Review Article

Altered brain metabolites in patients with diabetes mellitus and related complications – evidence from $^1$H MRS study

Xue Zhao1,*, Qing Han2,*, Xiaokun Gang1 and Guixia Wang1

1Department of Endocrinology and Metabolism, The First Hospital of Jilin University, Changchun 130021, Jilin Province, China; 2Department of Orthopedics, The Second Hospital of Jilin University, Changchun 130021, Jilin Province, China

Correspondence: Guixia Wang (gwang168@jlu.edu.cn)

In recent years, diabetes mellitus (DM) has been acknowledged as an important factor for brain disorders. Significant alterations in brain metabolism have been demonstrated during the development of DM and its complications. Magnetic resonance spectroscopy (MRS), a cutting-edge technique used in biochemical analyses, non-invasively provides insights into altered brain metabolite levels in vivo. This review aims to discuss current MRS data describing brain metabolite levels in DM patients with or without complications. Cerebral metabolites including N-acetylaspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (mI), glutamate, and glutamine were significantly altered in DM patients, suggesting that energy metabolism, neurotransmission, and lipid membrane metabolism might be disturbed during the progression of DM. Changes in brain metabolites may be non-invasive biomarkers for DM and DM-related complications. Different brain regions presented distinct metabolic signatures, indicating region-specific diabetic brain damages. In addition to serving as biomarkers, MRS data on brain metabolites can also shed light on diabetic treatment monitoring. For example, exercise may restore altered brain metabolite levels and has beneficial effects on cognition in DM patients. Future studies should validate the above findings in larger populations and uncover the mechanisms of DM-induced brain damages.

Introduction

Diabetes mellitus (DM) is a global burden with dramatic morbidity and mortality rates [1,2]. In 2013, the International Diabetes Federation (IDF) presented that the global DM incidence rate and impaired glucose tolerance (IGT) rate was 6.9 and 8.3%, respectively [3]. It was predicted that prevalence of DM and the IGT would reach 8.0 and 10.1% by 2035 [3,4]. In addition to the striking diabetic epidemiology data, DM contributes to numerous complications, such as diabetic retinopathy (DR), diabetic kidney disease (DKD), diabetic peripheral neuropathy (DPN), and diabetic cardiomyopathy (DCM) [5-8]. Poor glycemic control coupled with long DM duration induces rapid deterioration of these comorbidities and increases DM-induced mortality rate.

In recent years, emerging evidences have shown that DM affects brain metabolism during its progression, ultimately leads to DM-related metabolic disorders in central nervous system (CNS) [9-12]. The term ‘diabetic encephalopathy’ denotes an intimate relationship between DM and brain dysfunction, especially for aging, Alzheimer’s disease (AD), and depression [13-15]. However, the specific mechanism of DM-induced brain metabolic alterations was unclear. Studies have shown DM can induce significant differences in brain metabolite levels in both animal models and human subjects [4,10,16-18]. Thus, exploring the brain metabolite levels in different stages of DM during its progression may help to determine...
how DM mechanistically contributes to brain disorders, such as AD and depression. However, available reviews focussing on brain metabolites in patients with diabetes is limited.

Magnetic resonance spectroscopy (MRS), a cutting-edge technique of biochemical analyses, detects the alterations in metabolic neurochemical levels and energetics in different regions of the brain in vivo [10,19–22]. Given that MRS is both safe and non-invasive, researchers have applied MRS (especially 1H-MRS) to investigate the underpinnings of many neurological conditions, such as AD and DM [23–25]. To date, over 20 brain metabolites have been detected using high-field MRI/MRS systems in animal models. Those metabolites include, lactate (Lac), alanine (Ala), γ-Aminobutyric acid (GABA), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), glutamate (Glu), glutamine (Gln) glutathione (GSH) aspartate (Asp), phosphocreatine (PCr), creatine (Cr), phosphorylcholine (PC), glyc- erylphosphorylcholine, taurine (Tau), scyllo-inositol, myo-inositol (ml), glycine, ascorbate (Asc), glucose, and phosphorylethanolamine [10,19,20]. Monitoring changes in each metabolite level and ratios of metabolites could provide information about neuronal damages, membrane metabolism dysfunctions and neurotransmission defects occurring in neurological diseases. Additionally, the MRS platform can reflect the influences of neurological conditions on metabolic pathways in specific parts of the brain.

The present review summarizes available data on brain metabolite levels in patients with DM and DM-related comorbid complications, in order to identify non-invasive biomarkers for diabetes-induced brain metabolic changes during DM progression.

**Brain metabolite alterations in type 1 and type 2 DM patients**

DM is classified into type 1 diabetes (T1DM), type 2 diabetes (T2DM), gestational DM (GDM), and other specific types of diabetes, based on underlying disease mechanisms and the demand for insulin. In all diabetic patients, T1DM and T2DM patients make up a vast majority. Detailed characteristics of brain metabolite patterns identified by 1H-MRS in patients with T1DM or T2DM are given in Table 1.

**T1DM**

Amongst studies focussing on T1DM, the majority of patients were adults ranging from 25 to 45 years [26–29]. Only one study focussed on children with a mean age of 12–13 years [30]. Mangia et al. [26] performed hyperglycemic clamp tests on 13 patients with well-controlled T1DM (HbA1C (glycosylated hemoglobin): 7.5 ± 2%; DM duration: 22 ± 12 years) and 32 matched healthy controls. The results revealed that NAA and Glu were reduced by 6% (P<0.05) in the grey matter (occipital lobe) of T1DM patients. No significant differences were identified in other brain metabolite levels. A similar decrease in NAA (6%) was also demonstrated by Heikkilä et al. [28]. In that study, brain metabolite and glucose levels were detected in the frontal cortex, white matter and thalamic regions of 17 T1DM patients and 12 healthy controls. NAA and ml levels were elevated by 20% in the frontal white matter (P<0.001), and by 8% in frontal cortex (P=0.042) of T1DM patients. Moreover, brain glucose levels positively correlated with fasting plasma glucose (r = 0.88; P<0.01), indicating peripheral glucose was important determinants of brain energy expenditures. To explore affects of hyperglycemia on brain glucose levels, Heikkilä et al. [27] performed a 2-h hyperglycemic clamp test with glucose increase of 12 mmol/l. Significant decrease in glucose uptake was observed in thalamic region when compared with cortex and white matter regions (P=0.011), suggesting the involvement of thalamic region in the progression of hyperglycemia-induced brain disorders. Seaquist et al. [29] found that patients with poor glycemic control (HbA1C: 9.8 ± 1.7%) presented nearly 10% lower in brain glucose level in occipital cortex compared with healthy controls. These results demonstrated the influence of chronic hyperglycemia on brain glucose level as well as the presentation of distinct metabolite signatures. Moreover, the impaired glucose uptake may explain neurone loss and functional impairment comorbidities that occurred in elderly DM patients. Another study by Sarac et al. [30] revealed that NAA/Cr and Cho (Choline)/Cr ratios in the pons and NAA/Cr ratio in the left posterior parietal white matter region were reduced in children with T1DM (13.1 ± 3.5 years old) compared with healthy controls. Taken together, reduced NAA level was found both in T1DM adults and children. However, these results require confirmation in larger cohorts across ethnic backgrounds.

**T2DM**

Global incidence of T2DM has grown dramatically in the past decades. Several studies have reported significant brain metabolic alterations in patients with T2DM. Sinha et al. [31] showed that NAA level was significantly decreased in the right frontal and parieto-temporal region of ten patients with T2DM (P=0.03), while Glx level (Glu + Gln) was increased in the right frontal region (P=0.01) when compared with healthy controls. Consistent with the results presented above in T1DM, brain glucose levels were increased in all regions of the brain in patients with T2DM. Lin et
Table 1 Clinical parameters related to brain metabolite levels in T1DM and T2DM patients

| Study          | Year | Number                  | Age (years)                  | DM duration (years) | HbA1c (%) | Testing area                                      | Magnetic parameters | Identified brain metabolites                  |
|----------------|------|-------------------------|------------------------------|---------------------|-----------|--------------------------------------------------|---------------------|----------------------------------------------|
| Sinha et al. [31] | 2014 | Control: 7             | Control: 27.6 ± 2.8          | 3.5 ± 1.2           | 7.25 (6.8, 8.9) | Right frontal; right parieto-temporal; right parieto-occipital white matter | Strength: 1.5 T TR: 2000 ms TE: 30 ms | NAA; Cho; Cr; Glu; Gln; mI; glucose; Cr + PCr; Glu + Gin |
| Lin et al. [32] | 2013 | Control: 22            | Control: 52–80 T2DM: 51–85  | N/A                 | 5.8–14.3  | Lenticular nucleus; thalamus                    | Strength: 1.5 T TR: 1000 ms TE: 144 ms | NAA/Cr; Cho/Cr; lactic acid                  |
| Mangia et al. [26] | 2013 | Control: 32            | T1DM: 38 ± 10 T1DM: 41 ± 11 | 22 ± 12             | 7.5 ± 2   | Occipital lobe (grey matter); parieto-occipital region (white matter) | Strength: 4 T TR: 4.5 s TE: 4 ms | Ala; Asp; Asn; GPC + PC; Cr; PCr; GABA; Glc; Gln; Glu; GSH; myo-inos; scyllo-Ins; Lac; NAA; NAAG; PE; Tau |
| Heikkilä et al. [27] | 2010 | Control: 11            | T1DM: 30.8 ± 6.9 T1DM: 29.4 ± 4.5 | 12.4 ± 2.8          | 7.6 ± 0.8  | Frontal cortex; thalamus; white matter          | Strength: 1.5 T TR: 3000 ms TE: 30 ms | NAA; tCr; glucose                           |
| Heikkilä et al. [28] | 2009 | Control: 12            | T1DM: 29.5 ± 10 T1DM: 28.2 ± 4.4 | 7 ± 5               | 7.4 ± 1.1  | Frontal cortex; thalamus; white matter          | Strength: 1.5 T TR: 3000 ms TE: 30 ms | NAA; Cr; Cho; mI; glucose                    |
| Sahin et al. [16] | 2008 | Control: 14            | IGT: 41.5 ± 8.1 IGT: 44.5 ± 11.3 T2DM: 45.6 ± 14.1 | N/A                 | control: 7.9 ± 1.17 DM: 13.6 ± 1.5 | Frontal cortex; thalamus; parietal white matter | Strength: 1.5 T TR: 2000 ms TE: 31 ms | NAA/Cr; Cho/Cr; mI/Cr                        |
| Seaquist et al. [29] | 2005 | Control: 14            | T1DM: 37 ± 9 DM: 43 ± 13     | N/A                 | 9.8 ± 1.7  | Occipital cortex                                | Strength: 4.0 T TR: 4.5 s TE: 4–20 ms | Glc; Ins; Cho; Glu; NAA                      |
| Sarac et al. [30] | 2005 | Control: 14            | T1DM: 13.1 ± 3.6 T1DM: 12.1 ± 3.3 | N/A                 | 11.9 ± 3.4 | Pons, left basal ganglion; left posterior parietal white matter | Strength: 1.5 T TR: 2000 ms TE: 136 ms | NAA; Cho; Cr; NAA/Cr; Cho/Cr                 |

Abbreviations: Cho, choline; HbA1c, glycosylated hemoglobin A1c; ms, millisecond; N/A, none; PE, phosphoethanolamine; tCr, total Cr; TE, echo time; TR, repetition time.

al. [32] detected NAA, Cho, Cr levels in lenticular nuclei and thalamic regions. The results showed that NAA/Cr ratio was decreased (P=0.007) and Cho/Cr ratio was increased (P=0.001) in patients with T2DM. Additionally, NAA/Cr ratio was negatively correlated with fasting blood glucose (r1 = −0.573) and HbA1C levels (r2 = −0.564). Conversely, Cho/Cr ratio was positively correlated with the same variables. Thus, the DM-induced changes in brain metabolite levels were identified across extensive brain regions and these changes were associated with the peripheral glucose metabolism. However, it is still uncertain when these changes occur in the disease course and whether these changes happen before the diagnosis of DM. Sahin et al. [16] investigated brain metabolites in patients with IGT (n=13) and T2DM (n=25). The results showed that in the frontal cortex region, IGT patients had higher Cho/Cr when compared with healthy controls (P=0.038). However, in parietal white matter, Cho/Cr was significantly lower in T2DM patients than in controls (P=0.049). Patients with poorly controlled T2DM (HbA1C >10%) presented lower Cho/Cr ratio than patients with well-controlled T2DM patients (HbA1C < 10%; P=0.012). Based on these collective findings, brain metabolites differed amongst healthy controls and patients experiencing either IGT or T2DM patients. These findings provide evidence of specific MRS landmarks that indicate altered brain metabolism during DM progression. Future studies should focus on the primary changes in diabetic brain metabolism and identify the key changes to brain metabolites induced by hyperglycemia.
Table 2 Clinical parameters of studies focusing on brain metabolite levels in diabetic patients with chronic complications (DR, DKD, DPN and DR+DN)

| Study            | Year | Number | Age (years) | DM duration (years) | HbA1c (%) | Testing area | Magnetic parameters | Identified brain metabolites |
|------------------|------|--------|-------------|---------------------|-----------|--------------|---------------------|-------------------------------|
| **DR**           |      |        |             |                     |           |              |                     |                               |
| Tong et al. [37] | 2014 | Control: 30 | Control: 51.4 ± 5.3; T2DM: 51.2 ± 5; DR: 51.2 ± 5.7 | N/A | T2DM: 8.2 ± 1.7; DR: 8.2 ± 1.5 | Left frontal white matter, lenticular nucleus; optic radiation | Strength: 3.0 T | TR: 2000 ms; TE: 30 ms | NAA; Cho; Cr; ml; NAA/Cr; ml/Cr; NAA/Cho; Cho/Cr |
| Ozsoy et al. [17] | 2012 | Control: 15 | Control: 51 ± 7.4; DM: 50.8 ± 7.2; NPDR: 54.8 ± 8.4; PDR: 56.5 ± 8.3 | N/A | N/A | Left visual cortex | Strength: 1.5 T | TR: N/A; TE: N/A | NAA; Cho; Cr; NAA/Cr; Cho/Cr |
| **DKD**          |      |        |             |                     |           |              |                     |                               |
| Shi et al. [46]  | 2016 | NC: 30 | NC: 55.8 ± 9.2; DM: 53.6 ± 11.2; DN-IV: 56.9 ± 9.1; DN-IV: 57.3 ± 8.8 | N/A | DM: 9.1 ± 2.5; DN-III: 9.6 ± 2.9; DN-IV: 8.9 ± 2.3 | Basal ganglia; hippocampus | Strength: 1.5 T | TR: N/A; TE: N/A | NAA; Cho; Cr; NAA/Cr; Cho/Cr |
| Fiorina et al. [45] | 2012 | NC: 8; ESRO: 15 | ESRO: 31.6 ± 8.2; KD: 35.8 ± 6.6; KP: 30.1 ± 6.8 | N/A | ESRO: 7.9 ± 1.5; KD: 7.9 ± 1.3; KP: 5.8 ± 0.9 | Bifrontal cortex | Strength: 1.5 T | TR: 2600 ms; TE: 155 ms | NAA; Cho; Cr; NAA/Cr; Cho/Cr |
| **DPN**          |      |        |             |                     |           |              |                     |                               |
| Selvarajah et al. [43] | 2008 | DM: 6; DPN: 10 | Control: 42.5 ± 15.4; DM: 42.9 ± 8.7; DPN: 48.3 ± 11.5 | N/A | DM: 11.6 ± 7.7; DPN: 22.1 ± 8.8 | Thalamus | Strength: 1.5 T | TR: 5000 ms; TE: 20 ms | NAA; Cho; Cr; Glx; Lac; ml; NAA/Cr; NAA/Cho; Cho/Cr |
| Sorensen et al. [42] | 2008 | DM: 14 | Control: 48 (33, 60; DM: 57 (53, 63; DN: 61 (56, 68) | N/A | DM: 13.5 (8.8, 27; DN: 15 (10.3, 22.9) | Left thalamus; anterior cingulate cortex; dorsolateral prefrontal cortex | Strength: 1.5 T | TR: 1500 ms; TE: 25 ms | NAA; Cho; Cr; |
| **DR+DN**        |      |        |             |                     |           |              |                     |                               |
| Makimattila et al. [38] | 2004 | Control: 10 | Control: 36.2 ± 4.1; DR and DN: 36.2 ± 3.3 | 27.8 ± 3.1 | 9.1 ± 0.9 | Frontal cortex; thalamus; posterior frontal white matter | Strength: 1.5 T | TR: 3000 ms; TE: 35 ms | NAA; Cho; ml; glucose |

Abbreviations: DN, diabetic neuropathy; ESRD, end-stage renal disease; Ins, inositol; KD, kidney transplantation; KP, kidney-pancreas transplantation; N/A, none; NC, normal control; NPDR, non-proliferative DR; PDR, proliferative DR; TE, echo time; TR, repetition time.

Brain metabolite alterations in diabetic patients with chronic comorbid complications

Chronic hyperglycemia is an important risk factor for organ damages or dysfunctions in DM patients. The most frequently affected organs are kidney, eyes, and nervous system [33], namely, DKD, retinopathy, and neuropathy, respectively. Studies have shown significant changes in brain metabolite in DM comorbid complications. Details of study characteristics and identified brain metabolites are presented in Table 2.

**DR**

DR is a major cause of blindness in working-age adults around the world. DR is usually divided into two major stages: non-proliferative DR (NPDR) and proliferative DR (PDR) [34]. Previous studies reported a close relationship between retinopathy and both cerebral damage and stroke [35,36]. A study identifying the differences in brain metabolite levels amongst DR patients, DM patients and healthy controls revealed that NAA/Cr ratio in the frontal white matter (P=0.011) and optic radiation regions (P=0.037) were significantly lower in DR patients when compared with DM patients [37]. The ml/Cr ratio showed a 22% increase in DM patients when compared with healthy controls (P=0.009). Additionally, ml/Cr ratio was 24.6% lower in DR patients when compared with DM patients (P=0.001). The correlative analysis showed that NAA/Cr ratio was inversely correlated with DR severity (r = −0.511; P=0.005). Thus, decreases in NAA/Cr and ml/Cr ratios in DR patients may be associated with DR progression. Another study by Ozsoy et al. [17] in patients with NPDR and PDR showed that NAA/Cr and Cho/Cr ratios in the left visual cortex region were significantly different in patients with HbA1C > 8% and HbA1C < 8% (P<0.05). In addition, NAA/Cr...
and Cho/Cr were numerically reduced in PDR compared with NPDR patients although no significant difference was observed. Generally, the development of retinopathy was accompanied with other microvascular complications, including kidney disease and neuropathy. Mäkimattila et al. [38] detected the differences of brain metabolites in frontal cortex, thalamic and posterior frontal white matter between ten healthy controls and ten patients with both retinopathy and autonomic neuropathy. They found that Cho and mI levels were increased in the white matter by 21% ($P=0.02$) and 30% ($P=0.007$) in retinopathy and autonomic neuropathy patients, respectively, but NAA level was unaltered. These data suggested that Cho and mI levels might play a role in the progression of complex diabetic complications. Moreover, these variables were related to membrane damage and proliferation or activation of glia cells [39,40], further implicating brain metabolites in the development of diabetic complications.

DPN
Clinical manifestations of DPN include paresthesia, sensibility loss, ulcers, and osteomyelitis [41]. However, the underlying mechanism of DM neuropathy is unclear. Until recently, little evidence was available to establish a relationship between peripheral neuropathy and altered brain metabolite levels. Sorensen et al. [42] designed a study with three subgroups: T2DM patients with neuropathic pain ($n=12$), T2DM patients without pain ($n=14$), and healthy controls ($n=18$) to explore whether brain metabolite levels changed in patients with diabetic neuropathy. The results showed decreased NAA and Cr levels in dorsolateral prefrontal cortex region and thalamic region in DM group when compared with healthy controls ($P<0.05$). Patients with DPN presented lower NAA in the thalamic region than DM patients without pain. In the thalamic region, Selvarajah et al. [43] found that NAA/Cr and NAA/Cho ratio were lower in DPN patients compared with DM patients and healthy controls ($P=0.04; P=0.02$). Moreover, NAA/Cr level positively correlated with sural amplitudes ($r=0.61; P=0.004$) and nerve conduction velocities ($r=0.58; P=0.006$). Thus, DM (or high glucose concentrations) could affect both brain and peripheral nerve metabolism. The impact of changes in brain metabolite levels during DPN development should be researched carefully in future studies.

DKD
The incidence of patients with chronic kidney disease (CKD) has increased in the past 10 years. It has been considered as a great economic burden due to the striking number of patients with end-stage renal disease (ESRD) and increased requirement for renal replacement therapy [44]. Fiorina et al. [45] examined the brain metabolites of ESRD patients ($n=15$), kidney transplantation patients (KD, $n=9$) and kidney-pancreas transplantation patients (KP, $n=14$) and found decreased NAA/Cho ratio in ESRD, KD, KP groups when compared with healthy controls ($P<0.05$). NAA/Cr ratio was significantly lower in ESRD group than in KD and KP groups ($P<0.01$). These data showed that kidney and kidney-pancreas transplantation normalized the renal function and rescued DM-induced impaired brain metabolism to some extent. To identify the difference in brain metabolite levels in patients experiencing different DKD stages, Shi et al. [46] divided enrolled patients into four groups: DM patients (urinary albumin excretion rates (UAER) <20 μg/min) DN-III patients (20 μg/min ≤ UAER < 200 μg/min), DN-IV patients (UAER ≥ 200 μg/min), and healthy controls. When compared with DM patients, NAA and Cho levels were reduced in DN-III and DN-IV patients ($P<0.05$). Significant differences in NAA and Cho levels were observed between DN-III and DN-IV patients ($P<0.05$). Therefore, the findings support that brain metabolite levels are altered during different stages of DKD. Due to the limited evidence in this field, the underlying mechanism is unclear. But the degree of glucose control, and diabetic stages might be important factors influencing the alterations of metabolic molecules in brain detected by MRS [47,48]. Future studies should discover the mechanism further in a larger population.

Alterations of brain metabolites in diabetic patients with acute complications
Acute diabetic complications usually occur in T1DM patients, including diabetic ketoacidosis (DKA), hypoglycemia coma, hyperosmolar coma, and lactic acidosis. In the present review, studies were identified that investigated the influence of acute DM complications on brain metabolite levels. The characteristics of this collection of literature are given in Table 3.

DKA
DKA is one of the most devastating DM complications that leads to death in pediatric disease, which is usually complicated by cerebral edema [49]. Previous work has shown that there was neuron damage and loss during DKA progression. Wootton-Gorges et al. [50] detected brain metabolite levels during DKA treatment (2–12 h) and after recovery (> 72 h) in the occipital grey matter, basal ganglia, and periaqueductal grey matter regions. They found that
### Table 3 Clinical parameters of studies focussing on brain metabolite levels in diabetic patients with acute complications (hypoglycemia and DKA)

| Study                        | Year | Number | Age (years) | DM duration (years) | Hba1c (%) | Testing area                  | Magnetic parameters | Identified brain metabolites |
|-----------------------------|------|--------|-------------|---------------------|-----------|-------------------------------|---------------------|-----------------------------|
| Hypoglycemia                |      |        |             |                     |           |                               |                     |                             |
| Wiegers et al. [54]         | 2016 | Control: 7 | 27.6 ± 6.9 | 10 (2.5, 17.5)     | 7.5 ± 0.6 | White matter, grey matter; spinal fluid | Strength: 3.0 T TR: 3000 ms TE: 144 ms | NAA; tCho; tCr; m; Asp; Glu; Gln; Tau; inositol |
|                             |      | IAH: 7 | 24.7 ± 8.1  |                     | 7.3 ± 0.4 |                               |                     |                             |
|                             |      | NAH: 7 | 26.2 ± 5.8  |                     |           |                               |                     |                             |
| DKA                         |      | DKA: 29 | 11.9 ± 3.0  | New onset (45%)     | N/A       | Occipital grey matter; basal ganglia; periaqueductal grey matter | Strength: 1.5 T TR/TE: 1000/144 ms | NAA; Cr; Cho; NAA/Cho; Cho/Cr |
| Wootton-Gorges et al. [50]  | 2007 | DKA: 25 | 12 ± 2.7    | New onset (44%)     | N/A       | Periaqueductal region A and in the basal ganglia | Strength: 1.5 T TR/TE: 1500/144 ms | BHB; Lac; AcAc; NAA/Cho; PiCr; Cho; acetone |
| Cameron et al. [51]         | 2004 | DKA: 5  | 12.6 ± 3.2  | New onset           | N/A       | Frontal cortex; occipital region | Strength: 1.5 T TR: 1500 ms TE: 90 ms | Tau; m; glucose |
|                             |      | Non-DKA: 3 |            |                     |           |                               |                     |                             |

Abbreviations: AcAc, acetoacetate; BHB, β-hydroxy butyrate; IAH, impaired awareness of hypoglycemia; N/A, none; NAH, normal awareness of hypoglycemia; tCho, total Cho; tCr, total Cr; TE, echo time; TR, repetition time.

NAA/Cr ratios were significantly reduced in the basal ganglia during the DKA treatment period when compared with the recovery period (P=0.005). Similar trend was also found in the other regions tested. Cameron et al. [51] found that children with diabetes presented higher tau, mJ, and glucose levels in frontal region when compared with children without DKA (n=8). Another study from Wootton-Gorges et al. [52] discovered a peak of β-hydroxy butyrate in the first 4 h of DKA treatment, while an acetone/acetoacetate peak was detected more than 4 h after treatment. Thus, data from studies regarding brain metabolite levels during DKA were inconsistent. Future studies using more standardized designs are required to elucidate this interaction.

### Hypoglycemia

Impaired awareness of hypoglycemia (IAH) affects nearly 25% of patients with T2DM [53]. However, evidence describing brain metabolite levels in patients with hypoglycemia is quite limited. A recent study from Wiegers et al. [54] tested a two-step hyperinsulinemic euglycemic (5 mmol/l) - hypoglycemic (2.8 mmol/l) glucose clamp on seven patients with IAH, seven patients with normal awareness of hypoglycemia (NAH) and seven healthy controls. The results showed that brain lac was decreased by 20% in the IAH patients when compared with both NAH patients and healthy controls (P<0.001). Lac may be reduced due to increased lac oxidation during hypoglycemia, which can result in neurone loss and impaired awareness. It still remains unclear whether levels of other metabolites change in the brain during hypoglycemic events.

### Brain metabolite alterations in diabetic patients with associated diseases

Besides retinopathy, kidney disease, and peripheral neuropathy, DM also causes damages to other end organs and induces DM-associated diseases. Details about the studies about brain metabolites in patients with DM-associated diseases are presented in Table 4.

### Metabolic syndrome

Metabolic syndrome (MetS) refers to a confluence of conditions including hyperglycemia, hypertension, and dyslipidemia [55], which has been shown to affect brain functions and memory [56,57]. A study by Haley et al. [58] on 13 patients with MetS and normal cognition compared with 25 healthy controls revealed significantly increased mJ and glutamate levels in occipitoparietal grey matter regions. Additionally, mJ/Cr (P=0.031) and Glu/Cr (P=0.035) ratios were increased. Similar results were found when the sample size was expanded to 65 patients [59]. Therefore, brain
Table 4 Clinical parameters of studies focussing on brain metabolite levels in diabetic patients with associated diseases (metabolic syndrome, cognitive dysfunction, depression and hypertension)

| Study                  | Year | Number    | Age (years) | DM duration (years) | HbA1c (%) | Testing area                  | Magnetic parameters | Identified brain metabolites |
|------------------------|------|-----------|-------------|---------------------|-----------|--------------------------------|---------------------|-----------------------------|
| **Metabolic syndrome** |      |           |             |                     |           |                                |                     |                             |
| Karczewska-Kupczewska  | 2013 | High-IS: 8| High-IS: 21 ± 2; Low-IS: 27 ± 6 | N/A | N/A | Frontal cortex; thalamus; temporal region | Strength: 1.5 T TR: 1500 ms TE: 35 ms | NAA; Cho; Cr; ml; Glx; NAA/Cho; NAA/Cr; Cho/Cho; ml/ml; Cho/Cr; ml/Cho |
| et al. [60]            |      | Low-IS: 8 |             |                     |           |                                |                     |                             |
| Haley et al. [59]     | 2012 | Control: 46| Control: 50 ± 6.5 | MetS: 51.9 ± 7 | N/A | Occipitoparietal grey matter | Strength: 3.0 T TR: 3000 ms TE: 35 ms | NAA; Cho; Cr; ml; NAA/Cr; Cho/Cho; ml/ml; Cho/Cr |
|                       |      | MetS: 19  |             |                     |           |                                |                     |                             |
| Haley et al. [58]     | 2010 | Control: 25| Control: 51 ± 6 | MetS: 48 ± 6 | N/A | Occipitoparietal grey matter | Strength: 3.0 T TR: 3000 ms TE: 35 ms | NAA; Cho; Cr; NAA/Cr; Cho/Cho; ml/ml |
|                       |      | MetS: 13  |             |                     |           |                                |                     |                             |
| **Cognitive dysfunction** | |           |             |                     |           |                                |                     |                             |
| Wang et al. [65]      | 2015 | NC: 266   | NC: 60.5 ± 10.9 | DM: 61 ± 9.1 | 6.4 ± 3.2 | Left hippocampus; the frontal lobe | Strength: 3.0 T TR: 2000 ms TE: 9.4 ms | NAA; Cho; Cr; ml |
| DM: 188               |      |           |             |                     |           |                                |                     |                             |
| Tiehuis et al. [67]   | 2009 | NC: 40    | NC: 64.9 ± 5.4 | DM: 66.5 ± 5.4 | 8.7 ± 5.6 | White matter                   | Strength: 1.5T TR/TE: 2000/144 ms | NAA; Cho; Cr; NAA/NAA; Cho/Cho; Cho/Cho |
| DM: 72                |      |           |             |                     |           |                                |                     |                             |
| Lyoo et al. [66]      | 2009 | NC: 38    | NC: 30.8 ± 5.1 | DM: 32.3 ± 4.4 | 19.9 ± 3.5 | Prefrontal region     | Strength: 1.5 T TR: 1500 ms TE: 45 ms | Glc; NAA; glucose; Cr; Cho |
| DM: 123               |      |           |             |                     |           |                                |                     |                             |
| **Depression**        |      |           |             |                     |           |                                |                     |                             |
| Haroon et al. [69]    | 2009 | Control: 19| Control: 54.5 ± 9.6 | DM: 57.7 ± 7.9 | 7.7; DD: 56.9 ± 10.6 | Bilateral prefrontal white matter | Strength: 1.5 T TR: 3000 ms TE: 30 ms | NAA; Cho; Cr; Ala; Asp; GABA; ml; Glx; Tau; glc; Lac; scyllo-inositol |
| DM: 20                |      |           |             |                     |           |                                |                     |                             |
| DD: 18                |      |           |             |                     |           |                                |                     |                             |
| Ajlore et al. [70]    | 2007 | NC: 21    | NC: 55.7 ± 9.9 | DM: 58.1 ± 9.2 | 7.1 ± 1.1 | Dorsolateral white matter; subcortical nuclei region | Strength: 1.5 T TR: 3000 ms TE: 30 ms | NAA; Cho; Cr; NAA/Cr; Cho/Cho; ml; Glu; Gin |
| DM: 24                |      |           |             |                     |           |                                |                     |                             |
| DD: 20                |      |           |             |                     |           |                                |                     |                             |
| **Hypertension**      |      |           |             |                     |           |                                |                     |                             |
| Cao et al. [21]       | 2015 | Control: 30| Control: 59.7 ± 7.7; DHT: 62.8 ± 8.6 | N/A | N/A | Frontal cortex; parietal white matter | Strength: 1.5 T TR: 1500 ms TE: 35 ms | NAA; Cho; Cr; NAA/Cr; Cho/Cho |
| DHT: 33               |      |           |             |                     |           |                                |                     |                             |
| Kario et al. [72]     | 2005 | Control: 12| Control: 69 ± 9.4 | DHT: 69 ± 9.2 | 69 ± 9.2 | White matter                   | Strength: 1.5 T TR: 1500 ms TE: 30 ms | NAA; Cho; Cr; NAA/Cr |
| DHT: 20               |      |           |             |                     |           |                                |                     |                             |
| HT: 20                |      |           |             |                     |           |                                |                     |                             |

Abbreviations: DD, diabetic depression; DHT, diabetic hypertension; High-IS, high insulin sensitivity; HT, hypertension; IS, insulin sensitivity; MetS, metabolic syndrome; N/A, none; NC, normal control; TE, echo time; TR, repetition time.
pre-DM periods or in insulin-resistant patients. Future studies should focus on elucidating brain metabolite levels in prediabetic and insulin-resistant patients.

**Cognitive dysfunction**

Significant alterations of brain metabolites induced by cognitive impairment or AD were reported in both human and animal models [14,61,62]. Numerous studies have revealed that prolonged hyperglycemia contributes to cognitive dysfunction and increases the risk of dementia [63,64]. Wang et al. [65] explored brain metabolite signature in the left hippocampus and frontal lobe of 188 T2DM patients and 266 healthy controls. To detect cognitive function, Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were applied. DM patients had increased NAA, Cr, and mI levels in the left hippocampal region \((P<0.05)\) along with lower MMSE scores, especially in aspects of attention, and language \((P<0.05)\). Also, inverse correlations were found between MoCA score and both mI and Cr levels in the left hippocampal region of the T2DM patients \((P<0.05)\). Lyoo et al. [66] studied with T1DM where diabetic patients had higher cerebral glucose and Glu-Gln-aminobutyric acid (Glx) levels \((P=0.005)\), and lower cognitive performances in memory, executive functions, and psychomotor speeds \((P=0.003)\). An inverse relationship was found between high Glx levels and poor cognitive performance \((P<0.05)\). However, data from Tiehuis et al. [67] revealed that no differences were found in brain metabolites \((\text{NAA/Cr}, \text{Cho/Cr}, \text{NAA/Cho})\) ratios when 72 T2DM patients and healthy controls were compared \((P=0.13, 0.39, 0.89, \text{respectively})\). No obvious correlations were discovered between cognitive performances and the above-listed brain metabolite ratios, but T2DM patients did exhibit poorer cognitive performances in memory and information processing speeds. The explanation for these data inconsistencies may result from the differences in study population. For example, Tiehuis et al. [67] study was performed on patients with an HbA1C of 6.8 ± 1%, while the HbA1C level in patients from Wang’s study [65] was 8.5 ± 2% [65]. As described above, brain metabolite levels could be likely influenced by the glycemic control and HbA1C levels in patients. Moreover, patients of different ethnicities could have metabolic signatures that reflect environmental and dietary variances. Therefore, more studies are required to explore the effects of DM on brain metabolite levels in patients with DM-induced cognitive impairments.

**Depression**

DM and depression are linked by several etiologic factors [68]. Investigating altered brain metabolic signatures can elucidate mechanisms of the development of depression in DM patients. Haroon et al. [69] detected mI level in bilateral prefrontal white matter in depressed diabetic patients \((n=20)\). The results showed that mI and mI/Cr ratio in right frontal white matter were increased in DM patients when compared with healthy controls \((P<0.05)\). Correlative analyses identified an inverse relationship between mI levels and recall as well as recognition subtests \((P<0.05)\). Similar mI trend was reported by Ajilore et al. [70] in 20 diabetic patients with depression, 24 diabetic patients, and 21 healthy controls. In addition to increased mI levels, Glu and Gln levels were decreased significantly in subcortical regions of depressed diabetic patients when compared with DM patients and healthy controls \((P<0.001)\). Thus, these findings on mI, Glx levels might provide insights into the pathological underpinnings of the development of depression in DM patients.

**Hypertension**

Based on the data from World Health Organization (WHO), nearly 60% of patients with T2DM have concomitant hypertension, so brain metabolite alterations may be more pronounced in such patients [71]. Cao et al. [21] investigated brain metabolites in the frontal cortex and parietal white matter of 33 patients with diabetic hypertension (DHT) and 30 healthy controls. The results showed that both NAA/Cr and Cho/Cr ratios were significantly decreased in the frontal cortex regions \((P<0.01)\). Similarly, Kario et al. [72] showed that only NAA/Cr ratio was reduced in the parietal white matter when compared with healthy control patients \((P<0.001)\). The authors concluded that DHT could cause metabolic disorders in the brain in a region-specific pattern, and NAA/Cr and Cho/Cr ratios might be biomarkers indicative of this damage. Due to limited evidence investigating DHT patients and small sample sizes in the previous studies, it is doubtful that measurements of NAA/Cr and Cho/Cr ratios were powerful enough to serve as reliable biomarkers for DHT patients.

**The underlying mechanism of the altered metabolic molecules and their effects on brain functions**

Because \(^{1}H\) MRS technology can detect the altered metabolic molecules non-invasively, it is widely used for early diagnosis, and biomarker exploration for neurological diseases, including diabetes-induced brain dysfunction [73].
The alterations of metabolic molecules examined can convey much information about the underlying mechanism of diabetes-induced brain dysfunction, and the accumulations of these molecules can also aggravate the brain dysfunctions, resulting in a vicious circle. Studies have shown that when diabetes and its complications happen, glucose metabolism is affected and insulin resistance will happen [74,75]. Impaired insulin signaling will influence energy metabolism, inducing mitochondria dysfunction, enhanced oxidative stress, ROS accumulation, increased neuro-inflammation, apoptosis, gliosis, and decreased neurotrophic factors [76,77]. Long-term hyperglycemia can cause impaired osmotic adaptation, apoptosis, and so on (Figure 1). To reflect these pathological processes, the concentrations of metabolic molecules in brain will change. For specifically, NAA is frequently distributed in the brain. It is mainly located in neurones, especially in mitochondria. Thus, when neuronal density, function, or viability is affected, NAA level will change. And the change of NAA conveys the information about neurone loss [78,79]. Cho is involved in membrane metabolism, when the concentration of Cho changes, it means the cellular membrane is damaged [80]. Cr takes part in the energy metabolism, and increased Cr level means increased oxidative stress, ROS and mitochondria dysfunctions in both neurones and glial cells [81]. As for myo-inositol, it has been known as the marker of gliosis. When insulin resistance happens, inflammation will be enhanced, inducing the process of gliosis of glial cells [82]. And all above metabolic alterations will lead to disturbed neurotransmissions, accelerated the neurodegeneration and demyelination as well as induce brain atrophy. Finally, brain functions will be influenced in a large scale. Different stages of diabetes and different diabetic complications might present inconsistent levels of metabolic molecules, which might help to differentiate or diagnose the diabetic complications earlier. However, the exploration of the underlying mechanism of these metabolic molecules is at the initial stage, future study should validate above findings in larger-scale clinical studies.

Figure 1. The possible underlying mechanism of diabetes-induced brain dysfunctions.
Effects of diabetic treatment on brain metabolites

Since DM and its complications have effects on brain metabolites, application of diabetic treatments may restore brain metabolism and provide insights into treatments of other brain disorders. Most available studies have focussed on detecting brain metabolite levels in DM patients that are participating in physical exercise. Nagothu et al. [83] investigated 68 patients with T2DM, half of the patients did 6 months of yoga, while the rest half of the patients were not assigned a task. After 6 months, NAA levels were increased ($P<0.02$) and ml levels were decreased ($P<0.01$) in the right dorsolateral frontal lobe regions of the exercising patients when compared with the controls, suggesting that long-term exercise regimens restored altered brain metabolite levels in diabetic patients. However, Santhakumari et al. [84] reported that yoga had no effect on brain NAA and ml levels based on an analysis of five DM patients. Nevertheless, they found that yoga ameliorated memory decline in diabetic patients ($P=0.001$). Due to the small sample size in the latter study, it is difficult to conclude that exercise had a meaningful influence on brain metabolism, even in patients who experienced a significantly decreased HbA$_1$C ($P=0.03$). Thus, large-scale studies are required to elucidate this interaction. Moreover, future studies could explore the role of different DM treatments on brain metabolite levels to provide alternative explanations for the memory declines and brain dysfunctions observed in DM patients.

Conclusion

In the present review, associations between DM and its complications on brain metabolite levels were discussed. Cerebral metabolite levels including NAA/Cr, ml/Cr, Cho, Glu, and Gln were significantly altered in patients with DM when compared with healthy controls, suggesting that energy metabolism, neurotransmission, and lipid membrane metabolism were impaired in patients during DM progression. Monitoring changes in brain metabolites could be used as non-invasive biomarkers for progression of DM and development of related complications. Additionally, MRS data illustrating brain metabolite levels can elucidate and monitor the effects of DM treatment. Diabetic treatments such as exercise might restore altered brain metabolite levels and provide beneficial effects on cognition in DM patients. Future studies should validate the above findings in larger population samples and further uncover the mechanistic underpinnings of DM-induced brain damages.

Author contribution

X.Z. designed this review. Q.H. and X.G. performed the paper selection. X.Z. and G.W. wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the the National Natural Science Fund of China [grant number 81670732 (to G.W.), 81802174 (to Q.H.)]; the Jilin Province Development and Reform Commission [grant number 2013C036-4]; and the Jilin Province Science and Technology Department Project [grant numbers 20180520115JH, 20170204004GX (to Q.H)].

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AD, Alzheimer’s disease; Cho, choline; Cr, creatine; DHT, diabetic hypertension; DKA, diabetic ketoacidosis; DKD, diabetic kidney disease; DM, diabetes mellitus; DPN, peripheral neuropathy; DR, diabetic retinopathy; ESRD, end-stage renal disease; GABA, γ-aminobutyric acid; Glx, glutamate + glutamine; HbA$_1$C, glycosylated hemoglobin; High-IS, high insulin sensitivity; IGT, impaired glucose tolerance; IAH, impaired awareness of hypoglycemia; Lac, lactate; Low-IS, low-insulin sensitivity; MetS, metabolic syndrome; ml, myo-inositol; MMSE, mini-mental state examination; MoCA, Montreal Cognitive Assessment; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; NAH, normal awareness of hypoglycemia; NPDR, non-proliferative DR; PDR, proliferative DR; T1DM, type 1 DM; T2DM, type 2 DM; UAER, urinary albumin excretion rate.

References

1. Danaei, G., Finucane, M.M., Lu, Y., Singh, G.M., Cowan, M.J., Paciorek, C.J. et al. (2011) National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 378, 31–40, https://doi.org/10.1016/S0140-6736(11)60679-X
2. Yang, W., Lu, J., Wang, J., Xia, W., Ji, L., Xiao, J. et al. (2010) Prevalence of diabetes among men and women in China. N. Engl. J. Med. 362, 1090–1101

© 2018 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
International Diabetes Federation (2013) IDF Diabetes Atlas (6th edition). https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/19-atlas-6th-edition.html (Accessed 3 September 2018)

Duarte, J.M. (2015) Metabolic alterations associated to brain dysfunction in diabetes. Aging Dis. 6, 304–321, https://doi.org/10.14336/AD.2014.1104

Hu, X., Bai, T., Xu, Z., Liu, Q., Zheng, Y. and Cai, L. (2017) Pathophysiological fundamentals of diabetic cardiomyopathy. Compr. Physiol. 7, 693–711, https://doi.org/10.1002/cphy.c160021

Xu, X., Sun, J., Chang, X., Wang, J., Luo, M., Wintergerst, K.A. et al. (2016) Genetic variants of nuclear factor erythroid-derived 2-like 2 associated with the complications in Han descendents with type 2 diabetes mellitus of Northeast China. J. Cell. Mol. Med. 20, 2078–2088, https://doi.org/10.1111/jcmm.12900

Malecki, M.T. (2004) Type 2 diabetes mellitus and its complications: from the molecular biology to the clinical practice. Rev. Diab. Stud. 1, 5–8, https://doi.org/10.1901/REDS.2004.1.5

Gu, J., Cheng, Y., Wu, H., Kong, L., Wang, S., Xu, Z. et al. (2017) Metallothionein is downstream of Nrf2 and partially mediates sulforaphane prevention of diabetic cardiomyopathy. Diabetes 66, 529, https://doi.org/10.2337/db15-1274

Duarte, J.M.N., Carvalho, R.A., Cunha, R.A. and Gruetter, R. (2009) Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. J. Neurochem. 111, 368–379, https://doi.org/10.1111/j.1471-4159.2009.06349.x

Duarte, J.M. (2016) Metabolism in the diabetic brain: neurochemical profiling by 1H magnetic resonance spectroscopy. J. Diabetes Metab. Disord. 3, 011, https://doi.org/10.24966/DMD-201X/100011

Miles, W.R and Root, H.F. (1924) Psychologic tests applied to diabetic patients. J. Nerv. Ment. Dis. 60, 523, https://doi.org/10.1002/jnmd.192411000-00077

Nagalski, A., Kozinski, K. and Wisniewska, M.B. (2016) Metabolic pathways in the periphery and brain: contribution to mental disorders? Int. J. Biochem. Cell Biol. 80, 19–30, https://doi.org/10.1016/j.biocel.2016.09.012

Wang, Z., Huang, Y., Cheng, Y., Tan, Y., Wu, F., Wu, J. et al. (2016) Endoplasmic reticulum stress-induced neuronal inflammatory response and apoptosis likely plays a key role in the development of diabetic encephalopathy. Oncotarget 7, 78455

Lin, A.L., Laird, A.R., Fox, P.T. and Gao, J.H. (2012) Multimodal MRI neuroimaging biomarkers for cognitive normal adults, amnestic mild cognitive impairment, and Alzheimer’s disease. Neuror. Res. Int. 2012, 907409, https://doi.org/10.1155/2012/907409

Dejong, R.N. (1950) The nervous system complications of diabetes mellitus, with special reference to cerebrovascular changes. J. Nerv. Ment. Dis. 111, 181–206, https://doi.org/10.1002/jnmd.195011130-00001

Sahin, I., Alkan, A., Keskin, L., Cikim, A., Karakas, H.M., Firat, A.K. et al. (2008) Evaluation of in vivo cerebral metabolism on proton magnetic resonance spectroscopy in patients with impaired glucose tolerance and type 2 diabetes mellitus. J. Diabetes Complications 22, 254–260, https://doi.org/10.1016/j.jdiacomp.2007.03.007

Ozsoy, E., Doganay, S., Dogan, M., Alkan, A. and Firat, P.G. (2012) Evaluation of metabolite changes in visual cortex in diabetic retinopathy by MR-spectroscopy. J. Diabetes Complications 26, 241–245, https://doi.org/10.1016/j.jdiacomp.2012.03.007

Duarte, J.M., Ferreira, S.G., Carvalho, R.A., Cunha, R.A. and Kofalvi, A. (2012) CB(1) receptor activation inhibits neuronal and astrocytic intermediary metabolism in the rat hippocampus. Neurochem. Int. 60, 1–6, https://doi.org/10.1016/j.neuint.2011.10.019

Lin, A.L. and Rothman, D.L. (2014) What have novel imaging techniques revealed about metabolism in the aging brain? Future Neuro. 9, 341, https://doi.org/10.1016/j.fjftn.2013.11.013

Duarte, J.M., Do, K.Q. and Gruetter, R. (2014) Longitudinal neurochemical modifications in the aging mouse brain measured in vivo by 1H magnetic resonance spectroscopy. Neurobiol. Aging 35, 1660–1668, https://doi.org/10.1016/j.neurobiolaging.2014.01.135

Cao, Z., Ye, B.D., Shen, Z.W., Cheng, X.F., Yang, Z.X., Liu, Y.Y. et al. (2015) 2D-1H proton magnetic resonance spectroscopic imaging study on brain metabolite alterations in patients with diabetic hypertension. Mol. Med. Rep. 11, 4232–4238, https://doi.org/10.3892/mmr.2015.3305

Sherry, E.B., Lee, P. and Choi, I.Y. (2015) In vivo NMR studies of the brain with hereditary or acquired metabolic disorders. Neurochem. Res. 40, 2647–2655, https://doi.org/10.1007/s11002-015-1772-1

Duarte, J.M., Schuck, P.F., Wenk, G.L. and Ferreira, G.E. (2014) Metabolic disturbances in diseases with neurologic involvement. Aging Dis. 5, 238–255

Oz, G., Alger, J.R., Barker, P.B., Bartha, R., Bizzi, A., Boesch, C. et al. (2014) Clinical proton MR spectroscopy in central nervous system disorders. Radiology 270, 658–679, https://doi.org/10.1148/rad.13130531

Kantarci, K., Jack, C.R., Xu, Y.C., Campeau, N.G., O’Brien, P.C., Smith, G.E. et al. (2000) Regional metabolic patterns in mild cognitive impairment and Alzheimer’s disease: a 1H MRS study. Neurology 55, 210–217, https://doi.org/10.1212/00005053-195011130-00001

Manjila, S., Kumar, A.F., Moheet, A.A., Roberts, R.J., Eberly, L.E., Sequaist, E.R. et al. (2013) Neurochemical profile of patients with type 1 diabetes measured by 1H-MRS at 4 T. J. Cereb. Blood Flow Metab. 33, 754–759, https://doi.org/10.1038/jcbfm.2013.13

Heikkilä, O., Lundbom, N., Timonen, M., Groop, P.H., Heikkinen, S. and Makimattila, S. (2010) Evidence for abnormal glucose uptake or metabolism in thalamus during acute hyperglycaemia in type 1 diabetes: a 1H MRS study. Metab. Brain Dis. 25, 227–234, https://doi.org/10.1007/s11011-010-9199-5

Heikkilä, O., Lundbom, N., Timonen, M., Groop, P.H., Heikkinen, S. and Makimattila, S. (2009) Hyperglycaemia is associated with changes in the regional concentrations of glucose and myo-inositol within the brain. Diabetologia 52, 534–540, https://doi.org/10.1007/s00125-008-1242-2

Sequaist, E.R., Tkac, I., Damberg, G., Thomas, W. and Gruetter, R. (2005) Brain glucose concentrations in poorly controlled diabetes mellitus as measured by high-field magnetic resonance spectroscopy. Metabolism 54, 1008–1013, https://doi.org/10.1016/j.metabol.2005.02.018

Sarac, K., Akinci, A., Alkan, A., Aslan, M., Baysal, T. and Ozcan, C. (2005) Brain metabolite changes on proton magnetic resonance spectroscopy in children with poorly controlled type 1 diabetes mellitus. Neuroradiology 47, 562–565, https://doi.org/10.1007/s00234-005-1387-3

Sinha, S., Ekka, M., Sharma, U., R., Pandey, R.M. and Jaganathan, N.R. (2014) Assessment of changes in brain metabolites in Indian patients with type-2 diabetes mellitus using proton magnetic resonance spectroscopy. BMC Res. Notes 7, 1–7, https://doi.org/10.1186/1756-0500-7-41

© 2018 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
32 Lin, Y., Zhou, J., Sha, L., Li, Y., Qu, X., Liu, L. et al. (2013) Metabolite differences in the lenticular nucleus in type 2 diabetes mellitus shown by proton MR spectroscopy. *Am. J. Neuroradiol.* 34, 1692–1696, https://doi.org/10.3174/ajnr.A3492

33 Kikkawa, R. (2000) Chronic complications in diabetes mellitus. *Br. J. Nutr.* 84, 406–410, https://doi.org/10.1079/0966582017388653

34 Simo-Servot, O., Simo, R. and Hernandez, C. (2016) Circulating biomarkers of diabetic retinopathy: an overview based on physiopathology. *J. Diabetes Res.* 2016, 5263798, https://doi.org/10.1155/2016/5263798

35 Wong, T.Y., Klein, R., Sharrett, A.R., Couper, D.J., Klein, B.E.K., Liao, D.P. et al. (2002) Cerebral white matter lesions, retinopathy, and incident clinical stroke. *J. Am. Med. Assoc.* 288, 67–74, https://doi.org/10.1001/jama.288.1.167

36 Baker, M.L., Marino Larsen, E.K., Kuller, L.H., Klein, R., Klein, B.E., Siscovich, D.S. et al. (2007) Retinal microvascular signs, cognitive function, and dementia in older persons: the Cardiovascular Health Study. *Stroke* 38, 2041–2047, https://doi.org/10.1161/STROKEAHA.107.483586

37 Tong, J., Geng, H., Zhang, Z., Zhu, X., Meng, Q., Sun, X. et al. (2014) Brain metabolite alterations demonstrated by proton magnetic resonance spectroscopy in diabetic patients with retinopathy. *Science* 343, 1037–1042, https://doi.org/10.1126/science.1241457

38 Mäkimattila, S., Malmberg-Ceder, K., Häkkinen, A.M., Vuori, K., Salonen, O., Summanen, P. et al. (2005) Brain metabolic alterations in patients with type 1 diabetes-hyperglycaemia-induced injury. *J. Cereb. Blood Flow Metab.* 24, 1393–1399, https://doi.org/10.1097/01.WCB.0000143700.15489.B2

39 Shimoda, H., Iwasa, Y., Miyamoto, Y., Maniwa, Y. and Mitani, T. (2001) Proton MR spectroscopy in clinical routine. *JMRI* 13, 560–567, https://doi.org/10.1002/jmri.10179

40 Schweinsburg, B.C., Taylor, M.J., Videen, J.S., Ahlason, O.M., Patterson, T.L. and Grant, I. (2000) Elevated myo-inositol in grey matter of recently detoxified but not long-term abstinent alcoholics: a preliminary MR spectroscopy study. *Alcohol. Clin. Exp. Res.* 24, 699–705, https://doi.org/10.1111/j.1530-0277.2000.tb02042.x

41 Cakici, N., Fakkel, T.M., van Neck, J.W., Verhagen, A.P. and Coert, J.H. (2016) Systematic review of treatments for diabetic peripheral neuropathy. *Diabetes Metab. Syndr.* 10, 247–249, https://doi.org/10.1016/j.dsx.2016.06.005

42 Florina, P., Vezzulli, P., Bassi, R., Gremizzi, C., Falautano, M., D’Addio, F. et al. (2012) Near normalization of metabolic and functional features of the central nervous system in type 1 diabetic patients with end-stage renal disease after kidney-pancreas transplantation. *Diabetes Care* 35, 367–374, https://doi.org/10.2337/dc11-1697

43 Shi, X., Zhang, Y., Niu, H., Wang, R., Shen, J., Zhou, S. et al. (2016) Correlation between cognitive impairment and diabetic nephropathy in patients with Type 2 diabetes mellitus. *J. Central South Univ. Med. Sci.* 41, 143–150

44 Florina, P., Perseghin, G., De Cobelli, F., Gremizzi, C., Petrelli, A., Monti, L. et al. (2016) Altered kidney graft high-energy phosphate metabolism in kidney-transplanted end-stage renal disease type 1 diabetic patients: a cross-sectional analysis of the effect of kidney alone and kidney-pancreas transplantation. *Diabetes Care* 39, 597, https://doi.org/10.2337/dc16-1324

45 Florina, P., Filchi, F., D’Angelo, A., Finzi, G., Pellegratia, F., Guzzi, V. et al. (2004) Normalization of multiple haemostatic abnormalities in uremic type 1 diabetic patients after kidney-pancreas transplantation. *Diabetes 53*, 2291–2300, https://doi.org/10.2337/diabetes.53.9.2291

46 Turbridge, W.M. (1981) Factors contributing to deaths of diabetics under fifty years of age. On behalf of the Medical Services Study Group and British Diabetic Association. *Lancet* 2, 569–572, https://doi.org/10.1016/S0140-6736(81)90590-8

47 Woottong-Gorges, S.L., Buoncore, M.H., Kuppermann, N., Marcin, J.P., Barnes, P.D., Neely, E.K. et al. (2007) Cerebral proton magnetic resonance spectroscopy in children with diabetic ketoacidosis. *Am. J. Neuroradiol.* 28, 695–699

48 Cameron, F.J., Keen, M.J., Wellard, R.M., Werther, G.A., Neil, J.J. and Inder, T.E. (2005) Insights into the acute cerebral metabolic changes associated with childhood diabetes. *Diabet. Med.* 22, 648–653, https://doi.org/10.1111/j.1464-5491.2005.01453.x

49 Woottong-Gorges, S.L., Buoncore, M.H., Kuppermann, N., Marcin, J., DiCarlo, J., Neely, E.K. et al. (2005) Detection of cerebral β-hydroxybutrate, acetoacetate, and lactate on proton MR spectroscopy in children with diabetic ketoacidosis. *Am. J. Neuroradiol.* 26, 1286–1291

50 Cryer, P.E. (2013) Mechanisms of hypoglycaemia-associated autonomic failure in diabetes. *N. Engl. J. Med.* 369, 362–372, https://doi.org/10.1056/NEJma1215228

51 Wiegars, E.C., Rooijackers, H.M., Tack, C.J., Heerschap, A., de Galan, B.E. and Van, d.G.M. (2016) Brain lactate concentration falls in response to hyperglycaemia in patients with type 1 diabetes and impaired awareness of hypoglycaemia. *Diabetes 65*, https://doi.org/10.2337/db16-0068

52 Alberti, K.G.M.M., Eckel, R.H., Donato, K.A., James, W.P.T., Grundy, S.M., Zimmet, P.Z. et al. (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International A. *Circulation* 120, 1640–1645, https://doi.org/10.1161/CIRCULATIONAHA.109.192644

53 Akbaraly, T.N., Kivimaki, M., Shipley, M.J., Tabak, A.G., Jokela, M., Virtanen, M. et al. (2016) Metabolic syndrome over 10 years and cognitive functioning in late midlife: the Whitehall II study. *Diabetes Care* 33, 84–89, https://doi.org/10.2337/dc09-1218

54 Gatto, N.M., Henderson, V.W., St John, J.A., McCleary, C., Hodis, H.N. and Mack, W.J. (2008) Metabolic syndrome and cognitive function in healthy middle-aged and older adults without diabetes. *Aging Neuropsychol. Cogn.* 15, 627–641, https://doi.org/10.1080/13825580802036936

55 Haley, A.P., Gonzales, M.M., Tarumi, T., Miles, S.C., Goudarzi, K. and Tanaka, H. (2010) Elevated cerebral glutamate and myo-inositol levels in cognitively normal middle-aged adults with metabolic syndrome. *Metab. Brain Dis.* 25, 397–405, https://doi.org/10.1007/s11011-010-9221-y

56 Haley, A.P., Gonzales, M.M., Tarumi, T. and Tanaka, H. (2012) Subclinical vascular disease and cerebral glutamate elevation in metabolic syndrome. *Metab. Brain Dis.* 27, 513–520, https://doi.org/10.1007/s11011-012-9306-x
60 Karczewska-Kupczewska, M., Tarasow, E., Nikolajuk, A., Stefanowicz, M., Mutelewicz, N., Otziomek, E. et al. (2013) The effect of insulin infusion on the metabolites in cerebral tissues assessed with proton magnetic resonance spectroscopy in young healthy subjects with high and low insulin sensitivity. *Diabetes Care* **36**, 2787–2793, https://doi.org/10.2337/dc12-1437

61 Lin, A.L., Powell, D., Caban-Holt, A., Jicha, G., Robertson, W., Gold, B.T. et al. (2016) 1H-MRS metabolites in adults with Down syndrome: effects of dementia. *Neuromage Clin.* **11**, 728–735, https://doi.org/10.1016/j.nic.2016.06.001

62 Sanchez, H., Patil, I., Kanamori, K., Diaz Brinton, R., Zhang, W., Lin, A.L. et al. (2014) Hypermetabolic state in the 7-month-old triple transgenic mouse model of Alzheimer’s disease and the effect of lipic acid: a 13C-NMR study. *J. Cereb. Blood Flow Metab.* **34**, 1749–1760, https://doi.org/10.1038/jcbfm.2014.137

63 Perros, P. (2007) Long-term effect of diabetes and its treatment on cognitive function. *N. Engl. J. Med.* **356**, 1842–1852, https://doi.org/10.1056/NEJMoa06397

64 Biesels, G.J., Deary, I.J. and Ryan, C.M. (2008) Cognition and diabetes: a lifespan perspective. *Lancet Neurol.* **7**, 184–190, https://doi.org/10.1016/S1474-4422(08)70021-8

65 Wang, Y., Xu, X.Y., Feng, C.H., Li, Y.L., Ge, X., Zong, G.L. et al. (2015) Patients with type 2 diabetes exhibit cognitive impairment with changes of metabolite concentration in the left hippocampus. *Metab. Brain Dis.* **30**, 1027–1034, https://doi.org/10.1007/s11011-015-9670-4

66 Lyoo, I.K., Yoon, S.J., Musen, G., Simonson, D.C., Weinger, K., Bolo, N. et al. (2009) Altered prefrontal glutamate-glutamine-gamma-aminobutyric acid levels and relation to low cognitive performance and depressive symptoms in type 1 diabetes mellitus. *Arch. Gen. Psychiatry* **66**, 878–887, https://doi.org/10.1001/archgenpsychiatry.2009.86

67 Tiehuis, A., van der Meer, G., Mall, W., Pleizier, M., Biesels, G.J., Kappelle, J. et al. (2010) MR spectroscopy of cerebral white matter in type 2 diabetes: no association with clinical variables and cognitive performance. *Neuroradiology* **52**, 155–161, https://doi.org/10.1007/s00234-009-0598-4

68 Katon, W.J., Lin, E.H., Russo, J., Von, K.M., Checchanowski, P., Simon, G. et al. (2004) Cardiac risk factors in patients with diabetes mellitus and major depression. *J. Gen. Intern. Med.* **19**, 1192–1199, https://doi.org/10.1111/j.1525-1497.2004.30405.x

69 Haroon, E., Watari, K., Thomas, A., Ajilore, O., Mintz, J., Elderkin-Thompson, V. et al. (2009) Prefrontal myo-inositol concentration and visuospatial functioning among diabetic depressed patients. *Psychiatry Res.* **171**, 10–19, https://doi.org/10.1016/j.psychres.2008.03.006

70 Ajilore, O., Haroon, E., Kumaran, S., Darwin, C., Binesh, N., Mintz, J. et al. (2006) Measurement of brain metabolites in patients with type 2 diabetes and major depression using proton magnetic resonance spectroscopy. *Neuropsychopharmacology* **32**, 1224–1231, https://doi.org/10.1038/sj.npp.1301248

71 Chobanian, A.V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, J.L. et al. (2003) Seventh Report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension* **42**, 1206–1252, https://doi.org/10.1161/01.HYP.0000107251.49515.c2

72 Kario, K., Ishikawa, J., Hoshide, S., Matsui, Y., Morinari, M., Eguchi, K. et al. (2005) Diabetic brain damage in hypertension: role of renin-angiotensin system. *Hypertension* **45**, 887–893, https://doi.org/10.1161/01.HYP.0000163460.07639.3f

73 Aoki, Y., Kasai, K. and Yamasue, H. (2012) Age-related change in brain metabolite abnormalities in autism: a meta-analysis of proton magnetic resonance spectroscopy studies. *Transl. Psychiatry* **2**, e69, https://doi.org/10.1038/tp.2011.65

74 Park, C.R. (2001) Cognitive effects of insulin in the central nervous system. *Neurosci. Biobehav. Rev.* **25**, 311–323, https://doi.org/10.1016/S0149-7634(01)00016-1

75 Vrenken, H., Barkhof, F., Uitdehaag, B.M., Castelijns, J.A., Polman, C.H. and Pauwels, P.J. (2005) MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter.. *Magn. Reson. Med.* **53**, 256, https://doi.org/10.1002/mrm.20366

76 Luque-Contreras, D., Carvajal, K., Toral-Rios, D., Franco-Bocanegra, D. and Campos-Peña, V. (2014) Oxidative stress and metabolic syndrome: cause or consequence of Alzheimer's disease? *Oxid. Med. Cell. Longev.* **2014**, 497802, https://doi.org/10.1155/2014/497802

77 Sima, A.A. (2010) Encephalopathies: the emerging diabetic complications. *Acta Diabetol.* **47**, 279–293, https://doi.org/10.1007/s00592-010-0218-0

78 Rae, C.D. (2014) A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem. Res.* **39**, 1–36, https://doi.org/10.1007/s11064-013-1199-5

79 Gomez, R., Behar, K.L., Watzl, J. et al. (2012) Intravenous ethanol infusion decreases human cortical γ-aminobutyric acid and N-acetylaspartate as measured with proton magnetic resonance spectroscopy at 4 tesla. *Biol. Psychiatry* **71**, 239–246, https://doi.org/10.1016/j.biopsych.2011.06.026

80 Gujar, S.K., Maheshwari, S., Bjerkan-Burtscher, I. and Sundgren, PC. (2005) Magnetic resonance spectroscopy. *J. Neuro Ophthalmol.* **25**, 217, https://doi.org/10.1097/01.wno.0000177307.21081.81

81 Ross, B. and Bluml, S. (2001) Magnetic resonance spectroscopy of the human brain. *Anat. Rec.* **265**, 54–84, https://doi.org/10.1002/ar.1058

82 Fisher, S.K., Novak, J.E. and Agrafonoff, B.W. (2010) Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism, and functional significance. *J. Neurochem.* **82**, 736–754, https://doi.org/10.1111/j.1471-4159.2002.01041.x

83 Nagotto, R.S., Reddy, Y.J., Rajagopalan, A. and Varma, R. (2015) Right dorsolaterial frontal lobe N-Acetyl aspartate and myoinositol concentration estimation in type 2 diabetes with magnetic resonance spectroscopy. *J. Clin. Diagn. Res.* **9**, CC16–CC19

84 Santhakumari, R., Reddy, I.Y., Archana, R. and Rajesh, P. (2016) Role of yoga in alienating the memory decline and frontal lobe metabolite changes in type 2 diabetes. *Int. J. Res. Ayurveda Pharm.* **7**, 78–81, https://doi.org/10.7897/2277-4343.07116