ABSTRACT: From epidemiological studies it is known that diabetes patients display increased risk of developing dementia. Moreover, cognitive impairment and Alzheimer’s disease (AD) are also accompanied by impaired glucose homeostasis and insulin signalling. Although there is plenty of evidence for a connection between insulin-resistant diabetes and AD, definitive linking mechanisms remain elusive. Cerebrovascular complications of diabetes, alterations in glucose homeostasis and insulin signalling, as well as recurrent hypoglycaemia are the factors that most likely affect brain function and structure. While difficult to study in patients, the mechanisms by which diabetes leads to brain dysfunction have been investigated in experimental models that display phenotypes of the disease. The present article reviews the impact of diabetes and AD on brain structure and function, and discusses recent findings from translational studies in animal models that link insulin resistance to metabolic alterations that underlie brain dysfunction. Such modifications of brain metabolism are likely to occur at early stages of neurodegeneration and impact regional neurochemical profiles and constitute non-invasive biomarkers detectable by magnetic resonance spectroscopy (MRS).

Key words: Neurodegeneration, Diabetes, Alzheimer’s disease, Insulin, Metabolism
traditional complications, diabetes-associated brain dysfunction was observable. The concept that diabetes affects the central nervous system (CNS) was recognised in 1922, when evidence appeared for diabetes-induced cognitive dysfunction [4], and the term “diabetic encephalopathy” was introduced in 1950 to describe the complication of diabetes resulting on brain dysfunction and leading to cognitive impairment [5].

Several clinical studies reported lower performance on several cognitive domains in T1D patients, when compared to the general healthy population, including notably learning and memory impairment [6-10]. The magnitude of these cognitive deficits is often mild but severe cases can occur [11, 12]. Along with these deficits on brain function, diabetes was also reported to induce structural alterations, neuronal loss, demyelination and gliosis [13]. Accordingly, magnetic resonance imaging (MRI) and computed tomography showed general brain atrophy and increased occurrence of white mater hyper-intensities that are thought to result from small infarcts [14-16]. In T2D patients, impaired cognitive function was observed in particular when solving complex cognitive tasks [17-22]. Cognitive dysfunction is further accentuated in elderly T2D patients with reduced diabetes control [23, 24]. In summary, diabetes causes cognitive dysfunction that is moderated at younger age but can be accentuated in elderly diabetic patients and by poor control over blood glucose levels, and hampers daily functioning, thus reducing the quality of life. The neurochemical mechanisms underlying the development of diabetes-associated cognitive decline are poorly understood. Animal experimentations began aiming to understand the mechanistic processes leading to diabetic encephalopathy, but greater efforts must still be taken to achieve this goal.

Cognitive impairment in diabetes and AD

The prevalence of neurodegenerative disorders leading to dementia is raising worldwide due to aging of the population (increased lifespan), a situation that represents an enormous social burden [25]. While Alzheimer’s disease (AD) is the most common type of dementia, accounting for 60-80% of cases [26], about 8% of all cases of dementia can be attributable to diabetes mellitus [27] and this tends to increase with the growing prevalence of diabetes. The high incidence of Alzheimer's disease in T2D is linked to insulin resistance, hyperinsulinemia and hyperglycemia, as well as commonly accompanying hypercholesterolemia, hypertension and obesity [28, 29]. Cognitive dysfunction in patients with diabetes particularly impairs the ability to control glycaemia and thus is also associated with enhanced risk of severe hypoglycaemia episodes and cardiovascular diseases, relative to patients without cognitive abnormalities [30, 31]. Thus, preventing these events and their associated morbidity is of clinical importance. For that, the mechanisms of diabetic encephalopathy must be understood, and the discovery of reliable disease biomarkers and design of preventive strategies are necessary.

Diabetic encephalopathies in T1D and T2D differ in the underlying mechanisms and the nature of resulting cognitive deficits. Encephalopathy in T1D occurs in younger patients and involves impaired learning abilities, intelligence development and memory retrieval. Although uncontrolled sustained hyperglycaemia leads to brain alterations and functional deterioration due to degeneration of the microvasculature, another major underlying component leading to encephalopathy in T1D patients appears to be insulin deficiency with downstream effects on the expression of neurotrophic factors, on the efficiency of neurotransmission, and on oxidative and apoptotic stressors, resulting in loss of neuronal integrity and connectivity defects in the still developing brain [29]. Furthermore, T1D patients are at risk for developing hypoglycaemia unawareness and associated complications (see section “Hypoglycaemia and hypoglycaemia unawareness”).

In contrast to T1D, clinical research over the two last decades has established a striking link between encephalopathy in T2D and AD. T2D is associated with an increased risk of cognitive dysfunction and dementia, affecting the brain by mechanisms that include: (1) metabolic and vascular risk factors within the metabolic syndrome such as dyslipidaemia, hypercholesterolemia and hypertension, which were found to be associated with cerebrovascular disease, accelerated cognitive decline and dementia; (2) hyperglycaemia that has adverse effects on the brain and its vasculature; (3) insulin that can directly modulate energy metabolism, synaptic plasticity and learning and memory, as well as regulate the metabolism of the β-amyloid peptide (Aβ) and Tau protein, the building blocks of amyloid plaques and neurofibrillary tangles [28, 32, 33].

Brain metabolism and cognitive performance

Glucose catabolism is the major pathway of energy production in the mature brain, thus constituting the bioenergetic basis for neurotransmission. Therefore, increases in glucose metabolism during brain activation have been used for functional mapping by 2-deoxyglucose autoradiography and positron-emission tomography (PET), and indirectly influence hemodynamic changes observed with functional MRI. Moreover, pathways of brain glucose oxidation have been suggested to be linked to the glutamine-
glutamate/GABA cycling between astrocytes and neurons, i.e. the rates of glutamatergic and GABAergic neurotransmission [34]. For the sake of example, this was demonstrated by measuring a reduction of extracellular glucose in the hippocampus during a hippocampal-dependent spatial memory test [35, 36]. Moreover, blockade of brain glucose transport or astrocytic glucose metabolism inhibits memory consolidation (see [37] and references therein). In line with this, somatosensory stimulation increases the rate of glycogenolysis [38, 39] and thus reduces brain glycogen levels [40, 41], the glucose storage in the brain. Thus, impaired brain glucose homeostasis may compromise learning processes and memory consolidation, and this could constitute a link between diabetes and brain alterations leading to encephalopathy.

**Brain metabolism in aging**

Mitochondrial production of reactive oxygen species (ROS) is the main drive for accumulated cellular stress that primarily leads to aging-associated impairments of mitochondrial functions such as regulation of Ca\(^{2+}\) homeostasis and ATP synthesis, that is energy production (reviewed in [42]). In turn, defects in energy metabolism at the level of the tricarboxylic acid (TCA) cycle, electron transport chain and oxidative phosphorylation further enhance the ROS production and affects amino acid homeostasis (see [43] and references therein). Moreover, faulty mitochondrial ATP synthesis triggers higher demand from glycolysis. A \(^{13}\)C MRS study in the human brain demonstrated that the neuronal TCA cycle is indeed reduced in elderly subjects, resulting in deficient glutamate-glutamine cycling between neurons and astrocytes [44]. In contrast, glial oxidative metabolism was increased probably due to astrogliosis [44]. A large longitudinal MRS study in C57Bl/6 mice over 2 years further confirmed the age-induced impairment in amino acid homeostasis, with substantial reduction in the levels of neuro-active amino acids including the major excitatory and inhibitory neurotransmitters, respectively glutamate and GABA [43]. These metabolic modifications that occur in the brain due to cellular damage accumulated during lifespan are crucial for functional deterioration in aged subjects [45] and are further accentuated in neurodegenerative disorders [42].

**Brain metabolism in AD**

Impaired cerebral energy metabolism is known to occur in AD patients. PET with \(^{18}\)Ffluorodeoxyglucose in AD patients revealed progressive reduction in glucose metabolism, namely in parietal and temporal lobes [46]. This method has thus been employed to identify mild cognitive impairment and early stages of AD in living patients. Accordingly, after glucose administration, glucose was observed in the brain of AD patients at higher levels than healthy subjects [47], likely reflecting reduced glucose metabolism. Indeed, recent studies suggested that hypometabolism of glucose in the brain of AD patients is closely linked to cognitive decline [48] and can be indicative of larger subsequent cerebral atrophy [49]. Although cerebral atrophy is not generally related to the regional binding of the Pittsburgh compound B (measuring levels of Aβ aggregation), a relationship between Aβ deposition and atrophy was observed in early AD [49, 50]. Interestingly, it was also recently suggested that brain areas of high resting glucose metabolism (reflecting high neuronal activity) in healthy elderly individuals generally correspond to those where the level of regional Pittsburgh compound B uptake is greater in AD patients [51]. This suggests that Aβ deposition may be dependent on neuronal activity, probably because neuronal activity-associated high mitochondrial metabolism is prone to oxidative stress.

It is known that Aβ neurotoxicity involves induction of oxidative stress with concomitant damage to biomolecules including membrane lipids, proteins and nucleic acids, which promotes synaptic and mitochondrial dysfunction and eventually leads to apoptosis. These events are also promoted by intracellular neurofibrillary tangles that are formed by hyperphosphorylation of the microtubule-associated protein Tau. In brief, mitochondrial dysfunction involves electron transport chain dysfunction, opening of the mitochondrial permeability transition pore that disrupts oxidative phosphorylation, impaired mitochondrial dynamics that regulates axonal transport, and reduction of mitochondrial creatine kinase activity that decreases the energetic buffering effect of phosphocreatine [42, 52]. This then leads to synaptic dysfunction mainly due to insufficient energy supply for neurotransmitter recycling and for maintenance of cellular electrochemical gradients (Ca\(^{2+}\) and Na\(^{+}\) homeostasis). However, little is known about AD-induced specific alterations of neuronal and astrocytic metabolism involved in neurotransmission. Studies in vivo in AD patients [53] and in rodent models of the disease [54, 55] using \(^{13}\)C MRS pointed towards a reduction of glucose oxidative metabolism, namely in neurons with concomitant increased lactate production, and a reduction in the rate of glial pyruvate carboxylation that is necessary for de novo synthesis of amino acids, namely glutamine, glutamate, GABA and aspartate. Decreased glutamate levels were recently reported in the brain of AD patients [56]. In addition, Penner et al. found that glutamate concentration was increased in the
right hippocampus of AD patients after four months of galantamine treatment relative to pre-treatment levels, and those variations in glutamate levels were related to variations in N-acetylaspartate (NAA) content and cognitive performance [57]. Interestingly, it has been recurrently demonstrated that the regional levels of NAA and glutamate in the brain are positively associated since both are mainly located within neuronal cells (see [43] and references there in). NAA is believed to be produced in neuronal mitochondria and then used for myelin synthesis in oligodendrocytes [58]. Thus it is plausible that dysfunctional mitochondria are unable to produce sufficient NAA for adequate maintenance of myelin sheaths around long axonal fibres.

The majority of the clinical studies using $^1$H MRS consistently revealed that the brain of AD patients contains less NAA and more myo-inositol, compared to cognitively healthy elderly subjects, and that the concentration of these two neurochemicals is correlated to the decline in cognitive performance [42]. In general these modifications of NAA and myo-inositol are interpreted as loss of neuronal integrity and gliosis, respectively [58]. Consistent with neurodegeneration, hippocampal NAA levels were found to be positively associated to hippocampal volume across subjects presenting mild to moderate severity AD and age-matched healthy controls [59], i.e. loss of NAA correlates with atrophy of the cerebral tissue. Furthermore, the modifications of NAA and myo-inositol in the posterior cingulate of AD patients were associated to reduction of diffusion parameters measured by diffusion tensor imaging, namely mean diffusivity and fractional anisotropy [60], consistent with astrogliosis and/or axonal degeneration.

**Insulin signalling defects in aging and AD**

Insulin receptors are ubiquitously expressed in the brain, especially in the hippocampus, cortex, hypothalamus, olfactory bulb and pituitary, with particular high density in neurons. Insulin in the brain can reach local levels one order of magnitude greater than in plasma (due to local production) and exert its actions not only through insulin receptors but also via insulin-like growth factor 1 (IGF-1) receptors that are as well widely distributed throughout the brain [61, 62]. The insulin/IGF-1-mediated activation of complex signalling cascades serves the regulation a plethora of cellular processes including growth, differentiation and metabolism [63]. In the CNS, insulin exerts further control over neurotransmission, synaptic plasticity and cognitive processes, cellular proliferation and antiapoptotic mechanisms, and antioxidant defence [64, 65]. Therefore, defects in brain insulin signalling pathways result in altered brain function.

Cellular challenges during normal aging lead to many homeostatic alterations and ultimately result in neurodegeneration, impaired neuronal function and reduced cognitive performance [45]. Epigenetic factors modulated by aging and a sedentary lifestyle have been described to induce a metabolic shift by attenuating mitochondrial metabolism and increasing reliance on glycolysis [66]. This alteration starts a damaging cycle involving oxidized membrane receptors, signalling molecules, transcription factors, and epigenetic transcriptional regulators [62, 66]. Accordingly, decrease in insulin receptor levels and impaired insulin signalling was observed in the hippocampus, cortex and choroid plexus [62], and insulin resistance was reported to be associated with cognitive decline in non-demented aging [67].

Impaired insulin signalling is believed to be associated with extracellular Aβ deposition and with hyperphosphorylation of the microtubule-associated protein Tau and formation of neurofibrillary tangles, which are hallmarks of AD. Through the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway, insulin stimulates amyloid precursor protein metabolism, promotes Aβ release from neurons preventing its intracellular accumulation, and increases insulin-degrading enzyme (IDE) protein levels [68]. Thus, insulin resistance may contribute to increase intracellular Aβ and its extracellular accumulation. Since IDE degrades insulin and Aβ, as well as other peptides, altered insulin levels can also interfere with the clearance of Aβ [69]. On the other hand, insulin binding and receptor autophosphorylation are inhibited by Aβ [70]. Tau phosphorylation is inhibited by insulin, and impaired insulin signalling in a neuron-specific insulin receptor knockout mouse resulted in reduced phosphorylation of Akt and GSK3β, leading to increased Tau phosphorylation [71].

AD is in fact associated with impaired expression of insulin receptors [33] and altered insulin and IGF-1 levels [32, 72], which affects the cAMP response element-binding protein (CREB) pathway that controls memory formation, and the PI3K/Akt pathway signalling that regulates glucose homeostasis, thus increasing glucose levels and potentiating the formation of advanced glycation end-products [61, 73]. In line with this, intranasal insulin administration has been effective in enhancing cognitive performance in healthy humans and some patients with AD [74, 75] and in improving memory formation in rodents [76], which may include regulation of glucose uptake and/or metabolism [77]. In contrast, decreases in insulin or IGF-1 signalling in rodent models of AD triggered reductions in Aβ deposition and in cognitive performance [78-81]. In
summary, insulin signalling appears involved in the pathophysiology of AD, but the link between T2D, AD and cognitive dysfunction remains unclear.

**Neurodegeneration in animal models of diabetes**

Elderly-associated mild memory and cognitive impairment and AD are accompanied by atrophy of hippocampal formation [82-85]. When compared to healthy subjects, individuals with T2D display deficits in hippocampal-based memory performance with preservation of other cognitive domains, and show reduction of hippocampal volume but not other brain areas [86]. Cognitive dysfunction during diabetes is therefore particularly associated with significant changes in the integrity of the hippocampus, a brain region considered to mediate memory formation in animals, and electrophysiological analyses indicate that diabetes induces defects on long-term potentiation (LTP) in hippocampal slices, a form of synaptic plasticity considered to reflect learning and memory processes in mammals [87, 88]. Based on this, the vast majority of translational studies in animal models of diabetes were dedicated to the study of hippocampal structure and function. Impaired hippocampal-dependent spatial learning and memory have been demonstrated in different animal models of diabetes using the Morris water maze [89-93] and the Y-maze [94, 95].

**Insulin-sensitive diabetes**

Diabetic encephalopathy can derive from cellular damage caused by both glucose neurotoxicity upon hyperglycaemia and defective insulin signalling by either insulin deficiency or receptor desensitization [96]. Most translational/pre-clinical studies aiming to identify mechanisms of diabetic encephalopathy have investigated animal models characterised by insulin deficiency (being sensitive to insulin administration) and chronic hyperglycaemia. Such experimental diabetic conditions cause deficits in spatial learning and synaptic plasticity [90], synaptic degeneration [97-99], increased astrocyte reactivity and proliferation [98, 100, 101], oxidative stress [102] and altered metabolism [98, 103]. Furthermore, chronic hyperglycaemia and hypoinsulinemia in an animal model of T1D (streptozotocin-treated rats) lead to impairment of multiple neuromodulation systems in the hippocampus that control metabolism and synaptic activity [97, 98, 104, 105]. This may contribute to altered synaptic plasticity and memory performance, and constitute a potential target for neuroprotection. Given the close link between brain function and energy metabolism [34], metabolic alterations have been described in the brain of experimental models of T1D. These could involve both substrate delivery through the blood-brain-barrier (BBB) and intermediary metabolism in neurons and astrocytes.

Structural alterations were reported in the BBB of diabetic animals [106], consistent with increased BBB permeability observed in individuals with diabetes [107], suggesting that loss of BBB integrity may be involved in CNS dysfunction in diabetes. However, permeability of the BBB to glucose does not seem to be altered in the hippocampus and cortex of streptozotocin-induced T1D rats [98, 103]. Therefore, hyperglycaemia in uncontrolled diabetes results in proportionally increased glucose concentration in the brain. How the higher levels of glucose in diabetes are handled by neurons and astrocytes has not been clearly demonstrated.

Reduced hippocampal neurogenesis was reported to occur in two models of T1D, the non-obese diabetic (NOD) mice [108] and the streptozotocin-induced diabetic rats [109, 110]. Compared to non-diabetic controls, neuronal loss was observed in streptozotocin-induced diabetic rats [111] and BB/Wor diabetic rats [112]. Before the loss of neurons, synaptic deterioration was observed in the brain of diabetic Chinese hamster [113] and streptozotocin-induced diabetic rats [93, 97, 98, 114, 115]. In line with this, the efficiency of neurotransmission was found altered in diabetic brains. In particular, glutamatergic neurotransmission is strongly impaired in diabetes [88] and is probably associated with a reduction in energy metabolism supporting the glutamate-glutamine cycle [34]. Cortical slices but not synaptosomes prepared from the brain of streptozotocin-induced diabetic rats displayed reduced uptake of glutamate [116], suggesting impaired glutamate clearance by astrocytes that can result in higher levels of glutamate in the synaptic cleft and induce excitotoxicity. Also the content of dopamine, norepinephrine and serotonin was reported to be altered in certain brain regions in alloxan-induced [117] and streptozotocin-induced [114, 115] diabetic rats, which may be prevented by insulin treatment [115].

These structural and neurochemical alterations result in functional anomalies. The latencies of auditory and visual potentials were found to be prolonged in streptozotocin-induced diabetic rats [118, 119] and type 1 BB/Wor diabetic rats [120, 121] in relation to controls. Likewise, in hippocampal slices from streptozotocin-induced diabetic rats, LTP is impaired, whereas long-term depression (LTD) is enhanced when compared with control rats [90, 91, 122, 123]. These altered hippocampal synaptic transmission and plasticity were paralleled by reduced spatial learning and memory [91] that was prevented by glycaemia control with insulin treatment [122].

In insulin-deficient mice, intranasal insulin treatment
was shown to ameliorate synaptic degeneration and deficits in learning and memory, without preventing hyperglycaemia [124]. This suggests that impairment of central insulin signalling is an important factor for diabetes-induced brain injury. However, in contrast to hypoinsulinemia and associated hyperglycaemia, the mechanisms of impaired insulin sensitivity leading to altered brain homeostasis, synaptic activity and memory performance are much less understood.

**Insulin-resistant diabetes**

Animal models of insulin resistance displayed lower insulin levels in the brain. Namely, obese (fa/fa) and lean heterozygous (Fa/Fa) Zucker rats had lower immunoreactive insulin in the olfactory bulb, hypothalamus, hippocampus, cerebral cortex, amygdala, midbrain and hindbrain, in comparison to lean homozygous (Fa/Fa) Zucker rats [125]. Reduction in insulin levels was also found in the cerebrospinal fluid of high-fat fed, insulin-resistant dogs and were suggested to result mainly from impaired transport from plasma across the BBB [126]. However, insulin has been proposed to be produced in the brain [62] and could possibly counterbalance an eventual reduction in the uptake from blood. Nevertheless, aberrant insulin regulation in the brain will certainly have consequences for brain function. In pre-diabetes or T2D, insulin resistance is directly associated with reduced cortical glucose consumption and cognitive impairment [127]. Furthermore, like in AD, deficient insulin-PI3K/Akt signalling in animal models of diabetes may further induce hyperphosphorylation of the Tau protein and favour Aβ deposition [61] that leads to formation of neurofibrillary tangles and amyloid plaques.

In a mouse model of obesity-associated T2D, NONcNZO10/LtJ mice under an 11% fat diet, we recently reported impaired hippocampal-dependent spatial memory as indicated by decreased spontaneous alternation in the Y-maze, which was accompanied by decreased density of nerve terminal proteins (synaptophysin, SNAP25), glutamatergic markers (vesicular glutamate transporters), increased astrogliosis (immunoreactivity to glial fibrillary acidic protein, GFAP) and altered adenosinergic modulation system in the hippocampus compared to controls (NON/LtJ mice) kept under the same diet [95]. Interestingly, we failed to identify diabetes-induced neuronal loss in brain sections stained either cresyl violet or FluoroJade-C, which was corroborated by the lack of altered microtubule-associated protein 2 (MAP2) immunoreactivity, a marker of neuronal density [95]. This indicates that T2D primarily causes synaptic deterioration without neuronal death, which resembles the events occurring in aging [128] and models mimicking early AD [129, 130], thus corroborating the association of insulin resistance and T2D with increased incidence of AD. Moreover, compared to non-diabetic Wistar rats, also non-obese insulin-resistant Goto-Kakizyki (GK) rats displayed reduced spatial memory, which was accompanied by reduced pre-synaptic markers and increased glial specific proteins in the hippocampus [131]. Altered expression of genes involved in neurotransmission (among others) was found in the cortex of GK rats [132], indicating that diabetes-induced synaptic alterations are not region specific. A recent publication also reported that, relative to controls, GK rats display learning and memory deficits in a Morris water maze that are proportional to the grade of insulin resistance, reduction in dendritic spine density and neuronal viability in CA1 of the hippocampus, and impaired insulin-stimulated Akt/CREB phathway [133], consistent with AD-like pathology. Furthermore, synaptosomal preparations from the brain of GK rats show impaired mitochondrial function and increased vulnerability to oxidative stress and Aβ toxicity [134, 135], suggesting impaired intermediary metabolism at the level of the synapse. Although the brain of diabetic GK rats is more susceptible to Aβ toxicity and oxidative stress than control Wistar rats, it is not known if Aβ accumulation, Tau hyperphosphorylation and formation of neurofibrillary tangles occur in the brain of this model for non-obese insulin-resistant diabetes. In obese BBZDR/Wor rats that develop insulin resistance with hyperinsulinemia and hyperglycaemia, neuronal degeneration, reduced presynaptic densities and gliosis was reported in the frontal cortex, as well as down regulation of IGF-1 receptors and increased APP, β-secretase, Aβ and phosphorylated Tau [29, 136]. Insulin receptor substrate 2 knockout mice, a model of insulin-resistant diabetes, also displayed hyperphosphorylated Tau in the hippocampus [137].

In addition, glutamatergic and GABAergic neurons may adapt differently to diabetic encephalopathy, since there was a reduction in vesicular glutamate transporters (VGLUT1/2) in the hippocampus of NONcNZO10/LtJ mice compared to non-diabetic NON/LtJ mice, and a lack of diabetes-induced modification of vesicular GABA transporters [95]. The preservation of GABAergic nerve terminals in diabetes is also corroborated by Galanopoulus et al. who found reduced glutamate decarboxylase activity in cortical slices but not synaptosomes prepared from brains of streptozotocin-induced diabetic rats [116], which suggests that diabetes may impact the metabolism of GABA in glial cells but not in inhibitory nerve terminals. Interestingly, glutamatergic nerve terminals are also more susceptible to Aβ-induced toxicity than GABAergic terminals.
namely in the hippocampus [130]. Indeed, it is generally accepted that mainly glutamatergic and cholinergic pathways are disrupted in AD [138], and there is a growing body of evidence that GABAergic function is preserved in early phases of AD but becomes impaired when the disease is well established [139, 140].

In summary, although the mechanistic link from impaired oxidative glucose metabolism caused by insulin resistance to altered neurotransmission and impaired brain function remains to be fully elucidated, it may involve increased metabolic rates in glial cells due to astrogliosis and impaired neuronal metabolism, associated to synaptodegeneration. Probably, these metabolic alterations are caused by mitochondrial dysfunction that causes oxidative stress and affects neurotransmission efficiency at glutamatergic (excitatory) rather than GABAergic (inhibitory) nerve terminals, and which may occur much before appearance of neurofibrillary tangles/amyloid plaques. Insulin resistance-induced alterations of neuronal and glial energy metabolism remain to be investigated. Moreover, efforts should be devoted to the study of experimental models displaying insulin-resistance without the confounder of severe hyperglycaemia that causes microvasculature deterioration.

**Neurochemical profiling in models of diabetes by MRS**

Proton MRS became a tool of choice to investigate metabolic alterations induced by neurological disorders in a non-invasive manner [58] and it has also been employed to study brain metabolism in experimental models of diabetes. Diabetic rats under chronic hyperglycaemia, induced by streptozotocin administration, display a plethora of metabolic alterations in the hippocampus and cortex, most of which are normalized upon acute restoration of euglycaemia [98, 103]. Some of the metabolites more affected by hyperglycaemia were myo-inositol, taurine and creatine, which are considered major organic osmolytes regulating brain osmotic adaptation. Similar results were obtained in Goto-Kakizaki rats, an experimental model of insulin resistance and T2D [131]. High concentration of myo-inositol was also reported in the hippocampus of Zucker diabetic fatty rats compared to controls [141] and in the brain of diabetes patients [142, 143]. Thus, the study of the neurochemical profile supports the hypothesis that diabetes-induced hippocampal dysfunction involves deregulation of the osmotic balance. In contrast to AD, NAA levels do not appear altered in the diabetic brain, suggesting preservation of neuronal integrity, despite synaptic degeneration.

Similar to what is observed in AD patients, double transgenic mice expressing human mutant APP and human mutated PS1 (APP/PS1) display decreased glutamate and NAA concentrations in the hippocampus, that were observed at the time where hippocampal volume was already reduced but amyloid plaques were not yet present [144-146]. When amyloid deposits became detectable, these mice also presented increased brain myo-inositol levels, when compared to wild type mice [147], which were accompanied by astrogliosis [148]. Increased myo-inositol and glutamine and decreased NAA and glutamate were reported to occur in the cortex of these mice and, once amyloid plaques are observed, NAA levels were inversely associated to the area of cortex occupied by plaques [144].

Dedeoglu et al. but not Marjanska et al. reproduced these findings in mice possessing only the mutated APP gene. Both authors reported, however, higher cerebral taurine to creatine ratio in the brain of APP transgenic mice compared to wild type mice [145, 149]. These results were confirmed by MRS of brain extracts, in which glutathione levels were additionally found to be lower than in controls [149], consistent with redox deregulation [52].

**Hypoglycaemia and hypoglycaemia unawareness**

Postprandial hyperglycaemia periods are often prevented by insulin administration, principally in cases of T1D. However, episodes of severe hypoglycaemia are a major complication of this glycaemia control. Diminished brain function during acute hypoglycaemia leads to potential physical danger and, moreover, recurrent hypoglycaemia imposes long-lasting damaging effects on brain function [150, 151]. Cognitive performance is altered during experimentally induced hypoglycaemia [152-158], and some studies in patients with T1D suggested an association between recurrent hypoglycaemia and cognitive impairment [11, 16, 159-162].

During severe hypoglycaemia, the absence of sufficient glucose supply to the brain leads to neuroglycopenia that results in energy deprivation and thus other substrates are mobilised for ATP synthesis. Hypoglycaemia causes a reduction in brain glycogen and lactate levels and then depletes metabolic intermediates, namely glucose-6-phosphate, pyruvate, citrate, 2-oxoglutarate and malate [163], and leads to progressive reduction of brain glutamate, glutamine, GABA and alanine with concomitant increase in aspartate that can efficiently act on NMDA receptors [164-168]. The transition to encephalographic silence in hypoglycaemia (i.e. cessation of neural activity) is accompanied by a rapid decline in levels of phosphocreatine, nucleoside triphosphate, and an increase in inorganic orthophosphate, as measured in vivo by $^{31}$P MRS [166]. In this isoelectric state, most glycolytic and citric acid
cycle intermediates are very low and glucose-6-phosphate and pyruvate are virtually absent [163]. Only when glutamate and glutamine become substantially exhausted, aspartate becomes an important energy source [168]. Glucose administration to recover from hypoglycaemia allows for metabolic recovery and nearly restores amino acids to pre-hypoglycaemia levels [166, 168].

The occurrence of recurrent hypoglycaemia episodes impacts the body’s defence mechanisms against hypoglycaemia episodes. Many diabetes patients display a progressive decay in the counter-regulatory response over time, resulting in reduction of hypoglycaemia awareness [169, 170], where the glycaemia threshold for counter-regulation symptoms and cognitive dysfunction is shifted to lower plasma glucose levels and may be detected only upon severe neuroglycopenia [171]. Together, the hypoglycaemia-induced decrease in the sympathoadrenal response to blood glucose variations and the hypoglycaemia unawareness are termed hypoglycaemia-associated autonomic failure (HAAF). HAAF in diabetes patients leads to an increased risk for the occurrence of severe hypoglycaemia episodes.

Beyond the precise mechanisms that sense blood glucose and link the sensory machinery to the autonomic and endocrine responses which alert the patient to the low blood glucose levels and ultimately restore glucose homeostasis, very little is known about the mechanisms responsible for hypoglycaemia unawareness [172]. It has been proposed that adaptation to recurrent hypoglycaemia involves increased fuel availability to the brain from the blood stream, namely glucose [173-175] and acetate [176, 177]. In line with this, from studies in both humans and rodents, it has been proposed that recurrent hypoglycaemia leads to enhanced expression and density of glucose carrier proteins [178-180]. In addition to increased substrate uptake from the blood, hypoglycaemia unawareness could be linked to the so-called glycogen “supercompensation”, in which the brain adapts to hypoglycaemia by increasing glucose storage in the form of extra glycogen content [181, 182].

In neurons, the machinery for glycogen synthesis is believed to be physiologically inactive and glycogen phosphorylase nearly absent, rendering astrocytes the primary glycogen storage [183]. However, a recent study reported that cultured neurons have measurable glycogen levels and that its metabolism is important upon hypoxia [184]. Brain glycogen concentration lies between 3 and 7 μmol/g and has a turnover time of 5-10 hours [34]. For these concentration and turnover, a 10% reduction of glucose supply and a resting glucose consumption of 0.4-0.5 μmol/g/min would lead to depletion of glial glycogen in a couple of hours [185], emphasizing its role in buffering substrate delivery to the brain. Brain glycogen content is reduced upon insulin-induced hypoglycaemia due to increased glycogenolysis and, in contrast, it increases with circulating insulin and with brain glucose concentration [34]. Brain glycogen may also have a role in buffering fuel demands upon increased neuronal activity: it accumulates under deep anaesthesia but somatosensory stimulation upregulates glycogenolysis leading to a decrease in brain glycogen levels [40, 41]. Although suggesting a direct role of glycogen in brain function, this was not observed under visual stimulation in either rodents [41] or humans [186].

Using 13C MRS to measure enrichment from 13C-labelled glucose, Choi and co-authors submitted rats to hypoglycaemia and found that the rat brain displayed a 153% increase in glycogen compared to baseline after recovering for 7 hours under hyperglycaemia [181]. In mice, 6 hours after recovery at euglycaemia from insulin-induced acute hypoglycaemia caused a 25% increase in whole brain glycogen content [182]. However, 27 hours after the hypoglycaemia period, brain glycogen levels had return to baseline levels. The authors observed similar results when animals were submitted to daily recurrent hypoglycaemia insults for 9 days. In line with the effect of systemic hypoglycaemia, neuroglycopenia induced by repetitive intracerebroventricular injection of 2-deoxy-D-glucose for 3 days induced increases in brain glycogen content in the hypothalamus and cortex by 69% and 153%, respectively, in comparison to saline-injected rats [187]. Such glycogen modifications were associated to 5'-adenosine monophosphate-activated protein kinase (AMPK), a glucose sensor that is suppressed by high glucose [188] and activated by low glucose concentrations [189]. In contrast, Herzog et al. failed to detect glycogen supercompensation after acute or recurrent hypoglycaemia in the rat cerebellum, cortex and hypothalamus [190]. In line with this, after recovery from chronic and sustained hypoglycaemia bellow 3 mM for 2 weeks, brain glycogen levels and turnover were not different from control rats [191]. A recent study on patients with T1D did not support the glycogen supercompensation hypothesis [192]. Interestingly, brain glycogen supercompensation was also observed in rats after depletion upon exhaustive exercise [193]. In this study, brain glycogen levels were decreased with exhaustive exercise and were supercompensated at 6 hours after exercise in several brain areas, and it was sustained until 24 hours after exercise in both cortex and hippocampus (long-lasting supercompensation).

Increased glial glycogen stores can eventually sustain neuronal activity for a longer period [194] during a subsequent hypoglycaemia episode and, therefore, contribute to the shift in the glycaemia threshold at which counter-regulation initiates. However, it remains
to be addressed whether excessive content of glycogen can effectively buffer brain glucose supply to glucose-sensing neurons for longer periods.

All together, these data suggest that glycogen content and metabolism may have an important role in hypoglycaemia unawareness, even though very little is known on brain glycogen metabolism in diabetes conditions. Animal models of diabetes characterised by insulin deficiency and chronic hyperglycaemia show

unaltered glucose transport at the blood-brain-barrier [98], thus leading to high brain glucose concentration at hyperglycaemia. In chronic, sustained hyperglycaemia, the rodent brain does not display evident alteration of brain glycogen levels [195-198]. On the other hand, it is not clear whether insulin regulates whole brain glycogen deposition.

Figure 1. Events associated to impaired insulin signalling and leading to cognitive deterioration. Insulin regulates synaptic activity and glucose metabolism. Thus impaired insulin signalling leads to synaptic dysfunction and altered glucose homeostasis that impacts energy metabolism, osmolarity and redox balance. Furthermore, Aβ clearance and tau phosphorylation are under control of insulin/IGF-1 receptors. Hence, T2D leads to increased amount of amyloid precursor protein (APP), Aβ accumulation and tau hyperphosphorylation, leading to the formation of neurofibrillary tangles. Increased oxidative stress upon redox imbalance further affects mitochondrial metabolism and favours protein aggregation. In fact, advanced glycation end-products (AGE) in oxidative stress lead to a number of protein modifications that have functional consequences on metabolic pathways for signaling and energy production. While impaired energy metabolism may directly impact synaptic efficiency due to impaired membrane repolarisation and neurotransmitter synthesis/recycling, neurofibrillary tangles resulting from protein aggregation lead to degeneration of nerve terminals. This leads to cognitive impairment and is accompanied by astrogliosis and possibly by neuroinflammation.
Whether hypoglycaemia-induced brain glycogen supercompensation occurs in diabetes conditions remains to be clearly demonstrated. Recently, a study on a small sample of patients with insulin-dependent diabetes and hypoglycaemia unawareness suggested that brain glycogen concentration is even slightly lower than in healthy subjects (although not significantly different) [192]. However this was never studied in well controlled experimental models of diabetes, and the effects of severity and duration of the hypoglycaemia insult, as well as the conditions of recovery from hypoglycaemia, on the degree of glycogen supercompensation remain to be ascertained. Moreover, a possible involvement of glycogen metabolism in impaired insulin signalling in T2D was never investigated.

In summary, in addition to diabetes itself, the occurrence of recurrent hypoglycaemia episodes due to treatment are also responsible for CNS injury. While some studies reported that cognitive decline in diabetic patients treated with insulin can be largely attributed to recurrent episodes of hypoglycaemia rather than to hyperglycaemia, others do not support this notion [199, 200]. Because, T1D patients without hypoglycaemia episodes also display a decline in cognitive function [201, 202], insulin signalling defects and glucose neurotoxicity remain the most important contributors for the development of dementia.

**Conclusion**

Uncontrolled hyperglycaemia in diabetes mellitus leads to well described microvascular complications that affect many organs. While the development and severity of these complications is mostly dependent on the degree of glycaemia control and duration of the disease, treatment with insulin frequently results in hypoglycaemia episodes. Both hyper- and hypoglycaemia affect brain function and may lead to cognitive dysfunction and dementia, but impaired insulin signalling appears as a very important factor driving neurodegeneration. Experimental diabetic conditions cause deficits in spatial learning and synaptic plasticity, synaptic degeneration and increased astrocyte reactivity and proliferation particularly in the hippocampus and cortical areas involved in learning and memory processing (Figure 1). These structural and functional modifications are likely to involve alterations of cerebral metabolism and, because they occur in both insulin deficiency and insulin resistance, the lack of adequate cerebral insulin signalling seems to be crucial for the development of diabetic encephalopathy and AD, with which diabetes shares cellular features.

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