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Original experimental

The impact of the Standard American Diet in rats: Effects on behavior, physiology and recovery from inflammatory injury

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HIGHLIGHTS

- Chronic exposure to the Standard American Diet (SAD) leads to systemic inflammation.
- SAD consumption resulted in elevated blood glucose, and fat mass.
- Neither spontaneous pain nor open field locomotion was affected by the SAD.
- Recovery from inflammatory injury is prolonged by consumption of the SAD in rats.
- The SAD resulted in greater microglial activation in the spinal cord.

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ABSTRACT

Background and aims: Obesity is a significant health concern in the Western world and the presence of comorbid conditions suggests an interaction. The overlapping distributions of chronic pain populations and obesity suggests that an interaction may exist. Poor quality diet (high carbohydrates, saturated fats, omega-6 polyunsaturated fatty acids) can lead to increased adiposity which can activate immune cells independent of the activating effect of the diet components themselves. This dual action can contribute to chronic inflammation that may alter susceptibility to chronic pain and prolong recovery from injury. However, traditional examinations of diet focus on high-fat diets that often contain a single source of fat, that is not reflective of an American diet. Thus, we examined the impact of a novel human-relevant (high-carbohydrate) American diet on measures of pain and inflammation in rats, as well as the effect on recovery and immune cell activation.

Methods: We developed a novel, human-relevant Standard American Diet (SAD) to better model the kilocalorie levels and nutrient sources in an American population. Male and female rats were fed the SAD over the course of 20 weeks prior to persistent inflammatory pain induction with Complete Freund's Adjuvant (CFA). Mechanical and thermal sensitivity were measured weekly. Spontaneous pain, open field locomotion and blood glucose levels were measured during diet consumption. Body composition was assessed at 20 weeks. Following full recovery from CFA-induced hypersensitivity, blood was analyzed for inflammatory mediators and spinal cords were immunohistochemically processed for microglial markers.

Results: Chronic consumption of the SAD increased fat mass, decreased lean mass and reduce bone mineral density. SAD-fed rats had increased leptin levels and pro-inflammatory cytokines in peripheral blood serum. Following CFA administration, mechanical sensitivity was assessed and recovery was delayed significantly in SAD-fed animals. Sex differences in the impact of the SAD were also observed. The SAD increased body weight and common T-cell related inflammatory mediators in female, but not male, animals. In males, the SAD had a greater effect on bone mineral density and body composition. Long-term consumption of the SAD resulted in elevated microglial staining in the dorsal horn of the spinal cord, but no sex differences were observed.

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Conclusions: We demonstrate the negative effects of an American diet on physiology, behavior and recovery from injury. SAD consumption elevated pro-inflammatory mediators and increased microglial activation in the spinal cord. While there were sex differences in weight gain and inflammation, both sexes showed prolonged recovery from injury.
Implications: These data suggest that poor quality diet may increase susceptibility to chronic pain due to persistent peripheral and central immune system activation. Furthermore, consumption of a diet that is high in carbohydrates and omega-6 polyunsaturated fatty acid is likely to lead to protracted recovery following trauma or surgical procedures. These data suggest that recovery of a number of patients eating a poor quality diet may be expedited with a change in diet to one that is healthier.

Obesity in the Western world as a result of poor quality diet is a significant health concern. Aside from known cardiovascular and metabolic effects (i.e., Type 2 diabetes mellitus), poor quality diet is a risk factor for chronic pain [1–4]. While added weight puts additional strain on joints, the presence of pain in non-weight-bearing joints [5] suggests another underlying cause. We believe that chronic inflammation due to poor quality diet and increased adiposity interact to result in a chronic pro-inflammatory state that leaves individuals more susceptible to chronic pain. In fact, risk of chronic pain was associated with elevated body mass index (BMI) and inflammatory biomarkers in a large sample in Norway [6].

The immune system is recognized as playing an active role in inflammation and chronic pain [7–14]. Thus, immune system activation is likely to result in various chronic conditions, including pain. With respect to diet, saturated fatty acids activate toll-like receptor 4 (TLR4) [15,16], omega-6 polyunsaturated fatty acids (n-6 PUFAs) are precursors for prostaglandins [17] and carbohydrates elicit an insulin response and can result in significant oxidative stress [18–20]. A poor quality diet often leads to excess adipose tissue and can result in infiltration of pro-inflammatory macrophages [21,22] and the release of the adipokine leptin. Leptin has been shown to activate the innate immune system to prompt an inflammatory response [23]. Thus, poor quality diet and the resulting adiposity can contribute to an inflammatory state that is seen in obese humans [24–27] and animals [28–34]. This chronic inflammation is likely to alter pain.

Recently we reported that long-term consumption of a Total Western Diet (TWD) altered sensitivity to thermal and mechanical stimuli and resulted in a pro-inflammatory state in male mice. Additionally, the TWD prolonged recovery from injury following intraplantar Complete Freund's Adjuvant (CFA) [34]. These data support the notion that a Western diet results in a chronic pro-inflammatory state and may increase susceptibility to chronic pain. However, whereas the TWD was based on the median values of the National Health and Nutrition Examination Survey (NHANES), we wished to develop an animal model protocol to study the pain and inflammation responses to the standard American diet. Thus, we developed a diet that was based on the kilocalorie intake and food sources from a standard American diet [35] to be tested in male and female rodents. Here, male and female rats consumed our novel Standard American Diet (SAD) over the course of 20 weeks prior to induction of persistent inflammatory pain with CFA. Mechanical and thermal sensitivity was measured as well as physiological assessments following recovery from injury. The decision to use rats was based on our previous experience with harvesting brains and spinal cords from rats in order to perform immunohistochemistry and to test for the generalizability of the phenomena.

1. Methods

1.1. Animal subjects

Male ($n = 18$) and female ($n = 17$) Sprague Dawley rats (Charles River labs, 150–200 g at onset) were housed in groups of 2–3 per cage (10.25″ $W \times 18.75$″ $L \times 8$″ H), under a 12 h reversed light cycle (lights on at 21:00 h) and provided with standard chow (Harlan Teklad, Indianapolis, IN) and sterile water. Animal health was assessed daily for the duration of the experiment. Following the rat housing density policy, once rats reached a weight of 400 g, they were separated into additional cages. All rats were fed standard chow for two weeks before introduction to the experimental diet. After obtaining stable baseline measures, rats were assigned to either ad libitum regular chow (REG, NIH-31 Envigo, Madison, WI; $n = 18$, 9 males) or provided with our novel Standard American Diet (SAD, TD.140536, Envigo, Madison, WI; $n = 17$, 9 males) and a 20% solution of formula 55 high fructose corn syrup (Golden Barrel; Honey Brook, PA) to model sweetened beverage intake. The fructose solution was administered ad libitum via bottles. The SAD differs from commercially available high-fat diets in that it contains a human-relevant omega-6 to omega-3 PUFA ratio of 16:1 [36], has refined white flour (Gold Medal, General Mills, Minneapolis, MN), sugar and added trans fatty acids to mimic human intake [37]. These changes were chosen to make the diet more translatable to poor quality human diets, but based on the available Total Western Diet [38]. The SAD is composed of 16.7% protein (15.4% kcal), 52.9% carbohydrates (49.0% kcal), and 17.1% fat by weight (35.6% kcal). In contrast to traditional high-fat diets wherein there is a single source of fat, the SAD had multiple sources of fat, much like a human diet. The composition of the SAD is shown in Table 1. Table 2 lists the top 13 ingredients in the REG and SAD in descending order for comparison of components. The diet exposure lasted for 20 weeks prior to CFA treatment, for a total of 26 weeks. All of the animals used in the present study have been obtained, housed, cared for and used in accordance with the University of Alabama at Birmingham Institutional Animal Care and Use Committee guidelines.

1.2. von Frey testing

Rats were placed in individual transparent Plexiglas cubicles (custom made) atop a perforated metal floor and habituated for 20 min prior to behavioral testing. Nylon monofilaments (Stoelting Touch Test Sensory Evaluator Kit #2 to #9; ∼2.0–60 g; Wood Dale, IL) were firmly applied to the plantar surface of the hind paw. Both paws were tested and data presented represent an average for the two paws with the exception of the CFA data in which the data represent the ipsilateral, injected paw. The 50% withdrawal thresholds were estimated using the up-down method of Dixon [39]. Testing for mechanical sensitivity was performed at baseline and once per week during diet exposure.

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