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et al.

2018-04-06

10.1002/mrm.27204

Peer reviewed
Simultaneous pH-sensitive and oxygen-sensitive MRI of human gliomas at 3 T using multi-echo amine proton chemical exchange saturation transfer spin-and-gradient echo echo-planar imaging (CEST-SAGE-EPI)

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Funding information
American Cancer Society Research Scholar Grant (RSG-15-003-01-CCE) (B. M.E.); Art of the Brain (T.F.C.); University of California Research Coordinating Committee (B.M.E.); UCLA Jonsson Comprehensive Cancer Center Seed Grant (B.M.E.); UCLA SPORE in Brain Cancer (NIH/NCI)

Purpose: To introduce a new pH-sensitive and oxygen-sensitive MRI technique using amine proton CEST echo spin-and-gradient echo (SAGE) EPI (CEST-SAGE-EPI).

Methods: pH-weighting was obtained using CEST estimations of magnetization transfer ratio asymmetry (MTRsym) at 3 ppm, and oxygen-weighting was obtained using $R_2^*$ measurements. Glutamine concentration, pH, and relaxation rates were varied in phantoms to validate simulations and estimate relaxation rates. The values of MTRsym and $R_2^*$ in normal-appearing white matter, $T_2$ hyperintensity, contrast enhancement, and macroscopic necrosis were measured in 47 gliomas.

Results: Simulation and phantom results confirmed an increase in MTRsym with decreasing pH. The CEST-SAGE-EPI estimates of $R_2$, $R_2^*$, and $R_2^{**}$ varied linearly with gadolinium diethylenetriamine penta-acetic acid concentration ($R_2 = 6.2 \text{mM}^{-1}\text{sec}^{-1}$ and $R_2^{**} = 6.9 \text{mM}^{-1}\text{sec}^{-1}$). The CEST-SAGE-EPI and Carr-Purcell-Meiboom-Gill estimates of $R_2$ ($R_2 = 0.9943$) and multi-echo gradient-echo estimates of $R_2^{**}$ ($R_2^{**} = 0.9727$) were highly correlated. $T_2$ lesions had lower $R_2^{**}$ and higher MTRsym compared with normal-appearing white matter, suggesting lower hypoxia and acidity, whereas contrast-enhancement tumor regions had elevated $R_2^{**}$ and MTRsym, indicating high hypoxia and acidity.

Robert J. Harris and Jingwen Yao contributed equally to this work.
Abnormal metabolism is a hallmark of cancer. Notably, glycolysis is often enhanced in cancers, even in the presence of abundant oxygen (i.e., the Warburg effect) (Figure 1). This form of aerobic glycolysis results in a dramatic decrease in extracellular pH due to increased concentration of lactic acid. The increase in extracellular acidity comes with dramatic consequences, as it can be directly linked to the degree of tumor aggressiveness and increases tumor invasion. Interestingly, histological evidence suggests regions containing pseudopalisades, a pathological trademark of glioblastoma, are also hypoxic, express extracellular matrix proteases, and are the result of active tumor migration. Increased acidity within the tumor also has been shown to lead to decreased immune function. The acidic micro-environment in tumors is also conducive to elevated vascular endothelial growth factor expression and expression of platelet-derived endothelial cell growth factor, which has been shown to result in increased angiogenesis. This in turn leads to a positive feedback process, leading to further tumor growth, decreasing oxygen tension, increased hypoxia, and increasing glycolysis, resulting in increased lactic acid, decreasing extracellular tissue pH, and more mutations and/or adaptations of the tumor genome. Thus, extensive in vitro, preclinical, and clinical evidence appears to support the hypothesis that tumor acidity and oxygen metabolism both play a critical role in gliomagenesis. There remains, however, a critical need in furthering our understanding of the role of extracellular acidosis and oxygen metabolism in human gliomas and its clinical relevance due to the lack of a robust noninvasive tool for simultaneously estimating and localizing regions of low pH and oxygen consumption. Thus, the purpose of the study was to develop a new, noninvasive MRI technique that can provide high-resolution images with sensitivity to tumor acidity and oxygen metabolism that can be performed on clinical MRI systems.

1.1 pH-weighted metabolic MRI using CEST contrast from fast exchangeable amine protons on glutamine

In addition to glucose, glutamine is also a major source of fuel for malignant tumors. It is essential for cellular proliferation, tumor growth, and tumor cell survival. Glutamine is the most abundant amino acid in the body, circulating at concentrations of 0.6 to 0.9 mM and as high as 20 mM in tissue. Tumor cells often consume a significant amount of glutamine, acting at times like a “glutamine trap.” Glutamine demand is so high that transport systems are amplified to increase glutamate consumption. Glutamine has 2 nitrogen functional groups, an amine and an amide group, having hydrogen NMR resonance frequencies of 3.0 and 3.5 ppm, respectively, compared with water protons. The chemical exchange between amine and amide protons in bulk water has been shown to be pH dependent using a new imaging method called CEST imaging. The inherently elevated concentration of glutamine within tumors further increases the available proton exchange, resulting in a higher CEST signal at 3.0 ppm. The combination of increased protons (low pH) and increased glutamine makes pH-weighted MRI using CEST contrast from amine protons on glutamine particularly attractive as a noninvasive tool for assessment of microenvironment acidity within malignant brain tumors.

1.2 R2-based BOLD imaging

The level of glycolysis depends on both the accumulation of lactic acid and the inefficient use of oxygen. Thus, image maps that are sensitive to tumor oxygen metabolism are critically important for understanding the relative level of aerobic (pathologic) versus anaerobic (normal) glycolysis. Blood oxygen level–dependent imaging, based on the contrast mechanisms routinely used for functional imaging (fMRI), allows for noninvasive estimation of blood or tissue oxygenation. Simply stated, oxygenated blood containing oxyhemoglobin is diamagnetic, as the iron at the core is magnetically shielded from blood water, resulting in coherent MR spins and a high MR signal. Early studies from Ogawa et al confirmed this in mice breathing high concentrations of oxygen. In deoxygenated blood, the iron is exposed to blood water, resulting in magnetic interference with the proton magnetic moment on water molecules. This interference results in incoherent MR spins and signal dropout in the areas of high deoxyhemoglobin concentration. The paramagnetic nature of deoxyhemoglobin enhances the effective transverse relaxation rate R2. Changes in tissue R2 therefore reflect relative...
changes in concentration of deoxyhemoglobin, and thus an indirect measure of oxygen extraction fraction (OEF). Use of the reversible transverse relaxation rate, $R_2' = R_2 - R_3$, both isolates the local susceptibility effect while compensating for $R_3$ changes from factors such as water content variation. The sensitivity of $R_2'$ to concentration of deoxyhemoglobin has been reported by several studies and has been shown to correlate with the hypoxic state of tissue. Although $R_2'$ does not allow for a direct measurement of OEF, previous studies have demonstrated that OEF is proportional to $R_2'$, after normalization to relative blood volume fraction and static dephasing, which is expected for protons in blood at the specific magnetic field strength. Additionally, this approach has been used previously to explore oxygen metabolism in brain tumors as well as stroke. However, although higher measures of $R_2'$ may suggest higher concentration of deoxyhemoglobin, OEF, and/or local hypoxia, many other biological and/or technical influences (e.g., $B_1$ homogeneity) may influence this measurement.

In the current study, we introduce a new technique for obtaining fast, whole-brain, noninvasive, high-resolution pH-sensitive and oxygen-sensitive MR imaging contrast using multi-echo amine proton CEST echo spin-and-gradient echo (SAGE) EPI on a clinical 3T MRI system. pH-weighted image sensitivity was obtained through quantification of the z-spectral asymmetry in the magnetic transfer ratio (MTR) after selective saturation of the longitudinal magnetization of amine protons on glutamine at 3.0 ppm (MTR$_{asym}$ at 3.0 ppm), whereas oxygen sensitivity was obtained through quantification of $R_2'$ using a multi-echo SAGE-EPI readout to quantify relaxation rates $R_2$ and $R_2'$. Sensitivity and accuracy of this approach were confirmed using custom phantoms containing both gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) and glutamine and different concentrations and pH. Finally, both MTR$_{asym}$ at 3.0 ppm and $R_2'$ for tumor and normal tissue were characterized in patients harboring diffuse or malignant gliomas.

2 | THEORY

2.1 | pH-sensitive and oxygen-sensitive MR contrast using multi-echo amine proton CEST-SAGE-EPI

The magnetization of bulk water protons undergoing chemical exchange with amine protons can be described by the Bloch-McConnell equations in the form of

$$\frac{dM(t)}{dt} = X \cdot M(t) - c,$$

where

$$M(t) = e^{X t} \cdot M(0)$$

and

$$M(t) = e^{X t} \cdot M(0)/(X/c) - (X/c),$$

where $M_{ac}(t)$ represents the longitudinal magnetization of bulk water available for subsequent readout after CEST effects. Assuming the spoiler duration and water excitation pulse duration are negligible, $M_{ac}(t)$ immediately following excitation reflects the available longitudinal magnetization for subsequent readout. The chemical exchange between amine protons and bulk water protons can be characterized as a base-catalyzed process, governed by pH as follows:

$$k_b = k_0 + k_{base} \cdot 10^{-(14-\text{pH})},$$

where $k_0$ is the default exchange rate, $k_{base}$ is the base-catalyzed rate constant, and $k_b$ is the exchange rate of protons from the metabolite proton pool to the water pool.
Magnetic Resonance in Medicine

Described by the MTR given by bulk water magnetization following a saturation pulse is 

\[ MTR(\omega) = \frac{S(\omega)}{S_0} \]  

where \( S(\omega) \) is the amount of bulk water signal available after the saturation pulse with frequency \( \omega \), and \( S_0 \) is the signal available without application of RF saturation. Because MTR can be affected by symmetric effects of direct water saturation and conventional magnetization transfer effects, CEST contrast is often described by the asymmetry in the magnetization transfer ratio \( \text{MTR}_{\text{asym}} \) with respect to water proton resonance, as follows:

\[ \text{MTR}_{\text{asym}}(\omega) = \frac{S(-\omega) - S(\omega)}{S_0}. \]  \hspace{1cm} (6)

For amine proton CEST imaging, \( \text{MTR}_{\text{asym}} \) is evaluated at 3.0 ppm with respect to the bulk water resonance frequency.

Estimates of \( R'_2 \) were achieved through use of a SAGE-EPI readout consisting of 2 gradient echoes (TE1 and TE2), an asymmetric spin echo (TE3), and a spin-echo (TE4) EPI acquisition during a single excitation event (Figure 2). The solutions for \( R'_2 \) using the SAGE EPI were eloquently described by Schmiedeskamp et al. \( R'_2 \) was calculated as

\[ A = Y^{-1} S, \]  \hspace{1cm} (7)

where

\[ S = D \begin{bmatrix} \ln(S_1) \\ \ln(S_2) \\ \ln(S_3) \\ \ln(S_4) \end{bmatrix}, \quad Y = \begin{bmatrix} 1 & 0 & -TE_1 & 0 \\ 1 & 0 & -TE_2 & 0 \\ 1 & -1 & -TE_3 + TE_4 & T_E - 2 \cdot TE_3 \\ 1 & -1 & 0 & -TE_4 \end{bmatrix}, \quad A = \begin{bmatrix} \ln(S_0) \\ \ln(\delta) \\ R'_2 \end{bmatrix}. \]  \hspace{1cm} (8)

where \( \delta \) is the differences in residual signal differences caused by imperfectly matched slice profiles between echo trains before and after the refocusing pulse. \( R'_2 \) is calculated as

\[ R'_2 = R'_2 - R_2. \]  \hspace{1cm} (9)

3 | METHODS

3.1 | Amine proton CEST-SAGE-EPI

Simultaneous acquisition of pH-sensitive information and relaxometry measures of \( R'_2 \) were performed through modification of a previously described CEST-EPI sequence to include a SAGE-EPI readout (Figure 2). The SAGE-EPI readout consisted of 2 gradient echoes (TE1 = 14.0 ms; TE2 = 34.1 ms), an asymmetric spin echo (TE3 = 58.0 ms), and a spin echo (TE4 = 92.4 ms). All phantom and human CEST-SAGE-EPI data were acquired with a CEST saturation pulse train consisting of 3 (3×3) 100-ms Gaussian pulses with amplitude \( B_1 = 6 \mu T \), TR > 10 000 ms, FOV = 240 × 217, matrix size = 128 × 104, partial Fourier encoding = 6/8, GRAPPA = 3, bandwidth = 1630 Hz/pixel, and 25 contiguous slices with a 4-mm slice thickness. A total of 29 1 spectral points were acquired with data around ±3.0 ppm and 0.0 ppm with respect to water (from −3.5 to −2.5 in intervals of 0.1; from −0.3 to + 0.3 in intervals of 0.1; and from + 2.5 to + 3.5 in intervals of 0.1). An additional reference \( S_0 \) scan with identical parameters and no saturation pulse was acquired with number of excitations or averages = 4. The total acquisition time for CEST-SAGE-EPI was 7 minutes and 30 seconds, benchmarked on a 3T Siemens Prisma MR scanner (Software Versions VE11A-C; Siemens Healthcare, Erlangen, Germany).

3.2 | Phantom testing

To demonstrate the ability to simultaneously acquire pH-sensitive information along with relaxometry measures of \( R_2, R'_2, \) and \( R'_2 \), we performed CEST-SAGE-EPI, multi-echo gradient-echo (ME-GRE), and multi-echo spin-echo (Carr-Purcell-Meiboom-Gill [CPMG]) MR acquisition in a series of 36 glutamine phantoms (100 mM) with combinations of varying pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5) and gadopentetic acid (Gd-DTPA; Magnevist, Bayer HealthCare Pharmaceuticals, Berlin, Germany) concentration (0, 0.25, 0.5, 1.0, 1.5, and 2.0mM) in 50-mL falcon tubes. The 100-mM glutamine solution was prepared first using distilled water. The pH in each phantom was titrated using dilute acid (0.1N HCl) and base (0.1N NaOH) solution. The desired Gd-DTPA was then added to the phantom solution and vortexed. All samples were vortexed and pH was re-evaluated prior to MRI acquisition. The ME-GRE sequence used for \( R'_2 \) quantitation was...
collected with TE = 20/40/60/80 ms, TR = 10 000 ms, flip angle = 90°, FOV = 217 × 240 mm, matrix size = 116 × 128, slice thickness = 4 mm, and pixel bandwidth = 260 Hz. The CPMG sequence used for R2 quantitation was performed with TE = 49/98/147/196/245/294/343/392 ms, TR = 10 000 ms, flip angle = 90°, FOV = 217 × 240 mm, matrix size = 116 × 128, slice thickness = 4 mm, and pixel bandwidth = 260 Hz. Both the ME-GRE and CPMG sequences were repeated to improve SNR. All phantom experiments were physically repeated twice to ensure repeatability and compared with theoretical values using Bloch-McConnell simulations. Contrast-to-noise ratio (CNR) of MTR asym at 3 ppm between any 2 samples of differing pH a and b was calculated as

\[
\text{CNR} = \frac{|\mu_a - \mu_b|}{\left(\sigma_a^2 + \sigma_b^2\right)/2},
\]

### 3.3 Patients

A total of 47 histologically proven glioma patients (World Health Organization [WHO] IV, n = 20; WHO III, n = 14; WHO II, n = 13) were enrolled in the current study prior to initial surgical resection or at first recurrence. All patients provided informed, written consent to have advanced imaging, and this information included in our internal review board–approved research database. Patient characteristics are outlined in Table 1. In addition to CEST-SAGE-EPI prior to

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**FIGURE 1** The Warburg effect. A, Within normal tissues in the presence of oxygen, glucose is converted to pyruvate and then used for oxidative phosphorylation within the mitochondria. B, In normal tissues within a hypoxic environment, glucose is converted to pyruvate, then to lactate or lactic acid, decreasing extracellular pH. C, In cancer cells, glucose is converted to pyruvate then to lactate and lactic acid (80-85%) and a small portion of pyruvate enters the citric acid cycle (5-15%), regardless of whether oxygen is present. This altered metabolism, also known as the Warburg effect or aerobic glycolysis, results in increased extracellular acidity (lower pH) even when tumor tissue is well perfused.

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**FIGURE 2** Pulse sequence diagram for a multi-echo amine CEST spin-and-gradient echo (SAGE EPI (CEST-SAGE-EPI) sequence. After nonselective off-resonance CEST excitation consisting of 3 100-ms Gaussian pulses, a spectral-spatial water-only excitation pulse is invoked. Following excitation, 2 gradient-echo EPI trains are acquired (echoes 1 and 2), followed by a 180° refocusing pulse. Finally, an asymmetric spin-echo EPI train (echo 3) and a standard spin-echo EPI train (echo 4) are acquired. Partial Fourier k-space acquisition strategies were used to further reduce TEs (e.g., TE1/TE2/TE3/TE4 = 14.0/34.1/58.0/92.4 ms for the current study).
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(Continues)
contrast administration, all patients received the anatomic images according to the standardized brain tumor imaging protocol,\textsuperscript{32} including T2-weighted fluid-attenuated inversion recovery (FLAIR) images, T2-weighted turbo spin-echo images, and diffusion-weighted images with 3-mm slice thickness and no interslice gap, along with parameter-matched, 1-mm isotropic 3D T1-weighted MPRAGE scans before and following injection of 0.01 mg/kg Gd-DTPA.

### 3.4 Postprocessing

Clinical postprocessing of CEST-SAGE-EPI consisted of affine motion correction (mcflirt; FSL, FMRIB, Oxford, United Kingdom) and B0 correction via the WASSR (water saturation shift referencing) method,\textsuperscript{33} and/or creating B0 maps using phase information from the 2 acquired gradient echoes (Supporting Information Figure S1). An integral of width of 0.4 ppm was then taken around both the 2.3 ppm and 1.3 ppm (2.3 to 2.8 and 1.8 to 3.2, respectively) spectral points of the inhomogeneity-corrected data. These data points were combined with the S0 image to calculate MTR asym at 3.0 ppm as defined by Equation 5. Estimates of T1, T2, R2, R2', and R2'' from ME-GRE or CPMG were obtained by performing a mono-exponential fit to the gradient and spin echoes, respectively, whereas estimates of the same parameters were obtained using Equation 8. Maps of R2' were then calculated as

\[
R_2' = R_2 - R_2 = \frac{1}{T_2} - \frac{1}{T_2} \quad (11)
\]

with a higher value of R2' suggesting relatively higher concentration of hemoglobin, oxygen extraction fraction, and/or hypoxia.\textsuperscript{22-26}

### 3.5 Data analysis

For all data, the average MTR asym at 3.0 ppm calculated from the first (TE = 14.0 ms) and second (TE = 34.1 ms) gradient echoes were averaged to decrease increase the SNR of the resulting MTR asym images. The 2 gradient echoes were chosen instead of all echoes due to slightly higher variability in MTR asym measurements from asymmetric spin-echo and spin-echo measurements, due to the longer TE and additional signal loss from transverse relaxation. In phantom samples, regions of interest were drawn within each sample and the mean (\(\mu\)) and SD (\(\sigma\)) of MTR asym at 3 ppm for voxels within the sample were calculated.

When evaluating glioma patients, volumes of interest were drawn within normal-appearing white matter (NAWM) contralateral to the hemisphere containing evidence of tumor on T2-weighted FLAIR images. Lesions exhibiting abnormal T2 hyperintensity on FLAIR images (“T2 lesions” volumes of interest) were manually contoured on all patients. To reduce the influence of outliers, the median and median absolute deviation of MTR asym and R2' within these regions

### Table 1 (Continued)

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Note: M, male; F, female; MUT, mutant; WT, wild type; A, astrocytoma; O, oligodendroglioma; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma.
were calculated, and the median absolute deviation was used to define variability for all measurements. A Wilcoxon signed-rank test was used to determine whether averaging MTR\textsubscript{asym} from 2 gradient echoes resulted in a decrease in healthy-tissue MTR\textsubscript{asym} variability compared with a single gradient echo. For glioblastoma patients, volumes of interest of gadolinium contrast enhancement (CE) were segmented using T\textsubscript{1} subtraction maps and a semi-automated thresholding method outlined previously.\textsuperscript{34,35} Regions of central necrosis were also delineated and examined. A paired t-test was used to determine whether R\textsubscript{2} or MTR\textsubscript{asym} differed between T\textsubscript{2}-hyperintense lesions and NAWM. Within glioblastoma patients (WHO grade IV), a one-way repeated-measures analysis of variance (ANOVA) and Tukey’s test for multiple comparisons was used to determine whether R\textsubscript{2} or MTR\textsubscript{asym} at 3 ppm differed among regions of NAWM, T\textsubscript{2} hyperintense lesions, regions of CE, and areas of central necrosis. An additional one-way ANOVA and Tukey’s test for multiple comparisons was used to compare R\textsubscript{2} or MTR\textsubscript{asym} at 3 ppm for T\textsubscript{2} lesions across glioma grades II, III, and IV.

4 | RESULTS

The value of MTR\textsubscript{asym} at 3.0 ppm within each glutamine phantom, varying by pH, was similar across all 4 echoes, showing a characteristic increase in MTR\textsubscript{asym} at 3.0 ppm with decreasing pH (Figure 3A; ANOVA, p = .999; comparison across echoes, p > .99). These experimental results closely matched the simulation results using measured and theoretical relaxation and exchange rates (Figure 3A). The CNR was higher when averaging measurements from the 2 gradient echoes (TE = 14.1 ms and 34.1 ms) compared with a single gradient echo (TE = 14.1 ms) in phantom samples containing the same concentration of glutamine, but varying pH. In particular, CNR was approximately 13% higher when comparing pH = 7.5 to 7.0 (CNR\textsubscript{1&2} = 2.68; CNR\textsubscript{1} = 2.37), 7% higher when comparing pH = 6.5 and 7.0 (CNR\textsubscript{1&2} = 5.07; CNR\textsubscript{1} = 4.74), 15% higher when comparing pH = 6.0 versus 7.0 (CNR\textsubscript{1&2} = 17.2; CNR\textsubscript{1} = 15.0), and 6.5% higher when comparing pH = 6.0 versus 6.5 (CNR\textsubscript{1&2} = 6.72; CNR\textsubscript{1} = 6.31). Consistent with phantom results, the median MTR\textsubscript{asym} at 3.0 ppm in NAWM across all patients was not significantly different when using a single echo and the average of 2 gradient echoes (p = .31); however, the median absolute deviation, a measure of variance in the measurements, was significantly lower when averaging the 2 gradient echoes (p = .003).

In phantom samples containing varying concentration of Gd-DTPA, CEST-SAGE-EPI estimates of R\textsubscript{2}, R\textsubscript{2}', and R\textsubscript{2} varied linearly with concentration (Figure 3B), with estimates of transverse relaxivities of R\textsubscript{2} = 6.24 ± 0.04 mM\textsuperscript{-1}sec\textsuperscript{-1} (p < .0001), R\textsubscript{2}' = 6.86 ± 0.10 mM\textsuperscript{-1}sec\textsuperscript{-1} (p < .0001), and R\textsubscript{2} = 0.61 ± 0.08 mM\textsuperscript{-1}sec\textsuperscript{-1} (p = .0007). Phantom results identified a strong, significant linear correlation between R\textsubscript{2} measurements using CEST-SAGE-EPI and CPMG (Figure 3C; R\textsuperscript{2} = 0.9943, p < .0001) and did not differ by pH (p = .9915). Estimates of R\textsubscript{2} using CEST-SAGE-EPI, however, were lower than estimates of R\textsubscript{2} using standard CPMG measurements (slope = 0.8845 ± 0.006, p < .0001 compared with slope = 1), with CEST-SAGE-EPI estimates approximately 760 us lower on average than CPMG measurements (~7.1%). A strong linear correlation was also observed between R\textsubscript{2} measurements obtained using CEST-SAGE-EPI and ME-GRE measurements (Figure 3D; R\textsuperscript{2} = 0.9727, p < .0001), and these measurements also did not differ by pH (p = .2184). Estimates of R\textsubscript{2}' were not significantly different from measurements obtained using standard ME-GRE measurements (slope = 0.9862 ± 0.0155, p = .3819, showing no difference between slope = 1). Consistent with these results, calculated estimates of R\textsubscript{2} obtained through subtraction of sequential CPMG and ME-GRE measurements were congruent with SAGE-EPI measurements (Figure 3E; slope = 1.26 ± 0.1301, p = .0555, showing no difference between slope = 1) and did not differ by pH (p = .0533).

Although measurements of transverse relaxation rate were not affected by pH, the relationship between MTR\textsubscript{asym} at 3.0 ppm and pH was affected by transverse relaxation rate (Figure 3F). Specifically, as transverse relaxation rates R\textsubscript{2} or R\textsubscript{2}' increased as a result of increased concentration of Gd-DTPA, the sensitivity of MTR\textsubscript{asym} at 3.0 ppm to acidic pH decreased, particularly when concentrations were higher than 1 mM, corresponding to T\textsubscript{2} measurements of 150 to 170 ms and T\textsubscript{2}' = 130 to 170 ms. Repeated experiments showed higher coefficient of variation with higher concentrations of Gd-DTPA, or lower T\textsubscript{1}, as well as higher coefficient of variation with higher pH (Figure 3G).

Qualitatively, the T\textsubscript{2} hyperintense lesions in all patients exhibited heterogeneous areas of both elevated MTR\textsubscript{asym} at 3.0 ppm (acidity) and R\textsubscript{2} (hypoxia). Many areas of nonenhancing tumor in all patients exhibited uncharacteristically low measures of R\textsubscript{2} despite evidence of elevated acidity, suggesting nonenhancing tumor regions may be adequately oxygenated and undergoing aerobic glycolysis (Figure 1C). For example, Figure 4A illustrates a WHO grade II astrocytoma with an area of moderately elevated acidity in the medial frontal lobe; however, this region exhibited R\textsubscript{2} approximately 50% lower than surrounding NAWM. Figure 4B illustrates a large nonenhancing, isocitrate dehydrogenase (IDH) mutant WHO grade III astrocytoma with sizeable regions of macroscopic necrosis, as illustrated by T\textsubscript{1} hypointensity. These areas of necrotic tissue exhibited both high levels of acidity and hypoxia, whereas the surrounding nonenhancing components with largely intact blood–brain barrier had elevated acidity, as suggested by higher MTR\textsubscript{asym} at
FIGURE 3  Simulation and phantom testing. A, The CEST contrast increases with decreasing pH within a physiological pH range similarly for all 4 echoes in an amino acid phantom and simulation estimates. These measurements closely match simulation estimates with measured or estimated relaxation and exchange rates (dashed lines). B, R2 and R2' as measured by the multi-echo sequence show a linear increase with increasing gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) concentration. Estimates of R2 = 6.24 ± 0.04 mM⁻¹.sec⁻¹, R2' = 6.86 ± 0.10 mM⁻¹.sec⁻¹, and R2' = 0.61 ± 0.08 mM⁻¹.sec⁻¹. C, Comparison between measured R2 using CEST-SAGE-EPI and Carr-Purcell-Meiboom-Gill (CPMG) show strong correlation (R² = 0.9943, p < .0001), independent of pH (p = .9915). D, Comparison between measured R2' using CEST-SAGE-EPI and multi-echo gradient echo (ME-GRE) show a strong correlation (R² = 0.9727, p < .0001) and no dependence on pH (p = 0.2184). E, Estimates of R2' obtained through subtraction of sequential CPMG and ME-GRE measurements were not significantly different from those obtained using multi-echo SAGE EPI (slope = 1.26 ± 0.1301, p = .0555) and did not differ by pH (p = 0.053). F, Amine CEST has decreased sensitivity in the presence of contrast agents (Gd-DTPA) that shorten T1 and T2. Thus, edematous tumor tissue with longer T1 and T2 have higher CEST contrast in acidic environments. G, Coefficient of variation measured from multiple test-retest experiments is also higher in the presence of contrast agents or in environments with shorter T1 and T2 characteristics. MTRsym, magnetization transfer ratio asymmetry.
FIGURE 4  pH-sensitive and oxygen-sensitive MR images of human gliomas. A, A 42-year-old female with a nonenhancing recurrent World Health Organization (WHO) II isocitrate dehydrogenase (IDH) mutant astrocytoma (patient #43) exhibiting a region of focal acidity (high MTR$_{\text{asym}}$ at 3 ppm) and low oxygen consumption (low $R_2'$). B, A 51-year-old male patient with a newly diagnosed nonenhancing IDH mutant WHO III anaplastic astrocytoma (patient #22). This lesion showed large, heterogeneous regions of abnormally high and low hypoxia and acidity. C, A 53-year-old female patient with a recurrent IDH wild-type WHO IV glioblastoma exhibiting a region of focal acidity and large regions of low oxygen consumption (patient #15). D, A 53-year-old female with a newly diagnosed IDH wild-type WHO IV glioblastoma (patient #3) displaying ring enhancement, central necrosis, elevated acidity, and oxygen extraction (hypoxia) within the enhancing region as well as low oxygen consumption in surrounding nonenhancing tumor. $T_1w$, $T_1$-weighted image; $T_2w$, $T_2$-weighted image; FLAIR, fluid-attenuated inversion recovery.
3.0 ppm, but lower levels of hypoxia, or \( R_0 \), compared with regions of NAWM. Figure 4C,D shows patients with recurrent and newly diagnosed glioblastoma (WHO IV), respectively. Compared with the WHO grade II and III tumors with largely nonenhancing tumor with intact blood–brain barrier, patients with glioblastoma exhibiting extensive areas of CE displayed both high acidity (MTR asym at 3.0 ppm) and hypoxia (\( R_0 \)) as well as regions of moderate acidity and oxygenated tumor. (Additional information including T2 and T2* maps are illustrated in Supporting Information Figure S2).

Quantitative evaluation of various regions within these tumors confirmed these observations (Figures 5 and 6). Regions of T2 hyperintense lesions exhibited a significantly lower median \( R_0 \) (4.8 ± 0.2 sec\(^{-1}\)) compared with NAWM (6.2 ± 0.2 sec\(^{-1}\)) when pooling patients across all tumor grades (Figure 5A; paired t-test, \( p < .0001 \)). Within T2 hyperintense lesions, the median \( R_0 \) did not vary significantly across tumor grade (Figure 5B; ANOVA, \( p = .0537 \); WHO II = 4.6 ± 0.4 sec\(^{-1}\); WHO III = 4.2 ± 0.4 sec\(^{-1}\); WHO II = 5.4 ± 0.3 sec\(^{-1}\)). In glioblastoma patients, significant differences in median \( R_0 \) across tissue types were observed (Figure 5C; repeated-measures ANOVA, \( p = .0001 \)). In particular, the median \( R_0 \) was significantly lower in T2 hyperintense lesions (5.3 ± 0.3 sec\(^{-1}\)) compared with both NAWM (6.3 ± 0.2 sec\(^{-1}\); Tukey’s test, adjusted \( p = .0078 \)) and CE regions (10.0 ± 1.0 sec\(^{-1}\); Tukey’s test, adjusted \( p = .0002 \)), whereas no difference was detected between T2 lesions and areas of central necrosis (4.0 ± 0.5 sec\(^{-1}\); Tukey’s test, adjusted \( p = .1499 \)). Additionally, the median \( R_0 \) was significantly higher in CE lesions compared with NAWM (Tukey’s test, adjusted \( p = .0064 \)) and necrosis (Tukey’s test, adjusted \( p < .0001 \)), but significantly lower in necrosis compared with NAWM (Tukey’s test, adjusted \( p = .0064 \)). No differences in median \( R_0 \) were observed between recurrent or newly diagnosed tumor or normal tissues (\( p > .2 \)), nor were significant differences observed between IDH mutant and wild-type tumors (\( p = .12 \)); however, IDH mutant tumors tended to have lower \( R_0 \) compared with IDH wild-type tumors.
Median MTR_{asym} at 3.0 ppm within T2 hyperintense lesions (1.7 ± 0.1%) were significantly higher than NAWM (0.8 ± 0.03%) when pooling all patients across grade (Figure 5D; paired t-test, \( p < .0001 \)). Within T2 hyperintense lesions, the median MTR_{asym} at 3.0 ppm was significantly different across tumor grade (Figure 5E; ANOVA, \( p = .0297 \)), with WHO IV glioblastoma (2.0 ± 0.2%) exhibiting significantly higher acidity compared with WHO II gliomas (1.5 ± 0.1%; Tukey’s test, adjusted \( p = .0432 \)). No difference was observed between WHO III gliomas (1.6 ± 0.2%) compared with other grades (adjusted \( p > .05 \)). In a separate comparison, IDH mutant gliomas exhibited a slightly higher degree of tumor acidity compared with IDH wild-type tumors when correcting for grade (adjusted \( p = .0434 \)). Within WHO IV glioblastoma, significant differences in median MTR_{asym} at 3.0 ppm were observed among various tissue types (Figure 5F; ANOVA, \( p < .0001 \)). Areas of macroscopic necrosis exhibited the highest MTR_{asym} at 3.0 ppm degree of acidity (MTR_{asym} at 3.0 ppm = 4.4 ± 0.4%) compared with all other tissue types, including CE lesions (2.9 ± 0.1%; Tukey’s test, adjusted \( p < .0001 \)), T2 hyperintense regions (2.0 ± 0.2%, adjusted \( p < .0001 \)), and NAWM (0.8 ± 0.04%, adjusted \( p < .0001 \)). Additionally, CE tumor exhibited significantly higher median MTR_{asym} at 3.0 ppm compared with T2 hyperintense lesions (Tukey’s test, adjusted \( p = .0218 \)) and NAWM (Tukey’s test, adjusted \( p < .0001 \)), whereas T2 lesions showed higher median MTR_{asym} at 3.0 ppm (Tukey’s test, adjusted \( p < .0001 \)).

Examination of acidity and hypoxia characteristics within T2 hyperintense lesions pooled across tumor grade were markedly different from NAWM (Figure 6A), as T2 hyperintense lesions tended to be more acidic but slightly less hypoxic compared with NAWM. T2 hyperintense lesions did not show substantial separation across tumor grade (Figure 6B). Glioblastoma exhibited distinct characteristics across various tissue components (Figure 6C), with NAWM and T2 hyperintense lesions having relatively lower acidity and hypoxia combined with CE tumor and macroscopic necrotic tissue. The CE tumor exhibited a moderately high level of acidity, along with high degrees of oxygen extraction, whereas necrotic tissue showed low oxygen extraction yet high acidity.

5 | DISCUSSION

High-resolution pH-sensitive and oxygen-sensitive MR imaging contrast in brain tumors can be achieved on clinical 3T MR systems by implementing a multi-echo SAGE-EPI readout after off-resonance saturation or CEST preparation of amine protons (3.0 ppm). Comparable techniques for CEST imaging that take advantage of multiple echo readouts are relatively limited. Sun et al described a sequence that
uses a CPMG readout following CEST saturation for improving CNR in agarose phantoms by averaging images from a spin-echo train with TE = 60 ms. However, this sequence only measured R2 characteristics and could not correct for B0 using phase information available via multiple gradient echoes. The fast EPI readout available using CEST-SAGE-EPI vastly improves acquisition times over slower, single-slice, or volumetric approaches. Song et al and others have described single-echo EPI approaches to speed up acquisition time; however, these studies lacked concurrent measurement of subsequent echoes that would allow for estimation of R2, R2, R2 and B0. Thus, CEST-SAGE-EPI has many advantages including speed, in-line B0 mapping, whole-brain coverage, and simultaneous CEST, R2, R2, and R2 quantitation.

Mathematical simulations and phantom measurements confirmed the relationship between MTRasym at 3.0 ppm and pH reported previously. Measurements of transverse relaxivity for Gd-DTPA were found to be R2 = 6.24 ± 0.04 mM−1·sec−1 and R2 = 6.86 ± 0.10 mM−1·sec−1, which is consistent with the literature. Measurements of R2 and R2 also did not vary as a function of pH. As compared with CPMG measurements, CEST-SAGE-EPI tended to underestimate R2, consequently resulting in overestimation of R2; however, the magnitude of this difference averaged less than 1 ms (~760 us), or less than 10% difference, and increased with relaxation rate R2. This discrepancy may be related to inaccurate modeling of imperfections during RF inversion during SAGE-EPI readout, as has been suggested in previous SAGE-EPI studies. In addition to OEF, R2 value may also be influenced by venous blood fraction as reported by several studies. A more direct quantification of oxygen consumption or comparison may be achieved with high-field 17O MRI by detecting metabolically produced H217O or with 17O MRS. As demonstrated previously, edematous tissues and cancer tissues with long T2 tend to amplify pH-dependent CEST contrast, whereas tissues with shorter T2 exhibit only a few percentage changes in contrast, even in highly acidic and highly concentrated amino acid environments. In particular, with relatively short T2, including normal appearing white and gray matter may not have suitable sensitivity or CNR to provide meaningful pH information. Thus, the current CEST-SAGE-EPI sequence may not be immediately useful to explore functional or metabolic changes in otherwise normal neural tissues without additional considerations (e.g., higher field strengths, more averages), but it is uniquely suited for detection and assessment of brain tumor or oncologic metabolism. Although MTRasym contrast could be altered by effects from exchangeable proton pools other than amine protons, we have shown with phantoms (Supporting Information Figure S3) that the low pH results in elevation of MTRasym at 3.0 ppm despite the presence of amide protons and MT effect (introduced with 5% egg-white protein), and this is comparable to previous reports. The predominant pH dependence of MTRasym at 3.0 ppm could be explained by the high irradiation amplitude (B1 = 6 µT) preferably saturating fast-exchanging amine protons when compared with other proton pools. Another concern with using amine CEST contrast at 3.0 ppm is that it could suffer from an insufficient source of exchangeable protons if glutamine concentration is too low. In addition to glutamine, other amino acids and neurotransmitters possessing a similar amine functional group have similar pH dependence of exchange rate and MTRasym at 3.0 ppm. Large molecules such as bovine serum albumin were also reported to show characteristics of fast-exchanging amine protons at 3.0 ppm. With a total of about 20 to 25 mM amino acids within normal neural tissues and other contributions of amine proton pools, sufficient amine CEST contrast within tumor tissues is highly probable.

Combined information about both median tissue acidity (MTRasym at 3.0 ppm) and oxygen extraction (R2) helped to further delineate various tissue types and provide additional insights into the tumor microenvironment. Consistent with tumor biology and the Warburg effect, we observed elevated acidity in tumor tissues even when there was adequate oxygen delivery due to an intact BBB, high neovasculatry, and high blood flow. In particular, we observed lower levels of hypoxia and high acidity in regions of nonenhancing tumor (T2/FLAIR hyperintensity) (Figure 5A,D; Figure 6A,C), which can have elevated vascularity depending on the degree of malignancy and/or grade. This was similar across tumor grade, although glioblastoma had slightly higher levels of hypoxia compared with lower grades. In the same tumors we observed substantially higher levels of hypoxia in areas of CE (Figure 4D; Figure 5C; Figure 6A,C), which is known to be the most aggressive and hypoxic. These data suggest that tumor acidity and oxygen consumption are both complex and spatially heterogeneous, consistent with the known genetic, histopathologic, proteomic, and metabolic spatial heterogeneity. Hypoxic, nonhypoxic, and/or acidic tumors may also have differing therapeutic responses, as tumor hypoxia and acidity are both known to reduce sensitivity to chemoradiation. Thus, a tool that can colocalize areas of simultaneous pH and oxygen imbalance may allow for future therapeutic strategies to include radiation boost to regions containing the most hypoxic and acidic environments. Additionally, this tool may also allow for evaluation of direct inhibition of energetic pathways as a therapeutic targeted in these tumors.

In addition to tissue acidity and reduced oxygen consumption, the Warburg effect is also characterized by altered levels of glucose consumption. Dynamic glucose-enhanced MRI, which selectively saturates hydroxyl protons of glucose and allows for a dynamic detection of CEST contrast during glucose uptake, has shown promising results in mapping in
Mutations of isocitrate dehydrogenase (IDH1) are common in less aggressive gliomas and cause inhibition of the conversion of isocitrate to alpha-ketoglutarate, resulting in a buildup of 2-hydroxyglutarate (2HG), inhibiting oxidative phosphorylation and therefore inhibiting the ability of cells to use aerobic glycolysis. Therefore, we hypothesized that IDH1-mutated tumors may have lower $R_2$ measurements compared with IDH1 wild-type tumors, consistent with previous work by Stadtbauer et al. Although we observed slightly lower $R_2'$ in IDH1 mutated patients compared with wild-type patients, these effects were not statistically significant. Further studies in a larger patient cohort are needed to better understand this relationship and whether pH-sensitive and oxygen-sensitive MR signatures can identify IDH1 mutant tumors.

One potential limitation of our approach is using the conventional asymmetric analysis ($MTR_{asym}$) to interpret CEST effects. Although the $MTR_{asym}$ approach removes the symmetric effects from direct water saturation, the resulting contrast is still influenced by a number of other effects, including asymmetric magnetic transfer effect of semisolid tissue components, nuclear Overhauser enhancement 0 to 5 ppm upfield to water proton resonance frequency, and mixing effects from nearby exchangeable proton pools. Several alternative approaches have been developed to mitigate these problems, including chemical exchange rotation transfer, Lorentzian difference analysis, multiple-pool Lorentzian fit, and the 3-point method. Further investigation of pH-sensitive and oxygen-sensitive imaging with CEST-SAGE-EPI sequence could potentially benefit from applying these methods.

Additionally, proton exchange rates are influenced by temperature, with faster exchange rates of amine protons observed at higher temperatures, due to increased kinetic energy along with increased $T_2$ relaxation times resulting in increased available NMR signal at a given frequency. Thus, phantom results at room temperature (~18 °C) in the current studies are likely to underestimate $MTR_{asym}$ when compared with in vivo comparisons (~37 °C), as has been previously demonstrated. Thus, care should be given when interpreting absolute values of $MTR_{asym}$ observed in the phantom portion of the current study.

In vivo $R_2'$ mapping using SAGE-EPI readout can be influenced by a variety of technical issues, however, including $B_1$ inhomogeneity or mismatch between excitation and refocusing slice profiles, as well as biological factors including the effects of water diffusion and multiple water compartments within the tissues. The CEST contrast may also be influenced by multiple water compartments within tissues, although the water signal is thought to be predominantly from the extracellular water pool due to the very short relaxation times of bound intracellular water.

Technical imperfections including static magnetic field ($B_0$) and transmit RF field ($B_1$) inhomogeneity may also have led to altered image contrast. In our study, $B_0$ inhomogeneity was corrected with $B_0$ map generated from the WASSR method or from multi-echo $B_0$ mapping; however, $B_1$ inhomogeneities were not compensated for. $B_1$ inhomogeneity under 3T is reported to be relatively small (deviation < 10 to 20%) and have limited effect on $T_2$ quantification and pH-sensitive CEST contrast within the variation range in the current study. Nevertheless, validation of the effect of $B_1$ inhomogeneity on CEST-SAGE-EPI sequence and incorporation of $B_1$ correction in future work may be beneficial.

A final potential limitation of this study was the relative inhomogeneity of the patient population, which contained untreated and recurrent glioma patients on a variety of therapies. A larger study that separates out specific treatments along with tumor grade is necessary to better understand their influence on our measurements.

## 6 CONCLUSIONS

The current study presents a new CEST-SAGE-EPI sequence for obtaining high-speed, full-brain pH-sensitive and oxygen-sensitive image contrasts for brain tumor evaluation. Advantages of this technique include fast acquisition, in-line $B_0$ correction using phase information, whole-brain coverage, and simultaneous accurate estimation of CEST effects, $R_2$, $R_2^*$, and $R_2'$. Results in tumors showed a high degree of spatial heterogeneity, and measurements were consistent with known cancer biology.

## ACKNOWLEDGMENTS

American Cancer Society Research Scholar Grant (RSG-15-003-01-CCE) (B.M.E.); Art of the Brain (T.F.C.); University of California Research Coordinating Committee (B.M.E.); UCLA Jonsson Comprehensive Cancer Center Seed Grant (B.M.E.); UCLA SPORE in Brain Cancer (NIH/NCI 1P50CA211015-01A1) (B.M.E., L.M.L., P.L.N., A.L., W.B.P., T.F.C.); NIH/NCI 1R21CA223757-01 (B.M.E.)

## CONFLICTS OF INTEREST

Benjamin Ellingson is on the Advisory Boards for Hoffman La-Roche, Siemens, Nativis, Medicenna, MedQIA, Bristol Meyers Squibb, Imaging Endpoints, and Agios; he is a paid consultant for Nativis, MedQIA, Siemens, Hoffman La-Roche, Imaging Endpoints, Medicenna, and Agios; and he received grant funding from Hoffman La-Roche, Siemens, Agios, and Janssen. Timothy Cloughesy is on the Advisory...
Boards for Roche/Genentech, Amgen; Tocagen, NewGen, LPath, Proximagen, Celgene, Vascular Biogenics Ltd, Insys, Agios, Cortice Bioscience, Pfizer, Human Longevity, BMS, Merck, Notable Lab, and MedQIA.

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the supporting information tab for this article.

**FIGURE S1** Examples of B0 correction methods available for CEST-SAGE-EPI. A, B0 maps calculated for a single patient using phase differences from the first 2 gradient echoes acquired using CEST-SAGE-EPI. B, Corresponding B0 maps for the same patient using the WASSR (water saturation shift referencing) method. C, Resulting maps of MTR_{asym} at 3 ppm after B0 correction using multi-echo phase information. D, Value of MTR_{asym} at 3 ppm after the WASSR method for B0 correction. E, Value of MTR_{asym} at 3 ppm with no B0 correction.

**FIGURE S2** Anatomic and relaxometry MR images of human gliomas estimated using CEST-SAGE-EPI. Complimentary T2 and T2* relaxometry images estimated from CEST-SAGE-EPI for the 4 patients illustrated in Figure 3.

**FIGURE S3** Phantom results for 40 mM glutamine and 5% egg white protein. Results demonstrate increasing CEST contrast with decreasing pH, similar to those reported previously with pure glutamine phantoms. This suggests that fast-exchanging amine protons are the primary proton pool saturated using the current RF pulse scheme. Phantom preparation: Combined glutamine and protein phantoms with pH ranging from 6 to 7.5 were prepared by first adding glutamine into liquid egg white solution (5% protein) to reach concentrations of 40 mM. The pH within the sample solutions was then titrated to 6.0, 6.3, 6.6, 6.9, 7.2, and 7.4, with acid (1N HCl) and base (1N NaOH) solutions. Scan parameters: B1 = 6 uT, TR = 5000 ms, TE1/TE2/TE3/TE4 = 14.0/34.1/58.0/92.4 ms, FOV = 217 × 240 mm, matrix size = 116 × 128, slice thickness = 4.0 mm, slice number = 10, partial Fourier encoding = 6/8, GRAPPA = 3, bandwidth = 1628 Hz/pixel with number of excitations or averages = 2. A total of 36 offset frequencies were acquired: ±2.5 to ±4.0 ppm with 0.1-ppm interval (±6.0 and 20.0 ppm with respect to water proton Larmor frequency). Measurements were taken at room temperature (~20°C). Error bars represent SD within a region of interest within the Falcon tubes.