Obesity Increases Blood Pressure, Cerebral Vascular Remodeling, and Severity of Stroke in the Zucker Rat

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Abstract—Obesity is a risk factor for stroke, but the mechanisms by which obesity increases stroke risk are unknown. Because microvascular architecture contributes to the outcome of stroke, we hypothesized that middle cerebral arteries (MCAs) from obese Zucker rats (OZRs) undergo inward remodeling and develop increased myogenic tone compared with those in lean Zucker rats (LZRs). We further hypothesized that OZRs have an increased infarct after cerebral ischemia and that changes in vascular structure and function correlate with the development of hypertension in OZRs. Blood pressure was measured by telemetry in LZRs and OZRs from 6 to 17 weeks of age. Vessel structure and function were assessed in isolated MCAs. Stroke damage was assessed after ischemia was induced for 60 minutes followed by 24 hours of reperfusion. Although mean arterial pressure was similar between young rats (6 to 8 weeks old), mean arterial pressure was higher in adult (14 to 17 weeks old) OZRs than in LZRs. MCAs from OZRs had a smaller lumen diameter and increased myogenic vasoconstriction compared with those from LZRs. After ischemia, infarction was 58% larger in OZRs than in LZRs. Before the development of hypertension, MCA myogenic reactivity and lumen diameter, as well as infarct size, were similar between young LZRs and OZRs. Our results indicate that the MCAs of OZRs undergo structural remodeling and that these rats have greater cerebral injury after cerebral ischemia. These cerebrovascular changes correlate with the development of hypertension and suggest that the increased blood pressure may be the major determinant for stroke risk in obese individuals. 

Key Words: obesity ■ stroke ■ vascular remodeling ■ myogenic tone ■ hypertension

Stroke is the third leading cause of death in the United States and the major source of debilitation among adults.1 Obesity is a growing epidemic in the United States and is considered a risk factor for stroke.1 The mechanisms by which obesity affects stroke occurrence or outcome, however, have not been fully elucidated. Hypertension develops in many obese individuals, and according to the Heart Disease and Stroke 2008 Update, hypertension is the greatest risk factor for stroke.1 Although obesity is associated with hypertension and its related end-organ damage, the exact mechanism relating the 2 cardiovascular risk factors is not completely understood. Considering the coincident occurrence of hypertension and stroke risk in obese patients, it is possible that elevated arterial pressure is a major factor in cerebral vascular injury in the obese population.

One potential mechanism by which obesity may increase the risk of stroke is alteration in the perfusion of the cerebral circulation. Cerebral perfusion is regulated through active constrictor and dilator mechanisms and by the physical properties of the cerebral vasculature. Notable determinants of cerebral perfusion that correlate with stroke injury include myogenic tone and vessel structure. Increased myogenic tone has been demonstrated in middle and posterior cerebral arteries from spontaneously hypertensive rats, as well as vasopressin-deficient rats.2–4 Interestingly, Gonzalez et al5 demonstrated that 6-month-old but not 1-month-old spontaneously hypertensive rats have increased resting tone in the middle cerebral artery (MCA) compared with their normotensive counterpart, the Wistar-Kyoto rat. In addition, there is ample evidence of vascular remodeling in the cerebral circulation in several models of hypertension, focusing especially on spontaneously hypertensive rats.6–8 Of importance, however, is the limitation of these studies to models of severe malignant hypertension. Experimental evidence for cerebral vascular remodeling in more moderate forms of hypertension, such as those with obesity-induced hypertension, is lacking.

Common findings in hypertensive populations are changes in the mechanical and architectural properties of the cerebral vasculature, specifically an inward remodeling of the large arteries, such as the MCA.6,9,10 In addition to increasing the risk of flow obstruction, inward remodeling can be detrimental in ischemic conditions. After ischemia, vessels in the brain are near-maximally vasodilated, and, thus, reductions in maximum lumen diameter become a constraint on perfusion. Changes in arterial pressure are well documented to cause deleterious changes in vascular structure; as vessels become stiffer, wall thickness can increase, and lumen diameter can decrease.6,11 Because blood flow during ischemia is directly

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related to maximum lumen diameter, flow will be impaired as lumen diameter is decreased. Whether hypertension is the causal risk factor for alterations in cerebral vascular structure and, subsequently, whether these changes in cerebral vascular structure make up the mechanism of increased stroke risk in the obese population are unknown. Therefore, we tested the hypothesis that obese Zucker rats (OZRs) would have an increased infarct size after cerebral ischemia and demonstrate inward remodeling of the MCA and increased myogenic tone compared with lean Zucker rats (LZRs). We further hypothesized that changes in infarct size, vascular structure, and function would correlate with the development of hypertension in OZR.

Methods

Animals
Six-week-old male LZRs and OZRs were purchased from Harlan and were maintained on a 12-hour light/dark cycle with access to food and water ad libitum. Rats were housed in an American Association for Accreditation of Laboratory Animal Care–accredited facility, and all of the protocols were approved by the institutional animal care and use committee. Rats were considered young at an age of 6 to 7 weeks and adult at 14 to 16 weeks of age.

Telemetry
Rats were implanted with telemetry transmitters (Data Sciences International) in the abdominal aorta during the week before the initiation of the study. Rats were anesthetized using isoflurane in oxygen, and a midline incision was made to expose the abdominal aorta. The aorta was briefly occluded to allow insertion of the transmitter catheter that was secured in place with tissue glue. The transmitter body was sutured to the abdominal wall along the incision line as the incision was closed. The skin was closed with staples that were removed 7 days after surgery. Rats were allowed to recover from surgery and returned to individual housing. Individual rat cages were placed on top of the telemetry receivers, and arterial pressure wave forms were continuously recorded throughout the study.

Euglycemic Insulin Clamp
After an overnight fast, rats were anesthetized with isoflurane (2%) in oxygen and maintained at 37°C on a heating pad. The carotid artery was catheterized for blood sampling, and the jugular and femoral veins were catheterized for infusion of insulin and glucose, respectively. After measuring baseline blood glucose levels, rats were infused with insulin (10 μL/min) via the jugular vein, and blood glucose was measured every 5 minutes for 100 minutes. Although insulin infusion rates are typically adjusted for body weight in this protocol, a constant infusion rate was used in the current study based on a previous demonstration that LZRs and OZRs detected by a euglycemic insulin clamp as hyporesponsive that changes in infarct size, vascular structure, and function would correlate with the development of hypertension in OZR.

MCA Occlusion
Cerebral ischemia was induced by the intraluminal suture model developed by Longa et al. Rats were initially anesthetized with isoflurane in an induction chamber, and anesthesia was maintained with 2% isoflurane in oxygen; body temperature was maintained at 37°C. The skull was exposed by a small incision for the attachment of a laser Doppler flow probe (Perimed) to measure blood flow to the region supplied by the MCA. A midline incision was made to expose the carotid artery. The lingual and thyroid arteries were cannulated, and the external carotid and pterygopalatine arteries were ligated with 6-0 suture. A 3-0 nylon monofilament with a rounded end was inserted into the common carotid artery and was advanced through the internal carotid artery to block blood flow to the MCA, where it branches from the circle of Willis. MCA occlusion was verified by a drop in flow as measured by laser Doppler. After 1 hour of ischemia, the filament was pulled back to allow for reperfusion of the MCA. After 24 hours of reperfusion, rats were anesthetized and decapitated, and the brain was removed, sliced into 2-mm sections, and stained with 2% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich) to assess ischemic damage. Brains were fixed in 2% paraformaldehyde (Sigma-Aldrich), and digital images of brain sections were taken. The percentage of infarction was determined in all of the brain sections using the following equation: hemisphere infarcted = \[\frac{V_L - V_C}{V_C}\]×100, where \(V_L\) is the volume of the lesioned hemisphere, and \(V_C\) is the volume of the control hemisphere.

MCA Reactivity and Structure Assessment
Rats were euthanized with isoflurane and decapitation, and trunk blood was collected for the measurement of plasma insulin levels (ELISA; Alpco Diagnostics and Wako Chemicals). MCAs were dissected from the brain after decapitation and placed in Krebs buffer (contents in mmol/L: 118 NaCl, 23.8 NaHCO\(_3\), 4.7 KCl, 1.2 MgSO\(_4\), 1.18 KH\(_2\)PO\(_4\), 11.1 glucose, and 1.9 CaCl\(_2\)) on ice. Vessels were mounted on glass micropipettes in a pressure myograph (Living Systems Instrumentation) and secured using a nylon suture. Only vessels that held pressure at 80 mm Hg were used for experimentation. The pressure myograph was connected to a pressure servocontroller by a pressure transducer, allowing for pressure to be manipulated under 0-flow conditions. A video dimension analyzer connected to a monitor permitted measurement of lumen and outer diameters. After equilibration at 80 mm Hg in Krebs buffer heated to 37°C and gassed with 21% O\(_2\), 5% CO\(_2\), and 74% N\(_2\), the myogenic response was determined by measuring lumen diameter as pressure was increased by 20 mm Hg increments over a range of intraluminal pressures (0 to 140 mm Hg). The same measurements were made in the absence of calcium to determine passive vessel structure. Vessels were incubated in calcium-free buffer for 1 hour to deplete calcium from the vessels. Complete loss of active tone was verified by confirming the lack of a response in lumen diameter on the addition of papaverine. Comparing the lumen diameters under active and passive conditions, the percentage of myogenic tone was calculated as follows: % myogenic tone = \[1 - \frac{(ID_A / ID_P)}{100}\], where \(ID_A\) is inner diameter under active conditions, and \(ID_P\) is inner diameter under passive conditions.

Statistics
Physiological parameters, glucose infusion rates, and cerebral infarct size were compared between age-matched LZRs and OZRs using a Student \(t\) test. A 2-way ANOVA was used to compare lumen diameter and myogenic tone between LZRs and OZRs. A \(P\) value <0.05 was considered statistically significant. Values are presented as means±SEMs.

Results
The Table provides comparisons of baseline physiological parameters between LZRs and OZRs. Body weight was dramatically increased in adult OZRs compared with LZRs. Importantly, there was evidence of increased body weight in OZRs as early as 6 to 7 weeks of age; however, as indicated by the percentage of HbA1c, there was no difference in the glycemic load between adult LZRs and OZRs. The majority of HbA1c measurements in young rats yielded results that were lower than the detection of the kit. Therefore, these values cannot be reported. Plasma insulin levels were increased in both young and adult OZRs compared with age-matched LZRs, indicating that, even at a young age, OZRs demonstrate evidence of insulin resistance (IR). Furthermore, there is a difference in insulin sensitivity between LZRs and OZRs detected by an euglycemic insulin clamp as
early as 6 to 7 weeks of age (Figure 1). Although OZRs become more severely insulin resistant at an age beyond the scope of the current study, it is important to note that young and adult OZRs exhibit a similar degree of IR based on the insulin clamp data. Mean arterial pressure (MAP) was significantly elevated in adult OZRs; however, MAP was similar between young LZRs and OZRs. The time course of the increased blood pressure in OZRs is shown in Figure 2. Therefore, young OZRs represent a normotensive model of obesity and IR, whereas adult OZRs are obese, insulin resistant, and mildly hypertensive.

The response to cerebral ischemia was compared between adult male OZRs and LZRs using the MCA occlusion technique described by Longa et al. Rats were exposed to 1 hour of ischemia, followed by 24 hours of reperfusion. OZRs had a significantly larger infarct size compared with LZRs (10.6 ± 2.0% versus 27.4 ± 5.2% hemisphere infarcted, LZRs versus OZRs; *P<0.05), as indicated by the percentage of infarction in Figure 3. Laser Doppler flowmetry was used to confirm that the degree of occlusion was similar between the 2 groups (58.4 ± 4.6% versus 57.5 ± 5.2% change in cerebral blood flow, LZRs versus OZRs).

Alterations in myogenic tone and MCA structure were assessed to determine whether these important contributors to cerebral blood flow were altered in obesity. The percentage of myogenic tone was calculated from active and passive pressure response curves that were produced using isolated, pressurized vessels of adult male OZRs and LZRs, as described above. Myogenic tone was significantly increased in vessels from OZRs from 80 to 120 mm Hg compared with those from LZRs (Figure 4A). In addition, OZRs demonstrated a smaller lumen diameter over the entire range of pressures measured compared with LZRs (Figure 4B). There was also a decrease in the outer diameter in adult OZRs and a small but statistically significant decrease in wall thickness (data not shown). These findings suggest that MCAs from OZRs undergo an inward remodeling.

To determine whether infarct size, myogenic tone, and MCA structure in OZRs are associated with blood pressure, these parameters were assessed in young rats before the increase in MAP. As shown in Figure 5, after 1 hour of MCA occlusion and 24 hours of reperfusion, infarct size was similar between young LZRs and OZRs (7.5 ± 1.5% versus 8.3 ± 1.8% hemisphere infarcted, LZRs versus OZRs).
addition, myogenic tone was similar or decreased in MCAs from young OZRs, and there was no evidence of inward remodeling of the MCA according to measurements made in isolated vessels (Figure 6). These results indicate that deleterious changes in the cerebral circulation of adult OZRs are not present in young OZRs before the onset of hypertension, despite obesity and IR.

Discussion

The goal of the current study was to test the hypothesis that obesity and hypertension in the Zucker rat are associated with changes in cerebral vascular function and structure and increased stroke-induced injury. The key findings of this study are that adult OZRs with moderate hypertension and severe IR present with increased cerebral vascular myogenic tone, inward cerebral vascular remodeling, and increased cerebral tissue death after ischemia/reperfusion injury. Moreover, juvenile OZRs, obese and insulin resistant but not yet hypertensive, show none of these deficits. These findings indicate that prolonged, moderate hypertension, as evident in the adult OZRs, is a risk factor for cerebral vascular dysfunction and stroke. Relevant to these observations are the caveats of the model and the methods, effects of obesity on the cerebral circulation, and the role of hypertension in stroke.

OZRs have been widely used to investigate the cardiovascular effects of obesity and IR. The novel aspect of the current study, however, is that OZRs demonstrate increased susceptibility to cerebral ischemia/reperfusion injury. Other rodent models of obesity have also implicated that obesity worsens the response to ischemia. A recent study by Terao et al. demonstrated that leptin deficient ob/ob mice have a larger infarct size after MCA occlusion with an intraluminal suture than their littermate controls. In addition, photochemically induced thrombosis in the MCA resulted in increased stroke damage in ob/ob mice and diet-induced obese mice compared with controls. One caveat of the current study is that blood gases were not measured during ischemia. Although differential regulation of blood gases and pH cannot
be ruled out as an explanation of differences in stroke outcome, it should be noted that anesthesia during the ischemic period in the current study was brief; rats were conscious and breathing normally during the majority of the ischemic period, because rats were allowed to wake up during ischemia and during the entire 24-hour reperfusion period.

It is important to consider that, although the cause of obesity in the OZRs is not common among humans, the phenotype parallels human obesity in many ways. These rats have increased triglycerides, cholesterol, and insulin and eventually develop diabetes mellitus. It must be noted, however, that OZRs are morbidly obese, and there is evidence that stroke occurrence is directly related to body mass index. A prospective study by Kurth et al. reported that there is a significant increase in the incidence of total stroke, both ischemic and hemorrhagic, with each unit increase in body mass index, even after correcting for hypertension and diabetes mellitus.

The impact of obesity and it is associated risk factors on the cerebral vasculature is incompletely understood. Several studies have reported that adult OZRs have impairments in endothelium-dependent vasodilation of many vascular beds, including the cerebral circulation. In addition to reporting endothelial dysfunction of the MCA, Phillips et al. described that OZRs display evidence of increased constriction to 5-hydroxytryptamine and an increased myogenic response and increased myogenic tone at baseline pressures. These results are consistent with our findings of increased myogenic tone in OZRs over a range of intraluminal pressures. Contrary to what is reported here, Stepp et al. described remodeling of the hindlimb but not the cerebral circulation of OZRs. The reason for this discrepancy is unclear, but the lumen diameter measurements from Stepp et al. show a trend toward decreased diameter with a smaller sample size. Another novel aspect of the current study is the assessment of cerebrovascular structure and function and the response to cerebral ischemia in young OZRs.

Clinical and statistical evidence indicate that hypertension is the single greatest risk factor for stroke. A relationship between blood pressure and stroke has been clearly defined in experimental models; however, the majority of studies examining the effect of blood pressure on stroke have been in malignant hypertensive models, such as the spontaneously hypertensive rat. Less clear is the effect of a more moderate increase in pressure. The temporal correlation between blood pressure and ischemic injury, as well as alterations in cerebrovascular structure and tone, presented in the current study suggest that even a modest increase in pressure over time in the context of obesity may be detrimental to the cerebrovasculature and worsen the response to cerebral ischemia.

One caveat to consider is that hypertension may be necessary for the cerebrovascular complications in obesity but not sufficient in itself to drive the increased vascular and stroke injury. The extent to which other risk factors present in obesity, such as IR, amplify the role of increased arterial pressure in obesity remains to be determined. The results from the euglycemic hyperinsulinemic clamp experiments suggest that the extent of IR in young and adult OZRs is similar. Thus, a worsening of IR cannot explain the observed changes in the cerebrovasculature of OZR. Although IR is not worse in adult OZRs than in young OZRs, the importance and contribution of long-term sustained IR in this model is not clear. Moreover, metabolic perturbations, such as hyperinsulinemia and changes in plasma lipids, may have similar long-term consequences on the severity of stroke. The potential interaction of metabolic and hemodynamic factors in cerebral vascular function in obesity is an important area for future study.

**Perspectives**

This study further underscores the relationship between obesity and stroke. Furthermore, it provides new evidence that obesity is a risk factor for pathological alterations in the cerebral vasculature. Our results suggest that a chronic, moderate increase in blood pressure may be a major contributor to increased stroke risk and injury in the obese population. Additional studies are required to determine the exact role of obesity-induced hypertension in the pathology of stroke, as well as the contribution of other metabolic factors, such as chronic IR.

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**Disclosures**

None.

**References**

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