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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.

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; IκBa, I-kappa-B-alpha; LD, light–dark task; LFD, low fat diet; MKP-1, mitogen-activated protein kinase phosphatase-1; MR, mineralocorticoid receptor; NFκβ, 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 zelta/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. D12492), 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 CO_2 inhalation followed by decapitation. Whole brains were collected, flash-frozen in isopentane and stored at $-80\,^{\circ}C$. 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],