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Full-length Article

Voluntary exercise blocks Western diet-induced gene expression of the chemokines CXCL10 and CCL2 in the prefrontal cortex

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ABSTRACT

Obesity increases inflammation, both peripherally and centrally, and exercise can ameliorate some of the negative health outcomes associated with obesity. Within the brain, the effect of obesity on inflammation has been well characterized in the hypothalamus and hippocampus, but has been relatively understudied in other brain regions. The current study was designed to address two primary questions; (1) whether western diet (high fat/high sucrose) consumption would increase markers of inflammation in the prefrontal cortex and (2) whether concurrent voluntary wheel running would ameliorate any inflammation. Adult male mice were exposed to a western diet or a control diet for 8 weeks. Concurrently, half the animals were given running wheels in their home cages, while half did not have access to wheels. At the conclusion of the study, prefrontal cortex was removed and expression of 18 proinflammatory genes was assayed. Expression of a number of proinflammatory molecules was upregulated by consumption of the western diet. For two chemokines, chemokine (C-C motif) ligand 2 (CCL2) and C-X-C motif chemokine 10 (CXCL10), voluntary exercise blocked the increase in the expression of these genes. Cluster analysis confirmed that the majority of the tested genes were upregulated by western diet, and identified another small cluster of genes that were downregulated by either diet or exercise. These data identify a proinflammatory phenotype within the prefrontal cortex of mice fed a western diet, and indicate that chemokine induction can be blocked by voluntary exercise.

1. Introduction

Chronic consumption of a high fat/high sugar diet (the so-called Western Diet, WD) contributes to weight gain, and subsequent negative health outcomes, including increased adiposity, hyperlipidemia, and hyperglycemia. Additionally, obesity is associated with an increase in inflammation, both in the periphery (Xu et al., 2003) as well as in brain (De Souza et al., 2005). This peripheral inflammation is thought to play a causative role in at least some of the obesity-related health problems, such as insulin resistance (Xu et al., 2003), while central inflammation is believed to play a contributing role in obesity and related complications (Dorfman and Thaler, 2015; Zhang et al., 2008), through direct negative effects on neurons within circuits that regulate energy balance (Thaler et al., 2012).

Exercise is one of the most effective strategies to combat the adverse effects of weight gain. By increasing energy expenditure while maintaining or decreasing energy intake, weight loss is initiated, with a subsequent improvement in blood glucose and lipid levels, and a reduction in adipose tissue. Peripherally, weight loss can decrease inflammation. In a study following weight loss patients for 33 weeks, a decrease in circulating levels of adipokines and inflammatory markers was observed after weight loss (de Mello et al., 2008). In a rodent study in which a 3-week exercise

Abbreviations: CREB, cAMP response element binding protein; CNS, central nervous system; CCL2, chemokine (C-C Motif) ligand 2; CCR2, chemokine (C-C Motif) receptor 2; CTRL, control; CXCL10, C-X-C motif chemokine 10; COX-2, cyclooxygenase 2; EPM, elevated plus maze; EX RD, exercise-regular diet group; EX-WD, exercise-western diet group; GFAP, glial fibrillary acidic protein; GR, *glucocorticoid receptor*; HFD, high fat diet; IL-1β, interleukin 1 beta; IL-1, interleukin-1; IL-18, interleukin-18; IL-6, interleukin-6; Iba-1, ionized calcium-binding adapter molecule 1; *LPS*, lipopolysaccharides; MR, mineralocorticoid receptor; NOS2, nitric oxide synthase 2; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; IkBa, nuclear factor of kappa B inhibitor alpha; OF, open field; pCREB, phosphorylated cAMP response element binding protein; PFC, prefrontal cortex; PGES, prostaglandin E synthase; PGE2, prostaglandin E2; SED-RD, sedentary-regular diet group; SED-WD, sedentary-western diet group; SOCS3, suppressor of cytokine signaling 3; TLR4, toll-like receptor 4; TNF-a, tumor necrosis factor alpha; WD, Western diet.

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intervention followed a 12-week HFD exposure (and weight gain), exercise was found to reduce inflammation in skeletal muscle and liver, but interestingly, not adipose tissue (Jung et al., 2013). Inflammation in the brain may be similarly difficult to reverse. Following a 10 week exposure to HFD, hypothalamic inflammation was detectable, yet even after an 8 week return to chow feeding, adiposity levels normalized, but hypothalamic inflammation persisted (Wang et al., 2012).

With regard to HFD-induced inflammation in the brain, important regional differences exist. Inflammation associated with obesity and HFD has been consistently demonstrated in the hypothalamus (Thaler et al., 2012; Wu et al., 2014; Naznin et al., 2015), as well as in the hippocampus (Kang et al., 2016; Jeon et al., 2012). Fewer reports have examined the prefrontal cortex (PFC), and those that have generally failed to identify inflammation. In a two-week study with rats, in which animals were fed a cafeteria diet with or without the addition of sugar, there was no inflammation noted in cortex or hypothalamus (Beilharz et al., 2016). With a longer, 16 week HFD exposure in rats, increased expression of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) was detected in the hippocampus, but not in the cortex (Dutheil et al., 2015), while another study using 16 week exposure to HFD, found that levels of IL-1, IL-6, and TNF-α were not elevated in hypothalamus, hippocampus, nor cortex (Boitard et al., 2014). However, in a study which involved HFD feeding for 5 months in rats, an increase in prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) was detected in the cortex (Zhang et al., 2005). Similarly, 4 months of high fat feeding in mice led to an increase in IL-1 and TNF-α mRNA in cortex (Jayaraman et al., 2014), though, the precise region of cortex was not identified. It is difficult to draw definitive conclusions from the current literature, as a range of protocols (differing diets, length of exposure, species) makes direct comparison between studies impossible. Further, the majority of the experiments examined only a few proinflammatory cytokines.

Recent work has examined the synergistic effects of exercise and HFD. In a recent report, 20 weeks of high fat diet induced an increase in the numbers of microglia and astrocytes, and TLR4-related proteins in hippocampus, and treadmill exercise normalized these proinflammatory changes (Kang et al., 2016). Similar responses, where exercise reversed or normalized the HFD-driven inflammatory responses have also been seen in liver (Jeong et al., 2015), lung (Warren et al., 2015) and spinal cord (Yoon et al., 2016). For the PFC, the literature is limited and mixed. Microglia and astrocyte numbers in the cortex were normalized by treadmill exercise in the aforementioned study (Kang et al., 2016), however no other pro-inflammatory molecules were measured in cortex. In a recent report, treadmill running for 26 weeks was found to attenuate microglia activation in the arcuate nucleus, but had no effect on microglia in the PFC or hippocampus (Yi et al., 2012). Therefore, the primary goal of the present study was to examine how concurrent exposure to a western diet (high fat/high sugar) and voluntary wheel running would affect proinflammatory gene expression in the PFC using a broader panel of immune-related genes. Further, behavioral assays of anxiety and reward were completed, as there is some evidence that HFD exposure can be anxiogenic (Dutheil et al., 2015), and exercise can be anxiolytic (Dubreucq et al., 2015).

2. Methods

2.1. Materials and methods

2.1.1. Animals

C57BL/6 male mice (Charles River Laboratories International, Inc., Wilmington, MA) at 9 weeks of age were singly housed in standard polyethylene cages in an environmentally controlled room (22–24 °C) with a 12 h light/dark cycle. Mice were randomized to *ad lib* access to either standard control diet (#5001, 13% fat (35% from saturated fat), 29.8% protein, and 56.7% carbohydrate (3.8% from sucrose)) or a western-style diet (# D12079B, 17% protein, 41% fat (62% of dietary fat is saturated fat), 43% carbohydrate (29% is from sucrose), Research Diets, New Brunswick, NJ). Randomly chosen mice (n = 10/group) from each dietary group received *ad libitum* access to a voluntary running wheel (MiniMitter, Bend Oregon) in their home cages, generating 4 experimental groups; (1) control (CTRL) diet + sedentary (SED), (2) WD diet + SED, (3) CTRL diet + exercise (EX), and (4) WD + EX. Body weight was measured at the beginning (T0), weekly, and conclusion of the study (at sacrifice). Sucrose preference was measured at the beginning of the study and after 6 weeks on the diet/exercise protocol, and the other behavioral assays were completed at week 7. At the end of week 8, animals were euthanized by carbon dioxide overdose, followed by cervical dislocation; a method recommended by the Panel on Euthanasia of the American Veterinary Medical Association. Animal housing and behavioral experiments took place at University of California at Los Angeles. Flash frozen brain samples were shipped to University of Pennsylvania for further experimentation. Experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. The UCLA Chancellor's Animal Research Committee approved all procedures used in this study.

2.1.2. Elevated plus maze

The elevated plus maze (EPM) test to assess anxiety-like behavior was carried out according to a previously established protocol (Walf and Frye, 2007). The EPM apparatus was made of laminated wood and consisted of 2 opposing open arms (10 × 50 cm) and 2 opposing closed arms (10 × 50 cm with 30 cm high walls). The maze was placed 60 cm above the floor. White curtains surrounded the maze and behavior was recorded by an overhead video camera. Each mouse was placed in the middle of the maze facing the open arm that faced away from the experimenter. The video camera recorded the time each mouse spent in each of the arms over a period of 5 min. A closed arm entry was counted when the mouse placed all four paws in a closed arm. An open arm entry was recorded when the mouse placed all four paws in an open arm or when the mouse's hind-limbs were placed in the central area of the maze and both fore-limbs in an open arm with its head protruding into the open arm.

2.1.3. Open field test

The open field (OF) test to evaluate anxiety-like behavior was completed in a 1.2 m diameter circular tank with 60 cm walls. An inner circle, 80 cm in diameter, was marked on the tank floor to serve as a central arena. Testing began when each mouse was placed in the middle of the central arena and allowed to explore the field for 10 min. Mouse behavior was recorded by an overhead camera. Measurement included time spent and number of entries in central arena.

2.1.4. Sucrose preference

Consumption of sucrose engages the central reward circuitry, and sucrose preference is an indication of an animal's response to a naturally rewarding stimulus. Mice were individually housed (n = 10/group) in standard cages for 3 days with one 200 ml bottle of 4% sucrose solution (w/v), another 200 ml bottle of tap water and house chow available *ad libitum*. Sucrose (ml), water (ml), and food consumption (g), were measured and the placement of the bottles was reversed daily. Preference was calculated using the averages from the last 2 days only as follows: preference % = [(sucrose consumption/sucrose + water consumption) × 100].