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Full-length Article

# Chronic psychological stress and high-fat high-fructose diet disrupt metabolic and inflammatory gene networks in the brain, liver, and gut and promote behavioral deficits in mice

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ABSTRACT

The mechanisms underlying the association between chronic psychological stress, development of metabolic syndrome (MetS), and behavioral impairment in obesity are poorly understood. The aim of the present study was to assess the effects of mild chronic psychological stress on metabolic, inflammatory, and behavioral profiles in a mouse model of diet-induced obesity. We hypothesized that (1) high-fat high-fructose diet (HFHF) and psychological stress would synergize to mediate the impact of inflammation on the central nervous system in the presence of behavioral dysfunction, and that (2) HFHF and stress interactions would impact insulin and lipid metabolism. C57Bl/6 male mice underwent a combination of HFHF and two weeks of chronic psychological stress. MetS-related conditions were assessed using untargeted plasma metabolomics, and structural and immune changes in the gut and liver were evaluated. Inflammation was measured in plasma, liver, gut, and brain.

Our results show a complex interplay of diet and stress on gut alterations, energetic homeostasis, lipid metabolism, and plasma insulin levels. Psychological stress and HFHF diet promoted changes in intestinal tight junctions proteins and increases in insulin resistance and plasma cholesterol, and impacted the RNA expression of inflammatory factors in the hippocampus. Stress promoted an adaptive anti-inflammatory profile in the hippocampus that was abolished by diet treatment. HFHF increased hippocampal and hepatic *Lcn2* mRNA expression as well as LCN2 plasma levels. Behavioral changes were associated with HFHF and stress. Collectively, these results suggest that diet and stress as pervasive factors exacerbate MetS-related conditions through an inflammatory mechanism that ultimately can impact behavior. This rodent model may prove useful for identification of possible biomarkers and therapeutic targets to treat metabolic syndrome and mood disorders.



## 1. Introduction

Evolutionarily, acute stress and energetic balance are part of a successful pathway required for human survival; however chronic stress can modulate body composition by depleting non-adipose tissues or increasing adiposity via hypothalamus-pituitary-adrenal (HPA) axis alterations (Depke et al., 2008; Kuo et al., 2008). Accumulating evidence suggests that the interaction between stress exposure and diet imbalance can be an important connection between mood disorders and the global epidemic of obesity, but the mechanisms underlying this association are poorly understood (Basic et al., 2012; van Reedt Dortland et al., 2013; Blakemore and Buxton, 2014; Qi et al., 2014). Specific types of lipid

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contents in meals can stimulate the intestine–brain–liver neural axis that acts to regulate insulin sensitivity and circulating excess of nutrients (Wang et al., 2008). Furthermore, western diet associated with high-fat and high-fructose (HFHF) consumption can significantly impact fat storage contributing to the inflammatory status present in obesity (Appelhans et al., 2013; Bluher, 2013; Tchkonia et al., 2013).

Metabolic syndrome (MetS) is a set of diverse conditions including obesity, imbalance of cytokines, dyslipidemia, hepatic steatosis, and gut disorders, and is associated with an increased risk for psychiatric diseases (Capuron et al., 2008; Aschbacher et al., 2014; Bruce-Keller et al., 2014; Muller, 2014). Psychological stress, depression and negative life events are factors associated with increased risk of acute myocardial infarction (Xu et al., 2011). A relationship between anxiety, depression, and MetS in patients with type 2 diabetes (T2D) has been reported (Kahl et al., 2015). Furthermore, patients with chronic liver disease and depression disorders may be at higher risk for other medical complications and impaired quality of life (Sharma and Fulton, 2013; Davidson and Martin, 2014; Karagozian et al., 2014; Posadas-Romero et al., 2014).

Despite the accumulated evidence supporting a role for the immune system and cytokine signaling in the development of obesity and depression, it is not fully understood how HFHF, metabolic imbalance, and inflammation interact in obese states during a response to chronic stress (Ogden et al., 2012; Tekola-Ayele et al., 2013; Gallus et al., 2014; Wu et al., 2014). Chronic inflammation is a key factor in depressive and obesity states (Miller and Raison, 2015; Ramirez and Sheridan, 2016). Changes in central Lipocalin-2 (*Lcn2*) are associated with depression and anxiety (Mucha et al., 2011; Ferreira et al., 2013). The increased secretion of tumor necrosis factor (TNF) and other cytokines in the presence of HFHF diet or during psychological stress exposure can trigger additional changes and immune responses in metabolically active tissues such as gut, liver, and the adipose tissue that ultimately can affect the brain functions (Spruss et al., 2009; Jan et al., 2011; Milanski et al., 2012; Li et al., 2013a; Skrzypiec et al., 2013; Bailey, 2016).

The high degree of complexity related to obesity and stress described above demonstrates a critical need to use unbiased methodologies to assess how diet and stress can modulate centrally and peripherally metabolic, behavioral and immune outcomes in obesity. High-fat and high-carbohydrates consumption including fructose from beverages is much higher nowadays compared to past decades (Vos et al., 2008) and is associated with an increase in obesity (Dissard et al., 2013). Because diet and stress are two important exposures impacting human health in western society, a HFHF diet was used in this study to promote disturbances in metabolic profiles associated with obesity. We hypothesized that HFHF diet and chronic psychological stress would synergize to adversely mediate the impact of peripheral inflammation on the central nervous system and result in behavioral dysfunction. Secondly, we also expect an interaction between HFHF consumption and chronic psychological stress that could directly affect metabolic factors implicated in MetS, such as insulin resistance and dyslipidemia, both of which are often associated with mood disorders. For this purpose, HFHF intake was combined with two weeks of chronic psychological predatory stress to assess metabolic, inflammatory, and behavioral responses in wild type C57Bl/6 mice. MetS-related conditions were assessed using an untargeted metabolomics approach, and structural and immune changes in the gut and liver were evaluated. The results from this will assist in developing a well-characterized, animal model for screening interventions developed to prevent or treat MetS in westernized societies where chronic stress is an endemic problem.

## 2. Materials and methods

### 2.1. Animals

Male C57Bl/6 mice (n = 60), 7 weeks of age, were acquired from The Jackson Laboratory (Bar Harbor, ME). Each animal was singly housed in a colony room with a 12/12 h light-dark cycle (lights off at 7:00 p.m.) at an ambient temperature of 22–23 °C. Additional female C57Bl/6 mice, 4 weeks of age (n = 16), were acquired from the Jackson Laboratory for the Female Urine Sniffing Test (FUST). Adult male Long Evans rats (n = 30; 400+ grams upon arrival) purchased from Harlan (Indianapolis, IN, USA) served as stimulus (predator) animals. Rats were pair-housed and had free access to standard lab chow (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA) and water. Rats were housed in a separate room from mice and maintained on a 12/12 h reverse light/dark cycle (lights off at 07:00 a.m.) at an ambient temperature of 22–23 °C. Upon arrival at the animal facility, mice and rats were allowed seven days of acclimatization prior to experimentation during which they were all fed standard rodent diet (Lab Diet 5001). All studies and animal protocols were approved and guided by the Institutional Animal Care and Use Committee at Emory University (#2002041).

### 2.2. High-fat high-fructose (HFHF) diet

Mice received regular drinking water and standard chow diet (13% kcal from fat, #7001, Harlan-Teklad, Madison, Wisconsin) or high-fat diet (42% kcal from fat, TD.88137, Harlan-Teklad, Madison, Wisconsin) plus 30% (wt/vol) fructose solution (Sigma-Aldrich #F012, Sigma-Aldrich, St. Louis, MO) available *ad libitum* throughout the duration of the study (Fig. 1). The combination of high fat diet (Tables S1, S2) and fructose was chosen because it mimics the diet in developed countries with high rates of obesity and MetS

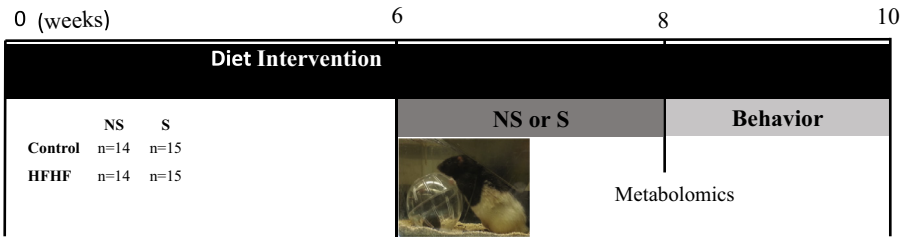


**Fig. 1.** Schematic and study timeline. Control groups consumed standard chow diet (4% kcal from fat and water) and HFHF groups consumed high-fat diet (42% kcal from fat + 30% fructose w/v) for 10 weeks. Groups (n = 14–15): Control diet/No Stress (Control NS), Control diet/Stress (Control S), High-fat high-fructose/No stress (HFHF NS) and High-fat high-fructose Stress (HFHF S). Predatory stress (PS): 15 min of daily predatory stress OR no stress (NS) for 2 weeks. Behavior: Marble burying and female urine test were performed to assess anxiety and anhedonia-like behavior; sociability test was used to evaluate social interaction. At 10 weeks mice were euthanized, and tissues were harvested.