

Adipose proinflammatory cytokine expression through sympathetic system is associated with hyperglycemia and insulin resistance in a rat ischemic stroke model

Ya-Yu Wang,^{1,2,3} Shih-Yi Lin,^{3,4} Yu-Han Chuang,⁴ Chun-Jung Chen,⁵ Kwong-Chung Tung,² and Wayne Huey-Herng Sheu^{3,4,6}

¹Division of Family Medicine, Taichung Veterans General Hospital, and ²Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung; ³School of Medicine, National Yang Ming University, Taipei; and ⁴Division of Endocrinology and Metabolism, and Departments of ⁵Medical Research and ⁶Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

Submitted 24 May 2010; accepted in final form 23 October 2010

Wang YY, Lin SY, Chuang YH, Chen CJ, Tung KC, Sheu WH. Adipose proinflammatory cytokine expression through sympathetic system is associated with hyperglycemia and insulin resistance in a rat ischemic stroke model. *Am J Physiol Endocrinol Metab* 300: E155–E163, 2011. First published October 26, 2010; doi:10.1152/ajpendo.00301.2010.—Patients who experience acute ischemic stroke may develop hyperglycemia, even in the absence of diabetes, but the exact mechanisms are still unclear. Adipose tissue secretes numerous proinflammatory cytokines and is involved in the regulation of glucose metabolism. This study aimed to determine the effects of acute stroke on adipose inflammatory cytokine expression. In addition, because sympathetic activity is activated after acute stroke and catecholamines can regulate the expression of several adipocytokines, this study also evaluated whether alterations in adipose proinflammatory cytokines following acute stroke, if any, were mediated by sympathetic system. Acute ischemic brain injury was induced by ligating the right middle cerebral artery and bilateral common carotid arteries in male adult Sprague-Dawley rats. Adipose tumor necrosis factor- α (TNF- α) and monocyte chemoattractant protein-1 (MCP-1) mRNA and protein levels were determined by RT-PCR and enzyme-linked immunoassay, respectively. The stroke rats developed glucose intolerance on days 1 and 2 after cerebral ischemic injury. The fasting blood insulin levels and insulin resistance index measured by homeostasis model assessment were higher in the stroke rats compared with the sham group. Epididymal adipose TNF- α and MCP-1 mRNA and protein levels were elevated one- to twofold, in association with increased macrophage infiltration into the adipose tissue. When the rats were treated with a nonselective β -adrenergic receptor blocker, propranolol, before induction of cerebral ischemic injury, the acute stroke-induced increase in TNF- α and MCP-1 was blocked, and fasting blood insulin concentration and homeostasis model assessment-insulin resistance were decreased. These results suggest a potential role of adipose proinflammatory cytokines induced by the sympathetic nervous system in the pathogenesis of glucose metabolic disorder in rats with acute ischemic stroke.

monocyte chemoattractant protein-1; sympathetic nervous system; tumor necrosis factor- α

A HIGH PROPORTION OF PATIENTS suffering an acute stress, such as stroke, may develop hyperglycemia, even in the absence of a preexisting diagnosis of diabetes (7, 20, 32). Both human and animal studies have suggested that stress-induced hyperglycemia

is not a benign occurrence and is associated with a high risk of mortality after acute stroke (7, 20, 32). Some studies have reported that decreased glucose can reduce ischemic brain damage (5, 24). In view of the importance of blood glucose on the prognosis of acute stroke, further elucidation of the pathogenetic mechanisms of poststroke hyperglycemia, whether due to transiently acute stress or a worsening of previously undiagnosed diabetes, is warranted. Over the last decade, adipose tissue has been found to secrete a number of cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), chemokines, such as monocyte chemoattractant protein-1 (MCP-1), and adipokines, such as leptin and adiponectin, involved in glucose metabolism, and the etiopathogenesis of obesity-related insulin resistance (4, 12, 21). It has been shown that subjects with obesity-related insulin resistance have a higher local expression of TNF- α in the adipose tissue (25). Furthermore, TNF- α can stimulate the production of other cytokines and chemokines, such as IL-6 and MCP-1, and in concert with them induce insulin resistance (14, 35). However, whether these adipose tissue-derived bioactive substances contribute to the development of hyperglycemia in acute stroke is less studied. To examine the relationship between adipocytokines and poststroke hyperglycemia, we used a rat model with ischemic brain injury and examined the changes of several adipose tissue-derived cytokines, including TNF- α , IL-1 β , IL-6, MCP-1, leptin, and adiponectin, after acute stroke. In addition, sympathetic tone has been shown to be activated by experimental cerebral ischemia (10, 34), and several studies have reported that sympathetic nervous system regulates the expression of several adipocytokines through adipocyte β -adrenergic receptors (3, 19, 26). Therefore, this study also determined whether alterations in adipose proinflammatory cytokines following acute stroke, if any, were mediated by sympathetic system using a nonselective β -adrenergic receptor blocker, propranolol.

MATERIALS AND METHODS

Animals and induction of cerebral ischemia. Adult male Sprague-Dawley rats (300–350 g) were anesthetized with phenobarbital (60 mg/kg body wt, intramuscular). The body temperature of each rat was maintained at $37.0 \pm 0.5^\circ\text{C}$ using a heating pad. Focal ischemic infarcts in the right lateral cerebral cortex were produced by ligating right middle cerebral artery (MCA) and bilateral common carotid arteries (CCAs), as described previously with some modifications (8, 9, 29). For ligation of right MCA, the animal was placed in a lateral

Address for reprint requests and other correspondence: S.-Y. Lin, Division of Endocrinology and Metabolism, Dept. of Medicine, Taichung Veterans General Hospital, No. 160, Sec. 3, Chung-Kang Rd. Taichung, Taiwan (e-mail: sylin@vghtc.gov.tw).

position, and a skin incision was made at the midpoint between the right lateral canthus and the anterior pinna. The temporal muscle was retracted, and a small (3 mm in diameter) craniectomy was made at the junction of the zygoma and squamosal bone using a drill cooled with saline solution. The dura was opened with fine forceps guided by a dissecting microscope to expose the right MCA. During the whole operative procedure, care was taken to avoid physical and thermal injury to the cortex. For ligation of CCAs, both the CCAs were exposed by midline anterior cervical incision. Both CCAs and the right MCA were ligated with a square knot using 10–0 suture. For sham operations, the animals underwent similar surgical procedure with isolation of both CCA and right MCA, and a needle was passed beneath the artery but without ligation.

Quantification of ischemic infarction. The brains were quickly removed 1, 2, 3, or 7 days after surgery and chilled in cold PBS for 5 min. Coronal sections (2-mm thick) were cut using a tissue slicer. Sections were immersed in PBS containing 2% triphenylterazolium chloride at 37°C for 30 min and then fixed in 10% phosphate-buffered formalin for 45 min (8). The triphenylterazolium chloride is reduced by certain enzymes in normal tissues to a deep red, fat-soluble, light-sensitive compound, clearly delineating ischemic areas. The areas of infarction were measured using a computer image analysis system (Alpha Innotech, IS1000).

Determination of glucose tolerance and insulin resistance. Insulin resistance was determined in fasting rats before and 1, 2, 3, and 7 days after inducing ischemic brain injury. Blood was sampled for glucose analysis before glucose loading and 30, 60, and 120 min after injecting glucose solution (2 g/kg, intraperitoneal). The total area under curve for glucose during intraperitoneal glucose tolerance test (2-h glucose area under curve) was calculated using the trapezoidal (trapezium) rule. Insulin resistance was evaluated according to the homeostasis model assessment (HOMA), described by Matthews et al. (31). The HOMA insulin resistance (HOMA-IR) index is calculated as [fasting insulin ($\mu\text{IU/ml}$) \times fasting glycemia ($\mu\text{mol/l}$)]/22.5. Blood glucose was measured from a tail vein using a hand-held Accucheck glucometer (Roche Diagnostics, Indianapolis, IN), and fasting blood insulin levels were measured in blood samples collected before intraperitoneal glucose injection using a rat/mouse ELISA (Linco Research, St. Charles, MO), according to the manufacturer's instructions.

Blood and adipose tissue preparation. On days 1, 2, 3, and 7 after ischemic brain injury, the experimental and sham-operated rats were anesthetized with intraperitoneal sodium phenobarbital and intra-arterial catheterization of the left femoral artery for blood sampling. Blood was immediately subjected to centrifugation, and plasma samples were stored at -70°C until analysis. In addition, the epididymal adipose tissue was rapidly dissected, weighed, and stored in liquid nitrogen until analysis.

Administration of propranolol. In some experiments, propranolol (2 mg/kg) was injected intraperitoneally 3 h before the induction of cerebral ischemia. Thereafter, the blood and adipose tissues were collected as described above.

Cytokine analyses in blood and adipose tissue. The epididymal fat was thoroughly homogenized in tissue protein extraction reagents (T-PER, Pierce Biotechnology), and the supernatant fluids obtained with centrifugation at 10,000 rpm for 10 min were collected. The adipose tissue and blood samples were assayed for TNF- α , IL-1 β , IL-6, MCP-1, leptin, and adiponectin using the rat inflammation kit (BD Bioscience).

RNA preparation and gene expression analysis. Total mRNA was extracted from frozen epididymal fat tissue samples using TriZol agents (Life Technologies), and the mRNA concentrations were determined by UV light absorbance at 260 nm. The expression of adipose proinflammatory cytokine mRNA was determined by semiquantitative RT-PCR analysis normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Briefly, 1–5 μg of total RNA were transcribed using reverse transcriptase, and poly(dT)_{12–18} primer, and then PCR reaction was

conducted in a DNA Thermal Cycler 480 (Perkin-Elmer, Norwalk, CT). The primer sets used in this study were as follows: forward 5'-CCCTCACACTCAGATCATCTTCTCAA and reverse 5'-TCTAAG-TACTTGGGCAGGTTGACCTC for TNF- α ; forward 5'-TGCAG-GTCTCTGTCACGCTTC and reverse 5'-TTCTCCAGCCGACTCAT-TGG for MCP-1; forward 5'-ACCACAGTCCATGCCATCAC and reverse 5'-TCCACCACCCTGTTGCTGTA for GAPDH. The target gene and GAPDH bands were measured by densitometry using Image Quant Analysis Software.

Western blot analyses. Adipose protein samples (100 μg protein) were subjected to electrophoresis and immunoblotted with an antibody to hormone-sensitive lipase diluted 1:1,000 (Cayman Chemical, Ann Arbor, MI). Then blotted membranes were stained with anti-rabbit or anti-goat IgG secondary antibodies labeled with horseradish peroxidase (Amersham, Arlington Heights, IL). Specific protein bands were visualized by enhanced chemiluminescence (Amersham). All measured protein levels were quantified by densitometry, and the percent change relative to sham-operated rats was determined after normalization by GAPDH, determined with anti-GAPDH antibody diluted 1:3,000 (Santa Cruz Biotech, Santa Cruz, CA).

Histology and immunohistochemistry. Excised adipose specimens were fixed in 10% formalin and embedded with paraffin for hematoxylin and eosin staining, and the average cell size of 100 adipocytes was measured in six continuous sections. For immunohistochemical examination, sections of formalin-fixed, paraffin-embedded epididymal adipose tissue were deparaffinized and incubated with anti-CD68 antibody. Then sections were washed three times in TBS-Tween 20 (20 mmol/l Tris, 137 mmol/l NaCl, 0.05% Tween 20, pH 7.5) and incubated with a biotinylated secondary antibody, followed by an avidin-biotin-peroxidase complex. The immunoreactive signal was developed by color deposition using diaminobenzidine as substrate. The average number of CD68-positive immunoactivity per observation was counted in six continuous sections.

Statistical analysis. All data were expressed as means \pm SE. Differences were determined by the Student's unpaired *t*-test. Comparisons among several groups were compared statistically by ANOVA, followed by the Bonferroni multiple-comparisons test. The correlation between two variables was analyzed by Spearman's rank-order correlation. Results were considered significant at $P < 0.05$. All data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 6.0 for Windows, SPSS, Chicago, IL).

RESULTS

Changes in glucose tolerance following acute cerebral ischemia. The body weight of the rats was significantly decreased 1, 2, and 3 days after the induction of cerebral ischemia compared with before surgery, and regained on day 7 (Table 1). The epididymal fat weight was also decreased significantly during the course of ischemic stroke. The infarct brain volume was enlarged maximally in the first 2 days after artery ligation and thereafter diminished progressively. The rats with acute cerebral ischemia had higher insulin and HOMA-IR levels on days 1, 2, and 3 after surgery compared with the sham-operated rats (Table 1). In addition, the stroke rats had higher postload glucose levels at 30, 60, 90, and 120 min after the intraperitoneal glucose injection compared with control rats during in the first 2 days following induction of acute ischemic stroke (Fig. 1). Although fasting blood glucose values in stroke rats were similar to that in the control group (Table 1), a moderate positive correlation was observed between infarct brain volume and fasting blood glucose levels ($r = 0.55$, $P < 0.05$).

Blood and adipose proinflammatory cytokine expression. To determine adipose proinflammatory cytokine expression following acute ischemic stroke, we quantified the levels of

Table 1. Serial change of metabolic characteristics in sham-operated and cerebral ischemia rats

	Baseline	Day 1	Day 2	Day 3	Day 7
<i>n</i>	6–8	6–8	6–8	6–8	6–8
Body weight, g					
Sham	317.8 ± 3.8	304.2 ± 3.9	309.4 ± 3.6	315.7 ± 3.5	328.3 ± 3.7
Ischemia	318.3 ± 3.8	289.2 ± 5.9*	290.0 ± 6.9*	266.0 ± 12.2*†	309.6 ± 13.8*
Epididymal fat, g					
Sham	3.2 ± 0.2	3.2 ± 0.1	3.2 ± 0.3	3.2 ± 0.5	3.3 ± 0.3
Ischemia	3.2 ± 0.2	2.5 ± 0.4*	2.2 ± 0.2*†	2.0 ± 0.3*†	2.4 ± 0.3*
Infarct volume, %					
Sham					
Ischemia		28.0 ± 3.4	36.3 ± 3.9†	22.7 ± 2.6†	8.3 ± 1.3†
Fasting glucose, mM					
Sham	6.1 ± 0.4	6.1 ± 0.4	6.1 ± 0.4	6.1 ± 0.4	6.1 ± 0.4
Ischemia	6.1 ± 0.3	5.6 ± 0.3	6.1 ± 0.6	5.7 ± 0.2	5.7 ± 0.1
Fasting insulin, μ IU/ml					
Sham	7.7 ± 0.2	7.7 ± 0.2	7.7 ± 0.2	7.7 ± 0.2	7.7 ± 0.2
Ischemia	7.7 ± 0.3	15.3 ± 3.8*	10.5 ± 1.2*†	9.9 ± 0.7*†	8.9 ± 0.3†
HOMA-IR					
Sham	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.2
Ischemia	2.1 ± 0.3	3.7 ± 0.7*	2.8 ± 0.3*†	2.5 ± 0.1*†	2.2 ± 0.1†

Values are means \pm SE; *n*, no. of rats. HOMA-IR, homeostasis model assessment-insulin resistance. * $P < 0.05$ vs. sham, unpaired Student's test. † $P < 0.05$ vs. ischemia day 1, one-way ANOVA and Bonferroni's test.

MCP-1, TNF- α , IL-1 β , and IL-6 in the epididymal adipose tissue, as well as the adipocytokines, including leptin and adiponectin. The TNF- α and MCP-1 mRNA and protein levels were significantly increased in the epididymal adipose tissue from the stroke rats on the first 3 days following surgery, with adipose TNF- α level peaked on *day 1* and MCP-1 on *day 2*

(Fig. 2, A and B), respectively, although circulating TNF- α and MCP-1 concentrations were not changed (data not shown). In addition, adipose and circulating leptin levels were significantly increased 1 day after the ischemic stroke, but progressively declined thereafter (Fig. 2C). However, adipose IL-6, IL-1 β , and adiponectin were not differentially expressed between the two groups (data not shown). A significant positive correlation was observed between adipose TNF- α and HOMA-IR ($r = 0.91$, $P < 0.01$) and adipose TNF- α and insulin ($r = 0.93$, $P < 0.01$).

Macrophage infiltration and hormone-sensitive lipase expression of adipose tissue. Hematoxylin and eosin staining of epididymal adipose tissue sections showed that the number of hematoxylin-positive nuclei was increased in acute stroke rats compared with sham-operated rats after induction of cerebral ischemia (Fig. 3A). The proportion of adipocyte size in the 25- to 50- μ m range from epididymal adipose in stroke rats was significantly higher compared with that in sham-operated rats ($P < 0.05$). Immune staining with an antibody against CD68, a macrophage-specific cell surface glycoprotein, showed that anti-CD68-positive macrophages were significantly elevated in the epididymal adipose tissue of rats with cerebral ischemia (Fig. 3B). The hormone-sensitive lipase protein levels were significantly elevated in the epididymal fat of stroke rats on the first 3 days following surgery, suggesting an enhanced lipolytic process in the adipose tissue of rats with cerebral ischemia (Fig. 4).

Propranolol effect on changes in proinflammatory cytokines, glucose tolerance, and brain infarct volume. To look after a potential role of the sympathetic nervous system in the increased TNF- α and MCP-1 expression in the adipose tissue following cerebral ischemic stroke, we administered propranolol before the induction of cerebral ischemia and showed that stroke-induced increase in TNF- α and MCP-1 24 h after cerebral ischemia was almost blocked (Fig. 5, A and B, respectively), but without an effect on the infarct volume (data not shown). In addition, the fasting insulin concentrations and HOMA-IR were also diminished in stroke rats that received

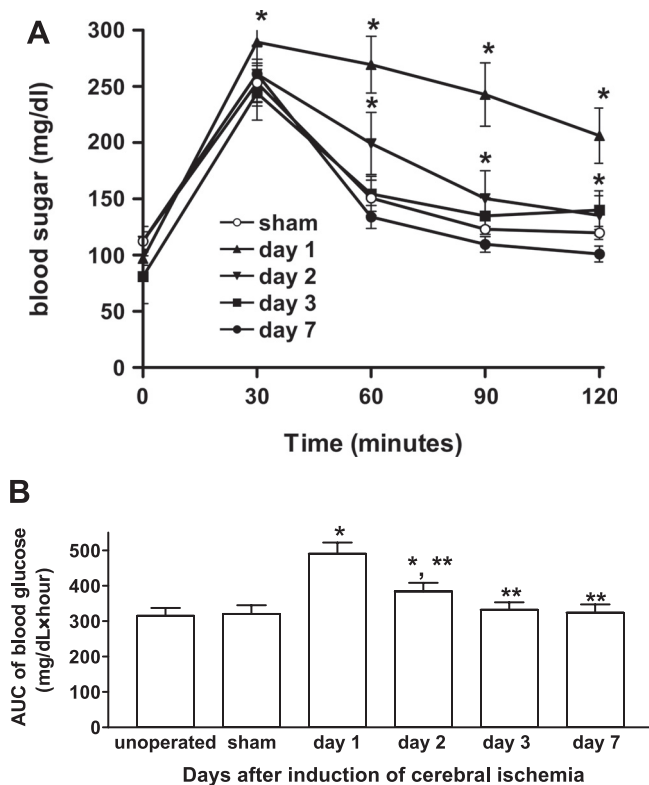


Fig. 1. Blood glucose concentrations (A) and areas under the curve (AUC; B) during intraperitoneal glucose tolerance test in sham-operated and cerebral ischemia rats on *days 1, 2, 3, and 7* after surgery ($n = 6-8$ for each experiment). Values are means \pm SE. * $P < 0.05$ vs. sham, unpaired Student's test. ** $P < 0.05$ vs. ischemia *day 1*, one-way ANOVA and Bonferroni's test.

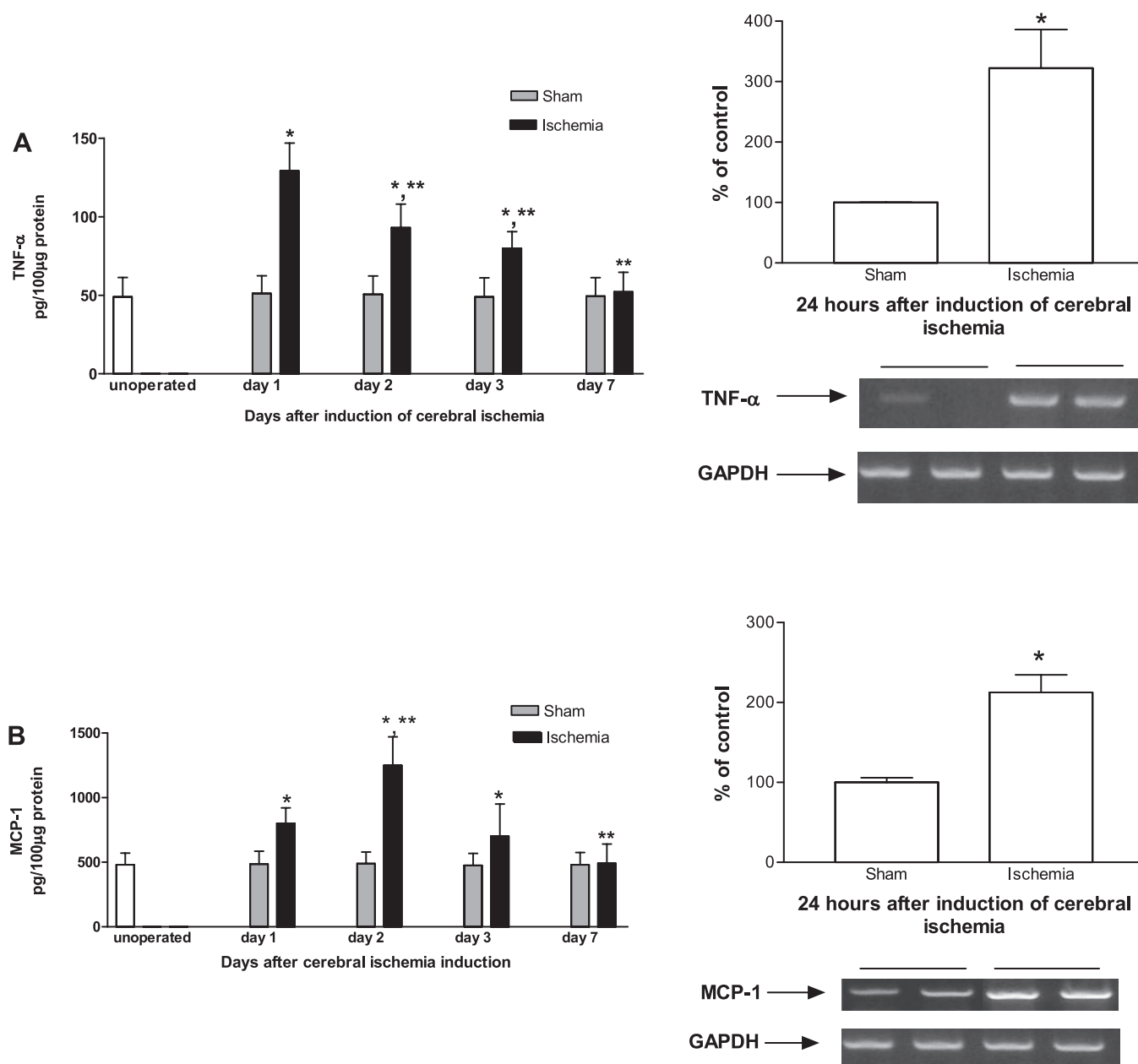


Fig. 2. Expression of adipocytokines in epididymal adipose tissue of sham-operated and cerebral ischemia rats on *days 1, 2, 3, and 7* after surgery ($n = 6-8$ for each experiment). *A*: relative changes of adipose TNF- α protein and mRNA levels. *B*: relative changes of adipose monocyte chemoattractant protein-1 (MCP-1) protein and mRNA levels. *C*: relative changes of adipose and serum leptin levels. * $P < 0.05$ vs. sham, unpaired Student's test. ** $P < 0.05$ vs. ischemia *day 1*, one-way ANOVA and Bonferroni's test.

propranolol (Fig. 6, *A* and *B*, respectively). To evaluate the role of another stress hormone, glucocorticoid, in poststroke hyperglycemia, we measured the circulating cortisol concentrations after inducing cerebral ischemia and found that blood cortisol values did not significantly differ between the stroke and control rats (data not shown).

DISCUSSION

Many factors, including counterregulatory hormones, aging, and bed rest have been proposed to be involved in development of stress illness-related hyperglycemia (6, 27, 33, 37). The present study was aimed to examine whether other possible

mediators, such as adipose proinflammatory cytokines, might also participate in the acute stress hyperglycemic reaction. Using an ischemic stroke rat model, we found that blood insulin, HOMA-IR levels, and postload glucose levels after intraperitoneal glucose injection were significantly elevated during the first 2 days following acute cerebral ischemia, and there was a positive relationship between fasting blood glucose and infarct brain volumes. Clinical studies have reported that hyperglycemia is prevalent in 20–50% of patients with acute ischemic stroke, even without a disease history of diabetes mellitus, and associated with stroke infarct volumes measured on cerebral computed tomography (7, 20, 32). Furthermore,

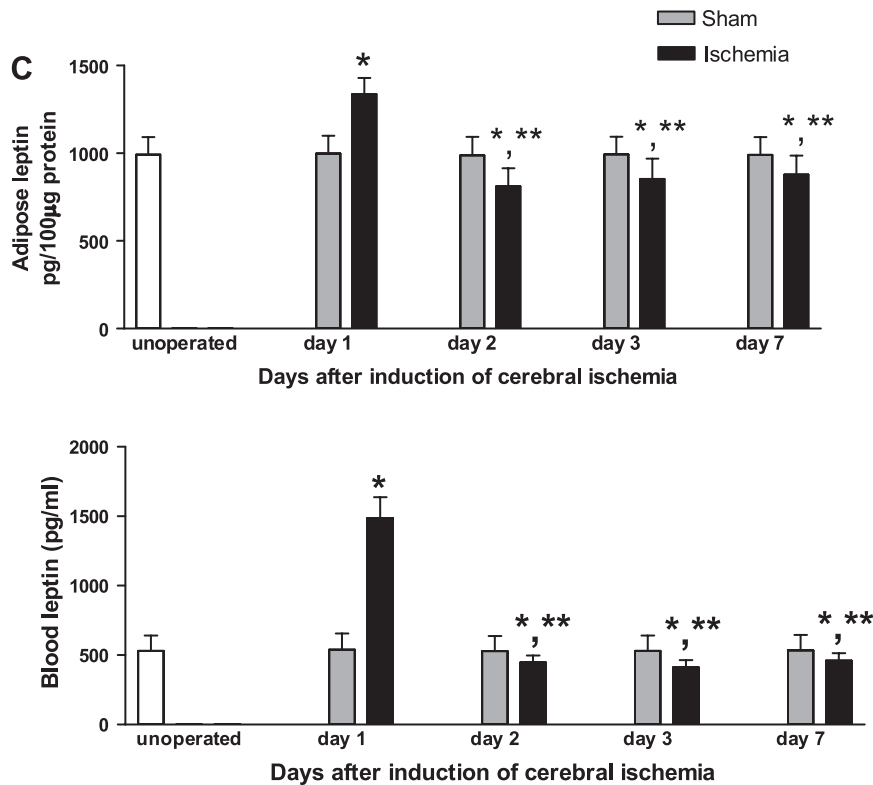


Fig. 2—Continued

among the patients with poststroke hyperglycemia, about one-third of them manifest only postload hyperglycemia with normal fasting blood glucose (11). In our acute stroke rat model, the blood glucose profiles and the relationship between glucose and infarct brain volume during the initial 2 days after cerebral ischemia appeared to resemble those observed in acute stroke patients. Although the postload glucose levels in stroke rats returned to the sham values on *day 3* after cerebral ischemia, the stroke rats still showed somewhat insulin resistance with elevated circulating insulin and HOMA-IR values. Of the cytokines/chemokines/adipokines measured, it was found that expression of TNF- α and MCP-1 mRNA and protein was increased in the adipose tissue of acute stroke rats concomitantly with macrophage infiltration into the adipose tissue and correlated with HOMA-IR and insulin levels. These data, all together, suggested a potential relationship between increased adipose proinflammatory cytokines and poststroke hyperglycemia and insulin resistance.

A large accumulation of data has documented the important role of adipose TNF- α in the development of insulin resistance in obesity and diabetes (4, 12, 21). Notably, some experimental studies propose that TNF- α can also be increasingly produced by adipose tissue during critical illness (2, 13, 17) and, consequently, participates in the development of insulin resistance in stress illnesses (39–41). Our study findings seemed to support that rationale. Although this study did not determine whether the increased adipose TNF- α expression was due to infiltrated macrophages or the adipocytes in the stroke rats, this increased adipose cytokine expression, regardless of its exact sources, would be expected to act locally in the adipose tissue and, in consequence, produce a variety of metabolic abnormalities, such as poststroke insulin resistance and hyperglycemia. However, it

should be noted that, in the stroke rats, the time pattern of adipose TNF- α and glucose changes did not match each other exactly at the same rate, suggesting that increased adipose TNF- α might just represent an epiphenomenon of the mechanisms underlying stroke-related insulin resistance, and further studies are necessary to elucidate a more precise role for TNF- α in metabolic alterations in acute stroke. In addition to participating in pathogenesis of insulin resistance, TNF- α is reported to be involved in fat mass loss in some wasting diseases, such as human immunodeficiency virus infection-associated lipodystrophy (22). It has been shown that TNF- α can downregulate several genes involved in adipogenesis, or stimulate lipolysis, and thus induce lipodystrophy (18). In the acute stroke rats, the epididymal fat mass was at the lowest level on *day 3* after acute cerebral ischemia, which was preceded by increased expression of adipose TNF- α and hormone-sensitive lipase. On *day 7*, when adipose TNF- α level returned to the sham value, the stroke rats began to regain the initial fat mass loss. These findings were in agreement with the potential role of TNF- α in the regulation of adipose mass.

MCP-1 has been suggested to be another novel adipocytokine related to the development of insulin resistance, and its levels in the adipose tissue and plasma are highly elevated in obesity (15, 35). In addition to the known *in vitro* effects impairing insulin signaling, MCP-1 can attract and activate circulating monocytes into the adipose tissue and turn them into macrophages, resulting in adipose inflammation and inducing insulin resistance in adipocytes (15, 35, 43, 44). In the acute stroke rats, the adipose MCP-1 expression was increased since *day 1* after induction of cerebral ischemia, likely contributing to subsequent adipose macrophage infiltration. Based on the physiological effects of MCP-1, it was supposed that increased MCP-1 expression and macrophage infiltration

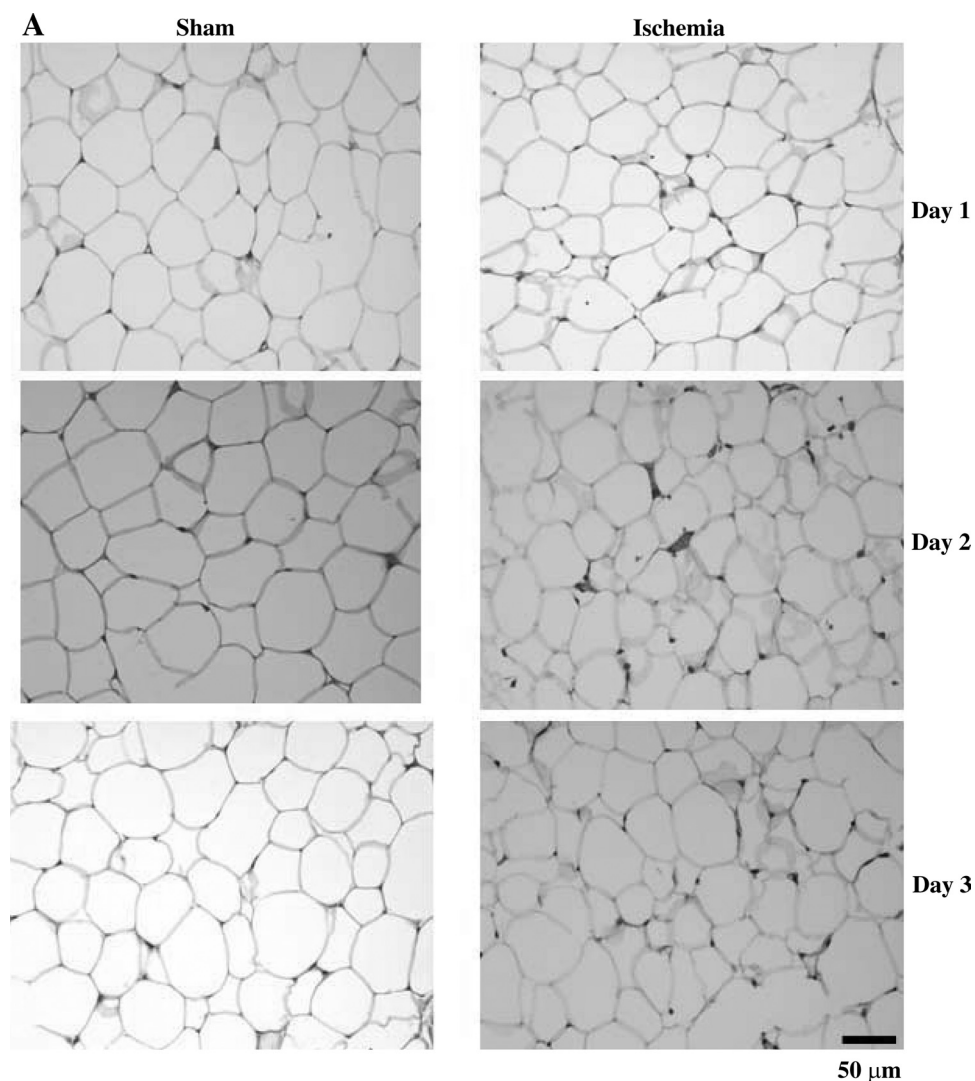


Fig. 3. Hematoxylin and eosin (A) and immunohistochemical staining using anti-CD68 (top; B) of adipose samples in sham-operated and cerebral ischemia rats obtained on days 1, 2, and 3 after surgery ($\times 400$). Scale bar = 50 μm . B, bottom: data are presented as the number of CD68-positive cells per field (left y-axis for ischemia rats, and right y-axis for sham rats). Values are means \pm SE. * $P < 0.05$ vs. sham, unpaired Student's test.

within the adipose tissue might also participate in the occurrence of poststroke hyperglycemia and insulin resistance, or even other pathological consequence, such as brain injury (39). MCP-1 has also been shown to be upregulated by TNF- α in vitro in adipocytes, at both the gene expression and protein secretion levels (35, 42). According to the time pattern of peak adipose TNF- α and MCP-1 expression in stroke rats, it was speculated that TNF- α might induce MCP-1 production, which thus further reinforced the inflammatory state in the adipose tissue after acute cerebral ischemia.

Leptin, a hormone secreted by adipose tissue in direct proportion to the amount of body fat, has main functions of reducing food intake, promoting energy expenditure, and balancing body weight through its effects in the central nervous system (28). Moreover, recent animal experimental study showed that leptin expression was also increased in ischemic brain parenchymal tissue and proposed that leptin could provide neuroprotection and inflammation modulation effects (36). Therefore, the early increased adipose leptin expression 1 day after acute ischemic stroke probably represented an adaptive response against the poststroke inflammatory cascade. However, it should be noted that the stroke rats on day 1 after surgery had a lower body fat

mass than sham-operated rats, implicating factors other than adiposity itself that may be responsible for this leptin upregulation. Administration of TNF- α has been shown to stimulate leptin production and secretion from adipose tissue (16). Given that TNF- α can induce leptin expression and secretion, and adipose TNF- α levels were increased after acute cerebral ischemia, it was thought that an interaction between TNF- α and leptin might exist in the initial phase of acute stroke. On days 2 and 3 after induction of ischemic stroke, adipose leptin expression was decreased concomitantly with loss of epididymal fat mass, despite increased TNF- α , suggesting that the stimulatory effect of TNF- α on leptin might be overridden by some other, as yet unidentified, factor(s). In addition, this decreased leptin was thought to have an adverse influence on poststroke glucose metabolism (23, 28).

Much experimental evidence has demonstrated that adipose tissue is innervated by the sympathetic nervous system in rodents, which can regulate lipolysis, fat cell number, proliferation, and the secretion of some adipocytokines, such as TNF- α and MCP-1 (3, 19, 26). Furthermore, the activity of the sympathetic nervous system has been shown to be increased in obese individuals and proposed to contribute to insulin resistance through the various effects of catecholamines on adipocytes (38). Accordingly, be-

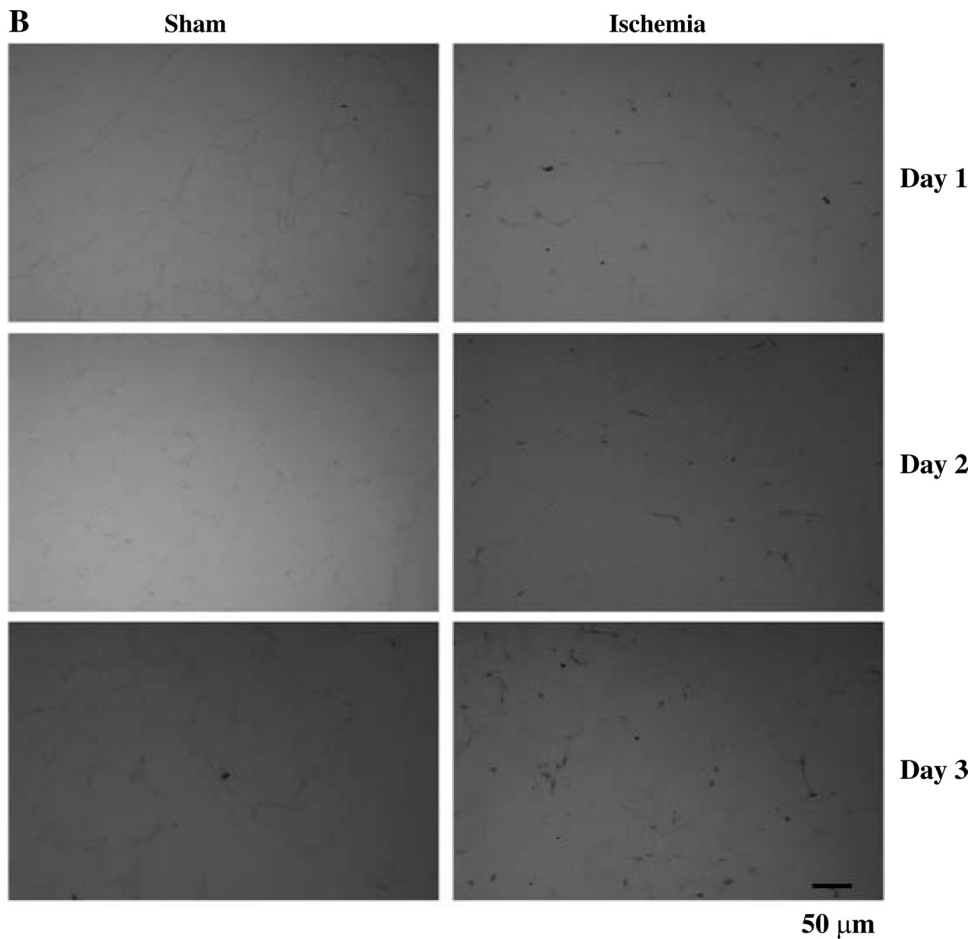
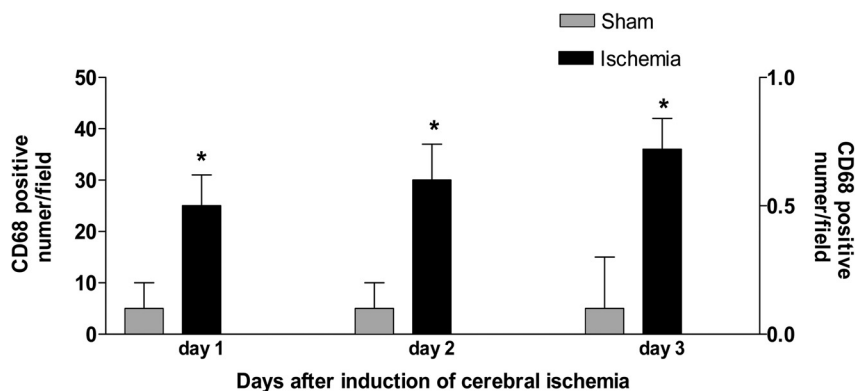


Fig. 3—Continued



cause acute cerebral ischemic infarction evoked an activation of sympathetic tone, it was hypothesized that the increased adipose TNF- α and MCP-1 expression might be related to the activation of the sympathetic nervous system. To test this hypothesis, the present study demonstrated that the administration of a β -adrenoceptor antagonist near completely suppressed the increase in adipose TNF- α and MCP-1, as well as the HOMA-IR and blood insulin levels after ischemic stroke. This finding suggested a role of sympathetic nervous system activation following acute stroke, especially β -adrenergic stimulation, in the upregulation of adipose

TNF- α and MCP-1 production, and thus the development of poststroke glucose metabolic disorder.

The present study had several limitations, and it is important to interpret the laboratory findings cautiously. First, only a cerebral ischemic model was used, and whether other stroke models, such as intracranial hemorrhage (7, 20, 32), have similar results is not known. Second, we did not directly address the exact causal relationship between the adipose proinflammatory response and hyperglycemia in the acute stroke rats. The increased adipose TNF- α and MCP-1 expression might be possible due to the

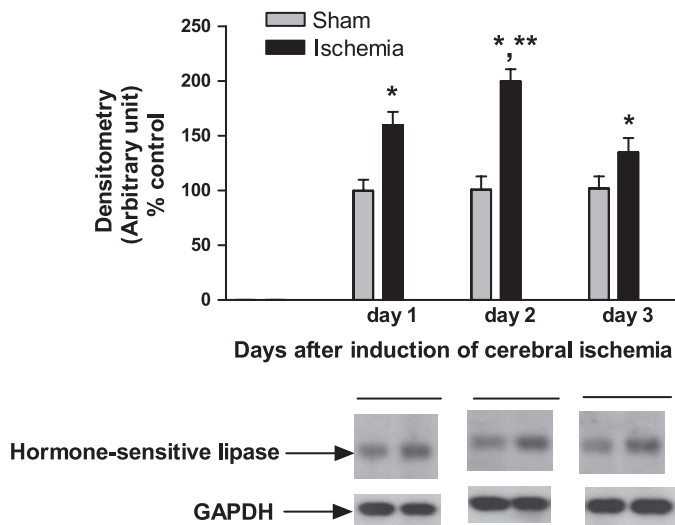


Fig. 4. Expression of hormone-sensitive lipase protein in epididymal adipose tissue of sham-operated and cerebral ischemia rats on *days 1, 2, and 3* after surgery ($n = 6-8$ for each experiment). Values are means \pm SE. * $P < 0.05$ vs. sham, unpaired Student's test. *** $P < 0.05$ vs. ischemia *day 1*, one-way ANOVA and Bonferroni's test.

poststroke hyperglycemia itself rather than its causes (30). In addition, this study only analyzed proinflammatory cytokine expression in the epididymal adipose. The sympathetic nervous system drive to the white adipose tissue has been reported to not be uniform across the different fat pads, although the systemic

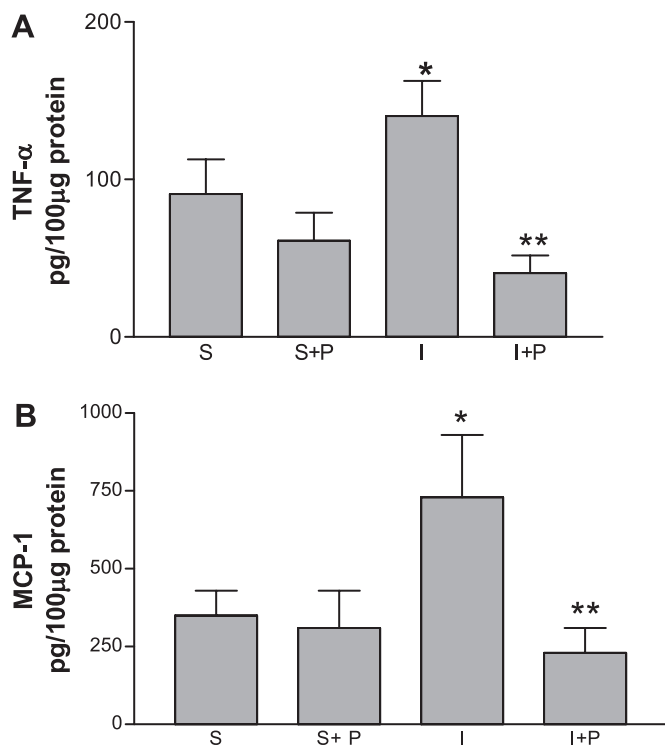


Fig. 5. Effects of administration of propranolol at 3 h before induction of cerebral ischemia on epididymal TNF- α (A) and MCP-1 protein levels (B) in sham-operated and cerebral ischemia rats on *day 1* after surgery ($n = 6-8$ for each experiment). Values are means \pm SE. S, sham; S+I, sham with propranolol; I, ischemia without propranolol; I+P, ischemia with propranolol. * $P < 0.05$ vs. sham. ** $P < 0.05$ vs. ischemia without propranolol.

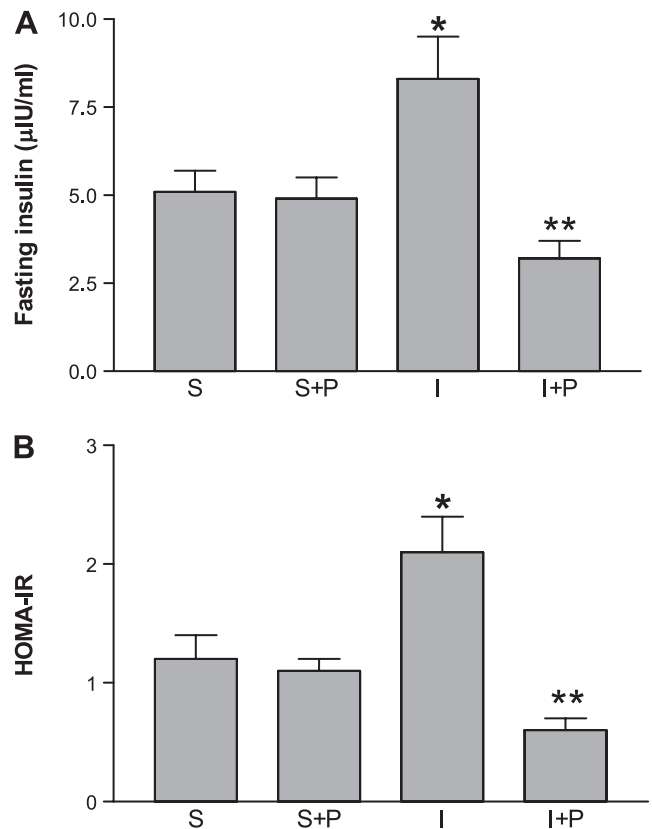


Fig. 6. Effects of administration of propranolol at 3 h before induction of cerebral ischemia on blood insulin (A) and homeostasis model assessment-insulin resistance (HOMA-IR) levels (B) in sham-operated and cerebral ischemia rats on *day 1* after surgery ($n = 6-8$ for each experiment). Values are means \pm SE. * $P < 0.05$ vs. sham. ** $P < 0.05$ vs. ischemia without propranolol.

leptin levels were increased in the stroke rats (3). Third, other stress-related factors possibly contributed to the development of insulin resistance in acute stroke. Lipolysis and free fatty acid release have been shown to increase following acute stroke, and both of them could affect the insulin sensitivity (1), although circulating cortisol levels were not increased after induction of cerebral ischemia in this study. Fourth, this study only used the β -adrenoreceptor antagonist at a single dose, and dose-dependent effects were not investigated. In addition, the beneficial effects of propranolol on blood glucose and adipose TNF- α and MCP-1 were possible due to its protection of neurons from ischemic injury, although this study did not show that β -adrenergic blockers reduced the cerebral damage area on *day 1* after surgery. Additional experiments are necessary to address all of these issues.

In summary, the present study demonstrated that the expression of TNF- α and MCP-1 and macrophage infiltration was increased in the adipose tissue of ischemic stroke rats, in association with glucose intolerance. In contrast, the administration of the β -adrenergic antagonist nearly reversed the poststroke inflammatory response in the adipose tissue, as well as insulin resistance. These findings suggest a potential role of the adipose proinflammatory cytokines induced by sympathetic nervous system in the pathogenesis of glucose metabolic disorder following acute ischemic stroke.

GRANTS

This work was supported by Taichung Veterans General Hospital, Taichung, Taiwan (Grant No. TCVGH-973503C), and National Science Council, Taipei, Taiwan (Grant No. 97-2314-B-075A-003-MY3).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

- Adibhatla RM, Hatcher JF. Altered lipid metabolism in brain injury and disorders. *Subcell Biochem* 49: 241–268, 2008.
- Atsumi T, Cho YR, Leng L, McDonald C, Yu T, Danton C, Hong EG, Mitchell RA, Metz C, Niwa H, Takeuchi J, Onodera S, Umino T, Yoshioka N, Koike T, Kim JK, Bucala R. The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. *J Immunol* 179: 5399–5406, 2007.
- Bartness TJ, Song CK, Shi HF, Bowers RR, Foster MT. Brain-adipose tissue cross talk. *Proc Nutr Soc* 64: 53–64, 2005.
- Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 96: 939–949, 2005.
- Bruno A, Kent TA, Coull BM, Shankar RR, Saha C, Becker KJ, Kissela BM, Williams LS. Treatment of hyperglycemia in ischemic stroke (THIS): a randomized pilot trial. *Stroke* 39: 384–389, 2008.
- Butler SO, Btaiche IF, Alaniz C. Relationship between hyperglycemia and infection in critically ill patients. *Pharmacotherapy* 25: 963–976, 2005.
- Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC. Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. *Stroke* 32: 2426–2432, 2001.
- Chen CJ, Liao SL, Chen WY, Hong JS, Kuo JS. Cerebral ischemic reperfusion injury in rat brain: effects of naloxone. *Neuroreport* 12: 1245–1249, 2001.
- Chen ST, Hsu CY, Hogan EL, Maricq H, Balentine JD. A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction. *Stroke* 17: 738–743, 1986.
- Cheung RT, Hachinski VC, Cechetto DF. Cardiovascular response to stress after middle cerebral artery occlusion in rats. *Brain Res* 747: 181–188, 1997.
- Dave JA, Engel ME, Freercks R, Peter J, May W, Badri M, Van Niekerk L, Levitt NS. Abnormal glucose metabolism in non-diabetic patients presenting with an acute stroke: prospective study and systematic review. *QJM* 103: 495–503, 2010.
- Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 444: 881–887, 2006.
- Desruisseaux MS, Nagajyothi Trujillo ME, Tanowitz HB, Scherer PE. Adipocyte, adipose tissue, and infectious disease. *Infect Immun* 75: 1066–1078, 2007.
- Fasshauer M, Klein J, Lossner U, Paschke R. Interleukin (IL)-6 mRNA expression is stimulated by insulin, isoproterenol, tumour necrosis factor alpha, growth hormone, and IL-6 in 3T3-L1 adipocytes. *Horm Metab Res* 35: 147–152, 2003.
- Ferrante AW Jr. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J Intern Med* 262: 408–414, 2007.
- Finck BN, Johnson RW. Tumor necrosis factor (TNF)-alpha induces leptin production through the p55 TNF receptor. *Am J Physiol Regul Integr Comp Physiol* 278: R537–R543, 2000.
- Franco-Pons N, Gea-Sorli S, Closa D. Release of inflammatory mediators by adipose tissue during acute pancreatitis. *J Pathol* 221: 175–182, 2010.
- Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab* 280: E827–E847, 2001.
- Fu L, Isobe K, Zeng Q, Suzukawa K, Takekoshi K, Kawakami Y. Beta-adrenoceptor agonists downregulate adiponectin, but upregulate adiponectin receptor 2 and tumor necrosis factor-alpha expression in adipocytes. *Eur J Pharmacol* 569: 155–162, 2007.
- Gray CS, Hildreth AJ, Alberti GK, O'Connell JE. Poststroke hyperglycemia: natural history and immediate management. *Stroke* 35: 122–126, 2004.
- Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 444: 860–867, 2006.
- Johnson JA, Albu JB, Engelson ES, Fried SK, Inada Y, Ionescu G, Kotler DP. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. *Am J Physiol Endocrinol Metab* 286: E261–E271, 2004.
- Kalra SP. Central leptin gene therapy ameliorates diabetes type 1 and 2 through two independent hypothalamic relays; a benefit beyond weight and appetite regulation. *Peptides* 30: 1957–1963, 2009.
- Kent TA, Soukup VM, Fabian RH. Heterogeneity affecting outcome from acute stroke therapy: making reperfusion worse. *Stroke* 32: 2318–2327, 2001.
- Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 280: E745–E751, 2001.
- Kralisch S, Klein J, Lossner U, Blüher M, Paschke R, Stumvoll M, Fasshauer M. Isoproterenol stimulates monocyte chemoattractant protein-1 expression and secretion in 3T3-L1 adipocytes. *Regul Pept* 135: 12–16, 2006.
- Kremen J, Dolinkova M, Krajickova J, Blaha J, Anderlova K, Lacinova Z, Haluzikova D, Bosanska L, Vokurka M, Svacina S, Haluzik M. Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: possible role in postoperative insulin resistance. *J Clin Endocrinol Metab* 91: 4620–4627, 2006.
- Lago F, Dieguez C, Gomez-Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. *Cytokine Growth Factor Rev* 18: 313–325, 2007.
- Lin TN, He YY, Wu G, Khan M, Hsu CY. Effect of brain edema on infarct volume in a focal cerebral ischemia model in rats. *Stroke* 24: 117–121, 1993.
- Lin Y, Berg AH, Iyengar P, Lam TK, Giacca A, Combs TP, Rajala MW, Du X, Rollman B, Li W, Hawkins M, Barzilay N, Rhodes CJ, Fantus IG, Brownlee M, Scherer PE. The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J Biol Chem* 280: 4617–4626, 2005.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419, 1985.
- McCormick MT, Muir KW, Gray CS, Walters MR. Management of hyperglycemia in acute stroke: how, when, and for whom? *Stroke* 39: 2177–2185, 2008.
- McCowan KC, Malhotra A, Bistrrian BR. Stress-induced hyperglycemia. *Crit Care Clin* 17: 107–124, 2001.
- O'Neill PA, Davies I, Fullerton KJ, Bennett D. Stress hormone and blood glucose response following acute stroke in the elderly. *Stroke* 22: 842–847, 1991.
- Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A* 100: 7265–7270, 2003.
- Signore AP, Zhang F, Weng Z, Gao Y, Chen J. Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials. *J Neurochem* 106: 1977–1990, 2008.
- Soop M, Nygren J, Thorell A, Ljungqvist O. Stress-induced insulin resistance: recent developments. *Curr Opin Clin Nutr Metab Care* 10: 181–186, 2007.
- Tentolouris N, Liatis S, Katsilambros N. Sympathetic system activity in obesity and metabolic syndrome. *Ann N Y Acad Sci* 1083: 129–152, 2006.
- Terao S, Yilmaz G, Stokes KY, Ishikawa M, Kawase T, Granger DN. Inflammatory and injury responses to ischemic stroke in obese mice. *Stroke* 39: 943–950, 2008.
- Trujillo ME, Scherer PE. Adipose tissue-derived factors: impact on health and disease. *Endocr Rev* 27: 762–778, 2006.
- Vachharajani V, Russell JM, Scott KL, Conrad S, Stokes KY, Tallam L, Hall J, Granger DN. Obesity exacerbates sepsis-induced inflammation and microvascular dysfunction in mouse brain. *Microcirculation* 12: 183–194, 2005.
- Wang B, Jenkins JR, Trayhurn P. Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-alpha. *Am J Physiol Endocrinol Metab* 288: E731–E740, 2005.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796–1808, 2003.
- Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821–1830, 2003.