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Chemical Exchange Rotation Transfer Imaging of Intermediate Exchanging Amines at 2 ppm

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Abstract

Chemical exchange saturation transfer (CEST) imaging of amine protons exchanging at intermediate rates and whose chemical shift is around 2 ppm may provide a means of mapping creatine. However, quantification of this effect may be compromised by the influence of overlapping CEST signals from fast exchanging amines and hydroxyls. We aimed to investigate the exchange rate filtering effect of a variation of CEST, named chemical exchange rotation transfer (CERT), as a means of isolating creatine contributions at around 2 ppm from other overlapping signals. Simulations were performed to study the filtering effects of CERT for selecting transfer effects from protons of specific exchange rates. Control samples containing the main metabolites in brain, bovine serum albumin (BSA), and egg white albumen (EWA) at their physiological concentrations and pH were used to study the ability of CERT to isolate molecules with amines at 2 ppm that exchange at intermediate rates, and corresponding methods were used for *in vivo* rat brain imaging. Simulations showed that exchange rate filtering can be combined with conventional filtering based on chemical shift. Studies on samples showed that signal contributions from creatine can be separated from those of other metabolites by using this combined filter, but contributions from protein amines may still be significant. This exchange filtering can also be used for *in vivo* imaging. CERT provides more specific quantification of amines at 2 ppm that exchange at intermediate rates compared to conventional CEST imaging.

Graphical abstract

Chemical exchange saturation transfer (CEST) imaging of intermediate exchanging amine at 2 ppm may provide a means of mapping creatine. However, quantification of this effect may be compromised by the influence of overlapping CEST signals from fast exchanging amines and hydroxyls. We developed an exchange filter effect, named AREX_{double, cpw}, to isolate creatine contributions at 2 ppm from other overlapping signals.

Keywords

Chemical exchange saturation transfer (CEST); Chemical exchange rotation transfer (CERT); creatine

INTRODUCTION

Chemical exchange saturation transfer (CEST) applies long, frequency selective RF pulses to saturate protons in exchanging pools and detect subsequent changes in water signals. Due to the accumulated effects of the exchange, CEST provides an indirect measurement of solutes with significantly enhanced sensitivity compared to direct detection via conventional magnetic resonance spectroscopy (MRS).

Amine protons at 2 ppm from water that exchange at intermediate rates (between slowly exchanging amides (1) and rapidly exchanging amines and hydroxyls, such as in glutamate (2) and glucose (3)) are found in important molecules such as creatine and proteins, and detection of these exchanging pools has potential practical applications, motivating the development of appropriate CEST methods. In previously developed approaches, CEST signals at 2 ppm have typically been assumed to represent creatine-weighted saturation transfer and have been applied to imaging of brain, muscle, and heart (4–7). However, distinct from the narrow and specific peaks prevalent in MRS, CEST peaks, especially those from fast exchanging pools (e.g. glutamate at 3 ppm (2)), are broad and often overlap (8,9). Therefore, the CEST signal at 2 ppm may have contributions from both intermediate exchanging amines at 2 ppm and other fast nearby exchanging pools.

Previously, a magnetization transfer ratio with asymmetric analysis (MTR_{asym}) has been applied to quantify CEST signals at 2 ppm. Ideally, this method requires only two sampling points and thus is very convenient. However, the results of such an approach potentially are compromised by the influence of B_0 errors, semi-solid magnetization transfer (MT) asymmetry effects (10), and relayed nuclear overhauser enhancement (rNOE) effects originating from the other side of the water peak (11–16). More recently, a multiple pool Lorentzian fitting method was used to quantify the CEST signal at 2 ppm without using upfield reference signals (6). However, this fitting method requires acquisition of the entire

Z-spectrum and thus is comparatively slow. In addition, as discussed above, these two methods cannot isolate the intermediate exchanging amines at 2 ppm from other overlapping fast exchanging pools. Moreover, these approaches, with direct subtraction of label and reference signals, cannot fully remove the effects of direct water saturation (DS), semi-solid MT, and T_{1w} effects, which contribute to the signal non-linearly (17). Thus their quantitative results may provide misleading information in pathologies in which these tissue parameters also change.

Here, we describe a novel approach to quantifying the CEST signal at 2 ppm which suffers less from these limitations. Conventional CEST acquisitions separate metabolites primarily based on their different NMR chemical shifts, which cover only a narrow range. However, in addition to NMR chemical shifts, metabolites may have exchangeable protons with a broad range of exchange rates. While conventional CEST signals depend on the exchange rate, we have developed a variation of CEST, named chemical exchange rotation transfer (CERT) (18), which has a stronger dependence on exchange rate, thereby allowing more specific exchange-rate filtering of the signal in addition to filtering based on chemical shift alone. Previously, we introduced a metric (MTR_{double}) based on the CERT approach with a duty cycle (dc) of 50% and demonstrated its ability to filter slowly exchanging protons. We applied this method to isolate slowly exchanging amides from nearby intermediate and fast exchanging amines (18). In this work, we designed a CERT method to select intermediate-exchanging protons by varying the dc while keeping constant pulse widths (pw) in the sequence. We apply this new metric ($MTR_{double, cpw}$) to isolate amines exchanging at intermediate rates at 2 ppm from other overlapping fast exchanging pools. In addition, we incorporate an inverse analysis method based on the previously published apparent exchange-dependent relaxation (AREX) approach (17), and label the combined metric $AREX_{double, cpw}$. This metric is designed to remove the DS, semi-solid MT, and T_{1w} effects, in addition to the previously mentioned exchange-rate filter effects. Overall, the new CERT filter provides a more specific quantification of intermediate exchanging amines at 2 ppm and should in particular increase the specificity of creatine imaging.

MATERIALS AND METHODS

CEST and CERT quantification metrics

In conventional CEST imaging, an off-resonance RF irradiation (continuous-wave (CW) or a series of shaped pulses) is applied at the solute resonance frequency and on the opposite side of water before data acquisitions. The subtraction of the signals at these two frequency offsets has been used to quantify the CEST effect, putatively removing DS and semi-solid MT effects. The CEST metric MTR_{asym} is defined by Eq. (1),

$$MTR_{asym} = \frac{S_+ - S_-}{S_0} \quad (1)$$

where S_+ is the reference scan signal and S_- is the label scan signal. However, MTR_{asym} has been shown to be influenced by rNOE saturation transfer and asymmetric MT effects (12,18).

To solve this problem, a multiple pool Lorentzian fit, MTR_{fit} , was proposed. The Z-spectra were fit to the sum of several Lorentzian peaks using the following equation (19):

$$S(\Delta) = 1 - \sum_{i=1}^N A_i \left(1 + \left(\frac{\Delta - \Delta_{0i}}{0.5 \cdot W_i} \right)^2 \right)^{-1} \quad (2)$$

where the Z-spectrum is a function of RF offset (Δ), peak full width at half maximum (W) and peak amplitude (A). Δ_{0i} is the resonance frequency offset of a solute pool. N is the number of coupled pools. Note that such fittings require acquisition at a large number of frequency offsets.

We have previously proposed a new acquisition and analysis approach, named CERT with a metric defined in Eq. (3) by subtraction of pulsed-CEST signals at irradiation flip angles (θ) of π and 2π , while keeping RF frequency offset, average irradiation power ($B_{avg \text{ power}}$), and dc constant.

$$MTR_{double} = \frac{S_{-}(\theta_2=2\pi, dc) - S_{-}(\theta_1=\pi, dc)}{S_0} \Big|_{B_{avg \text{ power}}} \quad (3)$$

where $(-)$ represents that the signal is acquired on the resonance frequency offset of the exchanging species. (π) and (2π) are the nutation angles of the individual pulses making up a long irradiation pulse train. S_0 is the signal acquired in the non-irradiated control case. $B_{avg \text{ power}}$ is the average irradiation power and is defined to be the square root of the average of the square of the amplitude over a pulse repetition period. MTR_{double} can quantify the CEST effect by removing DS and semi-solid MT effects and, by using the same frequency offset for both the label and reference scans, avoids the signal contributions from rNOE saturation transfer and asymmetric MT that affect conventional MTR_{asym} .

We here use a variant of MTR_{double} , called $MTR_{double, cpw}$. Instead of maintaining a constant dc as θ is varied, we maintain a constant pulse width (pw).

$$MTR_{double, cpw} = \frac{S_{-}(\theta_2=2\pi, dc_2) - S_{-}(\theta_1=\pi, dc_1)}{S_0} \Big|_{B_{avg \text{ power}}} \quad (4)$$

The dc is varied to maintain constant pw. This change increases the exchange sensitivity and maintains similar bandwidths for the two pulse trains, a relevant issue when addressing DS effects close to the water line.

We also make an additional change to the analysis, in light of a recent study that shows that CEST, DS, and semi-solid MT effects do not add linearly (17). Thus conventional CEST metrics, which linearly subtract the label and reference signals, cannot remove DS and semi-solid MT effects. In addition, CEST effects scale with T_{1w} (17,20). The AREX approach

addresses these issues by inversely subtracting the label and reference signals and normalizing with the apparent water longitudinal relaxation time ($T_{1\text{obs}}$) (17,21,22). Here, we combined the CERT and AREX approaches to define a new metric:

$$\text{AREX}_{\text{double, cpw}} = R_{1\text{obs}} \left(\frac{S_0}{S_-(\theta_1=\pi, \text{dc}_1)} - \frac{S_0}{S_-(\theta_2=2\pi, \text{dc}_2)} \right) \Big|_{B_{\text{avg power}}} \quad (5)$$

where $R_{1\text{obs}} = 1/T_{1\text{obs}}$.

Design of an intermediate exchanging pass filter for creatine imaging

Our goal is to be maximally sensitive and specific to exchange effects from amines in creatine. Table 1 lists the NMR chemical shifts and exchange rates (k_{sw}) of some of the metabolites present in the brain. The values of k_{sw} have a broader range than the NMR chemical shifts, which is the conventional parameter used in CEST for separating overlapping metabolites.

For our purposes, the major brain metabolites can be separated into three categories based on their exchange rates with water: slow exchange regime ($k_{\text{sw}} < 100 \text{ s}^{-1}$), intermediate exchange regime ($100 < k_{\text{sw}} < 1000 \text{ s}^{-1}$), and fast exchange regime ($k_{\text{sw}} > 1000 \text{ s}^{-1}$). From our literature survey (1,2,23,24), creatine has an exchange rate of around $500 - 950 \text{ s}^{-1}$ under physiological conditions, which is in the intermediate exchange regime; most neurotransmitters in brain are in the fast exchange regime; amides on proteins and peptides are in the slow exchange regime. Although some other metabolites are also in the intermediate exchange regime, their exchange rates are still widely separated from creatine. For example, the exchange rates of phosphocreatine (PCr) ($140, 120 \text{ s}^{-1}$) (23) and adenosine-5-triphosphate (ATP) (120 s^{-1}) (23) are around one-fifth that of creatine. Myo-inositol (MI), by contrast has an exchange rate of $\sim 600 \text{ s}^{-1}$, which is close to that of creatine, but MI is centered at around 0.6 ppm (25). Therefore, only creatine and MI are close within the intermediate exchange range. We reasoned that by considering the physiological concentrations of all metabolites as well as pH in brain, and by tuning the pass band of the exchange-rate filter to select creatine, a novel method that is relatively more specific for creatine imaging would be possible using CERT.

Numerical Simulation

To study exchange rate filter effects, MTR_{asym} , $\text{MTR}_{\text{double}}$, $\text{MTR}_{\text{double, cpw}}$, and $\text{AREX}_{\text{double, cpw}}$ were calculated as a function of the exchange rate (k_{sw}) by simulating a two-pool model (solute and water pool) with a solute offset of around 2 ppm (to match creatine) at 9.4 T. Simulation parameters included: $T_{1s} = 1.5 \text{ s}$, $T_{2s} = 15 \text{ ms}$, $T_{1w} = 1.5 \text{ s}$, and $T_{2w} = 60 \text{ ms}$, where the “s” and “w” subscripts correspond to solute and water, respectively; solute concentration (relative to water) was set to 0.001. These simulation parameters were chosen from a literature survey (8,26).

The simulation contains six coupled Bloch equations which can be written as

$\frac{d\vec{M}}{dt} = \mathbf{A}\vec{M} + \vec{C}$, where $\mathbf{A}$ is a 6×6 matrix (27). The water pool and solute pool each have three coupled equations representing the x, y, and z components of their magnetizations. Numerical estimates of the pulsed-CEST signals were obtained by integrating the differential equations through the pulse sequences (with sequence parameters matching the experimental work below) using the ordinary differential equation (ODE) solver in MATLAB (Mathworks, Natick, MA, USA).

Sample preparations

To test the relative sensitivities and specificities of different exchange metrics, we prepared samples containing a selection of brain metabolites and proteins. The major metabolites in the brain that can be detected by ^1H MRS at physiological concentrations are adenosine triphosphate (ATP), adenosine-5-diphosphate (ADP), aspartate (Asp), choline (Cho), creatine (Cre), gamma-aminobutyric acid (GABA), glutamine (Gln), glutamate (Glu), glucose (Glc), myo-inositol (MI), *N*-acetyl aspartate (NAA), phosphocreatine (PCr), and taurine (Tau) (2). These metabolites were included in our study, with the exceptions of Cho, which does not have any amine exchangeable protons, and ADP, which is at very low concentration in the brain. We also included two types of proteins, bovine serum albumin (BSA) and egg white albumen (EWA). In total, 13 solutions were prepared in $1 \times$ phosphate buffered saline (PBS) titrated to pH 7.0 and containing brain metabolites or proteins at their physiological concentrations, specifically 3 mM ATP, 2mM Asp, 6 mM Cre, 2 mM GABA, 2mM Gln, 10 mM Glu, 3 mM Glc, 10 mM MI, 10 mM NAA, 5 mM PCr, 2mM Tau, 10% (w/w) BSA, and 10% (w/w) EWA.

In order to test the creatine specificity of $\text{MTR}_{\text{double,cpw}}$ and $\text{AREX}_{\text{double,cpw}}$ under varying T_{1w} and semi-solid MT conditions, solutions containing 20 mM creatine in PBS were prepared with 0.05 mM or 0.1 mM MnCl_2 , and 1% or 2% agarose. The pH of MnCl_2 solutions was titrated to 7.0 at room temperature. The agarose solutions were heated by microwave and immersed in a water bath at 45 °C. Creatine was then added to the agarose solutions and pH was titrated to 7.0 at 45 °C. In order to test the creatine sensitivity of $\text{AREX}_{\text{double,cpw}}$, solutions containing 5 mM, 10 mM, and 15 mM creatine were prepared in PBS and pH was titrated to 7.0 at room temperature.

Animal preparations

Three rats bearing 9L tumors were studied. To induce brain tumors, each rat was injected with 1×10^5 9L glioblastoma cells in the right hemisphere, and was then imaged after 2 to 3 weeks. All rats were immobilized and anesthetized with a 2%/98% isoflurane/oxygen mixture during data acquisition. Respiration was monitored to be stable, and a constant rectal temperature of 37°C was maintained throughout the experiments using a warm-air feedback system (SA Instruments, Stony Brook, NY, USA). All animal experiments were approved by the Animal Care and Usage Committee of Vanderbilt University.

MRI and data analysis

All measurements on samples and animals were performed on a Varian DirectDrive™ horizontal 9.4T magnet with a 38-mm Litz RF coil (Doty Scientific Inc. Columbia, SC) at 37°C. CW-CEST Z-spectra with irradiation power (B_1) of 1 μ T were acquired with RF offsets from –4000 Hz to –2500 Hz with a step size of 500 Hz (–10 ppm to –6.25 ppm with a step size of 1.25 ppm at 9.4 T), –2000 Hz to 2000 Hz with a step size of 50 Hz (–5 ppm to 5 ppm with a step size of 0.125 ppm at 9.4 T), and 2500 Hz to 4000 Hz with a step size of 500 Hz (6.25 ppm to 10 ppm with a step size of 1.25 ppm at 9.4 T). Pulsed-CEST Z-spectra with average irradiation power ($B_{\text{avg, power}}$) of 1 μ T were acquired with RF offsets from –2000 Hz to 2000 Hz (–5 ppm to 5 ppm at 9.4 T) with an interval of 50 Hz (0.125 ppm at 9.4 T). A control scan was performed to measure S_0 by setting the RF offset to 100 kHz. For Z-spectra studies on solutions, the first points of the free induction decay (FID) were used for data analyses. For Z-spectra from rats *in vivo* and control samples, a single-shot SE-EPI imaging sequence was employed using a FOV of $30 \times 30 \text{ mm}^2$, a slice thickness of 2 mm, a matrix of 64×64 , and a receiver bandwidth of 250 kHz.

For CERT $\text{MTR}_{\text{double, dc}}$, dc was 50 %, pulse duration and pulse repetition time were 10.8 ms and 21.6 ms for π pulses, and 21.6 ms and 43.2 ms for 2π pulses, respectively. For CERT $\text{MTR}_{\text{double, cpw}}$ and $\text{AREX}_{\text{double, cpw}}$, dc, pulse duration, and pulse repetition time were 72%, 13 ms, and 18.1 ms for π pulses, and 18%, 13 ms and 72.2 ms for 2π pulses, respectively. The total irradiation duration was 5 s for animals and 8 s for samples, performed before acquisitions, with a 2s delay time before the next experiments. $R_{1\text{obs}}$ for animal experiments was obtained using selective inversion recovery (SIR) (28). $R_{1\text{obs}}$ for samples was obtained using an inversion recovery fast spin-echo (FSE) sequence.

MTR_{asym} was calculated using π irradiation Z-spectra with dc of 72%. MTR_{fit} was calculated by fitting the CW-CEST Z-spectra. In the present work, we used a five-pool (amide at 3.5 ppm, amine at 2 ppm, water, rNOE saturation transfer at –3.5 ppm, and semi-solid MT) Lorentzian fitting to process the CW Z-spectra. The fitting was performed voxel by voxel with a non-linear optimization algorithm to achieve the lowest root mean square of residuals (RMSR) between the data and model. All images were smoothed by a 3×3 median filter before fitting. Table 2 lists the starting points and boundaries of the fit.

The vendor (Varian) provided PRESS sequence with VAPOR water suppression was used to acquire high resolution ^1H spectra with the following parameters: spectral width = 2.5 kHz, number of points = 4096, TE1 = 8 ms, TE2 = 7 ms, TR = 3 s for samples and 2 s for animals, and number of acquisitions = 8 for phantoms and 200 for animals. To determine the relative creatine sensitivity of CERT $\text{AREX}_{\text{double, cpw}}$ and ^1H MRS, CERT irradiations were followed by a PRESS acquisition using the same sequence parameters as in the MRS experiments, but without water suppression and with only 1 acquisition (making the total acquisition time 30 s in both cases).

RESULTS

Exchange filter effect of CEST and CERT metrics

Figure 1 shows that the shape of the $\text{AREX}_{\text{double, cpw}}$ vs. k_{sw} curve obtained from simulations roughly matches that of $\text{MTR}_{\text{double, cpw}}$, which is not surprising given that they are functions of the same signal measurements, $S(\pi, dc_1)$ and $S(2\pi, dc_2)$. Figure 1 also shows there is a trade-off between sensitivity and exchange rate selectivity: the signal size and W decrease in parallel, going from MTR_{asym} ($W \approx 1350 \text{ s}^{-1}$) to $\text{MTR}_{\text{double, cpw}}$ ($W \approx 540 \text{ s}^{-1}$) to $\text{MTR}_{\text{double}}$ ($W \approx 260 \text{ s}^{-1}$). W for these curves is defined as the difference between two points with values half of the maximum value. $\text{MTR}_{\text{double, cpw}}$ (and $\text{AREX}_{\text{double, cpw}}$) provide a good compromise of sensitivity to creatine ($k_{\text{sw}} \approx 500 - 950 \text{ s}^{-1}$) and filtering of fast exchanging metabolites ($k_{\text{sw}} \approx 2000 \text{ s}^{-1}$). The ratios between CEST effects with k_{sw} of 2000 s^{-1} and those with 700 s^{-1} are 49%, 26%, 19%, and 18% for MTR_{asym} , $\text{MTR}_{\text{double}}$, $\text{MTR}_{\text{double, cpw}}$, and $\text{AREX}_{\text{double, cpw}}$ metrics, respectively. The current CERT approach can not filter out slow exchanging pools. However, in biological tissues, only amide protons at 3.5 ppm and rNOE effects at negative offsets are in the slow exchanging regime (1,14,29), both of which are far from the intermediate exchanging amines at 2 ppm. Furthermore, slow exchanging pools have corresponding small exchange-rate broadening of their peaks (27). Thus CEST effects from slow exchanging pools do not influence the quantification of intermediate exchanging amines at 2 ppm. In addition, rNOE at -3.5 ppm is likely further damped by its shorter transverse relaxation rate.

Isolating creatine using the CERT intermediate exchange filter effect

Figures 2a – 2d show MTR_{asym} , $\text{MTR}_{\text{double}}$, $\text{MTR}_{\text{double, cpw}}$, and $\text{AREX}_{\text{double, cpw}}$ spectra from samples consisting of different metabolite solutions at physiological conditions (pH 7 and 37°C). Figure 2a shows that although creatine is the largest MTR_{asym} contribution at around 2 ppm, glutamate still contributes roughly 40% of creatine's signal. Figure 2b shows that the creatine signal is removed in the CERT $\text{MTR}_{\text{double}}$ spectra. The isolation of the PCr peak at around 2.5 ppm is noteworthy and will be the subject of a separate investigation. Figure 2c shows that glutamate contributes roughly 20% of creatine's $\text{MTR}_{\text{double, cpw}}$ signal at around 2 ppm, indicating that the exchange filter effect of CERT $\text{MTR}_{\text{double, cpw}}$ can reduce the influence of glutamate. Myo-inositol has a similar exchange rate as creatine and therefore might be expected to contribute equally to the signal. However, its chemical shift at around 0.6 ppm is far from creatine and does not contribute much at around 2 ppm. Also note that PCr has relatively weak contribution at around 2 ppm (around 30% of signal from Cr using the $\text{MTR}_{\text{double, cpw}}$ metric). This diminished PCr contribution indicates a greater specificity for creatine compared to MRS, which measures total creatine = creatine + PCr. Figure 2d shows the CERT $\text{AREX}_{\text{double, cpw}}$ spectra. Note that apart from a scaling factor, the $\text{AREX}_{\text{double, cpw}}$ spectra matches those of $\text{MTR}_{\text{double, cpw}}$. This scaling factor can increase the specificity for creatine when there are relaxation rate changes, as measured in the next section.

Figures 3a – 3d show MTR_{asym} , $\text{MTR}_{\text{double}}$, $\text{MTR}_{\text{double, cpw}}$, and $\text{AREX}_{\text{double, cpw}}$ spectra from protein samples at physiological conditions (pH 7 and 37°C). There are four relevant sources of signal in Figure 3: slow-exchanging amides at ~ 3.5 ppm, intermediate-

exchanging amines at ~2 ppm, a fast-exchanging site (which may be from fast exchanging protein lysine amine protons (30,31)) that causes very broad contributions between ~0.5 and 5 ppm, and possibly hydroxyls at ~1 ppm. This last peak will be ignored in our analysis, since (due to the known rotation artifacts (18,32) that dominate the CEST metrics in Figures 3b, c, and d at low offsets) the possible hydroxyl peak can only be discerned in figure 3a. From figure 1, we expect the absolute magnitude of the broad fast-exchanging component to decrease as we go from MTR_{asym} , to $MTR_{\text{double,cpw}}$ to MTR_{double} , which appears to be the case in Figure 3, especially for EWA. Likewise, from Figure 1, we expect the ratio of the peak heights from intermediate-exchanging amines (at ~2 ppm) to those from slow-exchanging amides (at ~3.5 ppm) to also decrease as we go from MTR_{asym} , to $MTR_{\text{double,cpw}}$ to MTR_{double} , which again appears to be the case in Figure 3. In total, Figure 3 shows two key points: 1) that the measured results in proteins are consistent with the simulations in Figure 1, hence supporting the overall filtering approach underlying the proposed metric $AREX_{\text{double,cpw}}$, and 2) that proteins (in addition to creatine) may contribute to the measured signal near 2 ppm.

Specificity and sensitivity of CERT creatine imaging

Figure 4 shows the results of measurements on samples with different $T_{1\text{obs}}$ and semi-solid concentrations to evaluate the specificity of $MTR_{\text{double,cpw}}$ and $AREX_{\text{double,cpw}}$ when evaluating the peak near 2 ppm. When $T_{1\text{obs}}$ changes from 1.3 s to 2 s (Figure 4a), the $MTR_{\text{double,cpw}}$ metric changes by 33% (0.027 to 0.036) and the $AREX_{\text{double,cpw}}$ metric changes by -9% (0.023 to 0.021). When agar concentration changes from 1% to 2% (Figure 4b), the $MTR_{\text{double,cpw}}$ metric changes by -13% (0.078 to 0.068) and the $AREX_{\text{double,cpw}}$ metric changes by 3% (0.032 to 0.033). Thus, $AREX_{\text{double,cpw}}$ at around 2 ppm has a decreased dependence on both $T_{1\text{obs}}$ and the semi-solid concentration compared to $MTR_{\text{double,cpw}}$, indicating a greater specificity to changes in creatine content and exchange rate.

Figure 5a shows that $AREX_{\text{double,cpw}}$ at around 2 ppm is roughly proportional to the creatine concentration, with a small offset. To compare the creatine sensitivity of $AREX_{\text{double,cpw}}$ and conventional ^1H MRS, we acquired ^1H resonance spectra using a PRESS readout after saturating at around 2 ppm with irradiation flip angles of π and 2π (Figure 5b), as well as a conventional ^1H MRS PRESS water suppressed spectrum (Figure 5c) on a 15 mM creatine sample. Measurements of the signal-to-noise ratios (SNR) show that $AREX_{\text{double,cpw}}$ has a ~52-fold sensitivity advantage over conventional ^1H MRS at physiological concentrations and temperature. (The SNR was calculated with signal obtained from the maximum value of the peak and the noise from the standard deviation of a part of spectrum where there are no peaks.)

CEST and CERT imaging in tumors

Figure 6 shows the CEST and CERT spectra of rat brains bearing tumors. Note that MTR_{asym} at 2 ppm from contralateral normal tissue in Figure 6c is negative because of the rNOE saturation transfer effect on the opposite side of the water peak. Both MTR_{asym} at 2 ppm in Figure 6c and MTR_{fit} (from the five pool Lorentzian fit) at 2 ppm in Figure 6d have higher values in tumors than in the contralateral normal tissue. Figure 6e shows that the

contrast for $MTR_{\text{double, cpw}}$ at 2 ppm between tumor and normal tissues is diminished, while Figure 6f shows that for $AREX_{\text{double, cpw}}$ it is reversed. This indication by $AREX_{\text{double, cpw}}$ of lower creatine content in tumors is consistent with previous ^1H MRS measurements in animal tumor models (33), and confirmed by MRS in this study (Figure 7). (In addition to the peak at 2 ppm discussed above, Figures 6e and 6f show a peak at 3.5 ppm, due to APT effects, and a peak between 0 and 1 ppm, due to an artifact from water rotation effects (18,32)). These peaks likely do not affect the quantification of the peak at 2 ppm, as discussed above. Likewise, the rNOE peak at -3.5 ppm (not shown) is too far removed from 2 ppm and too slowly exchanging to contribute to the 2 ppm peak. Figure 8 shows an example image of $AREX_{\text{double, cpw}}$ at 2 ppm in which tumor is hypointense.

DISCUSSION

Separating intermediate exchanging amines from neighboring fast exchanging pools can increase the specificity of CEST imaging. However, fast exchanging pools have broad peaks when using conventional CEST acquisitions, and hence can not be well isolated by their resonance frequencies. In this work, we did not address the specific methods used previously to image creatine, as they vary significantly, e.g. references 1 to 4 use saturation pulses with durations between 250 ms and 3 s and powers between $1.2 \mu\text{T}$ and $3.6 \mu\text{T}$, and use both MTR_{asym} and Lorentzian line fitting metrics. We instead have compared the underlying exchange rate selectivity of CEST and CERT approaches. Furthermore, we developed a filter to select solutes with intermediate exchange rates using our previously described CERT approach. Measurements show that this technique can isolate creatine from other brain metabolites and thereby increase the specificity of CEST creatine imaging.

In our previous CERT works (18,32), we varied the pulse angle while maintaining constant integrated power and dc (at 50%). Here, we maintained constant integrated power and pw. The two primary benefits are a rough doubling of peak signal (as seen in the peak heights in Figure 1) and a simplification of pulse bandwidth issues (since all pulses have roughly the same bandwidth when pw is constant), which becomes increasingly important near the water resonance. In this work, our goal has been to selectively measure solutes near 2 ppm at intermediate exchange rates, and hence increase specificity for creatine. The choice of constant pw is reasonable in light of this goal, as is the choice of $B_{\text{avg power}} = 1 \mu\text{T}$, but a full optimization would require consideration of how $B_{\text{avg power}}$, dc, and pw affect sensitivity, specificity, and the generation of direct water rotation artifacts using a multi-pool tissue model, and is beyond the scope of the current work. Likewise, the creatine exchange rate has a strong dependence on temperature and pH (34), and hence variations in these biophysical parameters may significantly affect the exchange rate filtering, and hence the results, of all of the methods studied in this paper.

Previous MRS measurements have shown that the concentration of creatine usually decreases in tumors. The quantity $AREX_{\text{double, cpw}}$ at 2 ppm also has a lower value in tumor. In contrast, MTR_{asym} , MTR_{fit} , and $MTR_{\text{double, cpw}}$ do not have decreased values in tumor, but these quantities may be influenced by other effects such as semi-solid MT and T_{1w} . Note that our MTR_{fit} result in tumors differs from a previous multiple-pool fit of CEST signals at

2 ppm (6), which may be due to the different experimental parameters and different non-specific contributions.

Some other molecules, e.g. protein arginine side chains, also have intermediate exchanging amines at 2 ppm, which may contaminate CEST creatine imaging (30,31). Our method does not remove contributions from such proteins. Experiments on samples in Figure 2 and 3 indicate that contributions to intermediate exchanging amine signals at 2 ppm from proteins ($\text{AREX}_{\text{double, cpw}}$ at 2 ppm are around $3.9\% \text{ s}^{-1}$ for BSA and $7.2\% \text{ s}^{-1}$ for EWA) are significantly higher than that from creatine ($\text{AREX}_{\text{double, cpw}}$ at around 2 ppm is around $0.7\% \text{ s}^{-1}$). However, since our chosen proteins are two specific types that may contain high proportion of arginine side chains, the relative contribution from proteins and creatine *in vivo* may be significantly different from our sample studies. In any case, compared with several previous ways of quantifying CEST, our method significantly increases the specificity of CEST creatine imaging by removing contributions from neighboring fast exchanging sites. At the least, our method can be used to assess situations where no significant change in the protein concentration is expected.

In contrast with ^1H MRS, which measures total creatine (creatine plus phosphocreatine), the proposed CERT method is weighted towards creatine, which may provide a way to study the creatine kinase (CK) reaction. Our studies of samples also show that the proposed CERT metric has a much larger SNR compared to ^1H MRS.

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Abbreviations

CEST	chemical exchange saturation transfer
CERT	chemical exchange rotation transfer
MT	magnetization transfer
MTR	magnetization transfer ratio
AREX	apparent exchange-dependent relaxation
CW	continuous wave
rNOE	relayed nuclear overhauser enhancement
DS	direct water saturation
dc	duty cycle
pw	pulse width
W	peak full width at half maximum

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Fig. 1. Simulated MTR_{asym} , MTR_{double} , $MTR_{double,cpw}$, and $AREX_{double,cpw}$ at around 2 ppm vs. k_{sw} . Simulations were performed with a 2-pool model (solute and water pool) and B_{avg} power = 1 μ T. $AREX_{double,cpw}$ has different unit than the other metrics, and has been arbitrarily scaled in this figure to allow for easy comparison to $MTR_{double,cpw}$.

FIG. 2.

Experimental MTR_{asym} (a), MTR_{double} (b), MTR_{double,cpw} (c), and AREX_{double,cpw} (d) spectra from solutions containing the main metabolites in brain, irradiated with B_{avg} power = 1 μT at 9.4 T. T_{obs} were measured to be roughly 4 s for these samples.

FIG. 3.

Experimental MTR_{asym} (a), MTR_{double} (b), $MTR_{double,cpw}$ (c), and $AREX_{double,cpw}$ (d) spectra from solutions containing BSA and EWA, irradiated with B_{avg} power = 1 μ T at 9.4 T. T_{1obs} was measured to be roughly 2.2 s for these samples.

FIG. 4.

MTR_{double,cpw} and AREX_{double,cpw} at around 2 ppm on samples with different T_{1obs} (a) and agar concentration (b). T_{1obs} was measured to be roughly 4 s for the agar samples.

FIG. 5.

a) Linear dependence of $\text{AREX}_{\text{double,cpw}}$ on creatine concentration (5 mM, 10 mM, and 15 mM). b) The $\text{AREX}_{\text{double,cpw}}$ creatine signal at around 2 ppm (the difference of MRS spectra acquired after irradiation with 2π and π pulse trains (MRS)). Note that the peak is from water at 4.7 ppm, but gives creatine information via exchange. c) ^1H MRS point-resolved spectroscopy (PRESS) water-suppressed spectrum, isolating direct measurement of the creatine signal along with a residual water signal at 4.7 ppm. Both $\text{AREX}_{\text{double,cpw}}$ and ^1H MRS experiments were performed on the same creatine sample (with concentration

of 15 mM) and have roughly the same scan time of 30 s and receiver gain of 2 dB. The SNR of the AREX_{double,cpw} creatine signal at 4.7 ppm in (b) is roughly 52 times that of the ¹H MRS signal at 3.2 ppm in (c). AU, arbitrary unit.

FIG. 6. Experimental CW-CEST (a), pulsed-CEST (b), MTR_{asym} (c), MTR_{fit} (d), $MTR_{double,cpw}$ (e), and $AREX_{double,cpw}$ (f) spectra from tumors and contralateral normal tissues in rat brains. Error bars are standard errors of the mean.

FIG. 7.
MRS spectra from tumor and contralateral normal tissue in one rat brain.

FIG. 8. Anatomy (T_{2w} weighted) (a), R_{1obs} (b), and $AREX_{double,cpw}$ at 2 ppm (c) images from a representative rat brain bearing tumor.

Table 1

List of solute resonance frequency offsets (δ_0), exchange rates (k_{sw}), and concentration (f_s) of some molecules in brain.

Metabolite	δ_0 (ppm) / Hz at 9.4 T	k_{sw} (s ⁻¹)	f_s (mM)
MI ^a	0.6 / 240	600	10
Cr ^{b,c}	1.9 / 760	500 – 950	6
ATP ^c	2.0 / 800	120	3
PCr ^c	2.5 / 1000, 1.8 / 720	140, 120	5
GABA ^b	2.8 / 1120	2000	2
Glu ^b	3.0 / 1200	2000	10
Amide ^d	3.5 / 1400	30	100

^a _Ref (25)

^b _Ref (2)

^c _Ref (23)

^d _Ref (1,35)

Table 2

Starting points and boundaries of the amplitude, width, and offset of the five-pool Lorentzian fit. The unit of peak width and offset is ppm.

	Start	Lower	Upper
A_{water}	0.9	0.02	1
W_{water}	1.4	0.3	10
water	0	-1	1
A_{amide}	0.025	0	0.2
W_{amide}	0.5	0.4	3
amide	3.5	3	4
A_{amine}	0.01	0	0.2
W_{amine}	1.5	0.5	5
amine	2	1.75	2.25
$A_{\text{NOE}(-3.5)}$	0.02	0	1
$W_{\text{NOE}(-3.5)}$	3	1	5
NOE(-3.5)	-3.5	-4.5	2
A_{MT}	0.1	0	1
W_{MT}	25	10	100
MT	0	-4	4