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Illuminating the Effects of Stroke on the Diabetic Brain: Insights From Imaging Neural and Vascular Networks in Experimental Animal Models

Diabetes 2016;65:1779–1788 | DOI: 10.2337/db16-0064

Type 1 diabetes is known to cause circulatory problems in the eyes, heart, and limbs, and the brain is no exception. Because of the insidious effects of diabetes on brain circulation, patients with diabetes are two to four times more likely to have an ischemic stroke and are less likely to regain functions that are lost. To provide a more mechanistic understanding of this clinically significant problem, imaging studies have focused on how stroke affects neural and vascular networks in experimental models of type 1 diabetes. The emerging picture is that diabetes leads to maladaptive changes in the cerebrovascular system that ultimately limit neuronal rewiring and recovery of functions after stroke. At the cellular and systems level, diabetes is associated with abnormal cerebral blood flow in surviving brain regions and greater disruption of the blood-brain barrier. The abnormal vascular responses to stroke can be partly attributed to aberrant vascular endothelial growth factor (VEGF) signaling because genetic or pharmacological inhibition of VEGF signaling can mitigate vascular dysfunction and improve stroke recovery in diabetic animals. These experimental studies offer new insights and strategies for optimizing stroke recovery in diabetic populations.

Although type 1 diabetes (T1D) and type 2 diabetes (T2D) have different etiologies, they share many common features, such as chronically elevated blood glucose levels and vascular-related pathology of the heart, kidneys, limbs, and eyes. With respect to vascular dysfunction of the brain, both T1D and T2D confer an increased risk of ischemic stroke (1). Although improvements in acute stroke care have reduced mortality, the unintended consequence has been to increase the number of survivors living with disabilities, shifting the research priority from survivability to recovery. Patients with diabetes and stroke face the grim reality that their prognosis for recovering sensory, motor, and cognitive functions, not to mention independence in daily life, is poor (2,3). Therefore, a pressing need exists to intensify efforts at the basic science level to understand how diabetes impairs stroke recovery. To this end, the present review focuses on experimental insights on the deleterious effects of T1D on recovery from ischemic stroke. For reviews about molecular mechanisms of dysfunction after stroke associated with T2D models, the reader is directed to Ergul and colleagues (4,5). Furthermore, we emphasize findings from imaging studies that have generated new knowledge about the neural and vascular mechanisms that underlie poor functional outcome after stroke.

IMPAIRED STROKE RECOVERY IN RODENT MODELS OF DIABETES

Epidemiological studies have shown that patients with diabetes suffer higher stroke mortality (5). Those who survive are more likely to suffer severe disability and exhibit slower short- and long-term recovery than patients without diabetes (1,2,6). Many basic science studies have tried to replicate these clinical findings in the laboratory to better understand the cellular and molecular underpinnings. One widely used animal model of T1D involves the administration of streptozotocin (STZ). This drug selectively kills insulin-producing cells in the pancreas, rendering

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Received 13 January 2016 and accepted 31 March 2016.

mice hyperglycemic and leading to sequelae that mimic T1D (7). STZ-treated mice with chronic elevations in blood glucose levels (~2–4 weeks) show greater neurological deficits and are less likely to regain full use and dexterity of the stroke-affected limbs after focal ischemic or embolic stroke (8–10). One caveat with this finding is that it occurs after persistent hyperglycemia rather than after chronic low-grade dysregulation of glucose levels that would typically be present in human patients with diabetes. To better model the human condition, hyperglycemic mice were treated with insulin to lower blood glucose before or after the induction of stroke. If insulin was introduced immediately after the stroke, it did not normalize or improve functional recovery in previously hyperglycemic animals (9). However, treating rodents with insulin immediately after the induction of hyperglycemia can reduce ischemic damage and improve neurological function (11). Therefore, the longer experimental animals are hyperglycemic, the less likely they are to recover function after stroke.

CAN INFARCT SIZE EXPLAIN POOR STROKE RECOVERY?

One explanation for why diabetic animals may have a reduced rate of neurological recovery is that they simply have a larger cerebral infarction, as indicated in a meta-analysis by MacDougall and Muir (12). However, most studies induced stroke shortly (a few hours to days) after the induction of hyperglycemia (12,13). In this scenario, the brain and systemic vascular networks may be acutely compromised or abnormal before the stroke because of the sudden change in blood glucose levels. Studies examining infarct size after more prolonged periods of hyperglycemia (>2 weeks) in T1D models are somewhat mixed. Those that used transient or permanent occlusion of the middle cerebral artery (MCA) showed a general trend for hyperglycemia to increase stroke damage (12). However, this trend does not always hold for this stroke model (10,14) and not when photothrombosis is used (9,15,16). In this sense, various models of ischemic stroke may be more or less affected by hyperglycemia. For example, transient or permanent occlusion of the MCA produces a larger region of penumbra-like tissue (defined as potentially salvageable tissue in acute stroke) than the photothrombotic model where a sharp transition is seen between ischemic and perfused tissue. Therefore, in the MCA occlusion model, there may be more of a dynamic range to detect an influence of hyperglycemia on tissue damage. Of note, studies that used T2D models (e.g., the Goto-Kakizaki [GK] rat where hyperglycemia occurs gradually) found no difference or even smaller infarcts than normoglycemic controls (17), although this has not been found in the *db/db* mouse (18). Beyond the necrotic infarct, evidence shows that chronic hyperglycemia can increase the number of apoptotic cells labeled with caspase-3 (19). In this sense, quantifying infarct volume alone would not fully capture the extent of ischemic damage, something future studies could consider. In summary, these studies have indicated that an acute elevation in blood glucose is usually associated with greater infarction, but this is not necessarily the case when longer durations of hyperglycemia are used before the induction of stroke.

IMPACT OF DIABETES ON NEURAL CIRCUIT REWIRING DURING STROKE RECOVERY

The degree to which a person can recover from stroke depends not only on the amount of damage incurred but also on how well surviving tissues reorganize (20,21). The peri-infarct region is a well-established hotspot for growth factor production and neurovascular plasticity and has been shown to play a critical role in the restoration of brain functions after stroke (20,21). How T1D affects neuronal circuits in the peri-infarct region has not been well studied. To address this knowledge gap, Sweetnam et al. (9) used in vivo voltage-sensitive dye imaging to study forepaw-evoked cortical network activity and reorganization after stroke. Voltage-sensitive dye imaging is a powerful tool because it allows the visualization of neuronal activity across many cortical regions in real time (22). Normally, mechanical touch of the forepaw evokes a rapid and robust depolarization in the primary and secondary forelimb somatosensory cortex (Fig. 1*A*). In the first week after stroke, cortical responses to touch are greatly diminished in both diabetic animals and their respective controls (Fig. 1*B*). This loss of cortical responsiveness corresponds to the same period when functional deficits in forepaw use and dexterity are greatest. Over several weeks (~100 days), sensory-evoked cortical responses reemerge in peri-infarct cortical regions in nondiabetic animals (Fig. 1*C*) contemporaneously with the spontaneous recovery of paw function. By contrast, mice with chronically elevated blood glucose levels do not show this characteristic pattern of sensory cortex reorganization and recovery. In fact, hyperglycemic mice show very little improvement in cortical responses to touch from 7–100 days after stroke (Fig. 1*C*). Furthermore, treating hyperglycemic mice with insulin after the induction of stroke showed little benefit in improving cortical responsiveness (Fig. 1*C*). Accompanying these deficits in cortical plasticity, hyperglycemic and insulin-treated mice show persistent impairments in sensory function of the forepaw. These observations indicate that diabetes leads to profound impairments in cortical plasticity and recovery of paw function after stroke.

The reorganization of large-scale cortical networks after stroke involves extensive modifications at the synaptic level (21,22). How diabetes alters the rewiring of axon terminals and their postsynaptic targets onto dendritic spines is an open question. Of the few studies to address this issue in diabetic animals, Yan et al. (23) found that T1D rats have significantly reduced density of intracortically labeled axons (labeled with biotinylated dextran amines or axonal neurofilament markers) in the peri-infarct cortex relative to normoglycemic controls.

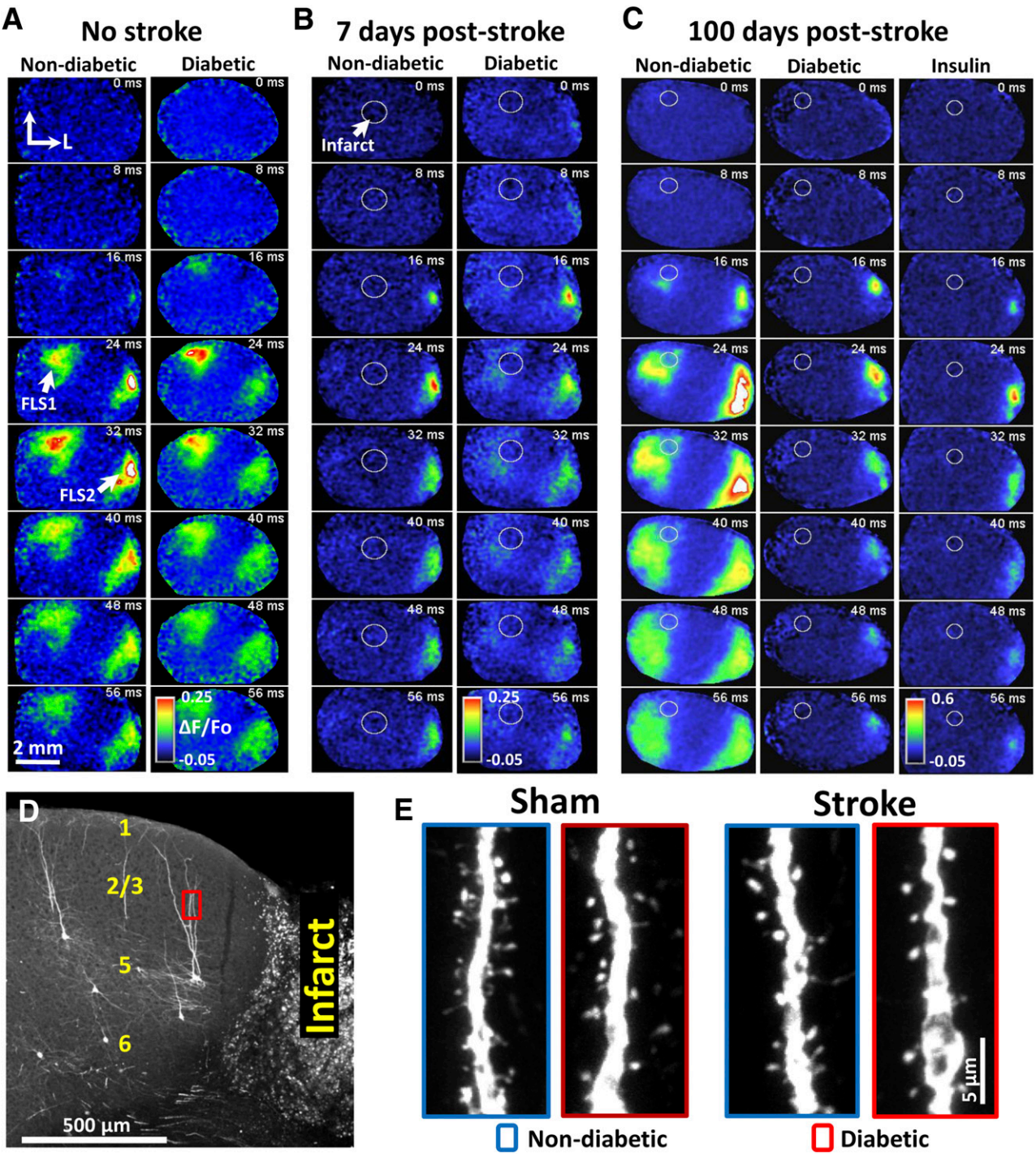

Figure 1— Diabetes impairs cortical plasticity after stroke and exacerbates the loss of synaptic connections. Voltage-sensitive dye imaging shows that diabetes impairs the reorganization of the forelimb sensory representation after stroke. *A–C*: Montages show the spatiotemporal dynamics of cortical responses to stimulation of the contralateral forepaw (initiated at 0 ms). In the absence of stroke damage (*A*), forelimb-evoked cortical responses were similar between nondiabetic and diabetic mice. Seven days after stroke (*B*) (circles denote infarct), the peri-infarct cortex shows little, if any, response to forelimb stimulation (FLS1), whereas responses are preserved in the secondary forelimb somatosensory cortex (FLS2). One hundred days after stroke (*C*), forelimb-evoked depolarizations reemerge in peri-infarct cortex of nondiabetic mice but not in diabetic or insulin-treated mice. The secondary forelimb somatosensory cortex also appears more responsive to forepaw touch in nondiabetic mice. Correlating with these impairments in cortical plasticity, diabetic mice also exhibit greater loss of apical dendritic spines on layer 5 neurons in peri-infarct cortex (*D* and *E*). Representative high-resolution confocal images illustrate that although stroke leads to a significant loss of spines, diabetes further exacerbates this synapse loss. Panels *A–C* were modified with permission from Sweetnam et al. (9).

Consistent with the idea that diabetes exacerbates the loss of synaptic connections in the peri-infarct cortex, our group and others have reported lower spine density in layer 5 cortical apical dendrites (Fig. 1*D*) as well as synaptophysin protein expression 1 week after stroke (15,24). In tandem with synapse loss, diabetic mice show an abnormal increase in hyperphosphorylated τ- and β-amyloid protein expression after ischemia (24,25), thereby raising an intriguing link among diabetes, impaired neural plasticity, and molecular signatures of dementia. This is significant because both stroke and diabetes are independent risk factors for cognitive impairment or dementia (26). Furthermore, elevated blood glucose levels in humans with stroke increases the risk of white matter disease (26,27). Many unanswered questions remain about the effect of diabetes on recovering neural circuits, such as how diabetes affects the rewiring of thalamocortical connections that deliver sensory information to the cortex or the function of inhibitory neurons that regulate cortical excitability. What impact diabetes might have on activity-dependent forms of synaptic plasticity that help to establish new routes of information processing when circuits are damaged by stroke needs further study. A study by Jing et al. (28) showed a reduction in the amplitude and slope of long-term potentiation in the CA1 hippocampal region in diabetic rats subjected to transient global ischemia. However, because there was not an ischemic nondiabetic control group, knowing the extent that diabetes exacerbates long-term potentiation deficits after stroke is difficult. Finally, because stroke can induce widespread alterations to cerebral blood flow and metabolism as well as functional and structural modifications in remote, but connected neurons (29,30), we have little knowledge of how hyperglycemia affects these distant networks.

EFFECTS OF DIABETES ON CEREBRAL BLOOD FLOW AND VASCULAR REMODELING AFTER STROKE

Because T1D leads to poorer stroke outcome, perhaps abnormal patterns of cerebral blood flow could explain this phenomenon. In nondiabetic animals, focal cerebral ischemia induced by photothrombosis or MCA occlusion leads to an immediate and severe reduction in blood flow in the infarct core (31). In the peri-infarct region of nondiabetic animals, blood flow is reduced ~50% within the first few hours, which recovers over several days or weeks, although not always to prestroke levels (32–34). In diabetic animals, cerebral blood flow exhibits a notably different pattern after stroke. Recent in vivo imaging studies have shown that both T1D and T2D exacerbate the acute reduction in regional blood flow and velocity of blood flow in peri-infarct arteries and microvessels after MCA occlusion (Fig. 2*A–E* and *I*) (35,36). These acute deficits in blood flow are evident in the first few hours after stroke and may be linked to an impaired recruitment of collateral cerebral blood flow (36) or a downregulation of clot-busting proteins, such as tissue plasminogen activator, in cerebral capillaries (37).

To our knowledge, only two studies have tracked blood flow patterns in cerebral vessels of diabetic animals for several days to weeks after focal ischemic stroke. In the first study, Tennant and Brown (16) used longitudinal two-photon imaging to repeatedly image blood flow in microvessels before and after photothrombotic stroke in the STZ model of T1D (Fig. 2*F*). They reported that blood flow velocity in hyperglycemic mice was unexpectedly, yet significantly elevated in the peri-infarct cortex 3–28 days after stroke relative to nondiabetic controls (Fig. 2*G* and *I*). Although this finding may seem counterintuitive, a study by Li et al. (38) that used laser Doppler imaging for regional estimates of cerebral blood flow also showed elevated blood flow 24 h after stroke in the GK T2D rat. In the Tennant and Brown study, administration of insulin to hyperglycemic mice immediately after stroke provided only a partial normalization of blood velocity levels (Fig. 2*G*). Blood flow in vessels more distant from the infarct was unaffected and not significantly different between experimental groups (Fig. 2*H*), proving that these abnormal peri-infarct vascular responses are related to the stroke and not to global complication of diabetes. Although there is no other longitudinal imaging study in T1D models for comparison, we believe that these results contrast with those recently obtained in a T2D model. Akamatsu et al. (36) used laser Doppler optical coherent tomography to track blood velocities in leptomeningeal surface vessels after MCA occlusion in the *db/db* mouse model. Their experiments revealed that T2D was associated with a reduction in collateral leptomeningeal blood flow to ischemic zones immediately after stroke and for up to 1 week later. Several experimental factors could explain the differences in blood flow changes between the two longitudinal imaging studies, most notably the model of diabetes used, the specific vessels studied, and the model of ischemia used. However, despite these differences in the direction of blood flow changes after stroke (i.e., abnormally increased or decreased), both studies reported the common theme of poor recovery of paw function. In this respect, perhaps the direction of abnormal blood flow after stroke is secondary to the fact that blood flow needs to be within a certain limit to most efficiently exchange oxygen and nutrients to the brain (39).

Stroke-related disruptions to cerebral blood flow and its restoration, particularly over the long term, could include vessel remodeling. In animal models of T2D, researchers have shown in postmortem specimens that the increase in vascular density normally observed after stroke is attenuated in hyperglycemic GK rats (4,40). By contrast, the nature of vessel remodeling after stroke in T1D models is less conclusive. For example, Ye et al. (10), reported that mice with T1D showed greater arterial density in the peri-infarct zone relative to nondiabetic mice 14 days after MCA occlusion. However, the abnormal

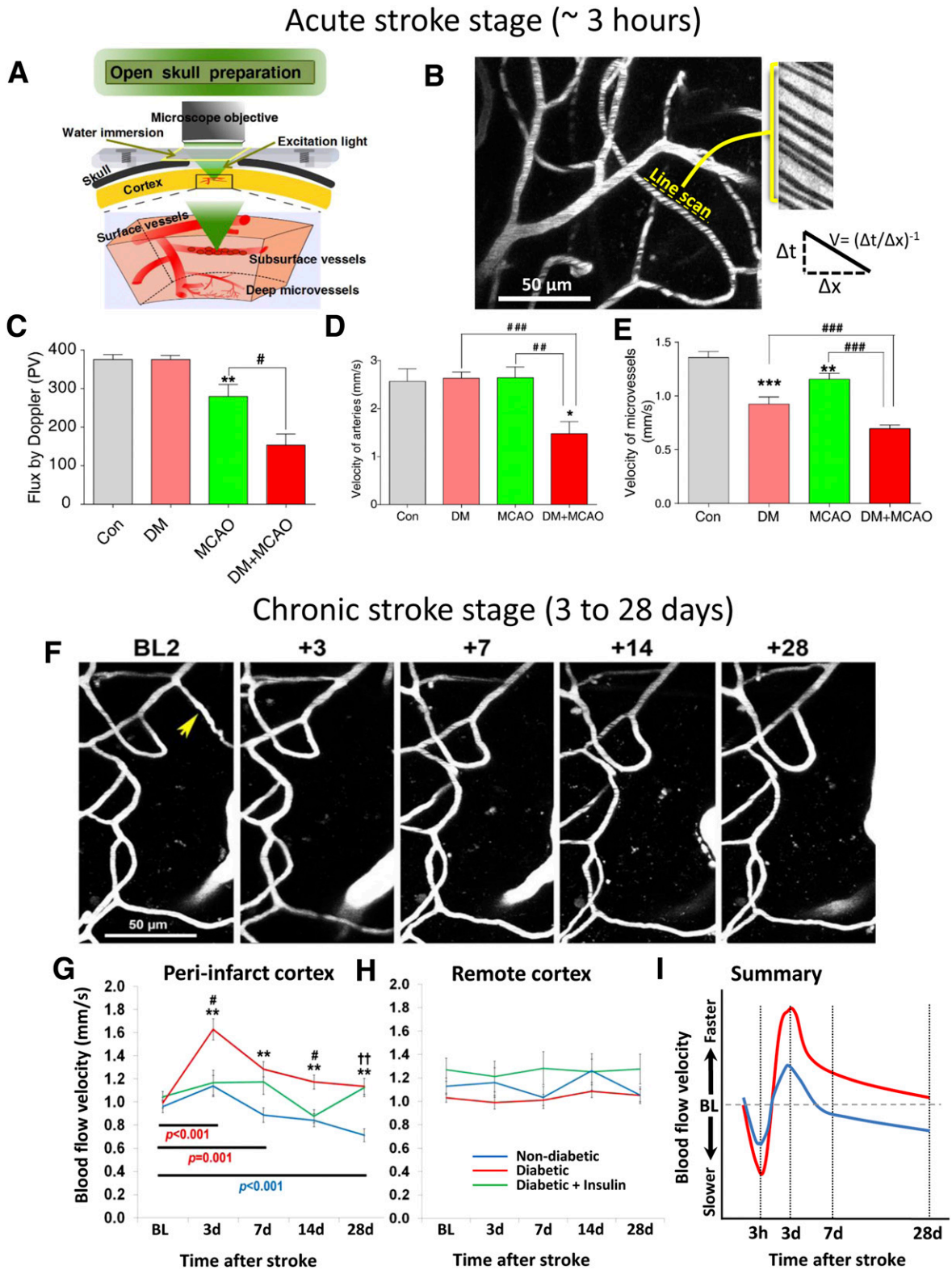

Figure 2—Abnormal patterns of blood flow in diabetic animals recovering from stroke. *A*: Experimental setup for in vivo two-photon imaging of acute changes in blood flow after stroke through a cranial window. *B*: A maximum-intensity z projection showing cerebral vessels labeled with a fluorescent dye injected into the bloodstream. Line scans across microvessels reveal a streaking pattern of diagonal lines created by nonfluorescent red blood cells moving through the vessel lumen. Velocity is determined by calculating the slope of the streaks. *C*: Changes in regional cerebral blood flow in control (Con) and diabetic (DM) mice after MCA occlusion (MCAO) by using a laser Doppler flow meter. Histograms show arterial (*D*) and microvessel (*E*) blood flow velocity in Con, DM, MCAO, and DM + MCAO mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Con; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$. Panels *A–E* modified with permission from Huang et al. (35). *F–H*: Diabetic animals also showed abnormal patterns of blood flow in the days to weeks after stroke. *F*: Representative in vivo two-photon

increase in vessel density in diabetic mice could not be explained by an increase in sprouting of the vascular endothelium because there were no group differences in the fraction of cells labeled with BrdU, a marker of cell division. More recently, our group explored the effect of T1D on vessel density and sprouting after stroke by using both histological and in vivo time lapse imaging. Although we also found that stroke led to an increase in the density of vessels in the peri-infarct cortex, no difference existed between diabetic and nondiabetic animals (16). This increase in vessel density was likely a result of the robust dilation of vessels commonly observed after stroke rather than the sprouting of vessels (16,34). The general absence of vessel sprouting in peri-infarct cortex was confirmed by directly imaging the same microvessels before and after stroke (Fig. 2*F*) (16), which is consistent with the Ye et al. study and other in vivo imaging studies in nondiabetic animals (33,41). Therefore, vessel sprouting could conceivably occur in T1D models near the ventricles after stroke where proliferating and migrating cells reside (42) or on the pial surface where obstructions to vessel growth (e.g., the dense extracellular matrix in brain parenchyma) would be less of a factor. On the basis of our examination of vessel branch points (15), one could argue that a slight trend exists toward greater vessel pruning in diabetic animals after stroke. This explanation fits with the reduction in vessel density observed in T2D rats after stroke (40). In summary, we believe that the primary effect of T1D on vascular structure in the stroke-affected hemisphere could be to promote vessel pruning rather than inhibit the sprouting of new vessels.

DYSFUNCTION OF THE BLOOD-BRAIN BARRIER

The blood-brain barrier (BBB) primarily comprises the vascular endothelium, basement membrane, pericytes, and astrocyte endfeet, although microglia modulate BBB function as well (43). The BBB plays a critical role in regulating the passage of blood-borne proteins, sugars, and ions into the brain. It is well established that stroke compromises the integrity of the BBB, and this exacerbates tissue injury and dysfunction (44). Many studies have examined the effects of diabetes on BBB function (45) and its response to stroke. The results have been in striking agreement, showing that diabetes exacerbates the disruption of the BBB (15,38). By using the STZ model of T1D, our group and others (8,10,15) have demonstrated that diabetic mice exhibit significantly greater hemorrhage volume and extravasation of blood-borne dyes in peri-infarct cortex 1–3 days after stroke (Fig. 3*A*). In some of these studies, greater BBB disruption correlated with greater infarct volume, suggesting that the BBB may enhance the spread of necrotic tissue damage into the penumbra (8,10). However, in our study (15), greater BBB disruption, even 3 days after stroke, did not equate with a larger infarct but instead exacerbated damage at the level of synapses/dendritic spines in peri-infarct regions (Fig. 1*D* and *E*). These studies revealed that diabetes has insidious effects on BBB integrity, which can damage the brain in obvious ways by enhancing the spread of cerebral infarction or in more subtle ways by stripping surviving neurons of their synaptic connections.

The defining characteristic of the BBB—the restriction of free flux between blood and the brain—arises primarily from two distinct properties of the brain's vascular endothelium: the presence of tight junctional complexes (TJCs) and low rates of vesicle movement or transcytosis (43). TJCs are adhesion-based connections between vascular endothelial cells, forming a seal around the vessel lumen and restricting the passage of anything larger than 180 Da (46). TJCs can be modified by phosphorylation of TJC-associated protein subunits (e.g., occludins), which can lead to TJC disassembly and possibly BBB permeability (46). In vitro studies have shown that hypoxia reduces TJC protein expression, which correlates with increased monolayer permeability (47). In animal models of T1D and T2D, several studies have shown a correlation between increased BBB disruption after stroke and an exaggerated loss of TJC protein expression in peri-infarct regions. However, direct visual evidence of tight junction disruption with electron microscopy were missing in these studies (8), which relied on Western blots or fluorescence microscopy (35). This caveat is significant because recent studies that combined electron microscopy with fluorescence imaging of TJC proteins in regions with confirmed BBB disruption noted that tight junction ultrastructure is generally unaltered or, at worst, contain small gaps at these sites (48,49). One unanimous finding of all electron microscopy studies has been the striking increase in transendothelial vesicle trafficking through the endothelium (48,49). In accordance with this, our own ultrastructural studies in T1D mice revealed a marked upregulation of vesicles and vacuoles in peri-infarct microvessels (Fig. 3*B*) without compelling evidence for TJC uncoupling (15). We confirmed the transendothelial route of permeability by finding blood-borne horseradish peroxidase or 5-nm gold particles in endothelial vesicles and beyond the vascular wall (Fig. 3*C*). We failed to detect blood-borne tracers trapped within TJCs after stroke, but the possibility that TJC

images reveal peri-infarct vascular networks before stroke (baseline [BL]) and various time points afterward. The vascular structure was stable, although the occasional vessel was permanently lost after stroke (arrow). Long-term measurements of microvessel velocity in peri-infarct cortex (*G*) and regions distant from the stroke (*H*). $^{**}P < 0.001$, non-DM vs. DM; $\dagger\dagger P < 0.001$, non-DM vs. DM + insulin; $\#P < 0.01$ DM vs. DM + insulin. Colored *P* values refer to within-group differences compared with BL velocity values. *I*: Summary of changes to peri-infarct blood flow velocity in non-DM (blue) and DM (red) mice relative to BL after stroke. Panels *F–H* modified with permission from Tennant and Brown (16). d, day; PV, portal venous.

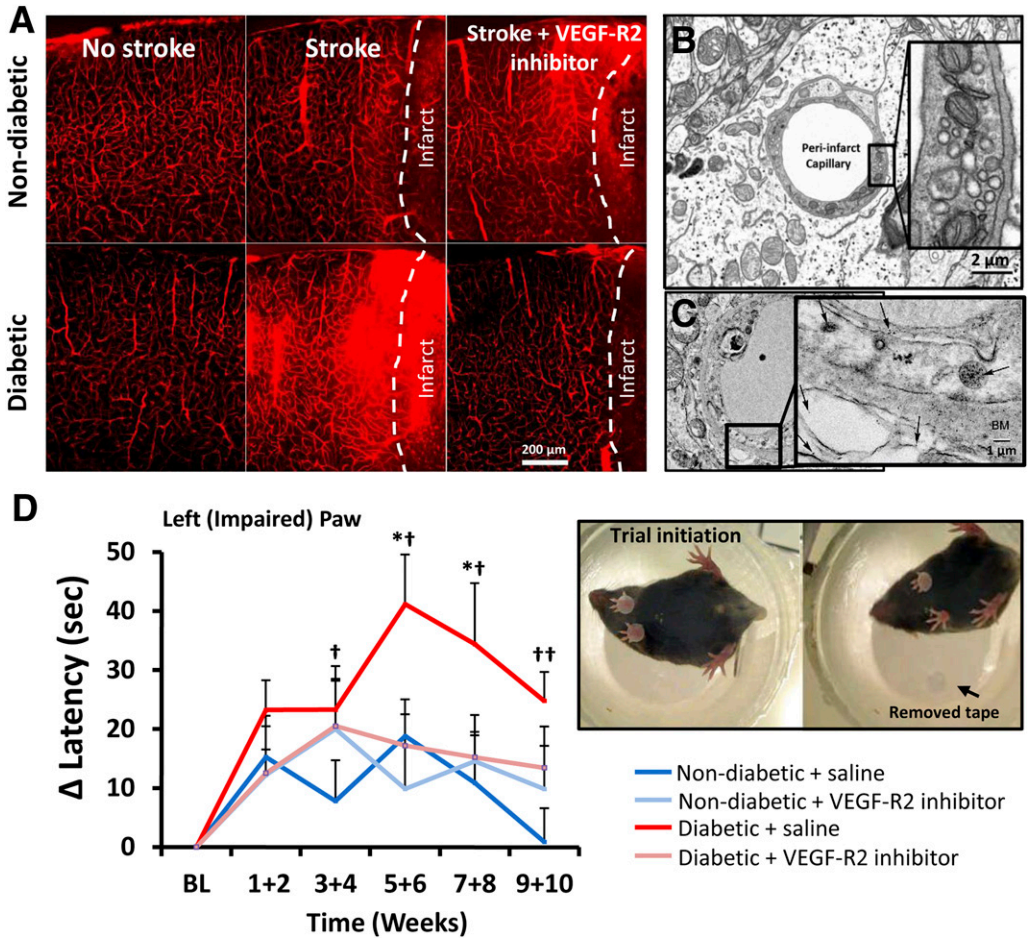

Figure 3—Aberrant VEGF-R2 signaling in diabetic mice plays a critical role in BBB disruption and diminished recovery of function. *A*: Representative confocal images showing cortical vascular networks labeled with Evans blue dye in diabetic and nondiabetic mice 3 days after sham surgery or stroke. In the absence of stroke, fluorescence was restricted to blood vessels (left). After stroke, extravascular dye fluorescence was evident in peri-infarct cortex, especially in diabetic mice. Treatment with the VEGF-R2 inhibitor SU5416 significantly attenuated BBB disruption in diabetic mice but had no beneficial effect and actually increased Evans blue extravagation in nondiabetic mice. *B*: An electron micrograph reveals increased transcytotic activity in a peri-infarct capillary 3 days after stroke. The inset shows a thickened endothelium laden with caveola-like vesicles. *C*: Diabetic peri-infarct capillary 3 days after stroke and a higher-magnification image of the boxed region show 5-nm gold particles (arrows) present in transcytotic vesicles within the endothelium, basement membrane (BM), and adjacent glial cells. *D*: Chronically inhibiting VEGF-R2 signaling (2–42 days poststroke) improves recovery of forepaw function in diabetic mice relative to those treated with saline. Sensorimotor function was tested by measuring the change latency to remove adhesive tape from the stroke-affected forepaw. Images show sample frames of trial initiation and removal of adhesive tape from the forepaws (arrow). $^*P < 0.05$, diabetic saline vs. diabetic SU5416; †$P < 0.05$, ††$P < 0.01$, diabetic saline vs. nondiabetic saline. Modified with permission from Reeson et al. (15). BL, baseline or prestroke tape removal latency.

opening is an ephemeral event, quickly disassociating and reassembling at a rate not captured by electron microscopy, still exists. Regardless of this uncertainty, endothelial transcytosis is a major player in BBB permeability and edema after stroke, particularly for animals with diabetes.

ABERRANT VEGF SIGNALING AND VASCULAR DYSFUNCTION AFTER STROKE

As described previously herein, cerebral blood flow and BBB function are highly dynamic after stroke, and diabetes adds to this complexity, involving many molecular factors that go beyond the scope of this review, such as inflammatory cytokines, matrix metalloproteases, and angiopoetin signaling (4,8,50). Briefly, we focus on the contribution of vascular endothelial growth factor (VEGF), which is an established regulator of vasculature structure and function (51). Several different VEGF ligands exist of which VEGF-A is the predominant variant (referred to as VEGF). VEGF binds to three receptor tyrosine kinases, VEGF receptor (R) 1, 2, and 3 (51). Although the full role of VEGF-R1 and -R3 remains an enigma, VEGF-R2 is known to mediate most of the effects of VEGF signaling (52). VEGF-R2 signaling is the major driver of angiogenesis and a survival signal for vessels in developing organs (52). After stroke, upregulation of VEGF-R2 signaling in peri-infarct regions is significant (15,53) and not fully understood, but it could protect vulnerable vessels from cell death or initiate angiogenic processes for restoring

blood flow. Increased VEGF-R2 signaling could also be detrimental by inducing BBB permeability and edema (53).

Two recent articles from independent groups studying a mouse model of T1D found abnormally elevated VEGF signaling after stroke (8,15). First, diabetes significantly amplified the upregulation of VEGF (8) and VEGF-R2 expression (15) on peri-infarct blood vessels compared with controls. This overexpression of VEGF signaling was somewhat delayed, evident 3–7 days after stroke, in the same region where enhanced BBB permeability was observed. Inhibiting VEGF-R2 signaling in diabetic mice with a clinically tested cancer drug (SU5416) or through an inducible gene knockdown in vascular endothelial cells (15) greatly reduced BBB permeability (Fig. 3*A*, right panel). Ameliorating BBB disruption in diabetic mice also significantly reduced the loss of synapses in peri-infarct cortical neurons and improved recovery of the stroke-affected limb (Fig. 3*D*). One unexpected finding from the VEGF-R2 inhibitor experiments was that the treatment benefit was only evident in diabetic mice because nondiabetic mice did not show improvements in BBB integrity, synapse density, or behavioral recovery (Fig. 3*A* and *D*). Collectively, these findings highlight the importance of finely balanced cell signaling after stroke, where too much VEGF signaling, as in the case of diabetic animals, exacerbates vascular dysfunction after stroke. Furthermore, the development of new stroke therapies cannot take a one-size-fits-all approach because comorbidities such as diabetes can dictate the efficacy of a particular treatment.

CONCLUSIONS AND FUTURE DIRECTIONS

Although the debate continues about the mechanisms by which hyperglycemia affects cell death in the ischemic core, the influence of diabetes extending beyond this region is now well established. This influence is perhaps most pronounced in surviving peri-infarct regions, which are critical for recovery of function (21). As illustrated in Fig. 4, T1D is associated with abnormal peri-infarct blood flow in both acute and chronic stages of stroke recovery, potentially reducing the efficiency of oxygen and nutrient delivery to surviving neurons (16). Concurrently, diabetes exacerbates the disruption of the BBB (8,15). A greater loss of BBB integrity is mediated primarily by an increase in endothelial transcytosis, and this further amplifies injury to peri-infarct neurons by stripping them of their synaptic connections. At a molecular level, our studies and those of others have indicated that aberrant VEGF signaling plays a central role in vascular pathology (8,15). Insulin treatment immediately after stroke was often insufficient to improve or normalize patterns of blood flow or BBB integrity in the diabetic brain (15,16). This unfortunate result suggests that better glucose control is not the only answer to improving stroke recovery, and combinatorial treatments will need to be considered in the future. Furthermore, it implies that hyperglycemia, even for relatively brief periods of time (on the order of days to weeks) could fundamentally alter the intracellular signaling milieu in the vascular endothelium. These signaling changes, some of which likely manifest at the epigenetic

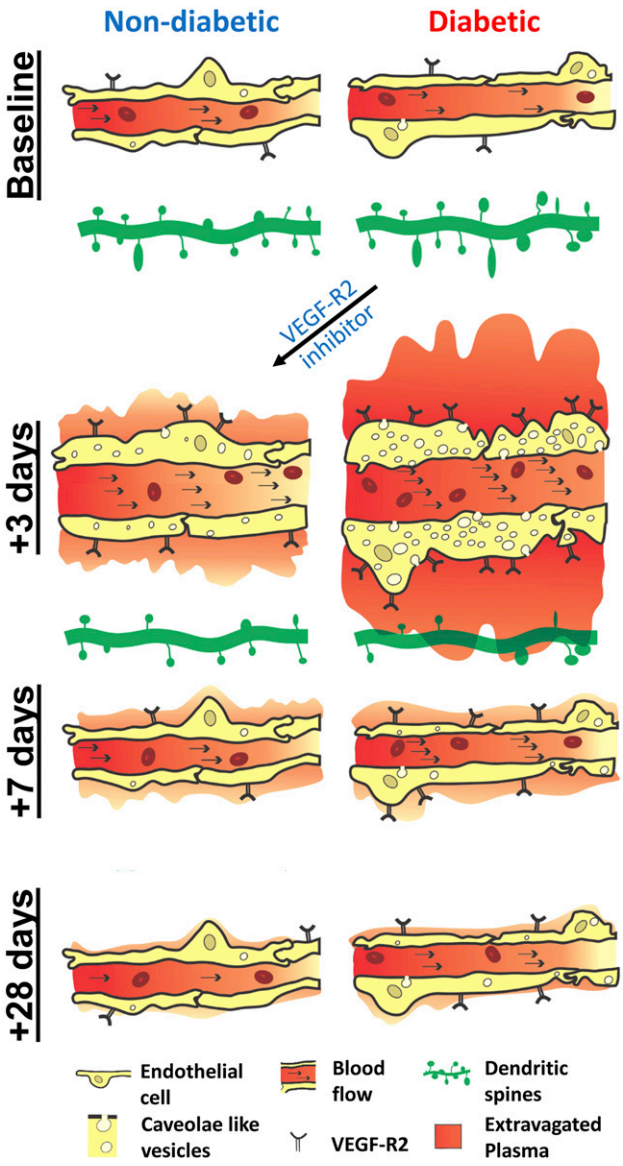

Figure 4—Summary of changes to peri-infarct blood vessels and dendritic structure after stroke. At baseline (before stroke), no significant differences were seen between diabetic and nondiabetic mice in blood flow velocity, vessel width, VEGF-R2 expression, or neuronal dendritic spine densities. Three days after stroke, microvessels in the nondiabetic peri-infarct cortex were dilated, with blood flow and VEGF-R2 expression increased. The endothelium was swollen and packed with caveola-like vesicles, which coincided with extravagation of blood-borne dyes through the BBB. In the diabetic peri-infarct cortex, vessels were also dilated, but blood flow was significantly increased and VEGF-R2 overexpressed compared with nondiabetic mice. Diabetic mice exhibited much greater disruption of the BBB (note extravagation of plasma) and loss of dendritic spines. Treatment with a VEGF-R2 inhibitor (arrow) attenuated the disruption of the BBB and loss of dendritic spines in diabetic mice. Seven to 28 days after stroke, VEGF-R2 expression and BBB permeability gradually returned to baseline levels, although blood flow in diabetic mice remained abnormally high during this time frame.

level, dictate how well the vascular networks adapt or not to an ischemic event.

Because the goal of preclinical research is to use animal models to first dissect the neurobiological mechanisms that mediate or explain a certain phenomenon and then exploit this knowledge to improve treatment options, the clinical realities of stroke and diabetes are important to keep in mind. These conditions, much like the experimental models used to study them, are highly heterogeneous. Therefore, more work is needed to understand how various models of stroke (e.g., embolic vs. hemorrhagic) affect recovery in patients with diabetes because each have a unique effect. Although this review focuses primarily on the STZ model of T1D, other genetic models in other species should be considered in future study. For example, NOD mice frequently are used in the study of diabetes pathophysiology in other organ systems (54), but little has been done with respect to stroke recovery. Even with the STZ model, we still do not know exactly what duration or magnitude of hyperglycemia can lead to poor stroke outcome. Thus, many important unresolved questions remain. Finally, we are encouraged by the development of new diagnostic tools for imaging vascular dysfunction in patients with stroke. Advanced imaging techniques like MRI are now capable of monitoring BBB permeability in patients with stroke. Before new BBB-targeting therapies can be responsibly applied in humans, we need to map the exact timing, location, and magnitude of BBB disruption in human patients with diabetes recovering from stroke. In conclusion, we are optimistic that the combined insights from imaging studies in humans and animal models will eventually provide a sufficiently nuanced and detailed roadmap for when, where, and how to intervene after stroke, with the ultimate goal of minimizing the burden of disability in the diabetic population.

Funding. This work was supported by operating, salary, and equipment grants to C.E.B. from the Canadian Institutes of Health Research, Heart and Stroke Foundation of British Columbia and Yukon, Michael Smith Foundation for Health Research, Natural Sciences and Engineering Council of Canada, and Canada Foundation for Innovation.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Baird TA, Parsons MW, Barber PA, et al. The influence of diabetes mellitus and hyperglycaemia on stroke incidence and outcome. J Clin Neurosci 2002;9:618–626
2. Megherbi SE, Milan C, Minier D, et al.; European BIOMED Study of Stroke Care Group. Association between diabetes and stroke subtype on survival and functional outcome 3 months after stroke: data from the European BIOMED Stroke Project. Stroke 2003;34:688–694
3. Kamouchi M, Matsuki T, Hata J, et al.; FSR Investigators. Prestroke glycemic control is associated with the functional outcome in acute ischemic stroke: the Fukuoka Stroke Registry. Stroke 2011;42:2788–2794
4. Ergul A, Abdelsaid M, Fouda AY, Fagan SC. Cerebral neovascularization in diabetes: implications for stroke recovery and beyond. J Cereb Blood Flow Metab 2014;34:553–563
5. Ergul A, Kelly-Cobbs A, Abdalla M, Fagan SC. Cerebrovascular complications of diabetes: focus on stroke. Endocr Metab Immune Disord Drug Targets 2012;12:148–158
6. Wei JW, Heeley EL, Wang JG, et al.; ChinaQUEST Investigators. Comparison of recovery patterns and prognostic indicators for ischemic and hemorrhagic stroke in China: the ChinaQUEST (QUality Evaluation of Stroke Care and Treatment) Registry study. Stroke 2010;41:1877–1883
7. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science 1976;193:415–417
8. Poittevin M, Bonnin P, Pimpie C, et al. Diabetic microangiopathy: impact of impaired cerebral vasoreactivity and delayed angiogenesis after permanent middle cerebral artery occlusion on stroke damage and cerebral repair in mice. Diabetes 2015;64:999–1010
9. Sweetnam D, Holmes A, Tennant KA, et al. Diabetes impairs cortical plasticity and functional recovery following ischemic stroke. J Neurosci 2012; 32:5132–5143
10. Ye X, Chopp M, Cui X, et al. Niaspan enhances vascular remodeling after stroke in type 1 diabetic rats. Exp Neurol 2011;232:299–308
11. Warner DS, Gionet TX, Todd MM, McAllister AM. Insulin-induced normoglycemia improves ischemic outcome in hyperglycemic rats. Stroke 1992;23: 1775–1780; discussion 1781
12. MacDougall NJ, Muir KW. Hyperglycaemia and infarct size in animal models of middle cerebral artery occlusion: systematic review and meta-analysis. J Cereb Blood Flow Metab 2011;31:807–818
13. Duverger D, MacKenzie ET. The quantification of cerebral infarction following focal ischemia in the rat: influence of strain, arterial pressure, blood glucose concentration, and age. J Cereb Blood Flow Metab 1988;8:449–461
14. Slivka AP. Hypertension and hyperglycemia in experimental stroke. Brain Res 1991;562:66–70
15. Reeson P, Tennant KA, Gerrow K, et al. Delayed inhibition of VEGF signaling after stroke attenuates blood-brain barrier breakdown and improves functional recovery in a comorbidity-dependent manner. J Neurosci 2015;35: 5128–5143
16. Tennant KA, Brown CE. Diabetes augments in vivo microvascular blood flow dynamics after stroke. J Neurosci 2013;33:19194–19204
17. Li W, Kelly-Cobbs AI, Mezzetti EM, Fagan SC, Ergul A. Endothelin-1-mediated cerebrovascular remodeling is not associated with increased ischemic brain injury in diabetes. Can J Physiol Pharmacol 2010;88:788–795
18. Vannucci SJ, Willing LB, Goto S, et al. Experimental stroke in the female diabetic, db/db, mouse. J Cereb Blood Flow Metab 2001;21:52–60
19. Muranyi M, Fujioka M, He Q, et al. Diabetes activates cell death pathway after transient focal cerebral ischemia. Diabetes 2003;52:481–486
20. Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. Ann Neurol 2006;59:735–742
21. Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci 2009;10:861–872
22. Brown CE, Aminoltejari K, Erb H, Winship IR, Murphy TH. In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J Neurosci 2009;29:1719–1734
23. Yan T, Chopp M, Ye X, et al. Niaspan increases axonal remodeling after stroke in type 1 diabetes rats. Neurobiol Dis 2012;46:157–164
24. Zhang T, Pan BS, Sun GC, Sun X, Sun FY. Diabetes synergistically exacerbates poststroke dementia and tau abnormality in brain. Neurochem Int 2010; 56:955–961
25. Zhang T, Liu X, Li Q, Wang J, Jia W, Sun X. Exacerbation of ischemia-induced amyloid-beta generation by diabetes is associated with autophagy activation in mice brain. Neurosci Lett 2010;479:215–220
26. Brands AM, Biessels GJ, de Haan EH, Kappelle LJ, Kessels RP. The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 2005;28:726–735

27. Manschot SM, Brands AM, van der Grond J, et al.; Utrecht Diabetic Encephalopathy Study Group. Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. Diabetes 2006;55:1106–1113
28. Jing XH, Chen SL, Shi H, Cai H, Jin ZG. Electroacupuncture restores learning and memory impairment induced by both diabetes mellitus and cerebral ischemia in rats. Neurosci Lett 2008;443:193–198
29. Carmichael ST, Tatsukawa K, Katsman D, Tsuyuguchi N, Kornblum HI. Evolution of diaschisis in a focal stroke model. Stroke 2004;35:758–763
30. Silasi G, Murphy TH. Stroke and the connectome: how connectivity guides therapeutic intervention. Neuron 2014;83:1354–1368
31. Zhang S, Boyd J, Delaney K, Murphy TH. Rapid reversible changes in dendritic spine structure in vivo gated by the degree of ischemia. J Neurosci 2005;25:5333–5338
32. Schrandt CJ, Kazmi SM, Jones TA, Dunn AK. Chronic monitoring of vascular progression after ischemic stroke using multiexposure speckle imaging and two-photon fluorescence microscopy. J Cereb Blood Flow Metab 2015;35:933–942
33. Mostany R, Chowdhury TG, Johnston DG, Portonovo SA, Carmichael ST, Portera-Cailliau C. Local hemodynamics dictate long-term dendritic plasticity in peri-infarct cortex. J Neurosci 2010;30:14116–14126
34. Shih AY, Friedman B, Drew PJ, Tsai PS, Lyden PD, Kleinfeld D. Active dilation of penetrating arterioles restores red blood cell flux to penumbral neocortex after focal stroke. J Cereb Blood Flow Metab 2009;29:738–751
35. Huang JY, Li LT, Wang H, et al. In vivo two-photon fluorescence microscopy reveals disturbed cerebral capillary blood flow and increased susceptibility to ischemic insults in diabetic mice. CNS Neurosci Ther 2014;20:816–822
36. Akamatsu Y, Nishijima Y, Lee CC, et al. Impaired leptomeningeal collateral flow contributes to the poor outcome following experimental stroke in the type 2 diabetic mice. J Neurosci 2015;35:3851–3864
37. Kittaka M, Wang L, Sun N, et al. Brain capillary tissue plasminogen activator in a diabetes stroke model. Stroke 1996;27:712–719
38. Li W, Prakash R, Kelly-Cobbs AI, et al. Adaptive cerebral neovascularization in a model of type 2 diabetes: relevance to focal cerebral ischemia. Diabetes 2010;59:228–235
39. Jespersen SN, Østergaard L. The roles of cerebral blood flow, capillary transit time heterogeneity, and oxygen tension in brain oxygenation and metabolism. J Cereb Blood Flow Metab 2012;32:264–277
40. Prakash R, Li W, Qu Z, Johnson MA, Fagan SC, Ergul A. Vascularization pattern after ischemic stroke is different in control versus diabetic rats: relevance to stroke recovery. Stroke 2013;44:2875–2882
41. Harb R, Whiteus C, Freitas C, Grutzendler J. In vivo imaging of cerebral microvascular plasticity from birth to death. J Cereb Blood Flow Metab 2013;33:146–156
42. Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. J Neurosci 2006;26:13007–13016
43. Daneman R. The blood-brain barrier in health and disease. Ann Neurol 2012;72:648–672
44. del Zoppo GJ, Mabuchi T. Cerebral microvessel responses to focal ischemia. J Cereb Blood Flow Metab 2003;23:879–894
45. Banks WA, Jaspan JB, Kastin AJ. Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. Peptides 1997;18:1577–1584
46. Sandoval KE, Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. Neurobiol Dis 2008;32:200–219
47. Mark KS, Davis TP. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. Am J Physiol Heart Circ Physiol 2002;282:H1485–H1494
48. Krueger M, Härtig W, Reichenbach A, Bechmann I, Michalski D. Blood-brain barrier breakdown after embolic stroke in rats occurs without ultrastructural evidence for disrupting tight junctions. PLoS One 2013;8:e56419
49. Nahirney PC, Reeson P, Brown CE. Ultrastructural analysis of blood-brain barrier breakdown in the peri-infarct zone in young adult and aged mice. J Cereb Blood Flow Metab 2016;36:413–425
50. Kumari R, Willing LB, Krady JK, Vannucci SJ, Simpson IA. Impaired wound healing after cerebral hypoxia-ischemia in the diabetic mouse. J Cereb Blood Flow Metab 2007;27:710–718
51. Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–676
52. Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P. Role and therapeutic potential of VEGF in the nervous system. Physiol Rev 2009;89:607–648
53. Hermann DM, Zechariah A. Implications of vascular endothelial growth factor for postischemic neurovascular remodeling. J Cereb Blood Flow Metab 2009;29:1620–1643
54. von Herrath M, Nepom GT. Animal models of human type 1 diabetes. Nat Immunol 2009;10:129–132