Maternal high-fat diet influences stroke outcome in adult rat offspring

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Abstract

Diet-induced epigenetic modifications in early life could contribute to later health problem. However, it remains to be established whether high-fat diet (HFD) consumption during pregnancy and the suckling period could predispose the offspring to stroke. The present study investigated the influence of maternal HFD on stroke outcome in adult offspring. Female Sprague–Dawley rats were fed a normal diet (5.3% fat) or a HFD (25.7% fat), just before pregnancy until the end of lactation. Male offspring were fed with the control diet or the HFD after weaning, to form four groups (control offspring fed with the control diet (C/C) or the HFD (C/HFD) and offspring of fat-fed dams fed with the control diet (HFD/C) or the HFD (HFD/HFD)). The offspring received middle cerebral artery occlusion on day 120 followed by behavioral tests (neurological deficit score, staircase-reaching test and beam-traversing test), and infarct volumes were also calculated. We found that the HFD/C rats displayed larger infarct volume and aggravated functional deficits (all $P < 0.05$), compared with the C/C rats, indicating that maternal fat-rich diet renders the brain more susceptible to the consequences of ischemic injury. Moreover, maternal HFD offspring displayed elevated glucocorticoid concentrations following stroke, and increased glucocorticoid receptor expression. In addition, adrenalectomy reversed the effects of maternal HFD on stroke outcome when corticosterone was replaced at baseline, but not ischemic, concentrations. Furthermore, expression of brain-derived neurotrophic factor (BDNF) in the ipsilateral hippocampus was decreased in the HFD/C offspring ($P < 0.05$), compared with the C/C offspring. Taken together, maternal diet can substantially influence adult cerebrovascular health, through the programming of central BDNF expression and the hypothalamic–pituitary–adrenal axis.

Keywords

- high-fat diet
- early life
- stroke
- corticosterone, brain-derived neurotrophic factor

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Introduction

Parental heredity and adult lifestyle are well known to contribute to development and health problems, but epidemiological and animal studies have suggested that specific environmental factors that a developing offspring experiences in early life may also have deleterious consequences on the health of adults (Barker 2004). Indeed, stress such as maternal separation or neonatal

bacterial challenge has been reported to increase adult susceptibility to obesity, insulin resistance, high blood pressure and cardiovascular disease (Gluckman & Hanson 2004, Craft 2006, Warner & Ozanne 2010, Liang *et al.* 2011). Moreover, it is known that challenges early in development can also alter the brain in a manner that makes it more susceptible to brain injury as an adult.

For example, brief mother–infant separation has been demonstrated to compromise functional recovery and long-term survival following stroke (Craft *et al.* 2006). It has also been reported that rat neonatal immune challenge alters adult responses to cerebral ischemia (Spencer *et al.* 2006).

The environment that offspring experiences in early life, including intrauterine and early postnatal environment, is highly influenced by maternal diet and metabolic status. Nowadays, the most common maternal dietary imbalance in many countries is the excessive intake of dietary fat. There is accumulating evidence supporting that perinatal overnutrition or high-fat diet (HFD) consumption induces health problems in adult offspring, such as the metabolic syndrome, hypertension and cardiovascular dysfunction (Khan *et al.* 2004, Alfaradhi & Ozanne 2011). Nevertheless, other studies have also reported that adult offspring of HFD-fed dams can have normal glucose tolerance and body composition (Miotto *et al.* 2013, Platt *et al.* 2014). More specifically, although numerous studies have indicated the cerebrovascular hazards of an increased intake of dietary fat in adults (Langdon *et al.* 2011) or beginning in childhood (Deutsch *et al.* 2009), it remains to be established whether HFD consumption during pregnancy and the suckling period can influence the cerebrovascular health of adult offspring.

Activation of the hypothalamic–pituitary–adrenal (HPA) axis is among the first measurable physiological responses to ischemic stroke (Slowik *et al.* 2002). Clinical studies have demonstrated that elevated post-stroke serum concentrations of cortisol are associated with increased morbidity and mortality (Christensen *et al.* 2004), while experimental evidence also suggests that elevated post-stroke corticosterone levels increase the infarct size in rodents (Balkaya *et al.* 2011). Thus, glucocorticoid exposure is a critical determinant of survival and functional outcomes following ischemic attack. Moreover, neuronal sensitivity to glucocorticoid, directly linked to glucocorticoid receptor (GR), is also an important factor determining the stroke outcome. It has been demonstrated that the response of HPA system activity to stress, including glucocorticoid concentrations (Tannenbaum *et al.* 1997) and GR expression (Sasaki *et al.* 2014, McNeilly *et al.* 2015), can be affected by dietary fat intake in adulthood or early life. In contrast, brain-derived neurotrophic factor (BDNF), which belongs to the neurotrophin family, plays an important role in various forms of neuroplasticity in the damaged brain (Kleim *et al.* 2003, Bekinschtein *et al.* 2008). Exposure to early maltreatment has been reported to induce long-lasting changes in the

methylation of the *Bdnf* gene in the prefrontal cortex in mice (Roth *et al.* 2009). Furthermore, Page *et al.* (2014) demonstrated that maternal fat-rich diet significantly decreases the levels of BDNF mRNA. Therefore, there is no reason to exclude the possibility that maternal HFD feeding may program the HPA system and BDNF expression and ultimately alter adult offspring susceptibility to ischemic stroke.

In the present study, we hypothesized that maternal HFD feeding compromises ischemic stroke outcome in adult offspring. To test this hypothesis, offspring of fat-fed dams were employed to establish the ischemic stroke model by the application of the middle cerebral artery occlusion (MCAO) procedure, and then histological and functional outcomes were determined. Additionally, comparisons were also made between maternal HFD offspring and animals fed with the HFD in adulthood. The further goal of the present study was to investigate whether the programming of BDNF expression, as well as the HPA system including glucocorticoid concentrations, neuronal sensitivity and GR expression, are associated with maternal HFD-induced alternation of stroke outcome.

Material and methods

Animals

Female Sprague–Dawley rats (aged 120–140 days), obtained from Wenzhou Medical University, were housed under controlled conditions (12 h light:12 h darkness, with lights on at 0700 h; temperature at $22 \pm 2^\circ\text{C}$) and provided with food and water *ad libitum*. The rats were fed with either a standard normal chow diet (5.3% fat (corn oil), 21.2% protein, 57.4% carbohydrate, 4.6% fiber; Mediscience Ltd, JiangSu, China) or a HFD (25.7% fat, 19.5% protein, 41.3% carbohydrate, 3.5% fiber; estimated fats: palmitic acid 4.5%, stearic acid 1.99%, palmitoleic acid 0.12%, oleic acid 6.86%, linoleic acid 2.58%, α -linolenic acid 0.25%, arachidonic acid 0.19%; Mediscience Ltd), for 10 days before mating and throughout pregnancy and lactation. The day of parturition was set as day 0, and litters were culled to eight pups per mother on day 1. Pups were kept with their mothers until weaning on day 21. Thereafter, weaned male rats were housed three per cage and fed the control diet or the HFD until day 120, to form four groups (control offspring fed with the control diet (C/C), offspring of fat-fed dams fed with the control diet (HFD/C), control offspring fed with the HFD (C/HFD) and offspring of fat-fed dams fed with the HFD (HFD/HFD)). Animal weight and food intake were recorded weekly in offspring after weaning.

In order to assess the impacts of maternal HFD on adult ischemic stroke outcomes, the offspring received MCAO in experiment 1: (1) C/C ($n=16$); (2) HFD/C ($n=16$); (3) C/HFD ($n=16$); (4) HFD/HFD ($n=16$). Another groups of offspring were used in experiment 2 to investigate the changes in the HPA profile following ischemic stroke in maternal HFD offspring: (1) C/C ($n=7$); (2) HFD/C ($n=8$); (3) C/HFD ($n=8$); (4) HFD/HFD ($n=8$). In experiment 3, neuronal sensitivity to corticosterone was determined by using adrenalectomized offspring that received high-dose corticosteroid replacement ((1) C/C ($n=8$); (2) HFD/C ($n=8$); (3) C/HFD ($n=8$); (4) HFD/HFD ($n=8$)) as well as low-dose corticosteroid replacement ((1) C/C ($n=7$); (2) HFD/C ($n=8$); (3) C/HFD ($n=8$); (4) HFD/HFD ($n=8$)). All animal procedures were performed in accordance with the Guidelines of the Chinese Council on Animal Care and approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University (Wydw2013-0110). All surgical procedures were carried out under ketamine anesthesia (100 mg/kg i.p.; Pharmacia and Upjohn) and xylazine (10 mg/kg i.p.; Bayer).

MCAO procedure

Transient focal cerebral ischemia was induced by 90 min of right MCAO followed by reperfusion as described previously (Longa *et al.* 1989), with minor modifications. Briefly, the right common carotid artery was exposed and dissected free of the vagus nerve. The right external carotid artery (ECA) and internal carotid artery (ICA) were also isolated. The ECA was then ligated at the distal end, which was cut off. A 4-0 nylon thread precoated with silicon was aseptically introduced into the right carotid artery in an anterograde fashion toward the carotid bifurcation. It was then directed distally up through the right ICA to a distance of ~ 20 mm from the carotid bifurcation to occlude the origin of the middle cerebral artery. After 90 min, the thread was withdrawn for reperfusion. Cerebral blood flow reduction of 80% of baseline after the onset of MCAO was confirmed in ischemic animals by laser Doppler flowmetry (Moor Instruments, Cambridge, UK).

Determination of stroke volume

The rats were decapitated at 1 week after MCAO, and brains were removed and placed in a metallic brain matrix for tissue slicing. The brain was dissected into coronal sections of 2 mm, then immersed sequentially in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in

normal saline at 37 °C for 10 min, and then fixed in 10% formalin for 10 s. The infarct area in the brain section was measured by National Institutes of Health Image software (Image J, NIH, Bethesda, MA, USA; <http://rsb.info.nih.gov/ij/>). The ischemic lesion volume was calculated as the sum of the infarct areas $\times$ thickness (2 mm), and was expressed as a percentage of the contralateral hemisphere.

Behavioral assessments

Behavioral testing was performed 1 day before and 1, 2, 3 and 4 weeks after MCAO in experiment 1 and 1 week after the surgery in experiment 3 by an investigator who was blind to the experimental groups.

Neurological deficit score Rats were evaluated using a modified neurological severity score (mNSS; Chen *et al.* 2001). The mNSS was a composite of motor (muscle status and abnormal movement), sensory (visual, tactile and proprioceptive), reflex and balance tests. Neurological function is graded on a scale of 0 to 18 (normal score 0; maximal deficit score 18). In the severity scores of injury, 1 point was awarded for the inability to perform the test or for the lack of a tested reflex; higher scores indicate more severe injuries.

Staircase-reaching test Two weeks prior to the surgery, offspring were mildly food restricted (90–95% *ad libitum* weight) and trained to reach for 45 mg food pellets (test diet) in the Montoya staircase task (Montoya *et al.* 1991, Langdon *et al.* 2011). Training occurred over a 10-day period with two 15 min trials/d until the animals successfully reached a minimum performance criterion of obtaining $\geq 12/21$ pellets and a standard deviation of < 2 over a period of eight trials with one forepaw (most animals normally obtain $> 17/21$ pellets with their 'good' forepaw). On post-surgery testing, reaching success was again assessed for two trials/d for two consecutive days and average success over these 2 days are presented. Each staircase should be modified to accommodate rats of varying sizes, ranging from 200 to 750 g.

Beam-traversing test Animals were trained to traverse a tapered beam (length 160 cm; widest portion 6 cm; narrowest portion 2 cm) with a ledge (1.5 cm in width located 2 cm below the top of the beam) elevated 75 cm from the floor to enter a darkened chamber. Performance was videotaped and analyzed by calculating the number of slips (forelimbs and hindlimbs separately). Steps onto the ledge were scored as a full slip (1.0) and if the limb touched

the side of the beam it was scored as a half-slip (0.5). Baseline performance was obtained prior to the surgery and the animals were assessed again after the surgery. The average number of slips over three trials was calculated at each time point and used for statistical analyses (Clarke *et al.* 2009, Langdon *et al.* 2011).

Determination of BDNF protein levels

The rats were decapitated and their brains were rapidly removed for the dissection of the ipsilateral hippocampus in experiment 1. The brain tissue was flash-frozen in isopentane and placed in -80°C until analysis. BDNF protein levels were quantified using a commercially available sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA). The tissue was homogenized in lysis buffer (100 mM Tris pH 7.0, 1 M NaCl, 4 mM EDTA, 0.1% sodium azide, 2% BSA, 2% Triton-X100, 5 μg aprotinin/ml, 0.1 μg pepstatin/ml A, 0.5 μg antipain/ml) at 20 volumes of the wet tissue weight (mg). The homogenate was centrifuged at 14 000g for 30 min at 4°C . The supernatant was removed and the amount of BDNF protein in each sample was analyzed in duplicate by ELISA. BDNF levels were normalized as pg/mg total protein.

Head screws and intravenous cannulation

The rats were implanted with intravenous cannulation for collection of blood samples without interruption in experiment 2, and fasted blood glucose/insulin levels were also determined. To allow the anchorage of a metal spring to protect exteriorized chronically implanted intravenous catheters, the animals were fitted with a tether screw on the skull. The rats were fitted with indwelling cardiac catheter via the left jugular vein, as described previously (Lin *et al.* 2011). The catheter was exteriorized at the back of the head and secured to a cranial attachment; the rats were fitted with a 30 cm-long metal spring tether (Instec Laboratories Inc., Boulder, CO, USA). The distal end of the tether was attached to a fluid swivel (Instec Laboratories), which allowed the rat freedom to move around the enclosure. The experiment commenced 4 days later.

Determination of fasted blood glucose/insulin levels

Before the HPA activity experiment, the rats were fasted overnight but were provided with water *ad libitum* throughout. Blood samples (0.15 ml) were collected via the cardiac catheter and centrifuged immediately, and plasma was separated and frozen at -70°C for later

determination of glucose (HK/G6P-DH enzymatic UV test; Roche Diagnostic Systems) and insulin (DGR Instruments, Marburg, Germany).

HPA activity following stroke

After 3 days of recovery, the rats were attached via the cardiac catheter to the blood sampling system. Basal blood samples (0.15 ml) were collected and centrifuged immediately, and plasma was separated and frozen at -20°C for later assay to determine corticosteroid concentrations. After controlled blood sampling for baseline corticosteroid levels, the animals were exposed to MCAO, and blood samples were collected at 1, 2, 3, 4, 6, 9, 12, 18, 24, 36 and 48 h after the onset of MCAO. The procedure was performed between 1000 and 1300, avoiding the elevated basal corticosteroid levels and peak HPA responses to stress associated with the dark phase of the cycle.

Determination of corticosterone concentrations

Total corticosteroid concentration was determined in plasma by RIA using a commercially available rat corticosterone kit (North Bio, Beijing, China). The sensitivity of the assay was 7.5 ng/ml. The intra-assay variation was 10.1% and the inter-assay variation was 14.2%.

Adrenalectomy and corticosterone replacement

To standardize peri-ischemic corticosteroid concentrations in offspring in experiment 3, a subset of rats were adrenalectomized and implanted subcutaneously with corticosterone pellets (Sigma) 48 h prior to ischemia. Behavioral tests were performed 1 week after the surgery, including the assessments as described above. The low-dose pellet (1 $\times$ 50 mg) and the high-dose pellet (2 $\times$ 100 mg) produced circulating corticosterone levels of approximately 170 and 380 ng/ml respectively. The pellets produced corticosteroid concentrations within the baseline (low dose) and post-ischemic (high dose) ranges observed in experiment 2. All adrenalectomized animals were provided with a water bottle containing 1% saline in addition to tap water.

Tissue collection and quantitative RT-PCR

Expression of GR mRNA in the hippocampus was determined by real-time quantitative RT-PCR. The rats were decapitated, and the hippocampus was collected and stored at -80°C until RNA extraction. The hippocampus

(0.1 g) was homogenized in 1 ml TRIzol reagent (Sigma–Aldrich) and total RNA was isolated. For the quantitative PCR, the following primers were used: GR – (sense) 5'-ACTGCAGGAGTCTCACAAAGACAC-3', (antisense) 5'-TGTCTGGAAGCAGTAGGTAAGGA-3'; 28S rRNA – (sense) 5'-TTGAAAATCCGGGGGAGAG-3', (antisense) 5'-ACATTGTTCCAACATGCCAG-3'. The LightCycler (Roche Biochemicals) was used for real-time quantitative analysis of GR mRNA expression. cDNA was synthesized using reverse transcriptase (Sigma–Aldrich) after RNA quality was verified by a spectrometer. The GR cycling conditions consisted of an initial single cycle of 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 55 °C and 60 s at 72 °C. The 28S rRNA reaction conditions were 10 min at 95 °C for one cycle, then 15 s at 95 °C, 10 s at 54 °C and 5 s at 72 °C for 28 cycles. Preliminary experiments were conducted to prepare the PCR products used to generate standard curves in real-time PCR. GR mRNA was quantified, against a standard curve of samples containing known GR PCR product concentrations, using the LightCycler program. 28S rRNA was quantified as a reference gene against a separate standard curve of samples containing known concentrations of the 28S rRNA product. The PCR product for GR mRNA was sequenced and analyzed using an ABI Stepone system (Applied Biosystems, Inc.). The values of GR mRNA were expressed as a ratio of mRNAs for GR and 28S rRNA.

Statistical analysis

All quantitative data are presented as mean $\pm$ S.E.M. Statistical comparisons in offspring body weight were made by repeated-measures ANOVA with Bonferroni correction for multiple comparisons. Behavioral data in experiment 1 were evaluated by repeated-measures ANOVA with a two-tailed independent *t*-test with Bonferroni correction for multiple comparisons used for *post hoc* comparisons. The statistical significance of brain infarct volume and BDNF levels in experiment 1, as well as behavioral data and GR mRNA levels in experiment 3, were evaluated by a one-way ANOVA followed by Dunnett's *t*-test. Corticosterone levels in experiment 2 were assessed by ANOVA followed by Dunnett's *t*-test to assess statistically significant differences within the same strain at different time points, as well as between different strains at the same time point. Integrated hormone levels were determined with the trapezoidal rule, and data were expressed over time of sampling. $P < 0.05$ was considered statistically significant.

Results

Offspring body weight

Repeated-measures ANOVA revealed that body weight was similar between the groups before week 13 (Fig. 1). The C/HFD animals showed increased body weight compared with the C/C animals ($P < 0.05$) from day 91 onward. Nevertheless, no significant difference was observed between the HFD/C and C/C offspring during the experiment (all $P > 0.05$). In addition, there was also no significant difference between the C/HFD and HFD/HFD offspring during the experiment (all $P > 0.05$).

Maternal HFD increased brain infarct volume

Seven rats randomly selected from each group in experiment 1 were killed 1 week after the surgery, and the extracted brains were analyzed for ischemic injury. The borders of the TTC stain enclosing the white infarct area were readily distinguishable in contrast to the red color of the normal area. The brain infarct volume in the experimental groups is shown in Fig. 2. Most importantly, the infarct volumes observed in the HFD/C group, occupying $42.17 \pm 5.75\%$ of the contralateral hemisphere, were significantly larger than $29.35 \pm 4.51\%$ of the C/C rats ($P < 0.05$). There was no significant difference in the infarct volume between the HFD/C and C/HFD groups ($P > 0.05$). The infarct volume, occupying $51.27 \pm 6.55\%$ of the contralateral hemisphere, was observed in the HFD/HFD group, but without significant difference when compared with those of the HFD/C or C/HFD offspring.

Figure 1

Growth rate in the male offspring of HFD-fed dams and normal diet (ND)-fed dams, maintained on the HFD or ND after weaning. HFD feeding in adult offspring significantly increased the body weight from day 91 onward. * $P < 0.05$ C/HFD group vs HFD/C group at the same time period.

Figure 2

Percent infarct relative to the contralateral hemisphere (mean $\pm$ S.E.M.) and representative examples. The HFD/C offspring had larger infarct sizes than the C/C rats, but had similar infarct volume to the C/HFD rats. No difference in infarct volume was observed between the HFD/C and HFD/HFD offspring. * $P < 0.05$ vs C/C group.

Effects of maternal diet on functional deficits following stroke

The influence of the diet on mNSS points following MCAO in the experimental groups is shown in Fig. 3. There were no significant differences in mNSS assessment between the groups before the MCAO surgery (all $P > 0.05$). Repeated-measures ANOVA of the animals' mNSS test revealed no effect of time ($P > 0.05$) but a significant effect of condition ($F(3,24) = 14.46$; $P < 0.01$) and no interaction ($P > 0.05$). Most importantly, *post hoc* analysis revealed that the deficit score was significantly

increased in the HFD/C group ($n = 9$) post-ischemia, when compared with the C/C group ($n = 9$, all $P < 0.05$). However, there was no significant difference in mNSS points between the C/HFD ($n = 9$) and HFD/C groups after the MCAO procedure (all $P > 0.05$). In addition, the deficit score of the HFD/HFD group ($n = 9$) was increased significantly after the surgery, compared with that of the C/C group (all $P < 0.05$), but showed no significant difference with the HFD/C and C/HFD groups (all $P > 0.05$).

Repeated-measures ANOVA of the animals' staircase performance also revealed no effect of time ($P > 0.05$) but a significant effect of condition ($F(3,24) = 17.41$; $P < 0.01$) and no interaction ($P > 0.05$). *Post hoc* analysis revealed that the reaching success in the staircase-reaching test was significantly decreased in the HFD/C group ($n = 9$), when compared with the C/C group ($n = 9$), on 7 and 14 days ($P < 0.05$) but not 21 and 28 days ($P > 0.05$) after the ischemic attack. This was unlikely due to motivational factors because the animals reached similarly with their contralateral ('good') paw prior to ischemia ($P > 0.05$) and with their ipsilateral (unaffected) paw post-ischemia ($P > 0.05$). However, there was no difference in the staircase performance between the HFD/C and C/HFD offspring ($n = 9$; $P > 0.05$).

As for the beam-traversing test, there were no significant differences in beam-traversing abilities between the groups prior to the MCAO procedure ($n = 9$ for each group; all $P > 0.05$). Repeated-measures ANOVA of the animals' beam-traversing performance showed no effect of time ($P > 0.05$) but a significant effect of condition ($F(3,24) = 13.16$; $P < 0.01$) and no interaction ($P > 0.05$). *Post hoc* analysis revealed that the beam-traversing ability was significantly impaired in the HFD/C group, when compared with the C/C group, only 1 week ($P < 0.05$) but

Figure 3

Effects of maternal diet on functional deficits following stroke, including mNSS points (A) in neurological deficit score, reaching success (B) in the staircase-reaching test and beam-traversing ability (C) in the beam-traversing test. Increased mNSS points (A), reduced reaching success (B) and

reduced beam-traversing ability (C) were observed in the HFD/C offspring, compared with the C/C rats. There was no significant difference in functional deficits between the HFD/C and C/HFD rats following stroke. * $P < 0.05$ HFD/C group vs C/C group at the same time period.

Figure 4

Effects of maternal diet on BDNF expression in the hippocampus of male offspring. The HFD/C offspring had reduced BDNF levels than the C/C rats, while the expression was similar between the HFD/C and C/HFD rats. No difference in hippocampal BDNF concentrations was observed in the HFD/C and HFD/HFD offspring. * $P < 0.05$ vs C/C group.

not 2–4 weeks ($P > 0.05$) after the onset of MCAO. There was also no significant difference in the beam-traversing performance between the HFD/C and C/HFD offspring after ischemic stroke (all $P > 0.05$). In addition, the beam-traversing performance of the HFD/HFD group was increased significantly 1 week after the surgery, compared with that of the C/C group ($P < 0.05$).

BDNF concentrations in the hippocampus

BDNF levels in the ipsilateral hippocampus in the experimental groups are shown in Fig. 4. The hippocampal BDNF concentration in the HFD/C rats ($n = 9$), with a group mean $\pm$ s.e.m. of 3.07 ± 0.43 pg/mg, was significantly decreased ($P < 0.05$), compared with 4.91 ± 0.51 pg/mg in the C/C group ($n = 9$). There was no significant difference in hippocampal BDNF levels between the C/HFD ($n = 9$) and HFD/C groups ($P > 0.05$). Moreover, compared with that of the C/HFD or HFD/C offspring, hippocampal BDNF levels in the HFD/HFD offspring ($n = 9$), with a group mean $\pm$ s.e.m. of 2.88 ± 0.39 pg/mg, displayed no significant changes after the onset of MCAO (all $P > 0.05$).

Determination of fasted glucose/insulin levels

After fasting overnight, mean blood glucose levels for the C/C, HFD/C, C/HFD and HFD/HFD groups were 4.19 ± 0.56 , 4.39 ± 0.61 , 4.47 ± 0.59 and 4.75 ± 0.61 mmol/l respectively, while insulin levels for each group were 1.09 ± 0.24 , 1.71 ± 0.62 , 2.12 ± 1.08 and 2.34 ± 0.79 mmol/l

respectively. No differences in plasma glucose or insulin concentrations were observed between the groups.

Post-stroke concentration of corticosterone

Basal plasma corticosteroid concentrations, as well as their responses during and up to 48 h after the onset of ischemic stroke are shown in Fig. 5. Basal plasma corticosteroid levels obtained before the MCAO procedure were all similar in the experimental groups (all $P > 0.05$). Compared with that of the C/C group ($n = 7$), the corticosterone levels were significantly increased in the HFD/C group ($n = 8$), from 24 h after the onset of MCAO to the end of the experiment (all $P < 0.05$). However, compared with the C/C rats, the C/HFD ($n = 8$) and

Figure 5

Effects of maternal HFD on HPA profile following stroke. (A) The HFD/C offspring had increased corticosterone levels than the C/C rats, from 24 h after the onset of MCAO to the end of the experiment. Compared with the C/C rats, the C/HFD and HFD/HFD offspring displayed increased corticosterone concentrations from 16 h after the onset of MCAO. (B) The HFD/C rats had increased integrated levels of corticosteroid than the C/C rats. * $P < 0.05$ HFD/C group vs C/C group at the same time period. # $P < 0.05$ vs C/C group.

Figure 6

Histological and functional injury in offspring that were adrenalectomized and replaced with high-dose corticosterone pellets on the 7th day after stroke, including infarct volume (A), mNSS points (B) in neurological deficit score, reaching success (C) in the staircase-reaching test and beam-traversing ability (D) in the beam-traversing test. (A) The HFD/C offspring

had larger infarct sizes than the C/C rats. Increased mNSS points (B), reduced reaching success (C) and reduced beam-traversing ability (D) were observed in the HFD/C offspring, compared with the C/C rats.

* $P < 0.05$ vs C/C group.

HFD/HFD offspring ($n=8$) also displayed increased corticosterone concentrations from 16 h after the onset of MCAO (all $P < 0.05$). In addition, there was no significant difference in the levels of corticosterone between the HFD/C and C/HFD groups ($P > 0.05$). Overall, the integrated levels of plasma corticosteroid were significantly increased in the HFD/C offspring ($P < 0.05$), when compared with the C/C rats. Moreover, compared with the C/HFD or HFD/HFD animals, the HFD/C group displayed the similar integrated levels of plasma corticosterone (all $P > 0.05$).

Neuronal sensitivity to corticosterone

The high-dose corticosterone replacement (producing approximate blood plasma concentrations of 380 ng/ml) reproduced in the offspring similar post-stroke outcomes in experiment 1. The HFD/C group ($n=8$) had a significantly larger mean infarct size than the C/C group ($n=8$; $P < 0.05$). The adrenalectomized HFD/C and C/HFD offspring ($n=8$) had a similar infarct volume after the high-dose corticosterone replacement ($P > 0.05$; Fig. 6A).

As for mNSS points, the deficit score was significantly increased in the HFD/C group, when compared with the C/C group on the 7th day after the onset of MCAO ($P < 0.05$; Fig. 6B). Moreover, compared with the C/C group, the reaching success in the staircase-reaching test and beam-traversing ability were significantly decreased in the HFD/C group (all $P < 0.05$). Nevertheless, compared with the C/HFD or HFD/HFD group ($n=8$), the HFD/C rats displayed similar performances in the behavioral assessments (all $P > 0.05$).

In contrast, when the adrenalectomized offspring were implanted with low-dose corticosterone pellets (producing approximate blood plasma concentrations of 170 ng/ml), no significant difference was observed in infarct size ($P > 0.05$; Fig. 7A) and mNSS points ($P > 0.05$; Fig. 7B) between the HFD/C ($n=8$) and C/C offspring ($n=7$). However, the HFD/HFD offspring ($n=8$) had larger infarct sizes and lower mNSS points than the C/C rats. Moreover, compared with the C/C group, the reaching success in the staircase-reaching test and beam-traversing ability were significantly decreased in the HFD/C group (all $P < 0.05$).

Figure 7

Histological and functional injury in offspring that were adrenalectomized and replaced with low-dose corticosterone pellets on the 7th day after stroke, including infarct volume (A), mNSS points (B) in neurological deficit score, reaching success (C) in the staircase-reaching test and beam-traversing ability (D) in the beam-traversing test. (A and B) The HFD/C

offspring had similar infarct sizes and mNSS points to the C/C rats respectively. (C and D) Reduced reaching success (C) and reduced beam-traversing ability (D) were observed in the HFD/C offspring, compared with the C/C rats. * $P < 0.05$ vs C/C group.

Figure 8

GR mRNA expression in the hippocampus of the offspring. The values are expressed as a ratio of GR mRNA and 28S rRNA (mean $\pm$ S.E.M.). The HFD/C offspring had an increased expression of GR mRNA than the C/C rats, while the expression was similar between the HFD/C and C/HFD rats. No difference in GR mRNA expression was observed in the HFD/C and HFD/HFD offspring. * $P < 0.05$ vs C/C group.

GR mRNA expression in the hippocampus

There was a significant increase in GR mRNA expression in the hippocampus of the HFD/C offspring, in comparison with the C/C group ($P < 0.05$; Fig. 8). In the hippocampus, no significant differences in GR mRNA expression were observed between the HFD/C and C/HFD offspring ($P > 0.05$). Moreover, compared with the HFD/C or C/HFD rats (all $P > 0.05$), the HFD/HFD group showed no significant difference in the hippocampal GR mRNA expression.

Discussion

Nutritional status during critical periods of early life has important influences on development, while modification of maternal diet has further been demonstrated to have consequences on later health of the offspring, changing their responses to environmental challenges and thus their predisposition to disease. The present study examined the stroke outcomes in a rat model of maternal fat dietary regimen, initiating just before pregnancy until the end of lactation. We found that, similar to HFD consumption in adulthood, fat-rich diet feeding during rat pregnancy and lactation also renders the brain more susceptible to the consequences of ischemic injury, supported by the evidence showing that maternal HFD increases functional deficits and infarct volume in adult offspring following focal ischemia. These results are

consistent with other data showing that a fat-rich diet during rat pregnancy and suckling induces vascular endothelial dysfunction, hypertension and cardiovascular dysfunction in adult offspring (Khan *et al.* 2003, 2005, Gray *et al.* 2015). Nevertheless, consistent with previous research (Khan *et al.* 2003, Elahi *et al.* 2009), the present study also demonstrated that maternal HFD had no effect on body weight and fasted glucose/insulin levels, indicating that the aggravated stroke outcome is not due to the changes in the mentioned factors.

The importance of the early-life challenges (e.g. stress) in determining the susceptibility to brain injury in adult offspring has been demonstrated in several animal models. Craft and his colleagues reported that brief mother–infant separation, regarded as psychological stress, can affect stroke recovery and survival, by increasing post-stroke pro-inflammatory cytokine expression and edema in mice (Craft *et al.* 2006). Moreover, rat neonatal immune challenge alters responses to cerebral ischemia and seizure susceptibility in adult (Spencer *et al.* 2006, Galic *et al.* 2008). The present study expands on these results by demonstrating that maternal HFD feeding, regarded as a metabolic stress in the early environment, can also substantially influence adult cerebrovascular health. Consistently, maternal HFD consumption also confers susceptibility to mental health and behavioral disorders in offspring, such as depression, anxiety, impairments in social behavior, cognitive deficit, reward-based behaviors and attention-deficit hyperactivity disorder in later life (Raygada *et al.* 1998, Bilbo & Tsang 2010, Sullivan *et al.* 2011, 2015, Giriko *et al.* 2013, Mendes-da-Silva *et al.* 2014). Additionally, we demonstrated that maternal fat-rich diet influences adult depressive disorder response to stressful challenge (Lin *et al.* 2015). Taken together, modification of maternal diet has profound effects on programming many aspects of physiology and behavior in offspring. Nevertheless, it was surprising that no differences were observed in any measure between the C/HFD and HFD/HFD groups, indicating that maternal and adult HFD feeding may share similar mechanisms. It was further supported by the evidence showing that there was no significant difference in infarct volume and functional recovery in the HFD/C and C/HFD offspring. However, the underlying mechanisms of invalidity of maternal HFD affecting the offspring fed with the HFD in adulthood require more investigations.

Functional recovery after stroke can potentially be induced by stimulation of endogenous neurogenesis (Zhang & Chopp 2009), which has been identified in the subgranular zone of the hippocampus. BDNF is an

important neurotrophic factor that stimulates adult neurogenesis and enhances the appearance and migration of new neurons in the subgranular zone. Thus, BDNF in the hippocampus plays an important role in neurotrauma repair and function recovery following stroke (Wu *et al.* 2004, Ploughman *et al.* 2009). The present study showed that the maternal HFD offspring exhibited reduced expression of BDNF in the hippocampus. Roth and his colleagues reported that exposure to early maltreatment can induce long-lasting changes in the methylation of the *Bdnf* gene in the prefrontal cortex and increases adult anxiety levels in mice (Roth *et al.* 2009). More specifically, it has been demonstrated that maternal obesity impairs hippocampal BDNF production and spatial learning performance in young offspring (Tozuka *et al.* 2010). To summarize, these results indicate that maternal HFD-induced aggravated histological injury and functional deficits following stroke are mediated, at least partially, by the reduced BDNF expression in the brain. Moreover, consistent with previous studies (Molteni *et al.* 2002, Wu *et al.* 2004), we also found that HFD consumption in adulthood decreases the hippocampal BDNF levels. Additionally, maternal HFD has also been demonstrated to impair hippocampal development of offspring, promoting decreased neuronal differentiation, and Notch 1 signaling in this brain region may be involved in the process (Mendes-da-Silva *et al.* 2015).

Glucocorticoid exposure is a critical determinant of stroke outcome. The present study demonstrated that the HFD offspring displayed elevated post-stroke corticosteroid levels, initiating 24 h after the onset of MCAO, without alteration of basal HPA activity. Moreover, increased fat intake during lactation modifies HPA responsiveness in developing rat pups (Trottier *et al.* 1998). Therefore, maternal HFD may program post-stroke corticosterone levels and subsequently affect the stroke outcome. Nevertheless, it has been reported that brief maternal separation mice experienced a dampened corticosteroid response to ischemia initially (Craft *et al.* 2006). The conflicting data presented above may result from the use of different animal species, different experimental models and different corticosterone measurement time points after the onset of MCAO. Although Trottier *et al.* (1998) reported that the effects of the HFD on stress responsiveness are mediated by changes in leptin exposure during development, the underlying mechanisms remain to be elucidated. Therefore, more work will be required to identify the changes in HPA regulatory mechanisms induced by maternal HFD.

Consistent with a previous study (Craft *et al.* 2006), our study showed that adrenalectomy reverses the effects of maternal metabolic stress on stroke outcome when corticosterone is replaced at baseline, but not ischemic, concentrations; thus, maternal HFD offspring are more sensitized as adults to the detrimental effects of elevated corticosterone concentrations during ischemia. Glucocorticoid sensitivity during ischemia may be directly linked to its receptor expression, which is extensively distributed throughout the brain (Adams *et al.* 2003). These results indicate that maternal diet may affect the distribution of GR in the brain. Indeed, the present study showed that maternal HFD increases the GR expression in the hippocampus. Consistently, previous research also demonstrated that brief maternal separation during neonatal development leads to increased expression of GR (Ladd *et al.* 2004). The surplus of GR increases the efficiency of the negative feedback regulation of the HPA axis during an acute stressor (Meaney *et al.* 1991), ultimately minimizing exposure to stress. This might provide a possible explanation for the similar concentration of corticosterone between the HFD/C and C/C offspring in the acute phase of ischemic stroke. Thus, maternal HFD offspring displayed not only elevated glucocorticoid concentrations following stroke but also increased neuronal sensitivity to corticosterone; the exposure to glucocorticoid makes them more susceptible to neuronal injury. Nevertheless, it has to be mentioned that, although no significant difference was observed in infarct size and mNSS points when the adrenalectomized offspring were implanted with low-dose pellets, there is a similar trend with high-dose supplementation. Therefore, we could not exclude the possibility that because of an increased expression of GR mRNA in the hippocampus, the adjustment of plasma corticosterone is not sufficient for reversing the adverse effects of HFD. The underlying mechanisms of invalidity of low-dose corticosterone supplementation affecting the stroke outcome in adrenalectomized maternal HFD offspring require more investigations.

In summary, these data suggest that a fat-rich diet during pregnancy and lactation can substantially influence offspring cerebrovascular health in adulthood. This study clearly demonstrated, for the first time, that maternal diet and metabolic status in early life can drastically affect the adult sensitivity to ischemic injury by altering the HPA axis and the BDNF system. Nevertheless, more studies need to be conducted to characterize the mechanisms by which maternal HFD influences the neuroendocrine responses and neurotrophin expression.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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