Research report

Chronic high fat feeding increases anxiety-like behaviour and reduces transcript abundance of glucocorticoid signalling genes in the hippocampus of female rats

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HIGHLIGHTS

• High fat diet in adult female rats for 10 weeks increased caloric intake weight gain.
• High fat diet increased anxiety-like behaviour in light dark and open field tasks.
• High fat diet decreased the expression of MR, GR and NFKB in the hippocampus.

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ABSTRACT

The consumption of diets high in saturated fats and obesity have been associated with impaired physical and mental health. Previous studies indicate that chronic high fat diet consumption leads to systemic inflammation in humans and non-human animal models. Studies in non-human animals suggest that altered physiological responses to stress are also a consequence of high fat diet consumption. Glucocorticoid signalling mechanisms may link immune and stress-related pathways in the brain, and were shown to be significantly altered in the brains of female rat offspring of mothers exposed to chronic high fat diet during pregnancy and lactation. For adult females, the consequence of chronic high fat diet consumption on these signalling pathways and their relationship to stress-related behaviour is not known. In this study, we examined the effects of chronic consumption of a high fat diet compared to a low fat control diet among adult female Long Evans rats. We found significant differences in weight gain, caloric intake, anxiety-related behaviours, and glucocorticoid-related gene expression over a 10-week exposure period. As expected, rats in the high fat diet group gained the most weight and consumed the greatest number of calories. Rats in the high fat diet group showed significantly greater levels of anxiety-related behaviour in the Light Dark and Open Field tasks compared to rats in the low fat diet group. Rats consuming high fat diet also exhibited reduced transcript abundance in the hippocampus of stress-related mineralocorticoid receptor and glucocorticoid receptor genes, as well as nuclear factor kappa beta gene expression, implicated in inflammatory processes. Together, these data indicate that chronic high fat diet consumption may increase anxiety-like behaviour at least in part via alterations in glucocorticoid signalling mechanisms in limbic brain regions.

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Abbreviations: 18s rRNA, 18S ribosomal RNA; Actin b, beta-actin; BMI, body mass index; CD11b, cluster of differentiation 11b; EPM, elevated plus maze; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GR, glucocorticoid receptor; HFD, high fat diet; IL-1ra, interleukin-1 receptor antagonist; IL-6, interleukin-6; IxBa, 1-kappa-B-alpha; LD, light–dark task; LFD, low fat diet; MKP-1, mitogen-activated protein kinase phosphatase-1; MR, mineralocorticoid receptor; NFkB, nuclear-factor kappa beta; OF, open field task; PND, post-natal day; qRT-PCR, quantitative real-time reverse transcriptase-polymerase chain reaction; UBC, ubiquitin C; Ywhaz, 14-3-3 protein zeta/delta.
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1. Introduction

Obesity remains at epidemic levels largely as a result of high caloric intake and sedentary lifestyles, and constitutes a major public health issue [1–3]. The preponderance of high fat western diet has been linked to a several health risks, including diabetes, cardiovascular disease and an overall increase in mortality [3–5]. The risk of psychiatric disorders is also greater for individuals of higher body mass index (BMI), and women show a more pronounced increase in the risk of affective disorders with increasing BMI compared to men, indicating sex differences in the impacts of dietary exposures on mental health [6–8]. For women of childbearing age, these constitute risks for their own health as well as for the health of potential offspring [1].

Studies in animal models have shown that exposure to chronic high fat diet (HFD) and the resulting obesity impact behaviour and brain function across the lifespan. Chronic exposure to HFD during the pre- and postnatal period prior to weaning in rats increases anxiety-like behaviour in the Open Field and Elevated Plus maze tasks in adulthood [9,10]. We have shown that these behavioural changes occur together with alterations in corticosterone receptor gene expression in the amygdala and pro-inflammatory and anti-inflammatory gene expression in the hippocampus and amygdala, effects that are particularly pronounced among adult female offspring [10,11]. Other studies in adult male rats indicate that chronic consumption of HFD is associated with a heightened hypothalamic pituitary adrenal (HPA) axis response to physical restraint and increased circulating corticosterone levels [12–14]. In addition, HFD consumption is associated with systemic inflammation, resulting in the dysregulation of inflammatory gene expression [15–17]. The behavioural impacts of alterations in corticosteroid receptors and associated downstream inflammatory processes in the context of dysregulated corticosterone receptor expression are increasingly recognized but remain poorly understood [18].

In this study, we sought to determine the effects of chronic consumption of HFD compared to a low fat control diet (LFD) in adult female rats. We examined body weight, caloric intake, anxiety-like behaviours and the expression of stress related, pro-inflammatory, and anti-inflammatory genes within the amygdala and hippocampus. We hypothesized that rats in the HFD group would weigh more and consume significantly more calories than rats in the LFD group. We also hypothesized that HFD consumption would be associated with increased anxiety-like behaviour, decreased corticosterone receptor and dysregulated pro- and anti-inflammatory gene expression in limbic brain regions.

2. Materials and methods

2.1. Subjects and diets

After one week of pair-housing and acclimation to the vivarium facility, 20 adult female rats (post-natal day [PND] 56) were provided ad libitum access to one of two diets: a high fat diet (HFD, \( n = 10 \)) and a low fat diet (LFD, \( n = 10 \)). The HFD was obtained from Research Diets, Inc. (New Brunswick, NJ: cat. No. D12429), and contained 5.24-kcal/g, composed of 20% protein, 60% fat (predominantly lard and soybean oil), 20% carbohydrate by kcal. The LFD was also obtained from Research Diets, Inc. (New Brunswick, NJ: cat. No. D12450B), and contained 3.8-kcal/g, composed of 20% protein, 10% fat, and 70% carbohydrates by kcal. Each rat was weighed three times per week and food was weighed daily for each cage of two rats. The females remained on their assigned diets for 10 weeks until sacrifice. All procedures were approved by the Local Animal Care Committee of the University of Toronto Scarborough.

2.2. Behavioural procedures

Behavioural experiments began after 8 weeks of exposure to the diets and occurred over a period of 2 weeks during the subjective light phase of the circadian cycle. Females determined to be in dioestrous through cytological analysis were moved to a holding room and allowed to habituate for 1 h prior to the start of any behavioural test or sacrifice. For each task, the cages were cleaned between trials with 70% ethanol and were left to air dry to reduce and homogenize odorants.

2.2.1. Elevated plus maze

The elevated plus maze (EPM) consisted of 2 open arms and 2 closed arms of equal sizes (45 × 10 cm) and a centre zone (10 × 12 cm), with the apparatus elevated 80 cm above the floor. The rat’s location within the EPM was tracked using Ethovision software (Ethovision, Noldus Information Technology Inc., Leesburg, VA) over a 5 min trial, where the frequency and duration within defined zones of the EPM was recorded. The Ethovision software also allowed for the manual coding of both duration and frequency of rearing and head dipping behaviours by volunteers blind to the diet conditions.

2.2.2. Open field

The open field (OF) task involved tracking the rat’s movement within an opaque square arena (40.3 × 40.3 cm) in a dimly lit room (33.7 lx) over a 5 min trial. The rat’s movements were converted to frequency of entries and time spent in predefined zones using Noldus Ethovision software. The predefined zones consisted of a centre (26.88 × 16.12 cm), an edge zone (8.51 cm around the walls of the arena) and a corner zone (four 8.96 × 8.06 cm zones, one in each corner).

2.2.3. Light–dark transition

The light–dark (LD) transition box consisted of 2 chambers of equal dimensions (30 × 30 cm) with a small opening (12 × 12 cm) that allowed passage of the animal between the 2 chambers. One of the boxes was black (dark), and the other was white (light). The trials were run for 10 min in a dimly lit room illuminated by a light bulb suspended over the LD task apparatus. The lighting conditions of the experiment prevented automatic tracking using Ethovision software, and therefore duration and frequency were manually coded.

2.3. Gene expression analysis

2.3.1. Tissue preparation, RNA isolation and cDNA conversion

Following the behavioural assays, 6 rats in each diet condition were quickly sacrificed by CO₂ inhalation followed by decapitation. Whole brains were collected, flash-frozen in isopentane and stored at −80 °C. Tissue punches containing the entire amygdala and dorsal hippocampus were collected using a cryostat, according to stereotaxic coordinates [30]. For each subject and brain region, RNA was extracted and purified using an RNA mini kit (Qiagen). The RNA was converted to cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). The quantity and quality of the RNA and cDNA were assessed using a nanodrop spectrophotometer (ND-2000C, ThermoScientific).

2.3.2. Gene expression analysis by quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR)

The primers used to interrogate 8 genes of interest along with 5 housekeeping genes (Beta-actin [Actin b], 18S ribosomal RNA [18S rRNA, Glyceraldehyde 3-phosphate dehydrogenase [GAPDH],
Ubiquitin C [UBC] , 14–3–3 protein zeta/delta [Ywhaz] were purchased from SA biosciences (Qiagen, Valencia, CA) or designed using sequence information from GenBank at the National Center for Biotechnology information (NCBI; www.ncbi.nlm.nih.gov; Table 1). The genes of interest were selected based upon previous studies of HFD effects on glucocorticoid signalling mechanisms, and included corticosterone receptors (mecolocorticoid receptor [MR], glucocorticoid receptor [GR]), pro-inflammatory signalling molecules (nuclear-factor kappa beta [NFκB], interleukin-6 [IL-6], and Cluster of Differentiation 11b [CD11b]) and anti-inflammatory signalling molecules (1-kappa-B-alpha [IkBa], mitogen-activated protein kinase phosphatase-1 [MKP-1], and interleukin-1 receptor antagonist [IL-1ra]). Gene expression in the amygdala and dorsal hippocampus was quantified using a StepOne Plus real-time PCR and fast SYBR Green PCR master mix (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). To enable relative quantification of gene expression, a standard curve was prepared from 11 serial dilutions starting with a mixture that contained cDNA from all the rats for each brain region. Quantification was performed in duplicate for the standard curve dilutions and in triplicate for the genes of interest and housekeeping genes. An analysis of the raw CT scores for each housekeeping gene using the NormQPCR R package in Bioconductor indicated that Actin b was the least variable housekeeping gene out of the 5 examined. Therefore, gene expression was quantified by calculating the ratio of the quantity of each gene of interest relative to Actin b for each subject and brain region.

2.4. Statistical analyses

Calorie consumption and rat weight data were analysed using a 2 (diet) × 10 (week) mixed-model repeated measures Analysis of Variance (ANOVA; SPSS v.22, IBM Corporation, Armonk, NY). Because we were also interested in possible direct effects of body weight and caloric consumption on behavioural performance, the average body weight and caloric consumption during the 2-week behavioural testing period was compared between the two diet groups using unpaired t-tests. Unpaired t-tests were also used for analyses of the behavioural measures (EPM, OF and LD) and gene expression between the two diet groups. Pearson correlations were used to test the relationships between the average body weight during the behavioural testing period and each of the significantly different behavioural measures, as well as the relationships among the three reported behavioural measures. Effects were considered statistically significant at \( P \leq 0.05 \) and non-significant trends at \( P \leq 0.10 \) are reported. Outliers that were greater or less than 2 standard deviations from the mean of each diet were eliminated from analysis (maximum 1 per diet condition).

3. Results

3.1. Body weight and caloric intake

All rats gained weight over the 9 week period \([F(1,8) = 319.32, P < 0.001]\), and differences in body weight between HFD and LFD rats accrued over time \([F(1,8) = 7.98, P < 0.001; \text{Fig. 1A}]\). Up to 6 weeks after arrival, no significant differences in body weight were found between the HFD group and LFD rats, after which HFD rats were significantly heavier than LFD rats. An analysis of the average weight between groups during the last 2 weeks of dietary exposure showed that HFD rats weighed more than LFD rats during the period of behavioural testing \([F(1,18) = 2.43, P = 0.02; \text{Fig. 1B}]\).

Caloric consumption decreased overall across weeks of exposure to the diets \( [F(1,8) = 5.45, P < 0.001] \), and HFD rats consumed more calories overall than LFD rats \( [F(1,8) = 18.52, P = 0.003; \text{Fig. 1C}] \). A significant effect of diet was also observed when the average caloric consumption during the two weeks of testing was examined, with HFD rats consuming more calories during the behavioural testing period compared to LFD rats \( [t(1,8) = 2.35, P = 0.04; \text{Fig. 1D}] \).

3.2. Anxiety-related behaviour

In the LD task, rats consuming LFD spent more time in the light zone than rats consuming HFD \([t(1,17) = 2.41, P = 0.02; \text{Fig. 2A}]\). In the OF, LFD rats entered the centre a greater number of times relative to the edges of the OF \([t(1,17) = 2.02, P = 0.05; \text{Fig. 2B}]\). Rats that spent more time in the lighted portion of the LD task also showed a greater relative number of entries into the centre of the OF \([R^2 = 0.68, P < 0.001]\). In the EPM, there was no effect of diet in the duration of time spent in the open relative to the closed zones \([t(1,17) = 0.31, P = 0.7; \text{Fig. 2C}]\). Body weight was not correlated with the significantly different behavioural measures, and there was no relationship between LD or OF performance and EPM performance.

3.3. Neural gene expression

Within the hippocampus, MR expression was significantly lower among rats consuming HFD compared to rats consuming LFD \([t(1,10) = 3.28, P = 0.008; \text{Fig. 3A}]\). Rats within the HFD group also showed significantly less transcript abundance of GR than LFD rats \([t(1,10) = 2.48, P = 0.03; \text{Fig. 3B}]\). Among pro-inflammatory genes, the expression of NfκB was significantly lower among HFD rats compared to LFD rats \([t(1,10) = 2.63, P = 0.02; \text{Fig. 3C}]\). For the other pro-inflammatory molecules interrogated, IL-6 and CD11b, and the anti-inflammatory signalling molecules IkBa, MKP-1 and IL-1ra there were no significant differences between diet groups.

Table 1
Prime specifications.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
</tr>
</thead>
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<tr>
<td>18S rRNA</td>
<td>ATGGTAGCTCGGCGTGCACTA</td>
<td>CTGCTGGCTTCTTTGATGT</td>
</tr>
<tr>
<td>Actin b</td>
<td>TTTGACACCTTTCAACACCCCC</td>
<td>ATGCCAGTCCTTCACACCAAG</td>
</tr>
<tr>
<td>CD11b</td>
<td>CGCTGATCTGCTTCAACTAGAG</td>
<td>ACGATACTGGGCAACAGAGAG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>ACATTCACAAAGGGGTGCT</td>
<td>GTGGTTCAACACCATCACAA</td>
</tr>
<tr>
<td>GR</td>
<td>SA biosciences cat. #PPRS2805B</td>
<td>–</td>
</tr>
<tr>
<td>IkBa</td>
<td>CAGATTCTTGCGCTCCTA</td>
<td>TGAGACCTTGGTACTTTC</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>CTGGGTACCTTACAGAGAACAATACC</td>
<td>TGGATGCCCAGAAGACATTCCGA</td>
</tr>
<tr>
<td>IL-6</td>
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<td>AGACAAAGCTCAGCACTGCA</td>
</tr>
<tr>
<td>MKP-1</td>
<td>GCCCTCACTTACAGCTTCTTTCCTCCTCAA</td>
<td>TGGACACTTGGTACTTTC</td>
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<tr>
<td>MR</td>
<td>GGCCGCTGCAAGAATCTCCTTT</td>
<td>GACAGTTCTTCCGGCAATC</td>
</tr>
<tr>
<td>NFκB</td>
<td>CA biosciences cat. #PPR42746A</td>
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<tr>
<td>UBC</td>
<td>CAACGAAGGTCACAAAGGAA</td>
<td>AAGACACCTCCCCACAAAAAC</td>
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<tr>
<td>Ywhaz</td>
<td>TTGACACAGACACAGGAAAGG</td>
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</tr>
</tbody>
</table>

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Table 1
Primer specifications.
within the hippocampus (IL-6: 0.78 ± 0.17 and 0.67 ± 0.06, CD11b: 0.82 ± 0.14 and 1.08 ± 0.13, IκBa: 1.08 ± 0.16 and 1.20 ± 0.11, MKP-1: 0.99 ± 0.19 and 1.54 ± 0.30, IL-1ra: 0.96 ± 0.11 and 0.98 ± 0.12; mean ± SEM of HFD and LFD, respectively). In addition, within the amygdala no significant differences were found between groups in corticosterone receptors, pro-inflammatory or anti-inflammatory gene expression (CD11b: 0.78 ± 0.07 and 0.84 ± 0.12, GR: 0.83 ± 0.80 and 0.98 ± 0.08, IκBa: 0.78 ± 0.92 and 0.73 ± 0.85, IL-1ra: 0.95 ± 0.10 and 0.93 ± 0.09, IL-6: 0.82 ± 0.13 and 0.68 ± 0.10, MR: 0.80 ± 0.08 and 0.94 ± 0.13, MKP-1: 1.23 ± 0.27 and 1.10 ± 0.13, NfκB: 0.75 ± 0.05 and 0.88 ± 0.08; mean ± SEM of HFD and LFD, respectively).

4. Discussion

This study was designed to assess anxiety-like behaviour and limbic gene expression in the glucocorticoid signalling pathway among adult female rats with chronic exposure to HFD containing 60% fat by kcal compared to a low fat diet containing 10% fat by kcal. Overall, HFD rats showed higher levels of anxiety-like behaviour compared to LFD rats, spending more time in the lighted portion of the LD task and showing relatively more entries into the centre than the edges of the OF. In addition, the expression of MR and GR corticosteroid receptors and NfκB pro-inflammatory gene expression in the hippocampus were decreased among HFD rats
relative to expression in LFD rats, implicating alterations in glucocorticoid signalling and related inflammatory processes in the effects of chronic diet HFD exposure. Together, these data indicate that chronic HFD consumption may alter anxiety-like behaviour at least in part via alterations in glucocorticoid signalling mechanisms in limbic brain regions.

We examined the body weight and caloric intake of HFD and LFD rats over the 10 weeks of diet exposure. We also explored the relationship between body weight and the behavioural measures to examine if behavioural changes associated with HFD exposure were directly attributable to the effects of the diet on body weight. As expected, HFD rats were showed greater caloric consumption than the LFD rats throughout the exposure period, including during behavioural testing. Body weight differences emerged by week 6, with HFD rats showing consistently greater body weight than LFD rats, including during the behavioural testing period. Body weight alone was not related to measures of anxiety-like behaviour.

We found that rats consuming HFD showed greater anxiety-like behaviour in the LD and OF tasks. Our data largely agree with our previous report of increased anxiety-like behaviour with perinatal exposure in female rats and support some reports in the literature in males of increased anxiety with chronic HFD consumption [10,19,20]. It is not clear why the results from the EPM did not support those of the LD and OF tasks. However, time in the lighted portion of the LD was highly correlated with the relative number of entries into the centre of the OF, suggesting these two measures may share underlying behavioural and/or neural processes. The expression of MR, GR and NFκB was significantly lower in the HFD group compared to the LFD group. Decreases in both MR and GR expression in the dorsal hippocampus are associated with a dysregulated HPA axis and an exaggerated response to physiological stress in part due to attenuated negative feedback suppression of hypothalamic endocrine cascades mediating corticosterone release [21–23].

There are potentially informative comparisons between the present study and our previous study of the effects of development HFD exposure. First, in the present study, we found that HFD consumption was associated with decreased MR and GR expression in the hippocampus of female rats. However, in a previous study, we found evidence of increased MR and GR levels in the amygdala in adult female rats exposed to HFD in the perinatal period, and no change in expression of MR or GR in hippocampus [10]. The glucocorticoid pathway is mediated by changes in MR and lower affinity GR with an altered HPA. Dysregulation of these receptors has been proposed to increase susceptibility to stress-related disease [24]. The hippocampus and amygdala have opposing roles in the regulation of the HPA axis, where levels of MR and GR refine circadian corticosteroid levels and adaptive responses to stress. For example, in the hippocampus GR activation plays an inhibitory role in the response to stress while in the amygdala GR activation enhances the response to stress [24]. We previously reported that exposure to HFD during the perinatal period was associated with a slower return to baseline levels of corticosterone following a stressor in adulthood [10], and several other studies have indicated the involvement of HFD consumption in HPA dysregulation [12,25–27]. Thus, the results of the present study are consistent with these results in indicating a role for dysregulation of corticosteroid receptors in an enhanced response to stress among HFD-exposed rats.

Second, with the exception of NFκB in the hippocampus (see below), no changes were detected among pro-inflammatory and the anti-inflammatory signalling molecules in the hippocampus or in the amygdala in the present study. This result differs from our previous developmental study, where changes in the expression of immune genes were observed in the hippocampus (IkBa, IL-1ra) and amygdala (NFκB, IL-6, IL-1ra) in the offspring of mothers consuming HFD during gestation and lactation. These differences may be attributable to several factors, including possible indirect effects on offspring of HFA dysfunction among dams consuming HFD and/or direct effects of exposure to triglycerides during critical periods of prenatal and neonatal development. Nevertheless, the results of the present study suggest that dysregulation of these immune genes are not required for the expression of differences in anxiety-like behaviour among HFD-exposed adult females.

To our knowledge this is the first report of decreased NFκB in the hippocampus with chronic HFD consumption. NFκB expression was shown to be increased in peripheral tissues in a chronic HFD-induced obesity mouse model [28]. NFκB is a primary regulator of the inflammatory response and its activity is known to be mediated by changes in GR expression [18]. Indeed, our previous developmental study found evidence for altered expression of both NFκB and GR in the amygdala of female exposed to HFD during the perinatal period [10]. GR expression has been also shown to have both inflammatory and anti-inflammatory roles in the brain, which may explain the similarity of the expression of NFκB and GR [18]. The expression of NFκB is also known to be neuroprotective in the hippocampus. Interestingly, a previous study showed that 4 weeks of HFD feeding in male rats lead to impaired hippocampal neurogenesis and increased circulating corticosterone [13]. Because expression was not examined in the previous study, it is unclear whether the reported changes were accompanied by decreased corticosterone receptors, decreased NFκB or both, and should be the subject of future study.

In sum, these data indicate that chronic HFD consumption may increase anxiety-like behaviour at least in part via alterations in
glucocorticoid signalling mechanisms in limbic brain regions. Because all rats were tested and sacrificed in dioestrous, the results cannot be attributed to differences in phases of estrous cycle between diet groups. Micro RNA-mediated downregulation of GR has been proposed as a model of stress-induced decreases in GR expression [29]. Future studies should be directed at examining these and other epigenetic mediators of the effects of chronic HFD on limbic gene expression.

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