Maternal high-fat diet induces sex-specific effects on inflammatory responses to corticosterone and lipopolysaccharide challenge in adult rat offspring

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ABSTRACT

Acute elevations in endogenous CORT caused by stress or exogenous administration potentiate inflammatory gene expression. Maternal obesity through high-fat diets (HFD) has been linked to higher basal levels of neuroinflammation, including increased expression of pro-inflammatory genes such as NFκB and interleukin-6 in the amygdala. These findings suggest that adult rats exposed to maternal HFD may elicit pro-inflammatory responses in the presence of an immune stressor and elevated CORT. To investigate, adult rat offspring exposed to maternal control fat house-chow diet (CFD) or HFD were administered exogenous CORT and lipopolysaccharide (LPS), a component of gram-negative bacteria. Transcript abundance of CORT receptors and downstream inflammatory genes were measured in the amygdala, hippocampus and prefrontal cortex, brain regions that mediate neuroendocrine and behavioural responses to stress. We found sex-specific responses, where HFD female offspring exhibited elevations in anti-inflammatory transcripts, and HFD male offspring responded with a larger pro-inflammatory response to simultaneous CORT and LPS administration. These findings suggest that exposure to maternal HFD leads to sex-specific alterations that could regulate the neural immune response, possibly as an adaptive response to basal inflammation.

INTRODUCTION

Obesity during pregnancy is an early life stressor that is becoming increasingly common in both developed and developing countries (Amugsi et al., 2017; Bhurosy and Jeewon, 2014; Forsyth et al., 2016). Maternal obesity can lead to metabolic and behavioural disorders involved with stress response in offspring (Catalano and deMouzon, 2015; Contu and Hawkes, 2017; King, 2006; Rivera et al., 2015; Van Lieshout et al., 2011; Zambrano et al., 2016). For example, epidemiological studies indicate that maternal obesogenic diets high in saturated fats are linked to increased risk anxiety-like disorders in offspring (Edlow, 2017; Rivera et al., 2015; Rofey et al., 2009).

The mechanisms by which exposure to maternal high-fat diet (HFD) alter inflammatory gene expression in brain regions involved with regulating anxiety behaviour is not well understood. In rodent models of maternal obesity, HFD exposure results in increased anxiety-like behaviour in the Open field and Elevated plus maze tasks at adulthood, accompanied by changes associated with the hypothalamic-pituitary-adrenal (HPA) axis, the neuroendocrine system that regulates stress responses (Bilbo and Tsang, 2010; Peleg-Raibstein et al., 2012; Sasaki et al., 2013; Sullivan et al., 2012). In events of stress, the HPA axis leads to the secretion of corticosterone (CORT) in rodents, which binds to glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) to mediate behavioural and immune stress responses in limbic brain regions including the amygdala, hippocampus (HPC), as well as the prefrontal cortex (PFC) (Munck and Náray-Fejes-Tóth, 1992; Silverman and Sternberg, 2012). In basal conditions, CORT binds to GR and leads to the expression of anti-inflammatory genes including, nuclear factor kappa beta-inhibitor alpha (IκBA) and mitogen-activated protein kinase phosphatase 1 (MKP-1) (Munhoz et al. 2010; Sorrells et al. 2009). However, acute elevations in

CORT caused by stress potentiate pro-inflammatory gene expression (Munhoz et al. 2006, 2010; Sorrells et al. 2009). Systemic stressors such as lipopolysaccharide (LPS), a component of gram-negative bacteria, activate inflammatory processes via NFκB, which leads to interleukin 6 (IL6), and cluster of differentiation molecule 11B (CD11B) expression.

The GR-NFκB pathway that regulates inflammatory stress is altered in the brain of adult rats exposed to maternal HFD. In basal conditions, there are increases in pro-inflammatory gene expression of NFκB and IL6 in the amygdala, along with downregulated serum CORT (Sasaki et al. 2013). These findings suggest that in adult rats exposed to maternal HFD, acute elevations in CORT will potentiate neuroinflammatory effects of LPS to a larger degree than in control animals. To investigate how maternal HFD impacts GR signalling and inflammatory gene expression in conditions of stress, adult female and male rat offspring exposed to maternal control or high-fat diet were administered exogenous CORT to simulate psychological stress, LPS to simulate systemic stress, or simultaneous CORT and LPS challenge. Transcript abundance of CORT receptors and downstream inflammatory pathway genes was measured in the amygdala, HPC, and PFC, limbic brain regions that mediate HPA axis responses to stress. Females and males were examined separately due to prominent sex differences in body weight and response to HFD, CORT, and LPS exposures as determined in past literature (Ashdown et al., 2007; Bilbo and Tsang, 2010; Chistyakov et al., 2018; Sasaki et al., 2013; Seale et al., 2004)

MATERIALS & METHODS

Animal Care

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. The adult rat offspring used for this study were untested littermates of the same cohort of animals in previous studies published by our group (Sasaki et al., 2014; Sasaki et al., 2013). For breeding, 7-week-old adult male and female Long Evans rats were purchased from Charles River Canada (St. Constant, QC), and housed with same-sex pairs, and maintained on a 12:12 h light-dark cycle (lights turned on from 7:00 am to 7:00 pm) with *ad libitum* access to food and water. Female breeders were placed on either a high-fat diet (HFD, 5.24 kcal/g, n=15) consisting of 60% fat, 20% protein, 20% carbohydrate (D12492; Research Diets, Inc. New Brunswick, NJ.), or a control fat house-chow diet (CFD, 3.02 kcal/g, n=14) consisting of 13.5% fat, 28.5% protein, and 58% carbohydrate (5001; Purine Lab Diets. St. Louis, MO) four weeks prior to mating, throughout gestation, lactation, and until weaning. Litters were weighed weekly during cage changes and were otherwise left undisturbed until weaning (post-natal day [PND] PND21), when they were all placed on a CFD diet and housed in same-sex pairs. At adulthood (PND90), body weights were measured prior to CORT and LPS injections (Figure 1).

Adult CORT and LPS challenge

CORT dissolved in propylene glycol (27840; Sigma-Aldrich) and LPS from *Escherichia coli* O111:B4, (L2630; Sigma-Aldrich) were used for subcutaneous and intraperitoneal injections respectively. Adult female and male offspring were handled 2 min/day for five consecutive days starting from PND85. At PND90, animals were divided into one of four experimental groups; 1)

subcutaneous dose of CORT (10 mg/kg of body weight), 2) an intraperitoneal dose of LPS (50 μg/kg of body weight), 3) a simultaneous dose of CORT and LPS (10 mg/kg, 50 μg/kg), or 4) handled controls (n=6 per diet, sex, and treatment). A 10mg/kg dose of CORT was previously shown to lead to heightened anxiety-like behaviour (Mitra and Sapolsky, 2008) and circulating plasma CORT levels similar to that of several hours of acute physiological stress (Stein-Behrens et al., 1994). The 50 μg/kg dose of LPS was shown to activate the HPA axis within 0.5-4 h, as shown by increased CORT levels in whole-blood (Vore et al., 2017) and induce pro-inflammatory cytokine expression in the hippocampus of offspring exposed to maternal HFD (Bilbo and Tsang, 2010). Animals were sacrificed 3 h post injection by CO_2 inhalation followed by rapid decapitation at the mid-point of the light phase (11-3 pm) to control for circadian-related changes in gene expression. Brains were dissected, flash-frozen in isopentane and dry ice, and stored at -80 °C.

RNA Extraction and cDNA Synthesis

Whole amygdala, dorsal hippocampus, and medial prefrontal cortex were cryo-sectioned using a Leica CM3050 cryostat and stereotaxic coordinates (Paxinos and Watson, 2007). RNA was extracted from the amygdala, dorsal hippocampus, and medial prefrontal cortex using TRIzol reagent (15596026; Invitrogen) in combination with RNeasy Plus Mini Kit (74134; Qiagen) as per the manufacturer's instructions. RNA quantification and quality assessments were done using a Nanodrop Spectrophotometer (ND-2000C; Thermo Scientific). 1 μg of total RNA was converted to cDNA using High Capacity cDNA Reverse Transcription Kit (4368814; Applied Biosystems) according to the manufacturer's instructions.

Gene expression Analysis by RT-qPCR

Relative mRNA expression of glucocorticoid receptor (GR), mineralocorticoid receptor (MR), nuclear factor kappa light chain enhancer of activated B cells (NFκB), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), interleukin 6 (IL6), interleukin 10 (IL10), cluster of differentiation molecule 11B (CD11B), mitogen activated protein kinase phosphatase 1 (MKP1), and insulin-like growth factor 1 (IGF1) in the three brain regions was measured using a StepOne Plus real-time thermocycler with a Fast SYBR Green PCR master mix (4385612; Applied Biosystems). Primers were purchased from Qiagen or Eurofins Genomics and designed according to GenBank sequence information at the National Center for Biotechnology Information (NCBI) (Table 1).

Relative gene expression was calculated using the quantity mean based on a standard curve of 11 serial dilutions ranging from 500 ng/μL to 0.49 ng/μL of cDNA. A standard curve was run per plate and per set of comparisons. Quantity means were normalized against the GEOmean of four reference genes, YWAZ, GAPDH, 18s, and Actin B. Reference genes were identified as stable internal controls based on geNORM analysis of stability across experimental groups, brain regions, and sex (Vandesompele et al., 2002). Stability M values calculated by geNORM: YWAZ = 0.322, Actin B = 0.49, GAPDH = 0.49, 18S = 0.68. Relative transcript levels were expressed as mean ± SEM representing n=6 biological replicates per experimental condition.

Statistical Analysis

Body weight of adult offspring was analyzed by 3-way (diet x drug x sex) ANOVA using SPSS (IBM Corp, 23). Transcript levels were analyzed using 1-way ANOVA with Scheffe post-hoc

analysis using R Statistical Software (R Foundation for Statistical Computing, Vienna, Austria, 3.4.2). Relationships were considered statistically significant at $p \leq 0.05$.

Table 1. Primer sequences used in RT-qPCR analysis.

Primer	Sequence
Reference genes	
Actin B	FW: TTTGAGACCTTCAACACCCC RV: ATAGCTCTTCTCCAGGGAGG
GAPDH	FW: ACATCAAATGGGGTGATGCT RV: GTGGTTCACACCCATCACAA
18S rRNA	FW: ATGGTAGTCGCCGTGCCTA RV: CTGCTGCCTTCCTTGGATG
YWHAZ	FW: TTGAGCAGAAGACGGAAGGT RV: GAAGCATTGGGGATCAAGAA
Glucocorticoid signalling	
GR	RT^2 qPCR Primer Assay (PPR52805B, Qiagen)
MR	FW: GGCAGCTGCAAAGTCTTCTT RV: GACAGTTCTTTCGCCGAATC
Pro-inflammatory	
CD11B	FW: GAAGCCTTGGCGTGTGATAG RV: GAGCAGTTTGTTCCCAAGGG
IL6	RT^2 qPCR Primer Assay (PPR06483B, Qiagen)
NFκB	RT^2 qPCR Primer Assay (PPR42746A, Qiagen)
Anti-inflammatory	
IκBα	FW: CAGGATTCTGCAGGTCCACT RV: TGGAGCACTTGGTGACTTTG
IGF-1	FW: GCTCTTCAGTTCGTGTGTGG RV: TGAGTCTTGGGCATGTCAGT
IL10	RT^2 qPCR Primer Assay (PPR06479A, Qiagen)
MKP1	FW: GCTCCACTCAAGTCTTCTTCCTCCAA RV: TGGACTGTTTGCTGCACAGCTCAG

RESULTS

Offspring body weight

As reported previously by our group for animals in this cohort, offspring with maternal HFD exposure showed no differences in body weight at birth, however weighed more than CFD

offspring through weaning (Sasaki et al., 2014; Sasaki et al., 2013). In adulthood (PND90), male offspring weighed more than female offspring (main effect of sex ($F_{(1,93)} = 220.20$, $p<0.001$, Figure 2), and both sexes with maternal HFD exposure weighed more than CFD offspring (females ($F_{(1,46)} = 19.71$, $p< 0.001$, males ($F(1,45) = 39.82$, $p< 0.01$, Figure 2). There were no significant differences in body weight between animals assigned to each of the treatment groups (CORT, LPS, CORT+LPS, control handled).

Transcript response to endocrine and immune challenge

CORT Challenge

In females, CORT challenge led to few differences in transcript abundance between HFD and CFD offspring (Figure 3A, 3B). In the amygdala, IκBα increased in HFD offspring (main effect of challenge ($F_{(3,19)} = 9.63$, $p<0.01$), Scheffe post-hoc $p=0.015$), but did not change among CFD offspring (Scheffe post-hoc $p= 0.195$, Figure 3C). In the hippocampus IκBα increased in both diet groups (main effect of challenge ($F_{(3,20)} = 13.35$, $p<0.01$), Figure 3D). Also in the hippocampus, MKP1 increased in HFD offspring (main effect of challenge ($F_{(3,20)} = 12.75$, $p<0.05$), Scheffe post-hoc $p=0.001$), but remained unchanged in CFD offspring (Scheffe post-hoc $p=0.985$, Figure 3E).

In male offspring, we observed more differences in transcript abundance in response to CORT challenge (Figure 4A, 4B). In the amygdala, GR decreased in both diet groups (main effect of challenge ($F_{(3,20)} = 9.006$, $p < 0.01$), Figure 4C), whereas MR decreased in HFD offspring (main effect of diet ($F_{(3,20)} = 4.147$, $p<0.01$), Scheffe post-hoc $p=0.047$, Figure 4D). Also in the amygdala, with CORT challenge, IκBα ($F_{(3,20)} = 52.35$, $p<0.01$, Figure 4E) and IL6 ($F_{(3,20)} = 4.82$, $p = 0.01$, Figure. 4F) increased in both CFD and HFD groups. MKP1 increased in

HFD offspring (main effect of challenge, ($F_{(3,20)}$ = 9.46, p<0.01), Scheffe post-hoc, p=0.02), but did not change in CFD offspring (Scheffe post-hoc, p=0.170, Figure 4G). In the hippocampus, GR decreased in both diet groups (main effect of challenge, ($F_{(3,20)}$ = 7.69, p<0.01), Figure. 4H)), whereas IκBα increased in both diet groups (main effect of challenge, ($F_{(3,20)}$ = 55.3, p<0.01); diet/challenge interaction ($F_{(3,20)}$ = 79.830, p<0.01), Figure 4I). In both diet groups, CORT challenge led to increases in IL6 ($F_{(3,20)}$ = 15.21, p<0.01, Figure 4J) and MKP1 ($F_{(3,20)}$ = 23.41, p<0.01, Figure 4K) in the hippocampus.

LPS challenge

In females, LPS challenge led to transcript abundance between HFD and CFD offspring (Figure 5A, 5B). Both diet groups showed decreased GR in the amygdala (main effect of challenge ($F_{(3,20)}$ = 6.74, p<0.01), Figure 5C). CFD offspring had decreased MR levels ($F_{(3,20)}$ = 3.55, p<0.01, Scheffe post-hoc p=0.05), where as MR levels in HFD offspring remained unchanged (Scheffe post-hoc p=0.749, Figure 5D). Also in the amygdala of both diet groups, there were increases in IκBα (main effect of challenge ($F_{(3,20)}$ = 10.07, p<0.01), Figure 5E), IL6 ($F_{(3,20)}$ = 6.12, p<0.01, Figure 5F), and MKP1 ($F_{(3,20)}$ = 22.63, p<0.01, Figure 5G). In the hippocampus of both diet groups, LPS challenge led to decreased MR transcript ($F_{(3,20)}$ = 8.70, p<0.01, Figure 5H), while there were increases in NFκB ($F_{(3,20)}$ = 26.80, p<0.01, Figure 5I), and IκBα ($F_{(3,20)}$ = 23.24, p<0.01, Figure 5J). IL6 transcript increased in both diet groups, however, there was a larger increase in HFD females (main effect of challenge ($F_{(3,20)}$ = 6.12, p<0.01); diet/challenge interaction ($F_{(3,20)}$ =2.75, p<0.05, Figure 5K)). MKP1 levels also increased in both diet groups in female offspring (main effect of challenge ($F_{(3,20)}$ = 17.57, p<0.01), Figure 5L). IGF1 transcript levels were lower in HFD females when compared to CFD in basal conditions (main effect of diet ($F_{(3, 20)}$ = 2.26, p<0.05), Figure 5M).

In males, there were fewer differences in transcript abundance between CFD and HFD offspring in response to LPS challenge (Figure 6A, 6B). In the amygdala of both diet groups there were increases in IκBα ($F_{(3,20)} = 28.29$, $p<0.01$, Figure 6C), IL6 ($F_{(3,20)} = 7.09$, $p<0.01$, Figure 6D), and MKP1 ($F_{(3,20)} = 8.84$, $p<0.01$, Figure 6E) in response to LPS challenge. Similar to changes in the amygdala, in the hippocampus of both diet groups, LPS challenge led to increases in IκBα ($F_{(3,20)} = 18.79$, $p<0.01$, Figure 6F), while IL6 levels ($F_{(3,20)} = 5.76$, $p<0.01$, Figure 6G) decreased in both diet groups. CD11B ($F_{(3,20)} = 14.95$, $p<0.01$, Figure 6H) and MKP1 ($F_{(3,20)} = 9.98$, $p<0.01$, Figure 6I) levels increased in both diet groups in female offspring in response to LPS. Lastly, IGF1 expression decreased in HFD males (main effect of challenge ($F_{(3,20)} = 3.73$, $p<0.05$, Scheffe post-hoc $p=0.017$), but remained unchanged in CFD counterparts (Scheffe post-hoc $p=0.410$, Figure 6J).

Combined CORT and LPS challenge

In females, CORT+LPS challenge lead to several differences in transcript abundance between HFD and CFD offspring (Figure 7A and 7B). In the amygdala, both diet groups showed decreases in GR (main effect of challenge ($F_{(3,20)} = 10.0$, $p<0.01$), Figure 7C), MR (main effect of challenge ($F_{(3,20)} = 9.42$, $p<0.01$); main effect of diet,($F_{(3,20)} = 5.596$, $p<0.05$), Figure 7D), and NFκB (main effect of challenge ($F_{(3,20)} = 12.52$, $p<0.01$), Figure 7E). In contrast, IκBα levels increased in HFD offspring ($F_{(3,20)} = 4.68$, $p<0.05$, Scheffe post-hoc $p=0.015$), but did not change in CFD offspring (Scheffe post-hoc $p=0.803$, Figure 7F) in response to CORT+LPS challenge. The ratio of IL6/IL10 was above 1 in both diet groups in the amygdala of female offspring (main effect of challenge ($F(3,20) = 9.48$, $p<0.01$); diet/challenge interaction ($F_{(3,20)=}4.36$, $p<0.05$), Figure 7G). Lastly, CD11b levels decreased in both diet groups ($F_{(3,20)} = 7.19$, $p<0.01$, Figure 7H) in response to CORT+LPS. In the hippocampus, IκBα ($F_{(3,20)} = 10.13$, $p<0.01$, Figure 7I)

and IL6/IL10 ratio ($F_{(3,20)}$ = 13.06, p<0.01, Figure 7J), increased in both diet groups, while CD11B decreased in CFD females ($F_{(3,20)}$ = 4.456, p<0.05, Scheffe post-hoc p=0.047) and no change was seen in HFD offspring (Scheffe post-hoc p=0.909, Figure 7K) in response to CORT+LPS.

In males, there were fewer differences in transcript abundance between diet groups in response to combined CORT+LPS challenge (Figure 8A and 8B). In the amygdala, GR decreased in HFD offspring (main effect of challenge ($F_{(3,20)}$ = 4.364, p<0.05), Scheffe post-hoc p=0.046), yet remained unchanged in CFD offspring (Scheffe post-hoc p=0.512, Figure 8C). MR levels decreased in CFD offspring (main effect of challenge ($F_{(3,20)}$ = 4.110, p<0.05), Scheffe post-hoc p=0.022), yet remained unchanged in HFD offspring (Scheffe post-hoc p=0.356, Figure 8D). NFκB levels decreased in both diet groups ($F_{(3,20)}$ = 4.44, p<0.05, Figure 8E), where as IL6/IL10 ratio increased in CFD males ($F_{(3,20)}$ = 4.458, p<0.05, Scheffe post-hoc p=0.024), but remained unchanged in HFD offspring (Scheffe post-hoc p=0.276, Figure 8F). Lastly, IGF1 levels decreased in both groups in response to CORT+LPS challenge ($F_{(3,20)}$ = 4.29, p<0.05, Figure 8G). In the hippocampus, there was increased GR levels in CFD offspring ($F_{(3,20)}$ = 4.90, p<0.05, Scheffe post-hoc p=0.044), yet remained unchanged in HFD offspring (Scheffe post-hoc p=0.999, Figure 8H). IκBα transcript levels increased in both diet groups ($F_{(3,20)}$ = 19.08, p<0.01, Figure 8I). Similar to that of GR, IL6/IL10 ratio increased in CFD offspring ($F_{(3,20)}$ =5.107, p<0.05, Scheffe post-hoc p=0.025) and did not change in HFD counterparts (Scheffe post-hoc, p=0.319, Figure 8J). Lastly, MKP1 levels increased in both groups ($F_{(3,20)}$ = 15.42, p<0.01, Figure 8K) in response to CORT+LPS challenge.

To better characterize the neural transcript response to combined CORT and LPS exposure, an additional brain region regulating the HPA axis was assessed; the medial prefrontal

cortex (PFC). In response to simultaneous CORT + LPS challenge, females in both diet groups showed increased levels of NFκB ($F_{(3,20)} = 6.85$, $p<0.01$, Figure. 9C) and IκBα ($F_{(3,20)} = 28.95$, $p<0.01$, Figure 9D), while IL6 increased in HFD offspring ($F_{(3,20)} = 4.347$, $p<0.05$, Scheffe post-hoc $p=0.040$), but did not change in CFD offspring (Scheffe post-hoc $p=0.784$, Figure 9E). MKP1 levels increased in HFD offspring with CORT+LPS challenge (diet/challenge interaction ($F_{(3,20)} = 2.08$, $p<0.05$), Figure 9F) when compared to CFD counterparts.

In males, GR levels decreased in the PFC of both diet groups ($F_{(3,20)} = 13.45$, $p<0.01$, Figure 9G) in response to combined CORT and LPS treatment. NFκB levels increased in HFD males (main effect of challenge ($F_{(3,20)} = 8.111$, $p<0.01$, Scheffe post-hoc $p=0.007$); diet/challenge interaction ($F_{(3,20)} = 5.486$, $p<0.01$), whereas no change was seen in CFD offspring (Scheffe post-hoc $p=0.994$, Figure 9H). Lastly, CORT+LPS challenge led to increased IκBα ($F_{(3,20)} = 42.56$, $p<0.01$, Figure 9I) and MKP1 ($F_{(3,20)} = 9.53$, $p<0.01$, Figure 9J) levels in both CFD and HFD males

DISCUSSION

The current study builds upon previous findings that adult offspring exposed to maternal HFD show evidence of sex-specific increases in inflammatory gene expression in the amygdala and the hippocampus (Bilbo and Tsang, 2010; Sasaki et al., 2014; Sasaki et al., 2013). As expected in CFD animals, CORT potentiated pro-inflammatory gene expression induced by LPS in both female and male offspring. This potentiation was also observed in HFD animals, however compared to the CFD animals, there were elevated levels of anti-inflammatory transcript in females, and a larger pro-inflammatory response in males. These findings suggest that maternal

HFD-exposed adult rats have sex-specific alterations to the programming of pro- and anti-inflammatory genes.

Enhanced anti-inflammatory responses to CORT challenge in male HFD offspring

CORT is classically associated with anti-inflammatory and immunosuppressive effects (Boumpas et al., 1993; Sorrells and Sapolsky, 2007). The anti-inflammatory actions of CORT include increased expression of IGF-1, IκBα, and MKP1, which supress the expression of inflammatory mediators. In this study, both diet groups and sexes showed increased expression of IκBα in the hippocampus in response to CORT challenge (Figures 3-4). However, in males, the increase in IκBα in the hippocampus in response to CORT was greater among HFD offspring when compared to CFD offspring. Overall, HFD males also showed reduced MR transcript in the hippocampus. It is possible that reduced MR transcript may have potentiated further GR-induced anti-inflammation with CORT administration, since reduced availability of MR and receptor saturation with CORT is associated with increased GR expression (Groeneweg et al., 2011; Smoak and Cidlowski, 2004; Sorrells and Sapolsky, 2007). This would suggest that male offspring exposed to maternal HFD may tolerate acute psychological or endocrine stressors more efficiently than CFD offspring in adulthood in order to initiate protective, anti-inflammatory signalling in limbic brain regions.

Enhanced pro-inflammatory response to LPS challenge in female HFD offspring

LPS binds to toll-like receptor 4, leading to the activation of MAP kinases that support inflammatory gene transcription. Over time, pro-inflammatory activation of the HPA axis leads to a return to homeostasis through GR-mediated expression of anti-inflammatory genes MKP-1 and IκBα that prevent further accumulation of cytotoxic pro-inflammation (Arenzana-Seisdedos

et al., 1997; Huang and Miyamoto, 2001; Lee and Hannink, 2001; Rodriguez et al., 1999; Sachdev et al., 2000; Silverman and Sternberg, 2012).

We found that LPS challenge largely led to increases in both pro- and anti-inflammatory expression in both sexes and diet groups, with a potentiated IL6 response in the hippocampus of HFD females (Figures 5-6). In both sexes, diet groups, and brain regions we found increased anti-inflammatory IκBα and MKP1 in response to LPS. Both sexes and diet groups also showed increased IL6 in the amygdala, and females showed increased NFκB transcript in the hippocampus. However, we found that, in the hippocampus, females exposed to maternal HFD had significantly higher levels of IL6 than CFD controls post-LPS. This discrepancy in IL6 response in females suggests that maternal HFD exposure may potentiate inflammatory mechanisms in conditions of immune stress. Notably, similar results have been reported in the hypothalamus, where chronic HFD *consumption* led to increased inflammation and neuronal apoptosis (Milanski et al., 2009; Zhang et al., 2008).

Sex-specific alterations to CORT+LPS challenge in HFD offspring

Basal levels of CORT dampen the pro-inflammatory effects of LPS through GC signalling and anti-inflammatory gene expression. Acute elevations in CORT through exogenous administration or psychosocial stress increases pro- and anti-inflammatory transcript expression, however, levels of pro-inflammatory transcript are higher in the presence of exogenous CORT compared to basal CORT levels (Munhoz et al., 2010, 2006) (Figure 11B).

HFD females had lower pro-/anti-inflammatory IL6/IL10 cytokine ratio relative to CFD females, indicating an enhanced anti-inflammatory transcriptional response in the amygdala (Figure 7). In the prefrontal cortex, only HFD females displayed an increase in MKP-1 (Figure 9). These findings suggest that maternal HFD exposure is predominantly associated with

increased anti-inflammatory transcriptional response in females. In contrast, CORT+LPS led to a significant increase in NFκB in the prefrontal cortex of HFD males (Figure 9). These findings suggest that maternal exposure to HFD in males may exacerbate a neuroinflammatory response to combined CORT+LPS challenge during adulthood.

CONCLUSION

Anxiety behaviour in rodents can be directly associated with inflammatory gene expression and inflammation in brain regions regulating HPA axis response to stress (Dantzer et al., 2008; Rodgers et al., 2012). Adult offspring exposed to maternal HFD exhibit increased anxiety during behavioural tests in the open field and elevated plus maze (Bilbo and Tsang, 2010; Sasaki et al., 2014; Sasaki et al., 2013; Sullivan et al., 2012). Future behavioural work is necessary to assess whether anti-inflammatory responses that are robustly activated upon physiological (CORT) and immune triggers (LPS) during adulthood seen in this study, may attenuate anxiety-like behaviour in offspring with perinatal HFD exposure. Our findings suggest the possibility that HFD-induced HPA axis programming during early life may convey neuroprotective and anti-inflammatory outcomes during adulthood when triggered by acute physiological and/or immune challenges.

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REFRENCES

Amugsi, D.A., Dimbuene, Z.T., Mberu, B., Muthuri, S., Ezeh, A.C., 2017. Prevalence and time trends in overweight and obesity among urban women: an analysis of demographic and health surveys data from 24 African countries, 1991 – 2014. BMJ Open 7, e017344.

Arenzana-Seisdedos, F., Turpin, P., Rodriguez, M., Thomas, D., Hay, R., Virelizier, J., Dargemont, C., 1997. Nuclear localization of I kappa B alpha promotes active transport of NF-kappa B from the nucleus to the cytoplasm. J. Cell Sci. 110 (Pt 3, 369–78.

Ashdown, H., Poole, S., Boksa, P., Luheshi, G., 2007. Interleukin-1 receptor antagonist as a modulator of gender differences in the febrile response to lipopolysaccharide in rats. Am. J. Physiol. Integr. Comp. Physiol. 292, R1667–R1674.

Bateson, P., Gluckman, P., Hanson, M., 2014. The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. J. Physiol. 592, 2357–68.

Bertini, R., Bianchi, M., Ghezzi, P., 1988. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. J. Exp. Med. 167, 1708–12.

Bethin, K.E., Vogt, S.K., Muglia, L.J., 2000. Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. Proc. Natl. Acad. Sci. U. S. A. 97, 9317–22.

Bhurosy, T., Jeewon, R., 2014. Overweight and obesity epidemic in developing countries: a problem with diet, physical activity, or socioeconomic status? ScientificWorldJournal. 2014, 964236.

Bilbo, S.D., Tsang, V., 2010. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J. 24, 2104–2115.

Boumpas, D., Chrousos, G., Wilder, R., Cupps, T., Balow, J., 1993. Glucocorticoid therapy for

immune-mediated diseases: basic and clinical correlates. Ann. Intern. Med. 119, 1198–208.

Brito, H., Radulski, D., Wilhelms, D., Stojakovic, A., Brito, L., Engblom, D., Franco, C., Zampronio, A., 2016. Female sex hormones influence the febrile response induced by lipopolysaccharide, cytokines and prostaglandins but not by interleukin-1β in rats. J. Neuroendocrinol. 28, 1–10.

Catalano, P., deMouzon, S.H., 2015. Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. Int. J. Obes. 39, 642–649.

Chistyakov, D., Azbukina, N., Astakhova, A., Goriainov, S., Chistyakov, V., Sergeeva, M., 2018. Sex-mediated differences in LPS induced alterations of TNFα, IL-10 expression, and prostaglandin synthesis in primary astrocytes. Int. J. Mol. Sci. 19, 2793.

Chrousos, G.P., 1995. The Hypothalamic–Pituitary–Adrenal Axis and Immune-Mediated Inflammation. N. Engl. J. Med. 332, 1351–1363.

Contu, L., Hawkes, C., 2017. A Review of the Impact of Maternal Obesity on the Cognitive Function and Mental Health of the Offspring. Int. J. Mol. Sci. 18, 1093.

Dantzer, R., O'Connor, J., Freund, G., Johnson, R., Kelley, K., 2008. From inflammation to sickness and depression: When the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56.

Edlow, A.G., 2017. Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. Prenat. Diagn. 37, 95–110.

Forsyth, S., Gautier, S., Salem Jr., N., 2016. Global Estimates of Dietary Intake of Docosahexaenoic Acid and Arachidonic Acid in Developing and Developed Countries. Ann. Nutr. Metab. 68, 258–267.

Groeneweg, F., Karst, H., de Kloet, E., Joëls, M., 2011. Rapid non-genomic effects of corticosteroids and their role in the central stress response. J. Endocrinol. 209, 153–167.

Huang, T., Miyamoto, S., 2001. Postrepression activation of NF-kappaB requires the amino-terminal nuclear export signal specific to IkappaBalpha. Mol. Cell. Biol. 21, 4737–47.

Huttunen, R., Syrjänen, J., 2013. Obesity and the risk and outcome of infection. Int. J. Obes. 37, 333–340.

King, J.C., 2006. Maternal Obesity, Metabolism, and Pregnancy Outcomes. Annu. Rev. Nutr. 26, 271–291.

Lawrence, C., Brough, D., Knight, E., 2012. Obese mice exhibit an altered behavioural and inflammatory response to lipopolysaccharide. Dis. Model. Mech. 5, 649–659.

Lee, S., Hannink, M., 2001. The N-terminal nuclear export sequence of IkappaBalpha is required for RanGTP-dependent binding to CRM1. J. Biol. Chem. 276, 23599–606.

Maniam, J., Antoniadis, C., Morris, M.J., 2014. Early-Life Stress, HPA Axis Adaptation, and Mechanisms Contributing to Later Health Outcomes. Front. Endocrinol. (Lausanne). 5, 73.

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D., Tsukumo, D., Anhe, G., Amaral, M., Takahashi, H., Curi, R., Oliveira, H., Carvalheira, J., Bordin, S., Saad, M., Velloso, L., 2009. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J. Neurosci. 29, 359–370.

Mitra, R., Sapolsky, R.M., 2008. Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. Proc. Natl. Acad. Sci. U. S. A. 105, 5573–8.

Munck, A., Náray-Fejes-Tóth, A., 1992. The ups and downs of glucocorticoid physiology. Permissive and suppressive effects revisited. Mol. Cell. Endocrinol. 90, C1-4.

Munhoz, C., Lepsch, L., Kawamoto, E., Malta, M., Lima, L. de S., Avellar, M., Sapolsky, R., Scavone, C., 2006. Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor- B in the frontal cortex and hippocampus via glucocorticoid secretion. J. Neurosci. 26, 3813–3820.

Munhoz, C., Sorrells, S., Caso, J., Scavone, C., Sapolsky, R., 2010. Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. J. Neurosci. 30, 13690–13698.

Nicolaides, N.C., Pavlaki, A.N., Maria Alexandra, M.A., Chrousos, G.P., 2000. Glucocorticoid Therapy and Adrenal Suppression, Endotext. MDText.com, Inc.

Paxinos, G., Watson, C., 2007. The rat brain in stereotaxic coordinates. Elsevier.

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.

Pohl, J., Sheppard, M., Luheshi, G., Woodside, B., 2014. Diet-induced weight gain produces a graded increase in behavioral responses to an acute immune challenge. Brain. Behav. Immun. 35, 43–50.

Rivera, H.M., Christiansen, K.J., Sullivan, E.L., 2015. The role of maternal obesity in the risk of neuropsychiatric disorders. Front. Neurosci. 9, 194.

Rodgers, K., Bercum, F., McCallum, D., Rudy, J., Frey, L., Johnson, K., Watkins, L., Barth, D., 2012. Acute neuroimmune modulation attenuates the development of anxiety-like freezing behavior in an animal model of traumatic brain injury. J. Neurotrauma 29, 1886–1897.

Rodriguez, M., Thompson, J., Hay, R., Dargemont, C., 1999. Nuclear retention of IkappaBalpha protects it from signal-induced degradation and inhibits nuclear factor kappaB transcriptional activation. J. Biol. Chem. 274, 9108–15.

Rofey, D.L., Kolko, R.P., Iosif, A.-M., Silk, J.S., Bost, J.E., Feng, W., Szigethy, E.M., Noll, R.B., Ryan, N.D., Dahl, R.E., 2009. A Longitudinal Study of Childhood Depression and Anxiety in Relation to Weight Gain. Child Psychiatry Hum. Dev. 40, 517–526.

Sachdev, S., Bagchi, S., Zhang, D., Mings, A., Hannink, M., 2000. Nuclear import of IkappaBalpha is accomplished by a ran-independent transport pathway. Mol. Cell. Biol. 20, 1571–82.

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

Sasaki, A., de Vega, W., Sivanathan, S., St-Cyr, S., McGowan, P.O., 2014. Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. Neuroscience 272, 92–101.

Seale, J., Wood, S., Atkinson, H., Bate, E., Lightman, S., Ingram, C., Jessop, D., Harbuz, M., 2004. Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. J. Neuroendocrinol. 16, 516–524.

Silverman, M.N., Sternberg, E.M., 2012. Glucocorticoid regulation of inflammation and its behavioural and metabolic correlates: from HPA axis to glucocorticoid receptor dysfunction. Ann. N. Y. Acad. Sci. 1261, 55–63.

Smoak, K., Cidlowski, J., 2004. Mechanisms of glucocorticoid receptor signaling during inflammation. Mech. Ageing Dev. 125, 697–706.

Sorrells, Shawn F., Caso, Javier R., Munhox, Carolina D., Sapolsky, R.M., 2009. The Stressed CNS: When Glucocorticoids Aggravate Inflammation. Neuron 64, 33–39.

Sorrells, S., Sapolsky, R., 2007. An inflammatory review of glucocorticoid actions in the CNS.

Brain. Behav. Immun. 21, 259–72.

Stein-Behrens, B., Mattson, M.P., Chang, I., Yeh, M., Sapolsky, R., 1994. Stress exacerbates neuron loss and cytoskeletal pathology in the hippocampus. J. Neurosci. 14, 5373–80.

Sullivan, E.L., Nousen, E.K., Chamlou, K.A., Grove, K.L., 2012. The Impact of Maternal High-Fat Diet Consumption on Neural Development and Behavior of Offspring. Int. J. Obes.

Vachharajani, V., 2008. Influence of obesity on sepsis. Pathophysiology 15, 123–134.

van Bodegom, M., Homberg, J.R., Henckens, M.J.A.G., 2017. Modulation of the Hypothalamic-Pituitary-Adrenal Axis by Early Life Stress Exposure. Front. Cell. Neurosci. 11, 87.

Van Lieshout, R.J., Taylor, V.H., Boyle, M.H., 2011. Pre-pregnancy and pregnancy obesity and neurodevelopmental outcomes in offspring: a systematic review. Obes. Rev. 12, e548–e559.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3, RESEARCH0034.

Vore, A.S., Doremus-Fitzwater, T., Gano, A., Deak, T., 2017. Adolescent Ethanol Exposure Leads to Stimulus-Specific Changes in Cytokine Reactivity and Hypothalamic-Pituitary-Adrenal Axis Sensitivity in Adulthood. Front. Behav. Neurosci. 11, 78.

Wacharasint, P., Boyd, J.H., Russell, J.A., Walley, K.R., 2013. One size does not fit all in severe infection: obesity alters outcome, susceptibility, treatment, and inflammatory response. Crit. Care 17, R122.

Watanobe, H., Yoneda, M., 2003. A mechanism underlying the sexually dimorphic ACTH response to lipopolysaccharide in rats: sex steroid modulation of cytokine binding sites in the hypothalamus. J. Physiol. 547, 221–232.

Zambrano, E., Ibáñez, C., Martínez-Samayoa, P.M., Lomas-Soria, C., Durand-Carbajal, M.,

Rodríguez-González, G.L., 2016. Maternal Obesity: Lifelong Metabolic Outcomes for Offspring from Poor Developmental Trajectories During the Perinatal Period. Arch. Med.

Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., Cai, D., 2008. Hypothalamic IKKβ/NF-κB and ER stress link overnutrition to energy imbalance and obesity. Cell 135, 61–73.

FIGURES

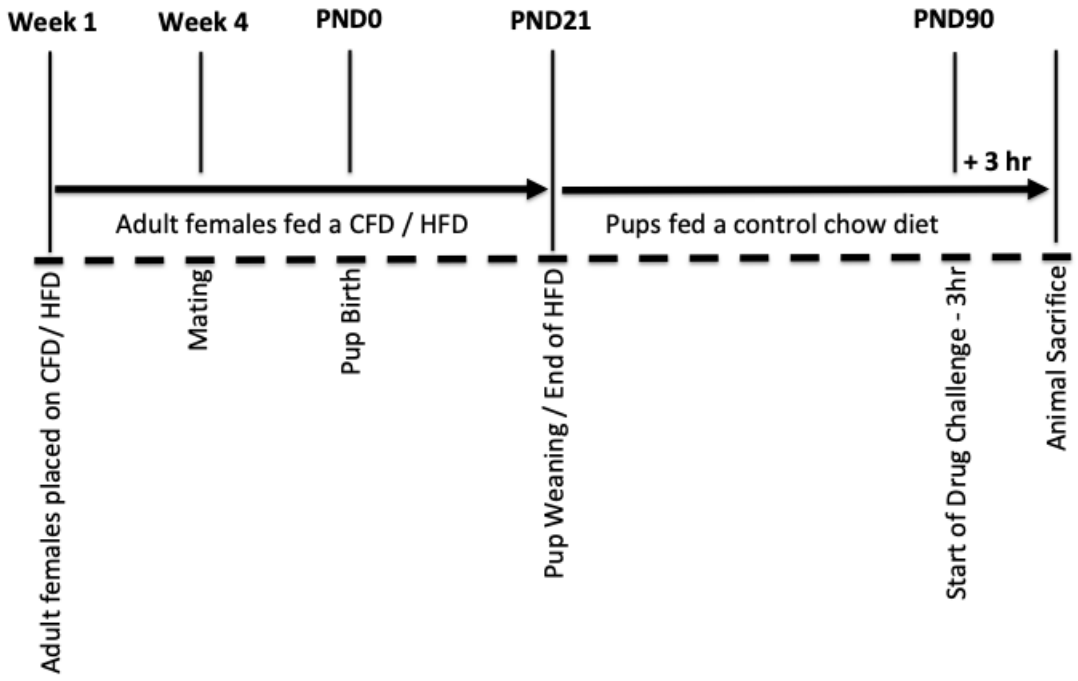

Figure 1. Overview of animal care and stress challenge. Adult females were placed on either CFD (control fat house-chow) or HFD (high fat diet) four weeks prior to mating and continued through gestation and lactation. Offspring were weaned at postnatal day (PND) 21 and fed control fat house-chow. On PND90 offspring were injected with corticosterone (CORT), lipopolysaccharide (LPS), or CORT+LPS combination, and sacrificed 3 hours later.

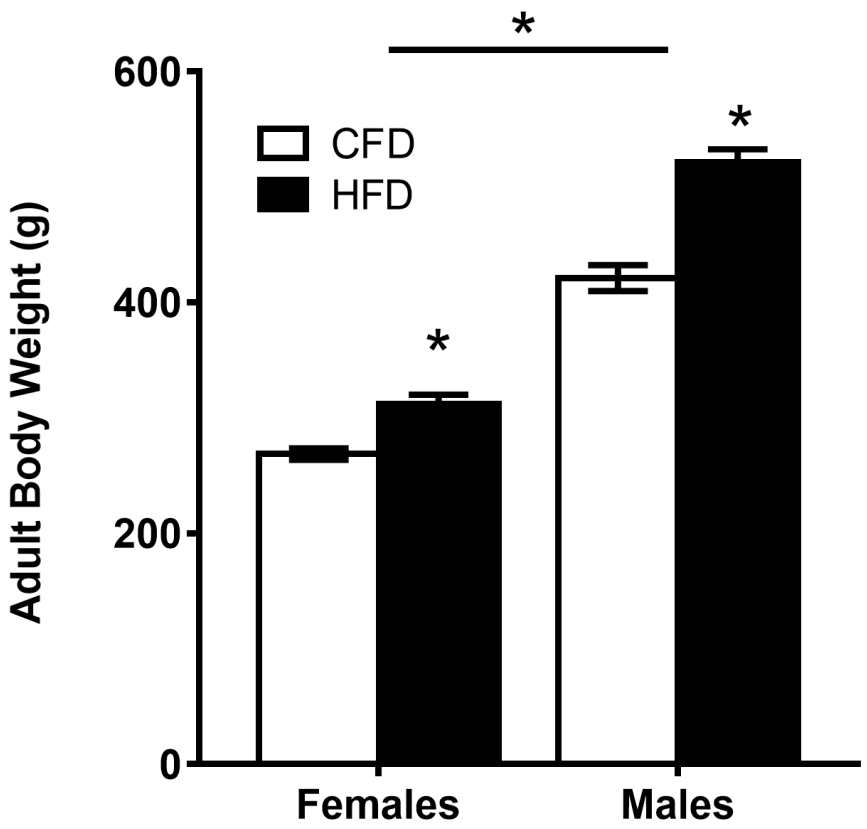

Figure 2. Offspring body weights in adulthood. Average body weight for PND90 offspring per sex and maternal diet condition ± SEM. n=24 for each group, CFD = control house chow die, HFD = high-fat diet. *$p < 0.0001$ for main effect of diet, and main effect of sex.

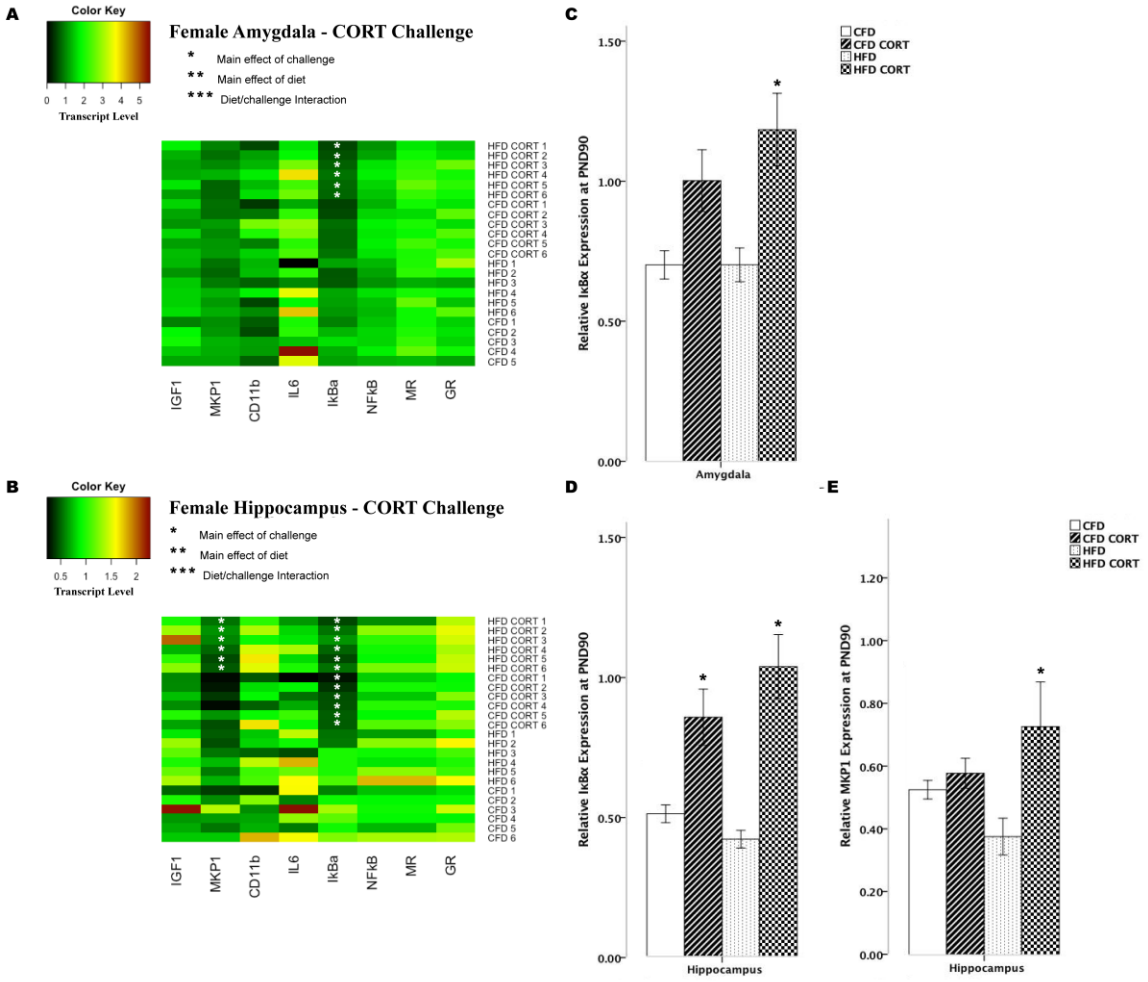

Figure 3. Pro and Anti-inflammatory gene expression is regulated by maternal high fat diet exposure combined with high dose corticosterone (CORT) administration in adult female offspring. A: Heatmap of mean ± SE relative expression of transcripts in the amygdala. **B:** Heatmap of mean ± SE relative expression of transcripts in the hippocampus. **C:** relative expression of IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) in the amygdala. **D-E:** relative expression of IκBα, and MKP1 (mitogen activated protein kinase phosphatase in the hippocampus. CFD = Control house chow, CFD HD CORT= Control house chow + high dose CORT, HFD = High fat diet, HFD HD CORT = High fat diet + high dose CORT. Bar * $p<0.05$ main effect of treatment. ** $p<0.05$ main effect of diet. *** $p<0.05$ diet/treatment interaction.

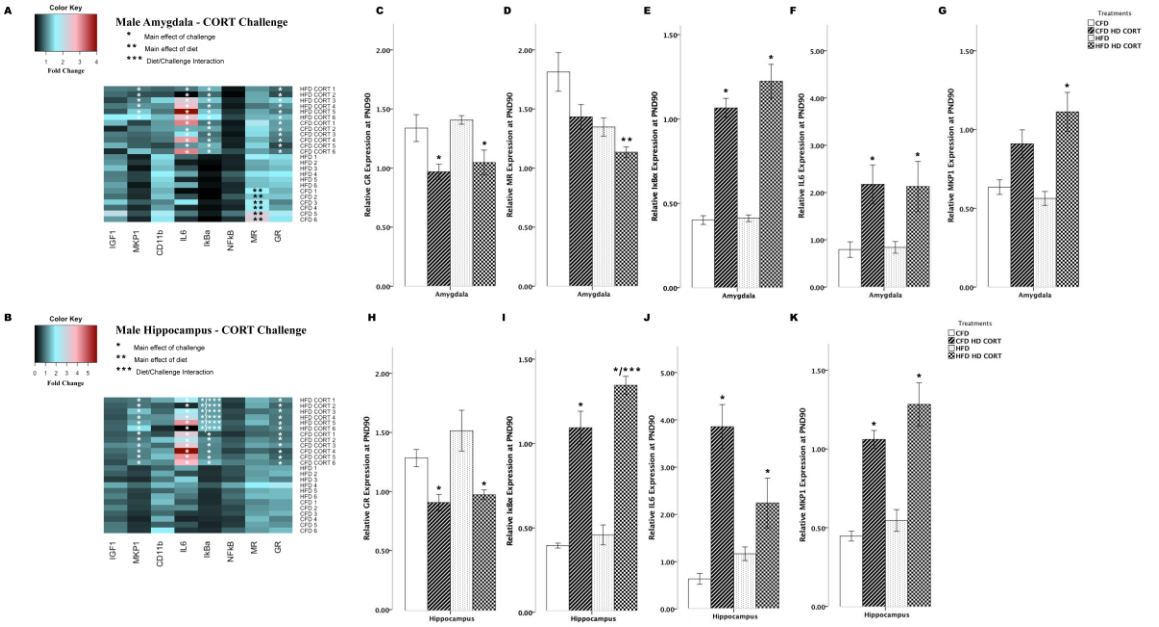

Figure 4. Pro and Anti-inflammatory gene expression is regulated by maternal high fat diet exposure combined with high dose corticosterone (CORT) administration in adult male offspring. A: Heatmap of mean ± SE relative expression of transcripts in the amygdala. **B:** Heatmap of mean ± SE relative expression of transcripts in the hippocampus. **C-G:** Relative expression of GR (glucocorticoid receptor), MR (mineralocorticoid receptor), IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), IL6 (interleukin 6), and MKP1 (mitogen activated protein kinase phosphatase in the amygdala. **H-K:** Relative expression of GR, IκBα, IL6, and MKP1 in the hippocampus.

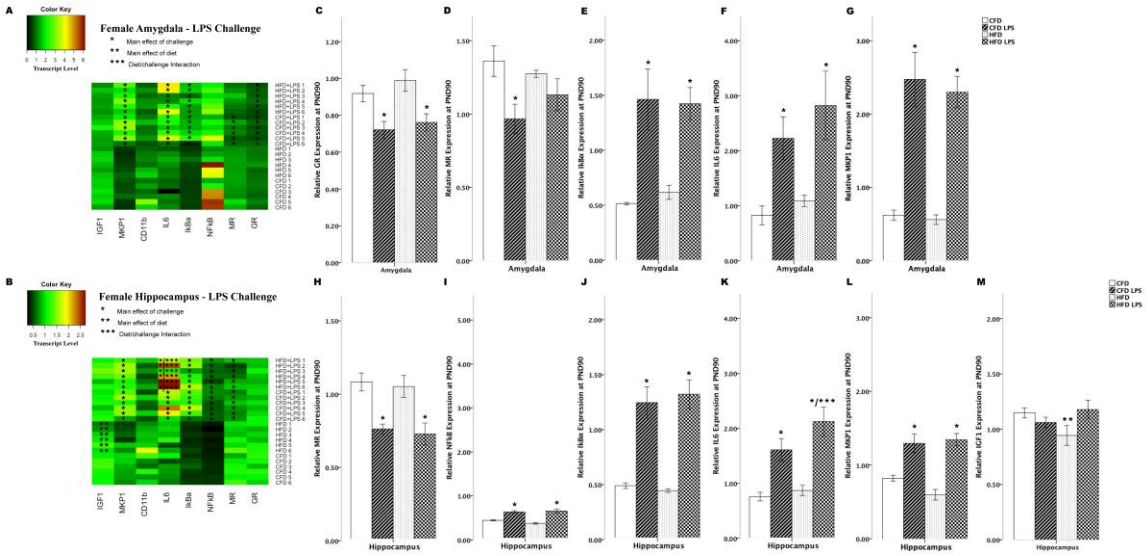

Figure 5. Pro and Anti-inflammatory gene expression is regulated by maternal high fat diet exposure combined with an immune challenge of lipopolysaccharide (LPS) in adult female offspring. A: Heatmap of mean ± SE relative abundance of transcripts in the amygdala. **B:** Heatmap of mean ± SE relative abundance of transcripts in the hippocampus. **C-G:** Relative abundance of GR (glucocorticoid receptor), MR (mineralocorticoid receptor), IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), IL6 (interleukin 6), and MKP1 (mitogen activated protein kinase phosphatase in the amygdala. **H-M:** MR, NFκB (nuclear factor kappa light chain enhancer of activated B cells), IκBα, IL6, MKP1, and IGF1 (insulin-like growth factor 1) in the hippocampus. CFD = Control house chow, CFD HD LPS= Control house chow + high dose LPS, HFD = High fat diet, HFD HD LPS = High fat diet + high dose LPS. Bar * $p<0.05$ main effect of treatment. ** $p<0.05$ main effect of diet. *** $p<0.05$ diet/treatment interaction. post-hoc comparisons within treatments.

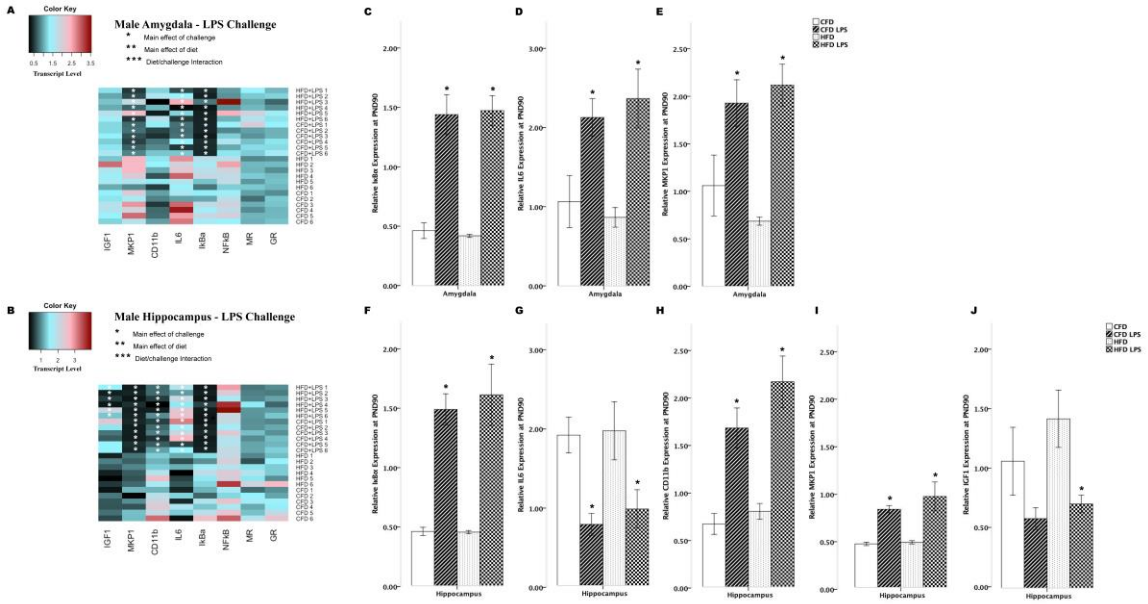

Figure 6. Pro and Anti-inflammatory gene expression is regulated by maternal high fat diet exposure combined with an immune challenge of lipopolysaccharide (LPS) administration in adult male offspring. A: Heatmap of mean ± SE relative abundance of transcripts in the amygdala. **B:** Heatmap of mean ± SE relative abundance of transcripts in the hippocampus. **C-E:** Relative abundance of IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), IL6 (interleukin 6), and MKP1 (mitogen activated protein kinase phosphatase 1) in the amygdala. **F-J:** IκBα, IL6, CD11b (cluster of differentiation molecule 11B), MKP1, and IGF1 (insulin-like growth factor 1) in the hippocampus.

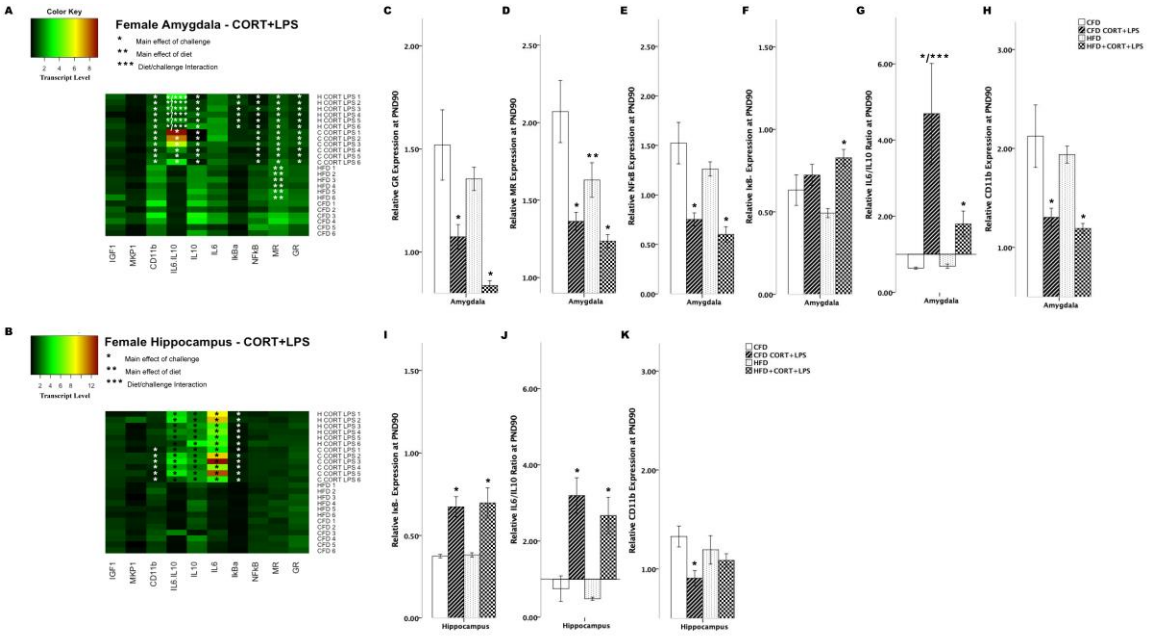

Figure 7. Pro and Anti-inflammatory gene expression is further exacerbated when maternal high fat diet exposure is combined with corticosterone (CORT) and lipopolysaccharide (LPS) treatment in adult female offspring. A: Heatmap of mean ± SE relative abundance of transcripts in the amygdala. **B:** Heatmap of mean ± SE relative abundance of transcripts in the hippocampus. **C-H:** Relative abundance of GR (glucocorticoid receptor), MR (mineralocorticoid receptor), NFκB (nuclear factor kappa light chain enhancer of activated B cells), IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), the ratio of pro versus anti inflammation designated by IL6/IL10 (interleukin 6/10), and CD11b (cluster of differentiation molecule 11B) expression in the amygdala. **I-K:** IκB, IL6/IL10 ratio, and CD11b in the hippocampus. CFD = Control house chow, CFD CORT+LPS= Control house chow + CORT+LPS, HFD = High fat diet, HFD CORT+LPS = High fat diet + CORT+LPS. Bar * $p<0.05$ main effect of treatment. ** $p<0.05$ main effect of diet. *** $p<0.05$ diet/treatment interaction. post-hoc comparisons within treatments.

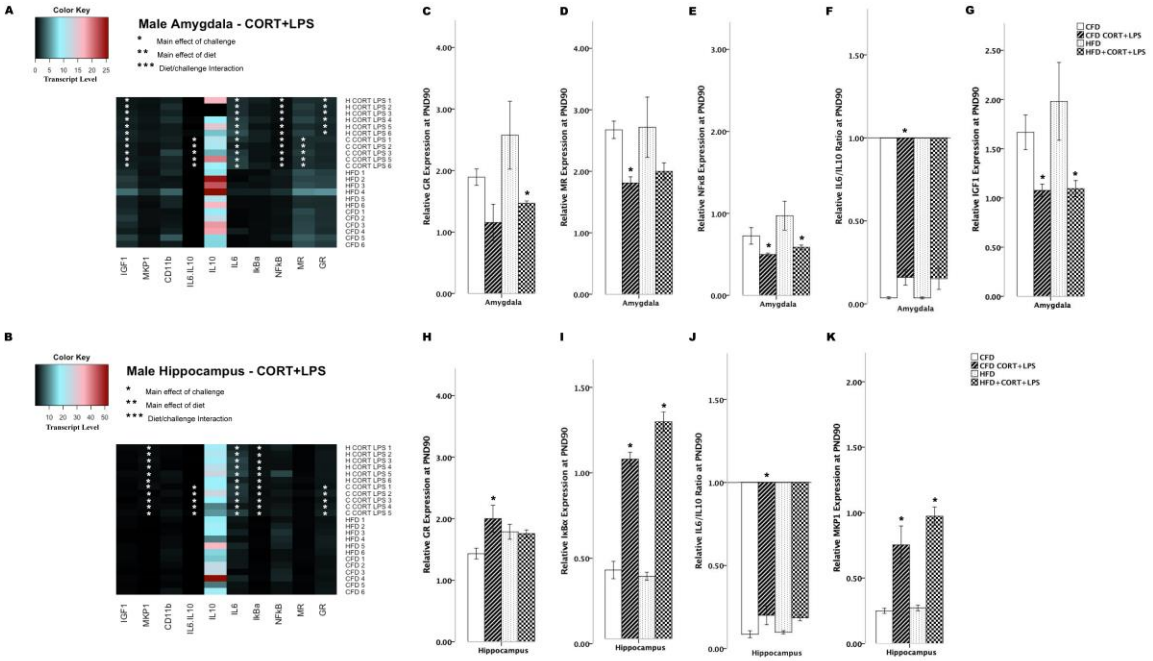

Figure 8. Pro and Anti-inflammatory gene expression is further exacerbated when maternal high fat diet exposure is combined with corticosterone (CORT) and lipopolysaccharide (LPS) treatment in adult male offspring. A: Heatmap of mean ± SE relative abundance of transcripts in the amygdala. **B:** Heatmap of mean ± SE relative abundance of transcripts in the hippocampus. **C-G:** Relative abundance of GR (glucocorticoid receptor), MR (mineralocorticoid receptor), NFκB (nuclear factor kappa light chain enhancer of activated B cells), the ratio of pro versus anti inflammation designated by IL6/IL10 (interleukin 6/10), and IGF1 expression in the amygdala. **H-K:** GR, IκBα, IL6/IL10 ratio, and MKP1 in the hippocampus.

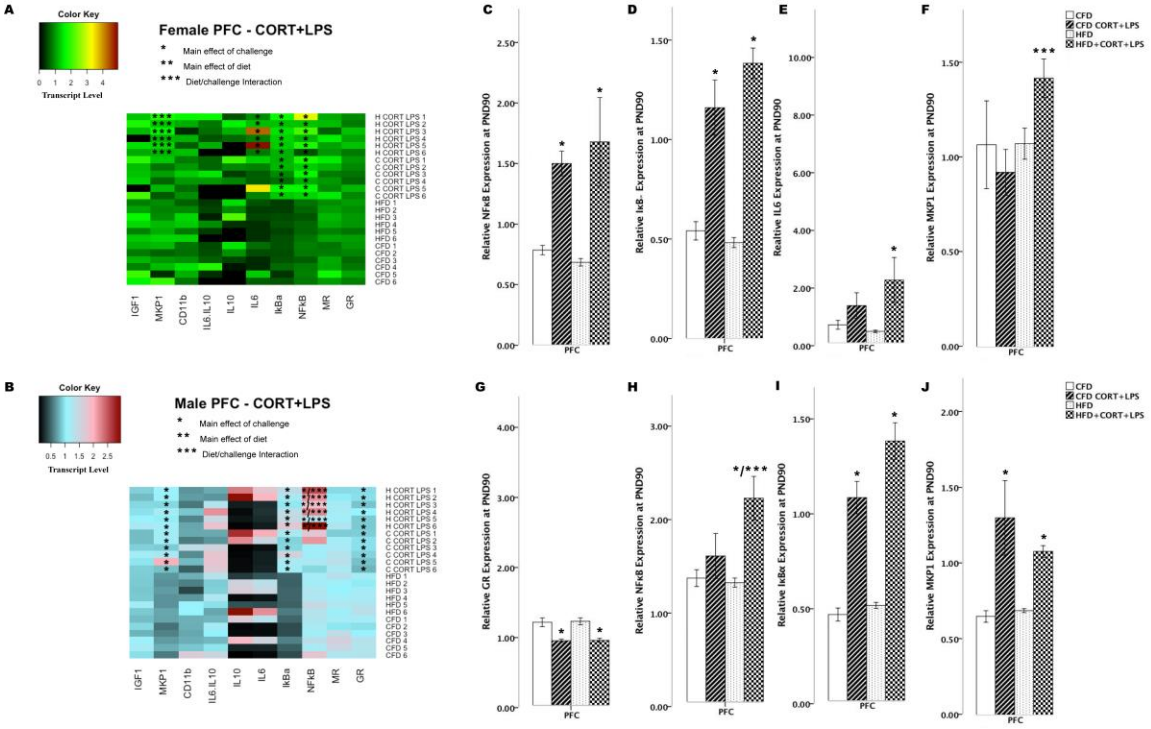

Figure 9. Pro and Anti-inflammatory gene expression is further exacerbated when maternal high fat diet exposure is combined with corticosterone (CORT) and lipopolysaccharide (LPS) treatment in the prefrontal cortex (PFC) of adult offspring. A: Heatmap of mean ± SE relative abundance of transcripts in the PFC of female offspring. **B:** Heatmap of mean ± SE relative abundance of transcripts in the PFC of male offspring. **C-F:** Relative abundance of NFκB (nuclear factor kappa light chain enhancer of activated B cells), IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), IL6 (interleukin 6), and MKP1 (mitogen activated protein kinase phosphatase 1) in the PFC of females. **G-J:** GR, NFκB, IκBα, and MKP1 in the PFC of males.

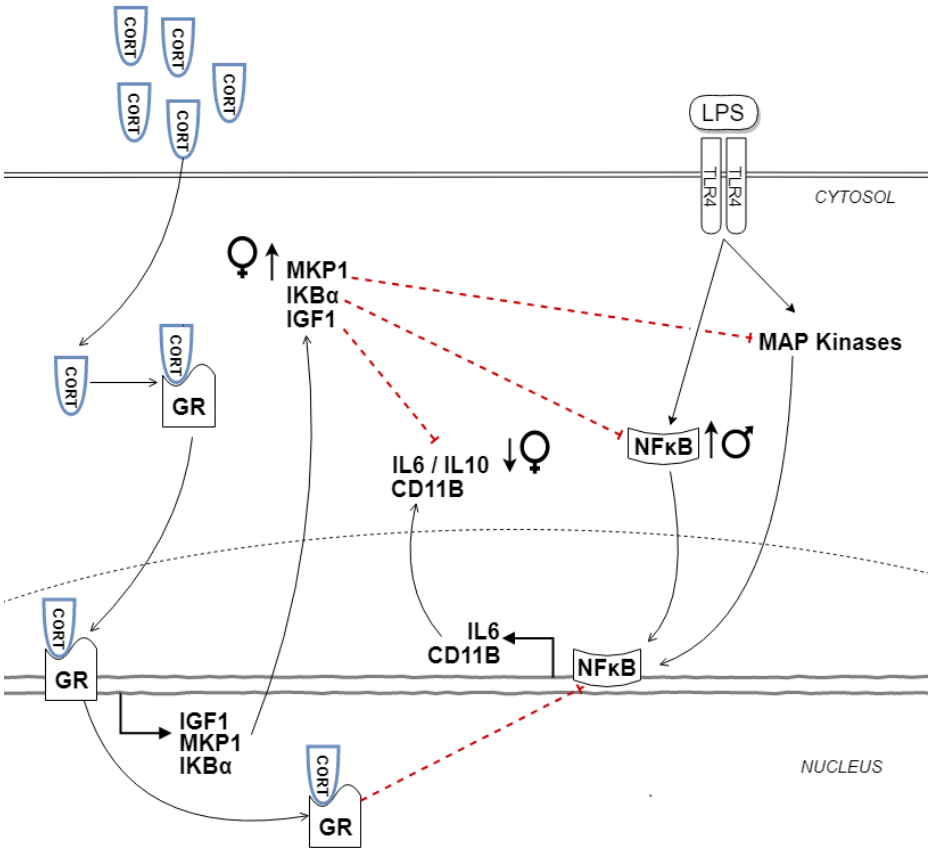

Figure 10. Maternal high-fat diet (HFD) induces sex-specific effects on inflammatory responses to corticosterone (CORT) and lipopolysaccharide (LPS) challenge in adulthood. CORT diffuses into the cytosol and binds to glucocorticoid receptor (GR). The CORT-GR complex induces the expression of anti-inflammatory molecules including IGF1, MKP1, and IκBα, which at basal levels inhibit pro-inflammatory expression of NFκB, IL6 and CD11B induced by LPS signalling to toll-like receptor 4 (TLR4) and mitogen-activated protein (MAP) kinases. However, with higher levels of CORT, anti-inflammatory effects are reduced. This system is altered in females exposed to maternal HFD, whereby there is increased anti-inflammatory MKP1 and reduced IL6/IL10 ratio. In males exposed to maternal HFD, there is increased pro-inflammatory NFκB.