MATERNAL HIGH-FAT DIET ALTERS ANXIETY BEHAVIOR AND GLUCOCORTICOID SIGNALING IN ADOLESCENT OFFSPRING

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Abstract—Maternal obesity and overconsumption of saturated fats during pregnancy have profound effects on offspring health, ranging from metabolic to behavioral disorders in later life. The influence of high-fat diet (HFD) exposure on the development of brain regions implicated in anxiety behavior is not well understood. We previously found that maternal HFD exposure is associated with an increase in anxiety behavior and alterations in the expression of several genes involved in inflammation via the glucocorticoid signaling pathway in adult rat offspring. During adolescence, the maturation of feedback systems mediating corticosteroid sensitivity is incomplete, and therefore distinct from adulthood. In this study, we examined the influence of maternal HFD on several measures of anxiety behavior and gene expression in adolescent offspring. We examined the expression of corticosteroid receptors and related inflammatory processes, as corticosteroid receptors are known to regulate circulating corticosterone levels during basal and stress conditions in addition to influencing inflammatory processes in the hippocampus and amygdala. We found that adolescent animals perinatally exposed to HFD generally showed decreased anxiety behavior accompanied by a selective alteration in the expression of the glucocorticoid receptor and several downstream inflammatory genes in the hippocampus and amygdala. These data suggest that adolescence constitutes an additional period when the effects of developmental programming may modify mental health trajectories. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: ANOVA, analysis of variance; CD11b, cluster of differentiation molecule 11B; CHD, control house chow diet; GR, glucocorticoid receptor; HFD, high-fat diet; HPA, hypothalamic–pituitary–adrenal; IkBa, I-kappa-B-alpha; IL-1Ra, interleukin-1 receptor antagonist; IL-6, interleukin-6; MKP-1, mitogen-activated protein kinase phosphatase-1; MR, mineralocorticoid receptor; NFkB, nuclear factor kappa beta; PD, postnatal day; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction.

Key words: glucocorticoid receptor, inflammatory, anxiety behavior, maternal, obesity, gene expression programming.

INTRODUCTION

Epidemiological studies suggest that maternal obesity and overconsumption of saturated fats during pregnancy have profound effects on offspring health, ranging from metabolic to behavioral disorders in later life (Godfrey et al., 2010). High-fat exposure during development has been linked with adverse health outcomes such as diabetes and coronary heart disease (Marx, 2002; Kahn et al., 2006; Van Gaal et al., 2006; Bersamin et al., 2008). Obesity has also been associated with an increased risk of developing behavioral disorders related to anxiety in humans (Boksa, 2004; Desai et al., 2009; Rofey et al., 2009; Peleg-Raibstein et al., 2012).

The influence of maternal high-fat diet (HFD) exposure on the development of brain regions implicated in anxiety behavior is not well understood. Non-human primates show increased fear response when faced with novelty after being developmentally exposed to HFD (Sullivan et al., 2010). Rodent studies have observed increased anxiety behavior in adult offspring perinatally exposed to HFD, accompanied by increased inflammation (Bilbo and Tsang, 2010; Peleg-Raibstein et al., 2012; Sasaki et al., 2013). In humans, maternal obesity predicts child obesity, and child obesity is associated with inflammation at an early age and with anxiety disorders (Weiss et al., 2004; Whitaker, 2004; Boney et al., 2005; Desai et al., 2009; Rofey et al., 2009).

Environmental factors during development have been shown to affect the long-term activity of the hypothalamic–pituitary–adrenal (HPA) axis, a primary mediator of the response to stress. Studies examining maternal care in early postnatal life and prenatal maternal stress have found long-term effects on offspring anxiety behavior and on the expression of HPA axis-associated genes in the brain (Meaney, 2001; Welberg and Seckl, 2001; McGowan et al., 2008; Brunton, 2010). These factors modify offspring HPA axis, in part, through changes in corticosteroid receptors in limbic regions such as the hippocampus and amygdala, which regulate circulating corticosterone levels during basal and stress conditions (Vazquez, 1998; Welberg and Seckl, 2001; Brunton, 2010). In addition to its role in inhibiting the stress response, as a transcription factor, the glucocorticoid receptor (GR) can influence downstream inflammatory processes, which are also affected

by altered HPA activity (Smoak and Cidlowski, 2004; Sorrells et al., 2009). Glucocorticoids have both pro- and anti-inflammatory roles in the brain (Sorrells et al., 2009). The expression of the inflammatory genes nuclear factor kappa beta (NFkB), interleukin-6 (IL-6), and cluster of differentiation molecule 11B (CD11b), has been previously linked to chronic alterations in glucocorticoid signaling (Sorrells et al., 2009) and maternal HFD exposure (Bilbo and Tsang, 2010; Sasaki et al., 2013). Likewise, the anti-inflammatory genes I-kappa-B-alpha (IkBa), mitogen-activated protein kinase phosphatase-1 (MKP-1), and interleukin-1 receptor antagonist (IL-1Ra) are known negative regulators of inflammatory response that are modified through chronic changes in GR, NFkB, and IL-6 signaling as well as in the context of maternal HFD exposure (Sorrells et al., 2009; Sasaki et al., 2013).

We previously examined the effects of perinatal HFD exposure on adult (postnatal day (PD) 90) anxiety behavior (Sasaki et al., 2013). Rats perinatally exposed to HFD exhibited an increase in anxiety behavior in the Open Field, Light–dark transition, and Elevated Plus Maze tasks. Females exposed to HFD appeared particularly vulnerable, as they showed an increase in the expression of corticosteroid receptors in the amygdala in association with increased anxiety. Male and female adult offspring showed alterations in the expression of several genes involved in inflammation via the glucocorticoid signaling pathway. It is known that the developing adolescent brain differs from the adult brain in terms of structure and function (Vazquez, 1998; Spear, 2000). Anxiety behavior and responses to stress in adolescent animals are distinct from those in adult animals, likely reflecting incomplete maturation of feedback systems mediating corticosteroid sensitivity (McCormick et al., 2008). To our knowledge, the influence of maternal HFD on adolescent anxiety behavior and related gene expression has not been examined.

In this study, we examined the effects of perinatal HFD exposure on adolescent (PD35) offspring anxiety behavior. Corticosteroid receptor and downstream inflammatory pathway genes were examined in the hippocampus and amygdala, as these two components of the limbic system are implicated in anxiety behavior and interact with the HPA axis to mediate the response to psychosocial stress.

EXPERIMENTAL PROCEDURES

Animals

Adult male and female Long Evans rats (7 weeks old) were obtained from Charles River Canada (St. Constant, QC). Rats were housed in same-sex pairs, maintained on a 12:12-h light–dark cycle (lights on from 7:00 AM to 7:00 PM), and had *ad libitum* access to food and water. All experimental protocols were approved by the Local Animal Care Committee at the University of Toronto Scarborough and were in accordance with the guidelines of the Canadian Council on Animal Care.

Diets

Female breeders were given access to either a HFD ($n = 10$) or control house chow diet (CHD, $n = 10$). The HFD (5.24-kcal/g) was obtained from Research Diets, Inc. (New Brunswick, NJ, USA: cat. No. D12492) and contained (by kcal): 60% fat, 20% protein, and 20% carbohydrate. The CHD (3.02 kcal/g) was obtained from Purina Lab Diets (St. Louis, MO, USA: cat. No. 5001) and contained (by kcal): 13.5% fat, 28.5% protein, and 58% carbohydrate. A comparison between similar formulations of HFD and CHD has been used to examine diet-induced obesity in several previous studies (e.g., (El-Haschimi et al., 2000; De Souza et al., 2005; Dunn and Bale, 2009; Tamashiro et al., 2009; Purcell et al., 2011; Sasaki et al., 2013). Dams remained on their respective diets for 4 weeks prior to mating and throughout pregnancy and lactation. Upon weaning at PD21, all offspring were given *ad libitum* access to CHD.

Subjects and general procedures

After mating, female breeders were housed individually. There were no significant differences in litter size or sex ratio among the diet groups. Offspring remained undisturbed until PD21 when they were housed in same-sex pairs. Body weights were measured at the start of the behavioral assays during mid-adolescence between PD35 and PD45 (Tirelli et al., 2003). A subset of male and female offspring (1–2 offspring/sex/litter) was used for behavioral assays. The offspring were run in squads, resulting in the following total numbers of offspring tested on the Light–Dark and Elevated Plus Maze tasks (HFD females $n = 19$; HFD males $n = 13$; CHD females $n = 19$; CHD males $n = 19$). Only the second squad was run on the Open Field task (HFD females $n = 13$; HFD males $n = 7$; CHD females $n = 13$; CHD males $n = 13$). Brains from $n = 6$ offspring per sex and per diet group were collected for gene expression analysis after completion of behavioral testing ($\sim$PD45). The order of behavioral testing for each sex and diet condition was counterbalanced within each task. All behavioral tasks were run in a dimly lit room (33.7 lux) that was illuminated through a single light bulb placed over the apparatus. After each behavioral test, the apparatus was cleaned using a 70% ethanol solution and allowed to air dry to remove or homogenize odorants. All behavioral testing and sacrifices occurred at the mid-point of the light phase of the circadian cycle (11 AM–3 PM) to control for potential confounding circadian effects.

Light–dark transition

The Light–dark transition task consisted of an opaque white Plexiglas box (light zone) connected to an opaque black box (dark zone) through a small (12 × 12 cm) opening to allow passage between the chambers. Both boxes were 30 × 30 cm. The rat was placed in the dark box at the beginning of each trial and allowed to explore the boxes for a period of 5 min. The task measured the duration and frequency of entries within the light zone

with the use of ANY-maze software (Stoelting Co., Wood Dale, IL, USA).

Open Field

The Open Field task consisted of a white opaque square box (40.3 × 40.3 cm). At the start of the session, a rat was placed in the edge of the maze facing the wall and allowed to freely ambulate for a period of 15 min. ANY-maze software was used to track the rats' movement and calculate number of entries and time spent in various predefined zones. The predefined zones included a center zone (8.96 × 8.06 cm), an edge zone of 8.51 cm wide along the wall of the box and a corner zone (8.96 × 8.06 cm in each of the corners). The duration and frequency of entries was calculated and analyzed for each zone and for the center zone relative to the edge.

Elevated Plus Maze

The Elevated Plus Maze task involved placing the rat in the center portion of the maze and tracking the rat's movement with ANY-maze software for a period of 5 min, during which the software calculated total duration and frequency of entries into predefined zones. The predefined zones within the Elevated Plus Maze arena consisted of a center zone (10 × 12 cm), two open arms (45 × 12 cm) attached to the center zone, and two closed arms (45 × 12 cm) attached to the center, with the apparatus elevated 80 cm above the floor. The open arms zone and closed arms zones were farther subdivided into proximal and distal zones of equal sizes. The amount of time spent and frequency of entries was calculated and determined for the open zones relative to the closed zones, the proximal zone within the open arms relative to the proximal zone within the closed arms, and the distal zone within the open arms relative to the distal zone within the closed arms.

Brain dissection and preparation

Rats were sacrificed by CO_2 inhalation followed by decapitation. Whole hippocampus and amygdala were rapidly dissected from the adolescent HFD- and CHD-exposed offspring using stereotaxic coordinates (Paxinos and Watson, 1997). Brain samples were flash-frozen and stored at −80 °C. RNA was extracted using the RNeasy plus kit (Qiagen, Germantown, MD, USA) according to the manufacturer's protocol and quantification and quality assessment was performed using a Nanodrop ND-2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Gene expression analysis by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

qRT-PCR analysis was performed on eight transcripts using the StepOne Plus real-time PCR machine and Fast SYBR Green PCR master mix (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). A standard curve was generated using 11 serial dilutions of a mixture of all offspring cDNA for each brain region examined. The expression of the eight transcripts was

normalized against the expression of ActinB. All qRT-PCR reactions were performed in triplicate for each subject. As such, the relative abundance of gene expression for each subject was calculated as the ratio of the triplicate average expression of each gene of interest against the triplicate average of ActinB expression.

Primers

Primers were purchased or designed according to GenBank sequence information at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm. nih.gov). ActinB: F: 5′-TTT GAG ACC TTC AAC ACC CC-3′ and R: 5′-ATA GCT CTT CTC CAG GGA GG-3′, GR: NR3C1 RT2 quantitative RT-PCR (qPCR) Primer Assay (PPR52805B, SA Biosciences, Qiagen, Valencia, CA, USA), mineralocorticoid receptor (MR): F: 5′-GGC AGC TGC AAA GTC TTC TT-3′ and R: 5′-GAC AGT TCT TTC GCC GAA TC-3′, NFkB: F: 5′-TCT GGT CAC CAA GCT CCA TGC TAA-3′ and R: 5′-TGG ATG TCA GCA CCA GCC TTT AGA-3′, IL-6: F: 5′-GGC AAA TTT CCT GGT TAT ATC C-3′ and R: 5′-AGA AAA GAG TTG TGC AAT GGC A-3′, CD11b: F: 5′-CTG GGA GAT GTG AAT GGA G-3′ and R: 5′-ACT GAT GCT GGC TAC TGA TG-3′, IkBa: F: 5′-CAG GAT TCT GCA GGT CCA CT-3′ and R: 5′-TGG AGC ACT TGG TGA CTT TG-3′, MKP-1: F: 5′-CAG GAT TCT GCA GGT CCA CT-3′ and R: 5′-TGG AGC ACT TGG TGA CTT TG-3′, IL-1Ra: F: 5′-CTG GGT ACT TAC AAG GAC CAA ATA CC-3′ and R: 5′-TGG ATG CCC AAG AAC ACA TTC CGA-3′.

Statistical analyses

Statview (SAS institute, Cary NC) was used to statistically analyze the results. Student's t-test was used to compare body weight between HFD- and CHD-exposed offspring. Behavioral measures (Light–dark transition, Open Field, and Elevated Plus Maze) were examined using a 2 × 2 diet-by-sex mix-model repeated measures analysis of variance (ANOVA) followed by Bonferroni post hoc testing when applicable. All behavioral measures were also tested for normality using the Shapiro–Wilk method in SPSS (IBM Corporation, Armonk, NY, USA). A 2 × 2 factorial ANOVA was used for gene expression analysis to examine the influence of diet and sex, followed by Bonferroni post hoc tests. As our previous study indicated that adult females were particularly sensitive to the influence of HFD on anxiety and related gene expression (Sasaki et al., 2013), one of the goals of our study was to examine sex-related effects of maternal HFD in adolescent offspring. We therefore performed planned comparisons to examine the effects of diet separately for males and females. Effects were considered statistically significant at $P \leqslant 0.05$.

RESULTS

Body weight

HFD-fed dams gained more weight than CHD-fed dams throughout gestation, and HFD-exposed offspring showed no difference in weight at birth and an increased rate of weight gain prior to weaning compared

to CHD-exposed offspring, as we reported previously (Sasaki et al., 2013). In adolescence at PD35, there was no difference in body weight between HFD-exposed and CHD-exposed male and female offspring ($P = 0.21$ and $P = 0.67$ respectively; Fig. 1).

Effect of maternal HFD on anxiety behavior in adolescent offspring

Light–dark transition task in offspring. Duration of time spent in light zone showed an overall significant main effect of sex [$F_{(1,65)} = 4.60$, $P = 0.03$], a main effect of diet [$F_{(1,65)} = 7.02$, $P = 0.01$], and a significant sex-by-diet interaction [$F_{(1,65)} = 5.77$, $P = 0.01$; Fig. 2]. Post hoc testing revealed that these effects were due to the fact that CHD-exposed male offspring spent significantly longer in the light zone than HFD-exposed males ($P < 0.01$). There was no difference between the CHD-exposed and HFD-exposed female offspring in the Light–dark transition task.

Open Field task in offspring. HFD-exposed offspring entered the center of the Open Field more than did CHD-exposed offspring [main effect of diet, $F_{(1,41)} = 6.65$, $P = 0.01$; Fig. 3A]. Post hoc testing showed that there was a significant effect of diet for center entries made by female HFD-exposed compared to female CHD-exposed offspring ($P = 0.01$). HFD-exposed offspring also showed a greater number of center entries relative to edge entries than did CHD-exposed offspring [main effect of diet, $F_{(1,40)} = 10.74$, $P < 0.01$, Fig. 3B] and, in addition, males displayed a greater relative number of center entries than did females [main effect of sex, $F_{(1,40)} = 3.97$, $P = 0.05$, Fig. 3B]. Both male and female HFD-exposed offspring showed a greater number of entries into the center zone relative to the edge than CHD-exposed male or female offspring ($P = 0.05$ and $P = 0.01$, respectively).

Elevated Plus Maze task in offspring. Overall, HFD-exposed offspring showed a greater number of entries into the open arm relative to the closed arm of the Elevated Plus Maze [main effect of diet, $F_{(1,63)} = 7.18$, $P < 0.01$; Fig. 4A]. Post hoc testing revealed that
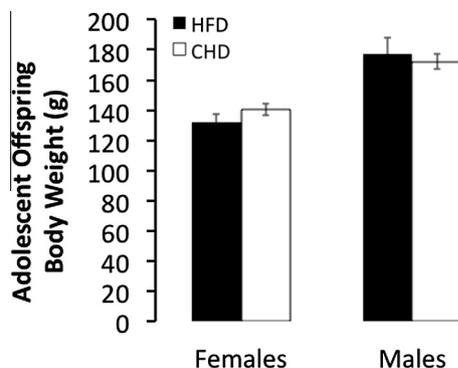

Fig. 2. Light dark transition. Mean ± SEM time spent (sec) by adolescent offspring in the lighted portion of the box. HFD = high-fat diet, CHD = control house chow diet. Bar *$P \leqslant 0.05$, main effect of diet, bar &$P \leqslant 0.05$, main effect of sex; **$P \leqslant 0.01$, post hoc comparison within sex.
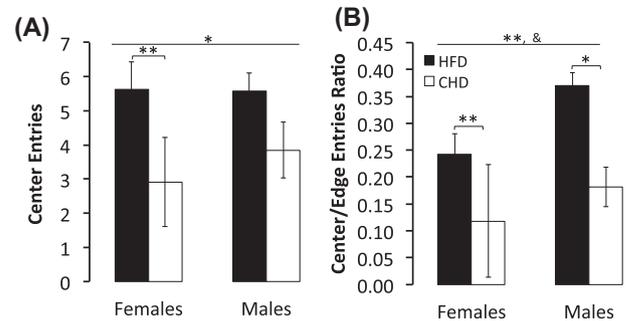
Fig. 3. Open Field. Mean ± SEM of (A) the number of entries into the center of the Open Field and (B) the proportion of entries into the center relative to the edge of the Open Field by adolescent offspring. HFD = high-fat diet, CHD = control house chow diet. Bar **$P \leqslant 0.01$, *$P \leqslant 0.05$, main effect of diet; bar &$P \leqslant 0.05$ main effect of sex; **$P \leqslant 0.01$, *$P \leqslant 0.05$ post hoc comparison within sex.

HFD-exposed female offspring entered the open arms more than did CHD-exposed female offspring ($P = 0.02$). When entries into the proximal zones (i.e. the portion of the arms closest to the center) were examined, the data showed that HFD-exposed offspring made more proximal open arm entries relative to proximal closed arm entries [main effect of diet, $F_{(1,64)} = 6.71$, $P = 0.01$; Fig. 4B]. Post hoc testing showed that HFD-exposed male offspring made more relative entries into the proximal open arms of the maze than did CHD-exposed male offspring ($P = 0.02$).

Effect of maternal HFD on expression of corticosteroid and inflammatory genes in adolescent offspring

Corticosteroid receptor gene expression in offspring. Transcript abundance of GR in the hippocampus was higher among HFD-exposed offspring compared to CHD-exposed offspring [main effect of diet, $F_{(1,20)} = 6.52$, $P = 0.02$; Fig. 5] with HFD-exposed females showing significantly increased hippocampal GR expression compared to CHD-exposed females ($P = 0.02$). In the amygdala, GR expression was not significantly different between diet groups or sexes. The

Fig. 1. Mean ± SEM body weight (g) of adolescent offspring. Maternal high-fat diet did not alter body weight in adolescent offspring. HFD = high-fat diet, CHD = control house chow diet.
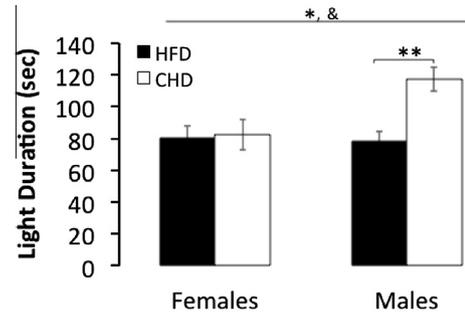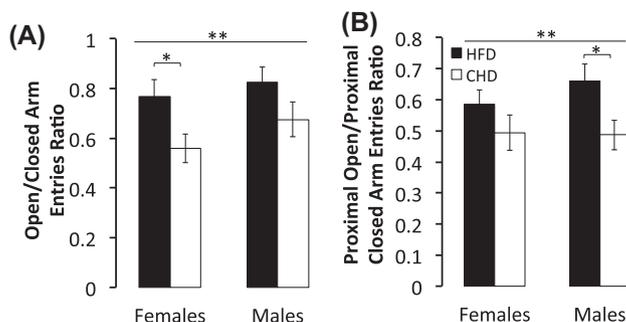
Fig. 4. Elevated Plus Maze. Mean ± SEM of (A) the proportion of entries into the open arms relative to the closed arms and (B) the number of entries into the proximal area of the open arms relative to the proximal area of the closed arms by adolescent offspring. HFD = high-fat diet, CHD = control house chow diet. Bar $^{**}P \leqslant 0.01$, main effect of diet; $^{*}P \leqslant 0.05$, post hoc comparison within sex.

expression of the other major corticosteroid receptor, MR, also did not differ significantly in the hippocampus or amygdala between diet groups or sex.

Pro-inflammatory gene expression in offspring. Fig. 6 shows transcript abundance of NFkB, IL-6, and CD11b. HFD-exposed offspring showed an increase in NFkB transcript abundance in the hippocampus [main effect of diet, $F_{(1,20)} = 3.98$, $P = 0.05$]. IL-6 expression in the hippocampus was significantly increased in HFD-exposed offspring [main effect of diet, $F_{(1,20)} = 4.41$, $P = 0.05$], but did not differ between diet groups or sex in the amygdala. In the amygdala, the HFD-exposed females showed a significantly decreased NFkB transcript abundance compared to CHD-exposed females ($P = 0.04$). CD11b did not show significant differences in expression between diet groups or sex in the hippocampus or the amygdala.

Anti-inflammatory gene expression in offspring. The expression of IkBa, MKP-1, and IL-1Ra was examined as they are known negative regulators of inflammatory response that are modified through chronic changes in

GR, NFkB, and IL-6 signaling (Sorrells et al., 2009). In the hippocampus, HFD-exposed offspring showed an increase in IkBa transcript abundance [main effect of diet, $F_{(1,20)} = 4.55$, $P = 0.04$; Fig. 7]. Post-hoc testing revealed that this difference in IkBa transcript was a result of increased transcript abundance among HFD-exposed males compared to CHD-exposed males ($P = 0.05$). HFD-exposed offspring also showed an increase in MKP-1 transcript abundance in the hippocampus [main effect of diet, $F_{(1,20)} = 6.75$, $P = 0.02$], which was significantly higher among HFD-exposed females compared to CHD-exposed females ($P = 0.02$). In the amygdala, HFD-exposed offspring showed a significant decrease in IL-1Ra transcript levels [main effect of diet, $F_{(1,20)} = 4.18$, $P = 0.05$]. No significant differences were found in IkBa and MKP-1 expression levels in the amygdala and in IL-1Ra expression levels in the hippocampus between diet groups or sex.

DISCUSSION

Our study yielded several major findings. First, perinatal HFD exposure resulted in an overall decrease in
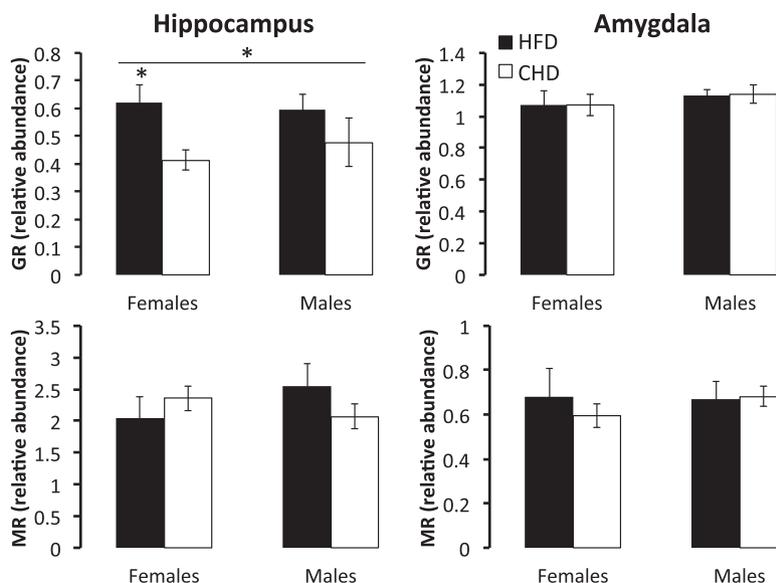

Fig. 5. Corticosteroid receptor gene expression. Mean ± SEM relative abundance of transcripts for glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in the hippocampus and amygdala of adolescent offspring. HFD = high-fat diet, CHD = control house chow diet. Bar $^{*}P \leqslant 0.05$ main effect of diet; $^{*}P \leqslant 0.05$ post hoc comparison within sex.
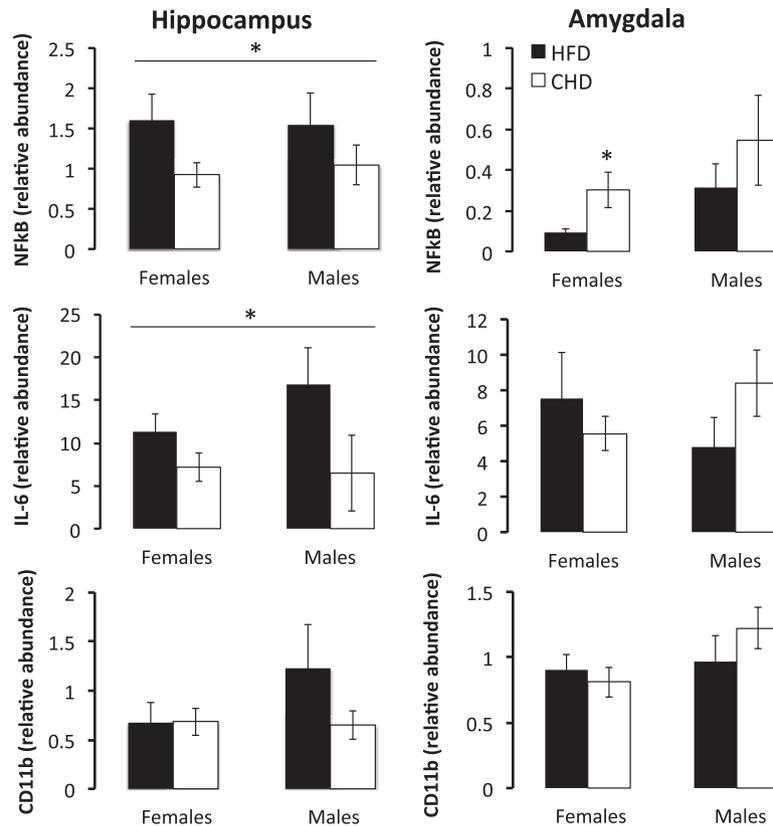
Fig. 6. Pro-inflammatory gene expression. Mean ± SEM relative abundance of transcripts for nuclear factor kappa beta (NFkB), interleukin-6 (IL-6) and cluster of differentiation molecule 11b (CD11b) in the hippocampus and amygdala of adolescent offspring. HFD = high-fat diet, CHD = control house chow diet. Bar *$P \leqslant 0.05$ main effect of diet; *$P \leqslant 0.05$ post hoc comparison within sex.

anxiety-like behavior on several measures of anxiety in adolescence. Second, perinatal HFD exposure was associated with a selective increase in GR transcript abundance in the hippocampus, particularly in female offspring. Third, in the hippocampus, the expression of NFkB and IL-6 pro-inflammatory genes and IkBa and MKP-1 anti-inflammatory genes were upregulated in HFD-exposed offspring, indicating a dysregulation of inflammatory gene expression. Fourth, in the amygdala, HFD-exposed females showed decreased expression of NFkB, and there was an overall decrease in IL-1Ra anti-inflammatory gene expression among HFD-exposed offspring. Finally, body weight was similar between the two diet groups at PD35, suggesting that our findings are attributable to perinatal HFD exposure rather than differences in current body weight. We did not observe a decreased body weight at birth among the HFD-exposed offspring, indicative of insufficient levels of protein. Together with the data indicating that HFD-fed dams gained more weight than CHD-fed dams throughout gestation, these data indicate that our model of diet-induced obesity likely did not involve protein restriction during prenatal development. Thus, under the conditions of the present study, the observed effects on anxiety behavior occurred independent of differences in offspring body weight in adolescence, with a decrease in anxiety behavior consistent with increased GR expression and differences in the expression of

inflammatory genes known to be sensitive to alterations in glucocorticoid signaling.

Overall, HFD-exposed offspring showed a decrease in anxiety-like behavior as indicated by increased exploration of the open arms of the Elevated Plus Maze and entries in the center portion of the Open Field. Differences among the diet groups were observed in relative measures of exploration between the open arms or center of the apparatuses and the closed arms or edges, suggesting that the effects of HFD on anxiety behavior were not due to differences in locomotor activity. These results in adolescent animals contrast with our previous study, where we found an increase in anxiety behavior in HFD-exposed offspring in adult animals (Sasaki et al., 2013). The Light–dark transition task was the only task to suggest that HFD-exposed off-spring, specifically males, displayed increased anxiety behavior, as males showed an increase in time spent in the lighted portion of the box. Several previous studies have indicated that behaviors characteristic of anxiety in adulthood are also consistent with impulsive/risk-taking exploratory behaviors in adolescent animals (Jacobson-Pick and Richter-Levin, 2010; Jacobson-Pick et al., 2011; McCormick and Green, 2013). For example, in a study by Colorado et al. (2006) adolescent offspring that experienced maternal separation, known to increase anxiety behavior in adulthood, spent an increased amount of time in the center of an Open Field (both a
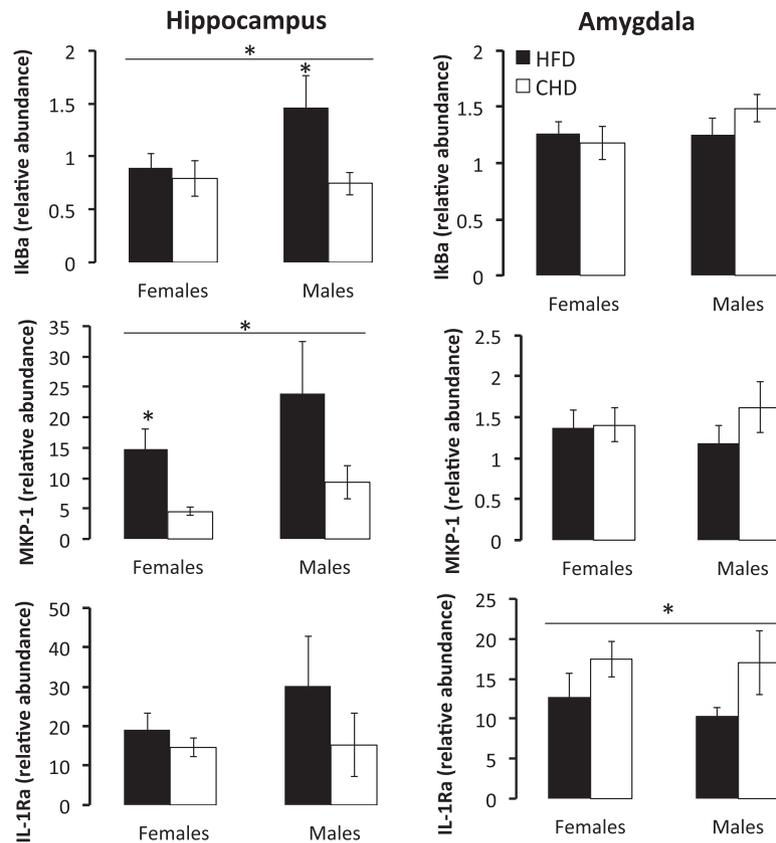
Fig. 7. Anti-inflammatory gene expression. Mean ± SEM relative abundance of transcripts for I-kappa-B-alpha (IkBa), mitogen-activated protein kinase phosphatase-1 (MKP-1) and interleukin-1 receptor antagonist (IL-1Ra) in the hippocampus and amygdala of adolescent offspring. HFD = high-fat diet, CHD = control house chow diet. Bars $^*P \leqslant 0.05$ main effect of diet; $^*P \leqslant 0.05$ post hoc comparison within sex.

novel Open Field and a familiar Open Field) and in the lighted portion of the Light–dark transition box (Colorado et al., 2006). Likewise, high levels of exploration of the open arms of the Elevated Plus Maze has also been suggested to be an indicator of impulsive behavior (Almeida et al., 1996). Distinct behavioral stress response profiles have been observed between adolescent and adult animals (Arborelius et al., 1999; Jacobson-Pick and Richter-Levin, 2010; Jacobson-Pick et al., 2011). Clinical studies in humans have also shown distinguishable symptom profiles between adolescents and adults in anxiety disorders, potentially due to differences in stress reactivity in adolescence compared to adulthood (Pine et al., 1998; Stein et al., 2001; Bostic et al., 2005). As impulsivity in adolescence is closely associated with anxiety behavior in adulthood, future work aimed at characterizing the effects of HFD on risk-taking choices and behavioral disinhibition using serial choice tasks in adolescents (Robbins, 2002) may shed light on mechanisms medicating the programming of impulsive behavior by HFD.

Corticosteroid receptor activation in the limbic system has been implicated in refining the response of the HPA axis, where increased levels of GR expression in the hippocampus are known to inhibit the HPA axis while increased levels of GR expression in the amygdala enhance the stress response (Groeneweg et al., 2011). Adolescent qRT-PCR analysis of corticosteroid receptors revealed that hippocampal GR expression was increased

in HFD-exposed offspring, particularly in females, which support our behavioral results indicating decreased anxiety among HFD-exposed offspring. These data differ from what was seen in adults (Sasaki et al., 2013), where we found an increase in GR and MR expression in the amygdala. No significant differences in GR or MR gene expression were found in the amygdala of HFD-exposed adolescents. The data indicate a developmental shift between adolescence and adulthood in the influence of HFD on limbic regions linked to HPA regulation and anxiety behavior.

In adult animals, chronic HFD exposure is well known to enhance the activation of the HPA axis due to increased secretion of ACTH and corticosterone (Tannenbaum et al., 1997), as well as enhancing the HPA response to stressors (Legendre and Harris, 2006). An obese maternal phenotype is associated with increased exposure of offspring to maternal leptin in the neonatal period, as leptin produced in the mammary gland is found in maternal milk (Walker, 2010). Pups receiving chronic leptin also show enhanced HPA-negative feedback associated with increased GR expression in the hippocampus and hypothalamus (Proulx et al., 2001). These data may indicate that maternal obesity leads to HPA dysregulation in offspring during development, possibly through a combination of altered maternal HPA during the prenatal period and altered maternal leptin exposure during postnatal life. Thus, although no

differences in body weight were found between HFD and CHD-exposed rats in adolescence, it remains possible that metabolic parameters such as altered central or peripheral leptin signaling, body composition, or insulin resistance as a result of perinatal HFD exposure may have contributed to the differences in anxiety behavior observed in this study.

Glucocorticoids have both pro- and anti-inflammatory roles in the brain (Sorrells et al., 2009), thus we investigated the expression of several inflammatory genes linked to glucocorticoid signaling (Sorrells et al., 2009) and perinatal HFD exposure (Bilbo and Tsang, 2010). Our results indicated a dysregulation of pro- and anti-inflammatory genes as a result of HFD exposure. NFkB and IL-6 transcripts were increased in the hippocampus in HFD-exposed offspring. Increased IL-6 expression in the hypothalamus (De Souza et al., 2005) and cortex (White et al., 2009) is an established consequence of prolonged HFD exposure. In conditions of chronic stress, increased GR expression is required for an enhanced inflammatory response when NFkB expression is elevated (Sorrells et al., 2009). In addition to GR being elevated in the hippocampus, increased expression of IkBa, a negative regulator of NFkB (Munhoz et al., 2010), was observed in the hippocampus of HFD-exposed adolescents, notably among male offspring. There was also an increase in MKP-1 transcript abundance in the hippocampus of HFD-exposed offspring, particularly in females, suggesting that these anti-inflammatory genes may be upregulated in response to pro-inflammatory gene upregulation. In the amygdala, NFkB transcript abundance was significantly decreased in HFD-exposed female offspring, and IL-1Ra showed decreased expression overall among HFD-exposed offspring. These results demonstrate brain region-specific effects of perinatal HFD exposure, and suggest that changes in hippocampal GR and inflammatory pathway genes may be relevant for adolescent anxiety behavior, at least in the present experimental context.

Maternal HFD is known to advance puberty onset, an effect possibly due to the increased presence of adipose tissue, which can serve as a site for estrogen aromatization (Hilakivi-Clarke et al., 1995). Maternal HFD also increases ER-a and -b levels in the hypothalamus (Cabanes et al., 2000). Given the role of estrogen in anxiety and the neuroendocrine response to stress, it is possible that variations in estrogen as a result of puberty onset may have contributed to the behavioral and neural response to HFD exposure among female offspring. Estrogen administration and estrous are associated with decreased defensive burying behavior, a measure of impulsivity, and decreased freezing behavior in a fear conditioning task, a measure of impulsive or anti-anxiety behavior (Llaneza and Frye, 2009). The recall of fear conditioning after extinction appears to be mediated by ER-b activation (Zeidan et al., 2011). These data indicate an influence of estrogens on the expression of impulsive and avoidance/anti-anxiety behaviors, with lower impulsive/anxious behaviors observed when estrogen levels are high. Alterations in response to developmental HFD exposure in estrogen-sensitive neural

pathways that could mediate these behavioral responses remain to be determined. However, given the known role of estrogens in mediating both HPA and inflammatory gene expression (Walker et al., 2009; Loram et al., 2012; Pyter et al., 2013) it is possible that the observed sensitivity of females to the effects of HFD on inflammatory gene expression may be partly a result of changes in circulating estrogen with HFD exposure.

Obesity has become a worldwide epidemic and we have yet to fully understand the effects of maternal diet on offspring health (Godfrey et al., 2010). In developed countries, approximately 30% of all pregnancies are now complicated by maternal obesity (Catalano, 2007). HFD exposure during development may lead to multiple deleterious health outcomes, including anxiety-related mental disorders. Additional studies are needed to establish the critical time window during perinatal development when HFD exposure influences the behavioral and neural responses observed in this study. Determining how maternal HFD specifically affects brain signaling pathways in offspring will allow for the development of effective interventions targeting the consequences of overnutrition on developmental programming.

REFERENCES

Almeida SS, Tonkiss J, Galler JR (1996) Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus-maze test. Physiol Behav 60:675–680.

Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB (1999) The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 160:1–12.

Bersamin A, Luick BR, King IB, Stern JS, Zidenberg-Cherr S (2008) Westernizing diets influence fat intake, red blood cell fatty acid composition, and health in remote Alaskan Native communities in the center for Alaska Native health study. J Am Diet Assoc 108: 266–273.

Bilbo SD, Tsang V (2010) Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J 24:2104–2115.

Boksa P (2004) Animal models of obstetric complications in relation to schizophrenia. Brain Res Rev 45:1–17.

Boney CM, Verma A, Tucker R, Vohr BR (2005) Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 115:e290–e296.

Bostic JQ, Rubin DH, Prince J, Schlozman S (2005) Treatment of depression in children and adolescents. J Psychiatr Pract 11: 141–154.

Brunton PJ (2010) Resetting the dynamic range of hypothalamic–pituitary–adrenal axis stress responses through pregnancy. J Neuroendocrinol 22:1198–1213.

Cabanes A, de Assis S, Gustafsson JA, Hilakivi-Clarke L (2000) Maternal high n-6 polyunsaturated fatty acid intake during pregnancy increases voluntary alcohol intake and hypothalamic estrogen receptor alpha and beta levels among female offspring. Dev Neurosci 22:488–493.

Catalano PM (2007) Management of obesity in pregnancy. Obstet Gynecol 109:419–433.

Colorado RA, Shumake J, Conejo NM, Gonzalez-Pardo H, Gonzalez-Lima F (2006) Effects of maternal separation, early handling, and standard facility rearing on orienting and impulsive behavior of adolescent rats. Behav Process 71:51–58.

De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA (2005) Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146: 4192–4199.

Desai RA, Manley M, Desai MM, Potenza MN (2009) Gender differences in the association between body mass index and psychopathology. CNS Spectr 14:372–383.

Dunn GA, Bale TL (2009) Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. Endocrinology 150:4999–5009.

El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS (2000) Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Investig 105:1827–1832.

Godfrey KM, Gluckman PD, Hanson MA (2010) Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends Endocrinol Metab 21:199–205.

Groeneweg FL, Karst H, de Kloet ER, Joels M (2011) Rapid non-genomic effects of corticosteroids and their role in the central stress response. J Endocrinol 209:153–167.

Hilakivi-Clarke L, Cho E, Raygada M, Onojafe I, Clarke R, Lippman ME (1995) Early life affects the risk of developing breast cancer. Ann N Y Acad Sci 768:327–330.

Jacobson-Pick S, Richter-Levin G (2010) Differential impact of juvenile stress and corticosterone in juvenility and in adulthood, in male and female rats. Behav Brain Res 214:268–276.

Jacobson-Pick S, Audet M-C, Nathoo N, Anisman H (2011) Stressor experiences during the juvenile period increase stressor responsivity in adulthood: Transmission of stressor experiences. Behav Brain Res 216:365–374.

Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840–846.

Legendre A, Harris RB (2006) Exaggerated response to mild stress in rats fed high-fat diet. Am J Physiol Regul Integr Comp Physiol 291:R1288–R1294.

Llaneza DC, Frye CA (2009) Progestogens and estrogen influence impulsive burying and avoidant freezing behavior of naturally cycling and ovariectomized rats. Pharmacol Biochem Behav 93:337–342.

Loram LC, Sholar PW, Taylor FR, Wiesler JL, Babb JA, Strand KA, Berkelhammer D, Day HE, Maier SF, Watkins LR (2012) Sex and estradiol influence glial pro-inflammatory responses to lipopolysaccharide in rats. Psychoneuroendocrinology 37:1688–1699.

Marx J (2002) Unraveling the causes of diabetes. Science 296:686–689.

McCormick CM, Green MR (2013) From the stressed adolescent to the anxious and depressed adult: investigations in rodent models. Neuroscience 249:242–257.

McCormick CM, Smith C, Mathews IZ (2008) Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. Behav Brain Res 187:228–238.

McGowan PO, Meaney MJ, Szyf M (2008) Diet and the epigenetic (re)programming of phenotypic differences in behavior. Brain Res 1237:12–24.

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 24:1161–1192.

Munhoz CD, Sorrells SF, Caso JR, Scavone C, Sapolsky RM (2010) Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. J Neurosci 30:13690–13698.

Paxinos G, Watson CR (1997) The rat brain in stereotaxic coordinates. San Diego: Academic Press.

Peleg-Raibstein D, Luca E, Wolfrum C (2012) Maternal high-fat diet in mice programs emotional behavior in adulthood. Behav Brain Res 233:398–404.

Pine DS, Cohen P, Gurley D, Brook J, Ma Y (1998) The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. Arch Gen Psychiatry 55:56–64.

Proulx K, Clavel S, Nault G, Richard D, Walker CD (2001) High neonatal leptin exposure enhances brain GR expression and feedback efficacy on the adrenocortical axis of developing rats. Endocrinology 142:4607–4616.

Purcell RH, Sun B, Pass LL, Power ML, Moran TH, Tamashiro KL (2011) Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. Physiol Behav 104:474–479.

Pyter LM, Kelly SD, Harrell CS, Neigh GN (2013) Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats. Brain Behav Immun 30:88–94.

Robbins TW (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl) 163:362–380.

Rofey DL, Kolko RP, Iosif AM, Silk JS, Bost JE, Feng W, Szigethy EM, Noll RB, Ryan ND, Dahl RE (2009) A longitudinal study of childhood depression and anxiety in relation to weight gain. Child Psychiatry Hum Dev 40:517–526.

Sasaki A, de Vega WC, St-Cyr S, Pan P, McGowan PO (2013) Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. Neuroscience 240:1–12.

Smoak KA, Cidlowski JA (2004) Mechanisms of glucocorticoid receptor signaling during inflammation. Mech Ageing Dev 125:697–706.

Sorrells SF, Caso JR, Munhoz CD, Sapolsky RM (2009) The stressed CNS: when glucocorticoids aggravate inflammation. Neuron 64:33–39.

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463.

Stein MB, Fuetsch M, Muller N, Hofler M, Lieb R, Wittchen H-U (2001) Social anxiety disorder and the risk of depression: a prospective community study of adolescents and young adults. Arch Gen Psychiatry 58:251–256.

Sullivan EL, Grayson B, Takahashi D, Robertson N, Maier A, Bethea CL, Smith MS, Coleman K, Grove KL (2010) Chronic consumption of a high-fat diet during pregnancy causes perturbations in the serotonergic system and increased anxiety-like behavior in nonhuman primate offspring. J Neurosci 30:3826–3830.

Tamashiro KL, Terrillion CE, Hyun J, Koenig JI, Moran TH (2009) Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. Diabetes 58:1116–1125.

Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD, Meaney MJ (1997) High-fat feeding alters both basal and stress-induced hypothalamic–pituitary–adrenal activity in the rat. Am J Physiol 273:E1168–E1177.

Tirelli E, Laviola G, Adriani W (2003) Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neurosci Biobehav Rev 27:163–178.

Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444:875–880.

Vazquez DM (1998) Stress and the developing limbic-hypothalamic–pituitary–adrenal axis. Psychoneuroendocrinology 23:663–700.

Walker CD (2010) Maternal touch and feed as critical regulators of behavioral and stress responses in the offspring. Dev Psychobiol 52:638–650.

Walker AK, Nakamura T, Byrne RJ, Naicker S, Tynan RJ, Hunter M, Hodgson DM (2009) Neonatal lipopolysaccharide and adult stress exposure predisposes rats to anxiety-like behaviour and blunted corticosterone responses: implications for the double-hit hypothesis. Psychoneuroendocrinology 34:1515–1525.

Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S (2004) Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 350:2362–2374.

Welberg LA, Seckl JR (2001) Prenatal stress, glucocorticoids and the programming of the brain. J Endocrinol 13:113–128.

Whitaker RC (2004) Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. Pediatrics 114:e29–e36.

White CL, Pistell PJ, Purpera MN, Gupta S, Fernandez-Kim SO, Hise TL, Keller JN, Ingram DK, Morrison CD, Bruce-Keller AJ (2009) Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: contributions of maternal diet. Neurobiol Dis 35:3–13.

Zeidan MA, Igoe SA, Linnman C, Vitalo A, Levine JB, Klibanski A, Goldstein JM, Milad MR (2011) Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biol Psychiatry 70:920–927.