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Research report

Diet-induced obesity attenuates cytokine production following an immune challenge

Katherine M. Baumgarner, Sharay Setti, Carolyn Diaz, Alyssa Littlefield, Amanda Jones, Rachel A. Kohman*

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

HIGHLIGHTS

- Diet-induced obesity (DIO) blunts the immune response to endotoxin.
- DIO impairs spatial learning that appears unrelated to changes in inflammation.
- Hippocampal BDNF and synaptophysin levels are reduced in diet-induced obese mice.

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ABSTRACT

Obesity increases susceptibility for numerous diseases and neurological disorders including cardiovascular disease, metabolic syndrome, and dementia. One factor that may contribute to the increased risk for these conditions is the development of chronic inflammation. The current study evaluated whether diet-induced obesity (DIO) affects cognitive performance by increasing neuroinflammation and prolonging the behavioral and inflammatory response to an immune challenge. Adult male C57BL/6 J mice were fed a high-fat (60% fat) or control diet (10% fat) for 2 or 5 months. After consuming their respective diets for two months, sickness associated behaviors were assessed 4 and 24 h after a lipopolysaccharide (LPS) or saline injection. In a separate experiment, DIO and control mice were tested for spatial learning in the water maze and challenged with LPS one month later. Peripheral cytokine production was assessed in adipose and spleen samples and the neuroinflammatory response was assessed in hippocampal, cortical, and brain samples. DIO impaired acquisition of a spatial learning task relative to control mice. However, these deficits are unlikely to be related to inflammation as DIO showed no changes in basal cytokine levels within the periphery or brain. Further, in response to LPS DIO mice showed comparable or attenuated levels of the proinflammatory cytokines interleukin-1β and interleukin-6 relative to control mice. DIO also reduced hippocampal expression of brain-derived neurotrophic factor and the pre-synaptic marker synaptophysin. Presently, the data indicate that DIO suppresses aspects of the immune response and that cognitive deficits associated with DIO may be related to reduced neurotrophic support rather than inflammation.

1. Introduction

The prevalence of obesity continues to rise in industrial nations, particularly within the United States of America which has shown a doubling of obese adults over the last 40 years [1]. The implications for health care and an individual's overall health are vast as obesity is a risk factor for several life threatening and debilitating diseases including type II diabetes, cardiovascular disease, stroke, several types of cancer and Alzheimer's disease among other forms of dementia [1–3]. The precise mechanism through which obesity promotes these diseases is yet unknown, but substantial evidence has indicated the obesity-induced changes in immune function may contribute to the onset and/or progression of several diseases. Assessment of immune activity in obese individuals has shown deficits in the ability to defend against an infection as well as recover following an injury [4,5]. For instance, obesity is associated with slower wound healing time following surgery or a burn

* Corresponding author at: University of North Carolina Wilmington, 601 S. College Road, Wilmington, NC 28403-5612, USA. Tel.: +1 910 962 3494.
E-mail addresses: kbaumgarner@uscupstate.edu (K.M. Baumgarner), ses8535@uncw.edu (S. Setti), diazc@uncw.edu (C. Diaz), aml8440@uncw.edu (A. Littlefield), aj3294@uncw.edu (A. Jones), kohmanr@uncw.edu (R.A. Kohman).

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[4,6]. Additionally, obesity is associated with an increase in susceptibility to infection after surgery [5]. Collectively, findings indicate that obesity is associated with compromised immune function that increases susceptibility to disease and infection.

Animal models of diet-induced obesity (DIO) have confirmed that the immune system is affected by an organism's diet. There is evidence that DIO leads to a basal increase in proinflammatory cytokines within the brain and periphery, particularly interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) that were found to be elevated in the cortex and hippocampus [7,8]. However, this is not always observed as others report no changes in basal levels of proinflammatory cytokines following high-fat diet consumption [9,10]. Similar variation has been observed in response to an immune challenge with the bacterial endotoxin lipopolysaccharide (LPS), the data indicate that rats fed a high-fat diet show increased plasma levels of IL-6 and TNF-α and increased expression of IL-6 and interleukin-1β (IL-1β) within the hypothalamus [11]. In contrast a recent report found that macrophages isolated from mice fed a high-fat diet showed lower IL-1β expression compared to cell from control mice [9]. Additionally, LPS-induced expression of IL-6 and TNF-α as well as toll-like receptor-4 (TLR-4), the primary receptor for LPS, was attenuated in peripheral macrophages from rabbits fed a high-fat diet compared to those on a control diet [12]. Clearly, the data support an altered immune profile as a consequence of DIO, however, whether the resulting response is suppression or enhancement of the inflammatory response, as well as the variables that may contribute to developing one over the other has not been fully delineated.

Activation of the immune system leads to a host of behavioral changes, collectively termed sickness behaviors, as well as deficits in cognitive function [13,14]. Sickness behaviors are considered an adaptive behavioral response that reflects an altered motivational state that facilitate recovery from an infection [13]. Transient expression of these behaviors is generally beneficial whereas enhancing the degree or duration of these behaviors can be aversive and potentially indicative of an overactive inflammatory response. Current evidence indicates that DIO increases the fever response following an immune challenge as prior work has shown a higher and/or prolonged fever response in DIO animals following LPS administration [9,11]. Additionally, reports indicate that DIO increases sensitivity to reductions in social behavior and anorexia following an immune challenge [9,11,15]. Interestingly, the prolonged anorexic response in the DIO mice was not associated with an increase in peripheral cytokine levels though changes within the brain were not assessed. Further the anorexic response was induced at a lower dose of LPS than what was required to induce a fever response indicating a dissociation of the reactions [9]. Though additional work is needed to clarify the DIO associated changes in the behavioral response to an immune challenge the existing data indicate the behavioral reaction is altered, but whether the changes reflect an exaggerated cytokine response is unknown.

Several reports have demonstrated that DIO results in cognitive deficits including spatial learning and working memory impairments [7,8,16]. Though limited, a few studies suggest a potential connection between DIO associated cognitive deficits and the altered immune prolife [7,8]. For instance, Pistell et al. [7] report that middle-aged mice fed a high-fat diet made more errors during the acquisition phase of the Stone T-maze compared to control diet mice. Further, consuming the high-fat diet increased expression of the microglial cell marker Iba-1 and expression of IL-1β, IL-6 and TNF-α in the cortex. These data indicate that DIO-induced changes in the immune system may have relevance to cognitive function even in the absence of an immune challenge. However, further work is needed to determine whether enhancement of the inflammatory response is the underlying cause of the DIO-associated cognitive deficits.

The objective of the current study was to elucidate the potential role of inflammation in the development of cognitive deficits in a DIO model. Additionally, we assessed whether DIO exaggerates and/or prolongs the duration of sickness behavior following an immune challenge and whether the corresponding inflammatory response as measured by changes in central and peripheral cytokine levels basally and following immune activation are associated with enhanced behavioral and cognitive deficits.

2. Materials and methods

2.1. Animals

Subjects were 72 male C57BL/6J mice. For Experiment 1, mice ($N = 34$) bred in the University of North Carolina Wilmington (UNCW) animal facility were used, with breeding stock obtained from The Jackson Laboratory (Bar Harbor, Maine). In Experiment 2, nineteen Diet-Induced Obese (DIO) C57BL/6J male mice and nineteen C57BL/6J control male mice were purchased from The Jackson Laboratory at 14 weeks old. These mice were fed the same high-fat or control diets employed in the current study (see below) at Jackson Laboratory and were started on the diets at 6 weeks of age. 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 the UNCW.

2.2. Diets and housing

Mice were assigned to the high-fat diet (HF; Open source diets, D12492) or control diet (Open source diets, D12450B). The HF 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. All mice in Experiment 2 were group housed for one month, but had to be individually housed for the remainder of the study due to fighting within cages. All cages were given a wooden chew toy. In Experiment 1 mice were fed the HF or control diet for 2 months. In Experiment 2 mice were fed the HF or control diet for 5.5 months (2 months prior to arrival and an additional 3.5 months at UNCW).

2.3. Experiment 1: effect of DIO on the duration of LPS-induced sickness behavior

Thirty-four male mice were divided into the DIO or control diet group. After consuming the high-fat or control diet for two months mice received an intraperitoneal (i.p.) injection of LPS (250 μg/kg) or saline. To ensure no differences in body weight within a dietary condition prior to LPS or saline treatment mice were assigned to their treatment condition based on body weight (heaviest to lightest). The dose of LPS administered is a commonly used dose in this strain of mouse that is more resistant to the effects of LPS than other strains [17]. Further, the 250 μg/kg dose of LPS reliably induces sickness behavior and cognitive deficits in variety of behavioral tasks [18–22]. Three hours after an LPS or saline injection mice were tested in the open field test (described below) for changes in locomotor activity. Twenty-four hours after the LPS or saline injection a portion of the mice ($n = 18$) were retested in the open field to assess any enduring changes in locomotor activity. Tissue collection was conducted after behavioral testing either 4 h ($n = 16$) or 24 h ($n = 18$) after LPS or saline treatment. Assessment of LPS-induced changes in total body weight (i.e., grams lost or gained) was 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.