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Saturated high-fat feeding independent of obesity alters hypothalamus-pituitary-adrenal axis function but not anxiety-like behaviour

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ABSTRACT

Overconsumption of dietary fat can elicit impairments in emotional processes and the response to stress. While excess dietary lipids have been shown to alter hypothalamus-pituitary-adrenal (HPA) axis function and promote anxiety-like behaviour, it is not known if such changes rely on elevated body weight and if these effects are specific to the type of dietary fat. The objective of this study was to investigate the effect of a saturated and a monounsaturated high-fat diet (HFD) on HPA axis function and anxiety-like behaviour in rats. Biochemical, metabolic and behavioural responses were evaluated following eight weeks on one of three diets: (1) a monounsaturated HFD (50%kcal olive oil), (2) a saturated HFD (50%kcal palm oil), or (3) a control low-fat diet. Weight gain was similar across the three diets while visceral fat mass was elevated by the two HFDs. The saturated HFD had specific actions to increase peak plasma levels of corticosterone and tumour-necrosis-factor-alpha and suppress mRNA expression of glucocorticoid and mineralocorticoid receptors, corticotropin-releasing hormone and 11β-hydroxysteroid dehydrogenase-1 in the paraventricular nucleus of the hypothalamus. Both HFDs enhanced the corticosterone-suppressing response to dexamethasone administration without affecting the physiological response to a restraint stress and failed to increase anxiety-like behaviour as measured in the elevated-plus maze and open field tests. These findings demonstrate that prolonged intake of saturated fat, without added weight gain, increases CORT and modulates central HPA feedback processes. That saturated HFD failed to affect anxiety-like behaviour can suggest that the anxiogenic effects of prolonged high-fat feeding may rely on more pronounced metabolic dysfunction.

1. Introduction

Western diets are characterized by excess fat intake that promotes obesity and increases susceptibility to major illnesses such as type 2 diabetes and cardiovascular disease. Another major complication of obesity is mood disorders (Hryhorczuk et al., 2013). Overweight and obesity are reported to significantly increase the risk of anxiety and depression (DeJesus et al., 2016; Gariepy et al., 2010; Luppino et al., 2010). In turn, the incidence of depression and anxiety increases the odds of developing obesity (de Wit et al., 2010; Luppino et al., 2010). A common feature of both metabolic and mood disorders is alterations in hypothalamus-pituitary-adrenal (HPA) axis function (Faravelli et al., 2012; Pasquali et al., 2006). Upon activation, the HPA axis induces a cascade of hormonal release events initiated by corticotrophin-releasing hormone (CRH) in the paraventricular nucleus (PVN) that leads to the release of glucocorticoids (GC). Corticosterone (CORT), the principal GC, acts on central GC receptors to affect immune, behavioural and metabolic processes (Charmandari et al., 2005; Sapolsky et al., 2000) and as a negative feedback to suppress further HPA activation (Nicolaides et al., 2015). Oversecretion of cortisol is associated with obesity (Kreze et al., 1995; Magiakou et al., 2006; Nieuwenhuizen and Rutters, 2008) and triggers the accumulation of abdominal (visceral) fat which is associated with greater cardiometabolic risk (Lopresti and Drummond, 2013). Hypercortisolism is also linked to anxiety disorders

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and atypical depression, hypofunctionality of the HPA axis and alteration in GC signaling in the PVN.

We and others have found that obesity in rodents caused by chronic high-fat feeding leads to anxiety- and depressive-like behaviours that are associated with anhedonia, increased plasma levels of CORT and neuroplastic adaptations in key brain regions controlling motivation and the response to stress (Dutheil et al., 2015; Martire et al., 2014; McNeilly et al., 2015; Sharma et al., 2013; Tannenbaum et al., 1997). Alterations in plasma free fatty acids (FA) have been shown to modulate HPA axis function (Widmaier et al., 1992). Interestingly, a specific drop in plasma levels of the saturated FA palmitic acid, but not in unsaturated FAs (oleic or linoleic), is reported to stimulate HPA activation (Oh et al., 2014). Despite these findings, it is not known if the effects of excess dietary lipids on HPA axis function are specific to saturated dietary lipids and if they rely on increases in body weight and adiposity. Consistent with the differential metabolic effects of saturated and unsaturated lipids in numerous tissues (Benoit et al., 2009; Kanoski and Davidson, 2011; Kien et al., 2014), we previously demonstrated that the long-term intake of a saturated (palm oil), but not a monounsaturated (olive oil), high-fat diet (HFD) can suppress motivated behaviour and mesolimbic dopamine function and signaling, independent of obesity and associated metabolic disturbances in the rat (Hryhorczuk et al., 2016).

Using the rat model of high-fat feeding developed previously that does not exhibit changes in body weight following different dietary interventions (Hryhorczuk et al., 2016) we sought to assess the impact of prolonged saturated or monounsaturated HFDs on HPA axis activity and anxiety-like behaviour to identify changes in the central expression of GC-relevant genes. Chronic intake of the saturated, but not the monounsaturated, HFD resulted in elevated peak plasma CORT and decreased the expression of several GC-related genes in the PVN. High-fat feeding also heightened HPA responses to a dexamethasone (DEX) challenge but did not affect the physiological response to a restraint stress or anxiety-like behaviour. These findings identify the specific impact of saturated dietary lipids on HPA tone and central feedback mechanisms that are independent of changes in body weight and suggest that the anxiogenic effects of a HFD does not manifest without profound diet-induced metabolic alterations.

2. Material and methods

2.1. Animals and diet

All procedures were approved by the Animal Care Committee of the CRCHUM. Male Wistar rats (Charles River, St Constant, QC, Canada) weighing 250–280 g upon arrival were group housed in a reverse 12 h light/dark cycle with *ad libitum* access to food and water. Rats were fed one of three diets as previously described (Hryhorczuk et al., 2016): a control diet (17% kcal soybean oil; “CTL”), an oleic acid-enriched HFD (50%kcal olive oil; “OLIVE”) or a palmitic acid-enriched HFD (50%kcal palm oil; “PALM”) for 8 weeks. HFDs were isocaloric and diverged only in fat source. Body weights were measured throughout and behavioural and biochemical experiments were carried out after 8 weeks. Six separate cohorts of rats (3 diet groups for a total of 165 rats) were used for (1) locomotor activity and CORT measurements, (2) plasma biochemistry, (3) fat pads collection and gene expression analysis, (4) dexamethasone suppression test (5) restraint stress challenge and (6) anxiety-like behaviour testing.

2.2. Plasma and tissue collection

Rats (n = 95) were sacrificed at the beginning of the dark phase under isoflurane anaesthesia following 8 weeks of diet. Brains were rapidly extracted and snap-frozen in isopentane. Adrenal glands, subcutaneous (dorsolumbar) and visceral (perirenal, retroperitoneal and perigonadal) fat depots were collected and immediately weighed. Trunk blood was collected in EDTA-coated tubes and centrifuged at 11000*g* for 15 min at 4 °C. Plasma was stored at −20 °C. Free fatty acids were measured by an enzymatic colorimetric method assay (Wako Chemicals USA, Inc.). C-reactive protein (CRP) was measured by ELISA (Life Diagnostics, Inc.) and TNFα by AlphaLISA (Perkin Elmer). Basal peak (nocturnal) CORT levels were measured by ELISA (Enzo Life Sciences, Inc.) from blood samples collected from the tail vein at the beginning of the dark phase after 7.5 weeks of diet.

2.3. Locomotor activity

Rats (n = 49) were introduced into acrylic chambers (42 × 42 × 21 cm, AccuScan Instruments, Columbus, OH, USA) equipped with light beam sensors to detect horizontal and vertical movements in the middle of the light period. Basal locomotor activity was recorded over a period of 6 h in the middle of the following dark phase using the Versamax Animal Activity Monitor.

2.4. Gas chromatography-mass spectrometry

Composition of plasma fatty acids, both free and bound to phospholipids, triglycerides and cholesterol was determined by gas chromatography-mass spectrometry (GC–MS) as previously described (Hryhorczuk et al., 2016).

2.5. Gene expression analysis

RNA was extracted from anterior pituitary and brain punches of the PVN, hippocampus (CA1, CA3 and dentate gyrus) and central (CeA) and basolateral (BLA) amygdala using QIAzol (Qiagen). Spectrophotometric quantification and visualization on a 1% agarose gel were systematically performed to ensure RNA quality. 0.5 µg of RNA were reverse transcribed into cDNA using the Moloney Murine Leukemia Virus Reverse Transcriptase (Life Technologies, Inc.). Genes of interest were amplified by quantitative PCR using the QuantiFast SYBR Green PCR kit (Qiagen) in the presence of appropriate primer pairs on a Corbett Rotor-Gene 6000. *11β-HSD1* forward: TGTCTCGC TGCCTTGAACTC, reverse: ATGTCCAGTCCACCCAAGAG; *11β-HSD2* forward: TGCTTCAAGACAGAGGCAGT, reverse: TGATGGCA TCTACAACGGGG; *β-actin* forward: TGAAGTGTGACGTTGACATCC, reverse: ACAGTGAGGCCAGGATAGAGC; *CRH* forward: GGAGAAGA GAGCGCCCCTAA, reverse: CGGATCAGAATCGGCTGAGG; *CRH-R1* forward: CAATGTGGCCTGGTGTAGGT, reverse: GGTG GAGTACGTGAGCACAA; *CRH-R2* forward: CCGAATCGC CCTCATCATCA, reverse: GGATACTCCGCAGCACTAGG; *Cyclophilin* forward: CTTGCTGCAGACATGGTCAAC, reverse: GCCATT ATGGCGTGTGAAGTC; *GR* forward: ACCAACGGAGGCAG TGTGAAA, reverse: GGGGACCCAGCGGAAAAC; *MR* forward: GACAATTCCAAGCCCGACACC, reverse: CTTGGCCCACTTCACGA CCTG. Expression levels were determined by the standard curve method and normalized to that of the most stable reference gene as assessed by the GeNorm and NormFinder algorithms for each region: cyclophilin for the hippocampus, β-actin for the CeA, BLA and pituitary and the geometric mean of β-actin and cyclophilin for the PVN.

2.6. Restraint stress test

Another cohort of rats (n = 22) was used to determine the CORT response to a restraint challenge. The test started at the beginning of the light phase. Each rat was restrained for 45 min in acrylic tubes. Blood samples were collected by tail-bleeding just before (T0) and right after the restraint (T45). CORT levels were measured as mentioned above.

2.7. Dexamethasone suppression test (DST)

Dexamethasone powder (DEX; Sigma-Aldrich) was reconstituted in