Physiology & Behavior 141 (2015) 1–8

Contents lists available at ScienceDirect

Physiology & Behavior

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

Diet-induced obesity attenuates endotoxin-induced cognitive deficits

Sharay E. Setti, Alyssa M. Littlefield, Samantha W. Johnson, Rachel A. Kohman *

University of North Carolina Wilmington, Department of Psychology, Wilmington, NC, USA

HIGHLIGHTS

- Diet-induced obesity blunts the cognitive deficits associated with inflammation.
- Diet-induced obesity attenuates LPS-induced interleukin-6 production in the brain.
- Basal expression of CD74 in the hippocampus is increased in diet-induced obese mice.

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form 19 December 2014
Accepted 22 December 2014
Available online 24 December 2014

Keywords:
High-fat diet
LPS
CD74
CD86
Spatial learning
Water maze
Cytokines
IL-6

ABSTRACT

Activation of the immune system can impair cognitive function, particularly on hippocampus dependent tasks. Several factors such as normal aging and prenatal experiences can modify the severity of these cognitive deficits. One additional factor that may modulate the behavioral response to immune activation is obesity. Prior work has shown that obesity alters the activity of the immune system. Whether diet-induced obesity (DIO) influences the cognitive deficits associated with inflammation is currently unknown. The present study explored whether DIO alters the behavioral response to the bacterial endotoxin, lipopolysaccharide (LPS). Female C57BL/6J mice were fed a high-fat (60% fat) or control diet (10% fat) for a total of five months. After consuming their respective diets for four months, mice received an LPS or saline injection and were assessed for alterations in spatial learning. One month later, mice received a second injection of LPS or saline and tissue samples were collected to assess the inflammatory response within the periphery and central nervous system. Results showed that LPS administration impaired spatial learning in the control diet mice, but had no effect in DIO mice. This lack of a cognitive deficit in the DIO female mice is likely due to a blunted inflammatory response within the brain. While cytokine production within the periphery (i.e., plasma, adipose, and spleen) was similar between the DIO and control mice, the DIO mice failed to show an increase in IL-6 and CD74 in the brain following LPS administration. Collectively, these data indicate that DIO can reduce aspects of the neuroinflammatory response as well as blunt the behavioral reaction to an immune challenge.

1. Introduction

A wealth of evidence indicates that obesity alters immune function [1–4]. These alterations in immune activity may contribute to the increased incidence of several diseases in obese individuals including cardiovascular disease and Alzheimer's disease [5–7]. In addition, obesity is associated with a higher rate of infection following surgery or an injury [2,8]. Further research has shown that obese individuals require a longer recovery time after surgery [8]. During influenza season, obese individuals have higher rates of hospitalization than normal weight individuals [9]. Given the substantial increase in the number of obese individuals, understanding the nature of obesity-related changes in immune function and their potential relevance to disease and infection susceptibility is of great importance.

Investigations using an animal model of diet-induced obesity (DIO) confirm that obesity modifies the response to an immune challenge. For instance, DIO has been shown to increase peripheral (i.e., plasma, spleen, and adipose tissue) levels of the cytokines interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and IL-1 receptor antagonist (IL-1ra) in response to the endotoxin, lipopolysaccharide (LPS) [3,4,10,11]. Further, DIO rats show higher expression of IL-6 and TNF-α in the hypothalamus following LPS administration when compared to control rats [3]. In contrast to these data, several reports have shown that DIO can suppress aspects of the response to an immune challenge. For example, Lawrence et al. [12] reported that DIO mice show reduced neural activation, as measured by the immediate early gene c-Fos, following LPS administration in select brain regions. In addition, macrophages from DIO animals show reduced interleukin-1β (IL-1β), TNF-α, nuclear factor-κB

* Corresponding author at: University of North Carolina Wilmington, 601 S. College Road, Wilmington, NC 28403-5612, USA.
E-mail addresses: ses8535@uncw.edu (S.E. Setti), aml8440@uncw.edu (A.M. Littlefield), swj4531@uncw.edu (S.W. Johnson), kohmanr@uncw.edu (R.A. Kohman).

http://dx.doi.org/10.1016/j.physbeh.2014.12.036
0031-9384/

(NF-κB), Toll-like receptor-4 (TLR-4), and inducible nitric oxide synthase (iNOS) levels following an immune challenge when compared to macrophages from control diet animals [13–16]. In addition, we found attenuated levels of IL-6 in the cortex of DIO male mice following LPS administration [17]. Presently, the reason for these divergent findings is unknown, but variations in the experimental procedure including differences in the animal's age, species, timing of tissue sampling, as well as the type and duration of the diet, among other factors, likely contribute to these differences. Despite these differences, the data collectively indicate that DIO alters the response to immune activation.

The body's response to an immune challenge includes a complementary shift in behavior. This behavioral response, commonly known as sickness behavior, includes a reduction in locomotor activity, alterations in sleep, depression of social and sexual behavior, anorexia, fever, as well as suppression of other species typical behaviors [18–20]. Induction of sickness behavior is mediated by the activity of several inflammatory molecules including prostaglandins and the proinflammatory cytokines IL-1β, IL-6, and TNF-α [18,20,21]. Prior work indicates that obesity, through alterations in immune function, may exacerbate the sickness behavior response. For example, Pohl et al. [22] report that rats fed a high-fat diet showed prolonged suppression of social behavior following LPS administration. However, DIO has no effect on LPS-induced reductions in locomotor behavior, as DIO animals show a similar reduction and recovery rate when compared to control animals [17,22]. Alterations in the fever response have also been observed, as some report an enhanced fever in DIO animals following an immune challenge [3,22]. However, this appears to be dose dependent as Lawrence et al. [12] report a blunted fever in DIO mice following a low LPS dose and an exaggerated fever following a higher dose. Taken together, these data indicate that DIO modifies aspects of the sickness behavior response to an immune challenge.

Beyond the development of sickness behavior, immune activation can disrupt cognitive function. Processes dependent on the hippocampus are particularly sensitive to disruption following an immune challenge. For instance, LPS administration impairs contextual, but not auditory, fear conditioning [23,24]. In addition, immune activation has been shown to disrupt spatial learning in the water maze [25–27]. Similar to sickness behavior, these cognitive deficits result from proinflammatory cytokines acting within the brain, as cytokine administration mimics the deficits induced by LPS and inhibition of IL-1β and IL-6 have been shown to block memory deficits following infection [28–32]. There has been some suggestion that the cognitive deficits associated with DIO may reflect basal increases in inflammation, however, this has not been consistently reported and may depend on the age of the animal [17,33,34]. While data exists on how DIO may alter the sickness behavior response to an immune challenge, whether DIO alters the development of cytokine-induced cognitive deficits is presently unknown. The objective of the current study was to assess alterations in spatial learning in DIO and control mice following an LPS challenge along with corresponding changes in the systemic and central inflammatory response.

2. Material and methods

2.1. Animals

Subjects were 52 female C57BL/6J mice bred in the University of North Carolina Wilmington (UNCW) animal facility, with breeding stock obtained from The Jackson Laboratory (Bar Harbor, Maine). Mice were 5 weeks old at the beginning of the study. Animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals and the experiments were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at UNCW.

2.2. Diets and housing

Mice were assigned to the high-fat diet (DIO; open source diets, D12492) or control diet (open source diets, D12450B). The high-fat diet contained 60% of calories from fat and the control diet contained 10% of calories from fat. Mice were group housed with two to four mice per cage. Mice started consuming the high-fat or control diet at 5 weeks of age and continued for 5 months until the mice were approximately 6 months old. Mice were weighed once a week throughout the experiment as well as assessed for weight loss following administration of LPS or saline (described below). The treatment-induced changes in total body weight (i.e., grams lost or gained) were determined by calculating a difference score for each mouse by subtracting the animal's body weight on the day of the injection from the animal's body weight 24 h after an LPS or saline injection.

2.3. Effect of DIO on LPS-induced spatial learning deficits

After consuming the high-fat or control diet for four months mice received a single intraperitoneal (ip) injection of LPS (250 μg/kg, obtained from *Escherichia coli*, serotype 0111:B4; Sigma, St. Louis, MO) or saline. Four hours later, mice were tested in the water maze to assess spatial learning. The maze consisted of a circular tub (121 cm diameter) and a white circular platform (12.7 cm). The platform was submerged 1 cm under the surface of the water. The water was made opaque with white tempera paint to conceal the platform. Water temperature was maintained at 19 °C ± 1 °C throughout testing. Extra-maze cues were located around the maze. Mice received three trials (up to 60 s) per day from different start locations for five consecutive days. If a mouse failed to locate the platform within the 60 s they were gently guided to the platform. All mice remained on the platform for 10 s at the end of each trial. A video tracking system (Topscan, Clever Systems, Reston, VA) was used to measure distance swam (mm), latency to locate the platform (s), and swim speed (mm/s). A single 60 s probe trial was conducted approximately 2 h after the subject's last trial on day 5. The platform was removed and the number of times the animal crossed the original location of the platform as well as percent time spent in the target quadrant that contained the platform during testing was recorded by the tracking system.

2.4. Effect of DIO on LPS-induced cytokine expression

One month following behavioral testing mice received a second ip injection of LPS (250 μg/kg) or saline. Mice received the same treatment as they received during the behavioral testing. Four hours after LPS or saline administration mice were sacrificed and tissue was collected to assess changes in gene expression via quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) and protein levels of proinflammatory cytokines via ELISA.

2.4.1. Tissue collection

Mice were sacrificed by rapid decapitation. Blood was collected in heparin-treated vacutainer tubes (Becton-Dickinson, Rutherford, NJ), centrifuged (2000 rpm for 30 min at 4 °C), and plasma collected and stored at −70 °C until assayed. Hippocampal samples were dissected on a chilled glass dish and immediately placed into RNAlater solution (Qiagen, Valencia, CA) and stored at −20 °C until RNA isolation. The remaining brain (i.e., everything, but the hippocampus) was placed in a separate tube and snap frozen. Additionally, the spleen and gonadal adipose pads from the abdominal cavity were collected and snap frozen for analysis with ELISA and qRT-PCR, respectively.

2.4.2. qRT-PCR

Hippocampal samples were sonicated and RNA purified by the RNeasy Mini Kit (Qiagen, Valencia, CA), then quantified and assessed for purity using a Gen5 Epoch spectrophotometer (BioTek Instruments,