Separation and Quantification of Lactate and Lipid at 1.3 ppm by Diffusion-Weighted Magnetic Resonance Spectroscopy

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INTRODUCTION

Spectral overlapping is a common problem that affects clinical and preclinical magnetic resonance spectroscopy (MRS) and hampers identification of important biomarkers for diagnosis and prognosis. Diffusion-weighted MRS (DW-MRS) is a noninvasive tool to probe the diffusion of metabolites (1–3). At short diffusion time regime, diffusion properties can be largely dependent on the molecular size. Therefore, it may be feasible to separate spectrally overlapped compartments with greatly different molecular sizes, such as lactate and lipid, by applying diffusion weighting.

Lactate is usually undetectable by MR in healthy tissues. In cancer studies, the increased glucose uptake, lack of oxygen supply, and accumulation of lactate are the main features of the tumor cells. Lactate content in the tumor tissue can predict metastases and overall survival of cancer patients (4–7). Lactate also reveals the recurrence of tumor cells after cancer treatment and the resistance to both radiotherapy and chemotherapy (6,8,9). The abnormal lactate elevation has an important role in both diagnosis and therapeutic management of cancer.

Mobile lipids are the MR visible neutral lipids accumulated in the form of small droplets under long-term stress. A number of in vivo and in vitro studies have demonstrated that accumulated mobile lipids are closely related to the cell death in response to cancer therapy (10). The concentration of mobile lipids indicates the extent of necrosis and apoptosis (11,12). Therapeutic outcomes such as radiation necrosis can be evaluated by the mobile lipid concentration, while the lactate concentration can serve as an indicator of tumor growth and recurrence. The accurate measurement of lactate and lipid concentrations is considered to be valuable for prognosis, discovering tumor recurrence, and evaluating the efficacy of therapeutic interventions (5).

Lactate is also related to hypoxia and hypoperfusion. In stroke and muscle ischemia, lactate content is an indicator of the time that tissue has been subjected to hypoxia and is closely related with pH value (13–15). As lactate has been reported to accumulate quickly in the acute phase in stroke, lactate concentration can be an important marker for grading or staging the disease or injury (13,14,16). Recent studies have also reported that mobile lipids are related to apoptosis signaling in stroke patients (17,18). The concentration of mobile lipids indicates the quantity of necrotic cell death in the injured tissue (14,17). In summary, monitoring lactate and lipid...
is essential for diagnosis and prognosis of a number of diseases.

Spectroscopy can provide direct observation and quantification of important biomarkers. However, in 1H-MRS, mobile lipids emit a strong signal from the methylene (–CH2–) protons at 1.2–1.4 ppm, and it overlaps with the lactate methyl (–CH3) protons at 1.3 ppm. These two signals can be separated using multiple echo times (TEs), because the lactate doublets at 1.3 ppm reverse in polarity with every TE increase of 144 ms (19). This method is simple and straightforward, but the resulting signal-to-noise ratio (SNR) is very low, and it is often compromised by the chemical shift displacement artifact (20,21). In the past two decades, lactate editing by J-difference has been developed and applied for the separation of lactate and lipid in tumor tissue (22,23). Spectral editing methods such as Meshcher-Garwood point-resolved spectroscopy and band selective inversion with gradient dephasing have demonstrated high sensitivity for detecting lactate. However, these methods are time consuming, require long TE, and usually take more than 30 min to acquire the difference spectra with reasonable lactate SNR (22–25). Zero-quantum coherence spectral editing combined with the stimulated echo (STE) sequence for spatial selection can also separate lactate from lipid (26). Furthermore, the implementation of selective multiple quantum coherence filtering with the chemical shift imaging sequence allows the sensitive detection of lactate without lipid contamination (27).

In the NMR community, there are various methods for resolving the overlapped resonances in certain dimensions based on different mechanisms (28,29). Separating the spectrally overlapped molecules or/macromolecules by diffusion has been previously proposed and demonstrated in ex vivo samples as two-dimensional diffusion-ordered spectroscopy (2D-DOSY) (30,31). By adding diffusion weighting to the overlapped resonances, DOSY is ideal for resolving different molecules in the dimension of diffusivity and characterizing their sizes (32–34).

Similar approaches can be applied in vivo to separate molecules and/or macromolecules with different diffusivities. Diffusion MR is a powerful tool for probing molecular diffusivity and tissue microstructure noninvasively. Most DW-MRS studies so far have focused on the resonances arising from relatively small metabolites (35,36). Recently, DW-MRS has been employed to investigate the diffusion behavior of macromolecules such as intramyocellular lipids (IMCLs) in skeletal muscles (37–39) and proteoglycans (PGs) in intervertebral discs (40). The diffusivities of macromolecules such as lipid, PG, and collagen have been shown to be much lower than those of the solutes in cytosol, such as NAA, choline, creatine, glucose, and lactate.

Lactate is a small metabolite (molecular weight of C3H6O3: 90 Da). It is expected to exhibit a relatively high diffusivity compared with lipid (molecular weight of an unsaturated triglyceride C55H98O6: 854 Da), especially given that the diffusion of mobile lipid in vivo is often restricted within the submicron-sized lipid droplets. A large difference in diffusivity between lactate and lipid may allow their robust separation and quantification from diffusion-weighted spectra. In this study, DW-MRS was applied as a tool for separating the overlapped lactate and lipid signals at 1.3 ppm. The feasibility of resolving their spectral overlapping by diffusion weighting was demonstrated in phantoms and rat brain tumors.

**METHODS**

**MR Instrument and DW-MRS Sequence**

All MR experiments were performed on a 7T MRI scanner (70/16 Pharmascan, Bruker BioSpin, Rheinstetten, Germany) equipped with a 370 mT/m gradient system along each axis. A receive-only quadrature surface coil and a circular polarized transmit-only coil were used. The DW-MRS sequence was implemented by modifying the stimulated echo acquisition mode (STEAM) based single-voxel MRS sequence with a pair of diffusion gradients applied along the shortest axis of the MRS voxel. Oil phantom experiments were first performed to evaluate and optimize the DW-MRS protocol by minimizing eddy current and maintaining adequate SNR while achieving adequate diffusion decay (38,40).

**Phantom Experiments**

All phantom experiments were conducted at room temperature (20 °C). One double-layer-cylinder phantom with 400 mM lactate solution in the outer layer and olive oil in the inner tube was made for demonstrating diffusion suppression of the lactate signal. The phantom was placed in parallel with the main magnetic field so that the lipid resonances in the inner tube would be shifted to the higher frequency as a result of the bulk susceptibility effect (41,42).

Six phantoms were made to demonstrate the lactate and lipid separation and quantification. The lipid droplet emulsion (~0.5-μm diameter, Intralipid 20%, Fresenius Kabi, Lake Zurich, Illinois) was mixed with lactate (L7022, Sigma-Aldrich, St. Louis, Missouri) in agarose gel (A9414, Sigma-Aldrich, St. Louis, Missouri). They were made with constant lactate concentration (50 mM) and varied lipid concentrations (lipid volume fraction from 0.1 to 0.6% in steps of 0.1%). These gel phantoms provided better imitation than the olive oil and lactate solution phantom of the live tissue where the mobile lipids are accumulated with lactate. DW-MRS experiments were performed on the gel phantoms with 11 diffusion weighting factors or b-values (0, 1.5, 2.5, 4, 6.5, 9, 30, 40, and 21 × 10^3 s/mm²). Other parameters were TE = 32 ms, repetition time (TR) = 1300 ms, mixing time (TM) = 64 ms, diffusion time (∆) = 80 ms, diffusion gradient duration (δ) = 9 ms, and number of excitations (NEX) = 32.

**Rat Brain C6 Glioma Experiments**

The brains of six Sprague-Dawley (SD) rats (body mass approximately 260 g) were injected with C6 glioma cells (43–45). In brief, the rats were anesthetized with 80 mg/kg ketamine and 10 mg/kg xylazine and placed in a stereotactic device. Their heads were shaved and an incision made in the anteroposterior direction to expose the bregma of the skull. A 1-mm hole was drilled through the skull at 1 mm anterior and 2.5 mm to the right of the
Bregma. 10 μL of cell culture medium (Dulbecco’s modified Eagle’s medium) containing approximately 10^6 C6 glioma cells were then injected into the cortex at 5 mm depth at 2 μL/min with a 26-G needle Hamilton syringe (Reno, Nevada). Afterward, the hole was filled with bone wax and the scalp was sutured.

DW-MRS experiments with nine b-values (0, 2, 4, 6, 10, 20, 30, 40, and 50 × 10^3 s/mm^2) were conducted on the 18th day after C6 glioma cell injection. The MRS voxel was placed at the tumor core and covered most of the tumor tissue based on the T_2-weighted images. MRS voxel sizes ranged from 151 to 215 μL, depending on the actual tumor sizes. The scan parameters were TE/TM/TR = 52/34/1300 ms, Δ/δ = 60/18 ms, and NEX = 128.

Oil red O staining was performed on the tumor tissue samples to confirm lipid accumulation in the tumor and to identify the lipid droplets (46–48). Rats examined by DW-MRS were sacrificed with transcardial perfusion using 4% paraformaldehyde in 0.1 M PBS (pH = 7.4) after an overdose of intraperitoneal pentobarbital. Brain samples postfixed with 4% paraformaldehyde were further processed by 30% sucrose overnight at 4°C for cryoprotection. They were embedded in optimum cutting temperature compound (OCT; Thermo Scientific, Waltham, Massachusetts) and 15-μm cryosections were prepared and stored frozen until staining. Frozen sections from OCT-embedded brain tissues were air-dried at room temperature, washed in 60% isopropanol and stained with 0.5% Oil red O solution (O1391, Sigma-Aldrich, St. Louis, Missouri) at room temperature for 1 h. Sections were washed through 60% isopropanol and mounted. Lipid droplets were visualized under the microscope and images were acquired.

**Data Analysis**

For all DW-MRS data, each individual free-induction decay (FID) was stored and phase-corrected before averaging to minimize the effect of zero-order phase variations caused by motion and gradient eddy current (38,40). Spectral analysis was performed using JMRUI software (http://www.mrui.uah.es/mrui/). Overlapped signals from lipid methyl (–CH_3) and lactate methyl (–CH_3) protons at 1.3 ppm were identified and integrated using AMARES algorithm. Fitting errors of the overlapping background signals were assessed by Cramér-Rao lower bounds (CRLBs) (49–51). Quantification was considered relevant only when the corresponding CRLB was below 25%. The DW signals of lactate and lipid were normalized by the spectral baseline noise. Their relative concentrations in arbitrary unit were calculated by normalizing the lactate and lipid signals with the MRS voxel volume.

Two calculation approaches were applied to quantify the lactate and lipid signals. For the biexponential fitting approach, the lactate and lipid signals and their apparent diffusion coefficients (ADCs) were computed by fitting the b-value-dependent DW signals to a biexponential model using least-squares regression according to the following equation:

\[
S = S_{lactate} \times e^{-b \times ADClactate} + S_{lipid} \times e^{-b \times ADClipid}
\]  

The spectral subtraction approach was also applied to estimate lactate and lipid signals from two or three DW spectra, which can offer the advantage of shorter acquisition time in practice. This way of estimation is justified because lipid diffusion is very slow and the lipid signal loss resulting from diffusion can be negligible at certain low b-values (6500 and 20000 s/mm^2 for gel phantoms and C6 glioma, respectively) where the lactate signal is already mostly suppressed by diffusion. Two DW spectra acquired at such b-value and b = 0 allow the direct estimation of lipid spectrum (ie, the DW spectrum acquired at such nonzero b-value), and indirect estimation of lactate spectrum by subtracting two spectra in the complex spectral domain.

Three DW spectra can further improve the lactate and lipid signal estimations and measure the lipid diffusivity using b = 0, and two nonzero b-values at which lipid diffusivity can be measured while the lactate signal is mostly suppressed. Such approach can yield more accurate lipid spectrum estimation by correcting for the lipid diffusion decay, and better lactate spectrum estimation. In this study, the two nonzero b-values were 6500 and 21000 s/mm^2 for gel phantoms, and 20000 and 50000 s/mm^2 for C6 glioma.

The accuracy of the lactate and lipid quantification in the gel phantoms was evaluated by the quality of the linear correlation between the relative lipid concentrations measured by DW-MRS and the known lipid volume fractions in the phantoms. Subtraction approaches using two and three b-values were also compared with the biexponential fitting approach for their quantification quality.

**RESULTS**

Figure 1 illustrates the robust separation of lactate and lipid by diffusion weighting. An axial T_2-weighted image of the double layer phantom is shown in the upper-right corner. As shown in the DW spectra, the 1.3-ppm signal from lipid methylene (–CH_2–) protons of the olive oil in the inner tube was shifted to 1.5 ppm and separated from the 1.3-ppm signal from lactate methyl (–CH_3) protons of the lactate solution contained in the outer layer by the bulk susceptibility effect. The effective suppression of lactate signal was achieved at a b-value of 6000 s/mm^2, whereas the lipid signal remained relatively unchanged.

Figure 2 demonstrates the separation and quantification of lactate and lipid signals in six gel phantoms. The lactate concentration was fixed at 50 mM, whereas the lipid volume fraction was varied. The location of the MRS voxel in the gel phantom is shown in the T_2-weighted image in Figure 2a. The representative DW spectra are also shown in Figure 2a. In these spectra, lactate doublets are clearly seen at the low b-value range (0–1500 s/mm^2), and they overlapped with the lipid resonance at 1.3 ppm. At high b-values, lactate doublets decayed to an undetectable level, but lipid resonance at 1.3 ppm only showed a very slow decay. The corresponding biexponential fitting of the integrated DW signals at 1.3 ppm is shown in the upper-right corner of Figure 2a. Figure 2b shows the relative lactate and lipid
concentrations in gel phantoms calculated from the biexponential fitting. Although the measured lactate concentrations maintained constant as expected, the measured lipid concentrations were highly correlated with their known lipid volume fractions. Figure 2c shows that lactate ADCs were relatively constant at \( (8.07 \pm 0.05) \times 10^{-4} \) mm\(^2\)/s, and that lipid ADCs exhibited a relatively large fluctuation \( (5.05 \pm 0.38) \times 10^{-7} \) mm\(^2\)/s). This fluctuation in lipid ADC likely resulted from the limited b-value range used in the experiments \( (0-21000 \) s/mm\(^2\)) that did not provide strong lipid signal decay for accurate lipid diffusivity estimation. The variation of the lipid droplet size in the gel phantoms might also contribute to the lipid ADC fluctuation.

Figure 3 shows the DW-MRS quantification of lactate and lipid in C6 glioma in six rat brains. In the upper-left corner of Figure 3a, the typical localization of the MRS voxel is shown as the rectangle superimposed on the tumor in a \( T_2 \)-weighted image. At lower b-value range \( (0-10000 \) s/mm\(^2\)), the overlapped resonances at 1.3 ppm showed a relatively faster decay; in contrast, the lipid resonance at 0.9 ppm exhibited little decay. The corresponding biexponential fitting of the DW signals at 1.3 ppm is presented in the upper-right corner of Figure 3a. Figures 3b and 3c summarize the quantification of lactate and lipid concentrations and ADCs. The lipid methylene (-CH\(_2\)-) proton signals were generally much stronger than the lactate methyl (-CH\(_3\)) signals, indicating a great extent of mobile lipid accumulation in C6 glioma. The presence of mobile lipids in C6 glioma was verified by the Oil red O staining as shown in Figure 4. The mobile lipid accumulation was observed in the tumor tissue but not in the normal tissue. The typical diameter of lipid droplets was approximately 0.5 \( \mu \)m.

Figure 5 demonstrates the use of spectral subtraction approach to calculate lactate and lipid contents in the gel phantoms. As shown in Figure 5a, clean separation of lactate and lipid spectra was achieved by directly subtracting the two spectra acquired at \( b = 0 \) and 6500 s/mm\(^2\), without and with the spectrum at \( b = 6500 \) s/mm\(^2\) corrected for diffusion loss. The lactate doublets can be clearly observed after subtraction. Two lipid resonances from methylene (-CH\(_2\)-) and methyl (-CH\(_3\)) protons were well removed from the lactate doublets. The lactate and lipid signals calculated using such spectral subtraction approaches and 11 b-value biexponential fitting approach are compared in Figures 5b and 5c. A spectral subtraction approach using either two b-values (0 and 6500 s/mm\(^2\)) or three b-values (0, 6500, and 21000 s/mm\(^2\)) produced results that were highly correlated with those by the biexponential fitting. Similarly, Figure 6a shows that lactate and lipid in the C6 glioma can also be separated by subtracting the spectra acquired at \( b = 0 \) and 20000 s/mm\(^2\). Figures 6b and 6c show that the accuracy of spectral subtraction approach using three b-values (0, 20000, and 50000 s/mm\(^2\)) was better than that of spectral subtraction using two b-values (0 and 20000 s/mm\(^2\)).

**DISCUSSION**

Separation and Quantification of Lactate and Lipid by DW-MRS

Lipids are a group of naturally abundant macromolecules in various tissue types in the human body (37, 52). The molecular weight of lipid is much larger than that of water-soluble small molecules, including lactate. Therefore, the diffusivity, characterized by the ADC, differs greatly between lipid and lactate (53). As shown in Figure 2c, the lactate ADC in gel phantoms was \( (8.07 \pm 0.05) \times 10^{-4} \) mm\(^2\)/s. It was three orders of magnitude higher than the lipid ADC \( (5.05 \pm 0.38) \times 10^{-7} \) mm\(^2\)/s). Figure 3c shows that lactate ADC in C6 glioma was \( (2.83 \pm 0.83) \times 10^{-4} \) mm\(^2\)/s, which was two orders of magnitude higher than the lipid ADC \( (2.68 \pm 1.29) \times 10^{-6} \) mm\(^2\)/s). These large diffusivity differences benefitted the effective separation and quantification of lactate and lipid by diffusing weighting.

As shown in Figures 5 and 6, the complex spectral subtraction approach allows the relatively simple estimation of the lactate spectrum by the difference spectrum of two or three DW spectra, thus incurring less acquisition time. For accurate lactate and lipid quantification, lipid diffusion decay needs to be considered. For example, based on the lactate and lipid ADC measurements in C6 glioma in the present study, approximately 94% of the lactate signal can be suppressed by diffusion weighting at \( b = 10000 \) s/mm\(^2\) when lipid signal loss is approximately 3%, and 99% of the lactate signal can be suppressed at \( b = 20000 \) s/mm\(^2\), whereas the lipid signal loss is approximately 5%. These estimations suggest that lipid signal loss resulting from diffusion should not be
neglected if lipid concentration is more than an order of magnitude higher than lactate concentration. In this scenario, the subtraction approach using two b-values would be problematic, and the three-b-value subtraction approach would become more desirable. As shown in Figures 6b and 6c, use of three b-values to include the lipid diffusivity estimation was necessary for accurate lactate and lipid quantification in C6 glioma. It should be further noted that the lactate phase evolution resulting from scalar coupling is neglected in Eq. 1 because of the relatively small TEs used in the present study. Future studies can account for such phase evolution in Eq. 1 for better quantification accuracy if the biexponential fitting approach is used.

The presence of lactate and lipid is not limited to tumor tissues. In fact, we also performed a pilot study to test the diffusion separation of lactate and lipid in a rat model of intracerebral hemorrhage (ICH) (Supporting Figure S1). In brief, collagenase solution was infused into the right striatum of six SD rats to induce the blood vessel dissolution and focal bleeding (54,55). The blood vessel disruption resulted in regional shortage of oxygen supply and gave rise to the lactate elevation. The cell death caused by hypoxia in the hematoma led to the increased mobile lipids. Biexponential decay of the DW signals at 1.3 ppm was observed as a result of the lactate and mobile lipids in the hematoma 20 h after collagenase injection. Compared with the rat brain C6 glioma, lipid concentration in hematoma was much lower, whereas the lactate concentration was similar.

Biological Insights from the ADCs of Lipid and Lactate

In this study, the lipid ADC in rat brain C6 glioma measured at a diffusion time of 60 ms ($2.68 \times 10^{-6}$ mm$^2$/s) is comparable with those reported in previous glioma studies (56,57). The lipid ADC varies between different tissue types and physiological conditions. For example, the intramyocellular lipid ADC measured in the rat hindlimb skeletal muscle is $1.01 \times 10^{-6}$ mm$^2$/s at a diffusion time of 80 ms and $0.25 \times 10^{-6}$ mm$^2$/s at 220 ms, revealing the strongly restricted diffusion behavior of lipid macromolecules within the micron-sized intramyocellular lipid droplets that are largely immobilized inside the muscle cells (37,40). The IMCL ADC is much lower than the mobile lipid ADCs measured in this study, both in C6 glioma and ICH hematoma. Note that the free and unrestricted lipid ADC was measured as $6.57 \times 10^{-6}$ mm$^2$/s in pure olive oil (40). This value was much higher than the lipid ADC measured in the gel phantoms in the present study ($5.05 \times 10^{-6}$ mm$^2$/s) and lower than the lipid ADC measured from the lipid droplet emulsion diluted

FIG. 2. DW-MRS measurements in the six agarose gel phantoms containing a constant lactate concentration but varied lipid droplet concentrations at 20 °C. DW spectra acquired from one phantom (lipid volume fraction 0.2%) are presented (a). The MRS voxel localization is shown as the box in the T$_2$-weighted image. The lactate doublets can clearly be seen at b-values lower than 4500 s/mm$^2$. The corresponding biexponential fitting of the 1.3-ppm DW signals is shown in the upper-right figure. Relative lactate and lipid concentrations calculated by the biexponential fitting (b). Lactate and lipid ADCs (c).
in saline (1.86 × 10⁻⁵ mm²/s, data not shown). All together, these large lipid ADC variations among different forms of lipids suggest that mobile lipid ADCs were determined by both size and mobility of the lipid droplets. Tissue type, cell size and shape, cytosol viscosity, and intracellular skeleton may all affect the mobility of lipid droplets in vivo, which can lead to the broad mobile lipid ADC variations in different tissues.

In this study, the lipid and lactate ADCs measured in C6 glioma showed a similar interanimal variation (Fig. 3c). This might arise from the inhomogeneous tumor microstructure among the six animals. The ADC differences could be also associated with tissue microstructure, such as heterogeneity in cell size, extent of edema, and blood coagulation in the lesion (1,2,58). The diffusion environment is complex in both tumor and ischemic tissues. Characterization of the lactate and mobile lipid diffusion behaviors may provide extra information about the tissue microstructural changes in diseased tissues.

Separation of Different Molecules and Macromolecules by Diffusion Weighting

In DOSY and DW-MRS experiments, diffusivity is often dominated by molecular size. Our recent study has shown that water can be suppressed by diffusion weight-
and be separated from PGs at 4.2–4.5 ppm in intervertebral discs. PGs are the important macromolecules in the extracellular matrix. This water and PG separation by diffusion weighting was based partly on the large difference in their molecular sizes (40). In the present study, DW-MRS was proved to be robust when applied to separate the overlapped resonances from the small lactate metabolite and large lipid macromolecules. Note that molecular diffusion is also sensitive to microstructural characteristics. Diffusion weighting has been applied to separate spectrally overlapped components that differed greatly in their microstructural characteristics. For example, IMCLs and extramyocellular lipids (EMCLs) at 1.2–1.5 ppm in skeletal muscles can be separated by diffusion weighting, because the difference in their microstructural distribution has dominated their diffusion properties (37,38,40). In fact, diffusion weighing of water molecules has been often applied to alter the contrast among vascular water, extracellular water, and intracellular water and separate these different components (59–61).

**Technical Issues in DW-MRS**

DW-MRS experiments are technically challenging. Non-water molecules are of very low concentrations in vivo. They exhibit large molecular weights and slow diffusion, and strong diffusion weighting is required to probe and utilize their diffusion properties. Consequently, DW-MRS experiments suffer from low SNR, require strong gradients, and are vulnerable to gradient eddy currents and motions. At present, the relatively low gradient strength in clinical MRI systems is one of the major
obstacles to robust DW-MRS implementation. Nevertheless, short TE and TM are generally desired to preserve SNR for accurate signal measurement in both preclinical and clinical DW-MRS studies. It is imperative to optimize the choice of TR and TE values, diffusion time, and diffusion gradient duration according to the T1 and T2 values of the molecule of interest and the b-value requirement. Compared to PRESS, STEAM-based DW-MRS loses half of the transverse magnetization, resulting in 50% SNR reduction. However, STEAM-based DW-MRS has an advantage over a PRESS-based one because the former offers the flexibility to increase diffusion time without increasing TE and significantly compromising SNR. In the present study, given the b-value requirement of 50000 s/mm² and the short lipid T2 value, a STEAM-based DW-MRS sequence was employed.

Strong diffusion gradient can induce substantial eddy current and contaminate spectral quantification in DW-MRS study. In DW-STEAM, adding two gradients between the second and third 90° RF pulses with the same amplitudes can reduce the eddy current effect (62). Furthermore, a short diffusion gradient duration is always preferred for the cancellation of eddy currents from the rising and falling edges of the gradient pulse. Using a prolonged TE will also reduce the short-term eddy current effect at the expense of SNR in practice.

DW signals at high b-values are highly sensitive to physiologic motions. In our study, the respiration was controlled by the mechanical ventilation system. In brain DW-MRS, the cerebrospinal fluid (CSF) pulsation and respiratory motion can easily lead to the ADC overestimation. For applying DW-MRS to study human brain
Separation of Lactate and Lipid by Diffusion-Weighted MRS

Lactate and lipid elevations are the hallmark of cancerous and ischemic tissues. Using diffusion weighting, robust separation of lactate and lipid signals at 1.3 ppm was demonstrated in the lactate/lipid phantoms and C6 glioma in vivo. In practice, it can be implemented by the simple spectral subtraction approach using two or three diffusion-weighted spectra acquired with carefully chosen b-values. Such DW-MRS presents a new approach to separate and quantify spectrally overlapped signals, such as lactate and lipid, by using the diffusivity difference associated with their different molecular sizes or mobility within tissue microstructure.

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