

2D L-COSY MR spectroscopy detects changes in brain glucose level in a mouse model of Alzheimer's disease

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Introduction: Glucose uptake and metabolism has been reported to be severely impaired in Alzheimer's disease [1]. Some of cellular/molecular risk factors for AD are changes in the composition of membranes and in glucose/energy metabolism [2]. However, how AD pathology effect the glucose metabolism is unclear. Transgenic mouse model of AD studies have in consistent results regarding glucose uptake into the brain. A few studies reported decreased cerebral glucose up take, but some others could not replicate these results [3,4]. The aim of this study is to investigate regional brain glucose levels changes with age using 1D MRS and to further quantify these changes with two dimensional correlated MR spectroscopy (L-COSY) at 9.4 T.

Methods: Tg2576 (N = 10) and none-transgenic littermates (N = 9) were used in this study [4]. *In vivo* ¹H NMR spectra were collected from same animals at four different ages, namely 10, 14, 16, and 18 months of age from hippocampus (Hipp), thalamus (Thal) and hippocampus cortex (Hipp + Ctx) regions. MRS data acquired using a 9.4 T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1000 mT/m gradients. The imaging coil used in this study was a 20-mm volume coil. The anatomical images for voxel positioning were acquired using the rapid acquisition with relaxation enhancement sequence [5]. *In vivo* spectroscopic data was acquired using the point resolved spectroscopy with TE = 15 ms, TR=3500 ms, number of averages (NA) = 512 (for hipp, hipp + Ctx region), NA = 312 average (for Thal region). First and second order shims were adjusted by FASTMAP which typically yielded a water linewidth between 16-20 Hz. Metabolite concentration were quantified using LC model and the unsuppressed water signal was used as an internal reference [6]. Metabolites quantified with Cramer-Rao lower bounds (CRLB, estimated error quantification) ≥ 50 % were classified as not detected. For 2D MRS, a localized 2D shift correlated spectroscopic sequence (L-COSY) was used (Fig. 1). The sequence consists of three RF pulses (90°, 180°, 90°), slice-selective along 3 orthogonal axes. The last slice-selective 90° RF pulse also served as a coherence transfer pulse for the L-COSY spectrum necessary for correlating the metabolites peak in the second dimension. A WT and TG mouse were sacrificed and brain slices were stained with anti- β 40, anti- β 42 [5].

Results: An age dependent highly significant decrease in glucose/ total creatine ratio in hippocampus cortex region of the brain was observed in AD mouse as compared to wild-type mice (Fig. 2A). Interestingly the decline in glucose was not evident in the thalamus region (Fig. 2B). These results correlate well with *in vivo* age dependent increase in plaque load in this AD mouse model [7]. The 2D L-COSY MRS results revealed clear unambiguous identification of the glucose peak. The decrease in the glucose in Tg2576 mice as compared to wild-type mice is clearly evident from the intensity of the cross-peak (H₃-H₄ at 3.62-3.4 ppm) at ~18.5 months of age (Fig. 3). The quantitative analysis of glucose from 2D spectra is shown in Fig. 3D. As is clear from this figure, highly significant decrease of glucose/creatinine ratio was observed in the transgenic mice but not in the control mice. The 2D MRS along with 1D MRS data showed that glucose uptake and or metabolism is severely impaired in this AD mouse model. These *in vivo* results revealing a decrease in glucose ratio are in concordance with previously reported *in vitro* results, which showed impairment of cerebral cortical glucose metabolism in old Tg2576 mice brain [8]. In conclusion our *in vivo* results demonstrated that long lasting heavy amyloid burden is associated with impairment of cerebral cortical glucose metabolism. Both 1D and 2D MRS approaches can serve as important non-invasive tools to detect subtle changes in brain metabolism associated with AD pathology.

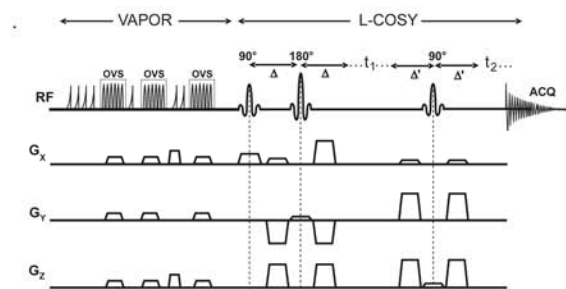


Fig. 1. The pulse sequence of 2D L-COSY preceded by a VAPOR sequence for global water suppression interleaved with outer volume suppression (OVS). The initial value of TE = 15 ms, consisting of 2 separate echo times; TE₁ (6ms) and TE₂ (9ms).

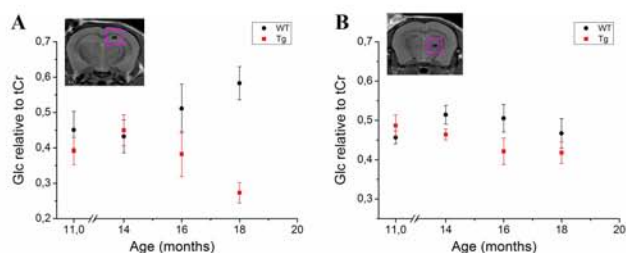


Fig. 2: Age dependent changes in glucose levels in (a) Hipp+Ctx and (b) Thal regions of wild type (WT) and the transgenic (TG) mice. Error bars shown are standard deviation of the mean. Glucose levels are significantly different ($p < 0.05$) in TG mice than control groups at 18 months of age. Student t test, ** $P < 0.001$. No significant changes were observed at thalamus regions.

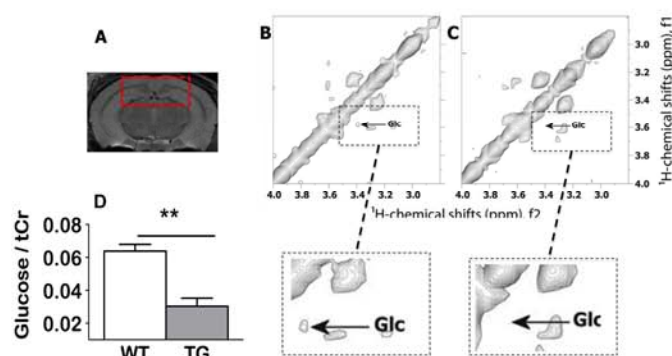


Fig. 3: The comparison of the 2D spectroscopy data between wild-type (WT) and transgenic (Tg) mice acquired from hippocampus cortex regions of the brain (A). The 2D peaks show clear differences in the level of glucose (Glc) at 18 months of age in WT (B) and Tg2576 mice (C). ROI: Hippocampus cortex, 27 μ l. (D) Quantitative analysis of glucose based on 2D data set. N (TG) = 8, N (WT) 4, Age average = 18.5.

References: [1] Cunnane et al. Nutrition 2011;27:3; [2] Poisnel et al., Neurobiol. Aging 2012;33(9):1995; [3] Nicholson et al. Brain Research 2010;1347:179; [4] Hsiao and Chapman et al. Science 1996; 274:99; [5] Braakman and Matysik et al., J Magn Reson Imaging 2006; 24: 530; [6] Pfeuffer J et al., J Magn Reson 1999; 141: 104; [6] Braakman N, Oerther T, de Groot HJM, Alia A. Magn Reson Med 2008; 60:449; [7] Thomas MA, Yue K, Binesh N, et al. Magn Reson Med 2001;46:58; [7] Braakman et al, J Magn Reson Imaging 2006;24:530; [8] Bigl et al. J Neural Transm 2003; 110:77.

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