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Chemical Exchange Saturation Transfer (CEST) MR Technique for Liver Imaging at 3.0 Tesla: an Evaluation of Different Offset Number and an After-Meal and Over-Night-Fast Comparison

Min Deng¹, Shu-Zhong Chen¹, Jing Yuan², Queenie Chan³, Jinyuan Zhou^{4,5}, and Yi-Xiáng J. Wáng¹

¹Department of Imaging and Interventional Radiology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR

²Medical physics and research Department, Hong Kong Sanatorium & Hospital, Happy Valley, Hong Kong SAR

³MR Clinical Science, Philips Healthcare Greater China

⁴Department of Radiology, Johns Hopkins University, Baltimore, MD, USA

⁵F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA

Abstract

Purpose—To explore whether Chemical Exchange Saturation Transfer (CEST) MRI can detect liver composition changes between after-meal and over-night-fast statuses.

Procedures—15 healthy volunteers were scanned on a 3.0 T human MRI scanner in the evening 1.5–2 hour after dinner and in the morning after over-night (12-hour) fasting. Among them seven volunteers were scanned twice to assess the scan-rescan reproducibility. Images were acquired at offsets ($n=41$, increment=0.25ppm) from -5 to 5ppm using a turbo spin echo (TSE) sequence with a continuous rectangular saturation pulse. Amide proton transfer-weighted (APT_w) and GlycoCEST signals were quantified with the asymmetric magnetization transfer ratio (MTR_{asym}) at 3.5ppm and the total MTR_{asym} integrated from 0.5 to 1.5ppm from the corrected Z-spectrum, respectively. To explore scan time reduction, CEST images were reconstructed using 31 offsets (with 20% time reduction) and 21 offsets (with 40% time reduction), respectively.

Results—For reproducibility, GlycoCEST measurements in 41 offsets showed the smallest scan-rescan mean measurements variability, indicated by lowest mean difference of -0.049% (95% limits of agreement: -0.209% to 0.111%); for APT_w, the smallest mean difference was found to be 0.112% (95% limits of agreement: -0.698% to 0.921%) in 41 offsets. Compared with after-meal, both GlycoCEST measurement and APT_w measurement under different offset number decreased after 12-hour fasting. However, as the offsets number decreased (41 offsets vs. 31 offsets vs. 21 offsets), GlycoCEST map and APT_w map became more heterogeneous and noisier.

Conclusion—Our results show that CEST liver imaging at 3.0 T has high sensitivity for fasting.

Keywords

Magnetic resonance imaging; Chemical Exchange Saturation Transfer (CEST); Liver; Glycogen; Amide Proton Transfer (APT)

Introduction

Chronic liver diseases, including simple steatosis (SS), nonalcoholic steatohepatitis (NASH), and liver fibrosis are major public health problems worldwide [1]. To date, noninvasive diagnostic tests clinically available are not yet sensitive or specific enough to detect non-symptomatic liver injury at early and sometimes intermediate stages. The diagnostic value of liver function examinations, serological tests of specific serum biomarkers, and liver stiffness measurement by ultrasound or by magnetic resonance imaging (MRI) in evaluation of liver fibrosis is still under investigation and validation [2–6]. Liver biopsy is invasive and associated with potential complications, sampling variability, and high level of interpretation variation [7, 8]. A noninvasive and quantitative technique for assessing liver fibrosis and monitoring disease progression or therapeutic intervention is highly desirable.

Chemical exchange saturation transfer (CEST) represents a novel MRI contrast mechanism [9–13]. CEST MRI has the advantage of high specificity to certain low-concentration biochemistry components such as protein [9, 10], glycosaminoglycan (GAG), glycogen [14], glutamate [15] and glucose [16, 17]. The pH-based amide proton transfer (APT) effect was first demonstrated in the rat brain during several physiological alterations in 2003 by Zhou *et al* [10]. After this, the protein content-based APT-weighted (APT_w) contrast was successively produced in the rat 9L gliosarcoma model and in patients with brain tumors [9, 18–20]. In 2007, van Zijl *et al* proposed the principle of endogenous Glycogen CEST (GlycoCEST) imaging and demonstrated that glycogen in the excised perfused mouse liver was detectable at 4.7T [14]. In 2008, Ren *et al* [21] demonstrated the distribution of glucose in livers using an exogenous paramagnetic CEST sensor, also on perfused mouse liver at 4.7T. Recently, Sagiya *et al* [22] for the first time reported the feasibility of *in vivo* GlycoCEST imaging in mice at 9.4T, and the observed temporal change in the MTR_{asym} at 1.25 ppm was suggested to reflect the alteration of hepatic glycogen levels by fasting and/or re-feeding. Bawden *et al* [23] reported that PRESS-CEST spectroscopy for simultaneously measuring local glycogen and lipid levels.

The feasibility of GlycoCEST imaging for *in vivo* liver has not been demonstrated at relatively lower magnetic field strengths for clinical scanners, such as 1.5T and 3T, where the GlycoCEST MR signal supposes to be much lower than at dedicated ultra-high field MRI scanner for research. Meanwhile, the GlycoCEST signal at clinical field strength is more likely contaminated by direct saturation (DS) of water and other CEST pools resonating at proximate frequencies due to the smaller absolute chemical shift. The reproducibility of GlycoCEST imaging for liver is crucial for its potential use as a reliable clinical imaging biomarker, but has not yet been well examined so far. The purpose of this study was to compare the measurements of liver APT and GlycoCEST effects at over-night

fasting status and post-meal status, as well as the impact of different offset number (41 vs. 31 vs. 21) on liver APT and GlycoCEST measurements.

Materials and Methods

Study subjects

The study was approved by the local human research ethics committee, and informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. A total of 15 volunteers were recruited in this study (8 males and 7 females; mean age: 32 years; age range: 25–52 years). All subjects were clinically healthy, with no liver disease history or alcoholism. Each volunteer underwent scan at two time points: 1.5–2 hour post-dinner and over-night (12-hour) fasting. In order to test the scan-rescan reproducibility of GlycoCEST and APTw MR imaging, 7 out of 15 volunteers underwent scan twice additionally in the morning after light breakfast with a time interval of 7 days.

MR data acquisition

MRI data were scanned using a Philips Achieva 3T scanner (Philips Healthcare, Best, the Netherlands). An 8-channel cardiac coil was used as the signal receiver, and a body coil was used as radiofrequency transmitter. After conventional T2 weighted images to localize the anatomy Turbo spin echo factor (TSE) = 54; TR (time of repetition)/TE (time of echo) = 1236 ms/70 ms; field of view (FOV) = 320×320 mm²; slice thickness=4 mm; pixel size = 1 × 1.5 mm², CEST images were acquired using a single-slice TSE sequence with chemical shift-selective fat suppression. A continuous-wave rectangular RF pulse was performed for saturation, with a B₁ field strength of 3 μT which was identical to the approach used by van Zijl *et al* [14] and a fixed duration of 300 ms, the longest allowable continuous RF duration on our scanner. Localized high-order shimming was applied for CEST imaging to reduce the static magnetic field inhomogeneity B₀. Major imaging parameters were as follows: TSE factor = 16; TR=2350 ms; TE = 6 ms; FOV = 320×320 mm²; pixel size = 2 × 2 mm²; slice thickness = 4 mm; NSA = 1; the total data acquisition time was 4 min and 58 sec. Baseline image was obtained first without using saturation pulse, and the saturated images were then acquired at the 41 offsets of 0, ± 0.25, ± 0.5, ± 0.75, ± 1, ± 1.25, ± 1.5, ± 1.75, ± 2, ± 2.25, ± 2.5, ± 2.75, ± 3, ± 3.25, ± 3.5, ± 3.75, ± 4, ± 4.25, ± 4.5, ± 4.75, ± 5 parts per million (ppm). The saturated images at positive and negative offsets were acquired in an interleaved fashion. The images were acquired with a breath-hold technique. The method for breath-hold technique has been described elsewhere [27].

Z-spectrum analysis

Data analysis was performed using home-developed Matlab (MathWorks, Natick, MA, USA) programs. The Z-spectrum was first least-square fitted by a 12th-order polynomial model and interpolated to a finer resolution of 0.001 ppm in each voxel. The actual water resonance was assumed to be at the frequency associated with the lowest intensity of the fitted Z-spectrum. To correct for the field inhomogeneity B₀, the interpolated Z-spectrum

was shifted to the 0 ppm of the offset axis. The magnitude of the CEST effect was quantified as a magnetization transfer asymmetry ratio (MTR_{asym}):

$$MTR_{asym}(\Delta\Omega) = \frac{S(-\Delta\Omega)}{S_0} - \frac{S(\Delta\Omega)}{S_0}$$

where Ω is the shift difference between irradiation frequency and the water resonance, S and S_0 donate the saturated and nonsaturated image intensities. The MTR_{asym} at 3.5ppm was quantified as APT-weighting (APT_w) value and the mean MTR_{asym} in the frequency range

$\Omega=0.5$ to 1.5 ppm was quantified as GlycoCEST value by referring to the reported ¹H MR spectrum peaks of liver Glycogen ranging from 3.25 to 3.94ppm (water resonance at 4.7ppm) at 3T [24]. The voxel-wise coefficient of determination R^2 was also calculated to evaluate the goodness-of-fit of Z-spectrum fitting. The voxel which showed relatively poor goodness-of-fit ($R^2 < 0.95$) would be excluded from analysis, thereby excluding the artifacts and blood vessels.

To explore the possibility of reducing the offset number to shorten scan time, specific offsets were retrospectively excluded manually in the Z-spectrum analysis. As shown in Fig. 1, in 31 offsets, 10 offset points ($\pm 1.75, \pm 2.25, \pm 2.75, \pm 4.25, \pm 4.75$ ppm) were excluded from the original 41 offsets. The offsets around 3.5 and 0.5–1.5 ppm were retained in order to ensure the accuracy of the GlycoCEST and APT_w calculation. In 21 offsets, every other offset point was excluded from the original 41 offsets until half of the offset points were eliminated. After excluding the specific offsets from the original 41 offsets, 31 offsets (0, $\pm 0.25, \pm 0.5, \pm 0.75, \pm 1, \pm 1.25, \pm 1.5, \pm 2, \pm 2.5, \pm 3, \pm 3.25, \pm 3.5, \pm 3.75, \pm 4, \pm 4.5, \pm 5$ ppm) and 21 offsets (0, $\pm 0.5, \pm 1, \pm 1.5, \pm 2, \pm 2.5, \pm 3, \pm 3.5, \pm 4, \pm 4.5, \pm 5$ ppm) were used in the Z-spectrum analysis and CEST map generation. The data acquisition time was 4:48 min for 41 offsets, and theoretically 3:58 min for 31 offsets, and 2:58 min for 21 offsets, respectively.

The data were analyzed by one Radiology trainee with more than 3 years' experiences reading abdominal MRIs. Five ROIs of approximately 200–300 mm² were manually placed on liver parenchyma region of T2 weighted anatomical images excluding observable artifacts and blood vessels, and then transferred onto the GlycoCEST image and APT_w maps (Fig. 2). The mean value of 5 ROIs was regarded as the liver GlycoCEST and APT_w value for the subject. To analyze the CEST image difference in different offset number, the GlycoCEST image and APT_w maps generated by 31 offsets and 21 offsets were also reconstructed. Same ROIs were copied onto the CEST maps of the same subject to measure the MTR_{asym} value in different offset number.

Statistic analysis

Data were presented as mean $\pm$ standard deviation, if not indicated otherwise. Wilcoxon Signed Ranks Test was used for MTR_{asym} value comparison in different status and offset number. Scan-rescan reproducibility was tested using intraclass correlation coefficient (ICC) and Bland-Altman analysis. According to Fleiss, ICC values > 0.75 represent a good agreement, and ICC values between 0.4 and 0.75 represent fair to moderate agreement [25].

Agreement of MTR_{asym} value measurements using different offset points were also evaluated by ICC. Bland-Altman analysis was performed with home-developed Matlab (MathWorks, Natick, MA, USA) program, all other statistical analyses were carried out using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA). A p -value < 0.05 was defined as statistical significance.

Results

Liver GlycoCEST and APTw MR scan-rescan reproducibility

For GlycoCEST, the ICC value for 41 offsets, 31 offsets, and 21 offsets was 0.758, 0.506, and 0.459, respectively (Table 1), while for APTw the ICC value for 41 offsets, 31 offsets, and 21 offsets was 0.835, 0.570, and 0.408, respectively (Table 1). The scan-rescan mean differences of GlycoCEST and APTw values are shown in Bland-Altman Plots (Fig. 3). For GlycoCEST, the smallest scan-rescan mean measurements variability was shown in 41 offsets, indicated by lowest mean difference of -0.049% (95% limits of agreement: -0.209% to 0.111%). For APTw, the smallest mean difference was found to be 0.112% (95% limits of agreement: -0.698% to 0.921%) in 41 offsets. These results show that liver CEST MRI with 41 offsets offered good scan-rescan reproducibility, while liver CEST MRI with 31 offsets and 21 offsets showed less satisfactory reproducibility. In addition, results obtained with 31 offsets and 21 offsets did not show a recognizable trend of measurement shift pattern (Fig. 5d).

Liver GlycoCEST and APTw MR at Post-meal and Post-Fasting

The results of post-meal and post-fasting GlycoCEST and APTw measurements in 15 subjects are given in Table 2, Fig. 4 and Fig. 5. Compared with post-meal, both GlycoCEST measurement and APTw measurement decreased post 12 h fasting. This downward trend was shown in all three numbers of offsets. GlycoCEST demonstrated greater decrease ratio of MTR_{asym} value than APTw post 12 h fasting, however, the difference of decrease ratio between GlycoCEST and APTw did not reach statistical significance ($= 0.427$ in 41 offsets, $p = 0.394$ in 31 offsets, $p = 0.191$ in 21 offsets). Typical GlycoCEST map and APTw map of a healthy volunteer with the corresponding Z-spectrum and MTR_{asym} spectrum is shown in Fig. 6. It can be visually observed that as the offset number decreases, GlycoCEST map and APTw map become more heterogeneous and noisier.

Discussion

Conventional MR cannot detect diffused liver diseases, or the liver parenchyma exhibit only subtle, nonspecific heterogeneity. A number of MR imaging techniques have been investigated to assess early liver parenchyma fibrosis, including $T_1\rho$ imaging [26–31], tagged MRI assessing liver strain [32], MR elastography [33, 34] and intravoxel incoherent motion (IVIM) technique [35, 36]. Our current study for the first time evaluated the feasibility of CEST imaging of *in vivo* liver at the clinical field strength of 3T, demonstrating the difference between over-night-fast and post-meal statuses. Consistent well with the previous *in vivo* liver GlycoCEST studies [22, 23], positive GlycoCEST MTR_{asym}

was observed after dinner, and GlycoCEST MTR_{asym} reduced considerably after 12 h fasting. This reduction of GlycoCEST MTR_{asym} after fasting is believed at least partially to reflect the reduction of hepatic glycogen levels, and such constitute a proof-of-concept of CEST imaging clinically. We previously showed that T1rho relaxation time may be a sensitive imaging biomarker for liver fibrosis [28]. However, T1rho did not show difference livers before breakfast and after dinner [27]. Our results in this study may incur that CEST imaging may be even more sensitive than T1rho in assessing biochemical change of liver compositions.

Besides the GlycoCEST effect, positive MTR_{asym} at 3.5ppm was also observed in human livers. Although this MTR_{asym} was termed as APTw by convention due to its Z-spectrum location at 3.5 ppm in the study, the chemical component source and mechanism attributed to this MTR_{asym} may be complicated. Particularly, this MTR_{asym} may be contaminated with the nuclear Overhauser enhancement (NOE) effect upfield at -3.5 ppm from the water resonance due to various mobile to less mobile molecules [37–39] or even with the pseudo NOE signal if the fat suppression is not used [40]. Ceramides (Cers) might be postulated to contribute to this MTR_{asym} , which are a lipid species that exert biological effects in nonalcoholic fatty liver disease through cellular proliferation, differentiation, and cell death [41]. However, since the fat suppression was applied prior to acquisition, this MTR_{asym} should not be dominated by the contamination of lipid at -3.5 ppm relative to water resonance, the opposite side on Z-spectrum [40]. Therefore, the contribution of possible upfield nuclear Overhauser enhancement (NOE) effects to the MTR_{asym} difference should be small [37, 38]. According to a 500 MHz proton NMR study of Cer and its analogs in deuterated chloroform ($CDCl_3$) at 25° C by Li et al [42], -NH in Cers has a chemical shift of ~6.3 ppm, about 1.6 ppm relative to water (4.7 ppm chemical shift). Considering the lower resonance of 128 MHz at 3T and the observation of broad baseline positive MTR_{asym} peak above 1.5 ppm (Fig. 6c), it is possible that -NH has concomitant spill-over effects at higher chemical shifts and induces positive MTR_{asym} . However, despite the benefit of pseudo-NOE effect minimization for APT by using fat suppression, it is also worth noting that fat suppression may induce extra water direct saturation and slightly reduce the general image SNR, and thus influence MTR_{asym} quantification, in particular for GlycoCEST as its resonance is so close to water. Consequently, the thorough investigation of the influence of fat suppression on CEST imaging and analysis is yet to be conducted.

Acquisition protocol is crucial for CEST studies and needs to be well determined. Ideally, APT and GlycoCEST should be acquired by individually optimized protocols based on their distinctive exchange properties. A same acquisition protocol for both APT and GlycoCEST was used mainly to save the total scan time and avoid the spatial mismatch due to motion, but would definitely compromise the analysis due to the quite different exchange characteristics associated with APT and GlycoCEST. The use of 3 μ T saturation strength was adopted from the previous GlycoCEST animal study at 4.7 T [14] and the longest allowable saturation pulse duration of 300ms was applied to achieve the CEST effects as much as possible. For APTw imaging, several previous studies suggested the optimized saturation strength of 2 μ T in the brain to achieve maximum tumor APTw contrast relative to normal tissues although 3 μ T saturation strength could achieve even higher absolute APTw signal. Furthermore, by considering the faster proton exchange rate of Glycogen than amide,

a slightly higher saturation strength of 3 μ T used in this study might be also preferable. Notably, as GlycoCEST effect is closer to the water resonance, the MTR_{asym} values of GlycoCEST could be more likely contaminated by direct saturation and magnetization transfer effects under high power saturation. It was also found (Fig. 6) that the saturated signal at -1 ppm was smaller than 10% of the baseline on the relatively flat Z-spectrum. As such, the predominant direct water saturation and magnetization transfer effects could drastically reduce the quantification accuracy of the small glycoCEST effect. In this aspect, a smaller saturation power should be useful to reduce the direct water saturation and magnetization transfer. Future study on saturation pulse optimization is warranted by investigating how the magnitudes of APTw and GlycoCEST signal change with different saturation powers and durations. An off-resonance spin-lock pulse with short irradiation duration may probably useful to reduce the direct water saturation and give higher sensitivity of GlycoCEST effect. Strategies to shorten the acquisition time while maintain CEST contrast-to-noise ratio should be further investigated [43–46]. One of the approaches is to reduce the offset number. However, our study demonstrated by reducing the offset number from 41 to 31 and 21 decreased scan-rescan reproducibility, while increased the coefficient of variation. Therefore, the results do not favor the reduction of offset number. It is worth noting that the reduction of offsets was conducted retrospectively, the reduced 31 and 21 offsets may not necessarily present the most optimized protocol for CEST acquisition which was beyond the purpose of the current study.

The measured CEST signal and contrast in tissue may be influenced by many factors, including but not limited to mobile proton content, proton exchange rate, tissue relaxation times, semi-solid magnetization transfer, imaging protocol as well as quantification method. As mentioned above, the quantification of the CEST effect by using MTR_{asym} , although effective and widely used, could be contaminated by the upfield NOE effect on Z-spectrum. In particular, at relatively low clinical field strengths like 3T, the intrinsic low CEST signal level and the spill-over effect due to the small absolute chemical shift from water could reduce the accuracy and precision of the MTR_{asym} quantification. In addition, MTR_{asym} quantification is also subject to B_0 and B_1 inhomogeneities. Consequently, we expect that the measured MTR_{asym} for both GlycoCEST and APTw in this study may primarily reflect the comprehensive overall change in liver protein and peptide, and glycogen levels. The individual contribution from each factor needs to be further assessed. Water saturation shift referencing (WASSR) has been proposed for better glycoCEST MTR_{asym} quantification via more accurate B_0 mapping [47]. However its utilization for moving organs like liver might be technically challenging due to motion. Currently, several alternative comprehensive processing approaches have been proposed for better CEST signal analysis [38, 39, 48–50]. However, these methods can be complicated and may require longer acquisition time, and their usefulness for routine practice remains to be further validated.

Conclusion

In summary, this study for the first time showed both APTw and GlycoCEST MR liver imaging on a clinical 3.0 T system is feasible for human subjects. Both APTw and GlycoCEST MR measurements showed high sensitivity for fasting. To translate CEST MR imaging into a practical tool and thereby positively influence clinical management,

technological advancements including acceleration of data acquisition remain to be further exploited [51, 52].

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References

- Wallace K, Burt AD, Wright MC. Liver fibrosis. *Biochem J*. 2008; 411:1–18. [PubMed: 18333835]
- Patel K, Shackel NA. Current status of fibrosis markers. *Curr Opin Gastroenterol*. 2008; 30:253–9.
- Anania C, Pacifico L, Ferraro F, et al. Aspartate transaminase to platelet ratio index (APRI) to assess liver fibrosis in patients with chronic liver disease *Hepat Mon*. 2011; 11:479–80. [PubMed: 22087183]
- Tatsumi C, Kudo M, Ueshima K, et al. Noninvasive evaluation of hepatic fibrosis using serum fibrotic markers, transient elastography (FibroScan) and real-time tissue elastography. *Intervirol*. 2008; 51(Suppl 1):27–33. [PubMed: 18544945]
- Sy S, Huang S, Wang YX, et al. Terahertz spectroscopy of liver cirrhosis: investigating the origin of contrast. *Phys Med Biol*. 2010; 55:7587–96. [PubMed: 21098916]
- Ligabue G, Besutti G, Scaglioni R, et al. MR quantitative biomarkers of nonalcoholic fatty liver disease: technical evolutions and future trends. *Quant Imaging Med Surg*. 2013; 3:192–5. [PubMed: 24040614]
- Janes CH, Lindor KD. Outcome of patients hospitalized for complications after outpatient liver biopsy. *Ann Intern Med*. 1993; 118:96–98. [PubMed: 8416324]
- Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med*. 2001; 344:495–500. [PubMed: 11172192]
- Zhou J, Lal B, Wilson DA, et al. Amide proton transfer (APT) contrast for imaging of brain tumors. *Magn Reson Med*. 2003; 50:1120–1126. [PubMed: 14648559]
- Zhou J, Payen J, Wilson DA, et al. Using the amide proton signals of intracellular proteins and peptides to detect pH effects in MRI. *Nat Med*. 2003; 9:1085–1090. [PubMed: 12872167]
- Yuan J, Chen S, King AD, et al. Amide proton transfer-weighted imaging of the head and neck at 3 T: a feasibility study on healthy human subjects and patients with head and neck cancer. *NMR Biomed*. 2014; 27:1239–47. [PubMed: 25137521]
- Kim M, Chan Q, Anthony MP, et al. Assessment of glycosaminoglycan distribution in human lumbar intervertebral discs using chemical exchange saturation transfer at 3 T: feasibility and initial experience. *NMR Biomed*. 2011; 24:1137–44. [PubMed: 21387446]
- Haneder S, Apprich SR, Schmitt B, et al. Assessment of glycosaminoglycan content in intervertebral discs using chemical exchange saturation transfer at 3.0 Tesla: preliminary results in patients with low-back pain. *Eur Radiol*. 2013; 23:861–8. [PubMed: 23052643]
- van Zijl PC, Jones CK, Ren J, et al. MRI detection of glycogen in vivo by using chemical exchange saturation transfer imaging (glycoCEST). *Proc Natl Acad Sci (USA)*. 2007; 104:4359–64. [PubMed: 17360529]
- Cai K, Haris M, Singh A, Kogan F, Greenberg JH, Hariharan H, et al. Magnetic resonance imaging of glutamate. *Nat Med*. 2012; 18:302–306. [PubMed: 22270722]
- Chan KW, McMahon MT, Kato Y, et al. Natural D-glucose as a biodegradable MRI contrast agent for detecting cancer. *Magn Reson Med*. 2012; 68:1764–73. [PubMed: 23074027]
- Walker-Samuel S, Ramasawmy R, Torrealdea F, et al. In vivo imaging of glucose uptake and metabolism in tumors. *Nat Med*. 2013; 19:1067–72. [PubMed: 23832090]
- Jones CK, Schlosser MJ, van Zijl PC, et al. Amide proton transfer imaging of human brain tumors at 3T. *Magn Reson Med*. 2006; 56:585–92. [PubMed: 16892186]

19. Zhou J, Zhu H, Lim M, et al. Three-dimensional amide proton transfer MR imaging of gliomas: Initial experience and comparison with gadolinium enhancement. *J Magn Reson Imaging*. 2013; 38:1119–28. [PubMed: 23440878]
20. Zhou J, Hong X, Zhao X, et al. APT-weighted and NOE-weighted image contrasts in glioma with different RF saturation powers based on magnetization transfer ratio asymmetry analyses. *Magn Reson Med*. 2013; 70:320–7. [PubMed: 23661598]
21. Ren J, Trokowski R, Zhang S, et al. Imaging the Tissue Distribution of Glucose in Livers Using A PARACEST Sensor. *Magn Reson Med*. 2008; 60:1047–1055. [PubMed: 18958853]
22. Sagiyama K, Zhang S, Dimitrov I, et al. In Vivo Monitoring of Liver Glycogen by Chemical Exchange Saturation Transfer Imaging (GlycoCEST) in Live Mice. *Proc Intl Soc Mag Reson Med*. 2014; 22:0762.
23. Bawden SJ, Mougin O, Hunter K, et al. Simultaneously Measuring Glycogen and Lipid Levels Using Localized CEST Spectroscopy at 3T. *Proc Intl Soc Mag Reson Med*. 2014; 22:3159.
24. Ouwerkerk R, Pettigrew RI, Gharib AM. Liver Metabolite Concentrations Measured with ¹H MR Spectroscopy. *Radiology*. 2012; 265:565–575. [PubMed: 22891360]
25. Fleiss, JL. The design and analysis of clinical experiments. New York: John Wiley & Sons; 1986. p. 1-31.
26. Zhao F, Wang YX, Yuan J, et al. MR T1ρ as an imaging biomarker for monitoring liver injury progression and regression: an experimental study in rats with carbon tetrachloride intoxication. *Eur Radiol*. 2012; 22:1709–16. [PubMed: 22752522]
27. Deng M, Zhao F, Yuan J, et al. Liver T1ρ MRI measurement in healthy human subjects at 3 T: a preliminary study with a two-dimensional fast-field echo sequence. *Br J Radiol*. 2012; 85:e590–5. [PubMed: 22422392]
28. Zhao F, Yuan J, Deng M, et al. Further exploration of MRI techniques for liver T1ρ quantification. *Quant Imaging Med Surg*. 2013; 3:308–15. [PubMed: 24404445]
29. Wang YX, Yuan J, Chu ES, et al. T1ρ MR imaging is sensitive to evaluate liver fibrosis: an experimental study in a rat biliary duct ligation model. *Radiology*. 2011; 259:712–719. [PubMed: 21436087]
30. Wang YX, Yuan J. Evaluation of liver fibrosis with T1ρ MR imaging. *Quant Imaging Med Surg*. 2014; 4:152–5. [PubMed: 24914415]
31. Allkemper T, Sagmeister F, Cicinnati V, et al. Evaluation of Fibrotic Liver Disease with Whole-Liver T1ρ MR Imaging: A Feasibility Study at 1.5 T. *Radiology*. 2014; 271:408–15. [PubMed: 24475807]
32. Mannelli L, Wilson GJ, Dubinsky TJ, et al. Assessment of the liver strain among cirrhotic and normal livers using tagged MRI. *J Magn Reson Imaging*. 2012; 36:1490–5. [PubMed: 22777877]
33. Salameh N, Larrat B, Abarca-Quinones J, et al. Early detection of steatohepatitis in fatty rat liver by using MR elastography. *Radiology*. 2009; 253:90–97. [PubMed: 19587308]
34. Bonekamp S, Kamel I, Solga S, Clark J. Can imaging modalities diagnose and stage hepatic fibrosis and cirrhosis accurately? *J Hepatol*. 2009; 50:17–35. [PubMed: 19022517]
35. Lu PX, Huang H, Yuan J, et al. Decreases in Molecular Diffusion, Perfusion Fraction and Perfusion-Related Diffusion in Fibrotic Livers: A Prospective Clinical Intravoxel Incoherent Motion MR Imaging Study. *PLoS One*. 2014; 9:e113846. [PubMed: 25436458]
36. Yoon JH, Lee JM, Baek JH, et al. Evaluation of hepatic fibrosis using intravoxel incoherent motion in diffusion-weighted liver MRI. *J Comput Assist Tomogr*. 2014; 38:110–6. [PubMed: 24378888]
37. Jones CK, Huang A, Xu J, et al. Nuclear Overhauser enhancement (NOE) imaging in the human brain at 7T. *Neuroimage*. 2013; 77:114–24. [PubMed: 23567889]
38. Jin T, Wang P, Zong X, Kim SG. MR imaging of the amide-proton transfer effect and the pH-insensitive nuclear over hauser effect at 9.4 T. *Magn Reson Med*. 2013; 69(3):760–70. [PubMed: 22577042]
39. Heo HY, Zhang Y, Lee DH, et al. Quantitative assessment of amide proton transfer (APT) and nuclear Overhauser enhancement (NOE) imaging with extrapolated semi-solid magnetization transfer reference (EMR) signals: Application to a rat glioma model at 4.7 T. *Magn Reson Med*. 2015; doi: 10.1002/mrm.25581

40. Lu J, Zhou J, Cai C, et al. Observation of true and pseudo NOE signals using CEST-MRI and CEST-MRS sequences with and without lipid suppression. *Magn Reson Med*. 2014; doi: 10.1002/mrm.25277
41. Pagadala M, Kasumov T, McCullough AJ, et al. Role of ceramides in nonalcoholic fatty liver disease. *Trends Endocrinol Metab*. 2012; 23:365–71. [PubMed: 22609053]
42. Li L, Tang X, Taylor KG, et al. Conformational characterization of ceramides by nuclear magnetic resonance spectroscopy. *Biophys J*. 2002; 82:2067–80. [PubMed: 11916863]
43. Zhao F, Deng M, Yuan J, et al. Experimental evaluation of accelerated T1rho relaxation quantification in human liver using limited spin-lock times. *Korean J Radiol*. 2012; 13:736–42. [PubMed: 23118572]
44. Wang YX, Zhao F, Yuan J, et al. Accelerated T1rho relaxation quantification in intervertebral disc using limited spin-lock times. *Quant Imaging Med Surg*. 2013; 3:54–8. [PubMed: 23482987]
45. Yuan J, Zhao F, Griffith JF, et al. Optimized efficient liver T(1rho) mapping using limited spin lock times. *Phys Med Biol*. 2012; 57:1631–40. [PubMed: 22398137]
46. Zhu Y, Zhang Q, Liu Q, et al. PANDA- T1p: Integrating principal component analysis and dictionary learning for fast T1p mapping. *Magn Reson Med*. 2014; doi: 10.1002/mrm.25130
47. Kim M, Gillen J, Landman BA, et al. Water saturation shift referencing (WASSR) for chemical exchange saturation transfer (CEST) experiments. *Magn Reson Med*. 2009; 61:1441–1450. [PubMed: 19358232]
48. Sun PZ, Benner T, Kumar A, Sorensen AG. Investigation of optimizing and translating pH-sensitive pulsed-chemical exchange saturation transfer (CEST) imaging to a 3T clinical scanner. *Magn Reson Med*. 2008; 60:834–841. [PubMed: 18816867]
49. Zaiss M, Schmitt B, Bachert P. Quantitative separation of CEST effect from magnetization transfer and spillover effects by Lorentzian-line-fit analysis of z-spectra. *J Magn Reson*. 2011; 211:149–155. [PubMed: 21641247]
50. Zaiss M, Bachert P. Chemical exchange saturation transfer (CEST) and MR Z-spectroscopy in vivo: a review of theoretical approaches and methods. *Phys Med Biol*. 2013; 58:R221–R269. [PubMed: 24201125]
51. Pang Y, Jiang X, Zhang X. Sparse parallel transmission on randomly perturbed spiral k-space trajectory. *Quant Imaging Med Surg*. 2014; 4:106–11. [PubMed: 24834422]
52. Pang Y, Yu B, Zhang X. Enhancement of the low resolution image quality using randomly sampled data for multi-slice MR imaging. *Quant Imaging Med Surg*. 2014; 4:136–44.

Fig. 1.
The 41, 31 and 21 offset schemes used in the Z-spectrum analysis and CEST map generation.

Fig. 2.
An example of ROIs placement on human liver parenchyma region of CEST image, excluding artifacts and blood vessels.

Fig. 3.

Bland-Altman plots for the liver GlycoCEST and APTw with 41 offsets, 31 offsets and 21 offsets in 7 volunteers. The scan-rescan differences of MTR_{asym} values are plotted against the average MTR_{asym} values of two scans for each subject. **a** For GlycoCEST in 41 offsets, the scan-rescan 95% limits of agreement ranges from -0.209% to 0.111% (mean difference: -0.049%). **b** For GlycoCEST in 31 offsets, scan-rescan 95% limits of agreement ranges from -0.519% to 0.561% (mean difference: 0.021%). **c** For GlycoCEST in 21 offsets, scan-rescan 95% limits of agreement ranges from -0.688% to 0.412% (mean difference: 0.138%). **d** For APTw in 41 offsets, scan-rescan 95% limits of agreement ranges from -0.698% to 0.921% (mean difference: 0.112%). **e** For APTw in 31 offsets, scan-rescan 95% limits of agreement ranges from -0.805% to 1.819% (mean difference: 0.506%). **f** For APTw in 21 offsets, scan-rescan 95% limits of agreement ranges from -1.607% to 2.092% (mean difference: 0.242%).

Fig. 4. Line plot of liver MTR_{asym} value comparison between post-meal and post-fasting for **a** GlycoCEST and **c** APTw ($n = 15$). Typical liver **b** GlycoCEST image and **d** APTw image of a healthy volunteer at post-meal and post-fasting. For this subject, the post-meal GlycoCEST MTR_{asym} value shows a decrease ratio of 48.2% after 12 h fasting (post-meal: 0.56% vs. post-fasting: 0.29%), while the decrease ratio for APTw MTR_{asym} value is 17.9% (post-meal: 1.51% vs. post-fasting: 1.24%).

Fig. 5. Line plot of human liver MTR_{asym} value of GlycoCEST and APTw at post-meal and post-fasting using **a** 41, **b** 31 and **c** 21 offsets ($n = 15$). **d** Line plot of post-fasting MTR_{asym} value of GlycoCEST and APTw using three different offset number compositions. No apparent trend is shown among the 41 offsets, 31 offsets, and 21 offsets.

Fig. 6.

Typical **a** GlycoCEST image and **b** APTw image of a 30 year old healthy woman using different offset number with the **c** corresponding Z-spectrum and MTR_{asym} spectrum. The black square marker represents the acquired raw data; the red line and pink dot line represent the fitting Z-spectrum and MTR_{asym} spectrum, respectively.

Table 1

Liver GlycoCEST and APTw scan-rescan MTR_{asym} values (%) from different offsets for each subject

Subject No	GlycoCEST 41 offsets		GlycoCEST 31 offsets		GlycoCEST 21 offsets		APTw 41 offsets		APTw 31 offsets		APTw 21 offsets	
	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2
1	0.24	0.40	0.02	0.38	0.05	0.58	2.39	2.93	2.90	3.10	2.46	3.41
2	0.53	0.63	0.48	0.75	0.16	0.67	1.89	1.30	3.62	1.84	2.60	1.36
3	0.34	0.33	0.21	0.35	0.21	0.40	2.03	2.00	1.67	2.05	1.61	2.62
4	0.16	0.24	0.66	0.40	0.62	0.65	0.98	0.42	1.55	0.63	1.52	0.63
5	0.45	0.67	1.03	0.72	1.09	0.78	2.45	2.12	3.12	2.67	3.78	2.41
6	0.56	0.53	0.62	0.63	0.84	0.81	2.49	2.90	2.91	2.27	2.45	2.88
7	0.50	0.51	0.45	0.07	0.40	0.43	1.60	1.39	1.54	1.21	1.68	1.11
mean	0.40	0.47	0.50	0.47	0.48	0.62	1.98	1.87	2.47	1.97	2.30	2.06
SD	0.15	0.16	0.33	0.24	0.38	0.16	0.55	0.91	0.86	0.84	0.80	1.03
CoV	0.39	0.33	0.66	0.52	0.80	0.26	0.28	0.49	0.35	0.43	0.35	0.50
ICC *	0.758		0.506		0.459		0.835		0.570		0.408	

* ICC= Intraclass correlation coefficient; CoV: coefficient of variation

MTR_{asym} values of GlycoCEST and APTw before and after 12 h fasting in different numbers of offsets

Table 2

	GlycoCEST				APTw			
	Post-meal (MTR _{asym} in %)	Post-Fasting (MTR _{asym} in %)	Decrease ratio(%)	p-value	Post-meal (MTR _{asym} in %)	Post-Fasting (MTR _{asym} in %)	Decrease ratio(%)	p-value
41 offsets *	0.71±0.31 (0.22–1.21)	0.19±0.29 (–0.35–0.73)	78.27 (14.29–155.82)	0.001	1.83±0.78 (0.71–3.11)	0.78±0.85 (–0.47–2.26)	65.04 (17.52–139.52)	0.001
31 offsets *	0.81±0.48 (0.10–1.73)	0.18±0.30 (–0.26–0.73)	69.25 (0.82–148.51)	0.001	1.93±0.69 (0.89–3.22)	0.78±0.73 (–0.34–1.87)	60.51 (3.79–129.91)	0.001
21 offsets *	0.67±0.56 (–0.13–2.01)	0.09±0.34 (–0.46–0.65)	97.21 (6.08–257.67)	0.001	2.00±0.90 (0.31–3.52)	0.61±0.81 (–0.75–1.75)	77.46 (2.56–281.82)	0.001

* Data are the means and data in parentheses are the range