Brain, Behavior, and Immunity 36 (2014) 35–45

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Named Series: Diet, Inflammation and the Brain

The effects of dietary saturated fat on basal hypothalamic neuroinflammation in rats

Tia Maric [a], Barbara Woodside [b], Giamal N. Luheshi [a,*]

[a] Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada
[b] Center for Studies in Behavioural Neurobiology, Concordia University, Montreal, Quebec, Canada

ARTICLE INFO

Article history:
Available online 25 September 2013

Keywords:
Obesity
Inflammation
Saturated fat diet
Sickness behavior
Cytokines

ABSTRACT

Recent evidence has demonstrated that consumption of high fat diets can trigger brain inflammation and subsequent injury in the absence of any peripheral inflammatory signaling. Here we sought to investigate whether a link exists between the concentration of highly saturated fats in the diet and the development of inflammation in the brain of rats and, whether the source of the saturated fat was an important factor in this process. Adult male rats had access to diets with a moderate level of total fat (32% of calories as fat) varying in level of saturated fat [low (20%) vs high (>60%)] and its source (butter or coconut oil). After 8 weeks of diet exposure peripheral and central tissues were collected for analysis of inflammatory signals. Neither blood nor white adipose tissue exhibited any changes in inflammatory mediators regardless of the saturated fat content or the source. In the brain however, we observed significant hypothalamic upregulation of the expression of markers of glial activation as well as of interleukin (IL)-1,6 and nuclear factor (NF)-IL-6, which were highest in the group fed the butter-based diets. The increase in these inflammatory mediators had no effect on basal body temperature or the temperature response to systemic lipopolysaccharide (LPS). The present results indicate that hypothalamic inflammation associated with consumption of diets high in fat is directly linked to the saturated fat content as well as the source of that fat. These effects are likely linked to other pathophysiological changes in the regulation of metabolism.

1. Introduction

Obesity is a health issue of global proportions due to increasing incidence of disorders such as type II diabetes, cardiovascular disease and even some cancers associated with chronic weight gain in humans (Barzilay et al., 2001; Hotamisligil, 2006; Mathieu et al., 2009; Pradhan et al., 2001; Schmidt et al., 1999). The development of these conditions is increasingly being linked with a sub-clinical inflammatory state, exemplified by higher than normal concentration of pro-inflammatory mediators such as the cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α in the circulation of chronically obese individuals (Mantzoros et al., 1997; Somm et al., 2006). It is suggested that the increase in these circulating inflammatory mediators is in part due to increased adiposity including white adipose tissue (WAT) expansion and adipocyte enlargement (Hotamisligil and Erbay, 2008; Odegaard and Chawla, 2008). This tissue, which until relatively recently has been considered to be a dormant energy store, is now known to be a major source of cytokines in addition to being the main source of cytokine-like energy regulating hormones including leptin and adiponectin (Trayhurn and Wood, 2005). White adipose tissue also constitutes a significant target to pathogens acting through toll like receptors (TLR), 2 and 4, which are expressed in fat, and when activated induce the release of inflammatory mediators (Kopp et al., 2009).

Using a rodent model of diet induced obesity (DIO), we reported that, *in vitro*, WAT extracted from obese rats produced significantly more cytokines in response to pathogenic challenge with the TLR 4 ligand lipopolysaccharide (LPS) than tissue extracted from lean counterparts (Pohl et al., 2009). In the same study, *in vivo*, we showed that the overall immune sickness-behavior response to LPS, including fever, was significantly exacerbated in magnitude and duration, in obese versus lean animals. The potentiation of these responses was accompanied by higher levels of cytokines both in the circulation and hypothalami of obese animals (Pohl et al., 2009). This study clearly demonstrated that DIO produced significant and fundamental changes in the innate immune response to pathogenic challenge; however, we found no evidence that baseline circulating cytokine expression was altered in these animals as a function of obesity.

* Corresponding author. Address: Douglas Mental Health University Institute, McGill University, Montreal, Quebec H4H 1R3 Canada. Tel.: +1 514 761 6131x4927; fax: +1 514 762 3034.
E-mail address: Giamal.Luheshi@McGill.ca (G.N. Luheshi).

0889-1591/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2013.09.011

The diet supplement used in our study to generate DIO contained only a moderate amount of fat (24% of calories) but did lead to prolonged hyperphagia, and doubling of fat pad weight after 8 weeks of diet exposure. By contrast, the majority of other reports on obesity-related basal inflammation in DIO rodents have used diets that have a high fat content (60% of calories as fat) to induce the obese state and the associated change in basal inflammatory markers (Cintra et al., 2012; Hariri et al., 2010). Some of these studies have demonstrated that exposure to such a high fat diet induces hypothalamic as well as peripheral inflammation. Indeed a recent study (Thaler et al., 2012) suggests that one day of exposure to a 60% fat diet is sufficient to increase hypothalamic cytokine expression and that this effect occurred in the absence of any increase in peripheral cytokines in both rats and mice.

In addition to the concentration of fat in the diet and the length of exposure to that diet, another important variable that can contribute to the development of an underlying inflammatory state are the specific dietary constituents used. Accumulating evidence has shown highly saturated fats, can directly promote inflammation both in the periphery and the brain (Ellis et al., 2002; Piers et al., 2003; van Dijk et al., 2009). Although the cellular and molecular pathway underlying these effects have not been fully elucidated, studies demonstrating direct stimulation of TLR-4 (Milanski et al., 2009) by saturated fatty acids in the diet provide the most likely pathway.

Thus it would appear from the available literature that the source, or type of dietary fat and its level of saturation may be a critical factor in the development of an underlying inflammatory condition (Hariri and Thibault, 2010). Based on these data, the goal of the present study was to investigate whether varying the source of fat content and the level of saturated fat in the diet would potentiate the effects of exposure to a diet with only moderate levels of total fat on basal peripheral and hypothalamic inflammation. Therefore, levels of expression of both pro-and anti-inflammatory markers in the circulation, WAT and the hypothalamus as well as markers of glia activation were compared among rats exposed for 8 weeks to a moderate fat diet (32% of calories as fat) that varied in the level of saturated fat and the source of the high saturated fats: butter or coconut oil. In addition, in a separate cohort we examined whether differences in the source and level of saturated fat in the diet would affect the response to an acute immune challenge (LPS administration).

2. Methods

2.1. Animals

Male Wistar rats (weighing 225–275 g at the start of the experiment; Charles River, St. Constant, Quebec, Canada) group housed (n = 4/cage) at ambient temperature of 22 °C (±1) on a 12-h light/12-h dark cycle (lights on at 0800 h) were used in these experiments. Rats had access to water and laboratory chow (Charles River Autoclavable Rodent Chow) *ad libitum*. Once a body weight range of 335–350 g was reached, rats were assigned to one of three diet conditions such that mean and range of body weight in each group were similar. Two cohorts of rats were used in these experiments, one to assess the effects of diet exposure on basal expression of pro-and anti-inflammatory markers and a second to evaluate the effects of diet exposure on the fever response to an acute immune challenge. In both cohorts, rats were given *ad libitum* access to one of these three diets for a period of 8 weeks. Diets were replaced with fresh pellets every second day and body weight was measured weekly. In the cohort tested for response to the immune challenge, using lipopolysaccharide (LPS) rats in each diet group was singly housed at the end of the 7th week of diet exposure to allow for measurement of daily food intake.

All protocols were approved by the Concordia University Animal Research Ethics Committee under the guidelines of the Canadian Council on Animal Care.

2.2. Diets

Groups of rats were given access to one of three nutritionally complete diets containing 32% of calories from fat, 17% calories as protein, and 51% as carbohydrate, with a 1:1 ratio of monounsaturated to polyunsaturated fat (Research Diets. INC, New Brunswick, NJ, USA). Diets differed, however in the amount and source of saturated fat content. Rats were assigned to either a low saturated fat diet group (LSF; D11012902) in which the source of dietary fat was corn and olive oil and only 20% of the fat calories were from saturated fat; a high saturated fat coconut oil based diet (HSF coconut; D11012901) in which 75% of fat calories came from saturated fat; or a high saturated fat diet in which butter was the primary source of fat (HSF butter; D11061301) and 60% of calories came from saturated fat (see Table 1 for details of diet composition).

2.3. Tissue collection and analysis

At the end of each study, rats were deeply anesthetized with a lethal dose of sodium pentobarbital (IP, 60 mg/kg body weight). Terminal blood samples were collected by intracardiac puncture and were centrifuged (10,000*g* for 10 min at 4 °C), allowing collection of serum which was then stored at −80 °C. Animals were then perfused through the heart with autoclaved physiological saline (1 ml/kg). Whole brains and weighed epididymal and retroperitoneal fat pads were collected, quick-frozen and stored at −80 °C until analysis.

Concentrations of circulating IL-6 as an example of a significant pro-inflammatory mediator (Cartmell et al., 2000) that has been

Table 1
Diet fatty acid profile.

	LSF	HSF coconut	HSF butter
% of calories from fat	32	32	32
% of fat as saturated	20	75	60
Butter	20	40.2	69
Coconut oil	0	92	46.2
Corn oil	93.1	30	47
Olive oil	49	0	0
Total	*162.1*	*162.2*	*162.2*
C4, Butyric	0.6	1.3	2.2
C6, Caproic	0.4	1.3	1.6
C8, Caprylic	0.2	7.5	4.3
C10, Capric	0.5	6.4	4.5
C12, Lauric	0.6	44.9	23.9
C14, Myristic	2	20.6	15.2
C14:1, Myristoleic	0.3	0.6	1
C16, Palmitic	21.6	21.8	27.2
C16:1, Palmitoleic	1	0.9	1.6
C18, Stearic	5.4	15.2	14.1
C18:1, Oleic	63.2	18.3	29.4
C18:2, Linoleic	61.5	19	29.8
C18:3, Linolenic	2	1	1.6
C20, Arachidic	0.2	0.4	0.7
C20:1,	0.1	0	0
C22, Behenic	0.3	0	0
C24, Lignoceric	0.2	0	0
Total	*160*	*159.2*	*157.2*
Saturated (g)	32	119.4	93.7
Monounsaturated (g)	64.6	19.8	32
Polyunsaturated (g)	63.4	20	31.5
Saturated (%)	20	75	59.6
Monounsaturated (%)	40.4	12.5	20.4
Polyunsaturated (%)	39.6	12.5	20