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Mapping Murine Diabetic Kidney Disease Using Chemical Exchange Saturation Transfer MRI

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Abstract

Introduction—Diabetic nephropathy (DN) is the leading cause of renal failure; however, current clinical tests are insufficient for assessing this disease. DN is associated with changes in renal metabolites, so we evaluated the utility of chemical exchange saturation transfer (CEST) imaging to detect changes characteristic of this disease.

Methods—Sensitivity of CEST imaging at 7T to DN was evaluated by imaging diabetic mice (db/db, db/db eNOS^{−/−}) that show different levels of nephropathy as well as by longitudinal imaging (8 to 24 weeks). Non-diabetic (db/m) mice were used as controls.

Results—Compared with non-diabetic mice, the CEST contrasts of hydroxyl metabolites that correspond to glucose and glycogen were significantly increased in papilla (P), inner medulla (IM), and outer medulla (OM) in db/db and db/db eNOS^{−/−} kidneys at 16 wks. The db/db eNOS^{−/−} mice that showed advanced nephropathy exhibited greater CEST effects in OM and significant CEST contrasts were also observed in cortex. Longitudinally, db/db mice exhibited progressive increases in hydroxyl signals in IM+P and OM from 12 to 24 weeks and an increase was also observed in cortex at 24 weeks.

Conclusions—CEST MRI can be used to measure changes of hydroxyl metabolites in kidney during progression of DN.

Keywords

MRI; chemical exchange saturation transfer (CEST); diabetic nephropathy; db/db mice; magnetization transfer (MT); nuclear Overhauser enhancement (NOE)

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

INTRODUCTION

Diabetic nephropathy (DN) is a major diabetic complication that leads to renal failure and determines the morbidity and mortality of many diabetic patients. Although clinical indicators or risk factors have been described for this disease, currently available tests do not reliably detect or predict its development and progression in individual patients (1,2), making it difficult to evaluate treatments targeted to high-risk populations. Thus, alternative techniques are required to better characterize patients with this disease.

In vivo MRI techniques are important tools for evaluating the functional, structural, and metabolic integrity of the compromised kidney in a wide variety of renal pathologies (3,4). Further, the application of non-invasive imaging methods to mouse models permits serial evaluation of disease progression and enables more detailed investigation of the pathological processes of renal disease (5,6). During the past decade, several MRI techniques have been applied to the assessment of DN. These include blood oxygenation level dependent (BOLD) MRI and diffusion weighted imaging (7–10). These techniques were shown to be useful for assessing diabetic renal injury; however, they do not evaluate specific events in DN. Diabetic kidney disease is associated with changes in tissue metabolites (e.g. glucose, glycogen, glycosaminoglycans) that should exhibit significant chemical exchange saturation transfer (CEST) effects in MRI. Therefore, CEST MRI may be a valuable approach for evaluating this disease.

CEST imaging detects metabolites with exchangeable solute protons, such as amine, amide, or hydroxyl groups. Through the measurement of water signal changes caused by a cumulative exchange effect between saturated solute protons and water protons, CEST shows an amplification effect enabling indirect detection of solute molecules in the millimolar range. CEST has been used to study amide levels in brain and spinal cord (11–14), tissue pH (15–18) and glycogen deposition (19,20). Recently, this method has been applied to the detection of glucose accumulation in tumors (21). The *in vivo* application of this method to renal disease models could facilitate our understanding of molecular and metabolic changes that occur during disease progression. To our knowledge, only one previous study evaluated pH levels in a rat model of acute kidney injury with this technique (22).

A reduction in endothelial nitric oxide synthase (eNOS) has been shown to be associated with advanced human diabetic nephropathy (23). Further, recent studies have shown that this effect can be recapitulated in mice (24). The db/db mice that carry leptin receptor deficiency are widely used as a model of type 2 diabetes, and db/db mice that lack the eNOS gene (db/db eNOS^{-/-}) have been shown to exhibit more advanced nephropathy that more closely resembles human DN (25,26). These strains provide a unique opportunity to evaluate the ability of CEST imaging to detect the severity of DN.

In the present study, we evaluated the utility of CEST imaging for the assessment of DN using the db/db and db/db eNOS^{-/-} models that show different levels of nephropathy. Quantification of CEST signal changes downfield from water (higher frequency) is challenging because of the influence of overlapping signals from different molecular groups,

nuclear Overhauser enhancement (NOE) effects upfield from water (lower frequency) that may influence conventional asymmetry analyses, and other tissue parameters (e.g. T_1 , T_2 , and solid component MT effects). In this work, we applied a multiple-pool fitting algorithm to quantify CEST effects. Here we demonstrate evidence that CEST may detect and quantify the changes in renal hydroxyl metabolites, which are associated with the progression of DN.

METHODS

Animals

The db/db and db/db eNOS $^{-/-}$ mice (30–65 g) were prepared as described previously (25). The db/m mice (20–28 g) with C57BLKS background were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Vanderbilt University.

Imaging

All MR images were acquired on a 7T Agilent horizontal bore imaging system. A Doty 38-mm inner diameter transceiver coil was used due to the large size of the diabetic mice. Anesthesia was induced and maintained with a 1.5%/98.5% isoflurane/oxygen mixture, and a constant body temperature of 37.5 °C was maintained using heated air flow. All imaging protocols used a field-of-view (FOV) of 35×35 mm². A fast spin-echo (FSE) sequence (TR = 2500 ms; effective TE = 52 ms; RARE-factor 8, resolution = 0.1×0.1×0.5 mm³) was used to achieve T_2 contrast. A one-dimensional navigator echo was employed to reduce motion artifacts.

CEST acquisitions were performed using a continuous wave (CW) CEST sequence with a 5.0 s irradiation pulse followed by 2-shot spin-echo echo-planar-imaging (TR = 7.5 s, TE = 17.6 ms, matrix of 64×64, resolution = 0.55×0.55×1 mm³, and number of excitations NEX = 2). Z-spectra were acquired with RF offsets from –1500 Hz to 1500 Hz with an interval of 50 Hz. Saturation pulse amplitude B_{cw} was 1.0 μ T. Fat saturation was applied at RF offset (–1042 Hz) using a sinc-shaped pulse. Reference scans were performed at the beginning and the end of the acquisition with RF offsets at 100 kHz, with averaged signal-to-noise ratio (SNR) across voxels in kidneys observed around 120 (SD was about 15 across voxels). To correct for B_0 inhomogeneity, a Water Saturation Shift Reference (WASSR) spectrum (27) was also collected using the CW-CEST sequence (with a 1000 ms CW saturation pulse and 0.1 μ T B_{cw}), and the saturation offset range was $\pm$ 120 Hz (15 Hz steps) with respect to water. Real-time respiratory-triggered acquisition was applied to minimize motion artifacts. The external trigger was located before the saturation pulse in the imaging sequence. The respiration waveforms dipped during animal inspiration and exhibited only small changes in amplitude during expiration (~500 ms stable expiration duration). The expiration gate output started 200 ms after the detection of the dip in the waveform that occurred at inspiration, and gate duration was shortened to 50 ms to ensure that the data acquisition for each volume started at similar positions in respiration phase cycles. CEST data acquisition time is about one and half hour.

Data analysis

All data were analyzed using MATLAB (Mathworks, Waltham, MA). Images were registered using a rigid registration algorithm based on mutual information (28). Kidneys and regional ROIs were manually segmented based on T₂-weighted images (29). The baseline correction (linear) was applied based on the reference scans performed at the beginning and the end of CEST acquisition.

The Z-spectra were B₀-corrected using WASSR and normalized to the control scan (S_o is the signal acquired with an RF offset of 100 kHz)

$$Z = \frac{S}{S_o} \quad (1)$$

Due to the B₀ inhomogeneity, nearest extrapolation and spline interpolation were applied for further quantification. Correlation coefficients (CC) were calculated based on the 2-D correlation analysis function in Matlab, with the averaged Z-spectrum across pixels in the cortex of kidneys selected as seed.

Conventional asymmetric magnetization transfer ratio (MTR_{asym}) (30) was calculated by

$$MTR_{asym} = \frac{[S(-\Delta\omega) - S(\Delta\omega)]}{S_o} \quad (2)$$

Where $S(\omega)$ and $S(-\omega)$ were the signals at positive offset and its counterpart at negative offset, respectively.

The MT effect from immobile macromolecules (MT_{IM}) was calculated at RF offset 1500 Hz by

$$MT_{IM} = \frac{[S_o - S(1500 \text{ Hz})]}{S_o} \quad (3)$$

NOE* map was calculated from three points in Z-spectrum

$$NOE^* = \frac{[Z(-600 \text{ Hz}) + Z(-1500 \text{ Hz})]}{2} - Z(-990 \text{ Hz}) \quad (4)$$

Due to the overlap of peaks, the CEST spectrum was fit to 5 components over the offset range from -1500 to 1500 Hz. The peak fitting algorithm operated by inverting the Z-spectra between -1500 to 1500 Hz and removing the remaining baseline to reduce solid MT effects such that the points at -1500 to 1500 Hz corresponded to 0 amplitude (31). A non-linear

optimization algorithm was applied to decompose the baseline-corrected signal into its overlapping components. The lowest root mean square (RMS) of residuals between the data and model in the selected segment was reached after the model fitting. In addition to the 4 peaks representing the direct effect, amide, amine and aliphatic peaks (31), an additional peak for hydroxyl was added. Signals were fit to the sum of 5 Lorentzian peaks:

$$signal(\Delta) = 1 - \sum_{i=1}^n A_i \left(1 + \left(\frac{\Delta - \Delta_{oi}}{0.5 * W_i} \right)^2 \right)^{-1} \quad (5)$$

where Z-spectrum is a function of RF offset (Δ), peak full width at half maximum (W) and peak amplitude (A). The peak resonance frequency (Δ_{oi}) was around -3.3, 0, 1.2, 2.2, and 3.5 ppm RF offset for the aliphatic, free water, hydroxyl, amine and amide proton pools, respectively. The peak amplitude of the resolved peak around ~1.2 ppm was associated with hydroxyl proton pool, and it was used to evaluate the relative level of glucose/glycogen in the kidney of DN. Student's t-tests were used for statistical testing, and were considered significant when $p < 0.05$. The correlation coefficient ρ was calculated using the Pearson correlation function.

RESULTS

Characteristic features of non-diabetic mouse kidney

The patterns of Z-spectra and distributions of MT_{IM} , NOE^* and MTR_{asym} values in the corresponding maps were regionally dependent on solid component MT effects from immobile macromolecules, NOEs from mobile aliphatic macromolecules, and CEST effects from mobile molecules with exchangeable protons. An average Z-spectrum and its corresponding MTR_{asym} curve of non-diabetic (db/m) mouse kidney were shown in Figure 1a. CEST effects were observed at RF offsets around 1.2, 2.2 and 3.5 ppm (asterisks), which are in general from exchangeable hydroxyl, amine and amide proton pools of mobile molecules, respectively. Non-CEST features were also apparent in the Z-spectrum of non-diabetic (db/m) kidney tissue, such as signal contributions from solid MT effect (MT_{IM} , indicated by 2-head blue arrow) and possible intramolecular and intermolecular NOEs (indicated by 1-head black arrow). Regional comparisons of Z-spectra and MTR_{asym} curves were conducted in the same kidney (Figure 1b). The cortex, outer medulla (OM), and inner medulla and papilla (IM+P) were identified from the T_2 -weighted image as shown in the top panel of Figure 1c. The IM+P region showed bigger MTR_{asym} values, lower NOE , and lower solid MT effects than OM and cortex (Figure 1c), though all the MTR_{asym} values observed were lower than 0.1.

CEST imaging of diabetic mouse kidney

CEST, NOE and solid MT effects were also observed in diabetic (db/db) mouse kidneys (Figure 2a), where the extra-renal space around IM+P showed very high MTR_{asym} contrast (Figure 2b–c). This is probably due to urine with high glucose content that is present in the renal pelvis. Blood vessels are also distributed along the IM+P (Supporting Figure S1); therefore, elevation of blood glucose may also contribute to the high CEST effects in IM+P.

However, in diabetic animals, urine glucose concentration is 10 times higher than the blood glucose level (32) and diabetic polyuria promotes urine retention in the pelvis. Therefore, it is more likely that high CEST effects in IM+P were caused by the urine. In this context, it is of note that CEST effects in extra-renal space were not significantly different from those in kidney tissue in the non-diabetic mice in which urinary glucose excretion is highly limited (Figure 1c). The Z-spectra became more asymmetric from cortex to IM+P (Figure 2b), and extra-renal space showed the most asymmetric Z-spectrum. This pattern was evident in MTR_{asym} curves (Figure 2b).

A pixel-by-pixel correlation analysis of the Z-spectrum can provide a global metric showing regional differences in molecular composition. With the averaged Z-spectrum of kidney tissue selected as the candidate reference, a correlation map was obtained for the global comparison of the features from Z-spectra. While the pixels in IM+P, OM and cortex regions showed correlation coefficients (CC) values close to 1, the extra-renal region showed lower CC values (Figure 2c). This region exhibited weaker correlation to kidney tissue perhaps because of the different molecular composition of urine in diabetic mice, which has much lower MT and NOE, and higher CEST effects than those of kidney tissue. This region was excluded from further regional comparison. Further, the fact that IM+P and extra-renal space showed low MT_{IM} and NOE^* (Figure 2c) indicates that these regions have less macromolecules and mobile aliphatic compounds, compared to OM and cortex. It was also noted that MTR_{asym} values are negative in some regions due to the contribution of NOE effects on the asymmetry analysis (Figure 2b).

In diabetic kidney, MTR_{asym} showed the strongest regional contrast at ~ 1.2 ppm RF offset. The IM+P showed the biggest MTR_{asym} values among the three ROIs, and OM showed higher MTR_{asym} at ~ 1.2 ppm RF offset than the cortex. Although the MTR_{asym} values at other RF offsets were not as big as those at ~ 1.2 ppm, the MTR_{asym} maps at 2.2 and 3.5 ppm RF offsets showed similar regional gradients in diabetic kidney. Given the fact that the MTR_{asym} distribution at ~ 1.2 ppm RF offset is associated with the composition of hydroxyls, primarily from glucose or glycogen, these findings suggest that glucose/glycogen content is increased in diabetic kidney.

Comparison of MTR_{asym} Maps in Murine Models of DN

We next characterized the CEST effects of DN in two mouse models, db/db and db/db eNOS $^{-/-}$ mice. The leptin receptor-deficient db/db strain develops hyperglycemia by 8 weeks of age and an increase in urinary albumin excretion is observed as early as 8 weeks (33). The nephropathy in this model slowly progresses and shows mild to moderate renal lesions. In contrast, eNOS-deficient db/db (db/db eNOS $^{-/-}$) mice exhibit advanced nephropathy that resembles human DN, although the severity of diabetes does not differ from that in db/db mice (25,26,34). This model is currently recognized as one of the best models of progressive DN (24,26). The heterozygous (db/m) mouse that shows no phenotype was used as a non-diabetic control. The MTR_{asym} maps were compared across the db/m, db/db and db/db eNOS $^{-/-}$ mice, around 1.2, 2.2 and 3.5 ppm RF offsets (Figure 3a). In the db/m mice, OM and cortex showed very small MTR_{asym} values, while the IM+P exhibited slightly higher MTR_{asym} . Compared with db/m mice, both db/db and db/db eNOS $^{-/-}$ kidneys showed

higher MTR_{asym} in IM+P and OM, especially at 1.2 ppm RF offset, and in db/db eNOS $^{-/-}$ mice an increase in MTR_{asym} was also observed in cortex. All the mice showed higher MT_{IM} (5 ppm RF offset) and NOE^* (−3.3 ppm RF offset) in OM and cortex than those in the IM+P region, and no significant differences were observed in MT_{IM} and the 3-point NOE^* values across models (data not shown). The level of solid and mobile macromolecular pool may not have evident changes, or current CEST imaging is not sensitive enough to detect the changes in solid macromolecular composition and NOE across DN models.

The progression of nephropathy in db/db mice was also studied by longitudinal CEST imaging from week 8 to week 24. As shown in Figure 3b, MTR_{asym} at 1.2 ppm was evidently increased in all regions on the progression of nephropathy in db/db mice, as compared with MTR_{asym} at 2.2 and 3.5 ppm RF offsets. At RF offsets 2.0 and 3.5 ppm, the MTR_{asym} was not altered in the cortex and OM over the period of study. Moderate increases of MTR_{asym} at 2.2 and 3.5 ppm were observed in the IM+P over this period.

Peak decomposition and selection for glucose/glycogen evaluation

To avoid the contamination of the CEST effect from solid component MT effects, NOEs, and overlapping peaks, multiple-pool fitting was applied to the averaged regional Z-spectrum to improve the precision and accuracy of measurements of metabolite variations in DN. The averaged regional Z-spectrum was decomposed into 5 fixed-position Lorentzian line shapes around −3.3, 0.0, 1.2, 2.2 and 3.5 ppm RF offsets, which represent the aliphatic (I), direct saturation on free water (DS), hydroxyl (II), amine (III), and amide (IV) respectively (Figure 4a). Lower panel shows the residuals computed between the sum of the fitted peaks and the original data (Figure 4a). The amplitude and width of the peaks were allowed to vary in achieving the best fit with the lowest root-mean-square (RMS) of residuals. Glucose/glycogen has several hydroxyls and could contribute to CEST effects between 0.5–2.8 ppm (19,35,36), so the high glucose/glycogen level in kidney could broaden peaks and interfere with the contributions of amines, especially at the RF offset ~2.2 ppm. Therefore, the decomposed peak amplitude at ~1.2 ppm should be appropriate for assessing glucose/glycogen levels because it is mainly from hydroxyls.

The averaged CESTR (CEST ratio) values, which were defined as the peak amplitudes of component peaks from the averaged regional Z-spectra, were compared and shown in Figure 4b–d. In non-diabetic mice (Figure 4b), CESTR values of aliphatic, amine, and amide decreased from cortex, OM to IM+P. In contrast, the hydroxyl peaks showed an opposite pattern, increasing from cortex, OM to IM+P (Figure 4b). The largest regional contrast was observed around ~1.2 ppm RF offset, showing relative level of 3.25:1.34:1 for peak amplitudes of IM+P, OM and cortex in the non-diabetic mice. The relative regional CEST contrasts are much smaller around ~2.2 ppm and ~3.5 ppm among IM+P, OM and cortex. The CESTR values for mobile aliphatic protons were high (~10%). The hydroxyl pool showed much lower levels than amides and amines in OM and cortex, with CESTR observed at $3.39 \pm 0.70\%$ and $2.53 \pm 0.68\%$ in OM and cortex respectively. The CESTR values for DS decreased from IM+P to cortex, with peak widths observed at 1.23 ± 0.08 (IM+P), 1.49 ± 0.08 (OM) and 1.61 ± 0.12 (cortex) ppm. The widths of DS peak showed about 2–3 times larger variability than CESTR values across subjects. The regional correlations between selected

parameters of different peaks were shown in Table 1. The aliphatic peak I had strong positive regional correlation with peak IV and negative regional correlation with DS. The regional correlation between peaks III and IV was relatively low. The CESTR of peak II was regionally correlated (Table 1) with DS amplitude ($\rho = 0.818$) and DS width ($\rho = -0.814$), but it showed weak correlation with peak IV ($\rho = -0.344$). In each region, the correlations between CESTR of peak II and DS parameters were weak.

Several changes in CESTR were observed in diabetic mice. First, the CESTR for aliphatic protons (peak I) was significantly ($p < 0.05$) decreased and that for amide (peak IV) was slightly ($p = \sim 0.1$) decreased in the IM+P of diabetic mice (Figure 4c&d), compared to those in db/m mice (Figure 4b). This may be due to increased urine volume in diabetic kidneys. Second, the CESTR at ~ 2.2 ppm RF offset in the diabetic mice qualitatively showed reversed regional gradient from the cortex to IM+P, compared with the non-diabetic mice. This observation is not significant, and it may be due to the increased glucose level in urine. Most importantly, the CESTR from hydroxyl protons (peak II) increased significantly in IM+P in both diabetic mice. Significant increases were observed in the OM and IM+P in db/db mice (Figure 4c) and in the cortex, OM, and IM+P in db/db eNOS $^{-/-}$ mice (Figure 4d), respectively. In diabetic mice, DS did not show significant change in CESTR (Figure 4b–d), but its width increased slightly in cortex and OM ($\sim 5\%$) and significantly in IM+P ($\sim 15\%$). This could be due to coalescence of peaks II and DS with the increased glucose level. Because evident changes were observed for hydroxyls, regional comparisons were focused on glucose/glycogen levels in subsequent analyses.

Regional comparison of glucose/glycogen levels

Glucose can be detected in millimolar concentrations by CEST imaging. Z-spectra of glucose at different concentrations (pH=7) are shown in Supporting Figure S2b. Peak height and area from fitting were compared to MTR_{asym} at ~ 1.2 ppm RF offset (Supporting Figure S2c). A correlation was observed between CEST effects at ~ 1.2 ppm RF offset and glucose concentrations, confirming that CEST contrast can provide an evaluation of glucose level (37).

The relative regional glucose/glycogen levels were compared in db/m, db/db, and db/db eNOS $^{-/-}$ kidneys (Figure 5). The db/m mice showed CESTR $8.21 \pm 1.51\%$, $3.39 \pm 0.70\%$, and $2.53 \pm 0.69\%$ in the IM+P, OM and cortex, respectively. Relative glucose/glycogen level was significantly increased in IM+P in diabetic kidneys, from $8.21 \pm 1.51\%$ (db/m mice) to $15.78 \pm 2.70\%$ (db/db mice) and $17.68 \pm 1.09\%$ (db/db eNOS $^{-/-}$ mice) (Figure 5a). The OM in db/db mice showed a significant increase in glucose/glycogen levels ($5.76 \pm 1.11\%$), while an increase was not observed in cortex ($3.28 \pm 0.83\%$). In db/db eNOS $^{-/-}$ mice, both OM and cortex showed significant increases in glucose/glycogen levels, with CESTR $9.53 \pm 1.01\%$ and $5.28 \pm 0.77\%$ respectively, and the increase in OM was more evident as compared with db/db mice. No significant difference was observed in blood and urine glucose levels between db/db and db/db eNOS $^{-/-}$ mice as reported previously [Blood glucose (mg/dL): db/m 194 ± 31.70 , db/db 384.7 ± 77.36 , db/db eNOS $^{-/-}$ 348.50 ± 81.15 ; Urine glucose (mM): db/m 7.2 ± 1.0 , db/db 582.8 ± 107.0 , db/db eNOS $^{-/-}$ 735.6 ± 38.0] (25,34).

The significant increase of CESTR at ~1.2 ppm was also observed over time in IM+P, OM and cortex in db/db mice (Figure 5b). It is of interest that temporal variation of glucose/glycogen level differs between regions, starting from the IM+P, then OM, and finally cortex. As compared with db/m mice ($9.03 \pm 2.05\%$), a significant increase in the peak amplitude of IM+P was observed in db/db mice at the age of week 12 ($11.77 \pm 2.00\%$, $p < 0.05$). OM was affected significantly in week 16, and its CESTR increased to $5.76 \pm 1.11\%$ compared with db/m control mice ($3.39\% \pm 0.70$). The cortex showed significantly increased CESTR ($5.08 \pm 1.09\%$) in week 24. Age-related changes were not observed in control db/m kidneys.

The conventional MTR_{asym} values at ~1.2 ppm showed quite similar distribution across models and longitudinal trend (Supporting Figure S3) in the IM+P region as what we observed for peak amplitude CESTR from fitting (Figure 5). However, the sensitivity of MTR_{asym} to glucose/glycogen was lower than CESTR, and this is more evident in OM and cortex (Supporting Figure S3). Among the three regions in kidneys, cortex had the lowest hydroxyl level. The B_0 inhomogeneity, MT asymmetry and NOE effects could induce negative MTR_{asym} values at ~1.2 ppm RF offset (Supporting Figure S3).

DISCUSSION

CEST is sensitive to compounds with hydroxyl, amine and amide groups and can provide information on variations of metabolic components during disease progression. Given the fact that DN is associated with changes in renal metabolites, we applied high-field CEST imaging to murine models of DN and evaluated its sensitivity and utility for characterizing this disease. Our data provide the first demonstration that *in vivo* CEST imaging could sense the severity and progression of type II diabetes-associated kidney disease based on the variation of glucose/glycogen composition.

Sensitivity of CEST measurements to DN and serial progress

Prominent CEST effects were observed in diabetic mice for the peak (~1.2 ppm RF offset) that corresponds to the glucose/glycogen (hydroxyl group). The CESTR at ~1.2 ppm RF offset was evidently increased in renal medulla (IM+P and OM) in diabetic (db/db and db/db eNOS $-/-$) mice. In kidney, CEST effects could be produced by metabolites in three tissue components: 1) blood (perfusion), 2) cellular and interstitial components, and 3) urine. The urine of diabetic animals contains a large amount of glucose as compared with non-diabetic animals (32,38). Also, urine is highly concentrated in medulla, especially in IM+P. Accordingly, the urine glucose concentration becomes very high in diabetic animals, about 10 times higher than blood glucose (32). Therefore, it is likely that the CEST effects observed in diabetic renal medulla largely result from high glucose urine. Urine is less concentrated in OM compared with IM+P. This may be the reason why the CEST effects are less evident in OM. Longitudinally, the CESTR at ~1.2 ppm RF offset was progressively increased in IM+P and OM in db/db mice. In the db/db model, hyperglycemia develops by 8 weeks of age and its level is progressively increased as the mice age, especially from 8 to 16 weeks (33,39,40). Therefore, this could be related to the increasing urine glucose levels caused by the progression of diabetes. It is of note that the medulla (IM+P and OM) in db/db eNOS $-/-$ mice showed higher CESTR at ~1.2 ppm RF offset than that in db/db mice,

although no difference was observed in blood glucose levels in these two groups. Severe tubular injury is observed in a part of cortical tubules in db/db eNOS^{-/-} mice (34,41), so this may be due to the increased urine glucose caused by the tubular damage that leads to the reduction of tubular glucose reabsorption. Indeed, the urine glucose level was slightly (~25%) higher in db/db eNOS^{-/-} mice as compared with db/db mice. On the other hand, it was shown that glycogen is predominantly deposited in OM in diabetic kidney (42,43). Therefore, this finding may also indicate the increased glycogen deposition in OM of db/db eNOS^{-/-} mice. In contrast to IM+P and OM, CEST effects (at ~1.2 ppm RF offset) were observed in cortex only in db/db eNOS^{-/-} and old db/db mice. The db/db eNOS^{-/-} mice are known to show lower blood glucose levels than db/db mice, due to prominent hyperinsulinemia (25,34). Also, in db/db mice, no difference was observed in blood glucose levels between 16, 20, and 24 weeks (data not shown)(40). These findings suggest that an increase in CEST effects in cortex may result from the increased glucose uptake by cortical tubules. Indeed, several studies have shown that tubular glucose uptake is increased in diabetic animals (44–46). Further, it was shown that glycogen deposition extends to proximal tubules as the DN progresses (42,43). It will be of interest to investigate whether eNOS deficiency or disease progression increases cortical tubular glucose uptake and glycogen deposition in diabetic mice. Thus, assessment of regional CEST effects in diabetic kidney may enable more comprehensive evaluation of DN and could be used as an imaging biomarker of this disease. The CESTR in cortex and OM may be used as a marker to distinguish moderate and advanced DN. In this context, it is of note that totally different pattern of CEST effects were observed in animal models of other renal disease (Wang F., unpublished data). Establishing the links between the CEST data and biochemical, functional, and histopathological renal changes should be a subject of future investigation.

Regional parameter correlation

A positive regional correlation between the amide and aliphatic CESTR (Figure 4 and Table 1) could be associated with the fact that every amino acid contains an aliphatic and amide group (31). When the glucose level is high, the correlations between CESTR values of peak III (~2.2 ppm RF offset) and other CEST peaks could be largely affected. The regional correlations between CESTR of peaks III and IV in kidneys are relatively weak. While peak IV is predominated by amides on the protein backbone, other non-protein sources such as creatine and glucose could have large contributions to peak III (36). Among the three regions, IM+P showed the longest T_1 and T_2 times for non-diabetic db/m mice (5). The amplitude of the CEST spectrum at different RF offset is a function of the resonance frequency, relaxation rates, concentration and exchange rate constant. The regional correlations between CESTR values of peaks I-IV and DS parameters could be related to the vast regional difference in tissue type and urine distribution in murine kidney.

Peak decomposition vs. conventional MTR_{asym}

The origin of this study is to assess the entire CEST spectrum and thereby evaluate the changes at different RF offsets associated with diabetic kidney disease. The CESTR from peak-fitting approach provides a semi-quantitative measure, without adjusting the relaxation effects and the MT contribution determined by quantitative MT (47). The effects of signal drift, B_0 inhomogeneity, MT, fixed-position approach, line shape and number of peaks in the

peak-fitting procedure have been assessed previously (31). Fast exchange rate, strong RF saturation pulse and the exchanging pool too close to the water resonance can result in coalescence and induce crosstalk among the pools. High saturation power could be less enlightening in resolving different pools using a peak-fitting approach, because of the reduced SNR and insufficient peak separation. To reduce the data acquisition time for assessing glucose/glycogen CEST effects using this procedure, larger intervals can be considered for RF offsets close to the 2 ends of Z-spectrum.

The MTR_{asym} can provide a quick means to determine CEST effects. However, the MTR_{asym} measure could be affected by B_0 inhomogeneity, asymmetry of MT and NOE effects in tissue. Thus the non-specific MTR_{asym} could conflict with its intended use as a metric assessing CEST effects, especially when NOE effects have big contribution. At high CEST saturation power, MT asymmetry, NOE, and APT effects are relatively small compared to CEST effects from fast exchanging pools (48). Thus higher saturation power is more ideal for detecting most CEST contrasts of hydroxyl protons using MTR_{asym} (36,48). With improved sensitivity of MTR_{asym} , data acquisition for assessing glucose/glycogen level could be narrowed down to a small range of RF offsets and data analysis would be simplified.

Challenge in CEST imaging of mouse diabetic kidney at high field

CEST imaging relies on the ability to acquire of high-resolution images free from artifacts from small mouse kidneys *in vivo*. We had several reasons for selecting respiration triggered multi-shot SE-EPI with fat saturation for studying mouse diabetic kidney disease. EPI is fast but signals from lipids can contaminate EPI data, so suppression of lipid artifacts is important (49). While kidney imaging is sensitive to body motion related to respiration, respiration triggered data acquisition approach helps to reduce motion artifacts. Local B_0 homogeneity is critical and multi-shot EPI can reduce B_0 -related artifacts (50). The RMS of B_0 was ~50 Hz ($10 \times 30 \times 30 \text{ mm}^3$ voxel), but B_0 could be larger than 120 Hz for pixels at the edge of kidney, especially for the left kidney adjacent to spleen, which stores red blood cells. Successful CEST imaging was restricted to the homogeneous B_0 region, because B_0 corrections were not efficient in regions when the deviations from nominal values were extremely large (Figure 2c).

Even though high SNR offered at high fields permits high resolution, partial volume averaging still may have impact on regional quantification in small mouse kidneys. While ROIs were selected based on T_2 -weighted images and CEST maps in this study, the regional analysis could be affected by motion and partial volume effects. We aim in future work to increase resolution to improve the precision and accuracy of CEST imaging.

CONCLUSIONS

In conclusion, murine diabetic kidney disease shows significant CEST effects at the peak (~1.2 ppm RF offset) that corresponds to glucose/glycogen. These CEST effects are associated with the severity and progression of the disease. This imaging technique may facilitate more comprehensive assessment of this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative features of CEST imaging in non-diabetic mouse kidney
 (a) Example Z-spectrum (○) and conventional MTR_{asym} curves (□) of kidney tissue. 2-head arrow: MT effect from immobile macromolecules (MT_{IM}), 1-head arrow: Nuclear Overhauser Enhancement (NOE). Asterisks indicate the CEST effects around 1.2, 2.2 and 3.5 ppm RF offsets. (b) Regional differences in B_0 -corrected Z-spectra and MTR_{asym} curves. IM+P (magenta), OM (blue), and Cortex (black). IM+P showed bigger CEST effects especially at ~1.2 ppm RF offset (arrow) than OM and cortex. (c) T₂-weighted image, MTR_{asym} maps at 1.2 ppm, 2.2 ppm, and 3.5 ppm RF offsets, 3-point NOE* map at -3.3 ppm RF offset, and map for solid MT effect at 5 ppm. 1–Cortex, 2–Outer Medulla (OM), 3–Inner Medulla and Papilla (IM+P), and 4–Extra-renal Space. The IM+P region (arrow) shows bigger MTR_{asym} values, lower NOE, and lower solid MT effects than OM and cortex. Non-diabetic db/m mouse at 16 weeks of age is shown.

Figure 2. Representative features of CEST imaging in diabetic mouse kidney

(a) Example Z-spectrum (○) and conventional MTR_{asyM} curves (□) of kidney tissue. 2-head arrow: MT effect from immobile macromolecules (MT_{IM}), 1-head arrow: Nuclear Overhauser Enhancement (NOE). Asterisks indicate the CEST effects around 1.2, 2.2 and 3.5 ppm RF offsets. (b) Regional differences in B_0 -corrected Z-spectra and MTR_{asyM} curves. Extra-renal Space (red), IM+P (magenta), OM (blue), and Cortex (black). (c) T₂-weighted image, correlation map showing correlation coefficient (CC) of Z-spectra at each pixel, with the averaged spectrum of kidney tissue selected as the seed for correlation analysis, MTR_{asyM} map at 1.2 ppm, 2.2 ppm, and 3.5 ppm RF offsets, 3-point NOE^* map at -3.3 ppm RF offset, and map for MT effect of immobile macromolecules (MT_{IM}) at 5 ppm RF offset. The db/db mouse at the age of 20 weeks is shown as an example. 1-Cortex, 2-Outer

Medulla (OM), 3–Inner Medulla and Papilla (IM+P), and 4–Extra-renal space. The white arrows indicate the artifacts from B_0 inhomogeneity, adjacent to spleen.

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Figure 3. Comparison of renal MTR_{asymp} maps in mouse models of DN

(a) Comparison of renal MTR_{asymp} maps between db/m, db/db, and db/db eNOS^{-/-} mice at ~16 weeks of age. (b) Longitudinal comparison of renal MTR_{asymp} maps in db/db mice.

Figure 4. Lorentzian decomposition and comparison of the averaged regional CEST features in mouse models of DN

(a) The peak decomposition of Z-spectrum in IM+P from model fitting, with peaks assigned at offset frequencies of -3.3 , 0 , 1.2 , 2.2 and 3.5 ppm for the aliphatic (I), direct saturation on free water (DS), hydroxyl (II), amine (III) and amide (IV) proton pools, respectively. The db/m mouse kidney is shown as an example. (b–d) Comparison of the averaged CESTR (percent peak amplitude of decomposed peak) across disease models ($n=12$ for db/m at the age from week 8 to week 24, $n=6$ for db/db and db/db eNOS^{-/-} at the age of 16 weeks). The data show mean $\pm$ SD. * $p<0.05$ vs. corresponding value in db/m mice.

Figure 5. Comparison of regional glucose/glycogen level

(a) Across disease models db/m (n=12, 8–24 weeks), db/db (n=6, 16 weeks), and db/db eNOS^{-/-} (n=6, 16 weeks). The data show mean $\pm$ SD. *p<0.05 vs. corresponding value in db/m mice. (b) Longitudinal comparison in db/db model (n=6). The data show mean $\pm$ SD. *p<0.05 vs. corresponding value in control (db/m) mice. The CESTR indicates the percent peak amplitude around ~1.2 ppm RF offset from model fitting, which is used for evaluating the relative glucose/glycogen level.

Table 1

Regional correlations between parameters

Parameter	CESTR I	CESTR II	CESTR III	CESTR IV	CESTR DS	Width DS
db/m	CESTR I	1.000	0.556	0.704	-0.777	0.553
	CESTR II	-0.588	1.000	-0.344	0.818	-0.814
	CESTR III	0.556	-0.579	1.000	-0.610	0.601
	CESTR IV	0.704	-0.344	0.580	-0.693	0.279 *
	CESTR DS	-0.777	0.580	1.000	1.000	-0.685
db/db	Width DS	0.553	-0.814	0.601	-0.685	1.000
	CESTR I	1.000	-0.750	0.614	-0.829	0.758
	CESTR II	-0.750	1.000	-0.219 *	0.733	-0.894
	CESTR III	-0.522	0.222 *	1.000	0.439 *	-0.259 *
	CESTR IV	0.614	-0.219 *	-0.431 *	-0.207 *	0.236 *
db/db eNOS-/-	CESTR DS	-0.829	0.733	1.000	1.000	-0.788
	Width DS	0.758	-0.894	-0.259 *	-0.788	1.000
	CESTR I	1.000	-0.800	0.236 *	-0.864	0.682
	CESTR II	-0.800	1.000	0.391 *	0.902	-0.538
	CESTR III	-0.057 *	0.223 *	-0.385 *	0.155 *	-0.382 *
	CESTR IV	0.391 *	-0.151 *	1.000	-0.305 *	0.317 *
	CESTR DS	-0.864	0.902	-0.305 *	1.000	-0.587
	Width DS	0.682	-0.538	-0.382 *	-0.587	1.000

Note: Regional Pearson correlation coefficients (ρ) between parameters of different peaks were calculated. Peaks I, DS, II, III, and IV were around -3.3, 0, 1.2, 2.2, 3.5 ppm RF offsets respectively. CESTR and Width were peak amplitude and width from fitting, and parameters of IM+P, OM and cortex for db/m (n=12), db/db (n=6), and db/db eNOS-/- (n=6) were included in the Pearson correlation analysis.

* The correlation is NOT significantly different from zero ($p > 0.05$).