed) components—and accompanying gradient. **b:** Spectral profile, with specified bands (dashed lines) for pyruvate (pyr) of a 3.3° flip, a 20° flip for alanine (ala) and lactate (lac), and no excitation of pyruvate-hydrate (pyr-H₂O). **c:** Spectral and spatial profile. Within the frequency range of interest, there is no chemical-shift misregistration. **d:** Pulse sequence. The spectral-spatial excitation was followed by a phase encoding gradient. A pair of adiabatic refocusing pulses is used for a B₁-insensitive spin-echo (33), which occurs halfway through the EPSI readout gradient. The

crushers around the adiabatic pulses and after the sequence are not shown, and the adiabatic pulse amplitude has been reduced for visualization.

FIG. 2.

Typical spectra and dynamic curves. **a:** The coronal T_2 -weighted FSE anatomical image shows the majority of the voxel locations. **b:** A typical single MRSI, acquired 25 sec following injection, demonstrates the spectral characteristics and typical SNR obtained with this method for 0.125-cc voxels. **c–e:** Dynamic curves for the metabolites. The liver (green box) contains all three metabolites and has a relatively large alanine signal. The tumor (red box) has very high lactate and no alanine, which is characteristic of these tumors. **f:** Dynamic curves from a normal mouse prostate and two TRAMP tumors. The lactate signal is greatly elevated with cancer and also is increased in the late-stage tumor.

FIG. 3.

Large, late-stage heterogeneous tumor. **a:** Coronal T_2 -weighted FSE anatomical image showing a subset of the voxel locations. **b:** Metabolite curves for pyruvate, lactate, and alanine in each voxel. The green box highlights the liver, while the red highlights the tumor. **c:** Overlay images of the metabolite tSNR, all identically windowed. **d:** FWHM of the pyruvate and lactate signal. **e:** MT of pyruvate and lactate. The lactate tSNR, MT, and FWHM were larger within the tumor when compared to the normal tissue.

FIG. 4.

Comparison of pyruvate and lactate characteristics between tumor voxels and liver voxels in TRAMP mice (circles) and normal mice (x's, liver only). The lines connect data points for the same mouse, and each color also represents a different mouse.

FIG. 5.

a–c: Low cellular density of proliferating cancer cells. **a:** The coronal T_2 -weighted FSE anatomical image suggests an ill-defined tumor. **b:** Histology of tumor tissue with Ki-67 (cell proliferation) staining, shown in brown at 10x magnification, indicates the relatively low density of proliferating cancer cells and scattered areas of proliferation. **c:** Peak amplitude curves. **d–f:** Same as (a–c), but for a more aggressive tumor with a higher proliferative index, as indicated by dense Ki-67 staining in (e). The lactate normalized SNR is much larger in the more aggressive tumor, while the pyruvate is relatively similar. No alanine was detected in any of these voxels.

FIG. 6.

Large, late-stage heterogeneous tumor. **a:** Axial T_2 -weighted FSE anatomical image. **b:** Hemotoxylin and eosin staining of a tumor cross-section at approximately the same location and orientation as **(a)**. **c,d:** Pyruvate and lactate curves. No alanine was detected. **e:** FWHM of pyruvate and lactate. **f:** MT of pyruvate and lactate. There was less lactate, as well as greater lac MT and FWHM, along the left and anterior portions of the tumor (wide green arrows), corresponding to regions of less cellular density in **(b)**. A lymph node metastasis can be seen (narrow red arrow) with very high lactate signal but very little pyruvate.

Table 1

Imaging Parameters and Tumor Statistics (Mean $\pm$ Standard Deviation) for TRAMP experiments With Histological Analysis.

TRAMP	Stage	pyr tSNR	ala tSNR	lac tSNR	lac MT (seconds)	lac FWHM (seconds)
1 ^a	Early-int.	2.46 $\pm$ 0.90	0.33 $\pm$ 0.12	7.12 $\pm$ 1.29	39.48 $\pm$ 1.60	32.96 $\pm$ 4.12
		3.12 $\pm$ 1.03	0.35 $\pm$ 0.39	7.56 $\pm$ 1.46	38.28 $\pm$ 0.08	34.02 $\pm$ 6.82
2	Early-int.	5.92 $\pm$ 2.48	1.41 $\pm$ 0.20	12.38 $\pm$ 3.14	36.25 $\pm$ 0.77	29.07 $\pm$ 0.18
3	Early-int.	6.55 $\pm$ 1.98	0.49 $\pm$ 0.27	9.69 $\pm$ 2.27	32.99 $\pm$ 2.01	26.99 $\pm$ 1.70
4 ^b	Late	14.62 $\pm$ 2.74	0.68 $\pm$ 0.39	13.43 $\pm$ 3.83	33.28 $\pm$ 1.12	30.83 $\pm$ 3.76
a,b		3.84 $\pm$ 1.83	0.62 $\pm$ 0.39	9.40 $\pm$ 2.22	31.85 $\pm$ 1.10	29.44 $\pm$ 5.27
5 ^{a,b}	Late	3.54 $\pm$ 1.49	0.46 $\pm$ 0.61	13.58 $\pm$ 8.94	38.24 $\pm$ 1.13	36.25 $\pm$ 4.43
a,b		1.41 $\pm$ 1.14	0.34 $\pm$ 0.34	18.88 $\pm$ 5.79	34.43 $\pm$ 1.82	30.62 $\pm$ 4.96
6	Late	7.79 $\pm$ 4.19	1.22 $\pm$ 0.59	29.26 $\pm$ 10.14	36.42 $\pm$ 1.29	31.27 $\pm$ 1.72
		9.10 $\pm$ 1.97	0.99 $\pm$ 0.44	24.62 $\pm$ 6.72	37.36 $\pm$ 1.48	31.38 $\pm$ 1.78

Two experiments were performed on four mice, all on the same or next day. There was statistical significance ($P < 0.01$) in the lac tSNR between the early- to intermediate- and late-stage tumor voxels.

^a $\theta_{\text{pyr}}: \theta_{\text{lac,ala}} = 2.5^\circ:15^\circ$.

^b Voxel size of 0.135 cc.