NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

G. Winther et al. / Neuroscience xxx (2018) xxx–xxx

Maternal High-fat Diet Programs Offspring Emotional Behavior in Adulthood

Gudrun Winther,[a]* Betina Elfving,[a] Heidi Kaastrup Müller,[a] Sten Lund[b] and Gregers Wegener[a,c,d]

[a] Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Risskov DK-8240, Denmark

[b] Department of Endocrinology and Internal Medicine Medical Research Laboratory, Aarhus University Hospital, DK-8000, Denmark

[c] Department of Clinical Medicine, AUGUST Centre, Aarhus University, Risskov DK-8240, Denmark

[d] Center of Excellence for Pharmaceutical Sciences, North-West University (Potchefstroom Campus), Hoffman Street, Potchefstroom 2520, South Africa

Abstract—Prenatal exposure to high-fat diet (HFD) might predispose offspring to develop metabolic and mental disorders later in life. Insight into the molecular and behavioral consequences of maternal HFD on offspring is sparse but may involve both neuroinflammation and a dysregulated neuroendocrine stress axis. Thus, the aim of this work was to: (i) investigate the influence of maternal HFD on memory, anxiety and depression-like behavior in adult offspring and (ii) identify possible biological biomarkers related to neuroinflammation and stress responses. Seven-week-old, female Sprague–Dawley rats received a control diet or a HFD eight weeks prior to conception and during gestation and lactation. We investigated the phenotype of the offspring in the in elevated plus maze, forced swim test, novel object recognition and open field test. Furthermore, hippocampal gene expression related to neuroinflammation and the stress axis was quantitated by real-time qPCR. We found that maternal HFD led to an anxiogenic offspring phenotype in the elevated plus maze, independent of sex. This behavioral alteration was accompanied by significantly higher mRNA levels of the hippocampal pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) mRNA and monocyte-chemoattractant protein-1 (MCP-1), both of which correlated with degree of behavioral change. Maternal exposure to HFD increased the offspring's levels of hippocampal, corticosteroid releasing hormone receptor 2 (CRHR2) and kynurenine mono oxygenase (KMO) mRNA, whereas kynurenine aminotransferase I (KAT1) mRNA levels were decreased. The present results suggest that neuroinflammatory and stress axis pathways in the hippocampus may contribute to anxiogenic effects of maternal HFD in offspring. © 2018 Published by Elsevier Ltd on behalf of IBRO.

Key words: maternal obesity, pregnancy, prenatal programing, anxiety, depression.

INTRODUCTION

Globally, a sedentary lifestyle combined with a diet characterized by low fiber and high caloric intake from fats and sugars might play a major role in the development of obesity and metabolic diseases (WHO, 2008). This constitutes an imminent problem as the increasing prevalence of obesity is a major public health concern (Flegal et al., 2010). Previous research suggests that obesity may be significantly associated with mood disorders (Rethorst et al., 2014; Milaneschi et al., 2018). Maternal obesity (body mass index (BMI) $\geq$ 30 kg/m^2) is a major health concern due to an increased risk for later health complications for both mother and child (Barker, 1998; Cedergren, 2004; Huang et al., 2017), with increased risk of offspring metabolic syndrome (Benjamini and Hochberg, 1995), cardiovascular diseases (Van Gaal et al., 2006; Hochner et al., 2012), obesity (Reynolds, 2013; Dudele et al., 2017) and type 2 diabetes (Marx, 2002; Kahn et al., 2006). Furthermore, maternal overconsumption of a diet high in saturated fats, a so called "Westernized diet" and obesity have been linked to increased risk of neurodevelopmental disorders and mental health problems including autism spectrum disorder (Bilder et al., 2013), attention hyper activity disorder (Rodriguez et al., 2008) emotional behavior (Deardorff

*Corresponding author.
E-mail addresses: gwinther@hotmail.com (G. Winther), betina.elfving@clin.au.dk (B. Elfving), heidi.muller@clin.au.dk (H. K. Müller), sten.lund@clin.au.dk (S. Lund), wegener@clin.au.dk (G. Wegener).

Abbreviations: BDNF, brain-derived neurotrophic factor; CON, control diet; EPM, elevated plus maze; FST, forced swim test; GR, glucocorticoid receptor; HFD, high-fat diet; HPA, hypothalamic–pituitar yadrenal; IGF, insulin-like growth factor; IL, interleukin; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MR, mineralocorticoid receptor; NFκB, nuclear factor kappa beta; NOR, novel object recognition; OFT, open field test; OGTT, oral glucose tolerance test; PND, postnatal day; TNF, tumor necrosis factor.

https://doi.org/10.1016/j.neuroscience.2018.07.014
0306-4522/

et al., 2017; Schaefer et al., 2017) and depression (Colman et al., 2012; Bergmann et al., 2016) in the children.

Evidence demonstrates that disturbed early life development, may have long-term impact on the hypothalamic–pituitaryadrenal (HPA) axis function, inducing downstream regulation of inflammatory mediators and thereby disrupt programing of offspring behavior (Sullivan et al., 2015). Preclinical studies with both non-human primates and rodents demonstrate that exposure to maternal HFD is involved in anxiety/fear manifestation in offspring (Sullivan et al., 2010; Ramirez-Lopez et al., 2016; Thompson et al., 2017), suggesting that the mechanisms involved are conserved across species. Few studies have evaluated the association between maternal obesity and offspring depression, but data indicate that male offspring born from HFD-exposed dams *in utero* or during lactation have an exacerbated depression-like phenotype compared to male offspring from lean dams (Giriko et al., 2013; de Noronha et al., 2017; Bayandor et al., 2018). It is currently not well-understood, how high-fat diet (HFD) may influence the development of psychiatric disorders in offspring. Interestingly, data indicate that maternal obesity or HFD consumption is associated with increased oxidative stress and neuroinflammation in offspring (Bilbo and Tsang, 2010; Peleg-Raibstein et al., 2012; Sasaki et al., 2013).

Obesity is associated with chronic low-grade systemic inflammation (Bray et al., 2002; Lumeng et al., 2007), which is suggested to be the precipitating factor for many obesity-associated complications such as mental health (Dantzer and Kelley, 2007; Sharma and Fulton, 2013). Chronic inflammation is characterized by cellular infiltration and activation of macrophages and T-lymphocytes, which are induced by overexpression of chemokines and cytokines (Wu et al., 2007; Makki et al., 2013). Obesity in humans and in the diet-induced obesity (DIO) rodent model demonstrate development of insulin resistance and type 2 diabetes and an increased level of several immunological factors. For example, tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 as well as monocyte chemoattractant protein-1 (MCP-1) are increased (Hotamisligil et al., 1993; Hotamisligil, 2006; Olefsky and Glass, 2010), causing an infiltration of M1 classically activated macrophages, exacerbating the inflammation in obesity (Weisberg et al., 2003; Kanda et al., 2006).

An imbalance in pro- versus anti-inflammatory cytokines influences the balance of the kynurenine pathway (KP), which is the major metabolic pathway of the essential amino acid tryptophan (TRP). TRP is an essential precursor to the neurotransmitter serotonin, which is implicated in mental disorders (Stockmeier, 2003). However, KP gives rise to several neuroactive metabolites and pro-inflammatory stimulation of the KP may trigger the dysregulation of several enzymatic steps, favoring the production of neurotoxic over neuroprotective metabolites (Stone and Darlington, 2013). Indeed, the KP has been proposed as a mechanistic link between the immune system and mental disorders (Asp et al., 2010; Schwarcz et al., 2012). However, it has not yet been investigated whether the maternal HFD may induce alterations in the KP, and thus be implicated in the consequences of altered behavior in adult offspring.

To elucidate the mechanistic understanding of how these pathways contribute to disease development in offspring, we designed the present study to investigate whether an obesogenic maternal diet alters (i) hippocampal-dependent behaviors (cognition, anxiety- and depression-like behavior), (ii) metabolism and expression of peripheral pro-inflammatory cytokines, (iii) expression of hippocampal genes associated with neuroendocrine function, neuroinflammation and the KP. Furthermore, the study aimed to assess possible gender differences.

EXPERIMENTAL PROCEDURES

Animals

Male and female Sprague–Dawley rats (7 weeks old) were obtained from Taconic Bioscience A/S (Ry, Denmark). Rats were housed in same-sex pairs until mating (Cage 1500U Eurostand Type IV S 480 × 375 × 210 mm, Techniplast, Buguggiate, Italy, including a tunnel shelter, pine bedding (Tapvei® Brogaarden, Aps, Lynge, Denmark) and a wooden stick). Animals were maintained at 22 ± 2 °C and 55 ± 10% relative humidity on a 12-h light/dark cycle (lights on at 07:00 a.m.). All experimental procedures complied with the EU Directive 2010/63/EU and the Danish Experimentation Act (LBK 1306 from 23/11/2007 with 2011 amendments). The protocol was approved by the Danish Animal Experimentation Committee (j.no 2012-15-2934-00254).

Diets

Rats received a control diet (CON) (D12492) and HFD (D12450J; for diet induced obesity) purchased from Research Diets Inc. (New Brunswick, NJ, USA). The CON diet consisted of 10% fat, 20% protein, 70% carbohydrate (complex carbohydrates as corn and sucrose starch) by kilo calories (3.85 kcal/g). The HFD consisted of 60% fat (mainly lard), 20% protein and 20% carbohydrate by kilo calories (5.24 kcal/g).

Study design

To investigate whether obesogenic maternal diet affects offspring metabolism and behavior, female breeders were randomly allocated into either a CON (n = 10) or HFD (n = 10) group, both groups receiving tap water *ad libitum*. Dams were placed on diet 8 weeks prior to mating and remained throughout gestation and lactation (see Fig. 1). Prior to mating, female rats were separated and pair-housed with a male rat (n = 20, fed with CON prior to breeding) throughout gestation and lactation. On postnatal day (PND) 2, litter size was randomly adjusted to eight, with equal sex distribution, to ensure standardized nutrition. Male and female offspring were weighed once a week from PND 2. At PND 21, male and female offspring were housed in same-sex pairs with *ad libitum* access to CON diet. Thus, the offspring