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Impact of Metabolic Diseases on Cerebral Circulation: Structural and Functional Consequences

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Abstract

Metabolic diseases including obesity, insulin resistance and diabetes have profound effects on cerebral circulation. These diseases not only affect the architecture of cerebral blood arteries causing adverse remodeling, pathological neovascularization and vasoregression, but also alter the physiology of blood vessels resulting in compromised myogenic reactivity, neurovascular uncoupling, and endothelial dysfunction. Coupled with the disruption of blood brain barrier (BBB) integrity, changes in blood flow and microbleeds into the brain rapidly occur. This overview is organized into sections describing cerebrovascular architecture, physiology, and BBB in these diseases. In each section, we review these properties starting with larger arteries moving into smaller vessels. Where information is available, we review in the order of obesity, insulin resistance and diabetes. We also tried to include information on biological variables such as the sex of the animal models noted since most of the information summarized was obtained using male animals.

Introduction

The brain is a highly metabolic organ with no energy reserves. As such, the brain requires relatively constant blood flow to deliver nutrients and remove waste products overall. Furthermore, cerebral perfusion is coupled to neuronal activity to ensure delivery of blood to

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Cross References

Blood Brain Barrier

Cerebral Microcirculation

Endothelium and Cerebral Circulation

Microvascular Consequences of Obesity and Diabetes

Neurovascular Coupling and Cerebral Circulation

where it is needed the most. To achieve such a high and complex demand, the brain has an extensive vascular network running approximately 400 miles long (22, 36, 207) with specialized architecture. Pial arteries that are at the surface of the brain are connected to the extensive smaller arterioles and capillaries by deep penetrating arterioles. Another unique feature of cerebral circulation is the presence of the blood brain barrier (BBB) formed by endothelium, basal lamina, astrocytes and pericytes. Accordingly, after the first introductory section that describes the arterial supply and the special angioarchitecture of the brain, the main section is organized into four subsections focusing on cerebral arteries and arterioles physiology (function), structure, cerebral blood flow (CBF), and BBB integrity in metabolic diseases, specifically obesity, insulin resistance and diabetes. We refer to small vessels as small brain surface arteries, parenchymal arterioles and capillaries as recently described (198). Readers are highly encouraged to read about small vessel disease in more detail in this book. Given that hippocampus, a critical domain of the brain for cognitive and memory tasks, is highly susceptible to hypoxia, metabolic disease-mediated changes in hippocampal cerebrovascular function and structure are also reviewed under a separate subheading. The final section reviews the consequences of these cerebrovascular changes in central nervous system complications of metabolic diseases focusing on stroke and cognitive impairment. A summary of this organizational flow is illustrated in Figure 1.

Cerebrovascular Anatomy and Architecture

In order to better evaluate our current understanding of the impact of metabolic diseases on cerebral circulation and also identify gaps that can drive further research in cerebrovascular physiology, we will briefly review the arterial blood supply of the brain and its topological organization. We believe this is important because as we review alterations in cerebrovascular function and structure in metabolic diseases in the following sections, it will be evident that most of our current knowledge is based on studies involving certain vascular segments such as middle cerebral artery (MCA) or brain regions such as the frontal cortex.

The arterial blood supply of the brain is delivered by two major routes, which give rise to two unique connections in the brain (Fig. 2A): internal carotid arteries (ICA) and basilar artery (BA). The ICAs, including left and right ICAs, branch into to anterior cerebral artery (ACA) and MCA on each side of the brain supply the anterior circulation of the brain. Posterior cerebral artery (PCA) arises from the basilar artery, which is formed by the merge of left and right vertebral arteries. While the vertebrobasilar route principally provides the blood supply of the posterior brain, these two major networks are not independent of each other. BA and ICAs along with communicating arteries form a complete ring known as Circle of Willis at the base of the brain and downstream branches of ACA, MCA and PCA form extensive collateral networks on the surface of the brain (Fig 2B). Through the organization of these collaterals at the base and surface of the brain, blood flow can be diverted when necessary.

It has been suggested that blood has to travel through three levels to supply the brain (Fig 3) (94, 227). Surface pial arteries and veins form the top level providing circulation of the surface of the brain. Penetrating arteries stemming from this network are the second level and give rise to penetrating arterioles that dive deep into the brain parenchyma connecting to

subsurface capillary circulation. Surface arteries lose their extrinsic innervation upon their entry into the brain parenchyma and become intrinsically innervated from neurons located within the brain itself (108). Penetrating arterioles exhibit increased myogenic tone compared to pial arteries (42, 46). Another unique feature of these arterioles is that they do not branch out and they are known as the “bottleneck” of cerebral perfusion (192). The third level is the 3-dimensional capillary network. Intracerebral arterioles and capillaries are part of the neurovascular unit where there is constant bidirectional communication among neurons, glial cells and the vasculature to couple neuronal signals to vascular responses. Most of our current knowledge of the angioarchitecture of the brain has been gained through assessment of the cortical layers by multiphoton imaging modalities coupled with electrophysiology studies in male animals (73). Organization and functional regulation of deeper layers of the vascular networks under flow conditions remains to be determined and warrants intense future research.

Cerebrovascular Physiology (Function)

As briefly discussed above, adequate cerebral perfusion is essential for brain function. Being an organ with high metabolic demands, improper blood supply to the brain could lead to ischemia and brain damage within minutes (27). Therefore, tight regulatory mechanisms operate to ensure optimum delivery of sufficient blood containing oxygen and nutrients to the brain all the time. Cerebrovascular autoregulation and neurovascular coupling together with metabolic, chemical and endocrine factors represent different processes that aim to achieve adequate and stable cerebral perfusion (27, 151). This section will review various components of cerebrovascular physiology, namely myogenic response, neurovascular coupling and ligand-mediated vasoreactivity with an integrated discussion of the impact of metabolic diseases on these functional properties.

Autoregulation and myogenic behavior

Cerebral autoregulation is one of the well-developed mechanisms in the brain to protect it from fluctuations in perfusion pressure (130, 151, 167). Unlike other vascular beds, relatively larger conduit arteries like basilar artery as well as pial arteries and deeper smaller arterioles all contribute significantly to autoregulation by either dilating or constricting in order to protect downstream microvessels (36, 90, 196). According to Poiseuille’s law, the size of the vessel is the most powerful determinant of blood flow. Therefore, a small change in radius will have a dramatic effect on flow. Impairment of cerebral autoregulation has been reported following stroke, intracranial hypertension and with chronic diabetes (167, 196). Once this autoregulatory function is lost, CBF elevation will couple the increase in pressure and significant brain injury could take place. Several mechanisms contribute to the process of cerebral autoregulation, the most prominent one is the myogenic response.

Myogenic response describes the ability of smooth muscle cells (SMCs) to react to changes in blood pressure in order to keep blood flow constant. SMCs usually contract as result of stretching, which is due to an increase in blood pressure, and dilate upon blood pressure reduction. Myogenic tone is used to describe the extent of contraction at a particular pressure point. Changes in vessel diameter in response to pressure changes are known as

myogenic reactivity. Myogenic response was first described by Bayliss in 1902, when he observed a powerful contraction in dog carotid artery following exposure to high pressure (19). After that, several studies provided further support to these findings, in which they showed that myogenic nature of the arterioles is the determinant of vascular resistance in response to pressure elevation (131, 224). Moreover, myogenic response remained intact in endothelium denuded arterioles (88, 154) and even after the elimination of nervous system influence on the blood vessel (110). These findings provide evidence that myogenic response is an intrinsic property of SMCs. There is a general agreement that myogenic response mediated by small arteries contributes significantly to the regulation of blood flow (25, 57, 69, 179, 195, 234, 235). However, unlike most vascular beds, it has been shown that large pial arteries contribute to ~50% of cerebral vascular resistance (90, 118). This unique nature of the brain allows tight regulation of blood flow despite changes in perfusion pressure (58, 221).

Mechanisms of myogenic response

Before we review how metabolic disorders could impair myogenic response, we need to explain the process of myogenic response development. It requires a series of events that start with the detection of wall/stretch and end eventually with SMC contraction.

Membrane depolarization is an essential step in the beginning of myogenic response (Fig. 4). Changes in transmural pressure and wall tension lead to the opening of voltage gated calcium channels causing an increase in calcium influx, which is essential for myosin light chain kinase (MLCK) activation (147) and initiation of SMC contraction. MLCK is responsible for phosphorylating MLC, which once phosphorylated can bind to the actin filament and generate contraction. Actin is a family of multifunctional proteins, which are the most abundant protein in eukaryotic cells. It exists in both a soluble free monomer (globular G-actin) and part of a linear polymer microfilament (filamentous F-actin). Actin polymerization through the association of globular G-actin is a pivotal step for muscle contraction. It has been reported that inhibiting actin polymerization with cytochalasin made the cerebral arteries incapable of holding pressure and caused them to experience forced dilation (40, 43). Despite extensive research, the initial factors that sense the changes in transmural pressure and act as mechanosensors remain controversial and an active area of research. We should also keep in mind that calcium influx through voltage gated calcium channels is not the only way to achieve the required calcium concentration for contraction. For example, activation of phospholipase C on the plasma membrane leads to the production of inositol 1,4,5-triphosphate (IP3) and 1,2 diacylglycerol (DAG). Then, IP3 diffuses into the cytosol and stimulate the release of calcium from the sarcoplasmic reticulum (190, 252) causing the activation of MLCK and muscle contraction.

Once the myogenic response is started, other mechanisms contribute to myogenic reactivity. PKC or Rho kinase mediated pathways are involved in achieving SMC constriction via increasing calcium sensitization by inhibiting myosin light chain phosphatase (MLCP) without changing intracellular levels of calcium (119). MLCP is an important enzyme that regulates SMC vasodilation via MLC dephosphorylation. Therefore, inhibition of MLCP results in maintaining phosphorylated MLC and in turn SMC contraction. PKC could also

alter calcium sensitivity via phosphorylating calponin and caldesmon, which are involved in excitation-contraction coupling (181, 254). Interestingly, the activation of large-conductance calcium activated potassium channels (BK_{Ca}), via the generation of calcium sparks, is responsible for membrane hyperpolarization and hence myogenic tone attenuation (148, 149).

Myogenic tone and reactive oxygen species (ROS)

While excessive generation of ROS, often associated with metabolic diseases, is believed to contribute to detrimental effects on cerebral vasculature, we should keep in mind that ROS in physiological amounts contribute significantly to normal signaling and the metabolic process for myogenic response development.

There are various enzymatic sources of superoxide anion within the vasculature including, mitochondria, nitric oxide synthase, xanthine oxidase and to a greater extent NADPH oxidase. The breakdown of superoxide anion by superoxide dismutase results in the formation of hydrogen peroxide, which has been shown to induce cerebral vasoconstriction (136) and vasodilation (126, 230, 253). The discrepancy in the results of the aforementioned studies could be attributed to the difference in species and hydrogen peroxide concentration. Gebremedhin *et al.* showed that the increase in intraluminal pressure results in myogenic constriction of cerebral arteries, which was associated with enhanced superoxide anion and hydrogen peroxide production. They further demonstrated that dismutation of superoxide anion or hydrogen peroxide significantly reduced the myogenic tone. ROS-induced myogenic constriction occurs via the inhibition of phosphatase and tensin homolog (PTEN), which led to increased phosphoinositide-3-kinase (PI3K) activity, increased intracellular calcium levels and vasoconstriction (100). Superoxide anion has a biphasic effect on vascular tone, in which vasorelaxation or vasoconstriction can occur according to its concentration (63). Superoxide anion could indirectly affect blood vessels via interacting with nitric oxide (NO). Therefore, it reduces the availability of NO, a potent vasodilator, leading to vasoconstriction.

The reaction between superoxide anion and NO produces peroxynitrite, which is a powerful oxidizing and nitrating agent. The impact of peroxynitrite on vascular reactivity is complex and poorly understood. Peroxynitrite at concentrations ranging between (10^{-2} to $10 \mu\text{M}$) induced vasorelaxation in intact and denuded isolated canine cerebral artery (156, 157). However, it has been shown that low concentration of peroxynitrite was accompanied with vasoconstriction while high concentrations ($100 \mu\text{M}$) led to cerebral artery vasodilatation (28, 79). Those findings were in agreement with a recent study, which demonstrated the importance of maintaining optimum levels of peroxynitrite in myogenic response (49). While physiological levels were essential for myogenic tone development, pathological levels of peroxynitrite following ischemia/reperfusion led to loss of myogenic tone.

Myogenic tone in arteries vs arterioles

It is important to note that our understanding of the cerebral myogenic reactivity and tone relies on isolated pressurized arteries studies using MCA segments. Recent findings suggest that myogenic response of penetrating arterioles may differ from pial arteries. For example,

it was reported that the negative feedback mechanism, which dampens pressure-mediated vasoconstriction via generation of calcium sparks by BK_{Ca} channels, is not operational in penetrating arterioles. Experimental studies have also shown that penetrating arterioles display intact myogenic reactivity after ischemic injury, (37, 46, 47) unlike pial arteries which experience myogenic dysfunction (8, 38, 41, 45). Furthermore, Cipolla *et al.* found that penetrating arterioles retain myogenic response after hyperglycemic ischemic stroke while pial arteries rapidly lose tone (39), which could explain why hyperglycemia does not worsen lacunar stroke outcomes (242) where penetrating arterioles are the main sites of occlusion. These rigorously conducted past studies emphasize the need for future studies to advance our understanding of the mechanisms contributing to myogenic response in different tiers of the cerebral circulation.

Metabolic diseases and myogenic response

Obesity is a risk factor for several cardiovascular disorders including type 2 diabetes, hypertension and stroke. The mechanisms underlying obesity-induced vascular dysfunction are not fully understood. Many studies have been conducted to explore the effect of obesity on autoregulation, since it is crucial for maintaining optimum organ perfusion. To date, the impact of obesity on myogenic response is still elusive. Howitt *et al.* (120) found that MCAs isolated from male rats fed with high fat diet for up to 10 weeks experienced myogenic tone similar to control. In this particular study, high fat diet increased plasma insulin levels four-fold suggesting the existence of insulin resistance but the increase in glucose levels was modest. The Zucker rat, in which there is a mutation in the leptin receptor, is the most widely used rat model of genetic obesity. Cerebral arteries (30, 201) from obese male Zucker rats (17 weeks of age) that had fasting hyperglycemia and insulin resistance experienced enhanced myogenic tone compared to their lean control rats, which could explain the increased risk for poor stroke outcomes.

Consistent with the previous studies, Osmond *et al.* (193) showed that adult obese male Zucker rats (14–16 weeks of age) displayed an increase in cerebrovascular myogenic tone and a decrease in lumen diameter. Moreover, they reported that if stroke was superimposed on this pathology, infarct size was increased compared to lean Zucker rats. However, there was no evidence of increased myogenic tone or vascular remodeling in juvenile obese Zucker rats, which developed insulin resistance but no hypertension. Those findings lend further support to a previous study done by Katakam *et al.* (134), in which they showed that there was no difference in myogenic tone between young Zucker obese rats (7–10 week range) and their lean control. They reported that the young Zucker obese rats used in the study were insulin resistant, but with normal blood glucose and blood pressure. Altogether, both studies suggest that myogenic response remains intact in early obesity and that obesity-mediated prolonged moderate blood pressure elevation may be responsible for cerebrovascular changes. It is important to reiterate that most of these studies were conducted with pial arteries and that there is a gap in our understanding of how penetrating and parenchymal arteriole tone is regulated in obesity and in females. Further research is definitely needed in this area.

Diabetes is associated with endothelial dysfunction, elevated blood pressure and morphological changes in the cerebral arteries, which set a platform for myogenic response impairment. Clinical studies have shown impaired cerebral autoregulation with diabetes (24, 142, 166). In the late 80s, Duckrow *et al.* reported that acute or chronic hyperglycemia resulted in significant reduction in regional CBF in rats (75). Various experimental studies using streptozotocin-induced type 1 diabetes (T1D) (66, 265) and genetic models of Type 2 diabetes (T2D) (78, 128, 139) have demonstrated that arteries isolated from diabetic animals tend to develop more tone than control animals in response to increase in pressure. For example, male Goto-Kakizaki (GK) rats, a lean model of T2D that has insulin resistance, show greater MCA tone shortly after (6 weeks) the spontaneous onset of diabetes (Fig. 5A) (139). Jarajapu *et al.* reported that BBZDR/Wor rats, another model of T2D with insulin resistance, also show changes in myogenic reactivity of the PCA starting after 4 weeks of diabetes and progress to develop more tone after 5 months of diabetes (128). Those findings could explain the early compromised CBF observed in diabetic animals compared to control. Interestingly, Sauve *et al.* have recently shown that T2D induced by the high fat diet plus low dose streptozotocin (HFD/STZ) model resulted in myogenic tone elevation of olfactory cerebral arteries. However, there was no change in PCAs (219).

Diabetes progresses as the animal ages. While BBZDR/Wor rats discussed above developed greater myogenic tone with age, in the GK model there was a significant reduction in myogenic tone as animals got older (Fig. 5B) (2, 137, 150). Inappropriate myogenic control was associated with vascular remodeling, manifested in increased media thickness, cross sectional areas and reduction in lumen diameter in diabetic animals compared to control (2, 150). Specifically, MCAs isolated from GK rats rapidly lose their myogenic tone after ischemic injury (139) which may be in part responsible for increasing the incidence of hemorrhagic transformation (77, 78). Interestingly, Ding *et al.* have demonstrated that loss of retinal myogenic response was associated with poorer memory in diabetic patients (65). Retinal and cerebral microcirculation shares anatomical and physiological properties (199). That's why the eye is considered the window into the brain; retinal vasculature is considered a potential marker for the state of cerebral vasculature. Therefore, based on the previous findings, it is possible that cerebral myogenic abnormalities together with structural remodeling contribute to dysregulation of CBF in diabetes. While there are some differences with respect to arterial segments affected and/or the degree of changes in myogenic response among the different models of metabolic disease, it is clear that cerebral circulation is an early target in metabolic diseases. Improvement of myogenic behavior and CBF could be a powerful prevention strategy for complications such as cognitive impairment and stroke.

Mechanisms of altered myogenic response in metabolic diseases

Despite the extensive efforts in studying the impact of diabetes on vasculature, the mechanisms underlying diabetes-induced changes in myogenic reactivity are still not fully characterized. Diabetes could lead to increased myogenic tone via multiple mechanisms including, decreased tonic release of NO from the endothelium (265), increased tumor necrosis factor expression (TNF) (219), increased phospholipase C (PLC) activation (128). Moreover, Dong *et al.* found that increased myogenic tone in PCAs of diabetic mice was due to decreased BK_{Ca} currents (66, 67). BK_{Ca} are large-conductance calcium activated

potassium channels, which play an important role in the negative feedback response that limits myogenic vasoconstriction (148, 149).

Recently, Abd-Elrahman *et al.* reported that cerebral arteries isolated from T2D diabetic rats experienced an increased basal myogenic tone at low pressure points (1). They demonstrated that increased basal Rho-associated protein kinase (ROCK2) activity was responsible for the observed elevation in myogenic tone via inhibiting MLCP as well as mediating actin polymerization. On the other side, they showed that myogenic behavior changes as animal age, manifested by the reduction in myogenic tone of arteries of rats diabetic for 18–20 weeks. They pointed out that myogenic response impairment was the result of the loss of ROCK-mediated myogenic constriction. Kold-Petersen *et al.* also attributed the diminished myogenic tone in 22 week old diabetic rats to reduced calcium sensitivity as a result of impaired Rho-kinase pathway (150).

Endothelin-1 (ET-1), the most potent endogenous vasoconstrictors known, contributes to diabetes-mediated vascular dysfunction. Dumont *et al.* demonstrated that diabetic rats treated with bosentan, a dual ET receptor antagonist that blocks both ET receptor subtypes, exhibit myogenic tone similar to control (76). Our group reported that bosentan or angiotensin II type 1 receptor antagonist could reverse the established myogenic dysfunction in aged diabetic rats (5). In addition, we showed that proper glycemic control with metformin or blocking angiotensin II type 1 receptor by azilsartan medoxomil was able to prevent the vascular remodeling and normalized the myogenic tone in diabetic animals (Fig. 5B) (2, 137). Interestingly, Blum *et al.* (26) demonstrated that myogenic response of retinal arterioles was significantly improved by proper metabolic control in diabetic patients. Taken together, both experimental and clinical studies provide evidence of the impact of poor glycemic and metabolic control during diabetes on vascular myogenic function. However, it is important to note that despite the best efforts, animal models do not represent the complexity of clinical metabolic diseases and readers are encouraged to consider changes in blood pressure, insulin sensitivity and blood glucose in published studies.

It is uncertain whether sudden acute elevation in glucose levels will have similar effects on myogenic reactivity as diabetes. Acute exposure to high glucose levels (44 mmol/dl) was shown to induce vasodilation and loss of basal tone in rat PCAs. Moreover, loss of tone made the arteries irresponsive to changes in pressure (44, 48, 106). These findings lend further support to a previous study in which they reported that acute hyperglycemia led to CBF elevation and cerebrovascular resistance reduction in nondiabetic dogs (228). Taken together, studies involving acute or chronic elevation of glucose levels suggest that myogenic response is one of the physiological mechanisms that are susceptible to modifications by hyperglycemia. Therefore, more attention should be given to elucidate the possible mechanisms by which diabetes and/or acute hyperglycemia would impair cerebral myogenic behavior.

Neurovascular Coupling

The brain requires delicate mechanisms to achieve continuous vascular supply of oxygen and glucose to meet its metabolic demands. As it was mentioned earlier cerebral

autoregulation contributes significantly to maintaining constant blood flow despite changes in cerebral perfusion pressure. Communicating neuronal signals to the vasculature, namely neurovascular coupling, results in functional hyperemia, another crucial mechanism by which the brain matches blood flow to the neuronal activity by enlarging vessel diameter in response to increased metabolic demand (102, 124, 200, 246). Neurovascular coupling requires close communication in the neurovascular unit which is comprised of vascular cells (endothelial cells, VSMCs and pericytes), astrocytes, and neuronal cells (Fig. 3). The components of the neurovascular unit differ across the 3 levels of the cerebrovascular network. At the capillary level, endothelial cells, pericytes, neurons and astrocytes make up the neurovascular unit. Upstream in levels 2 and 3, VSMCs are also involved. Emerging evidence suggests that the communication within neurovascular unit is bidirectional and the vasculature also sends signals to the neurons, a new concept defined as vasculo-neuronal coupling, which is discussed further below.

Mechanisms of bidirectional neurovascular coupling

Mechanisms of neurovascular coupling are still evolving and involve coordinated action of multiple mediators that originate from different cells and act at different levels of the cerebral vasculature discussed above (18, 33, 72, 94, 152, 212). At the pial surface where there is extrinsic innervation, various neurotransmitters released from neurons can directly alter vascular function and CBF. It was later shown that at arteriole level (level 2), neuronal activation triggers an increase in Ca^{2+} in astrocytes which leads to dilatory and constrictor responses via the generation of prostaglandin E2 (PGE2), epoxyeicosatrienoic acids (EETs), and 20-hydroxyeicosatetraenoic acid (20-HETE) (18). A recent study, however, reported that while an increase in astrocytic Ca^{2+} is necessary for neuronally evoked dilation at the capillary level (level 3), arteriole dilation depends on NMDA receptor-mediated NO release and not mediated by astrocyte Ca^{2+} signaling (183). It is clear that our understanding of the mechanisms contributing to neurovascular coupling is still incomplete and presents a fertile area of research.

In this review, we would like to draw attention to the relatively new concept of “vasculo-neuronal coupling”. Kim *et al.* showed that changes in parenchymal arteriole tone can affect neuronal activity (Fig 6) (141). When parenchymal arteriole tone is increased, astrocytic Ca^{2+} is elevated and cortical neuronal activity is decreased. Conversely, a decrease in arteriolar tone leads to increased resting cortical pyramidal neuron activity. These findings led authors to propose that neurons may be able to efficiently adjust their activity according to perfusion levels and hence protect themselves. This is a very active area of research where mechanisms are not fully understood.

Metabolic diseases and neurovascular coupling

Very few studies investigated the impact of HFD on neurovascular coupling. Functional hyperemia was measured upon whisker stimulation in male rats fed with HFD for 8 weeks. CBF change was significantly reduced in HFD compared to control due to impaired potassium (K^+)-induced vasodilation. Rats displayed an increase in body weight and adiposity, but there was no change in blood pressure, blood glucose or insulin levels. Those findings suggest that the impaired neurovascular coupling was due to HFD and not any

metabolic abnormalities (158). Interestingly, the neurovascular uncoupling reported with HFD containing high levels of high saturated fatty acids was not observed with polyunsaturated fatty acids. On the contrary, several experimental studies (reviewed in (105)) have shown an improved neurovascular coupling following administration of omega 3 fatty acids. These findings are very important as they highlight the importance of following a healthy life style by maintaining optimum balance in our nutrition.

Diabetes is considered a major risk factor for various cerebral complications including stroke, different forms of dementia such Alzheimer's disease, and cognitive impairment. Different studies showed the link between acute or chronic hyperglycemia and neurovascular uncoupling (68, 74, 185). Dorner *et al.* reported that the increase in human retinal blood flow as a result of flickering light stimulation is due to increased neuronal activity, which was significantly reduced by acute hyperglycemia (68). Moreover, flicker response of retinal arteries was significantly reduced in diabetic patients, indicating an impaired functional hyperemia (99, 165). In agreement with the aforementioned studies, Mishra and Newman found that retinal arteriole dilation in response to light stimulation was reduced in STZ-induced diabetic rats compared to control. They also showed that the amplitude of light evoked vasodilation was restored by inhibiting inducible nitric oxide synthase (182).

Unsurprisingly similar findings were reported regarding cerebral functional hyperemia, in which Duarte *et al.* pointed out that neurovascular coupling was impaired in diabetic patients as determined by functional MRI (74). Similar to clinical studies, T2D male rats displayed compromised functional hyperemia upon whisker stimulation, evidenced by the decrease in blood flow in somatosensory cortex compared to the control (Fig. 7C and D) (139). Furthermore, Vetri *et al.* demonstrated that neurovascular coupling was significantly impaired in T1D animal model. They showed that pial arteriole dilation was significantly reduced in diabetes compared to control following sciatic nerve stimulation (247, 248). Interestingly, they demonstrated that neurovascular uncoupling could be prevented by pancreatic islets transplantation or reversed by the administration of PKC inhibitor.

One should keep in mind that coordinated flow response should take place to avoid the drop in microvascular pressure. This means that vasodilation of downstream vessels in response to increased neuronal activity needs to be coupled with upstream vessel relaxation as well. Indeed retrograde vasodilation, which is also referred as vasoconductance, has been reported in a couple of studies, showing its importance in increasing blood flow during functional hyperemia (102, 125, 163). However, whether this mechanism is impaired in metabolic diseases is not known.

Agonist-Induced Cerebrovascular Reactivity

In addition to myogenic reactivity, regulation of the caliber of arteries and arterioles, hence vascular resistance and blood flow in the central nervous system, depends on a delicate balance of constrictor and dilatory responses mediated by vasoactive agents. Endothelium is an early target in metabolic diseases and endothelial dysfunction contributes significantly to increased cerebrovascular resistance and compromised flow.

Metabolic diseases and endothelial dysfunction

Three pathways contribute to the generation of a dilatory response in the cerebral circulation: 1) NO produced by endothelial nitric oxide synthase (eNOS or also known as NOS3), 2) prostacyclin (PGI₂), an arachidonic acid metabolite generated by cyclooxygenase and 3) endothelium-derived hyperpolarizing factor (EDHF), which is now referred as endothelium-dependent hyperpolarization (EDH) and includes multiple mediators (91, 92). These pathways converge on the activation of K⁺ channels leading to the hyperpolarization of VSMCs as well as the generation of cyclic nucleotides by adenylate and guanylate cyclase to induce relaxation. Metabolic diseases can induce alterations in these dilator pathways that lead to impaired cerebrovascular reactivity. As will be apparent in the following sections, dysregulation of NO-dependent relaxation is the most extensively reported mechanism, yet our knowledge of the regulation EDH in cerebral circulation in metabolic diseases is very limited. While studies investigating STZ-induced T1D effects on the cerebrovasculature predominate the literature, there are some reports in genetic or diet-induced models of T2D.

Insulin resistance and endothelial dysfunction

Effect(s) of insulin resistance on cerebral relaxation is mostly studied using lean and obese Zucker rats. In adult obese rats (12 weeks), NO-mediated relaxation responses of basilar arteries and side branches were greatly diminished in an oxidative stress-dependent manner as determined by measurement of vascular responses through a cranial window (81). A follow-up study by the same group showed that rosuvastatin improves this attenuated relaxation response of basilar arteries by inhibiting NADPH oxidase activity (82). Another study using an *ex vivo* approach showed that MCA relaxation is also diminished in 17-week old obese male Zucker rats (201). However, cerebral microcirculatory responses were preserved as demonstrated by no changes in cortical blood flow in response to topical bradykinin application or carbon dioxide ventilation in 12-week old obese and lean animals suggesting that early preventive strategies can offer vasculoprotection for the microcirculation (127). Interestingly, two groups reported that impairment of endothelium-dependent relaxation occurred despite an increase in eNOS protein suggesting that enzyme activity was dysregulated in obese Zucker rats (223). In this model, impaired basilar artery relaxation also occurs in female rats (133) and an NO-donor does not restore dilation. However, it is important to note that in this study animals were relatively older (36 weeks). Regardless, these studies emphasize the need for research in regulation of dilatory responses in the microcirculation, especially in the female sex.

K⁺ channel opening is critical for hyperpolarization leading to vasorelaxation. Relaxation induced by cromakalim, an ATP-sensitive K⁺ channel (K_{ATP}) activator, is diminished in basilar arteries and side branches in 12-week old obese Zucker rats (81). A recent report showed that activation of mitochondrial K_{ATP} channels leads to relaxation of the PCAs in normal rats (135). While mitochondrial function is not altered in young obese Zucker rats (178), whether this mitochondrial depolarization-dependent relaxation is altered in disease is not known.

EDH is an important mechanism of vasodilation in the brain (56, 260). Until recently, the identity of a factor that causes hyperpolarization was a subject of intense debate. It is now recognized that there are many factors including arachidonic acid metabolites derived from the cyclooxygenases, lipoxygenases and cytochrome P450 pathways as well as H₂O₂, CO and H₂S that can cause smooth muscle hyperpolarization (91, 92). For recent advances in this field, readers are highly encouraged to refer to a special series of articles on endothelium-dependent hyperpolarizations in health and disease that were published in *Acta Physiologica* (issue 219) in 2017 (109). The impact of metabolic diseases on EDH mediators, however, is not fully understood as this field is rapidly evolving with the identification of novel factors.

Diabetes and endothelial dysfunction

GK rats are a lean model of T2D that present with moderate hyperglycemia and insulin resistance without obesity. Relaxation of basilar arteries but not mesenteric arteries was impaired in diabetic rats after 10 weeks of diabetes. Inhibition of eNOS decreased the relaxation response of basilar arteries to a greater extent in GK rats suggesting a compensatory increase in NOS-mediated responses at this point of the disease (215). As discussed below, this model also displays hyperreactivity to ET-1 as evidenced by greater contraction. While chronic ET_A receptor blockade restored relaxation to control values in the GK animals with no significant effect in control Wistar rats, ET_B selective blockade A-192621 caused paradoxical constriction of basilar arteries in diabetes (114). Interestingly, the same group showed that dual blockade of both ET_A and ET_B receptors with bosentan improved the maximum relaxation response (160). These studies suggested that the ET system plays an important role in cerebrovascular dysfunction in this model.

In the Zucker diabetic fatty obese model of T2D, mitochondrial dynamics were reported to be altered in the cerebral vasculature. Voltage-dependent anion channel levels were much lower and superoxide production was significantly increased in the microvessels of obese rats (177). As discussed above, depolarization of mitochondria in endothelial cells promotes cerebral vasodilation in regular Sprague Dawley rats (135). Whether this endothelium-dependent relaxation mechanism is impaired in diabetes is an intriguing question that remains to be answered.

The duration of the disease influences vascular reactivity to vasoactive agents in T1D as well. In addition, reactivity of cerebral macro- and microvasculature are differentially affected. In this model of diabetes, acetylcholine-mediated dilator response of cerebral arterioles was impaired after as little as 5–6 weeks of diabetes as measured by a cranial window approach (145). Responses to sodium nitroprusside were similar in diabetic and nondiabetic mice at both time points. In contrast, the vasomotor function of the carotid artery was not affected after 5 to 6 weeks of diabetes. In diabetic iNOS-deficient mice, cerebral arteriolar vasomotor function was not impaired, even after 4 months of diabetes. On the other hand, acetylcholine-induced dilation in basilar arteries from rats was impaired after 8–12 weeks of diabetes and reversed by insulin treatment (173). Cerebral arterioles developed endothelial dysfunction sooner than cerebral macrovessels such as the basilar artery and extracranial arteries such as the carotid artery (145, 243). Dilator responses to

acetylcholine, adenosine 5'-diphosphate, and beta-adrenergic receptor activation were reduced in cerebral arteries (14, 171). In PCAs, long-term diabetes (20–32 weeks) decreases dilation to histamine and mediates increased myogenic tone in diabetic BBZDR/Wor rats (128).

Through a series of studies, Arrick and colleagues showed that T1D impairs endothelium-dependent vasorelaxation by dysregulation of both eNOS and nNOS in an oxidative stress manner (9, 12–15, 169, 170). They did not observe any differences in the relaxation response upon stimulation with a NOS-independent agonist. In one study, it was found that inhibition of the poly(ADP-ribose) polymerase inhibited vascular superoxide generation in pial arterioles and improved eNOS-dependent dilatory response (13). In another study by the same group, nNOS-mediated vasorelaxation was also diminished in cerebral arterioles in a superoxide-dependent manner (14). Furthermore, inhibition of ET-1 signaling by blockade of ET_A receptors, as well as exercise or resveratrol treatment, reduced oxidative stress and restored cerebrovascular function (12, 15, 170). Enhancement of NOS activity by tetrahydrobiopterin treatment also improved vascular function (169). Involvement of NOS dysregulation in cerebral endothelial dysfunction was also shown in the Akita T1D mouse model. Chandra *et al.* reported that targeted overexpression of eNOS improved cerebrovascular relaxation and CBF in this model (35).

CBF

As discussed above, adaptive and maladaptive changes in autoregulation and vascular reactivity in metabolic diseases ultimately affect CBF. Reductions in CBF have been demonstrated in patients with T1D compared to healthy controls (217). Single-photon emission computed tomography (SPECT) performed in both children and adults with T1D demonstrated areas of both increased and decreased CBF relative to non-diabetics. The most affected regions were the cerebellum, frontotemporal brain and frontal brain. These changes in CBF correlated with poor glycemic control and the presence of microvascular complications (129, 208, 217). Additionally, the duration of hyperglycemia was a factor (222).

In T2D, a number of studies using both SPECT (188, 214) and arterial spin labeling (ASL) MRI (155) have reported decreases in CBF. Poor glycemic control as indicated by increased H_{A1C} was found to be associated with loss of cerebral autoregulation (174) and lower CBF (155). Utilizing PET imaging, it has been observed that T2D is associated with reductions in CBF to the insular cortex (20), and that overall glucose intolerance leads to reductions in perfusion to the orbitofrontal cortex, inferior parietal lobe, and the superior temporal gyrus (239). A recent study showed that basal NO activity is attenuated in the cerebral circulation of established T2D patients and this endothelial dysfunction can potentially explain the reductions in CBF observed in patients (191).

Other studies in T1D have reported increases in total CBF compared to non-diabetic patients when controlled for relative brain atrophy (244). It was suggested that this process in the brain is similar to the hyperperfusion that occurs in the kidney in the early stages of diabetes, thus leading to additional pulsatile flow damaging the microvasculature. Experimental

models have also shown diabetes-mediated temporal and spatial alterations in CBF. In STZ-induced T1D model, CBF is lower early in the disease process whereas the same model later shows increased perfusion (111) (Fig. 8). It was also reported that STZ-mediated changes in cerebral perfusion and metabolism are region specific (261). A two-photon fluorescence microscopy study reported disturbed cerebral capillary blood flow in STZ-induced T1D mice increases the susceptibility to ischemic insults (123). Another group reported similar findings and specifically showed that diabetes-mediated changes in CBF are associated with impaired recovery after stroke (209). It was also reported that the decrease in CBF as a result of nanoparticle exposure was greater in diabetic animals, again demonstrating increased susceptibility to harmful stimuli in diabetes.

Our understanding of CBF changes in T2D models is relatively understudied compared to T1D models. In the GK model of T2D, CBF was lower compared to control Wistar rats even shortly after the onset of disease (139) (Fig. 7A and B). As discussed under Vascular Structure in the following section, these animals develop pathological neovascularization, which renders them more susceptible to intracranial bleeding after stroke (78, 159). In the db/db mouse model of T2D, impaired collateral blood flow contributes to poor functional outcomes after stroke as compared to wild-type control mice (257).

Cerebrovascular Structure

Cerebrovasculature undergoes structural changes not only during development but also in adulthood in response to various stimuli. This dynamic restructuring of the vascular networks encompasses several processes including neovascularization stemming from angiogenesis and arteriogenesis as well as remodeling of the vascular wall and vasoregression. While in some conditions, cerebrovascular restructuring is beneficial to maintain physiological integrity, in disease settings adverse remodeling can occur. In this section, we will first briefly define these vascularization processes and then review how metabolic disorders such as obesity, insulin resistance or diabetes alter these processes at parenchymal and pial circulation levels.

Vasculogenesis is usually used to describe the process of the formation of a primitive endothelial network from mesenchymal stem cells or endothelial progenitor cells. Vasculogenesis occurs during the third week of embryogenesis in response to the newly formed tissue hypoxia (71). Additionally, vasculogenesis can occur through the recruitment of endothelial progenitor cells into adult tissue in response to local cues, mainly hypoxia (220). In both cases, vasculogenesis starts by the migration of endothelial progenitor cells to the avascular areas as an unorganized capillary plexus that is pruned and shaped later on by angiogenesis (32).

Angiogenesis describes an orchestrated cascade of events that leads up to the formation of new vessels from already existing vessels (Fig. 9). Tissue hypoxia stimulates angiogenesis through the activation of hypoxia inducible factor-1 α (HIF-1 α), which increases tissue expression of vascular endothelial growth factor-A (VEGF-A) and VEGF receptor 2 (VEGFR-2) (flt-1) (93). Increased tissue VEGF levels stimulate the quiescent endothelial cells in the adjacent vessel, tip cell, to sprout and migrate. Tip cells degrade the extracellular

matrix by matrix metalloproteinases (MMP)-mediated proteolytic degradation (61). Propagating tip cell suppresses the adjacent cells through Del/Notch signaling from sprouting (245) and migrates along the VEGF-A gradient pulling behind the stalk endothelial cells that start dividing to form a small channel. Endothelial cells secrete angiopoietins (Ang-1 and -2) and cognate receptor Tie-2, neuropilin-1, platelet-derived growth factor B (PDGF-B) and basic fibroblast growth factor to help in the recruiting of pericytes. High endothelial/pericyte ratio in the brain induces further capillary stabilization (32) and is crucial for BBB (229). Newly formed capillaries may remain as capillaries, undergo vasoregression or recruit VSMCs and undergo arteriogenesis depending on the microenvironment and the stimuli.

Arteriogenesis refers to a cascade of molecular and cellular events leading to the remodeling of pre-existing vessels by increasing lumen and wall thickness. Arteriogenesis increases the vascular stability of the newly formed vessel as well as the oxygen and nutrient supply to the tissue. Arteriogenesis is used to describe the remodeling of newly formed sprout endothelial cell channels to become a capillary covered by pericytes (117). Arteriogenesis also refers to the increase of the capillary size into arterioles or arterioles into a bigger artery to meet the nutrient and oxygen demands of the tissues. Vascular remodeling takes place through the increase in VSMC coverage within the intima media. The process starts when the flow and shear stress stimulates the endothelial cells release of VEGF-A, PDGF-B, S1P-1, angiopoietins and TGF- β 1 that mediate the differentiation of PDGFR- β +PC progenitor cells and/or VSMCs (31). Moreover, increased shear stress activates the endothelial production of endothelial adhesion molecules, chemokines, and chemokine receptors. Chemokines trigger the accumulation of leukocytes at inflammatory sites which further mediates endothelial cell adhesion and migration (Fig. 9).

Collateralization is a form of arteriogenesis, in which arteriole-to-arteriole anastomoses are formed to cross-connect the arterial trees in response to occlusion or tissue ischemia (117, 250). The basal level of cerebral collateralization as well as collateral remodeling after an ischemic insult greatly alters the ischemic outcomes. It is worth mentioning that the extent of the pial collateral formation differs among different strains as well as different pathological conditions but there is no sex effect (87, 262).

Vasoregression describes the process of obliteration and/or pruning of the capillaries network. During development, physiological vasoregression usually occurs after the formation of the primary vascular plexus into a more mature and trimmed vascular tree. A good example is the vasoregression of the retinal capillaries to prune out the initial retinal plexus into a mature one. On the other hand, under pathological conditions, progressive vasoregression could aggravate microvascular complications and worsen the outcomes especially after ischemic insult where reparative angiogenesis is required. A good understanding of the vasoregression mechanisms may have a profound effect on development of therapeutic strategies and treatment options for multiple vascular pathologies. Oxidative stress, endothelial dysfunction, inflammation, and hyperglycemia are the most accepted mechanisms for endothelial death contributing to the vasoregression observed in metabolic disorders (83, 86).

Acellular capillary or string vessel formation is one of the easily detected forms of vasoregression. It is usually observed in the retinal capillaries of aged patient or under pathological conditions. Acellular capillaries describe the endothelial cell death in retinal capillaries leaving only a thin layer of non-perfused basement membrane. In addition to the loss of endothelial cells, there is also evidence for degeneration of SMCs and pericyte loss (34). Altogether, this leads to a narrowing of vascular lumen and loss of a capillary that is left with a ghost basement membrane. Acellular capillaries, also known as string vessels, have become a surrogate marker for endothelial cell death in diabetic and age-related macular degeneration patients (Fig. 10).

Vascular remodeling is the process of changes in the vessel wall that ultimately affects the lumen and mostly refers to blood vessels that have the intimal, medial and adventitial layers. This process, which starts as an adaptive response to changes in hemodynamic stimuli, locally generated growth factors and vasoactive substances, may subsequently contribute to the pathophysiology of target organ complications in many diseases. Vascular remodeling is coordinated by complex interactions between endothelial cells, VSMCs, fibroblasts and infiltrating inflammatory cells (86). In addition, vascular remodeling involves cell growth, death, and migration as well as production or degradation of extracellular matrix. Remodeling is classified based on the changes in the vessel wall (wall thickness and wall: lumen ratio), the lumen (lumen diameter) and/or cross sectional area. Based on lumen diameter, it is classified as inward vs. outward remodeling and based on changes in vessel wall area, it is referred to as hypotrophic, eutrophic or hypertrophic remodeling (101, 187).

Metabolic diseases and cerebrovascular architecture

Metabolic diseases have been shown to induce cerebrovascular remodeling and alteration of the structure of cerebrovasculature (84, 85). In a model of diet-induced obesity, rats that were fed with HFD developed visceral obesity, mild hypertension, increased fasting blood glucose, and insulin resistance. These animals presented with reduced lumen diameter and outer diameter of the MCA as well as increased wall-to-lumen ratio and wall thickness. This caused an increase in the MCA wall stiffness that was associated with an increased expression of collagen type I and an increase in the activity of MMP-2 (62). In the obese Zucker rat model, obesity had a significant effect on MCA structure and caused extensive MCA inward remodeling, decrease of lumen diameter and decrease in the outer diameter. MCA inward remodeling was associated with greater severity of infarction if the animals were subjected to ischemic brain injury (193, 194). Moreover, obesity produces endothelial dysfunction that leads to further decrease of cerebral vessel relaxation in response to acetylcholine. This effect was reported to occur in a temporal manner and appeared much earlier in cerebral arterioles than that in carotid arteries and via the increase in superoxide anion production by NADPH oxidases (64, 164).

Similar results were reported in GK rats, a lean and insulin resistant model of T2D characterized by mild to moderate increases in blood glucose as discussed before. In this model, mild hyperglycemia caused significant medial thickening and hypertrophic remodeling in a relatively short period (159). There was significant extracellular matrix deposition and increased wall thickness of MCAs that occurred in an ET-1-dependent

manner (115, 138). A significant decrease in the cerebral perfusion came in parallel with the increase in media:lumen ratio. It was later shown that these changes were independent of changes in blood pressure (137). Furthermore, adverse remodeling of MCAs could be reversed by glycemic control or ET receptor antagonism (4). While numerous growth factors can be involved in cerebrovascular remodeling of the micro and macrovasculature in diabetes, one potential target is ET-1, the most potent vasoconstrictor with proliferative properties. The role of ET-1 has been recently reviewed as it plays a very important role in vascular complications of diabetes (85).

Diabetes affects other forms of cerebrovascular patterning. Leptomeningeal arteries including pial arteries perfuse the superficial surface of the brain and provide collateral circulation. In a comprehensive study, different mouse model of hyperlipidemia, obesity, and diabetes were examined for pial collateral number as well as diameter in response to an ischemic insult. In obese mice ($Lep^{ob/ob}$) pial collateral numbers were equivalent to wild type but collateral diameter was reduced. However, fewer native pial collaterals were observed in mice with hyperlipidemia and moderate obesity ($Ldlr^{-/-}$ fed a Western diet), hyperlipidemic mice ($Apoe^{-/-}$ fed a normal diet), and T1D ($Ins2^{Akita}$). Mice with metabolic syndrome ($Lep^{ob/ob}$; $Ldlr^{-/-}$) that have T2D, hypertension, moderate obesity, and hyperlipidemia had pronounced reduction in perfusion as well as number and diameter of pial collaterals (186).

On the other hand, in the GK T2D rat model, pial vasculature showed increased tortuosity as well as collateral numbers and size (159). Diabetes significantly increased the number and diameter of the collaterals and intra-tree arteriole-to-arteriole anastomoses between pial MCAs and ACAs. Increased capillary density and neovascularization occurred within 5–6 weeks after the spontaneous onset of diabetes in this model. Diabetes increased the leakiness and the BBB permeability through the decrease in tight junction proteins (159). A follow-up study showed that these changes were accompanied by parallel increases in MMP-2 and -9 activity that promoted cerebrovascular remodeling and affected vascular myogenic reactivity (78). This remodeling was associated with higher vascular damage after ischemia/reperfusion injury. The study also showed that glycemic control and inhibition of MMPs prevented pial remodeling and reduced associated vascular damage after ischemic brain injury in diabetes (Fig. 11A) (78).

In T1D, significant vascular remodeling as early as 4 weeks after the induction of diabetes has been reported (176). Pial arteries were found to be tortuous and dilated with increases in arterio-venous shunting. Moreover, vascular remodeling was accompanied with reduced vasoconstrictive responses to local increases of PO₂ that were no longer reversible as the animal aged (176). As summarized above, the impact of metabolic diseases on pial vascular architecture varies. These differences may be due to the difference in animal strains, experimental models, duration of insult and the number of metabolic risk factors present within each of these models.

Our knowledge of the impact of metabolic diseases on the structure of pial arteries originating from surface arteries is limited. The branch density and tortuosity of pial arteries were found to be increased in GK T2D rats (204). Yet, this increase in the pial artery

tortuosity and increased branching density was accompanied by reduced cerebral blood flow. On the other hand, diabetes has been shown to increase cortical and striatal vascularization as evidenced by greater vascular density, volume, surface area, and branching in the GK model as compared to control rats. As reported in other species, vascularization was greater in the cortex than striatum and progressively increased from front to the back of the brain. These measurements relied on the visualization of the vasculature by fluorescein isothiocyanate (FITC)-tagged high molecular weight dextran (202, 204). Furthermore, colocalization of FITC-signal with isolectin B4, an endothelial cell marker, showed that there was relatively more isolectin staining in diabetic sections. This finding indicates the presence of more non-perfused vessels in both the cortex and striatum of diabetic animals (204). These alterations that occurred early in the disease process did not progress as animals aged (4). Glycemic control with metformin reversed and prevented deranged neovascularization in diabetic animals as shown by decreased vascular and branch density (Fig. 11B and C).

In a different model of T2D, the $Lepr^{db/db}$ model, branch density and tortuosity were enhanced with a trend for decreased lumen diameter of the penetrating arterioles. The non-perfused/perfused vessel ratio was not different between control and diabetic animals. Although no significant difference in vascular density in $Lepr^{db/db}$ mice as compared to control mice, there was an increase in the vascular volume and surface area that was more dominant in the microvasculature (202, 204). More recent studies by the same group reported that vascularization is increased in HFD/STZ model of diabetes as well suggesting that increased cerebral vascularization is a common finding in diabetes (264).

Increased new vessel formation in the GK T2D model was associated with increased VEGF protein levels in micro but not macro-vessels as well increased VEGF-A signaling in brain microvascular endothelial cells isolated from this model (204). A recent study showed that inhibition of augmented VEGF signaling in diabetes using anti-VEGFR-2 compound caused a significant decrease in cerebral vascularization in diabetic rats neovascularization (3). The same study also showed that diabetes-augmented VEGF signaling caused angiogenic imbalance via deactivation and decreased availability of roundabout protein (ROBO-4), an endothelial cell guidance protein. The use of anti-VEGF therapy restored the ROBO-4 availability and decreased diabetes induced pathological neovascularization (3). Yet, functional outcomes of cerebral ROBO-4 restoration in diabetes, such as improvement of cognitive function and attenuation of bleeding after stroke remains to be determined (Fig. 12).

As described above, metabolic disorders such as obesity and diabetes alter both the structure and function of the cerebrovasculature that in turn disrupt BBB and CBF. These changes in the cerebrovasculature not only increase the risk but also worsen the recovery and functional outcome when the brain is challenged with a second insult such as stroke (83, 86, 237). It is now well accepted that protection of neuronal death is not sufficient for a recovery from stroke and neuro-restoration should be accompanied and supported by vascular restoration. It has been shown that T2D male GK rats that exhibit pathological excessive vascularization as described above develop vasoregression and profound loss of vasculature after stroke in all areas measured including peri-infarct area, cortex and striatum relatively distal to the

injured zone (203). This loss is associated with increased endothelial apoptosis which is driven by peroxynitrite-mediated switch of VEGF-akt1-cell survival pathway to VEGF-MAPK-cell death pathway (6). Treatment with metformin during the 14 days recovery period following stroke regulates blood glucose levels and prevents this dramatic loss of vasculature resulting in better functional outcomes.

On the other hand, in T1D, vascular density was reported to be increased in the peri-infarct area 14 days after stroke (258). Another study measured vessel density and sprouting after photothrombotic stroke in T1D using elegant histological and *in vivo* time lapse imaging (238). Stroke injury increased vascular density in both control and diabetic animals and this was postulated to be a result of the robust dilation rather than the sprouting of vessels. Interestingly, this study also reported lesser branch points indicative of increased pruning after stroke as seen in T2D GK rats but to a lesser extent (238). Initial results from ongoing studies with HFD/STZ model of diabetes suggested that the loss of vasculature after stroke also occurs in this model (264). These studies do suggest that metabolic diseases have a profound effect on cerebrovascular architecture and these effects depend on the severity of metabolic diseases as well as severity of secondary injuries such as stroke.

Hippocampal Vascularization

The hippocampus is a critical domain for learning and memory (146). The hippocampal formation is comprised of the hippocampus proper (CA1–4), dentate gyrus (DG) and the subiculum. Inputs from the entorhinal cortex synapse in the DG, which then synapse in the CA3 region. Axons from the CA3 project to the CA1 region and some leave the hippocampus through the fornix. The formation of new episodic memories is one of the functions of the DG and has also been implicated in the spontaneous exploration of novel environments (213).

Neurons rely on a constant flow of blood for energy, which means the vasculature in the hippocampus is critical to proper neuronal function. Blood is supplied to the hippocampus primarily through PCA, with a small percentage of arteries originating from the anterior choroidal artery (AChA) (Fig. 13). Rake-like arteries arise from the PCA and enter the hippocampus through the DG, fimbriodentate sulcus and hippocampal sulcus (50, 80). The internal traverse arteries mainly penetrate into the CA3 region, while the external traverse arteries branch less and enter the molecular layer of the DG. The CA1 region is infiltrated by arteries deriving from the longitudinal vessels or the internal traverse. The major veins surrounding the hippocampus include the basal, deep transverse, internal traverse and external traverse veins (50). Overall, the hippocampus has lesser number of capillaries compared to the cortex and striatum (51, 104). Given that the hippocampus is severely impacted by ischemic events in remote areas, such as middle cerebral artery occlusion (MCAO) (89, 143), and in metabolic diseases as evidenced by deficits in cognition (161, 175, 232), we will review the limited information about hippocampal vascularization in metabolic diseases.

Metabolic diseases and hippocampal vascularization

Studies in Sprague-Dawley rats showed no difference in capillary density in the CA3 and DG regions after being fed a HFD (60% kcal from fat) for 8 months (95). While capillary density after 8 months of diet was not different in this study, microvascular perfusion in the hippocampus was reduced and acute insulin treatment improved hippocampal blood flow suggesting capillary recruitment. A study by Tucsek *et al.* suggested prominent changes in capillary density occur in aged animals. This study measured capillary density through CD31 staining in relatively young (8 months old) and aged (24 months old) mice fed HFD and found a decline in capillary density in the hippocampus that was associated with cognitive impairment in aged obese mice (Fig. 14). Furthermore, they reported larger decreases in capillary density in the CA1 region of aged obese mice (241).

Beauquis *et al.* reported a reduction in microvessels in the hippocampus, in particular the DG region, in 4-month-old spontaneously T2D GK rats (21). A more recent study using a HFD-induced model of T2D showed that there is an increase in surface area, volume and branch density in the CA1 and DG region of the hippocampus in both male (251) and female animals (unpublished data). Interestingly, the increase in vascularization indices were greater in female diabetic rats. While male diabetic animals showed deficits in hippocampus-dependent cognitive tasks, there was no change in female diabetic rats.

The combined effect of stroke and diabetes on hippocampal vascularization was investigated in young male and female control and T2D (HFD/STZ-induced diabetes model) rats that were stroked using the 60-minute transient MCAO model. 14 days post-stroke, vascular surface area and volume were greater in the DG of male animals and this effect was more profound in diabetic rats (unpublished results). Branch density was also increased but this effect was similar in both control and diabetic animals. In female animals, however, there was a decrease in vascularization of the DG. Whether these changes are unique to the hippocampus or similar patterns are observed in other regions in the brain remain to be determined.

Cerebrovascular Integrity

The cerebrovasculature provides the unique structure of BBB through tight junctions on endothelial cells, the basal lamina, astrocytic endfeet and pericytes (Fig. 15). These important structures are critical in maintaining homeostasis within the brain by providing a selective barrier to gases and lipid-soluble steroids. Three important proteins essential to maintaining the tight junctions of endothelial cells include zona occludens-1 (ZO-1), occludin and claudin-5 (162). The supporting cells such as pericytes, astrocytes and VSMCs provide stability and maintenance to the BBB. Pericytes are essential to stability of cerebrovasculature (236, 255) and have been implicated in CBF regulation (107, 144). Pericytes are ensheathed by basal lamina, which separates them from the endothelial cells and astrocytes; yet maintain contact with the endothelium through N-cadherin and connexins (10, 52). Loss of pericytes has been reported to lead to vascular-mediated neuronal degeneration (23). Pericytes and astrocyte signaling have also been shown to play a role in cerebrovascular integrity. Armulik *et al.* reported that loss of pericytes resulted in the loss of aquaporin-4 polarization in the astrocytic endfeet (11). Changes to astrocyte reactivity can

contribute to neurovascular uncoupling, leading to elevated inflammation and subsequent BBB breakdown (29, 197). Disruption of BBB integrity can lead to increased immune cells infiltration, glial and neuronal cell damage, and further perpetuate BBB breakdown. Here we will discuss changes to BBB integrity in metabolic diseases starting with obesity.

Numerous studies have shown that western diet (high in sugar and saturated fat) increases BBB permeability in the hippocampus (54, 55). After 90 days on a western diet, BBB permeability increased in the hippocampus, which suggests a gradual breakdown of the barrier (113). Hargrave *et al.* went on to report increases in the dorsal, but not ventral, striatum. This suggests that BBB permeability in obesity is not global, but sub-regions should be further explored. Tight-junction proteins have also been seen to be downregulated on a western diet (132). Kim *et al.* explored the impact of maternal obesity in the arcuate nucleus of the hypothalamus, an area key in body weight control, and found increased BBB permeability and differentially expressed tight junctions than pups born from normal weight mothers (140). Supporting cells of the BBB are critical in determining the relative permeability of the cerebrovasculature. Aged obese animals showed reduction of pericyte coverage on the microvessels in the hippocampus (241).

Diabetic subjects have been reported to have increases in BBB permeability as detected by gadolinium magnetic resonance imaging (231). Numerous experimental studies have reported that both T1D and T2D induce BBB permeability and dysfunction in hippocampus, striatum, frontal cortex, thalamus and cerebellum (7, 116, 172, 256). Studies in STZ-induced diabetic animals observed increased BBB permeability and reduced tight junction proteins such as occludins, zona occludens and claudins (59, 116) (Fig. 15). ZO-1, occludin and claudin-5 specifically have lower expression in hyperglycemic conditions through upregulation of superoxide anions and angiogenic factors, such as VEGF (162, 216). Similar results were reported in the *db/db obese* mice model of T2D as well (96, 233).

As briefly discussed above, the number and coverage of pericytes contribute to relative permeability of capillaries (11, 53). Hyperglycemia has been reported to cause pericyte dysfunction and loss through production of ROS both *in vivo* and *in vitro* (205, 206, 225). High glucose-treated pericytes showed elevated activation of the receptor for advanced glycation end products (RAGE), resulting in increased cell death. RAGE-induced apoptosis was inhibited when treated with ascorbic acid (168). It was shown that T2D GK rats have lower endothelial cell/pericyte ratio in the brain (159). A recent study from the same group showed a reduction in anti-angiogenic ROBO-4 in the microvascular endothelial cells of diabetic rats as well as in human brain microvascular pericytes grown in diabetic conditions (high glucose + palmitate). In rats overexpressing ROBO4, pathological neovascularization was prevented while pericyte coverage was improved (3). These results suggest that pericytes are an important target in barrier dysfunction mediated by metabolic diseases.

Astrocytic endfeet are part of the BBB. Oxidative stress was shown to be elevated in astrocytes grown in high glucose conditions, which interfered with gap junction communication after prolonged exposure (98). Moghaddam *et al.* reported chronic treatment with berberine ameliorated astrogliosis through reductions in oxidative stress in STZ-

induced T1D rats (184). T1D caused increases in reactive astrocytes in the hippocampus, which became more pronounced in aged animals (218).

The underlying mechanisms of BBB impairment in diabetes are likely to be multifaceted. In addition to the cellular mechanisms discussed above, there are insights into molecular and inflammatory pathways that may be involved. MMPs, a family of zinc-dependent proteolytic enzymes, have been implicated in degradation of the extracellular matrix (211). MMP-2 and MMP-9 specifically have been implicated in compromising BBB integrity. Aggarwal *et al.* observed improvement in BBB function through reductions of MMP-9 when treated with S-nitrosogluathione, an agent that protects against oxidative/nitrosative stress (7). Peroxisome proliferator-activated receptor- γ activation restored tight junction expression, reduced MMPs and improved overall BBB function in T2D mice (180). Studies in *db/db* mice and *in vitro* models showed increased activation of protein kinase C β (PKC β), and when treated with the selective PKC β inhibitor LY-317615, BBB leakiness and macrophage infiltration were prevented (226, 233). Fukuda *et al.* observed inhibition of ROS and improvement in the integrity of the BBB in hyperglycemia when treated with glucagon-like peptide 1 (GLP-1) agonist, a newer class of antidiabetic agents, suggesting this might be a BBB-protective treatment for diabetic patients (97).

The combination of stroke and diabetes exacerbates BBB injury through multiple pathways. High-mobility group box 1 (HMGB1)-mediated inflammatory response and its receptor RAGE has been implicated in BBB dysfunction in T1D (259) and T2D after ischemia (121). Furthermore, treatment of T2D rats with bone marrow stromal cells reduced HMGB1 and RAGE expression as well as improved BBB permeability (121). MMPs have also been implicated in diabetic stroke pathology (153). It has been reported that MMP-9 has a more rapid increase following stroke in *db/db* than the heterozygous littermates, resulting in higher breakdown of tight junction proteins. The endogenous inhibitors (TIMPs) for MMPs were upregulated prior to MMP upregulation in heterozygous mice, which was ameliorated in *db/db* mice. These results suggest that there is an imbalance of MMP/TIMPs in diabetic stroke, and may provide a target for future studies (153). HIF-1 α upregulation after diabetic stroke has also been seen to play a role in the breakdown of the BBB. Knockout of HIF-1 α ameliorated BBB permeability as well as tight junction disruption (263). A downstream target of HIF-1 is VEGF. Indeed, increased VEGF receptor (VEGF-R2) correlated with increased BBB permeability after stroke and delayed inhibition of VEGF-R2 improved stroke outcome and BBB function in diabetic mice (210). Collectively, there is no doubt that barrier function of cerebral circulation is a unique property of this vascular bed and BBB integrity is compromised in metabolic diseases.

Consequences and Conclusions

It is well established that the risk and severity of neurological diseases including stroke, vascular cognitive impairment and Alzheimer's disease are increased in metabolic diseases (16, 17). Yet, the underlying molecular, cellular and pathophysiological mechanisms are less understood and less studied complications associated with metabolic diseases. Given that 1) constant CBF and BBB integrity are essential for brain homeostasis, and 2) cerebral circulation is an early target in these conditions, it is highly likely that changes in

cerebrovascular structure and physiology play an important role in the onset and progression of these diseases. We would like to direct readers to two excellent articles that review the impact of cerebrovascular disease on ischemic stroke and cognitive impairment (60, 122) as well as to a scientific statement from the American Heart Association that presents evidence on vascular contributions to cognitive impairment and dementia (103). This important statement identified the need for two lines of research: imaging of neurovascular changes over the disease course and studies on intensive reduction of **vascular** risk factors in high-risk groups.

We would like to draw attention to several review articles that discuss the impact of metabolic diseases on ischemic stroke and cognitive impairment risk and outcomes from both a clinical and experimental point of view. Dorrance *et al.* summarize the studies on obesity and address the obesity paradox (70), that is while obesity increases the stroke risk, the outcomes are better in obese patients. On the other hand in experimental models, ischemic injury and outcomes are worse in obesity. This paradox needs further clarification. The impact of T1D and T2D on vascular contributions to cognitive impairment was also recently reviewed (112) and highlighted a role for the innate immunity-mediated inflammation in neurovascular changes in diabetes. Lastly, cerebrovascular complications of diabetes were discussed with a focus on stroke (85).

Experimental models give us the opportunity to dissect mechanisms and identify new therapeutic targets to mitigate dysfunction of the cerebral circulation in metabolic diseases. Furthermore, cerebrovascular protection and restoration strategies should be part of the extensive research efforts focused on protecting and restoring neurons. We would like to emphasize that with the exception of a few studies (74, 99, 165, 186, 265), most of the preclinical studies referenced in this review used only male animals. Moving forward, we need to do a better job incorporating biological variables such as age and sex into our experimental approaches to investigate cerebral circulation in health and disease.

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Didactic Synopsis

Major Teaching Points:

1. Brain has no energy reserves and depends on constant blood flow for oxygenation.
2. Brain is a highly vascularized organ in which blood vessels are organized into 3 tiers: superficial pial network, penetrating arterioles and deep microvascular network, all contributing to cerebrovascular resistance and autoregulation of cerebral blood flow (CBF).
3. Metabolic diseases including obesity, insulin resistance and diabetes disrupt autoregulation of CBF.
4. Impairment of myogenic and ligand-mediated reactivity of cerebral blood arteries and arterioles as well as neurovascular coupling are the underlying causes of compromised autoregulation in metabolic diseases.
5. Diabetes changes vascularization patterns in the brain as well as the structure of cerebral arterial tree and their interaction with neighboring cells resulting in compromised cerebrovascular architecture and integrity.
6. Pathological changes in the structure and function of cerebral circulation contribute to development and progression of complications such as stroke and cognitive impairment in metabolic diseases.

Figure 1.

Schematic illustration depicting the impact of metabolic diseases on structural and functional properties of cerebral circulation. Obesity, insulin resistance and diabetes alter physiology and structure of the cerebrovasculature leading to dysregulation of cerebral blood flow (CBF) as well as increase the risk and severity of central nervous systems complications including stroke, cognitive impairment and neurodegeneration.

Figure 2.

(A) Illustration of the carotid and vertebrobasilar arteries, main arterial supplies of the brain. (B) The arteries at the base of the brain. Internal carotid arteries (ICA) and basilar artery (BA) join to form the Circle of Willis which gives rise to cerebral arteries: anterior (ACA), middle (MCA) and posterior (PCA) cerebral arteries. Used with permission from (189).

Figure 3. Representative illustration of the angioarchitecture of the cortex demonstrating the top level of surface arteries and arterioles feeding into the subsurface capillary network via penetrating arterioles. Modified and used with permission from (94).

Figure 4.

Illustration of the mechanisms of myogenic tone development. Pressure increase in the arterial wall is sensed by mechanoreceptors leading to an increase in intracellular Ca^{2+} . Subsequently, myosin light chain kinase (MLCK) is activated leading to myosin phosphorylation and actin polymerization culminating in vasoconstriction. Rapid dephosphorylation of myosin heavy chain by myosin light chain phosphatase (MLCP) promotes relaxation. Activation of Rho associated kinase (ROCK) inhibits MLCP and contributes to sustained contraction. Modified and used with permission from (249).

Figure 5.

(A) MCA isolated from (10 to 12 week) diabetic rats experienced increased myogenic tone compared to control. (* $p < 0.05$ vs control, $n = 6-8$). Used with permission from (139). (B) 22 week old diabetic rats experienced impaired myogenic reactivity compared to age matched control rats. Treatment with azilsartan restored myogenic tone of diabetic rats. (* $p < 0.05$ vs Wis, # $p < 0.05$ vs vehicle, $n = 6-8$ /group). Modified with permission from (2).

Figure 6.

The working model for vasculo-neuronal coupling. Increased luminal pressure causes constriction of parenchymal arteriole leading to increased Ca^{2+} in astrocytes via the activation of TRPV4 channels. This signal contributes to maintenance of vascular tone as well as inhibition of pyramidal neuron firing activity. It is postulated that pressure-evoked parenchymal arteriole constriction and increased astrocytic Ca^{2+} can further modulate pyramidal neuron resting activity via somatostatin interneurons (dotted lines). Modified with permission from (141).

Figure 7.

(A) Representative image of CBF in both diabetic and control rats generated by magnetic resonance imaging. Scale bar for color-coding is shown below. (B) CBF was reduced in the parietal cortex and striatum of diabetic rats compared to control. (C) Time course of cortical laser-Doppler flow in both control and diabetic rats during whisker stimulation in anesthetized animals (D) Bar graph showing the increase in CBF during whisker stimulation, which was significantly reduced in diabetic vs control rat. (*, $p < 0.05$ vs. cortex; ***, $p < 0.001$ vs. control n=4–12). Modified with permission from (139).

Figure 8.

CBF is different in early and late stage diabetic mice. Representative images of cortical perfusion obtained with laser speckle (A) and whole brain perfusion obtained with MRI FAIR-RARE (B) imaging are shown on top and collective data are summarized in bar graphs below. Laser speckle imaging reveals that at 6 weeks cortical perfusion in brain areas supplied by the MCA is decreased in diabetic animals. At 14 weeks after the onset of diabetes, diabetic mice exhibit an increase in whole-brain perfusion compared to control mice. $n=4/\text{group}$, $*=p<0.05$ vs. Control. Used with permission from (111).

Figure 9.
Illustration of the angiogenesis and arteriogenesis cascade. Used with permission from (83).

Control

Diabetes

Figure 10.

Illustration of the retinal acellular capillary, a model of vasoregression in metabolic diseases.

Acellular capillaries in the retinal vasculature are indicated by yellow arrows. Diabetes increases retinal acellular capillaries compared to control retina.

Figure 12.

Inhibition of VEGF signaling repairs pathological cerebral neovascularization in diabetes.

Diabetic rats with established pathological neovascularization were assigned for treatment with vehicle or the VEGFR-2 inhibitor, SLKB1002 (10 mg/kg/day) for 2 weeks. SLKB1002 significantly decreased all neovascularization indices (n=5–6, *, Ψ P<0.05). Used with permission from (3).

Figure 13.

Illustration of blood supply to the hippocampus. The main supply to the hippocampus usually arises from the posterior circulation via single or multiple middle and posterior hippocampal arteries. ACA, anterior cerebral artery; ICA, internal carotid artery; MCA, middle cerebral artery; PcomA, posterior communicating artery; PCA, posterior cerebral artery. Used with permission from (240).

Figure 14.

Representative images of CD31-positive hippocampal capillary endothelial cells (red) in young and aged mice fed a standard diet (SD) or HFD (A-D). Aged animals fed with a HFD showed reduced capillary length density in the CA1 region (E and F). (* $p < 0.05$ vs young SD, # $p < 0.05$ vs aged SD, \$ $p < 0.05$ vs young HFD). Used with permission from (241).

Figure 15.

Schematic representation of BBB in health and metabolic diseases. The tightly sealed monolayer of endothelial cells in BBB are achieved by tight junction and adherens junction complexes and further supported by pericytes, astrocytes, and microglia to keep components of the circulating blood separated from neurons and brain parenchyma.