Diabetes Mellitus and Blood-Brain Barrier Dysfunction: An Overview

Shikha Prasad1, Ravi K Sajja1, Pooja Naik1 and Luca Cucullo1,2*
1Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas, USA
2Vascular Drug research Center, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

Abstract

A host of diabetes-related insults to the central nervous system (CNS) have been clearly documented in type-1 and -2 diabetic patients as well as experimental animal models. These host of neurological disorders encompass hemodynamic impairments (e.g., stroke), vascular dementia, cognitive deficits (mild to moderate), as well as a number of biochemical, electrophysiological and behavioral alterations. The underlying causes of diabetes-induced CNS complications are multifactorial and are relatively little understood although it is now evident that blood-brain barrier (BBB) damage plays a significant role in diabetes-dependent CNS disorders. Changes in plasma glucose levels (hyper- or hypoglycemia) have been associated with altered BBB transport functions (e.g., glucose, insulin, choline, amino acids, etc.), integrity (tight junction disruption), and oxidative stress in the CNS microcapillaries. Last two implicating a potential causal role for upregulation and activation of the receptor for advanced glycation end products (RAGE). This type I membrane-protein also transports amyloid-beta (Aβ) from the blood into the brain across the BBB thus, establishing a link between type 2 diabetes mellitus (T2DM) and Alzheimer’s disease (AD, also referred to as “type 3 diabetes”). Hyperglycemia has been associated with progression of cerebral ischemia and the consequent enhancement of secondary brain injury. Difficulty in detecting vascular impairments in the large, heterogeneous brain microvascular bed and dissecting out the impact of hyper- and hypoglycemia in vivo has led to controversial results especially with regard to the effects of diabetes on BBB. In this article, we review the major findings and current knowledge with regard to the impact of diabetes on BBB integrity and function as well as specific brain microvascular effects of hyper- and hypoglycemia.

Keywords: Diabetes mellitus; Blood–brain barrier; Glucose transporter; P-glycoprotein; Oxidative stress; Permeability; Tight junctions; in vitro.

Introduction

Diabetes mellitus (DM) is a multi-faceted metabolic syndrome and currently one of the major health concerns in public health across the globe. DM is characterized by high rates of mortality and morbidity, especially in relation to T2DM [1]. Both, insulin-dependent (type 1) and independent (type 2) DM, have detrimental effects on the structure integrity and function of vascular beds, underlying the pathophysiology and development of various peripheral and CNS disorders. Hyperglycemia-elicited complications at the microvascular level include increased superoxide production, thickening of capillary walls and abnormal proliferation of endothelial cells with increased vascular permeability (both in vitro and in vivo including DM patients). The pathophysiology of microvascular complications in diabetes encompasses major biochemical pathways while the common endpoint appears to be mitochondrial superoxide overproduction in the endothelial cells lining the vascular walls of the blood vessels. The increased superoxide production causes the activation of four major pathways involved in the pathogenesis of complications: increase in polyol and hexosamine pathways flux, activation of Protein Kinase C (PKC) and increased formation of advanced glycation end product (AGE) ligands originating from proteins, lipids and nucleic acids (e.g., LDL) [2,3]. RAGE activation initiates a vicious cycle eliciting more oxidative stress generation [3,4] and subsequently evolving vascular inflammation [5] and thrombosis [6], thereby implicating a potential vascular damage [7,8]. Furthermore, the overproduction of reactive oxygen species (ROS) inactivates endothelial nitric oxide synthase (eNOS) and prostacyclin synthase, thereby impairing the vascular tone [2,9,10].

A growing body of evidence from recent clinical and experimental studies suggests that prolonged hyperglycemic conditions, particularly in type 2 DM, elicit a progressive impairment of neuronal function in the brain [10]. Stroke and cerebral ischemia are typical CNS complications related to diabetes due to the impairments in cerebral vascular supply [11]. Diabetic patients are also at higher risk of experiencing stroke than normal population [11-13] and 50% of stroke-affected individuals have been diagnosed with hyperglycemia [14]. It is also reported that subjects with type 2 DM have significantly lower brain volume and are more likely to have single or multiple cerebral infarcts compared to normoglycemic individuals [13]. In addition, preclinical studies in mice suggest that vascular injury occurring in response to an ischemic insult following stroke is significantly exacerbated in diabetic subjects [15] and the situation is further worsened by recurrent hypoglycemia [16]. Type 2 diabetes can negatively impact the outcome of stroke (ischemic brain damage): in fact increases the risk of stroke, as demonstrated in vivo in type 2 diabetic mice [15]. Conversely, hyperglycemia is also associated with high levels of mortality and morbidity during cerebral ischemia, perhaps, caused by increased cerebral hematoma expansion [14] and higher risk of cerebral hemorrhage due to tissue Plasminogen Activator (tPA) activation and superoxide production damaging the BBB [17]. Recent studies also evoke a role for the AGE-RAGE system activated by hyperglycemia leading to a further enhancement of oxidative stress and amplification of inflammatory signals from nearby leukocytes [18,19]. Improved glyemic control in these patients

*Corresponding author: Luca Cucullo, Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, 1300 S. Coulter Street, Amarillo, TX 79106, USA, Tel: 806-356-4015 x293; Fax: 806-356-4034; E-mail: luca.cucullo@ttuhsc.edu

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seem to ameliorate these pathological conditions [10] however, rapid normalization of plasma glucose levels in hyperglycemic subjects can lead to cerebral hypoglycemia thus favoring cognitive decline [20-25]. Other studies have demonstrated an association between altered glycemic conditions and alterations of the electrophysiological, structural and neurochemical profiles of brain function [26] which can impair neuronal plasticity and synaptic transmission [9,10].

T2DM has been strongly associated with mild cognitive impairments [24,27] and is considered a predisposing factor for developing vascular dementia [28] and Alzheimer disease [22,29]. Furthermore, DM has also been associated with increased severity of epileptic seizures [30] and risk of mortality following traumatic brain injury (TBI) [31]. Frequent co-occurrence of psychiatric disorders (such anxiety and depression) [32] have also been recorded in DM patients. These large onset of CNS comorbidities in diabetic patients is not entirely surprising considering the cerebrovascular (such as reduced vascular tone, BBB leakage, inflammation, thrombosis [4,33-38]) and metabolic (hyper/hypoglycemia and hyperinsulinemia [34,39,40]) abnormalities that characterize DM.

Altered glycemic conditions such as those observed in diabetic patients are prodromal to blood-brain barrier (BBB) impairment [34,41-46]. This is of paramount relevance for the pathogenesis of brain disorders in DM since the BBB act as a gate keeper of the brain with a range of interrelated functions including protecting the CNS from potentially harmful substances, regulate transport of essential molecules, maintain the brain homeostasis, and provide immune regulatory functions [47].

Unfortunately, despite the existence of a large body of data regarding DM-induced microvascular complications of the kidney and retina, the impact of DM at the level of the cerebrovascular system is still poorly understood from a mechanistic point of view and under-investigated. Thus, advancements in this specific field can reveal unique pharmacological targets for the development of novel and more effective drugs for the treatment and/or prevention of diabetes-associated CNS complications.

**BBB: Structure, Function and Glucose Transport**

The BBB has been described in detail both physiologically and morphologically [48]. At the cellular level, the BBB is constituted by vascular endothelium lining the cerebral microvessels with the closely apposed astrocytic end-feet processes (Figure 1) [49]. The BBB endothelium is characterized by distinctive expression patterns of trans-membrane transport systems to regulate traffic of substances in and out of the brain parenchyma [50,51]. In addition, expression of inter-endothelial tight junctions, lack of fenestrations and minimal pinocytic transport which concur in the regulation and maintenance of the brain microenvironment are the unique features of the BBB endothelium. Tight junctions between adjacent endothelial cells form a diffusion barrier, which selectively excludes most blood-borne (including electrolytes and other water soluble compounds) and xenobiotic polar substances from entering the brain through paracellular routes. Specialized efflux transport mechanisms (e.g., P-glycoprotein, breast cancer resistant protein/BCRP, multidrug resistance protein-4/MRP-4 etc.) are also in place to regulate the passage of amphipathic and hydrophobic molecules and protect the brain from potentially harmful substances.

![Figure 1: Structure and location of brain microvessels.](image)
Glucose is the primary brain bioenergetic source. While the CNS utilizes approximately 25% of the total glucose, it only accounts for ~2% of the total body mass [52]. Besides glucose, lactate (from astrocytes) also provides energy to neurons [52,53]. Glucose crosses the BBB through two independent groups of transporter proteins: facilitative sodium independent transporters (GLUT) and sodium dependent glucose co-transporters (SGLT) [54,55]. Facilitative glucose transporters (GLUT) include 14 proteins [56] of which GLUT-1 is the first identified member and a major transporter of glucose across BBB [55,57,58]. This is evident from the clinical reports in which patients with GLUT-1 deficiency syndrome had CSF/blood glucose ratio of 0.19 - 0.35 (vs. the normal value of 0.65), developed seizures and other development disorders [57]. In contrast to SGLT, GLUT proteins are saturable transporters and help in movement of glucose along the concentration gradient [53,59]. While GLUT-1 transporters in brain microvascular endothelial cells and astrocytes carry glucose across the BBB, GLUT-3 is considered the main (although not the exclusive) neuronal glucose transporters [57]. GLUT-1 transporters in mature and fully differentiated BBB endothelium are heterogeneously distributed with studies reporting higher luminal to abluminal ratio in human brain microvessels [60,61], while the opposite holds true in rats and other species [62,63]. This heterogeneity correlates to the variable energy demands, cerebral glucose utilization and maintenance of normal glucose level [64,65] with reports suggesting an increase in their local demands, due to increase in local cerebral glucose utilization [53]. More specifically, increased glucose transport across BBB correlated with increased luminal density of GLUT-1. On the other hand, higher abluminal expression (lower luminal/abluminal ratio) was observed when GLUT-1 is down-regulated [66] suggesting that luminal-abluminal redistribution and/or expression of GLUT-1 modulates glucose entry into the brain (Figure 2). SGLT-1 plays a major role in transport of glucose across intestinal membrane and renal proximal tubule. Although the role of SGLT-1 in CNS glucose transport has not been fully investigated, their presence on brain microvascular endothelial cells and involvement in transporting glucose in certain pathophysiological conditions (e.g., oxygen glucose deprivation-ischemia) has been observed [59]. Unpublished data from our group revealed up-regulation of SGLT-1 expression in a well-established human BBB endothelia cells line (hCMEC/D3 [67]) following either acute or chronic exposure to hypoglycemia.

Insulin-sensitive glucose transporters (GLUT-4) and insulin receptors on BBB endothelial cells also may play a central role in regulating glucose transport into CNS. Very little or no insulin is synthesized by the brain in normal conditions; although it has been synthesized by the brain in normal conditions; although it has been

Figure 2: Glucose transport across the BBB: Glu1 transporters located on microvascular endothelial cells and astrocytes transport glucose across the BBB, while Glu3 transports glucose in neurons. Heterogeneous distribution of Glu1 transporters is a typical hallmark of a fully mature BBB and correlates to the variable energy demands, glucose utilizations and maintenance of normal glucose level.
shown that DM increases its transport across the BBB [39,68-70]. \textit{In vivo}, insulin can access the brain cells both via the cerebrospinal fluid reaching through regions lacking proper BBB (e.g. circumventricular organs), and directly through the BBB via specific insulin receptors that can act as transporters [71,72]. The effect of insulin on CNS is of particular interest in view of a recent body of evidence suggesting that DM could affect the pathogenesis of AD through mechanisms involving alterations in downstream signaling of the insulin receptor at BBB. Although additional studies are required to shed full light on the mechanisms involved, insulin may have a role in regulating phosphorylation of tau in tauopathies - via activation of glycogen synthase kinase-3 and amyloid-β peptides [73]) as well as the expression (up-regulation) of soluble receptors for advanced glycation end products (sRAGE) [74]. High levels of sRAGE have been associated with incident cardiovascular disease and all-cause mortality in type 1 [75] and 2 diabetes [76] and may also reflect tissue RAGE expression in DM [77]. Thus, according to recent studies, activation of the AGE-RAGE system seems to plays a central role in the pathogenesis of vascular abnormalities and thrombotic insult observed in DM patients [4,6].

Ultimately, alteration of BBB function and integrity can have a profound impact on the CNS being prodromal to the pathogenesis and progression of major neurological disorders. Thus diabetes-dependent impairment of BBB function can severely impact the CNS.

Effect of hyperglycemia on BBB glucose transport in DM

On the basis of common understanding it is believed that when a substrate is in excess, body will down-regulate the receptor expression for it, in order to balance its demand and supply cycle. This suggests that the biological answer to chronic excess of glucose in the systemic circulation should be down regulation of its transporters. In this line, many experimental studies report a down-regulation of BBB glucose transporters in hyperglycemic animals [63,78,79], indicating the measures taken by brain from preventing excessive glucose intake. However, this is not a universal scientific consensus since other studies (in both animals and humans) did not reveal significant changes in the BBB glucose transporters expression in DM [41,80-83].

Glucose transporter expression studies performed in streptozotocin (STZ)-induced diabetic rats showed that chronic hyperglycemia down-regulates both mRNA and protein expression of GLUT-1 and GLUT-3. Down-regulation of glucose transporters was independent of the method used to induce DM \textit{in vivo} [78]. Local cerebral glucose utilization during chronic but not acute hyperglycemia was increased in DM Sprague–Dawley rat models. This was paralleled by a moderate (yet significant) decrease in expression of GLUT-1 in brain vessels although no changes in GLUT-3 were observed [63].

\textit{In vivo} studies in pig models undergoing continuous glucose monitoring showed alteration of brain glucose similar to that measured locally in muscle and subcutaneous fat tissue in response to hyperglycemia (although in hypoglycemic conditions the glucose levels in the brain decreased on a similar time scale as the other tissues but to a much lesser degree) [82]. On the other hand, no significant changes in glucose uptake and GLUT-1 expression were observed in STZ-induced diabetic rats [41,84]. Further, high-field magnetic resonance spectroscopy study in humans by Seaquist and colleagues did not reveal major changes in global cerebral blood flow (CBF) or regional glucose metabolism (including maximal transport velocity of glucose) after acute hyperglycemia [83]. Nevertheless, such conflicting \textit{in vivo} data with regard to BBB GLUT expression can be plausibly explained by differences in experimental approaches, animal models and methods of analyses. Further investigation based on common experimental ground with regard to model platforms, method, and analysis will be required to validate the results and reach a consensus over the final conclusions. To this end, \textit{in vitro} human models could certainly help dissecting out specific aspects of DM-related alteration of glucose transport at the BBB which are currently impractical or unfeasible in \textit{in vivo} or in human studies.

**Hypoglycemia and glucose transport across the BBB**

Hypoglycemia is of major concern in diabetes as it leads to severe impairment of CNS function. Frequent treatment regimen to reduce glucose plasma concentration in DM patients, triggers repetitive hypoglycemic insults to the brain cells and induce hypoglycemia-associated autonomic failure (HAAF). The general concept underlying HAAF is that defective glucose counter-regulation (lack of adrenomedullary epinephrine response which under normal circumstances would reduce insulin concentration and increase glucagon) and hypoglycemia unawareness lead to a vicious cycle of recurrent hypoglycemia and further impairment of glucose counter-regulation. The clinical relevance of this phenomenon is now well established, but the precise mechanisms and mediators remain largely unknown [85,86].

Majority of diabetic patients face a great difficulty in maintaining normal blood glucose level due to HAAF and hypoglycemia unawareness. Using \textit{[1H]} nuclear magnetic resonance (NMR) spectroscopy, Criego et al. [87] measured the \textit{in vivo} steady state brain glucose concentrations under controlled metabolic conditions to understand whether subjects with hypoglycemia unawareness would have higher brain glucose concentrations than control subjects. They concluded that brain glucose concentration was higher in the hypoglycemia unaware group by 17 ± 6% in comparison to the control group [87]. However, Segel et al. reported contradictory data (measured by using 1-\textit{13C} glucose Positron Emission Tomography) showing no changes in glucose transport from blood to brain, blood flow to brain and cerebral glucose metabolism between healthy subjects and patients subjected to 24 hrs interprandial hypoglycemia [88]. Brain glucose concentration in sixteen human subjects subjected to recurrent hypoglycemia (three hypoglycemic clamps for 30 min at 0 hr, 9 hr, and 24 hr intervals) was not different from the results obtained from the same subjects during a control study [89]. Even, prior exposure to recurrent hypoglycemia in diabetic rats did not result in an increase in extra cellular fluid glucose concentration in the inferior colliculus [90]; thereby suggesting short episodes of recurrent hypoglycemia do not alter transport or metabolism of brain glucose. However, increased expression of GLUT-1 mRNA and protein at the BBB in rodent model of chronic hypoglycemia suggest the existence of a compensatory mechanism to increase glucose transport activity across the BBB in response to chronically low circulating blood glucose levels [91].

Increased brain glucose uptake under hypoglycemic condition seems to be due to both increased GLUT-1 synthesis and redistribution across BBB [91]. Brain glucose extraction [measured by Brain Uptake Index] increased in experimental hypoglycemia group versus controls (independently from the method used to induce hypoglycemia \textit{in vivo} such as implanting insulin secreting tumors, insulin osmotic mini-pumps or repeated injections [92]). Similar observations were made by Lei et al. in rats following 12-14 days of hypoglycemia. 48% increase in brain glucose levels was observed, while brain glycogen...
concentration remained unaffected (although, others have reported decreased brain glycogen level in hypoglycemic conditions) [93]. Further, hypoglycemia resulted in 25-45% increase in glucose uptake, 23% increase in total GLUT-1 expression and redistribution to the luminal (vascular) side of BBB endothelium [41]. Increased GLUT-1 expression and luminal redistribution further aggravates the condition by increasing the transport of glucose across the BBB. In addition, acute (or mild) hypoglycemia was shown to up-regulate, GLUT-1, GLUT-4, angiotensinogen and mitogen-activated protein kinase phosphatase-1 [94]. Increase in angiotensinogen expression can favor vasodilation, thereby increasing local blood flow. Alternatively, increased local blood flow raises glucose level locally leading to hypothalamic overestimation of cerebral blood glucose which results in counter regulatory imbalance [94]. See Table 1 for a summary of glycemia-dependent alteration of glucose transport.

### Transport of amino acids across the BBB in DM

Choline is the precursor for the neurotransmitter acetylcholine, which is involved in variety of functions including memory and muscle control. Choline enters the brain through a saturable transport mechanism at the BBB. Reduced choline transport across the BBB was observed in streptozocin-induced diabetic rats when the kinetics of transport was compared to age-matched vehicle-injected controls [95]. The effect was statistically significant only in long standing diabetic animals (9 wks) thus, suggesting a time dependent effect of diabetes on choline transport. In parallel studies using continuous infusion quantitative autoradiograph methods, Mans and colleagues determined the regional permeability-times-surface area (PS) product and influx for choline transport was compared to age-matched vehicle-injected controls [95].

On similar lines, inhibition of VEGF expression improved occludin and ZO-1 expression thereby attenuating inter-endothelial leakage. In accordance, down-regulation of the HIF-1α activity by the use of inhibitors improved BBB integrity and tightness [105].

Impact of diabetes on BBB integrity and other pathophysiological changes

Experimental evidence from in vitro and in vivo studies has shown that BBB integrity in diabetes is somewhat compromised resulting in increased barrier permeability [34,45,46,97]; although current data do not provide conclusive results and outcomes remain controversial. in vitro BBB studies using co-culture of human brain microvascular endothelial cell (HBMEC) with juxtaposed human astrocyte (HA) showed loss of BBB integrity (measured in terms of trans-endothelial electrical resistance, TEER) under hyperglycemic culture conditions (25 mM D-glucose) maintained for five days. BBB integrity normalized upon re-establishment of normoglycemic conditions (5mM D-Glucose) or upon treatment with antioxidants [98]. A significant increase in expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1, IL-4), followed by activation of Nuclear Factor kappa-light chain-enhancer of activated B cells (NF-κB) and Signal Transducer Activator of transcription 3 (STAT3) inflammatory pathways was reported in other studies investigating the impact of hyperglycemia on human astrocytes (HA) [99]. Closely associated astrocytic end-foot processes contribute to modulate ECs differentiation in addition to induction and maintenance of the BBB properties. There is also evidence showing inhibition of astrocytic gap junctional communication in tissue culture and brain slices obtained from diabetic rats. Increased production of reactive oxygen-nitrogen species was also noted although the exact mechanism remains unclear [100,101]. Further, increased level of vascular endothelial growth factor (VEGF; a pro-angiogenic factor) in response to advanced glycation end-products was also reported [102].

Protein expression and transcriptional activity of hypoxia-inducible factor-1α (HIF-1α) was up-regulated in endothelial cell cultures by high glucose levels (30mM). In addition, the expression level of VEGF - a downstream vascular effector of HIF-1α was also increased. VEGF enhances and supports the translocation of GLUT-1 to the cell surface at the BBB besides promoting angiogenesis and decreasing expression of inter-endothelial tight junction proteins (e.g., ZO-1 and occludin [103]), thus increasing BBB permeability. Morphometric analysis using colloidal gold particles (GPs), showed that the immunodensity for occludin was significantly lower in the brain microvessels of diabetic mice in comparison to controls [104].

Impact of diabetes on BBB TJ proteins.

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<td>GLUT-1</td>
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<td>GLUT-4</td>
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Table 1: Effects of hyperglycemia and hypoglycemia on BBB glucose transporter expression.

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Experimental studies with contrasting results have been reported in humans where plasma VEGF levels decreased during hyperglycemia and increased during hypoglycemia; with a convincing explanation that this is probably a neuroprotective mechanism to maintain a constant cerebral glucose supply [106]. Overall, these findings implicate the critical role for VEGF, as a vascular permeability factor, in hyper and hypoglycemia-induced BBB dysfunction.

In addition to VEGF, recent studies provide evidence for a major role of matrix metalloproteinases (MMP) in altered glycaemia-induced loss of BBB integrity. For example, BBB permeability to 14C sucrose (a well-known paracellular marker) increased in response to increased expression of MMP-2 (also paralleled by down-regulation of occludin and ZO-1) in diabetic rats [34,107-109]. Similar to VEGF, advanced
glycation end-products were shown to stimulate the release of MMP-2 [102]. See Table 2 for a summary of glycemia-dependent alteration of BBB tight junction and EC adhesion molecules.

Acute transient hyperglycemia has also been reported to cause early inflammation and endothelial injury. This was clearly outlined in middle cerebral carotid artery occlusion (MCAO) rat model of ischemia-reperfusion injury. Increase in high-mobility group box 1 (HMGB1) and intercellular adhesion molecule-1 (ICAM-1) levels during ischemia-reperfusion were observed in both mild hyperglycemia (blood glucose ~150 mg/dL) and transient severe hyperglycemia (blood glucose ~400 mg/dL) with significant impact on BBB integrity [110]. These results were further reiterated by subsequent studies showing a marked increase of ICAM-1 positive stained cortex microvessels in diabetic rats after 3 days of reperfusion which was also paralleled by increase in IL-1β expression [109]. It is possible that the increased HIF-1α and VEGF expression in response to hyperglycemia synergistically adds to the detrimental BBB response elicited by flow-cessation [111], thus further increasing the loss of barrier integrity observed during reperfusion. However this hypothesis needs to be confirmed.

Regional as well as whole brain permeability increased by more than 100% in diabetic ketoacidosis patients (13 children) under observation right from initiation of study through the treatment period. Level of inflammatory cytokines also significantly increased in these patients. Diabetic ketoacidosis is a result of type 1 diabetes in children wherein increased BBB permeability results in complications like cerebral edema [112]. Absence of tight junction proteins like occludin, claudin-5, ZO-1 and JAM-1; albumin extravasation; and presence of increased neuroinflammatory markers chemokine CC ligand 2 (CCL2), NF-xB and nitrotyrosine were also observed in a similar study. This clearly suggests that neuroinflammation combined with loss of BBB integrity (TJ proteins) plays a primary role in the pathogenesis of brain edema in these patients [108]. All together, these data strongly support a major impairing role of diabetes on BBB integrity and maintenance of brain homeostasis prodromal to the onset of major neurological disorders.

Hyperglycemia also hinders the supply of vitamin C (ascorbic acid) to both retina and brain. Vitamin C is mainly transported across the BBB by GLUT-1 as dehydroascorbic acid (DHA) and is accumulated in the form of ascorbic acid. Vitamin C is involved in the biosynthesis of Kreb’s Cycle.

**Figure 3: Glucose metabolism and oxidative stress:** Glucose metabolism and energy production starts with glycolysis where glucose undergoes a series of metabolic steps to produce pyruvate. Pyruvate can either enter Kreb’s cycle for further metabolism processing and energy production via aerobic pathways or get converted to lactate (ending its metabolic transformation). The intermediates formed during glycolysis can enter into different pathways of metabolism such as Pentose phosphate pathway (PPP), Hexosamine biosynthetic pathway (HBP), Protein kinase C pathway and advanced glycation end-product pathway (AGE). Glucose itself can also enter polyol pathway instead of glycolysis to form fructose. Altered glycaemia can hamper/affect any of these other pathways of glucose metabolism, leading to oxidative stress and potential cell damage.
of collagen, catecholamine and peptide neurohormones. It acts as an antioxidant and scavenges the free radicals, thereby detoxifying the brain [113-115]. 14C DHA transport was reduced by 84.1% in streptozotocin-induced diabetic rats. In addition, P-glycoprotein (P-gp: a member of ATP-binding cassette transporter glycoprotein) expression levels in diabetic mice have been contrastingly reported to decrease [116,117], increase [118] or remain unaffected [119] by others. Further, Insulin therapy was reported to restore the increased P-gp levels in STZ-induced diabetic rats [117]. Additional histological changes and vascular abnormalities observed at the level of the brain microcirculation in diabetes include thickening of the capillary basal membrane, collagen deposition, accumulation of lipid peroxidation by-products and endothelial degeneration at the level of the cerebral microvasculature [120]. Ultimately diabetes can lead to abnormal cerebral neovascularization and remodeling [121], which may further contribute to vascular damage and risk of hemorrhage associated with stroke or neurodegenerative processes in diabetes.

**Oxidative stress at BBB in DM**

Understanding the brain metabolic pathways involved in energy production is imperative to unravel how diabetes ultimately causes oxidative stress which can lead to early inflammation and endothelial injury.

Glucose metabolism (Figure 3) and energy production starts with glycolysis where glucose undergoes a series of metabolic steps to produce lactate (anaerobic metabolism end product) or pyruvate which can be further processed to extract more energy. Under normal condition the extensive bioenergetic demand of the BBB machinery is met by the conversion of pyruvate into carbon dioxide (CO2) and water (H2O) in an oxygen-dependent 8-step enzymatic process along the tricarboxylic acid cycle (TCA cycle). The theoretical energetic yield of the process from the complete oxidation of one glucose molecule to the end product generation of CO2 and H2O is of 6X NADH, 2X FADH2, and 2X ATP (equivalent to 36X ATP molecules). The aerobic pathway also provides reducing equivalents (such as NADH and NADPH) to counteract oxidative stress caused by both endogenous and exogenous reactive oxidative species (ROS) [2,122,123].

Neurons and astrocytes interact to fulfill the energy requirements, with glycolysis occurring in astrocytes while TCA cycle taking place in neurons. Each cell type contains both sets of enzymes, thereby making it a subject for validation. During glycolysis itself, the intermediates formed can enter into different pathways of metabolism (Figure 3) - namely pentose phosphate pathway (PPP), hexosamine biosynthetic pathway (HBP), protein kinase C pathway (PKC) and AGE pathway. Glucose itself can also enter polyol pathway instead of glycolysis to form fructose [2,122,123]. We have subsequently discussed how these pathways abnormally get regulated in DM.

Hyperglycemia aids in production of ROS which leads to variety of microvascular and macrovascular complications [2,122]. Although preliminary studies have shown that human brain endothelial cells exhibit vulnerability to hyperglycemic stress (associated with notable cytosolic and mitochondrial redox shifts) [124,125] characterization of molecular and physiological responses of the BBB to DM-associated oxidative stress (ROS generation) has been barely initiated and is still under-investigated. This is extremely important since oxidative stress has been associated with the pathogenesis and progression of many neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) [126,127].

Takahashi et al. [128] reported that acutely and chronically induced hyperglycemia increased PPP activity and glutathione (GSH) levels in astrocytic culture, in turn decreasing ROS levels. Higher PPP activity facilitates the regeneration of GSH such that cells are able to combat the higher oxidative stress level (protective role). To this end, nuclear translocation of Nrf2 (nuclear factor-erythroid 2 p45 subunit-related factor 2) in association with BIP (immunoglobulin heavy-chain-binding protein) was also observed. Nrf2 (the primary cellular defense against the cytotoxic effects of oxidative stress) normally lies in the cell cytoplasm but translocates into nucleus under oxidative stress to initiate the Nrf2 antioxidant response pathway of transcription of anti-oxidant genes and proteins (e.g., NAD(P)H quinone oxidoreductase 1, Heme oxygenase-1, glutathione S-transferase, etc.).

Generation of excess superoxide during hyperglycemia suppresses GAPDH, thereby promoting glucose utilization by alternative pathways (e.g., processing of Glyceraldehyde-3-P through PKC pathway and AGE pathway) [2]. Pronounced vasogenic edema that occurs during hyperglycemic stroke has been investigated to be mainly due to PKC activation. Further PKC activation affects BBB permeability through ZO-1 phosphorylation, TJ disruption and increase in VEGF expression [129]. Increased intracellular levels of AGE products damage the cells by altering the function of various proteins (modified by AGEs), including their interaction with surface membrane components (e.g., integrins) and AGE receptors. This occurs in macrophages, vascular endothelial and smooth muscle cells. Activation of AGE receptors (RAGE) increases ROS formation and leads to activation of NF-kB pathway. This ultimately promotes the expression of a variety of pro-inflammatory mediators [2,122,130,131] thus adjuvanting/strengthening the immune and pro-inflammatory responses. In fact increased accumulation of AGEs in Alzheimer's patients has proven to cause neuronal death and degeneration, thereby supporting the fact that diabetes increases the risk of AD and any shift in normal glucose metabolism is deleterious for BBB integrity. In addition, oxidative stress has been shown to activate matrix metalloproteinases (MMP-1, -2, and -9) while decreasing tissue inhibitors of MMPs (TIMP-1 and -2) in a protein tyrosine kinase (PTK)-dependent manner [126].

Even though all cells are exposed to elevated glucose level in DM, hyperglycemic damage is restricted to only certain subtypes (retinal cells, endothelial cells) that are unable to down-regulate glucose transporter expression. As previously discussed, five major mechanisms are believed to be responsible for hyperglycemic damage: 1) Increased polyol pathway flux; 2) increased intracellular production of advanced glycation end-products; 3) increased expression of AGEs receptors and its activating ligands; 4) activation of protein kinase C; 5) increased flux through the hexosamine pathway. The upstream triggering event shared by all these mechanism is mitochondrial overproduction of ROS [2,132].

**Conclusions**

Although the DM-dependent vascular damage has been clearly associated to downstream mechanisms generating oxidative stress and inciting inflammation, the impact of DM on the blood-brain barrier has only been marginally addressed. Due to the unique characteristics of the BBB the effects of DM on the brain microcapillaries are, perhaps, different from other microvascular beds such as the retina and peripheral nerves. Most of these adverse effects can build over time with each insult being clinically unnoticed until neurological damage has occurred.

Diabetes effects on BBB function have been approached and
documented with respect to transport of glucose across the BBB in pathophysiological conditions like hyperglycemia and hypoglycemia. BBB regulates the efflux of metabolic byproducts of the CNS metabolism thereby allowing the transport functions to adapt to alterations in blood glucose levels. Chronic alteration in blood glucose content (hyper- and hypoglycemia) is a characteristic hallmark of diabetes and has been shown to trigger corresponding/compensatory alteration in the expression of glucose transporters at the BBB. Specifically, up-regulation of glucose transporters has been correlated to hypoglycemia suggesting an increase in BBB glucose extraction [83,92,93]. By contrast, hyperglycemia appears to down-regulate glucose transporters in the expression of glucose transporters at the BBB. Specifically, up-regulation of glucose transporters has been correlated to hypoglycemia suggesting an increase in BBB glucose extraction [83,92,93].

Overall, the current common consensus is that diabetes-dependent alteration of systemic glucose level play a significant role in the pathogenesis and/or progression of major neurological disorders by altering the structural and functional properties of the BBB [2,122,134]. However, this field of research is still severely under-explored. In depth studies aimed at unraveling how DM progressively alters BBB function and structure, the molecular players and mechanisms involved are still very much needed.

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References


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