Hyperglycemia, Insulin, and Acute Ischemic Stroke
A Mechanistic Justification for a Trial of Insulin Infusion Therapy

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Background and Purpose—Hyperglycemia is associated with increased mortality and morbidity in acute ischemic stroke. Hyperglycemia induces a pro-oxidative and proinflammatory state that can cause direct neuronal toxicity. Hyperglycemia-mediated increase in matrix metalloproteinase-9 can cause neuronal damage by an increase in cerebral edema. Moreover, hyperglycemia may be responsible for a procoagulant state that can further compromise blood supply to the penumbral areas in acute ischemic stroke. Insulin infusion has an effect that is opposite to that of hyperglycemia. It not only lowers blood glucose levels but also exerts an antioxidant and anti-inflammatory effect. Insulin also improves NO production and results in improved blood circulation to the ischemic areas. This article focuses on the potential mechanisms underlying the injurious effects of glucose and the beneficial effects of insulin.

Conclusions—In the absence of other potential beneficial therapies, there is an urgency to institute trials with insulin infusion in acute ischemic stroke. (Stroke. 2006;37:000-000.)

Key Words: hyperglycemia • insulin • inflammation • stroke

Hyperglycemia without pre-existing diabetes mellitus has been recognized in acute stroke for a long time.1,2 Whereas diabetes mellitus is clearly a risk factor for the occurrence of stroke1 and for its poor prognosis,3 hyperglycemia without pre-existing diabetes mellitus is also linked to increased mortality and morbidity in stroke patients.2 However, there is no consensus on whether hyperglycemia per se is the cause of poor stroke outcomes.3 Results of the recent clinical trials on insulin infusion therapy in other acute conditions such as myocardial infarction6,7 and critically ill patients8 have rekindled interest in the possible therapeutic efficacy of insulin in hyperglycemia associated with acute stroke. Although insulin lowers blood glucose levels, it has other important effects that may influence the underlying pathogenic mechanisms in stroke. This article reviews the proposed mechanisms underlying adverse outcomes of ischemic stroke with hyperglycemia and possible effects of insulin infusion in improving stroke outcomes. Although hyperglycemia is relevant in intracerebral hemorrhage,9 the pathogenic mechanisms leading to intracerebral hemorrhage are different from those leading to ischemic stroke, and intracerebral hemorrhage is not a focus of this review.

Hyperglycemia and Stroke Outcome
In a retrospective study in 1976, Melamed showed that hyperglycemia was present in 28% of patients with stroke in the absence of a previous history of diabetes.1 The incidence and degree of hyperglycemia were related to the severity of acute stroke and hospital mortality was significantly higher in hyperglycemic patients.1 Since then, the association between hyperglycemia and stroke outcome has been published in many more studies and reviewed in a meta-analysis by Capes et al.2 This meta-analysis found that the relative risk of death after ischemic stroke with admission blood glucose levels >6.1 to 7.0 mmol/L (110 to 126 mg/dL) was 3.28 (95% CI, 2.32 to 4.64).2 There was also a greater risk of poor functional recovery in the hyperglycemic patients.2 Several recent studies in patients treated with thrombolytic therapy for acute ischemic stroke have again demonstrated a profound effect of hyperglycemia on stroke outcomes10–15 (Table). Hyperglycemic effects are more prominent in nonlacunar stroke than lacunar stroke. In a large study involving 1259 patients with acute ischemic stroke, hyperglycemia was associated with worse clinical outcomes only in nonlacunar stroke.16 Although in some other studies, the investigators concluded that hyperglycemia was simply a stress response to acute stroke,17,18 poor clinical outcome in hyperglycemic patients is quite evident.

Etiology of Hyperglycemia in Acute Stroke
Hyperglycemia after acute stroke may be attributable to several underlying mechanisms. These include: a nonspecific reaction to acute stress; autonomic, hormonal, and metabolic alterations as a result of tissue injury; uncovering of underlying latent diabetes by the acute stroke; activation of the hypothalamo-hypophyseal-adrenal axis attributable to a di-
Mechanisms of Hyperglycemia-Mediated Brain Damage

The possible mechanisms of glucose-mediated increase in cerebral infarct size include poor blood flow to the ischemic penumbra;20,21 changes in cerebral metabolism;22 increase in N-methyl-D-aspartate (NMDA) receptor–mediated calcium entry into the neurons;23 increased local edema;24 and, most important, glucose-mediated increase in oxidative stress and inflammation.25,26 These mechanisms may well be interrelated.

Hyperglycemia-Associated Reduction in Perfusion

In experimental rats, the injection of intraperitoneal glucose to produce hyperglycemia during induction of ischemia of the brain was associated with a 24% reduction in regional blood flow, whereas injection of D-mannitol to produce an equivalent elevation of plasma osmolality reduced cerebral blood flow by only 10% when compared with controls that received normal saline.20 Additionally, hyperglycemia is seen to cause reduction in blood circulation to the marginal ischemic areas after occlusion of the middle cerebral artery.21 This suggests that penumbra around the infarct area converts to infarct in hyperglycemia. This phenomenon is analogous to the defect in cerebral blood flow in diabetics. CO2-induced increase in cerebral blood flow is decreased in diabetics.27 CO2-induced cerebral vasodilatation is mediated through NO, and diabetics are known to have decreased endothelial NO production. Moreover, glucose-induced reactive oxygen species (ROS) can neutralize NO in the vessel wall (Figure 1). Hyperglycemia-induced reduction in cerebral blood flow may be mediated through neutralization of NO or its impaired production.

Studies on the Effect of Hyperglycemia in Acute Stroke Patients Treated With Thrombolytic Therapy

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<tr>
<td>Leigh et al10</td>
<td>201</td>
<td>8.3 mmol/L at admission</td>
<td>Change in NIHSS at 24 h &amp; 4 = good outcome; &amp; 4 = poor outcome</td>
<td>OR for hyperglycemia in poor outcome compared with good outcome = 5.67 BGT in good outcome: 138 mg/dL and in poor outcome: 173 mg/dL</td>
<td>0.009 0.024</td>
<td>TT within 6 h</td>
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<td>Saposnik et al11</td>
<td>212</td>
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<td>0.003 0.003</td>
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<td>Alvarez-Sabin et al12</td>
<td>73</td>
<td>7.7 mmol/L at admission</td>
<td>Change in NIHSS at 24 h mRS at 3 mo</td>
<td>Correlation between NIHSS and BGT in patients with successful reperfusion: ( r = -0.43 ) OR for poor outcome (mRS 3–6) at 3 months for hyperglycemic reperfused group = 8.4</td>
<td>0.019 0.005</td>
<td>TT in 3 h</td>
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<tr>
<td>Bruno et al15</td>
<td>624</td>
<td>Admission BGT</td>
<td>NIHSS change of &amp; 4 at 3 mo</td>
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<td>Infarct size by diffusion-weighted MRI at admission, day 3 and day 7 NIHSS at 28 days</td>
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<td>&lt;0.05 &lt;0.05</td>
<td>TT in 3 h</td>
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TT indicates thrombolytic therapy; BGT, blood glucose test; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; mRS, modified Rankin scale.

rect effect of brain ischemia on the pituitary, and irritation of the glucose regulatory centers in the brain by a stroke. By far, the most popular belief is that stroke related hyperglycemia is a stress response with activation of the hypothalamo-hypophyseal-adrenal axis, which leads to an increase in cortisol and catecholamines. According to this simple explanation, the poor stroke outcome in patients with hyperglycemia may be because more severe stroke induces higher levels of catecholamines and corticosteroids and represents an epiphenomenon associated with a poor outcome rather than having any causal relationship.18 However, data have not been consistent on the relationship between hyperglycemia and stress hormones in stroke; 1 study showed that hyperglycemia may be a stress response,17 whereas another showed an absence of such an association.19 Hyperglycemia in acute stroke is probably the result of multiple factors, including cytokine-induced resistance to insulin action.
Hyperglycemia-Associated Metabolic Alteration
Changes in cerebral metabolism may be the other mechanism of increased cerebral damage by hyperglycemia. Hyperglycemic cats had significantly reduced cerebral high-energy phosphates, elevated lactic acid, and larger ischemic lesions in the occluded middle cerebral artery territory. Hypometabolism of the ischemic focus is seen early and extends more into the penumbra in hyperglycemic animals when compared with normoglycemic animals. In a study on humans, cerebral hypometabolism assessed by positron emission tomography in acute ischemic cerebral infarction was more severe in patients with glucose concentrations >6.7 mmol/L. Hypometabolism may be related to lactic acid accumulation leading to mitochondrial dysfunction in the ischemic tissue. Hyperglycemia during cerebral ischemia leads to higher lactic acid accumulation in the ischemic tissue because the tissue glucose levels get depleted during ischemia in the normoglycemic state. Hyperglycemia may also directly affect mitochondrial function in the ischemic penumbra and cause significant intracellular brain acidosis. Cortical acidosis leads to the recruitment of the ischemic penumbra into the infarct zone.

Hyperglycemia-Associated Impaired Calcium Homeostasis
Excitatory amino acids, notably glutamate, play a central role in neuronal death by activation of postsynaptic glutamate receptors, particularly NMDA receptors. This activation leads to an excessive influx of calcium through ion channels, mitochondrial injury, and eventual cell death. It has been shown that the rise in extracellular glutamate concentrations after forebrain ischemia was more pronounced in hyperglycemic rats than in normoglycemic animals. The difference was observed in the neocortical regions of the brain and correlated with increased cell damage. Thus, hyperglycemia, by increasing the availability of glutamate, may induce calcium-mediated neuronal cell death. Hyperglycemia may also be harmful to calcium recovery during the early perfusion period after focal cerebral ischemia, thereby increasing intracellular calcium for a longer time.

Inflammation and Free Radical–Associated Injury
Hyperglycemia is known to be associated with inflammation and oxidative stress (Figure 1). A 75-g glucose challenge has been shown to induce an increase in superoxide generation by leukocytes by 140% over the basal in addition to increasing p47phox expression, a subunit of NADPH oxidase, the enzyme that converts molecular O2 to the superoxide radical. Glucose intake also results in comprehensive inflammation as reflected in an increase in nuclear factor κB (NF-κB) binding and a decrease in inhibitor kappa B (IκB) expression. NF-κB is a nuclear transcription factor that normally stays in the cytoplasm in association with IκB. In response to an inflammatory stimulus, there is an increase in IκB kinase-α and IκB kinase-β, which phosphorylate IκB and result in its ubiquitination and proteosomal degradation. Degradation of IκB results in release of NF-κB and in its translocation from the cytoplasm to the nucleus, where it stimulates the transcription of proinflammatory cytokines. Activation of NF-κB and superoxide generation have been shown to be involved in tissue injury after occlusion of middle cerebral artery. NF-κB activation leads to increased production of...
inflammatory cytokines and chemokines such as tumor necrosis factor-α and monocyte chemoattractant protein (MCP-1). This attracts leukocytes to the ischemic area. Superoxide radicals can cause direct cell damage through lipid peroxidation, protein carbonylation, and DNA damage. Superoxide also neutralizes NO produced by endothelium by converting NO to peroxynitrite. NO is critical in maintenance of blood flow to the ischemic brain tissue by causing vasodilatation of arteries. Glucose intake also causes an increase in 2 other proinflammatory transcription factors: activator protein-1 (AP-1) and early growth response-1 (Egr-1). AP-1 regulates the transcription of matrix metalloproteinases (MMPs), whereas Egr-1 modulates the transcription of tissue factor (TF). Thus, glucose intake increases the expression of MMP-2 and MMP-9 as well as that of TF. MMP-9, also involved in the process of central spreading depression after an acute stroke, plays a significant role in brain damage by increasing brain edema. Central spreading depression is characterized by neuronal and glial depolarization, which is followed 3 to 6 hours later by an increase in the expression of MMP-9 initially in the cortical blood vessels, spreading later to neuronal layers and finally to the pia and the arachnoid. The increase in MMP-9 results in a reduction of laminin, endothelial barrier antigen, and the zona occludens. These 3 proteins are important in the maintenance of blood–brain barrier. The decrease in their concentration affects the integrity of the blood–brain barrier and an increase in the permeability of the barrier, resulting in edema with the leakage of plasma proteins and inflammatory cells. Stroke patients with hyperglycemia indeed develop more pronounced cerebral edema. Glucose-induced increase in TF can activate the extrinsic pathways of coagulation. Plasminogen activator inhibitor-1 (PAI-1), an inhibitor of fibrinolysis, is known to be increased in hyperglycemia. The increase in TF and PAI-1 can worsen ischemic damage by promoting coagulation in local capillaries. Thus, hyperglycemia may increase the cerebral damage by disrupting the microcirculation and upregulating the inflammatory and the related thrombotic/fibrinolytic mechanisms in brain.

**Insulin Treatment of Acute Stroke**

It has been demonstrated in animal models that during acute focal and global ischemia, insulin therapy reduces ischemic brain damage and can be neuroprotective. Whereas insulin lowers the glucose levels and thus reverses the damaging effects of glucose, there is an emerging body of evidence that there may be direct benefits of insulin itself. In a global ischemic model, insulin reduced neuronal necrosis regardless of its effect on glucose levels. In addition, insulin, and to a lesser extent insulin-like growth factor-1, reduced ischemic damage when injected directly into the brain ventricles. Therefore, it has been suggested that insulin has a direct neuroprotective effect on central nervous system parenchyma.

Insulin has recently been shown to possess a potent anti-inflammatory effect in vitro and in vivo (Figure 2). Insulin has been shown to suppress several proinflammatory transcription factors, such as NF-κB, Egr-1, and AP-1, and the corresponding genes regulated by them that mediate inflammation. Insulin has also been shown to suppress ROS generation, p47phox expression in the circulating mononuclear cells, as well as plasma concentrations of intercellular adhesion molecule-1 (ICAM-1) and MCP-1. In addition to its inhibitory effect on AP-1 and Egr-1, insulin suppresses...
their regulated gene products as indicated by a fall in plasma concentration of MMP-9, TF, and PAI-1,\textsuperscript{45–47} an effect diametrically opposite to that of glucose. MMP-9 is involved in the phenomenon of central spreading depression as noted above. MMP-9 null mice have smaller brain infarct volumes after the experimental induction of a stroke.\textsuperscript{38} Thus, clearly, MMP-9 is a cardinal mediator of these effects and a reduction in its activity or expression by insulin could be a rational therapeutic approach in the prevention or the limitation of ischemia-related damage to the brain. Insulin also causes a similar reduction in the plasma concentration of vascular endothelial growth factor (VEGF), a cytokine that induces an increase in the expression of MMP-9.\textsuperscript{47} It has also been shown that VEGF can cause the loss of endothelial cell tight junctions.\textsuperscript{48} It is possible that VEGF and MMP-9 may act in a synergistic fashion to cause a disruption of the blood–brain barrier during ischemia because hypoxia is the major factor inducing an increase in the expression of VEGF.\textsuperscript{49} The fact that insulin suppresses MMP-9 and VEGF, both of which are the mediators of ischemic damage, suggests strongly that it may have a beneficial role in the treatment of an acute stroke. Moreover, insulin-mediated suppression of TF and PAI-1 can produce an anticoagulant effect. High catecholamine levels in the circulation during acute stroke can increase the production of free fatty acids. Free fatty acids decrease the generation and the stability of prostacyclin,\textsuperscript{50} which is important for not only vasodilatation but also for preventing platelet aggregation. Insulin inhibits lipolysis, leading to a decrease in plasma-free fatty acids and thus may exert an antiplatelet and anticoagulant effect. The anti-inflammatory effect of insulin has been confirmed by us in acute myocardial infarction.\textsuperscript{51}

In addition to suppressing the mediators of inflammation and coagulation, insulin has also been shown to increase endothelial NO release and the expression of NO synthase (NOS) expression in the endothelial cells.\textsuperscript{52,53} Insulin was also recently shown to increase the expression of neuronal NOS in astrocytes as well as neurons.\textsuperscript{54} Generation of NO would potentially help in vasodilatation and improved blood flow to the penumbra but also result in decreased production of free fatty acids. Free fatty acids decrease the generation and the stability of prostacyclin,\textsuperscript{50} which is important for not only vasodilatation but also for preventing platelet aggregation. Insulin inhibits lipolysis, leading to a decrease in plasma-free fatty acids and thus may exert an antiplatelet and anticoagulant effect. The anti-inflammatory effect of insulin has been confirmed by us in acute myocardial infarction.\textsuperscript{51}

Conclusions
Hyperglycemia is detrimental in acute stroke, whereas insulin infusion can reverse this effect. Hyperglycemia induces an inflammatory state, whereas insulin has an anti-inflammatory effect. Insulin may help protect the brain not only through its glucose-lowering effect but also through its direct anti-inflammatory effect. Strongest evidence in favor of insulin use has come from the clinical trials in intensive care units and in acute myocardial infarction patients.\textsuperscript{7,4} Insulin infusion can be safely administered to acute stroke patients.\textsuperscript{56,57} In a recent study, intravenous insulin infusion effectively brought down blood glucose from a mean of 14.7±4.9 to 7.3±1.1 mmol/L without any problems.\textsuperscript{57} Although results from controlled clinical trials assessing insulin therapy in patients with stroke are still lacking, the available data favor the need for avoiding hyperglycemia in patients with acute stroke. The ongoing Glucose Insulin in Stroke Trial (GIST)—UK trial\textsuperscript{58} was set up to answer the question whether correction of hyperglycemia is beneficial in acute stroke. The trial started in 1999 with a target to enroll 1200 patients by 2002.\textsuperscript{59} The demonstration of mortality benefit, the primary end point of this trial, requires a large number of patients. However, GIST has been slow in patient recruitment. By the time the results of GIST trial come out, it may no longer be relevant because the control group has a very high target blood glucose level (17 mmol/L or 306 mg/dL), which is unacceptable in the current guidelines for any hospitalized patient. If the primary end points were nonmortality outcomes or cerebral infarct size measured by diffusion perfusion–weighted MRI, much smaller numbers will be needed. A trial of insulin infusion involving 400 patients will have enough power to detect any nonmortality clinical benefits as well as 20% difference in infarct size, assuming a mean infarct size of 15 cm\textsuperscript{2} and an SD of 10. We suggest excluding intracerebral hemorrhage from such a trial and conduct a separate study for this condition. In the absence of potential beneficial therapies for acute ischemic stroke, there is an urgency to institute these trials with a drug that has no side effects other than potential hypoglycemia.

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References


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