# Imaging of glucose uptake in breast tumors using non-labeled D-glucose

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## Introduction

Tumors generally have higher glucose utilization and uptake than normal tissue, which increases following malignant transformation. Increased glucose uptake is a marker of tumor aggression, which can be measured by 18FDG-PET. This radioactive imaging has become the radiological modality of choice for detecting malignancy. Studies have shown the suitability of FDG-PET for detecting and staging primary breast carcinomas and for monitoring response to chemotherapy (1). Other important biomarkers of malignancy are increased permeability of the vascular bed and increased microvessel density (2). Tumor vascular status can be assessed using dynamic contrast-enhanced MRI (DCE-MRI) (2,3), which has been used to determine tumor grade, extent of disease, and treatment response. We proposed to use CEST (Chemical Exchange Saturation Transfer) MRI to detect glucose *in vivo* through the OH resonance (4). This so-called glucoCEST imaging was studied before and after intravenous infusion of glucose at 9.4T in xenografts of a highly aggressive and metastatic experimental breast cancer cell line (MDA-MB-231) in mice.

## Methods

Animal Preparation: MDA-MB-231 human breast cancer xenografts were grown orthotopically in the mammary fat pad of female SCID mice (n =3) Animals were anesthetized and positioned in the RF coil of a 9.4T horizontal bore Bruker Biospec scanner. The tail vein of the animal was catheterized for infusion of 500 mM solution of D-glucose in saline. An initial loading bolus of 0.1 ml was followed by continuous infusion with exponentially decreasing rates from 0.5 ml/h to 0.05 ml/h to maintain a target blood glucose concentration of 20 mM. The protocol was optimized in a bench experiment using serial samples of venous blood analyzed on our blood analyzer (Radiometer ABL700). CEST imaging was followed by GdDTPA at 0.2 mmol/kg to measure tumor viability and contrast uptake pattern.

*In Vivo* CEST: CEST imaging was conducted through collection of two sets of saturation images, which are a water saturation shift referencing (WASSR) set for $B_0$ mapping (5) and a glucoCEST set for characterizing contrast. The method was first optimized with glucose phantoms. For the WASSR images, the saturation parameters were $t_{sat}$=250 ms, B1 =1.0 μT, TR=1.5 sec with saturation offset incremented from -2 to +1ppm with respect to water in 0.1ppm steps, while for the CEST images: $t_{sat}$=3.5 sec, B1 =3.0 μT, TR=6 sec, with offset from -4.9 to -2.0 (0.4 ppm steps), from -1.8 to +1.8 (0.2 ppm steps), and from 2.0 to +4.9ppm (0.4 ppm steps) with a fat suppression pulse (3.4 ms hermite pulse, offset=-3.5 ppm). The acquisition parameters were: TR=5.0 sec, effective TE= 21.6 ms, RARE factor=8, pixel size: 0.2x0.5mm$^2$, slice thickness=1.5 mm; 29 frequency points.

Data Analysis: Data were processed using Matlab scripts and the CEST contrast was quantified by calculating the asymmetry in the magnetization transfer ratio ($MTR_{asym}$ =$S(-\Delta\omega) - S(\Delta\omega)/S_0$) ROI masks were drawn manually based on the T2w images to cover the areas of increased $MTR_{asym}$ after infusion, and $\Delta MTR_{asym}$ was calculated from $MTR_{asym}$ (post-infusion) - $MTR_{asym}$ (pre-infusion).

## Results

Phantoms: Fig. 1 illustrates glucoCEST detection for a phantom of D-glucose in PBS buffer (pH = 7.3, T = 37˚C). Notice that the shape of the $MTR_{asym}$ spectra after asymmetry analysis changes with concentration. It will also change with power level (higher B1 will broaden the direct saturation line shape in Z-spectra). At the power level used our detection sensitivity is about 0.5% of water signal per mM. In vivo, the plasma concentration will be ramped up to about 20 mM. If total plasma and EES is 20-40% of the voxel volume, this should give an effect of a few % (a few molar of signal representing a few mM of glucose).

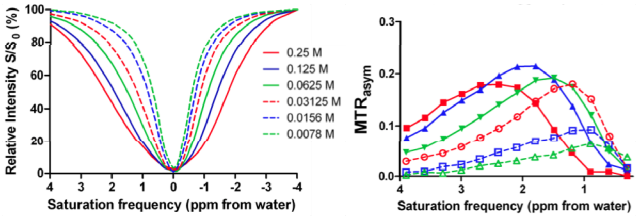


**Fig. 1.** GlucoCEST data for a phantom at 9.4T ($B_1$: 3.6μT; tsat: 3.5s) as a function of glucose concentration. Notice the change in lineshape for the Z-spectra (left) and MTRasym spectra (right) with concentration. Effect size at 1.2ppm is about 0.5% (0.55M water) per mM D-glucose at this $B_1$.

In vivo: Fig. 2 shows glucose delivery and uptake data in a mouse inoculated with MDA-MB-231 (2.5 weeks). GlucoCEST MRI was performed during steady state before infusion (40-min scan) and after blood glucose stabilization (40 min scan). Z-spectra and $MTR_{asym}$ images before and after infusion show clear differences. Subtracted $MTR_{asym}$ images show a profile resembling the glucose data in phantoms. The effect is 3.5% at 1.2 ppm offset, in line with expectations based on the phantom studies. The average increase for the 3 mice studied was 1.9±1.2%. Fig. 3 shows the steady state image 3 min after GdDTPA infusion, clearly highlighting the tumor.

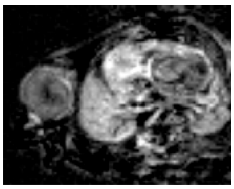

**Fig. 3. GdDTPA image 3 min after injection**

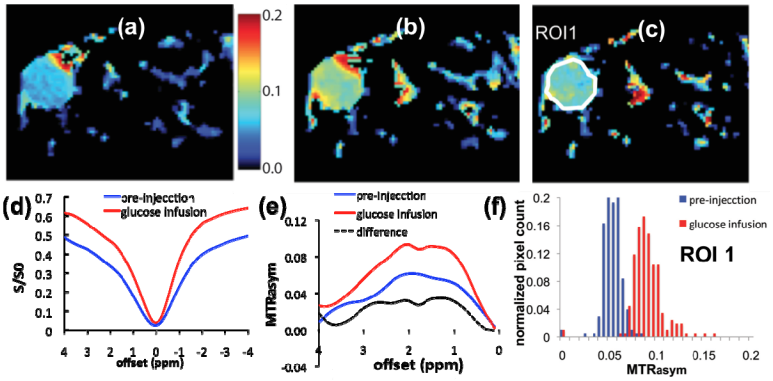


**Fig. 2.** (TOP) MTRasym images at offset 1.2ppm for a mouse inoculated with MDA-MB-231 (a) pre-infusion; (b) post-infusion with D-glucose (c): b – a difference image. (BOTTOM) (d Z-spectra; (e) MTRasym spectra for ROI 11. (f) MTRasym histograms.

## Discussion and Conclusion

The data show that it should be possible to measure the uptake of non-labeled D-glucose with MRI. The spatial distribution of glucose resembled the Gd-contrast distribution (Figs. 2,3). The glucose difference signal is expected to reflect effects of permeability, perfusion and metabolism of the tumor. It would require fast time dependent studies to assess this. This new method for *in vivo* visualization of glucose distribution can be readily implemented on clinical MR scanners. Although the method requires infusion of exogenous glucose, this is a safe agent, already available in infusion bags in the clinic.

[1] Escalona et al. Med Oncol 27, 114, (2010). [2] Knopp et al. JMRI 10, 260, (1999). [3] Degani H, Nat. Med. 3, 780, 1997. [4] van Zijl et al, PNAS 104, 4359 (2007). [5] Kim et al MRM 61, 1441 (2009). Supported by NIH grant 5P50CA103175-05.