Mapping brain glucose uptake with chemical exchange-sensitive spin-lock magnetic resonance imaging

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INTRODUCTION

Glucose is the body’s fuel; its uptake and metabolism are sensitive biomarkers of cellular dysfunction in afflictions such as cancer, stroke, Alzheimer’s, and psychiatric diseases. Direct detection of glucose uptake in vivo has been established in human and animal studies by $^{13}$C and $^{1}$H nuclear magnetic resonance spectroscopy, but low glucose concentrations severely limit sensitivity. Clinical positron emission tomography scans with radioisotope-labeled glucose is much slower than their resonance frequency separation ($\delta$) ($i.e., k/\delta \ll 1$); however, this condition is not satisfied under physiologic conditions where exchange between water and glucose hydroxyl protons is rapid, and their resonance frequencies are close. Therefore, high glucose doses are required for CEST detectability in normal brain.

These encouraging studies indicate that ‘glucoCEST’ has a wide range of applications; however, as will be shown below, technical challenges include relatively low sensitivity, susceptibility to $B_0$ inhomogeneity and fluctuation, low temporal resolution, and difficulty in quantification. A main concern is that optimal CE contrast is obtained by CEST only when the exchange rate ($k$) between protons in water and those in the hydroxyl groups of glucose is much slower than their resonance frequency separation ($\delta$) ($i.e., k/\delta \ll 1$).

An alternative technique to indirectly map the CE of biomolecules with water utilizes on-resonance spin-lock (SL) MRI. This CE-sensitive SL (CESL) technique offers promise as a tool to enhance sensitivity to faster exchange processes (versus CEST). We recently reported that CESL contrast is optimal for the intermediate CE regimes relevant to glucose ($i.e., k/\delta \sim 1$), and showed high glucose detection sensitivity in phantom studies. In addition, feline and human applications of SL functional MRI have shown high-temporal resolution, which can improve the detectability to glucose changes. On-resonance SL signals are usually quantified by $T_{1\rho}$, the spin-lattice relaxation time in the rotating frame. Although $T_{1\rho}$ has contributions from all CE and non-CE relaxation pathways, and thus lacks specificity to any particular type of labile protons, recent in vivo studies have shown that tissue $T_{1\rho}$ is insensitive to variations in blood flow, volume,
and intravascular susceptibility; this lack of chemical specificity (versus CEST) is alleviated in applications with glucose administration (referred to as ‘glucoCESL’), where the signal response is expected to be dominated by elevated concentrations of glucose and its metabolic products.

In the current work, we methodically examined the feasibility of mapping brain glucose changes with CESL after intravenous injection of Glc or 2DG. Assessment included 9.4T experiments and simulations incorporating SL frequency variation. Phantom experiments of biomolecular solutions characterized CESL for hydroxyl groups with different k values, and for varying pH, concentration, and water longitudinal (T₁) and transverse (T₂) relaxation times. Computer simulations of CESL versus CEST compared sensitivity features and investigated the dependence on B₀ shifts. Three separate groups of in vivo rat-brain glucoCESL studies with high-temporal resolution were performed with intravenous injections. First, the dose dependence and detection threshold with our settings was determined for Glc. Next, glucoCESL responses were compared for matched doses of Glc, 2DG, and mannitol, and with measurement of arterial blood Glc and 2DG levels. The latter studies served to characterize dynamic properties and to assess ΔR₁ responses from changes in tissue water content after intravenous injection of hypertonic solutions; mannitol served as a control, since it induces large elevations in plasma osmolality, has a long half-life in blood (60 minutes in rats), and does not cross the blood–brain barrier. And lastly, glucoCESL responses were obtained for varied SL power levels at steady-state blood glucose levels to further examine the origin and quantification of glucoCESL responses.

**MATERIALS AND METHODS**

**Theoretical Background**

Although CEST and CESL each incorporate an RF preparation period to encode the exchange processes between water and labile protons, their labeling approaches are different. In the on-resonance CESL technique, water proton spins are excited (i.e., magnetization is flipped to the transverse plane) and then SL irradiation is applied at the same water resonance frequency. During the SL period, protons exchange with labile protons (e.g., the hydroxyl groups of glucose). Water proton magnetization is then returned to the longitudinal plane, where the CEST-reduced magnetization can be detected. The SL frequency in CESL is usually a few hundred Hertz (Hz) to effectively ‘lock’ the water magnetization in the presence of B₀ inhomogeneity, and this requires higher power (equivalent to an RF magnetic field strength B₁ of ∼10 μT) versus CESL studies, where B₁ is usually <2 μT. However, on the resonance SL duration in CESL is usually comparable to water T₂ (on the order of tens of milliseconds for in vivo studies), and this duration is much shorter than required for the off-resonance saturation of CEST, which should be comparable to water T₁ (on the order of seconds). Thus, the specific absorption rate for CESL is often similar to that of CEST, and has already been applied to several human studies.

Signal intensity in CESL is determined by R₂₁ (1/T₁), the spin-lattice relaxation rate in the rotating frame, and R₂₀ is obtained by varying the SL duration (TSL) and fitting the data to S = S₀ exp(−R₂₀ TSL), where S₀ and S are signal intensities with and without SL irradiation, respectively. Assuming a two-pool exchange process where the relative population of labile to water protons, p, is much smaller than 1, R₂₀ can be expressed as:

\[ R_{20} = R_{2a} + R_{2b}, \]

where R₂₀ is the transverse relaxation rate of water protons without CE contributions. The R₂₀ term fully characterizes the CE process and is proportional to labile proton concentration as defined by:

\[ R_{2a} = \frac{k \cdot (\gamma B_0 / 2\pi)^2}{\gamma^2 + 4\pi^2 B_0^2 + k^2}, \]

where \( \omega_o \) is the SL frequency equal to \( \gamma B_0 / 2\pi \) (units of Hz), and \( \gamma \) is the gyromagnetic ratio. Determination of R₂₁ at multiple \( \omega_o \) levels yields a ‘dispersion curve’, where R₂₁ can then be approximated as the R₂₁ value at high \( \omega_o \) and CE-related values p, k (units of per second), and \( \Delta (units of rad/second) \) can be determined by fitting the dispersion data to Equations 1 and 2.\(^\text{1,8}\) Equation 1 quantitatively describes both CESL and CEST data for glucose and other biomolecules, as confirmed by our present phantom studies.\(^\text{1,8}\)

In glucoCESL applications, ΔR₁ with administration of Glc or 2DG should be dominated by ΔR₂₀ and relate to the change in the ratio of hydroxy to water protons (Δp), as expressed by:

\[ \Delta R_1 = \Delta R_{2a} + \Delta R_{2b} + \Delta R_{2a} = \frac{\Delta p / k \cdot \gamma^2}{\gamma^2 + 4\pi^2 \omega_o^2 + k^2} \]  \[ \text{(3)} \]

**Simulations**

Characterizations of CESL and CEST were performed by computer simulations with Bloch–McConnell equations as follows.

(1) Sensitivity to different exchange rates was compared for CESL versus CEST by simulating the maximum CE contrast achievable by each of these approaches for various k₁ values, for water R₁ = 1/T₁ = 0.5 per second, and water R₂₀ values of 5 and 20 per second. For comparison with in vivo glucoCESL data, we also assumed a chemical shift, δ, between water and labile protons of 3.770 rad/second, equivalent to +1.5 ppm relative to water at 9.4 T, and a labile proton concentration of 10 mmol/L with water R₁ = 0.5 per second and water R₂₀ = 20 per second. Contrast for CESL was calculated as the difference (ΔS) between T₂₁-weighted signals (S) with CE (i.e., p ≠ 0) and without CE (i.e., p = 0), normalized by S₀. Contrast for CEST was obtained from the asymmetric magnetization transfer (MT) ratio, which is the difference (ΔS) between signals with irradiation frequency offsets, Ω, at the reference frequency (Ω = −δ) and at the hydroxy proton frequency (Ω = +δ), normalized by S₀. Maximum contrast at each k value was simulated for CESL at optimal (k₁) and TSL values, and for CEST at optimal k₁ for steady-state conditions (saturation duration → ∞).

(2) The effect of errors on CESL versus CEST measurements owing to variations in water resonance frequency was simulated for B₀ shifts ranging from 0 to 20 Hz, assuming water R₁ = 0.5 per second, water R₂₀ = 20 per second, and no CE (p = 0). The CEST asymmetric MT ratio errors were calculated at Ω = 200, 400, and 600 Hz with B₁ = 1.6 μT and 4-second saturation duration (typical for glucoCESL studies).\(^\text{1,6,11,13}\) For comparison, the errors in CESL contrast (ΔS/ΔS₀) and CESL R₁, owing to B₀ shifts, were simulated for k₁ = 300, 400, and 500 Hz. Since B₀ shifts cause CESL signal oscillations, the magnitude of signal oscillations was first calculated as ΔS = ΔS₀ with B₀ shift 500 Hz (without B₀ shift) for each of 100 equally spaced TSL values ranging between 45 and 55 milliseconds (typical in vivo tissue T₂₁ values at 9.4 T) and then averaged.

Chemical Exchange-Sensitive Spin-Lock Magnetic Resonance Imaging Experiments—General

All MRI experiments were performed on a 9.4-T MR system (Agilent Technologies, Santa Clara, CA, USA). For phantom experiments, a 3.8-cm ID quadrature volume coil (Rapid Biomedical, OH, USA) provided RF transmission and detection; on-resonance SL irradiation of TSL duration at the desired (k₁) level was sandwiched between a 90° hard excitation pulse and a 90° hard flip-back pulse. For the in vivo experiments of paradigms 1 and 2 (see below), RF transmission was provided by a volume coil (6.4 cm diameter) and received by a surface coil (2.2 cm diameter; coil combination from Nova Medical, MA, USA). For the in vivo experiments of paradigm 3, RF was transmitted and received by a home-built surface coil (1.6 cm diameter), since these studies required higher (k₁) values.\(^\text{1,9}\) In all in vivo studies, 50 milliseconds of SL irradiation at the desired (k₁) level was sandwiched between an adiabatic half-passage pulse and an inverse adiabatic half-passage pulse (each of 1.5 millisecond duration).\(^\text{26}\) Immediately after SL preparation, MR images were acquired by single-shot echo planar imaging (EPI).

Chemical Exchange-Sensitive Spin-Lock Phantom Magnetic Resonance Imaging Studies

Biochemicals dissolved in phosphate-buffered saline were placed in individual vials, and data from multivial phantoms were acquired at 37°C to characterize various CESL properties. (i) Effects of hydroxyl groups from different molecules were studied with 30 mmol/L solutions of Glc, 2DG, glycogen, and myo-inositol at pH = 7.0. (ii) Dependence on pH was determined with 20 mmol/L Glc at pH = 6.8, 7.0, 7.2, and 7.4. (iii) Water T₁ and T₂ effects on quantification were examined with 5 and 20 mmol/L Glc.
solutions of Glc at pH = 7.0, both with and without addition of 0.1 mmol/L MnCl₂. (iv) Linear dependence of $R_{1\rho}$ on glucose concentration was validated with 5, 10, 15, 20, 30, and 40 mmol/L solutions of Glc and 2DG at pH = 7.0, with addition of 0.15 mmol/L MnCl₂ to reduce $T_2$ to values closer to tissue, and also to speed acquisition. For all phantom, dispersion data were acquired with spin-echo EPI for 11 $\omega_2$ values between 125 and 4,000 Hz, with $64 \times 64$ matrix size, $4 \times 4$ cm field of view, 5 mm slice thickness, echo time = 25 milliseconds, and repetition time = 15 seconds.

Chemical Exchange-Sensitive Spin-Lock In Vivo Magnetic Resonance Imaging Studies

Animal preparation. With approval by the Institutional Animal Care and Use Committee at the University of Pittsburgh, 32 male Sprague-Dawley rats were studied (26 for MRI experiments, and 6 for bench-top measurements of blood glucose). Animals were anesthetized with isoflurane (5% for induction and 2% for surgery) in a mixture of O₂ and air, with the O₂ concentration maintained at 30%. The right femoral vein was catheterized to deliver maintenance fluid and Glc (or 2DG or mannitol). The right femoral artery was catheterized to ensure arterial blood pressure was maintained within a normal physiologic range. After surgery, the isoflurane level was reduced to ~1.5%, and maintained at this level. End-tidal CO₂ levels were monitored and kept within 3.0 to 4.0%. Rectal temperature was maintained at 37.2 ± 0.5°C using a feedback-controlled heating pad.

Animal magnetic resonance imaging experiments. GlucoCESL rat-brain studies were performed for three separate paradigms with intravenous injection of Glc, 2DG, or mannitol. General EPI parameters were matrix size = $64 \times 64$, field of view = $3.2 \times 3.2$ or $2.56 \times 2.56$ cm², 2 mm slice thickness, repetition time = 3 seconds, echo time = 30 milliseconds for single- or double-spin-echo EPI, and echo time = 12.5 milliseconds for gradient-echo EPI. Determinations of $R_{1\rho}$, time courses in each run were made from interleaved acquisitions of images with TSL = 0 and with TSL = 50 milliseconds at the corresponding $\omega_2$ values; when TSL was 0, delays after data acquisition were increased by 50 milliseconds to maintain constant repetition time. Solutions of Glc, 2DG, and mannitol were prepared at 20% weight by volume, and each dose was injected over a 1- to 5-minute period, except for the additional infusion of Glc in paradigm 3. Specific details are as follows:

Paradigm 1: The detection threshold of glucoCESTM MRI with our settings was determined by administering consecutive doses of 0.25, 0.5, and 1.0 g/kg body weight Glc (n = 5 rats) at 40, 100, and 180 minutes, respectively, after initiation of scanning. The 60- and 80-minute intervals between doses allowed Glc and glucose to recover to baseline levels (as shown below in Figure 5D for 1 g/kg injection). Maps of allowed blood glucose to recover to baseline levels (as shown below in initiation of scanning. The 60- and 80-minute intervals between doses.

Paradigm 2: The dynamic properties of glucoCESL MRI were assessed for Glc, 2DG, and mannitol (4 mmol/L each) for mannitol doses of 1 g/kg body weight, both to compare sensitivity, and to characterize the source of $\Delta R_{1\rho}$, changes. Data were acquired for 100 minutes after Glc or 2DG administration; responses after mannitol injection were small, so acquisition in those studies was terminated at 60 minutes. Maps of $R_{1\rho}$ (and $\Delta R_{1\rho}$) were generated as in paradigm 1. In separate bench-top studies, blood glucose levels were sampled every 10 minutes with administration of Glc (n = 3 rats) or 2DG (n = 3 rats), and time courses were compared with those of $\Delta R_{1\rho}$.

Paradigm 3: The origin of glucoCESL contrast was further assessed by acquiring time-dependent data for $\Delta R_{1\rho}$ at varied $\omega_2$ values in Glc and in 2DG studies with data acquired at steady state. If the origin of signal change is dominated by CE effects, then $\Delta R_{1\rho}$ should decrease with $\omega_2$, as expected in Equation [3]. A second goal was to compare steady-state responses to 2DG and Glc administration, where the correlations between blood and brain glucose levels for the latter have been established. As blood glucose levels decrease faster after Glc versus 2DG injection, steady-state levels were attained in Glc studies (n = 4 rats), by injecting 0.3 g/kg body weight Glc intravenously over 1 minute period, followed by constant infusion of 2.0 g/kg per hour Glc for 1 hour; four double-spin-echo EPI $T_2^*$-weighted images were acquired in an interleaved manner, one without and three with SL weighting ($\omega_2 = 500, 1,000$, and 2,000 Hz). Blood glucose levels were sampled shortly before and ~60 minutes after onset of the first Glc injection. In 2DG studies (n = 5 rats), a single injection of 1 g/kg 2DG was administered; three $T_2^*$-weighted images were acquired in an interleaved manner either by double-spin-echo or gradient-echo EPI, one without SL preparation and two with SL weighting of $\omega_2 = 500$ and 2,000 Hz (results from both EPI techniques were similar, so they were combined). To ensure that $\omega_2$ was similar in all studies and across the region of interest (ROI), a midcortical ROI in the dorsal area close to the surface coil was first selected from anatomic images (see orange pixels of Figure 5C inset image for an example), and $\omega_2$ was restricted so that its spatial average over the ROI reached the target value.

Data Analysis

Data were analyzed with in-house Matlab programs and STIMULATE software. For phantom studies, quantitative analyses were performed for large circular ROIs defined within each sample vial. Values of $\omega_2$, $R_{1\rho}$, and $R_2$ for each solution were first obtained by fitting the $R_{1\rho}$, dispersion data to Equations [1] and [2]. Then $R_{1\rho}$ values were determined at $\omega_2 = 500$ Hz and at $\omega_2 = 2,000$ Hz, and the ratio between them was calculated. For fitting to Equation [2], the relative population (p) is the number of labile protons per biomolecule (N) times its concentration divided by 110 mmol/L (proton concentration in the water pool), where N is 5 for Glc, 4 for 2DG, 3 for glycosgen, and 6 for myo-inositol. Although different hydroxyl protons within each molecule have their own chemical shifts and exchange rates, it is the average $\omega_2$, $R_{1\rho}$, and $R_2$ values from all hydroxyl resonances that were determined here.

For in vivo experiments where images with varied $\omega_2$ and TSL values were acquired in an interleaved manner, images with same $\omega_2$ and TSL values were extracted from each run. Time series of $R_{1\rho}$ images were calculated by mono-exponential fitting of signal intensities from TSL = 0 milliseconds and TSL = 50 milliseconds, as $R_{1\rho} = \ln [(S_{TSL=0} / S_{TSL=50\,\text{milliseconds}})] / 50$ milliseconds. Statistical maps corresponding to $R_{1\rho}$ changes resulting from Glc or 2DG injection were calculated by performing Student’s t-tests on a pixel-by-pixel basis, where a threshold of P < 0.05 (uncorrected for multiple comparisons) was chosen and a minimum cluster size of 3 pixels was applied. Baseline data were averaged from the period spanning 10 to 0 minutes before start of injection. Data for glucose-induced changes were from periods spanning 10 to 25 minutes post injection for paradigms 1 and 2, and 50 to 70 minutes post injection for paradigm 3. Quantitative analyses were performed for ROIs encompassing all brain pixels (paradigm 1) and from small midcortical ROIs (paradigms 2 and 3), as determined from anatomic images. Time-dependent series of $\Delta R_{1\rho}$ maps are presented as averages over 10-minute intervals before subtraction from the baseline average, while $\Delta R_{1\rho}$ time courses from within ROIs are presented as averages over 1-minute intervals and are shown as mean ± s.e.m.

RESULTS

Chemical Exchange-Sensitive Spin-Lock Magnetic Resonance Imaging Studies in Phantoms

Figure 1 shows dispersion data characterizing CESL properties in phantoms. In Figure 1A, 30 mmol/L solutions of Glc, 2DG, myo-inositol, and glycogen all show high $R_{1\rho}$ values when $\omega_2 \leq 500$ Hz, but this CE effect is attenuated as $\omega_2$ increases, and $R_{1\rho}$ is much slower when $\omega_2 \geq 2,000$ Hz; this $\omega_2$ dependence on CE is an important property of CESL. The arrows in the dispersion curve approximately indicate the half-width at half-maximum value of $\omega_2$, where the condition $\omega_2 = k^2 + \delta^2$ is satisfied. Assuming similar $\delta$, the higher half-width at half-maximum values for Glc and 2DG suggest that $k$ is faster than for glycogen and myo-inositol.

Figure 1B shows $R_{1\rho}$ dispersion data for 20 mmol/L Glc solutions spanning the range of physiologic pH values (6.8 to 7.4); the half-width at half-maximum values of $\omega_2$ increase with pH. Characteristics of Figure 1B dispersion curves can be appreciated from Equation [2], given that $\delta$ is constant for all four solutions (pH-independent), whereas $k$ increases with pH. Namely, at very small $\omega_2$ values, the largest $\omega_2$, is seen for the pH 7.0 solution, meaning that $\delta$ is closest to the $k$ value of that solution (i.e., in the intermediate CE regime), whereas at very large $\omega_2$, $R_{1\rho}$ increases with $k$. Note that at $\omega_2 \approx 500$ Hz (vertical dashed line), $R_{1\rho}$ and therefore $R_{1\rho}$ are relatively insensitive to this physiologic pH range, with a variation of <8%. However, when $\omega_2 \geq 2,000$ Hz, $R_{1\rho}$ and $R_{1\rho}$ are much more sensitive to pH. From Equation [2], the
Table 1. Values derived by fitting phantom $R_{1p}$ dispersion data to Equations [1] and [2]

|          | Glc | Glc | Glc | 2DG | Gly | Ins |
|----------|-----|-----|-----|-----|-----|-----|
| pH       | 6.8 | 7.0 | 7.2 | 7.4 | 7.0 | 7.0 |
| $k$ (per second) | 4,280 | 4,710 | 6,100 | 8,140 | 4,500 | 2,155 | 3,388 |
| $\delta$ (rad/second) | 3,572 | 3,648 | 3,773 | 3,824 | 3,522 | 3,322 |
| $R_{1w}(\omega_1 = 500 \text{ Hz})$ | 3.5 | 3.0 | 2.6 | 2.1 | 3.4 | 6.5 | 6.1 |
| $R_{1w}(\omega_1 = 2000 \text{ Hz})$ | 3.0 | 2.6 | 2.1 | 3.4 | 6.5 | 6.1 |

2DG, 2-deoxy-D-glucose; Glc, D-glucose; Gly, glycogen; Ins, myo-inositol; $k$, chemical exchange rate between protons in water and those in the hydroxyl groups; $\delta$, resonance frequency separation between protons in water and those in the hydroxyl groups; $R_{1w}$, exchange-mediated relaxation term. Results are mean values for all OH groups.

Figure 1. Chemical exchange-sensitive spin-lock magnetic resonance imaging data at 9.4 T (Tesla) from biochemical solutions prepared in phosphate-buffered saline, and measured at 37°C. (A–C) Dispersion curves (rotating-frame spin-lattice relaxation rates, $R_{1p}$), versus spin-lock frequencies, $\omega_1$) are displayed for solutions with (A) D-glucose (Glc), 2-deoxy-D-glucose (2DG), glycogen (Gly), and myo-inositol (Ins), all at 30 mmol/L concentration at pH = 7.0; (B) 20 mmol/L Glc at four pH values; and (C) 5 and 20 mmol/L Glc at pH = 7.0 without and with MnCl$_2$. Color-matched arrows in panel A indicate the approximate half-width at half-maximum (HWHM) values of $\omega_1$ for each solution, suggesting that the chemical exchange rates ($k$) between protons in water and those in the hydroxyl groups are faster for Glc and 2DG versus Gly and Ins (see text for rationale). The dispersion curve in panel B is steepest for samples at pH = 6.8 and 7.0, with HWHM values for $\omega_1$ increasing with pH value; the $R_{1w}$ values are similar for all pH values at $\omega_1 = 500$ Hz (vertical dashed line). The nearly identical $R_{1w}$ span between arrows at $\omega_1 = 500$ Hz in panel C indicates that Glc concentration-dependent $R_{1w}$ differences are independent of $T_1$ and $T_2$ relaxation. (D) As expected from Equation [2], $R_{1w}$ is shown to be linearly proportional to glucose concentration in Glc and 2DG solutions (at pH = 7.0 with 0.15 mmol/L MnCl$_2$; acquired with $\omega_1 = 500$ Hz); the slope of the linear fit is 0.066 per second per mmol/L for Glc and 0.050 per second per mmol/L for 2DG.

Simulations of Chemical Exchange Effects in Chemical Exchange Saturation Transfer and Chemical Exchange-Sensitive Spin-Lock with Bloch–McConnell Equations

Values for maximum contrast with CEST and CESL were calculated for different $k/\delta$ values. Contrast with CEST is optimal for slow CE regimes ($k/\delta << 1$), but very low for fast CE regimes ($k/\delta >> 1$). However, CESL contrast is optimal for intermediate CE regimes ($k/\delta \approx 1$), and is higher than CEST when $k/\delta > 1$. Contrast with both CESL and CEST decreases as $R_{2w}$ increases. For a more specific example relevant to glucose in vivo, the $R_{2w} = 20$ per second data from the dotted region of Figure 2A were replotted as a percentage change in Figure 2B, where both Glc and 2DG are represented at 9.4 T by the blue vertical line ($k/\delta = 1.29$, from Table 1), and at 3 T by the green vertical line ($k/\delta = 4.05$). These results suggest that CESL MRI is a good choice for glucose mapping at 9.4 T, and an even better choice at lower fields where $k/\delta$ is larger.

Computed errors owing to water resonance frequency variations in CEST and CESL experiments appear in Figure 3. Simulations for typical in vivo CEST saturation (i.e., $B_1 = 1.6 \mu$T applied for 4 seconds) show that the asymmetric MT ratio signal at an irradiation offset ($\Delta$) of 400 Hz has an error of 0.5% for the 2-Hz $B_0$ shift often encountered in brain studies, and 5% for a 20-Hz shift (Figure 3A). Simulations for typical in vivo CESL studies (i.e., TSL ~ 50 milliseconds) show that errors from $B_0$ shifts are greatly reduced (versus CEST) for $\omega_1 \geq 300$ Hz, with negligible effects on either contrast ($\Delta S/S_0$; Figure 3B) or on calculated $R_{1w}$ values (Figure 3C), even for $B_0$ shifts as large as 20 Hz.
Contrast in both CEST and CESL depends on $k_{\text{CESL}}$ outperforms CEST in the fast-exchange regimes (with CESL highest for intermediate-exchange regimes (separation, $d$) in the slow-exchange regimes (i.e., when $k$ relaxation rate in the absence of exchange effects), but scales linearly with $\delta$ and with relative population (i.e., glucose hydroxyl protons to water protons, $p$) when $\rho-\delta<<1$, so contrast is normalized by $(\rho-\delta)$. (B) Simulations are plotted over a smaller range of $\Delta k/\delta$ values, for reasonable in vivo values of $R_{2,0}=20$ per second, 10 mmol/L of labile protons ($P=0.00091$), and $\delta=3,770$ rad/second; vertical lines indicating the $k/\delta$ values appropriate for both D-glucose and 2-deoxy-D-glucose at 9.4 T (Tesla; blue line) and at 3 T (green line) show that CESL outperforms CEST at both field strengths; however, the sensitivity enhancement is substantially higher at 3 T.

Dynamic glucoCESL Response Comparisons for Glc, 2DG, and Mannitol in Rat Brain

Time-dependent glucoCESL Figure 5 shows in vivo postinjection responses that are much larger and persist for a longer duration for 1 g/kg 2DG versus 1 g/kg Glc (paradigm 2) in serial $\Delta R_{1p}$ maps typifying 0 to 90 minutes post injection (Figures 5A and 5B, respectively). These dynamic properties are further shown in $\Delta R_{1p}$ time course averages from all the four animals (Figure 5C). Peak $\Delta R_{1p}$ values were reached in $\sim 20$ minutes for both 2DG and Glc, but with $\sim 2.5$ times higher magnitude for 2DG, which is in good agreement with a glucoCEST report. Recovery of $\Delta R_{1p}$ to preinjection baseline levels occurred around 60 minutes post-Glc injection, but recovery for 2DG still had not occurred at the end of the 100-minute window. The higher-intensity and longer-duration $\Delta R_{1p}$ responses to 2DG (versus Glc) can partially be explained by the difference in time courses of blood glucose levels after single 1-g/kg dose injections (Figure 5D). Blood glucose levels peaked immediately after injection, with magnitudes similar for 2DG and...
GlucoCESL data show that increases for Glc versus 2DG studies were similar: Further assessment of contributions to Frequency and Blood Glucose Levels that blood glucose levels should be raised above baseline by more than 300 mg/dL (~17 mmol/L). With the near-linear relationship between brain and blood glucose levels established by nuclear magnetic resonance spectroscopy, these studies suggested a brain glucose elevation of 6 to 7 mmol/L as a detection threshold for glucoCEST at 9.4 T. However, our in vivo glucoCEST rat-brain studies at 9.4 T show robust detection at doses as low as 0.25 g/kg Glc, meaning ΔR₁₀ ~0.1 per second is the detection threshold for our conditions, which corresponds to ~1.5 mmol/L increase in brain glucose concentration. We reported in a prior fMRI study that with sufficient signal averaging to enhance the sensitivity, neural stimulation induced a small CESL ΔR₁₀ response of ~0.05 per second in the cat visual cortex, which may be attributed to a submillimolar decrease in glucose. This enhancement in glucose detection sensitivity for glucoCEST versus glucoCEST may be explained by three major factors—differing sensitivity to exchange rates, differing temporal resolution, and

**DISCUSSION**

Enhancement in Glucose Detection Sensitivity for glucoCESL versus glucoCEST

Previous glucoCEST rat-brain studies at 9.4 T found no detectable responses for a dose of 0.5 g/kg Glc, and even at 1 g/kg Glc the responses were weak and had diminished after 10 minutes, despite the fact that blood glucose levels remained hyperglycemic. Reliable detection of brain glucose with glucoCEST requires that blood glucose levels should be raised above baseline by more than 300 mg/dL (~17 mmol/L). With the near-linear relationship between brain and blood glucose levels established by nuclear magnetic resonance spectroscopy, these studies suggested a brain glucose elevation of 6 to 7 mmol/L as a detection threshold for glucoCEST at 9.4 T. However, our in vivo glucoCEST rat-brain studies at 9.4 T show robust detection at doses as low as 0.25 g/kg Glc, meaning ΔR₁₀ ~0.1 per second is the detection threshold for our conditions, which corresponds to ~1.5 mmol/L increase in brain glucose concentration. We reported in a prior fMRI study that with sufficient signal averaging to enhance the sensitivity, neural stimulation induced a small CESL ΔR₁₀ response of ~0.05 per second in the cat visual cortex, which may be attributed to a submillimolar decrease in glucose. This enhancement in glucose detection sensitivity for glucoCEST versus glucoCEST may be explained by three major factors—differing sensitivity to exchange rates, differing temporal resolution, and
different effects from $B_0$ shifts. First, $k$ values for Glc were determined to be 4,280 to 8,140 per second for a physiologic range of pH values (6.8 to 7.4), and this falls between the intermediate and fast exchange regimes ($k/\delta > 1$, Table 1), where CESL contrast is expected to exceed that of CEST (see Figure 2B). However, this sensitivity advantage of glucoCESL will be reduced if $k$ is much slower, such as the case for the lower pH of ischemic tissues. Second, temporal resolution in our glucoCESL sensitivity tests was 12 seconds, while typically for glucoCEST it is ~10 minutes to accommodate the long saturation pulse and the need to acquire images at multiple RF offsets. 10,11,13 This much-higher temporal resolution of glucoCESL provides a higher statistical power for glucose detection as compared with glucoCEST. Third, the effect of any $B_0$ shift is much smaller for glucoCESL versus glucoCEST. Because the saturation frequency for CEST is quite close to water, $B_0$ shift of just a few hertz can cause significant signal errors, making an effective pixel-wise $B_0$ correction scheme with acquisition of redundant data essential.10,11,13 Unfortunately, accurate pixel-wise $B_0$ corrections could be very difficult in the presence of the large physiologic or instrumental fluctuations that may occur during long CESL acquisitions. 30

Sensitivity enhancement of glucoCESL versus glucoCEST is expected to be even higher at lower magnetic fields for two reasons. (i) Since $\delta$ is proportional to the magnetic field, the increase in $k/\delta$ at lower clinical fields (such as 3T) as compared with ultra-high fields ($\geq 9.4$ T) will consequently make the relative sensitivity gain of CESL versus CEST even more prominent (Figure 2B). (ii) Although, CESL only have minimal errors originating from $B_0$ shifts, these errors in CEST may intensify at lower fields where the RF saturation frequency offset is proportionately closer to water (see Figure 3A, $\Omega = 200$ versus 600 Hz).

Origin of glucoCESL Signals
A glucose increase in three separate pools can contribute to glucoCESL signals: intravascular blood and extracellular and intracellular spaces in tissue. The intravascular blood glucose contribution to $\Delta R_{1p}$ is minimal at 9.4 T because of (1) a small arterial vascular volume fraction (~1%) and (2) suppression of the venous component in EPI acquisitions at relatively long echo times, due to short $T_2$ and $T_2^*$ values. 33 In addition, insight about the contribution from intravascular glucose can be obtained by comparing the temporal dynamics of blood glucose levels and the high-temporal resolution time course of $\Delta R_{1p}$. Blood glucose peaks immediately after injection, whereas $\Delta R_{1p}$ increases gradually and peaks ~15 to 20 minutes post injection. This temporal mismatch indicates that $\Delta R_{1p}$ should have minimal contributions from intravascular glucose.

A decrease of extravascular water content induced by the elevation of plasma osmolality can contribute to a small $\Delta R_{1p}$ term, as evidenced after mannitol injection (Figure 5C). However, this contribution is exaggerated with mannitol, since it remains in the vasculature with a half-life of ~60 minutes. 35 The osmolality-induced $\Delta R_{1p}$ for glucoCESL is dependent on the difference between blood and tissue glucose concentrations. Tissue uptake of Glc and 2DG makes their osmolality contributions to $\Delta R_{1p}$ even smaller, since there will be rapid equilibration of blood and tissue glucose concentrations. In addition, osmolality effects should be further reduced by rapid metabolism of Glc to glucose-6-phosphate, and metabolism of 2DG to 2DG-6-phosphate (2DG6P) with the latter accumulating in tissue.

As glucoCESL vascular contributions are minimal, the change in $R_{1p}$ mostly arises from the extracellular and intracellular spaces in tissue, where relative contributions depend on glucose concentration, pool size, $k$, and $\omega_1$. Intracellular pH is usually 7.0 to 7.1, 6,32 while extracellular pH is ~7.2 to 7.3. 33,34 Our phantom data suggest that at $\omega_1 = 500$ Hz, glucoCESL sensitivity is similar for this range of pH values (Figure 1B), and therefore the $\Delta R_{1p}$ change would have similar weighting from both extracellular and intracellular spaces and reflect the total glucose change. The ratio of in vivo $\Delta R_{1p}$ at 500 to 2,000 Hz is ~40% smaller than for the aqueous phantoms at pH 7.0, suggesting that overall in vivo $k$ values are higher (according to Equation [3] and Table 1). It is possible that in vivo $k$ values are higher than our phantom at matched pH because of a different concentration of exchange catalysts. Further studies will be necessary to quantify in vivo $k$ values and to determine whether $\Delta R_{1p}$ is negligible in Glc and 2DG results, possibly by fitting in vivo dispersion data acquired with more $\omega_1$ levels to Equations [1] and [2].

Quantification of Glucose Concentration Changes
One critical question is whether a change in Glc concentration can be determined with CE-MRI. In both CEST and CESL, the CE...
sensitivity is coupled with non-CE relaxation mechanisms including T1 and T2 effects, and MT from semisolid macromolecules, as seen in Figures 2A for varied T2. Consequently, different T2 and MT effects will alter CE signal intensities, even when metabolite concentrations are the same. Therefore, in vivo glucoCEST signals cannot be directly compared with those measured in aqueous phantoms to determine the glucose concentration. Unlike CEST and CESL signal intensities, our phantom results indicate that R1 changes in CESL are independent of T1 and T2 and provide a quantitative index of glucose concentration changes (Figure 1C). In addition, the non-CE MT effect is also incorporated into the R2,0 term in Equation (1) with minimal contribution to ΔR1. Therefore, the assumption that the k value is the same in vivo as for our aqueous phantoms (Figure 1C and Table 1), the brain glucose change can be estimated. In the steady state, the ΔR1, value of 0.30 per second averaged between 50- and 70-minutes post-Glc injection at ω1 = 500 Hz in paradigm 3 (Figure 6A) yields an estimate of 4.5 mmol/L increase in brain glucose concentration, or 3.7 μmol per gram tissue, assuming 0.83 g water per gram tissue. This is in good agreement with the conversion of our 160 ± 25 mg/dL (i.e., 8.9 ± 1.5 mmol/L) blood glucose levels (obtained at 60 minutes post injection) to brain glucose levels of 4 ± 1 μmol per gram tissue, using the blood–brain glucose relationship. Although, blood glucose levels were similarly elevated in Glc and 2DG studies (160 versus 144 mg/dL) when ΔR1, reached steady state (60-minute point of Figure 6), the increase in brain 2DG concentration is estimated to be ~13 mmol/L from ΔR1, of 0.65 per second, much higher than for Glc. It should be noted that glucose-administered CE-MRI techniques detect an overall increase in the number of hydroxyl groups, which predominantly arise from concentration changes of glucose (Glc or 2DG) and its metabolic products containing hydroxyl groups, such as glucose-6-phosphate and 2DG6P. Given a minimal contribution from intravascular blood, the higher steady-state ΔR1, values in 1-g/kg 2DG versus 1-g/kg Glc studies (Figure 5) are primarily because of a larger extravascular (i.e., neural tissue) concentration of 2DG and its metabolic products (versus Glc). It is known that unlike glucose-6-phosphate, 2DG6P accumulates in cells. It was reported that with a 0.5-g/kg 2DG injection, the brain concentration of 2DG6P was approximately the same as that of 20 minutes post injection and was approximately four times that of 2DG at 100 minutes post injection. Thus, with similar increases of blood Glc and 2DG concentrations, the much higher ΔR1, of 2DG may be mainly because of the increased concentration of intracellular 2DG6P. This observation and interpretation are consistent with the recent glucoCEST results, where the signal from 2DG is much higher than Glc, even when the steady-state blood glucose concentration with Glc infusion is higher than that of 1-g/kg 2DG injection.

CONCLUSION
Recent glucoCEST MRI studies show potential for widespread applications in diseases with altered glucose transport and metabolism, but sensitivity is limited, especially at clinical magnetic fields, and quantification is difficult. Our alternative glucoCESL approach substantially increases sensitivity and provides an index for potential quantitative mapping of glucose concentration changes that can be applied to dynamic studies with subminute temporal resolution. These advantages make glucoCESL MRI a viable tool for studies of glucose transport and potentially metabolism in clinical settings and in functional studies.

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