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Original Article

# Metabolism in the Diabetic Brain: Neurochemical Profiling by $^{1}$H Magnetic Resonance Spectroscopy

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## Abstract

Diabetes is associated with decrements in cognitive function and with abnormalities in brain morphology. In addition, alterations of metabolism have been reported in the diabetic brain. Magnetic Resonance Spectroscopy (MRS) is a non-invasive technique that can be employed to determine the concentration of metabolites in a fully non-invasive manner under normal physiological conditions. The present article reviews major findings from $^{1}$H MRS studies in the brain of diabetes patients, and of pre-clinical models of both insulin-dependent and insulin-resistant diabetes. Metabolic alterations measured *in vivo* by MRS are closely associated to events of the neurodegenerative process at cellular level, and thus allow understanding the pathophysiology of diabetes-associated brain complications. Moreover, MRS constitutes an excellent tool for tracking outcomes of therapeutic interventions. However, further studies are required to clearly establish the links between diabetes-induced alterations of metabolism, structure and function in the brain.

**Keywords:** Brain metabolism; Diabetic encephalopathy; Neurochemical profile; MRS

## Abbreviations

| | | |
|---|---|---|
| MRI | : | Magnetic Resonance Imaging |
| MRS | : | Magnetic Resonance Spectroscopy |
| NAA | : | N-acetylaspartate |

## Introduction

While type 1 diabetes is linked to autoimmune destruction of insulin-releasing β-cells, type 2 diabetes is closely associated with obesity, a pandemic that in western societies is favoured by a sedentary lifestyle and the widespread consumption of palatable food products rich in saturated fat and refined carbohydrates [1]. Both type 1 (insulin-dependent) and type 2 (insulin-resistant) diabetes affect brain structure and function. Diabetes is associated with chronic hyperglycaemia, microvascular complications, insulin resistance, dyslipidaemia, and hypertension, which are all important risk factors for cognitive dysfunction [2,3]. A plethora of studies in rodent models of diabetes suggest that both glucose neurotoxicity and deficient insulin signalling trigger a neurodegenerative process that leads to behavioural and cognitive alterations. In particular, diabetic conditions cause synaptic deterioration that is accompanied by alterations of neuromodulation systems namely in the hippocampus [2]. These modifications likely result in defective neurotransmission and synaptic plasticity, with behavioural consequences. Notably, the brain of diabetes patients also displays important atrophy of the hippocampus, which can be detected by Magnetic Resonance Imaging (MRI) [3-6]. While neurodegeneration has been largely studied in diabetes, metabolic modifications require elucidation. Neuronal loss and tissue atrophy are measurable by conventional imaging modalities. However neuronal dysfunction begins with biochemical modifications much before symptoms and irreversible tissue damage occur [7,8]. Since the impact of diabetes on brain metabolic pathways may precede synaptic degeneration, neuronal loss and tissue atrophy, the ability of detecting such brain metabolic alterations early in the neurodegenerative process will allow for effective pharmacological and/or behavioural interventions. Early interventions exerting metabolic control may prevent future irreversible tissue deterioration, and halt the cognitive decline in diabetes.

## Neurochemical Profiling by Proton Magnetic Resonance Spectroscopy

Magnetic resonance techniques provide unique capabilities for studying brain function in living tissues and thus received considerable attention in the past couple of decades. The physical mechanism by which nuclei with magnetic moment produce magnetic resonance signals is out of the scope of this review. A simple explanation of Magnetic Resonance Spectroscopy (MRS) principles and techniques can be found elsewhere [9].

MRI is usually employed to detect protons ($^{1}$H) of water. Given the large concentration of water $^{1}$H in brain tissue, it has been possible to image structure and function of healthy and diseased central nervous system in high detail. On the other hand, $^{1}$H MRS is a non-invasive technique based on the $^{1}$H resonance of carbon-bound hydrogen atoms in metabolites. Each $^{1}$H in the sample experiences a slightly different magnetic field depending on its chemical environment, therefore resonating at a slightly different frequency. Most of the signal in $^{1}$H MRS will come from the bulk tissue water, but by suppressing the signal from water protons, one is then able to observe a spectrum containing signals from a variety of molecules occurring in the μmol/g, that is, at concentrations several orders of magnitude smaller than tissue water. The assessment of metabolite concentrations *in vivo* by MRS thus provides information complementary to MRI.

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In the particular case of the brain, $^{1}$H MRS provides a set of biomarkers - the neurochemical profile - that can be employed for non-invasive assessment of disease development and outcome of therapeutic interventions [8]. The number of quantifiable metabolites depends on many factors in the MRS acquisition process, namely the pulse sequence parameters, and the spectral signal-to-noise ratio and spectral resolution [7]. To simplify the spectral analysis at low magnetic fields, many studies performed $^{1}$H MRS with long echo times. With this approach, the major resonances observed are from *N*-Acetylaspartate (NAA), total creatine (creatine plus phosphocreatine), choline-containing compounds, glutamate plus glutamine (often called "Glx"), and *myo*-inositol. The role of each MRS-detectable metabolite in the brain was reviewed and discussed previously [7]. Importantly, NAA is present in neurons but not in glial cells of the mature brain, which makes it an important biomarker for neuronal integrity. In contrast, elevated *myo*-inositol has been generally considered to represent astrogliosis, and choline has been referred as a marker for increased membrane turnover, cellular proliferation, or inflammation. Since glutamate is mostly present in neurons and glutamine is synthetized in glial cells, "Glx" variations are of difficult interpretation. Total creatine is generally assumed to be uniformly distributed across brain cells and thus has been used as normalisation factor for the remaining MRS signals.

Over the last couple of decades, major methodological improvements allied to increases in sensitivity and spectral resolution at high magnetic fields provide absolute quantification of an extended number of metabolites in the brain in both humans and small animals (Figure 1). At high magnetic field, nowadays considered 7 T and above, state-of-the-art brain MRS provides a neurochemical profile composed of the chemical species present in the brain at a concentration above ~0.2 µmol/g, in practice about 20 metabolites. The concentrations of metabolites measured *in vivo* likely reflect the activity of metabolic processes in the living tissue [10], and the ability to measure an extended neurochemical profile affords insight into key biochemical processes at the cellular level. In line with this, it is known that neurochemical profiles (1) are modified during brain development, maturation and aging reflecting the structural and functional changes in the cerebral networks, (2) are region specific reflecting cell populations, (3) reflect brain functional states, and (4) are affected by environmental factors and pathological conditions [7].

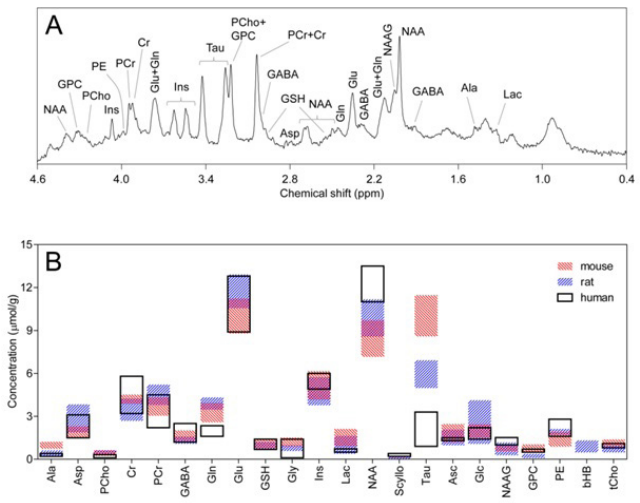


**Figure 1:** Neurochemical profiling by $^{1}$H MRS. Panel A shows a typical $^{1}$H spectrum of the mouse cortex at 14.1 T. Spectrum was acquired using SPECIAL with TE = 2.8 ms and TR = 4 s (for details see, e.g., [11]), and processed with a shifted Gaussian function (gf = 0.12, gfs = 0.02) prior to Fourier transformation. Panel B shows the normal range of neurochemical profiles in the mouse, rat and human cortex. The plotted concentration ranges are based on the mean concentrations from published $^{1}$H MRS studies in the cerebral cortex of adult C57BL/6 mice at 14.1 T [11-15], Sprague-Dawley rats at 9.4 T and 14.1 T [16,17] and humans at 3.0, 7.0 T, and 9.4 T [18-21].

Ala: Alanine; Asc: Ascorbate; Asp: Aspartate; bHB: β-Hydroxybutyrate; Cr: Creatine; GABA: γ-aminobutyrate; Glc: Glucose; Gln: Glutamine; Glu: Glutamate; Gly: Glycine; GPC: Glycerylphosphorylcholine; GSH: Glutathione; Ins: *myo*-inositol; Lac: Lactate; NAA: *N*-Acetylaspartate; NAAG; *N*-Acetylaspartylglutamate; PCho: Phosphorylcholine; PCr: Phosphocreatine; PE: Phosphorylethanolamine; Scyllo: *scyllo*-inositol; Tau: Taurine; tCho: total choline (PCho + GPC)

## MRS in Diabetes Patients

### Type 1 diabetes

MRS studies investigating the brain of diabetes patients have mostly been performed at low magnetic field, namely 1.5 T. Although there is a limited number of metabolites that can be quantified at 1.5 T, changes observed in spectral lines in expertly conducted experiments surely reflect metabolic alterations. A study at 1.5 T revealed lower NAA-to-creatine and choline-to-creatine ratios in the pons and lower NAA/creatine in the left posterior parietal white matter of children (8 to 19 years old) with poorly controlled type 1 diabetes than in healthy individuals [22]. Heikkilä et al., [23] reported increased *myo*-inositol in the frontal cortex of type 1 diabetes patients relative to non-diabetic controls, without any modification in the levels of NAA, choline or creatine. In a subsequent study, the authors failed to identify diabetes-induced alterations of the neurochemical profile in the cerebellum [24]. Other MRS studies at 1.5 T identified a reduction of NAA in the visual cortex of diabetes patients with Hemoglobin A1c over 8% relative to those below 8% [25], and a reduction of NAA/choline in the frontal cortex of type 1 diabetes patients with end-stage renal disease relative to control subjects [26]. Decrease in NAA may indicate neuronal damage, either loss of processes, cells, or simply metabolic and functional impairment. In contrast, increased *myo*-inositol has been associated with astrogliosis. An alteration in choline-containing lipids may be caused by dynamic changes in cellular membranes, such as upon an inflammatory response.

Seaquist et al. [27] employed MRS at a higher magnetic field (4 T) to measure cortical glucose levels in type 1 diabetes patients and control subjects, and found no significant difference between the glucose concentrations in patients with poorly controlled diabetes and those in healthy volunteers studied at the same plasma glucose concentration [27]. Thus it appears unlikely that chronic hyperglycaemia in diabetes has clinically meaningful effects on brain glucose concentration at steady-state, as was also found in streptozotocin-induced diabetic rats [28,29]. On the other hand, patients with type 1 diabetes and hypoglycaemia unawareness displayed higher brain glucose concentrations compared to that in controls under identical experimental conditions [30], suggesting that recurrent hypoglycaemia episodes may result in increased blood to brain glucose transport. Although these studies did not report alterations on other brain neurochemicals [27,30], a later re-analyses of a carefully selected subset of subjects revealed small diabetes-induced reductions in NAA and glutamate concentrations in grey but not white matter of the cortex [31]. Interestingly, an earlier study investigated the frontal and parietal cortex, and reported no differences in metabolite levels (namely NAA/creatine and choline/creatine) between of type 1 diabetes patients with and without recurrent hypoglycaemia [32].

Recent studies reported no difference in baseline concentrations of neurochemicals in the brain of type 1 diabetes patients relative to healthy control subjects, while the brain metabolic response to hypoglycaemia may be impaired in diabetes [33,34]. In healthy subjects a hypoglycaemia insult leads to a reduction of brain glutamate levels, a response that is blunted in diabetes patients depending on their degree of hypoglycaemia-associate autonomic failure, a syndrome that includes hypoglycaemia unawareness [33,34]. In addition, brain lactate levels drop during hypoglycaemia in diabetes patients (depending on whether awareness of hypoglycaemia is impaired or not), suggesting stimulated utilisation of lactate as metabolic substrate [33,34]. Interestingly, during hypoglycaemia with lactate infusion, type 1 diabetic patients displayed increased brain lactate levels relative to healthy subjects [35]. Altogether, these reports suggest that, even if basal metabolism is unaltered in the diabetic brain, metabolic adaptations to hypoglycaemia differ from those in the brain of healthy subjects. Moreover, diabetic patients exposed to repeated hypoglycaemia episodes may display specific brain metabolic adaptations [2] (further discussed in [2]), which may be a confounding effect on neurochemical profiling.

Other studies included patients with both type 1 and type 2 diabetes. In these studies, there diabetes was found to increase choline/creatine [36,37] and to reduce NAA [38] in cortical areas of patients relative to controls. These three studies reported an increase in cortical *myo*-inositol levels induced by diabetes [36-38]. Another MRS study at 1.5 T with type 1 and type 2 diabetes patients reported a diabetes-induced reduction in NAA levels in the left thalamus and dorsolateral prefrontal cortex, but not in the anterior cingulate cortex [39].

### Type 2 diabetes

A MRS study at 1.5 T reported lower NAA concentration in the right frontal and right parieto-occipital cortex but not in the right parieto-temporal cortex of type 2 diabetes patients relative to controls [40]. This study reported a diabetes-induced increase in glutamate and glutamine levels exclusively in the right frontal region of the brain, and choline, *myo*-inositol and creatine levels were normal in all brain regions examined [40]. Another study at 1.5 T compared type 2 diabetic patients with hypertension and hypertensive control subjects, and showed a diabetes-induced reduction of NAA levels, without modification of creatine and choline [41]. Sahin et al., [42] performed an MRS study at 1.5 T on the frontal cortex, thalamus, and parietal white matter of type 2 diabetes patients and patients with impaired glucose tolerance. When compared to control subjects, glucose intolerant patients only displayed increased choline/creatine in the frontal cortex. On the other hand, diabetes patients showed higher *myo*-inositol/creatine in the frontal cortex, and higher choline/creatine in the parietal white matter. Modi et al., [43] also found increased choline/creatine in the left occipital lobe of type 2 diabetes patients, relative to controls. However, from many brain areas investigated, this was the sole neurochemical modification.

Ajilore et al., [44] performed MRS at 1.5 T on type 2 diabetes patients exhibiting also major depression. The authors found that glutamine and glutamate concentrations were reduced in a region encompassing the subcortical nuclei of depressed diabetic patients, relative to both healthy subjects and diabetes patients without depression [44]. Moreover, levels of *myo*-inositol were increased in the frontal white matter of diabetes patients with or without depression, relative to controls subjects [44]. In this study, NAA and choline levels were similar in all subjects. This study suggests that confounding factors may underlie the heterogeneity of MRS findings in diabetes patients. Tong et al., [45] studied metabolite changes of brain tissue in the left frontal white matter, left lenticular nucleus, and left optic radiation of type 2 diabetes mellitus patients with and without retinopathy with MRS at 3 T. Interestingly, the authors found that NAA/creatine ratios were reduced only in the frontal white matter and optic radiation of patients with retinopathy, relative to healthy subjects. However, *myo*-inositol/creatine was higher in the left lenticular nucleus of diabetes patients than of controls, but not in patients with retinopathy. A 3-T MRS study reported increased glutamate and *myo*-inositol levels in the occipitoparietal grey matter in subjects with metabolic syndrome relative to controls [46]. However, in a subsequent study, the authors suggested that peripheral atherosclerosis can be a confounder in MRS studies: individuals with metabolic syndrome were found to have increased glutamate concentration in the occipitoparietal grey matter, relative to controls, but only if they also display high carotid artery intima-media thickness [47].

The brain is composed of many anatomical structures with different functions. The heterogeneity of MRS findings in the diabetic brain is likely linked to the investigation of different brain areas in each independent study. On the other hand, most MRS studies in the brain of diabetes patients were performed at low magnetic field, which may not provide sufficient spectral resolution for reliable neurochemical profiling. Furthermore, the little control over comorbidities of the recruited subjects in MRS studies hampers the ability to detect small diabetes-induced changes in the concentration of neurochemicals. Nevertheless, it appears that a reduction in the levels of the putative neuronal marker NAA, as well as an increase in *myo*-inositol content, accompany the neurodegeneration process in diabetes. Interestingly, a recent study suggested that insulin sensitivity is associated to cortical levels of these two metabolites [48]. In other neurodegenerative disorders, and notably in Alzheimer's disease, reduced NAA and increased *myo*-inositol concentrations have been associated with brain dysfunction [8].

## MRS in Experimental Models of Diabetes

Proton MRS became a tool of choice to investigate metabolic alterations induced by brain disorders in a non-invasive manner, and it has also been employed to study brain metabolism in experimental models of diabetes. Diabetic rats exposed to several weeks of chronic hyperglycaemia, induced by streptozotocin administration (widely used experimental model of type 1 diabetes), display a plethora of metabolic alterations in the hippocampus and cortex, relative to control rats [28,29]. Interestingly, these studies also found that most hyperglycaemia-induced metabolic alterations are normalized upon acute restoration of euglycaemia. Some of the metabolites more affected by hyperglycaemia were the brain osmolytes *myo*-inositol, taurine and creatine, suggesting that alterations of the neurochemical profile are mainly related to osmolarity regulation. Similar results were obtained in Goto-Kakizaki rats, an experimental model of insulin resistance and type 2 diabetes [49]. High concentration of *myo*-inositol was also reported in the hippocampus of Zucker diabetic fatty rats compared to controls [50]. Recently, Zang et al. found that chronic hyperglycaemia in streptozotocin-induced diabetic rats leads to a reduction of NAA in the striatum and hippocampus but not in the cortex [51]. While this NAA reduction was installed 4 days after diabetes induction, one

month later diabetic rats also displayed higher taurine and *myo*-inositol levels in the hippocampus, when compared to controls [51]. Thus, the study of the neurochemical profile in these animal models supports the hypothesis that diabetes-induced hippocampal dysfunction involves an osmolarity shift, probably due to continuous exposure to high brain glucose levels.

Classical models of diabetes rarely provide a complete phenotype of type 1 or type 2 diabetes in humans. Models of diabetes induced by hypercaloric intake are nowadays being preferred for translational research. Indeed, current knowledge regarding the energy imbalances in diet-induced obesity and insulin resistance has been strongly supported by basic research in animals fed hypercaloric diets. For example, mice fed a high-fat diet (60% kcal from fat) show increased body weight, larger adipocytes, higher concentration of lipids in liver and skeletal muscle, and insulin resistance in less than one week after initiating the diet, relative to a control diet [52-54]. Diets with high levels of both fat (58% kcal) and sucrose (26% kcal) also lead to obesity and insulin resistance [55]. Perhaps not surprisingly, hypothalamic injury in mice is evident within a few days of high-fat diet feeding, preceding significant weight gain [56]. Early hypothalamic alterations are thus important determinants for the loss of whole-body metabolic control in these diabetes models.

In the context of diet-induced obesity, rats exposed to one week of high-fat and fructose diet displayed impaired hippocampal insulin signalling, and smaller hippocampal volume with synaptic degeneration, reduced neuronal processes, and astrogliosis [57]. Rats under a similar diet for 5 days displayed impaired performance in place but not object recognition tasks [58], which are dependent on the function of hippocampus and perirhinal cortex, respectively. Furthermore, synaptic deterioration and impaired learning and memory induced by high-fat and high-sucrose diet were found to be dependent on neurotrophic factors that modulate synaptic plasticity [59]. High-fat diet alone is also able to impair hippocampal-dependent spatial memory [60,61]. A recent MRS study in mice exposed to cafeteria diet for 2 months showed that brain choline levels are altered in diet-induced obesity and may reflect the known obesity-induced neuroinflamation in the hippocampus [62]. Auer et al. [62] further found reduced levels of NAA in the hippocampus, consistent with neuronal dysfunction. This study, however, lacked the power to clearly detect alterations on metabolites involved in energy metabolism and neurotransmission, which are indicative of brain dysfunction. In the same study, however, obesity was associated with a reduction of glutamate in the prefrontal cortex, without any other metabolic alterations. Further studies are required to characterise brain metabolic alteration in upon exposure to hypercaloric diets, rich in fat and/or sugars.

## Conclusion and Future Directions

Diabetes-induced metabolic derangements result in altered neurochemical profiles measured by $^{1}$H MRS. However, the link between appearance of cerebral neurochemical alterations and deterioration of brain function in diabetes is not clearly defined. Future studies in patients should include sufficiently large groups of individuals, with a good characterisation of the diabetes phenotype and comorbidities. Importantly, clinical research should be paralleled by translational research in animal models of diabetes in which multi-modal magnetic resonance protocols can provide longitudinal assessment of brain metabolism, structure and function. Such studies will allow identifying the relation between metabolic alterations and brain dysfunction at stages when alterations of brain morphology are not yet present.

At high magnetic fields, neurochemical profiling by state-of-the-art $^{1}$H MRS allows to precisely determine the concentration of about 20 metabolites in the living brain [7]. MRS is thus an excellent toll to probe metabolic alterations in the brain of diabetes patients and of animal models in well controlled preclinical studies. Moreover, rather than single-voxel MRS, more advanced MRS methods are available to map the neurochemical profile throughout the brain even in the small rodent brain [63]. In addition to this mapping of the neurochemical profile, one can also map single metabolites throughout the brain with high spatial resolution with Chemical Exchange Saturation Transfer (CEST) MRI, a technique that can, for example, be employed for detection of lactate [64], glucose [65] or glutamate [66]. Thus, in combination with functional magnetic resonance imaging techniques, neurochemical mapping may provide the link between functional and metabolic networks across the brain, as well as their impairment in diabetes. Indeed, connectivity at functional and structural level, as assessed by means of functional and diffusion MRI, are known to be altered in diabetes and pre-diabetes states, and linked to decrements in cognitive performance [6].

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