Early effects of high fat diet on neurovascular function and focal ischemic brain injury

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Running title: Effect of high fat diet on ischemic stroke

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ABSTRACT

Obesity is a risk factor for stroke but the early effects of high fat diet (HFD) on neurovascular function and ischemic stroke outcomes remain unclear. The goal of this study was to test the hypotheses that HFD beginning early in life: 1) impairs neurovascular coupling, 2) causes cerebrovascular dysfunction, and 3) worsens short term outcomes after cerebral ischemia. Functional hyperemia and parenchymal arteriole (PA) reactivity were measured in rats after 8 weeks of HFD. The effect of HFD on basilar artery function after middle cerebral artery occlusion (MCAO) and associated O-GlcNAcylation were assessed. Neuronal cell death, infarct size, hemorrhagic transformation (HT) frequency/severity and neurological deficit were evaluated after global ischemia and transient MCAO. HFD caused a 10% increase in body weight and doubled adiposity without a change in lipid profile, blood glucose, and blood pressure. Functional hyperemia and PA relaxation were decreased with HFD. Basilar arteries from stroked HFD rats were more sensitive to contractile factors and acetylcholine-mediated relaxation was impaired. Vascular O-GlcNAcylated-protein content was increased with HFD. This group also showed greater mortality rate, infarct volume, HT occurrence rate, HT severity and poor functional outcome as compared to control diet (CD) group. These results indicate that HFD negatively affects neurovascular coupling and cerebrovascular function even in the absence of dyslipidemia. These early cerebrovascular changes may be the cause of greater cerebral injury and poor outcomes of stroke in these animals.

Key words: cerebral ischemia, high fat diet, hemorrhagic transformation, neurovascular coupling, vascular dysfunction.
Obesity is an independent risk factor for acute ischemic stroke (AIS) (19, 36). An alarming recent report showed that the prevalence of AIS dramatically increased in children and young adults, which positively correlated with increases in risk factors including obesity, lipid disorders and diabetes (13). Clinical studies also suggest that obesity is an independent predictor of unfavorable functional outcome and mortality in AIS patients treated with tissue plasminogen activator (tPA), the only therapeutic option these patients have (39-40). Given that stroke is the leading cause of disability and that obesity epidemic is on the rise, these clinical and social problems are expected to get worse and therefore early interventions are necessary. While experimental studies in genetic or diet-induced obesity models showed increased cerebral infarct size and poor outcomes of stroke (7, 25, 32-33, 41), the early impact of a high fat diet (HFD) before the development of obesity on AIS injury and functional outcomes is not known.

It is known that the brain heavily relies on the constant blood flow for proper function. Two important mechanisms that contribute to the regulation of cerebral blood perfusion are autoregulatory behavior of cerebral vessels and functional hyperemia upon increased neuronal activity (11, 16, 20). HFD can negatively affect vascular function as demonstrated by increased myogenic tone and endothelial dysfunction in diet-induced as well as genetic models of obesity (7-8, 24, 32-33). The effect of a HFD on neurovascular coupling and cerebrovascular reactivity after an ischemic insult especially in the absence of metabolic abnormalities is unknown. To address this key deficit in our knowledge, the current study tested the hypotheses that HFD 1) impairs neurovascular coupling, 2) causes cerebrovascular dysfunction, and 3) worsens outcomes after cerebral ischemia even in the absence of obesity.
METHODS

Animals

This study was conducted in accordance with the National Institute of Health guidelines for the care and use of animals in research and approved by the Division of Laboratory Animal Services at the Georgia Health Sciences University. Male Wistar rats (Harlan Laboratories Inc., Indianapolis, IN; 4-5 wk old, n=64) were fed either an isocaloric control diet (CD, 10% fat) or HFD (45% fat from Research Diets, New Brunswick, NJ) for 8 wk ad libitum. Blood pressure was measured with tail cuff (Kent Scientific, Torrington, CT) and blood glucose levels were measured with a glucometer (Freestyle, Abbott Diabetes Care Inc., Alameda, CA).

Metabolic parameters

At sacrifice, blood was collected and processed for plasma analyses. Adipose tissue from the subcutaneous, peritoneal, and epididymal depots was collected and weighed separately. Total adiposity (all depots combined) was normalized to body weight and expressed as percent (%) of body weight. Plasma insulin (ALPCO Diagnostics, Salem, NH), triglycerides and cholesterol (Wako USA, Richmond, VA) were measured, respectively.

Measurement of functional hyperemia

Functional hyperemia was assessed 2 days prior to ischemia injury by measuring the cerebral blood flow (CBF) change in the somatosensory cortex upon whisker stimulation (21-22). Animals were anesthetized with ketamin/xylazine (100/10 mg/kg)
injection and trimmed contralateral whiskers were gently stroked at a frequency of 2.5 Hz using a cotton tip attached to a vortex. The PIM3 laser Doppler scanning system (LDS, Perimed Inc., Ardmore, PA) was programmed to scan an area covering somatosensory cortex, which is supplied by the MCA, without tissue contact. CBF changes were expressed as percentage (%) increase relative to resting levels.

**Brain slice preparation**

Parenchymal arteriole function was assessed using a well-established brain slice preparation (4-5, 15). After sacrifice, the brain was removed and 300 μm thick coronal slices were cut in ice-cold artificial cerebrospinal fluid (aCSF) containing 3 mM KCl, 120 mM NaCl, 1 mM MgCl2, 26 mM NaHCO3, 1.25 mM NaH2PO4, 2 mM CaCl2, 10 mM glucose and 0.4 mM L-ascorbic acid, equilibrated with 95% O2/5% CO2 (3). Ascorbic acid was added to reduce cell swelling associated with oxidative stress. An aCSF with identical composition was used for bath perfusion in all experiments, except for those assessing the effects of high external [K+] in which control aCSF contained 4.2 mM KCl, and KCl replaced NaCl to increase [K+] to 10 mM. Osmolality of aCSF was ~290 mosm/kgH2O. Following the slicing procedure, slices were kept at room temperature in aCSF equilibrated with 95% O2/5% CO2 (pH ~7.45) until used.

**Video microscopy**

Diameter changes in cortical arterioles (< 30 μm internal diameter) were recorded using an upright Zeiss Axioscope 2FS microscope (Carl Zeiss USA, Thornwood, NY) equipped with infrared Differential Interference Contrast (IR-DIC) optics, a water-
immersion objective and an EMCCD camera (iXon+885, Andor Tech, South Windsor, CT). Images were acquired at 1 frame/s, visualized and stored using IQ software (Andor Tech). The slices were perfused with aCSF (35 ± 2 °C) gassed with 95% O₂/5% CO₂, and were allowed to equilibrate for ≥ 10 min prior to beginning of recording. Only one arteriole per slice was recorded. Slices were perfused with the thromboxane A₂ receptor agonist U46619 to induce vasoconstriction, and test solutions were applied in the constant presence of U46619 after a stable preconstriction was attained. Vessels that did not respond to U46619 were not included in the analysis. Data from arteriolar diameter (IR-DIC) experiments were analyzed with custom software created by Dr. Adrian D. Bonev (Univ. of Vermont). Changes in the internal (luminal) diameter of arterioles were determined from averaged measurements taken from multiple points across the arteriolar lumen. Baseline diameter (represented as 100%) was determined during the first ~10 min of sampling, before any experimental stimulation. All arteriolar diameter values are expressed as percent relative to baseline. Vascular tone is expressed as “degree of constriction” relative to baseline.

Models of ischemia

Focal cerebral ischemia was induced by transient middle cerebral artery occlusion (MCAO) as previously described (10). Briefly, all animals were anesthetized with 2% isoflurane via inhalation. The right MCA was occluded for 3 h with a 19-21 mm 3-0 surgical nylon filament, which was introduced from the external carotid artery lumen into the internal carotid artery to block the origin of the MCA. The rectal temperature was maintained at 37°C with a heating pad (Fine Science Tools, Foster City, CA). The
cerebral perfusion was monitored with LDS to confirm successful occlusion or reperfusion. In a subset of animals, global cerebral ischemia (GCI) was induced (10 min occlusion/7 day reperfusion) as an alternative method of ischemia. For GCI, all animals (except sham control) underwent 4-vessel occlusion (4-VO) performed as described previously (43). Briefly, 24 h after electrocautery of the vertebral arteries, the common carotid arteries (CCAs) were occluded with aneurysm clips to induce 10 min forebrain ischemia. Animals which lost their righting reflex within 30 s and whose pupils were dilated and unresponsive to light during occlusion were selected for the experiments. The clips were then removed, and the blood flow through the CCAs was confirmed before the wound was sutured. The animals of the sham group underwent identical procedures except that the CCAs were not occluded. Rectal temperature was maintained at 36.5 to 37.5°C throughout the experiment with a thermal blanket.

**Isolated vessel studies**

At 24 h after focal cerebral ischemia, 2 mm-basilar artery segments were isolated and mounted in myograph for isometric tension recordings as described previously (27). Concentration-response curves to serotonin [5-hydroxytryptamine (5-HT), 1 nM to 100 µM], endothelin-1 (ET-1, 0.01 nM to 0.1 µM) and the stable analog of the endoperoxide prostaglandin H₂ (U46619, 0.1 nM to 10 µM) were performed to evaluate vascular contractility. Endothelium-dependent relaxation to acetylcholine (Ach, 1 nM to 1 µM) was assessed after vessels were constricted to 60% of the baseline tension with phenylephrine (PE). Sensitivity (EC₅₀) and maximum response (R_max) values were calculated from the respective concentration-response equations (27).
Evaluation of O-GlcNAcylation

O-GlcNAcylation-modified protein levels in the basilar arteries were determined by immunoblotting as previously described (28) using anti-O-GlcNAc antibody, CTD 110.6 (1:2000; Pierce Biotechnology, Rockford, IL). All membranes were stripped and re-blotted with anti-actin antibody to ensure equal protein loading.

Infarct, edema, and hemorrhagic transformation analysis

Brains from animals that died overnight after MCAO before sacrifice at 24 h were not processed for evaluation of ischemic injury but were included in the mortality data. The infarct volume was measured after 2,3,5-triphenyltetrazolium chloride (TTC) staining as previously described (10). Edema is reported as % increase in the ischemic hemisphere size vs. the contralateral hemisphere. After staining, the hemispheres were separated and deep frozen for tissue hemoglobin quantification with QuantiChrom kit (BioAssay Systems, Hayward, CA) (35). HT occurrence rate (presence of macroscopic bleeding) and severity (excess hemoglobin, Hb, µg/mg protein in the ischemic hemisphere) were reported.

TUNEL Staining

Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end (TUNEL) staining was performed on the free-floating coronal sections of GCI groups at 7 days after reperfusion using the In Situ Cell Death Detection Kit (Roche Diagnostics, Indianapolis, IN) as described previously (43). Samples were analyzed with a LSM510
Meta confocal microscope. For quantitative analyses, the number of TUNEL-positive cells per 250 µm length of medial CA1 pyramidal cell layer was counted bilaterally in 4 to 5 sections per animal to provide a single value for each animal. A mean ± SEM was calculated from the data.

**Behavioral measurements**

Neurobehavioral tests were assessed and scored in a blinded fashion by video recording before MCAO surgery and before sacrifice on each animal. The items tested in Bederson’s score included: 1) spontaneous ipsilateral circling, graded from 2 to 0; 2) hindlimb retraction and 3) forelimb flexion, graded from 1 to 0, respectively; and 4) resistance to push, graded from 1 to 0. Beam walking ability tested the stability of the animal traversing a 2.4 cm wide, 80 cm long beam and graded to 7 point scale method. Composite score was given by combining all the above tests with a greater score representing better neurological outcome. Grip strength was measured with a standard grip strength meter (Columbus Instrument, Columbus, OH). The rat was gently held with their forepaws grasping the pull-bar and then pulled back consistently. The digital recording obtained from 3 trials was averaged.

**Statistics**

Data were expressed as mean ± SEM or as scattered plots with median where appropriate. Contractile responses were calculated as a percentage of KCl (120 mmol/L)-induced contraction. Concentration-response curves were fitted using a nonlinear interactive fitting program (GraphPad Software Inc., La Jolla, CA) and two
pharmacological parameters were obtained: the maximal effect generated by the agonist (or $E_{\text{max}}$) and $-\log EC_{50}$ (or $pD_2$). Statistical analyses of $E_{\text{max}}$ and $pD_2$ values as well as infarct size, Hb, grip strength, % change in CBF were performed using Student’s t-test. HT occurrence was compared using Chi square test and neurological deficit by composite score was analyzed using Mann-Whitney test. For analysis of mortality, Fisher’s exact test was used. Values of $p<0.05$ were considered statistically significant.

RESULTS

Metabolic parameters

Eight weeks HFD significantly increased the body weight and adiposity without affecting plasma lipids (Table 1). Adipose tissue in all depots (subcutaneous, peritoneal and epididymal) was increased. There were no differences in blood glucose, blood pressure or plasma insulin levels.

Effect of HFD on cerebrovascular function

Effect of HFD on cerebrovascular function was assessed by several methods looking at vessels of different caliber. First, functional hyperemia was measured to evaluate the response of smaller arterioles by using the relative change in CBF upon whisker stimulation. As shown in Fig 1A and B, HFD animals displayed blunted change in CBF indicating impaired neurovascular coupling.

Next, the tone and relaxation properties of PAs were measured in slice preparations. We previously showed that the degree of tone in PAs dictates the polarity of the vascular response to vasoactive signals released by activated astrocytes with
decreased tone favoring constrictions and increased tone favoring dilations (3). To determine if the HFD induced any change in vascular tone, cortical PAs were exposed to 150 nM U-46619 to induce arteriolar constriction. While no statistical significant differences were achieved, arterioles from the HFD group showed lower baseline tone (23.9 ± 4.8%, n=11) compared to controls (35.7 ± 9.9%, n=6). The values of the control group were comparable to those previously reported by us in Sprague-Dawley rats fed chow diet (3). In agreement with our previous observations, reduced tone as that observed in the HFD group resulted in a reduced vasodilatory response to 10 mM K⁺ (Fig 1C).

Third, the contractile and dilatory responses of basilar arteries before and after focal ischemic injury were determined. There was no effect of HFD on these functions if the animals were not subjected to stroke (data not shown). However, when basilar arteries were tested at 24 h after MCAO, the concentration-response curves to several vasoconstrictors including 5-HT, ET-1, and U-46619 were left-shifted, indicating enhanced sensitivity, as well as greater maximum responses (Fig 2A-C). The endothelium-dependent relaxation was also significantly impaired in the HFD group (Fig 2D). O-GlcNAc levels in the basilar arteries of HFD-fed animals were significantly greater suggesting that this post-translational modification can be the underlying mechanisms of increased contractility in basilar arteries (Fig 3A and B).

**Effect of HFD on neurovascular injury after ischemia/reperfusion**

When focal ischemic injury was induced by 3 h MCAO/21 h reperfusion, the percent drop in CBF after occlusion (40±5% in CD and 38±4% in HFD compare to baseline) or
recovery after reperfusion (17±3% in CD and 20±11% in HF compare to occlusion) was similar in both groups, but infarct size was higher in the HFD group than in controls (Fig 4A). Mortality rate was 11% (2 out of 18) and 33% (6 out of 18) in CD and HFD groups, respectively (p=0.09). When ischemic injury was induced by 10 min global cerebral ischemia followed by 7 days reperfusion, mortality was 50% in the HFD group. Hippocampal CA1 sections were collected from animals that survived the surgery and TUNEL staining performed to access apoptotic cell death. There was no difference in apoptotic cell death between sections from control and HFD rats (Fig 4B). In the focal ischemia model, there was no difference in edema between the groups but the incidence of macroscopic HT as well as tissue hemoglobin levels were increased in the HFD group (Fig 5A-C).

**Effect of HFD on functional outcome after ischemia/reperfusion**

A composite score derived from Bederson’s score and beam walking showed that HFD rats performed poorer on the behavioral tests as compared to the CD group (Fig 6A, 7.8±1.3 in CD vs. 4.0±0.8 in HFD, *p<0.05). However, there was no difference on grip strength (Fig 6B, 1.11±0.07 kgF in CD vs. 0.90±0.08 in HFD, p=0.07).

**DISCUSSION**

This study provides novel information about the early impact of HFD on cerebrovascular function and stroke outcomes in the absence of overt metabolic changes. First, HFD impairs communication between neurons and penetrating arterioles even in the absence of an ischemic insult. Second, ischemic injury serves as a second
hit and causes large artery dysfunction in stroked HFD rats that is not otherwise detectable in HFD animals alone. Third, stroke in HFD-fed animals that do not have obesity or metabolic derangement worsens neurovascular injury and functional outcomes. Collectively, these data suggest that detrimental effects of HFD start early in the disease process and preventive measures should be implemented as early as possible.

HFD or obesity is a major risk factor for vascular dysfunction. It has been realized several decades ago that high intake of saturated fat in the diet significantly enhanced the development of the atherosclerotic and autoimmune lesions in aorta of the autoimmune-prone B/W mice, which were known to develop severe glomerulonephritis and vasculitis (12). Numerous studies thereafter have demonstrated that HFD impaired the structure and function and increased the lesion in different vascular beds (18, 31, 37, 42). Recent studies which focused on the cerebral vasculature have found that the dilator response to ACh was impaired in cerebral arterioles of HFD-fed apoE-/- mice (24) or in basilar artery of HFD-fed PPAR-γ knockdown mice (2). In the present study, we found that relatively short-term administration of nonatherogenic HFD impaired the ability of smaller arterioles to dilate, and altered the contractile and dilatory properties of basilar arteries only after ischemic injury. Interestingly, these detrimental changes in cerebrovascular function were in the absence of overt obesity. There is no definition of obesity in animal models as it clearly defined in humans as body mass index (BMI) over 30. A person has traditionally been considered to be obese if s/he is more than 20 percent over their ideal weight. In our animals total body weight increased by 10% and
this was mainly adipose mass. Thus, the changes we observed in this model are mainly the effect of HFD and not obesity *per se*.

Cerebral vascular function is closely regulated by the central nervous system activity, especially astrocytes which processes are in direct contact with both synapses and blood vessels (20). Previous reports demonstrated the contribution of astrocytes in neurovascular coupling through $K^+$ signaling (3, 9). In the current study, we evaluated if $K^+$-mediated vasodilation is disrupted following HFD treatment. We found that whisker stimulation-induced functional hyperemia (in vivo) and $K^+$-induced vasodilation (in vitro) are reduced in the HFD group. The data suggest that parenchymal arterioles from the HFD group had impaired vascular function. Given the lack of increased blood flow response following whisker stimulation, future studies addressing the role of astrocytes in activity-dependent vascular responses are needed to better define whether HFD only affected vascular function or if it also altered the activity of upstream mechanisms such as that of $K^+$ signaling by astrocytes.

In the current study we found no significant effect of HFD on surviving neuronal cell number in the GCI model even with different methods (NeuN staining, data not shown), which is consistent with another report that used 60 day Western high fat diet in Sprague Dawley rats and also found no effect of HFD on neuronal cell death or survival after GCI (1). While we did not assess functional endpoints after GCI in our study, the study by Arvanitidis et al. did assess functional outcome using the Morris Water Maze and found no significant effect of HFD, a finding consistent with the lack effect of HFD on neuronal cell death or survival in both our and their study. In a preliminary study, we utilized an even longer HFD period of 10 weeks, with the thought that a longer duration
may be needed to observe an effect in the GCI model. However, 10 week HFD also had no significant effect on neuronal cell death/survival after GCI. It is not clear as to why HFD increased neuronal damage in the FCI but not GCI model of cerebral ischemia. It is known that the pathophysiological mechanisms differ between the two models (e.g. a more delayed neuronal cell death occurring in vulnerable brain regions following GCI), which might contribute to the difference. In addition, the duration of ischemia between the two models (3h in FCI vs. 10 min in GCI) is also quite different, which might contribute to differences in effects. While the mechanisms underlying the differential effect of HFD on neuronal cell death/survival in the two ischemia models requires further study, the significant effect of HFD observed in the FCI model is of potential translational importance. This is especially true considering that, of the two ischemia models, the FCI (MCAO) model is generally accepted as the most translationally relevant model of ischemic stroke, as more than 75% of strokes in human involve occlusion of the middle cerebral artery.

Obesity is an independent risk factor and may affect other risk factors for stroke such as hypertension, diabetes, and hyperlipidemia. Experimental studies have showed that either HFD or genetically induced obesity was accompanied with increased cerebrovascular remodeling, promoted hypertension and increased infarct size in either transient or permanent focal ischemia models (7, 32). HFD-fed apoE-/- mice with hyperlipidemia also had increased infarct volume (23). However, there is another report which showed that one-month HFD had no effect on the cerebral ischemia outcome (26). In the present study, 8 weeks HFD resulted in significantly larger infarct volume after transient focal ischemia induced by suture occlusion of MCA, which is comparable
to previous reports. When a global ischemia model was employed, there was no difference in hippocampal neuronal death between the groups which was also reported by another group (1). These findings suggest that the duration of the diet and method of ischemia are important in the extent and localization of neuronal injury. While we do not know the potential mechanisms contributing to greater neurovascular injury and poor outcomes in our model, it is possible that proper regulation of cerebral perfusion after stroke contributes to unfavorable outcomes. Since large arteries like basilar artery contribute significantly to total cerebrovascular resistance and are major determinants of microvascular pressure, dysregulation of basilar artery function may worsen stroke injury by altering cerebral perfusion after stroke. In this context, it is highly possible that exacerbated release of vasoactive factors, such as endothelin-1 (ET-1), released into the circulation may be mediating this response. In a recent elegant study from Dr. Cipolla’s group, investigators showed that plasma from stroked hyperglycemic animals can affect cerebrovascular function through peroxynitrite generation and ET-1 (34). In another study, we showed that stroke decreases the dilatory ability of basilar arteries in regular chow-fed animals as compared to sham (6) and administration of atrasentan, an ET receptor antagonist, at reperfusion prevented this response. While the experimental conditions of that particular study were different, maximum relaxation observed in sham rats (~50%) was reduced to ~25% and this was normalized by ET receptor antagonism.

In the present study, we do not have a sham group, but it is possible that even control diet-fed animals may be displaying some degree of dysfunction and this is exacerbated in HFD. We have previously shown that high fat diet increases plasma ET-1 (38). Taken
together, the ET system may play a role in exacerbated stroke injury in our model and will be further pursued.

Along the same lines, based on our recent studies showing augmented O-GlcNAcylation increases vascular reactivity to ET-1 (28), we next assessed whether this post-translational modification is a potential downstream mechanism contributing to HFD-induced vascular dysfunction. As recently reviewed, there are multiple targets are regulated by O-GlcNAcylation in the vasculature (30). A positive correlation between phosphorylation of the MAPK cascade (ERK1/2 and p38) and nuclear O-GlcNAcylation was observed in fetal human cardiac myocytes exposed to high glucose (14). The previous work from our group has shown that O-GlcNAcylation-induced increased reactivity of aorta to phenylephrine was prevented by a PKC inhibitor or Rho-kinase inhibitor, respectively (17, 29). Our current finding of increased O-GlcNAc levels in basilar arteries of HFD-fed animals after stroke merits further studies to determine the mechanisms linking HFD to increased O-GlcNAcylation as well as linking O-GlcNAcylation to cerebrovascular dysfunction. In a preliminary study, we found that HFD alone caused a small increase (1.5 fold) in O-GlcNAc levels as compared to 4-fold increase observed in this study with HFD + MCAO. It is of great interest to determine whether blockade of increased O-GlcNAc levels prevents vascular dysfunction and improves stroke outcomes.

**Perspectives and Significance**

In the current study, the important findings of impaired neurovascular communication, large artery dysfunction and augmented neurovascular injury suggest
that even short term HFD without obesity or metabolic imbalance may be detrimental to the cerebrovasculature and exacerbate the response to cerebral ischemia. We recognize that there are limitations to this study such as evaluation of the outcome only at 24 h and inclusion of only male rats. Given that acute ischemic stroke dramatically increased in children and young adults, which positively correlated with increases in risk factors including obesity, lipid disorders and diabetes (13), further studies are warranted to explore the underlying mechanisms by which HFD worsens short and long term stroke outcome in both female and male animal models.
FUNDING SOURCES

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DISCLOSURE

None
REFERENCES


TABLE AND FIGURE LEGENDS

Figure 1. **Functional hyperemia is compromised in HFD.** (A) Tracing of changes in CBF as measured by laser Doppler directed at 2mm posterior and 5 mm lateral to the bregma during whisker stimulation demonstrate that HFD blunts neurovascular coupling (n=11/group). (B) Quantitative analysis of total change in cerebral blood flow (CBF) upon neuronal stimulation. (C) K⁺-induced relaxations of parenchymal arterioles in brain slices are decreased in HFD-fed animals (n=4/group). Values=mean ± SEM, *p<0.05.

Figure 2. **Effect of HFD on basilar artery function after focal cerebral ischemia/reperfusion.** HFD increased the contractile response to multiple agonists (A-C) and also reduced endothelium-dependent relaxation (D). Experimental values of contraction were calculated relative to the contractile response produced by KCl 120mM, which was taken as 100% (n=8/group). Values=mean ± SEM, *p<0.05.

Figure 3. **O-GlcNAcylation in basilar artery after focal cerebral ischemia/reperfusion.** An increase in total O-GlcNAc-protein content was seen in HFD fed group after MCAO (n=6/group). A representative Western blot image of O-GlcNAc-modified proteins and actin control are given in panel A and cumulative data is summarized in panel B. Representative images were selected from the same membrane, and splices are indicated by dashed lines. Values=mean ± SEM, *p<0.05, n=4.
Figure 4. Effect of HFD on neuronal injury in different models of cerebral ischemia. Focal ischemia (A) induced by a 3 h MCA occlusion/21 h reperfusion increased infarct size in HFD group but 10 min global ischemia (B) did not impact neuronal death in the hippocampus (n=10-18/group). Values are median in A, and mean ± SEM in B *p<0.05.

Figure 5. Effect of HFD on vascular function after focal cerebral ischemia/reperfusion. The balanced edema percentage (A) was not significantly higher but the occurrence rate and the severity of HT (B, C) determined by excess Hb in the brain was greater in the HFD group (n=18/group). Values are median in A, and mean ± SEM in B, *p<0.05.

Figure 6. Effect of HFD on functional outcomes after focal cerebral ischemia/reperfusion. A composite neurological deficit score includes Bederson’s score [including 1) spontaneous ipsilateral circling, graded from 2 to 0, 2) hindlimb retraction, 3) forelimb flexion, and 4) resistance to push, scored either 1 or 0, respectively] and beam walk graded from 1 to 7 was measured. (A) Composite score was lower in HFD-fed rats indicating poor outcome. (B) Grip strength was not affected. (n=18/group). Values are median in A, and mean ± SEM in B, *p<0.05.
**Table 1. Metabolic parameters of CD and HFD groups.** SBP: Systolic blood pressure. Values=mean ± SEM. *p<0.05.

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<th>CD (n=18)</th>
<th>HFD (n=18)</th>
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<td>Adiposity (% body weight)</td>
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<td>SBP (mmHg)</td>
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<td>115±3</td>
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Fig. 1

A

CBF (%) vs Time (sec)

whisker stimulation

CD
HFD

B

Δ CBF (% increase)

CD
HFD

C

Δ Diameter (%)

CD
HFD

*
Fig. 2

A

% Contraction (KCI) vs. 5-HT (Log [M])

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<td>HFD + MCAO</td>
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B

% Contraction (KCI) vs. ET-1 (Log [M])

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<td>HFD + MCAO</td>
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C

% Contraction (KCI) vs. U-46619 (Log [M])

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<td>HFD + MCAO</td>
<td>6.9±0.1*</td>
<td>41±5*</td>
</tr>
</tbody>
</table>

D

% Relaxation (PE) vs. ACh (Log [M])
Fig. 3

CD + MCAO  HFD + MCAO

O-GlcNAc

β-actin

```
CD+MCAO
HFD+MCAO

<table>
<thead>
<tr>
<th>Protein</th>
<th>CD+MCAO</th>
<th>HFD+MCAO</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-GlcNAc</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>β-actin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
```

*Significant difference
Fig. 5

A

![Edema (% of contralateral)]

- Edema (% of contralateral)
- CD
- HFD

B

![Δ Hb (μg/mg protein)]

- Δ Hb (μg/mg protein)
- CD
- HFD

C

![HT occurrence rate (%)]

- HT occurrence rate (%)
- CD
- HFD

4/18

10/18

(*)
Fig. 6

A

![Graph showing composite score for CD and HFD groups.](image)

B

![Bar chart comparing grip strength in CD and HFD groups.](image)