INTRODUCTION
Type 2 diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia, insulin resistance and relative insulin deficiency. Poorly controlled or chronic hyperglycemia is associated with end-organ damages in the eyes, kidneys, peripheral nerves and the brain. Cognitive deficits have been observed in type 2 diabetic patients [1], and impairment of learning and memory in diabetic rats [2, 3]. In vivo 1H magnetic resonance spectroscopy (MRS) is a non-invasive technique to measure the tissue contents of a number of brain metabolites. In this study, we used in vivo 1H MRS to study the biochemical changes in two brain regions, hippocampus and striatum, of mice with type 2 diabetes.

METHODS
A group of male mice with type 2 diabetes (db/db) and their age-matched heterozygous control (db+) mice were studied at ages of 16 and 24 weeks. The diabetic mice were homozygous for the diabetes spontaneous mutation (Lepr/db), and became obese at approximately 3–4 weeks of age. All mice underwent MR scans performed on a Varian 9.4 T MR scanner. A two-loop quadrature surface RF coil placed on the animal head was used for transmission and reception of the NMR signals. During the experiments, the core temperatures the anesthetized animals were 37 °C. A spectroscopic voxel of ~6 mm was placed in the left hippocampus or left striatum using T2-weighted MR images (Fast spin echo sequence, ETL = 16, echo spacing/TE/TR = 11/11/4000 ms, matrix = 256 x 256, FOV = 25.6x25.6 mm, slice thickness = 0.5 mm, NT = 2). FASTMAP [4] was employed to adjust first- and second-order shim currents. The spin echo, full intensity acquired localized (SPECIAL) spectroscopy (TE = 3 ms, TR = 4 s, TM = 20 ms) [5] was used for all 1H MRS data acquisition. Water suppression was performed using the VAPOR technique [6]. Metabolite concentrations were estimated using the LCModel [7] with unsuppressed water signals as an internal concentration reference. The t-test was performed to compare neurochemical concentrations in the dbdb and db+ mice.

RESULTS AND DISCUSSION
Excellent localization and shrinking lead to water linewidth of 13 – 17 Hz. Figure 1 shows the spectra obtained from the hippocampus of (A) a db+ mouse and (B) a dbdb mouse at ages of 16 months. Reliable quantification of up to 18 metabolites from the highly resolved spectra was assessed by using the LCModel analysis [6]. The metabolites include ascorbate (Asc), γ-aminobutyric acid (GABA), glutamine (Gln), glutamate (Glu), glutathione (GSH), myo-inositol (Ins), lactate (Lac), N-acetyl aspartate (NAA), taurine (Tau), creatine (Cr), phospho-creatine (PCr), glycerophosphoryl-choline (GPC), and phosphocholine (PCho). Figure 2 (A, B) shows the concentrations of the metabolites in the hippocampus of db+ and dbdb mice of (A) 16 and (B) 24 weeks old (n = 3 for each group). The levels of Glc in the db+ mice were 10.9±1.1 μmol/g and 10.4±1.3 μmol/g at age of 16 and 24 weeks, representing increases of 196% (p = 0.001) and 126% (p = 0.02), respectively, compared with those in db+ mice. At the age of 16 weeks, the levels of Gln and Glu in the db+ mice were 66% (p = 0.004) and 14% (p = 0.09) higher than those in the db+, respectively. At the age of 24 weeks, they became 61% (p = 0.05) and 21% (p = 0.006). For Ins and Tau, the respective increases were 16% (p = 0.07) and 42% (p = 0.001) at 16 weeks, and 33% (p = 0.005) and 52% (p = 0.04) at 24 weeks. The levels of Lac changed -54% (p = 0.02) at the age of 16 weeks. The levels of GPC were significantly increased (p=0.04) while the total levels of GPC+PCho did not show significant changes at 24 weeks. Figure 2 (C, D) shows alterations of the metabolite levels in striatum, which were similar to those in hippocampus. The levels of Glc, Asp, Gln, Ins, Tau, and Cr+PCr in the db+ mice were significantly higher compared with those in db+ mice. The Gls levels in the db+ mice were higher: 23% (p = 0.1) at 16 weeks. The levels of Lac were 68% decreased (p = 0.01) at 16 weeks. The levels of GPC increased by 32% (p = 0.001) at 16 weeks and 27% (p = 0.1) at 24 weeks. The total levels of GPC+PCho in the db+ mice were consistently higher than those in the db+ mice and the differences between two groups were up to 17%, e.g., at 24 weeks (p=0.06). The NAA levels showed no significant changes in both brain regions. The neurochemical profiles of the db+ mice were in agreement with those reported by Tkac et al [8]. The significant increases in the Glc and Ins levels in the db+ mice were consistent with elevated brain Glc due to hyperglycemia and changes in osmolyte contents due to alterations in osmoregulation in hyperglycemia as reported in diabetic patients.

REFERENCES

Figure 1. Spectra obtained from the hippocampus of a wt (A) and a dbdb (B) mouse at age of 16 weeks. The spectra were processed with only zero-order phase correction.

Figure 2. Hippocampal (A, B) and striatal (C, D) metabolite concentrations in the dbdb and the db+ mice at age of 16 (A, C) and 24 (B, D) weeks. Error bars denote standard deviation and * denotes statistical significance level p < 0.05.