Maternal high fat diet programs stress-induced behavioral disorder in adult offspring

ChengCheng Lin a, Bei Shao b, Huanjie Huang b, YuLei Zhou b, YuanShao Lin b,a

a Department of Surgery Laboratory, First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
b First Department of Neurology, First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

HIGHLIGHTS
- The effects of maternal HFD on offspring behavior did not persist into adulthood.
- Maternal HFD sensitizes offspring to the detrimental effects of stress on behavior.
- CUMS-treated offspring of HFD-fed dams have increased CGRP levels in hippocampus.
- Central infusion of CGRP antagonist produced antidepressant effect in HFD + CUMS rats.
- Maternal HFD attenuate the habituation of HPA profile responses to repeated stress.

ABSTRACT
Early life exposure to specific environmental factors can contribute to development of behavioral disorders in adulthood. Although maternal high fat diet (HFD) consumption during the perinatal period has been reported to program offspring behavior, the underlying mechanisms remain to be elucidated. The present study was designed to evaluate the influence of maternal HFD on offspring behavior under nonstressed and stressful conditions, using male Sprague-Dawley offspring, which mothers were fed with HFD or normal diet (ND), receiving chronic unpredictable mild stress (CUMS) in the adulthood. We found that although the detrimental effects of maternal HFD consumption on offspring depressive behavior did not persist into adulthood, it markedly aggravated the behavioral disorder response to stressful challenge in adult offspring. Moreover, calcitonin gene-related peptide (CGRP) concentration in CSF and hippocampus were increased in the HFD + CUMS rats, compared to the ND + CUMS subjects. Another separate groups were fitted with intracerebroventricular (icv) cannulae. Central infusion of αCGRP8–37, a CGRP antagonist, produced antidepressant effects in HFD + CUMS rats, implying that the programming of maternal HFD on offspring behavior responses to stress may be mediated partially by endogenous central CGRP signaling. Moreover, we found that maternal HFD significantly exacerbated HPA profile response to acute restraint stress and attenuated the habituation of HPA responses to repeated restraint stress, suggesting that maternal HFD may program the changes of HPA-regulatory mechanisms. Overall, our findings suggest that maternal HFD influence adult depressive disorder response to stressful challenge, through the modulation of endogenous central CGRP signaling and HPA-regulatory components.

1. Introduction
The parental heredity and adult lifestyle are well-known to contribute to developmental and health problems, but epidemiological and animal studies suggest specific environmental factors that a developing offspring experiences during the perinatal period also play an important role in programming many aspects of physiology and behavior. Indeed, evidence suggests that epigenetic events initiated during the prenatal period can lead to persistent adaptations in structure, metabolism and physiology that predispose offspring towards disease and impaired physiology [1]. Moreover, maternal separation has been reported to increase susceptibility to obesity, insulin resistance, high blood pressure and cerebrovascular diseases in adult offspring [2–4], while newborn infant challenge can also induce cardiovascular and cerebrovascular dysfunction in adult offspring [3–5]. Furthermore, specifically related to neurological or psychological function, maternal rejection or separation has been implicated in the development of depressive phenotypes in adult humans and animals [6,7].
The environment that offspring experiences in the early life, including intrauterine and early postnatal environment, is highly influenced by maternal diet and metabolic status. Indeed, perinatal overnutrition or consumption of high fat diet (HFD) has been demonstrated to lead to health problems of adult offspring, such as metabolic syndrome, hypertension and cardiovascular dysfunction, in animal studies and epidemiological investigations [8–10]. In addition to the metabolic and endocrine systems, 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 [11–14]. Based on above evidence, maternal HFD consumption plays an important role in offspring physiology and behavior, it should be pointed out, however, that other studies reported that adult offspring of HFD-fed dams can have normal glucose tolerance, body composition, as well as normal behavior and cognitive ability [11,15–17]. More importantly, in the light of the current stressful environment that many humans live in, it is important to address whether perinatal HFD may exacerbate the adverse effects of the stressful challenge on adult offspring physiology and behavior.

Calcitonin gene–related peptide (CGRP), a 37-amino-acid neuropeptide, best known for its potent vasodilatory properties [18,19], has been implicated in many aspects of the behavior. Functional studies suggest that CGRP may contribute to the modulation of many psychological and behavioral processes, such as stress responses, anorectic and fear-related behaviors [20–22]. Moreover, CGRP–immunoreactive (CGRP-ir) in the hippocampus is elevated in “genetically depressed” Flinders Sensitive Line rat, while maternal separation (a behavioral model of depression) could even exacerbate this elevation [23]. More specifically, a recent study shows that, through methylation of the CGRP gene, gestational environment programs adult depression-like behavior [24]. These results strongly indicate that genetic disposition and development stress may contribute to the susceptibility to depression, at least partially, by exerting CGRP-specific effects on adult neurobiology. Nevertheless, whether CGRP participates in the programming of maternal HFD on the adult behavior has not been investigated.

The present study was designed to evaluate the effects of maternal HFD on offspring behavioral phenotype at different developmental stages, with additional emphasis on the contributions of maternal diet to the psychological and neurological consequences of stress. Moreover, the involvement of CGRP in the programming of maternal HFD was investigated by examining the levels of CGRP in the central nervous system in the models. Furthermore, we investigated whether intracerebroventricular (icv) administration of CGRP antagonist CGRP 3– 7 would attenuate depression-like behaviors in adult stressed HFD offspring. In addition, the neural pathways that regulate stress responses are also involved in the modulation of behavior, and the available literature strongly suggests interactions between the two systems. Thus, we further examined the involvement of hypothalamic–pituitary–adrenal (HPA) axis activity in the processes, by investigating the HPA profile responses to acute psychological stress (restraint stress) and the repeated stress-induced habituation of HPA activity in HFD offspring.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley (120–140 days) rats, obtained from Wenzhou Medical University, were housed under controlled conditions (12 h:12 h light–dark, with lights on at 0700 h; temperature at 22 ± 2 °C) and provided with food and water ad libitum. The rats were fed with either a standard normal chow diet (ND; n = 8; 21% kcal fat, 17% kcal protein, 63% kcal carbohydrate; Medicience Ltd., Jiangsu, China) or high fat diet (HFD; n = 12: 45% kcal fat, 20% kcal protein, 35% kcal carbohydrate; Medicience Ltd.), for 10 days before mating and throughout pregnancy and lactation. The day of parturition was set as day 0, and litters, including females and males, were evenly culled to 8 per mother on day 1. Pups were kept with their mothers until weaning on day 21. Thereafter, weaned male rats were housed 3 per cage and fed a normal chow diet. All male offspring from one dam were used in the same experiment and distributed randomly in the groups. Animal weight and food intake were recorded weekly in offspring post-weaning.

In order to assess the impacts of high-fat feeding during pregnancy and lactation on offspring depressive behavior under nonstressed or stressful conditions, the male ND and HFD offspring were subjected to 14-day CUMS or normal circumstance in experiment 1: (1) ND rats with normal circulation (n = 9); (2) ND rats treated with CUMS (n = 9); (3) HFD rats with normal circulation (n = 9); (4) HFD offspring treated with CUMS (n = 9). Another sets of HFD + CUMS rats were used in experiment 2 involving the drug administration: (1) HFD + CUMS rats treated with vehicle (n = 8); (2) HFD + CUMS animals treated with CGRP antagonist (n = 9). Moreover, another groups of rats were used in experiment 3 to investigate the changes of HPA profile responses to acute and repeated restraint stress in offspring: (1) ND rats (n = 9); (2) ND rats treated with restraint (n = 9); (3) HFD rats (n = 9); (4) HFD animals treated with restraint (n = 9). All animal procedures were performed in accordance with the Guidelines of the Chinese Council on Animal Care and approved beforehand by the Institutional Animal Care and Use Committee of Wenzhou Medical University. All surgical procedures were carried out under ketamine anesthesia (100 mg/kg i.p.; Pharmacia and Upjohn, Crawley, UK) and xylazine (10 mg/kg i.p.; Bayer, Leverkusen, Germany).

2.2. CUMS procedures

At the age of 120-day, the CUMS procedure was performed on animals as described previously [25], with minor modifications. The procedure contained 9 different stressors randomly arranged day and night across 14 consecutive days: 18 h water deprivation, 20 h food and water deprivation, 12 h of 45° cage tilt, 21 h wet cage, overnight illumination, 2 min swimming in water at 4 °C, 2 min swimming in water at 45 °C, 1 min tail pinch and 2 h immobilization.

2.3. Behavioral assessments

Sucrose preference test (SPT), open-field test (OFT) and forced swimming test (FST) were employed five times to assess the depressive-like behaviors. The behavioral assessments were conducted at the age of 56, 120, 127, 134 and 141 days to observe the effects of maternal HFD on behaviors at the different stages. SPT and OFT were used to investigate the effects of drugs on depressive-like behavior in rats.

2.3.1. Sucrose preference test

The SPT was used to operationally determine anhedonia. In the SPT which was conducted between 0900 h and 1000 h, the animals were allowed to consume water and 1% sucrose solution for 1 h after 20 h food and water deprivation. The sucrose preference index was calculated according to the following ratio: sucrose preference = sucrose intake (g)/sucrose intake (g) + water intake (g). The sucrose preference was monitored with nonstressed (young and adult) and stressful conditions.

2.3.2. Open-field test

The OFT was performed to evaluate general locomotor and rearing activity of rats. The apparatus consisted of a dark varnished wooden box (100 cm square chamber, 40 cm high walls) with the floor divided into 25 equal squares. Locomotor activity was defined as at least three paws in a quadrant and rearing behavior defined as the animal standing upright on its hind legs were tallied over a 3-min period. The OFT was
administered with nonstressed (young and adult) and stressful conditions.

2.3.3. Forced swimming test

The FST was conducted as described previously [26]. Briefly, rats were placed individually for 15 min in a glass cylinder (50 cm tall and 20 cm in diameter) containing tap water to 35 cm depth (25 ± 1 °C). Twenty-four hours after their first exposure (training purposes, with no data collected), the animals were replaced in the swim apparatus for 5 min, and the session was recorded by two trained observers. Climbing behavior consisted of upward movements of the forepaws in and out of the water, while immobility was assigned when the rat remained afloat in the water without additional activity other than that required to keep the rat’s head above the water. The FST was performed with nonstressed (young and adult) and stressful conditions.

2.4. Measurement of CGRP levels in central nervous system

To evaluate the possible central changes of CGRP in subjects, CSF was extracted from rats and CGRP-ir levels were measured at the age of 142-day, after the CUMS procedure. After behavioral test, rats were anesthetized and placed in a stereotaxic frame. CSF was extracted from the cisterna magna of the rats and centrifuged (4000 × g for 5 min) to remove blood contamination from the puncture point. CSF (0.2 ml) was acidified with 16 μl of a mixture consisting of 5.0% formic acid, 1.0% trifluoroacetic acid (TFA), 80% 1 M HCl, and 1.0% NaCl. The acidified CSF was centrifuged at 7000 × g and 4 °C for 20 min. The supernatant was put into an activated C-18 Sep-Pak disposable cartridge column (Amersham Pharmacia Biotech) and eluted with a mixture (2 ml) of methanol/water/TFA (90:9:1). The eluate was vacuum dried and stored at −80 °C until assay. On the day of the assay, the frozen sample was thawed in a small amount (−20 μl) of 0.1% TFA at 4 °C over a 30-min period. The CGRP level was measured in duplicate using a rat CGRP Radioimmunoassay Kit (Purevalley Biotech., Beijing, China).

CGRP-ir in the hippocampus was extracted by a modification of the method described previously [27]. In brief, the rats were decapitated and hippocampus was collected and homogenized in 2 ml of ice-cold 15% TFA buffer containing 1 M HCl, 1 M NaCl, 5% formic acid, and 100 kU/ml aprotinin. The homogenate was centrifuged at 7000 × g for 20 min (4 °C). The pellet was homogenized in the same volume of the TFA buffer and centrifuged again. The supernatant was put into an activated C-18 Sep-Pak disposable cartridge column (Amersham Pharmacia Biotech) and eluted with a mixture (2 ml) of methanol/water/TFA (90:9:1). The eluate was vacuum-dried and stored at −80 °C until assay. CGRP-ir level in brain homogenate was also measured in duplicate using a rat CGRP Radioimmunoassay Kit (Purevalley Biotech.) [28]. The total protein content in each sample was determined by the Bradford method (Thermo Scientific, IL, USA).

2.5. Brain cannula implantation

For experiment 2 involving the central administration of CGRP receptor antagonist in the HFD + CUMS animals, groups of HFD rats were fitted with unilateral ivc guide cannula (22 gauge; Plastic One, USA) positioned towards the left lateral cerebral ventricle, the coordinates for implantation being 0.6 mm lateral, 1.5 mm posterior to Bregma, and 4 mm below the surface of the dura [29]. The guide cannula was secured using dental cement (Dental Filling Ltd., UK), and fitted with a dummy cannula (Plastics One) to maintain patency. Brain cannulae were implanted just after the CUMS procedures.

2.6. Central administration of CGRP antagonist into HFD + CUMS rats

To investigate the involvement of CGRP signaling in behavioral disorder response to stressful challenge in adult HFD offspring, CGRP receptor antagonist αCGRP8–37 (Tocris Bioscience, Ellisville, MI, USA) were centrally administrated into HFD + CUMS rats. The drugs were prepared fresh on the day of infusion in artificial cerebrospinal fluid (aCSF). On the morning of infusion, an ivc injection cannula (Plastics One) with extension tubing, preloaded with drug or vehicle, was inserted into the guide cannula. HFD + CUMS rats were treated with CGRP antagonist αCGRP8–37 (6 μg in 500 nl aCSF, n = 9) or aCSF (500 nl, n = 8) by ivc injection. CGRP antagonist infusion was conducted on the 4th day after the termination of CUMS. The behavioral assessments were conducted 1 day before and immediately after the drug infusion.

2.7. Head screws and intravenous cannulation

The ND and HFD animals were implanted with intravenous cannulations for collection of blood samples without interruption in experiment 3. In order to allow the anchorage of a metal spring to protect exteriorized chronically implanted intravenous catheters, animals were fitted with a tether screw on the skull. Rats were fitted with two indwelling cardiac catheters via the jugular veins, as previously described [30]. The catheters were 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 (Insect Laboratories Inc, Boulder, CO). The distal end of the tether was attached to a fluid swivel (Insect Laboratories), which allowed the rat freedom to move round the enclosure. Experimentation commenced 4 d later.

2.8. HPA profile response to acute or repeated restraint

On the morning of experimentation, the rats were attached via one of the two cardiac catheters to blood sampling system. Once connected, the animals were left undisturbed for 1 h. Basal blood sample (0.15 ml) were collected and centrifuged immediately, the plasma was separated and frozen at −20 °C for later assay to determine corticosterone (CORT) concentrations. After 1 h of recovery period, the animals were placed in restraint devices for 60 min [30], and blood samples were collected immediately before, during restraint and the 3-h postrestraint period, at 0, 15, 45, 75, 105, 135 and 240 min after the onset of restraint stress. Restraint stress was performed between 1000 and 1300, avoiding the elevated basal CORT levels and peak HPA responses to stress associated with the dark phase of the cycle. Control animals underwent the same procedures but were not restrained. The same experimental regimen was repeated four times in all rats at 5-day intervals.

2.9. Hormone assay

Total CORT was determined in plasma by radioimmunoassay using rat commercially available CORT kit (North Bio, Beijing, China). The sensitivity of the assay was 7.5 ng/ml. The intra-assay variation was 10.4% and the interassay variation was 15.0%.

2.10. Statistical analysis

All quantitative data were represented as the mean ± SEM. Statistical comparisons in offspring body weight and food intake were by repeated-measures ANOVA with Bonferroni correction for multiple comparisons. Data and interactions of behavior in experiment 1 were evaluated by a two-way ANOVA with treatment and time as two factors, followed by the Bonferroni for post-hoc tests. The statistical significance of CGRP levels in experiment 1 and behavioral data in experiment 2 was evaluated by a one-way ANOVA followed by Dunnett’s t-test. The significance of the effect of restraint stress on CORT in different groups was assessed using ANOVA followed by Dunnett’s t-test to assess statistically significant differences within the same strain at different time points, as well as between the different strains at the same time point. Integrated hormone levels during restraint and 3 h recovery period were
determined with the trapezoidal rule, and the data were expressed over time of sampling. \( P < 0.05 \) was considered statistically significant.

3. Results

3.1. Offspring food intake and body weight

Repeated ANOVA revealed that food intake and body weight were similar between groups before the CUMS procedure (Fig. 1). CUMS significantly decreased food intake in both ND and HFD offspring during the procedure \( (P < 0.01) \). At the end of CUMS and thereafter, the body weight was significantly decreased in HFD + CUMS group, as compared with the HFD group \( (P < 0.05) \). There were no significant differences in body weight and food intake between ND + CUMS and HFD + CUMS rats, during and after the CUMS procedure \( (P > 0.05) \).

3.2. The effects of maternal HFD consumption on offspring behavior with nonstressed or stressful conditions

To determine the effects of maternal HFD consumption on offspring behavior in nonstressed or stressful circumstances, behavioral tests were performed for several times with different conditions. In the SPT, maternal HFD consumption significantly decreased the sucrose preference in rats on the 56-day with nonstressed condition \( (P < 0.01) \), indicating maternal HFD feeding led to reduced sucrose preference in young rats. Nevertheless, there were no significant differences in sucrose preference between male adult offspring exposed to maternal HFD or ND consumption \( (P > 0.05) \) on the 120-day. Two-way ANOVA showed that HFD + CUMS significantly decreased the sucrose preference in rats \( (F(3,24) = 19.22, P < 0.01) \) (Fig. 2A). Post-hoc analysis revealed that the sucrose preference of HFD + CUMS animals was decreased significantly, compared with that of ND, ND + CUMS, and HFD rats \( (P < 0.05) \) at the age of 127, 134 and 141 days, during and after the CUMS procedures. The sucrose preference index was significantly reduced in ND + CUMS group, as compared with the ND group \( (P < 0.05) \). No significant difference was observed in sucrose preference between HFD rats and ND rats on the 120-day after the birth and thereafter.

Moreover, maternal HFD consumption significantly decreased the rearing activity \( (P < 0.01) \) but not locomotor activity \( (P > 0.05) \) in the OFT on the 56-day. There were no significant differences in either locomotor or rearing activity between adult male offspring exposed to maternal HFD or ND \( (P > 0.05) \) on the 120-day. Two-way ANOVA showed that there was a significant effect of HFD + CUMS on locomotor activity \( (F(3,24) = 21.37, P < 0.01) \) and rearing activity \( (F(3,24) = 7.24, P < 0.05) \) in the OFT (Fig. 2B and C). Post-hoc analysis revealed that, compared with ND, ND + CUMS, and HFD animals, the HFD + CUMS offspring showed a reduction in locomotor activity \( (P < 0.05) \) and rearing activity \( (P < 0.05) \) at the age of 127, 134 and 141 days, during and after the CUMS procedures. Compared with ND rats, HFD ones displayed no significant changes in locomotor and rearing activity \( (P > 0.05) \) on the 120-day after the birth and thereafter.

The climbing and immobility time in FST in the groups of rats were illustrated in Fig. 2D and E. There was a significant effect of maternal HFD consumption on immobility time \( (P < 0.01) \) but not the climbing behavior \( (P > 0.05) \) of young offspring, with an age of 56-day. However, there were no significant differences in either climbing behavior or immobility time between adult male offspring exposed to maternal HFD or ND consumption \( (P > 0.05) \) on the 120-day. Two-way ANOVA showed that there was a significant effect of HFD + CUMS on climbing \( (F(3,24) = 10.17, P < 0.05) \) and immobility time \( (F(3,24) = 17.37, P < 0.01) \) in the FST. Post-hoc analysis revealed that on the 14th and 21th day after the onset of CUMS, the HFD + CUMS offspring showed a reduction in climbing behavior \( (P < 0.05) \) and an increase in immobility time \( (P < 0.05) \), compared with ND, ND + CUMS, and HFD animals. There was significant difference in immobility time \( (P < 0.05) \) but not climbing behavior \( (P > 0.05) \) between HFD + CUMS and ND + CUMS groups on the 7th day after the onset of CUMS.

3.3. The CGRP concentrations in the central nervous system

The CGRP concentrations of CSF in the groups of rats were illustrated in Fig. 3A. The CSF CGRP concentration in the HFD + CUMS rats, with a group mean \( \pm \) SEM of 48.27 \( \pm \) 6.55 pg/ml, was significantly elevated \( (P < 0.05) \), compared with 18.29 \( \pm \) 3.51, 22.17 \( \pm \) 4.25, 32.35 \( \pm \) 5.23 (pg/ml) in the ND, HFD and ND + CUMS groups, respectively. Moreover, compared with ND rats, rats in ND + CUMS group have higher concentration of CGRP in the CSF \( (P < 0.05) \). There was no significant difference between the CGRP levels in CSF of ND and HFD rats.

The CGRP levels in the hippocampus in groups were shown in Fig. 3B. The hippocampal CGRP concentration in the HFD + CUMS rats, with a group mean \( \pm \) SEM of 3.42 \( \pm \) 0.41 pg/mg, was significantly elevated \( (P < 0.05) \), compared with 0.95 \( \pm \) 0.27, 1.14 \( \pm \) 0.34, 2.35 \( \pm \) 0.43 (pg/mg) in the ND, HFD and ND + CUMS groups, respectively. Moreover, compared with ND rats, rats in ND + CUMS group \( (P < 0.05) \) but not HFD group \( (P > 0.05) \) have higher concentration of CGRP in the hippocampus.

3.4. The involvement of central CGRP signaling in programming of maternal HFD on offspring

Fig. 4 showed that central infusion of CGRP receptor antagonist \( \alpha \text{CGRP}_{37} \) significantly attenuated the depressive-like behaviors in HFD + CUMS model. The HFD + CUMS animals treated with \( \alpha \text{CGRP}_{37} \) demonstrated an increased sucrose preference, with a group mean \( \pm \) SEM of 62.14 \( \pm \) 5.73%, compared with 49.12 \( \pm \) 4.52% in vehicle-treated HFD + CUMS rats. Moreover, central infusion of \( \alpha \text{CGRP}_{37} \) significantly attenuated HFD + CUMS-induced suppression of climbing behavior in FST \( (P < 0.05) \). Moreover, HFD + CUMS rats

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**Fig. 1.** Food intake (A) and growth rate (B) in male offspring of HFD fed dams and ND-fed dams, with or without 14 days CUMS procedure start at the age of 120-day. CUMS significantly decreased the body weight and food intake. \( * P < 0.05 \). HFD + CUMS group compared with HFD group at the same time period.
treated with αCGRP_8–37 showed less immobility time (P < 0.05) in FST, compared with vehicle-treated HFD + CUMS rats.

3.5. The effects of maternal HFD on HPA responses to acute or repeated restraint stress

Basal plasma CORT concentrations, as well as their responses during and up to 3 h after the termination of restraint stress during the repeated stress experiment were shown in Figs. 5A–D. During the several episode of repeated stress experiment, basal plasma CORT levels obtained before the restraint stress were all similar in groups. For the first restraint stress episode, HFD offspring showed a significant (P < 0.05) elevation in plasma CORT 15 min into the restraint and during the 1 h procedure, but there were no significant differences in CORT compared with ND offspring. Moreover, HFD offspring showed significant (P < 0.05) elevation in plasma CORT at 15, 45, 75 and 180 min post-stress, compared with ND offspring. Moreover, during the third restraint stress episode, the difference of CORT levels showed statistical significance 15 min but not 45 min after the onset of restraint procedure. For the fourth restraint stress, HFD offspring showed significant (P < 0.05) elevation in plasma CORT during and after the restraint procedure.
**Discussion**

The present study provides the first evidence revealing that although the detrimental effects of maternal HFD consumption on offspring depressive behavior did not persist into adulthood, it markedly aggravate the behavioral disorder response to stressful challenge in adult offspring. We found that perinatal HFD feeding led to reduced sucrose preference, reduced locomotor and exploratory activity, as well as decreased struggling behavior in young rats, which representing some depressive-like behavior. However, these mental health disorders did not sustain into adulthood [36]. Conversely, some studies demonstrated maternal HFD have a long-term impact on offspring behavior [12–14]. The conflicting data presented above, which highlight a controversial role for maternal HFD on adult offspring behavior, may result from the use of different animal species, different fat sources (lard, vegetable oil, margarine, etc.) and the different durations of diet exposure. In our experiments, HFD offspring were fed a normal chow diet after weaning. Therefore, although the related mechanism remains to be established, it may be reasonable to speculate that, by long-term feeding of normal diet after weaning, maternal HFD-induced changes in behavior-regulatory neural circuitry would be recovered in adult offspring.

Although there is no difference in depressive phenotype between adult HFD and ND offspring under nonstressed conditions, our study further demonstrated that, compared with ND + CUMS rats, HFD + CUMS offspring showed more serious depressive-like behavior. These findings raise the possibility that prenatal exposure to maternal HFD increases the sensitivity of offspring to the deleterious effects of stress. It is supported by findings that the combination of adult HFD consumption with a maternal history of HFD administration, but not administration of HFD alone for the designated periods of time (either perinatal or postnatal), could encumber memory retention in Morris maze test, implying the interaction of maternal and offspring HFD could alter cognitive function and perturb brain homeostasis in vivo [35]. Metabolically, Tamashiro and colleagues also reported that prenatal stress and/or HFD during the intrauterine or postnatal environment affects offspring in a manner that increases their susceptibility to diet-induced obesity and leads to secondary adverse metabolic consequences [37]. Overall, our data suggest that specific perinatal factors can alter the susceptibility of offspring to future psychological challenge. The present study further supported “two-hit” hypothesis which proposed that genetic or environmental factors disrupt early development and consequently produce increased susceptibility to disease, including Parkinson’s disease and schizophrenia [37–39]. The “first hit” disruptions which occur during early development set the stage for long-term vulnerability to a “second hit” that occurs later in life and leads to health problem. Thus, it appears that maternal HFD may be not the direct cause of behavior abnormality in adults, but instead increases future susceptibility.

Identification of the mechanisms and pathways that produce long-term vulnerability in response to perinatal environmental factors will facilitate development of clinical intervention and prevention strategies to reduce the incidence and severity of disease. Although the neuroanatomical circuitry involved remains to be fully elucidated, the present study indicate endogenous central CGRP signaling may involve in the programming of maternal HFD on adult behavior responses to stress. CGRP has been implicated in mediating the depression, showed in both clinic [40,41] and animal [23,24] studies. Our present study show
Central CGRP concentrations was increased in HFD + CUMS rats which showed depressive-like behavior. More importantly, we found that central administration of CGRP receptor antagonist produced antidepressant effects in HFD + CUMS rats. To our knowledge, this is the first report regarding the involvement of endogenous central CGRP signaling in the programming of maternal HFD on the behavior. Consistently, Jiao and colleagues reported that gestational environment programs adult depression-like behavior through methylation of the CGRP gene [24]. Moreover, although infusion of αCGRP₈⁻⁻³⁷ significantly reduced depressive symptoms in the HFD + CUMS rat, we found that αCGRP₈⁻⁻³⁷ alone did not change behaviors in normal rats (unpublished observations). Therefore, perhaps, maternal HFD sensitizes offspring to the influence of stressful experiences on CGRP release in the hippocampus and other limbic components [42,43], which mediates behavioral effects such as those observed upon exogenous CGRP infusion [22,24]. Nevertheless, it is worth noting that the depression-like behavior in the HFD + CUMS rats was only attenuated by CGRP₈⁻⁻³⁷ infusion, not completely eliminated, putting forward the possibility that the CGRP signaling might just play a partial modulatory role in the programming of maternal HFD on behavioral disorder response to stress.

It is well established that the neural pathways that regulate stress responses are also involved in the modulation of behavior [44,45]. The present study for the first time showed that, without alteration of basal HPA activity, maternal HFD significantly increases HPA profile response to an acute restraint stress in adult offspring. More importantly, our results further indicate that the maternal HFD consumption could attenuate the habituation of the HPA response evoked by repeated restraint stress [31–33], as shown by a prominent corticosterone profile response to repeated stress exposure. The observed normal basal HPA profile and aggravated stress response of adult HFD offspring may provide reasonable explanation to the behavior under nonstressed and stressful conditions, respectively. Moreover, it is worth noting that, consistent with other study [46], maternal HFD-induced changes of the neuroendocrine profile overlap with those caused by prenatal stress, such as chronic restraint stress [47] or neonatal immunological challenge [48]. The overlapping consequences of maternal HFD and stress

Fig. 5. Effects of maternal HFD on HPA profile response to acute and repeated restraint (1 h) stress. (A–D) showing maternal HFD significantly increased CORT levels responses to restraint stress in the 1st, 2nd, 3rd and 4th restraint episode, respectively. (E) showing the integrated levels of CORT, during restraint and 3 h recovery period, were significantly decreased in ND rats in the 3rd and 4th restraint stress. *P < 0.05, HFD group compared with ND group at the same time period. #P < 0.05. HFD group compared with ND group in the same restraint episode. + P < 0.05 versus 1st restraint stress in the same group.
exposure in neuroendocrine response raise the possibility that perinatal HFD may mimic stress exposure, as both interfere with the neuroendocrine development, altering the HPA axis activity in later life. Furthermore, although the aggravated HPA response to acute or chronic stress reflect alternations in the regulatory components of the HPA axis, the underlying mechanism remains to be elucidated. Therefore, more work will be required to indentify the changes of HPA-regulatory mechanisms induced by maternal HFD.

5. Conclusions

The results of this study suggest that the detrimental effects of maternal HFD consumption on young offspring behavior did not persist into adulthood, but it markedly affect the behavioral appearance under stressful conditions. Moreover, our results indicate the programming effect of maternal HFD on the neuroendocrine activity and the expression of CGRP in hippocampus, may involve in mediating the detrimental effects of maternal HFD consumption on behavior disorder response to stress in adults. Healthy diet during the perinatal period may be clinically beneficial in the prevention of behavior disorder, especially under stressful conditions. Nevertheless, more studies need to be conducted to characterize the mechanisms by which maternal HFD influences behavior.

Conflict of interests

The authors declare no conflict of interests.

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