Metabolic profiles in the brain of a transgenic mouse model of type 2 diabetes assessed using in vivo 1H MRS at 9.4 T

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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 leaning and memory in diabetic rats [2, 3]. *In vivo* ^{1}H 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* ^{1}H 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 (dbdb) and their age-matched heterogygous 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 (air:oxygen = 1:0.5 with 1-2% isoflurane) were maintained at 37 °C. A spectroscopic voxel of ~6 µl was located 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 ^{1}H 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.

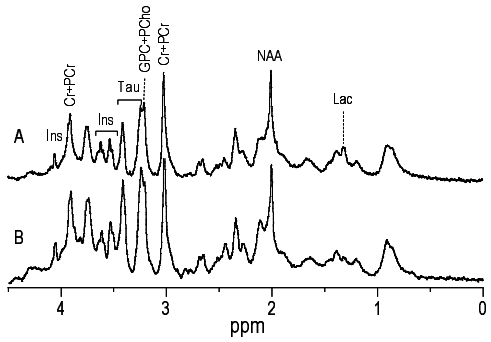

Figure 1. Spectra obtained from the hippocampus of a wt (A) and a dbdb (B) mouse at age of 16 weeks. The spectra were processed with only zero-order phase correction.

RESULTS AND DISCUSSION

Excellent localization and shimming 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 phosph-choline (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 dbdb 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 dbdb 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 was 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 dbdb mice were significantly higher compared with those in db+ mice. The Glu levels in the dbdb 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 dbdb 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.

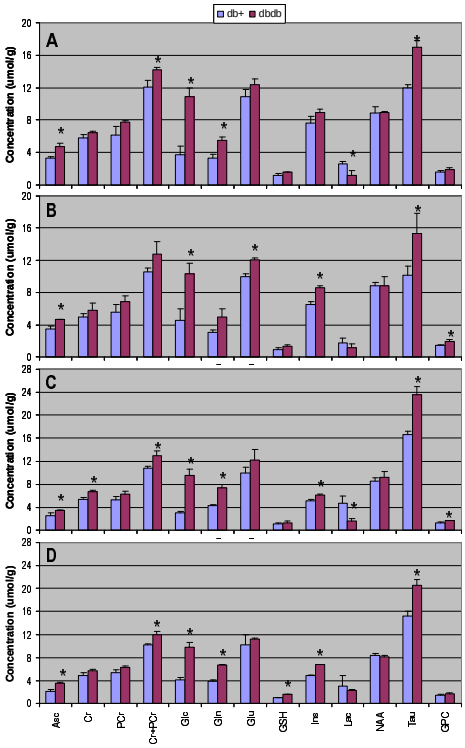

Figure 2. Hippocampal (A, B) and striatal (C, D) metabolite concentrations in the dbdb and the db+ mice at age of 16 (A, C) and 24 (B. D) weeks. Error bars denote standard deviation and * denotes statistical significance level p < 0.05.

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 dbdb mice compared with 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

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