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Evaluation of in vivo cerebral metabolism on proton magnetic resonance spectroscopy in patients with impaired glucose tolerance and type 2 diabetes mellitus

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Abstract

The aim of this study was to investigate possible metabolic alterations in cerebral tissues on magnetic resonance spectroscopy (MRS) in patients with impaired glucose tolerance (IGT) and with type 2 diabetes mellitus (T2-DM). Twenty-five patients with T2-DM, 13 patients with IGT, and 14 healthy volunteers were included. Single-voxel spectroscopy (TR: 2000 ms, TE: 31 ms) was performed in all subjects. Voxels were placed in the frontal cortex, thalamus, and parietal white matter. *N*-acetylaspartate (NAA)/creatine (Cr), choline (Cho)/Cr, and myo-inositol (MI)/Cr ratios were calculated. Frontal cortical Cho/Cr ratios were increased in patients with IGT compared to control subjects. Parietal white matter Cho/Cr ratios were significantly higher in patients with IGT when compared to patients with T2-DM. In the diabetic group, frontal cortical MI/Cr ratios were increased, and parietal white matter Cho/Cr ratios were decreased when compared to the control group. Frontal cortical NAA/Cr and Cho/Cr ratios and parietal white matter Cho/Cr ratios were decreased in diabetic patients with poor glycemic control (A1C>10%). A1C levels were inversely correlated with frontal cortical NAA/Cr and Cho/Cr ratios and with parietal white matter Cho/Cr ratios. T2-DM and IGT may cause subtle cerebral metabolic changes, and these changes may be shown with MRS. Increased Cho/Cr ratios may suggest dynamic change in membrane turnover in patients with IGT. Diabetic patients with poor glycemic control may be associated with neuronal dysfunction/damage in brain in accordance with A1C levels and, in some, extend with insulin resistance.

Keywords: Diabetes; Impaired glucose tolerance; Magnetic resonance spectroscopy; Brain

1. Introduction

It is becoming increasingly evident that the brain is one of the targets for diabetic end organ damage (Biessels, Kappelle, Bravenboer, Erkelens, & Gispen, 1994; Brands, Henselmans, Haan, & Biessels, 2003). The underlying mechanism causing brain damage in diabetes has not been fully explained. It seems that the fluctuation in blood glucose level, as well as acute and/or chronic metabolic and vascular impairment, such as deficits in cerebral blood flow, may cause functional and structural cerebral changes in diabetic patients (Biessels, van der Heide, Kamal, Bleys, & Gispen, 2002; Brands, Kessels, de Haan, Kappelle, & Biessels, 2004).

Magnetic resonance spectroscopy (MRS) is a very powerful diagnostic modality that gives information on neuronal/axonal viability, cellular energetic, and cellular membrane status (Bitsch et al., 1999). It may provide neurochemical information on subtle and overt brain parenchymal changes. This information is used to discriminate normal and pathological tissues. Therefore, it is increasingly being used to diagnose and manage various cerebral diseases (Alkan, Kutlu, Halac, et al., 2004; Alkan, Kutlu, Kocak, et al., 2004; Alkan et al., 2003). However,

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Table 1
Clinical and laboratory characteristics of the study groups and the control group

			DM		
	Control (n=14)	IGT (n=13)	A1C<10 (n=10)	A1C≥10 (n=15)	P
Age (years)	41.5±8.1	44.5±11.3	45.6±14.1	53.7±10.7	>.05
Gender (F/M)	9/5	8/5	4/6	9/6	>.05
Duration of diabetes (years)			9.0±5.4	8.2±5.1	>.05
A1C (%)	5.2±0.2	5.7±0.3	7.9±1.17	13.6±1.5	<.05
Fasting plasma glucose (mmol/l)	5.5±0.6	6.3±0.5	7.7±2.1	8.6±1.4	<.05
Postprandial plasma glucose (mmol/l)	6.3±0.7	8.1±1.2	11.5±3.7	12.8±2.5	<.05
Plasma insulin (μU/ml)	10.4±2.7	20.3±15.1	12.0±3.2	16.1±5.8	<.05
C peptide (ng/ml)	2.6±0.4	3.8±1.6	2.3±1.4	2.7±1.5	<.05
HOMA-IR	2.5±0.7	5.7±3.8	4.0±1.2	6.2±2.5	<.05

few studies investigated brain metabolite changes using MRS technique in patients with diabetes mellitus, and these changes have not been documented in detail (Biessels et al., 2001; Kreis & Ross, 1992; Perros, Deary, Sellar, Best, & Fier, 1997). Moreover, no one has yet investigated brain metabolite changes in patients with impaired glucose tolerance (IGT). This study was designed to contribute to a better understanding of cerebral metabolism on MRS in patients with IGT and type 2 diabetes mellitus (T2-DM).

2. Materials and methods

Twenty-five patients with T2-DM, 13 patients with IGT, and 14 age- and gender-matched healthy volunteers were included in this cross-sectional study. None of the subjects had systemic or cerebrovascular disease, head trauma, and overt cognitive dysfunction (in the diabetic group, three patients were diagnosed as having cerebrovascular disease and one patient was diagnosed as having meningioma; all of them were excluded from the study). The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The study protocol was approved by the institutional ethical board. All subjects were fully informed and gave their written informed consent.

Glucose levels were measured via hexokinase method (enzymatic UV test) using Olympus AU 2700 (Olympus Diagnostica GmbH, Hamburg, Germany). Insulin levels were measured via chemiluminescent method using Immulite 2000 system (DPC, California, USA). A1C was measured by high-performance liquid chromatography method using Agilent 1100 (Agilent Technologies, California, USA). Insulin resistance was calculated by way of the homeostatic model of assessment formulation-insulin resistance (HOMA-IR) using the following formula: HOMA-IR=[Fasting plasma insulin (μU/ml)×fasting plasma glucose (mmol/l)]/22.5 (Matthews et al., 1985).

T2-DM and IGT were diagnosed according to the criteria established by the American Diabetes Association (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). IGT was considered when the fasting plasma glucose level was lower than 7 mmol/l and the second-hour glucose value was between 7.8 and 11.1 mmol/l. A fasting plasma glucose value greater than 7 mmol/l or greater than 11.1 mmol/l at the second hour after the glucose load was diagnosed as DM. For further evaluation, the diabetic group was divided into two subgroups according to hemoglobin A1C levels (A1C; Subgroup I: 10 patients, A1C<10%; Subgroup II: 15 patients, A1C≥10%).

The MRI examination consisted of routine imaging and single-voxel spectroscopy (SVS). MRI was performed on a 1.5-T system (Philips, Gyroscan Intera Master, Best, Netherlands). T_1-weighted images (TR: 560 ms, TE: 15 ms) were obtained in the axial and sagittal planes (with 5-mm-thick

Table 2
Mean (±S.D.) NAA/Cr, Cho/Cr, and MI/Cr ratios of the frontal cortex, thalamus, and parietal white matter in subjects

	Location								
	Frontal cortex			Thalamus			Parietal white matter		
Study groups	NAA/Cr	Cho/Cr	MI/Cr	NAA/Cr	Cho/Cr	MI/Cr	NAA/Cr	Cho/Cr	MI/Cr
IGT (n=13)	1.94±0.53	1.03±0.48[a]	0.82±0.32	2.66±0.77	0.99±0.39	1.01±0.65	2.35±0.67	1.17±0.36[b]	1.34±0.60
DM									
Total	1.98±0.97	0.80±0.37	1.03±0.59[c]	2.46±0.89	0.83±0.38	0.92±0.6	2.70±0.68	0.82±0.37[c]	1.24±0.59
Subgroup I (n=10)	2.58±0.85[d]	1.06±0.29[d]	0.88±0.28	2.68±0.81	0.87±0.31	0.65±0.31	3.02±0.93	1.08±0.35[d]	1.09±0.52
Subgroup II (n=15)	1.63±0.87	0.65±0.35	1.11±0.71	2.35±0.94	0.81±0.44	1.07±0.67	2.53±0.45	0.67±0.29	1.32±0.63
Controls (n=14)	1.84±0.78	0.69±0.27	0.72±0.35	2.68±0.80	0.84±0.36	0.69±0.28	2.72±0.63	1.07±0.44	1.14±0.45

Subgroup I=A1C<10; Subgroup II=A1C≥10.
[a] Differences between patients with IGT and the control group.
[b] Differences between patients with IGT and those with T2-DM.
[c] Differences between patients with T2-DM and the control group.
[d] Differences between Subgroups I and II.

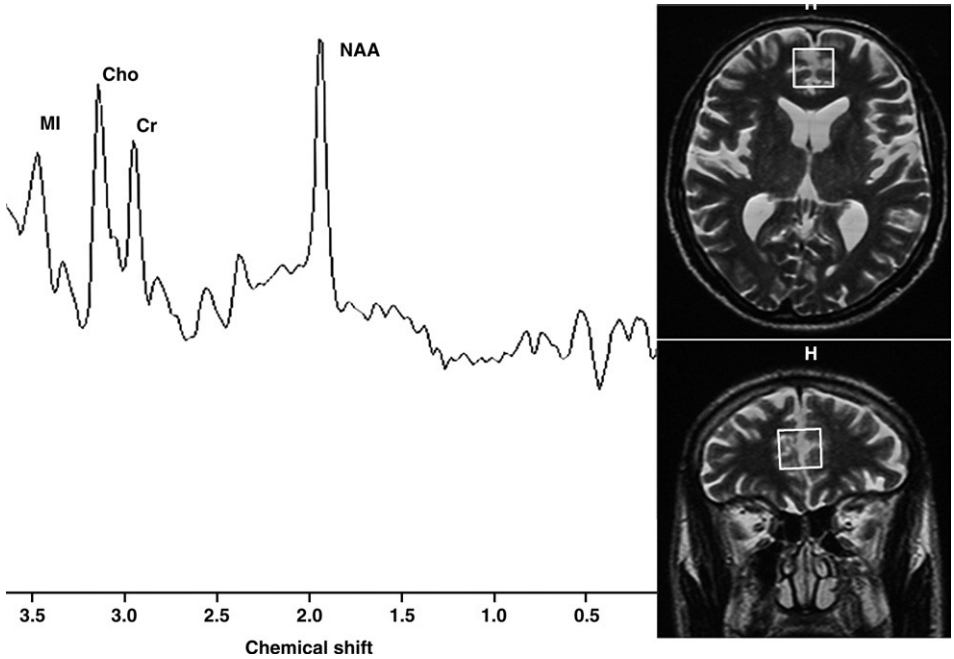

Fig. 1. The MR spectrum obtained from the frontal cortex shows increased Cho/Cr and MI/Cr ratios and normal NAA/Cr ratio in a patient with T2-DM.

slices). T_2-weighted images (TR: 4530 ms, TE: 100 ms) were obtained in the axial and coronal planes. SVS was performed in all patients using a point-resolved spectroscopy sequence (PRESS; TR: 2000 ms, TE: 31 ms) with 256 averages. Voxels were placed in the parietal white matter (20×20×20 mm), thalamus (15×15×15 mm), and frontal cortex (20×20×20 mm) since these locations were studied previously in diabetic patients (Makimattila et al., 2004). Due to the increased signal/noise ratio of short TE, and the visualization of additional compounds like myo-inositol (MI), glutamate–glutamin, glycine, and lipid seen by short TE, the short TE PRESS was chosen as the primary pulse sequence (Alkan et al., 2003; Barba et al., 2001; Ernst & Hennig, 1991; Ernst, Kreis, & Ross, 1993; Govindaraju, Young, & Maudlsey, 2000). Prior to MR spectroscopy, shimming was performed to optimize field homogeneity, and water suppression was optimized using automated routines. A chemical-shift-selective saturation pulse suppressed the water signal. A spectral sweep width of 1000 Hz was used with a data size of 1024 points. All data postprocessing were performed with software provided by the manufacturer. Resonance peaks were assigned as follows: *N*-acetylaspartate (NAA), 2.0 ppm; creatine (Cr), 3.02 ppm; choline (Cho) 3.2 ppm; MI, 3.56 ppm. Peak area metabolite

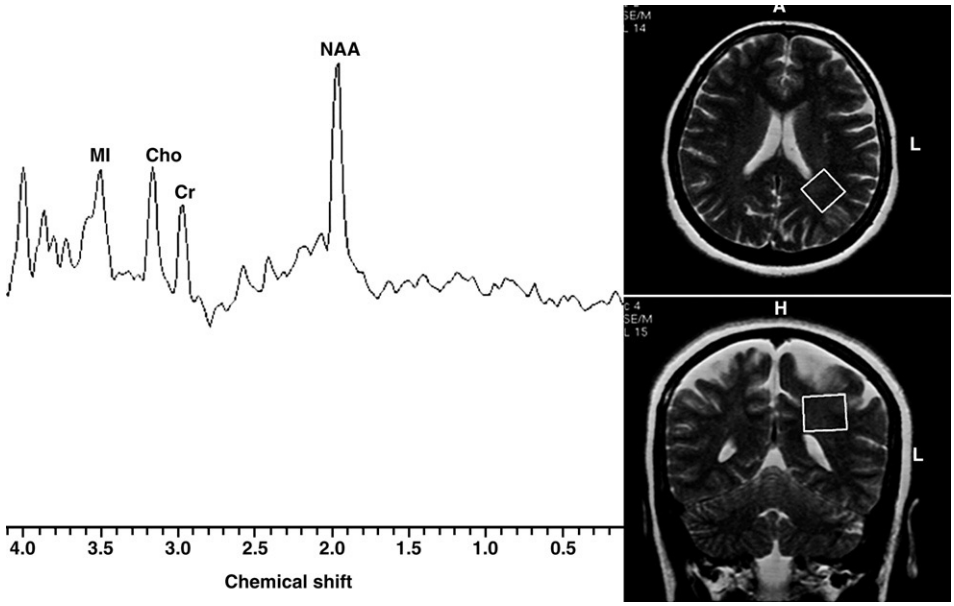

Fig. 2. The MR spectrum obtained from the parietal white matter shows increased Cho/Cr and MI/Cr ratios in a patient with IGT.

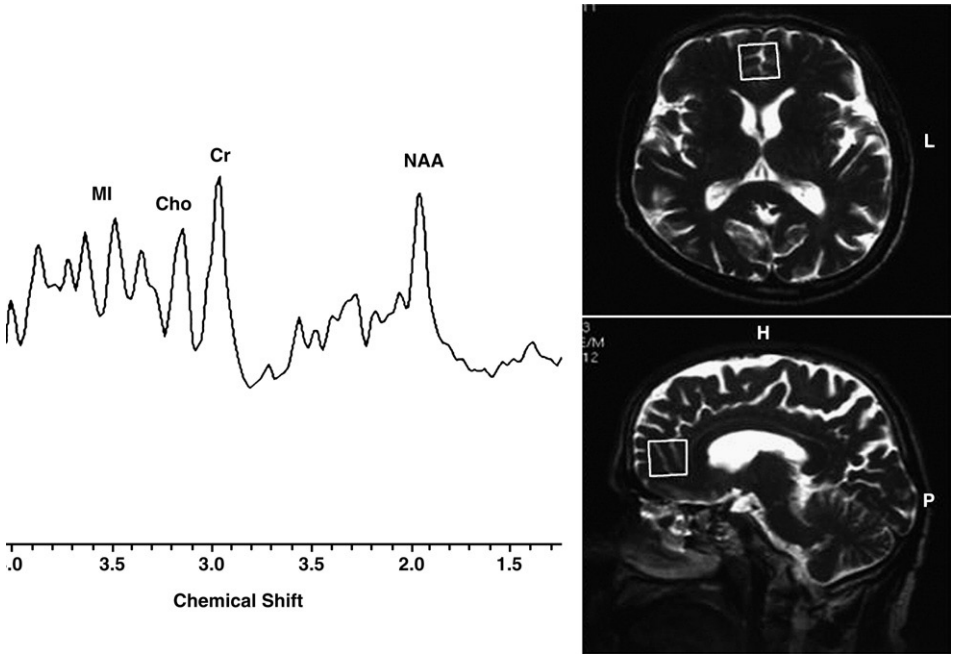

Fig. 3. The MR spectrum obtained from the frontal cortex shows decreased NAA/Cr and Cho/Cr ratios in a diabetic patient with poor glycemic control (A1C>10%).

ratios (mean integral values of metabolites; NAA/Cr, Cho/Cr, and MI/Cr) were calculated.

Statistical analysis was performed with SPSS for Windows Version 13.0 (SPSS Inc., Chicago, IL, USA). All values in text, tables, and figures were expressed as mean±standard deviation unless stated otherwise. Distributions of values, whether normally distributed or not, were determined by Kolmogorov–Smirnov test. Unpaired *t* test and Mann–Whitney *U* test were used to compare continuous variables. χ^2 test was used to compare categorical variables. Spearman's test was used for correlation analysis. $P<.05$ was considered as the level of statistical significance.

3. Results

Mean age of patients with T2-DM, patients with IGT, and control subjects were 49.8±12.4, 44.5±11.3, and 41.5±8.1 years, respectively. Study groups and the control group did not differ significantly regarding their mean ages and

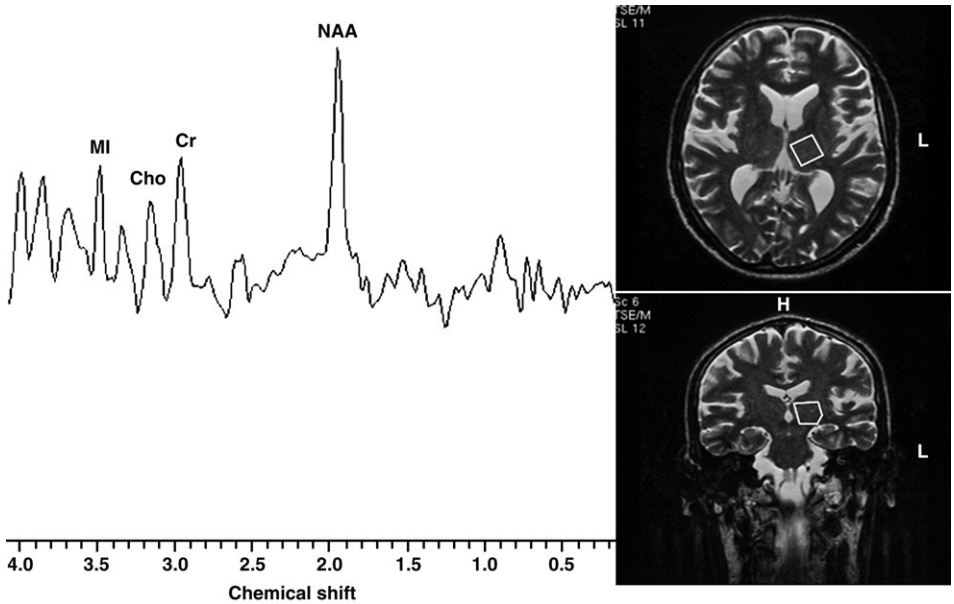

Fig. 4. The MR spectrum obtained from the thalamus shows increased MI/Cr ratio and decreased Cho/Cr ratio in a diabetic patient with high A1C level (A1C=14%).

gender distributions. Clinical and laboratory characteristics of the study groups and the control group are given in Table 1. Conventional MR imaging was normal in all subjects.

Parietal white matter, frontal cortex, and thalamic metabolite measurements are presented in Table 2. Frontal cortical Cho/Cr ratios of patients with IGT were significantly higher than the ratios of control subjects (P=.038; Fig. 1). Parietal white matter Cho/Cr ratios of these patients were significantly higher than the ratios of patients with T2-DM (P=.011; Fig. 2). In the diabetic group, frontal cortical MI/Cr ratios were higher than the ratios of the control group (P=.033). Parietal white matter Cho/Cr ratios of these patients were significantly lower than the ratios of the control group (P=.049). Subgroups were also compared regarding their metabolite ratios. Frontal cortical NAA/Cr (P=.014) and Cho/Cr (P=.017) ratios (Fig. 3) as well as parietal white matter Cho/Cr (P=.012) ratios were lower in Subgroup II than in Subgroup I (Table 1). There was no significant difference between the study groups and controls as regards to NAA/Cr, Cho/Cr, and MI/Cr ratios obtained from the thalamus.

Metabolite ratios, diabetes duration, and gender and age of patients were not significantly correlated. A1C levels were inversely correlated with frontal cortical NAA/Cr (r=−.39, P=.048) and Cho/Cr (r=−.49, P=.024) ratios, as well as with parietal white matter Cho/Cr (r=−.39, P=.048) ratios. Increased A1C levels were associated with thalamic MI/Cr ratios (r=.51, P=.008; Fig. 4). Insulin levels were inversely correlated with frontal cortical NAA/Cr ratios (r=−.43, P=.031). Fasting plasma glucose and HOMA-IR levels were inversely correlated with parietal white matter NAA/Cr ratios (r=−.43, P=.031 and r=−.43, P=.031, respectively).

4. Discussion

Clinical features, epidemiology, and pathophysiology of the peripheral diabetic neuropathy are well established. However, adverse effects of the diabetes on the central nervous system are less described and the underlying pathophysiology is largely unknown (Brands et al., 2003). Neuropsychological studies of diabetics reveal cognitive impairment, including complex information processes such as learning and memory (Tun, Nathan, & Perlmuter, 1990). Neuroradiological studies report mild cerebral atrophy and subcortical and brainstem lesions (Araki et al., 1994; Dejgaard et al., 1991).

Major MRS resonances (short TE: 31 ms) of normal brain tissue include NAA, Cho, Cr, and MI. Among them, NAA is the most sensitive CNS metabolite. It is an important predictor of neuronal dysfunction and abnormalities of neuronal structures, such as reduced neuronal density or viability. Cr, the second most important metabolite, plays an important role in the cellular energy metabolism, and it is mainly concentrated in glial cells (Alkan, Kutlu, Halac, et al., 2004; Alkan, Kutlu, Kocak, et al., 2004; Alkan et al., 2003; Cecil & Jones, 2001). Except in trauma, stroke, tumor, and Cr deficiency syndromes, Cr levels tend to remain relatively unchanged. Therefore, Cr is often used as a putative internal standard against which the other metabolites can be compared (Cecil & Jones, 2001). Only limited MRS data are available for DM, and most of them are confined to T1-DM and streptozotocin-induced diabetic rats. Kreis and Ross (1992) reported a significant reduction of *N*-acetyl metabolites in the parietal white matter, but no change in the occipital cortex, in subacute and chronic DM patients. Biessels et al. (2001) found a reduction in NAA/Cr ratios in streptozotocin-induced diabetic rats. Lai et al. (2001) showed a decrease in NAA levels of the basal ganglia of hyperglycemic diabetic patients with chorea-ballismus. Recently, Sarac et al. (2005) demonstrated a significant decrease in NAA/Cr ratio obtained from pons and posterior parietal white matter of poorly controlled T1-DM patients. In this study, we found decreased NAA/Cr ratios in the frontal cortex of diabetic patients with higher A1C levels (>10%). Increased A1C levels were associated with decreased NAA/Cr ratios.

Increased fasting plasma glucose and increased insulin resistance (HOMA-IR) were associated with decreased NAA/Cr ratios in the parietal white matter in the diabetic group. These findings indicate that a poor glycemic control may be related to neuronal loss/dysfunction in the frontal cortex and parietal white matter. Although underlying pathophysiological mechanisms are not clear, it is speculated that cerebral free radicals are increased during hyperglycemia (Tallroth, Ryding, & Agardh, 1993; Vincent, Brownlee, & Russell, 2002). These radicals may contribute to neuronal dysfunction (decrease in NAA) by oxidizing proteins and damaging DNA (Kumar & Menon, 1993). Further studies are needed to demonstrate whether this neuronal dysfunction is permanent or not.

Cho is a constituent of the phospholipid metabolism of cell membranes. Major components of the Cho resonance are Cho-containing compounds with small molecular weight, such as phosphocholine and glycerophosphocholine, which form a pool involved in membrane synthesis and degradation. Therefore, increased Cho primarily reflects an increased membrane synthesis and/or an increased number of cells. An increase in Cho/Cr ratio may also indicate an inability to properly incorporate Cho-containing molecules into myelin. Loss or disruption of normal myelin also increases the availability of Cho-containing compounds. The increased Cho/Cr ratio, which could indicate increased membrane turnover, as well as myelin breakdown, might correspond to glial cell proliferation (Alkan, Kutlu, Halac, et al., 2004; Alkan, Kutlu, Kocak, et al., 2004; Alkan et al., 2003; Bitsch et al., 1999; Cecil & Jones, 2001; Ross & Michaelis, 1994). Cho is also a precursor for acetylcholine, which is a critical neurotransmitter involved in memory, cognition, and mood. Kreis and Ross (1992) showed an increase of Cho on the order of 10%, in both the white matter and the gray matter of T2-DM patients when compared to patients with T1-DM. In another study, diabetic

patients with nonketotic hyperglycemia and chorea-ballismus were shown to have increased Cho levels in their basal ganglia (Lai et al., 2001). In our study, we showed an increase in frontal cortical Cho/Cr ratio in patients with IGT. This finding may indicate a demyelinating process. Cho/Cr ratios in the parietal white matter and frontal cortex were inversely correlated with A1C levels. A similar finding was also found by Sarac et al. (2005) who have reported a decreased pontine Cho/Cr ratio in T1-DM. However, they found normal Cho/Cr levels in the left basal ganglia and posterior parietal white matter (Sarac et al., 2005). Authors suggested that dynamic changes in membrane lipid and/or decreased turnover may possibly cause these variations in Cho levels. Furthermore, differences in cerebral blood flow in different sites of the brain, as an end organ in diabetic patients, may also contribute different metabolite changes.

MI is a metabolite involved in hormone-sensitive neuroreception, and its triphosphorylated derivative is believed to act as a second messenger of intracellular calcium mobilizing hormones. Decreased MI content in the brain has been associated with the development of diabetic neuropathy (Winegrad & Greene, n.d.). Elevated level, on the other hand, may indicate osmolar changes in glia and glial alterations (Winegrad & Greene, n.d.). In this study, there was an increase in the MI/Cr ratio of the frontal cortex in the diabetic group. A1C levels were positively correlated with MI/Cr ratios in thalami, which may be related to osmotic stress and/or glial activation. Since MI appears to be almost exclusively located in astrocytes, it is now recognized as the most important osmolyte or cell volume regulator. Increase in MI/Cr ratios may be the result of an increased cerebral osmolality in diabetic patients (Winegrad & Greene, n.d.). In our study, the change in MI/Cr ratios obtained from the thalamus and parietal white matter was similar for both the IGT and T2-DM. These findings may suggest the role of increased insulin resistance/decreased insulin sensitivity in causing the observed changes.

In conclusion, T2-DM and IGT may lead to cerebral metabolic changes. These alterations, which are imperceptible to conventional MRI, may be shown with MRS. Increased Cho/Cr ratios may suggest dynamic change in membrane turnover in patients with IGT. Diabetic patients with poor glycemic control (HbA1C≥10) may be associated with neuronal dysfunction/damage in brain in accordance with A1C levels and, in some, extend with insulin resistance.

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