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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.

Table 1
Composition of the Standard American Diet. Amounts are given as dry weight where appropriate.

Ingredient	g/kg
Casein	141.0
L-cystine	1.8
White flour	410.0
Corn starch	25.0
Maltodextrin	60.0
Sucrose	126.8
Soybean oil	17.3
Corn oil	10.6
Cottonseed oil	20.9
Lard	18.0
Beef tallow	16.0
Anhydrous milkfat	23.3
Vegetable shortening	59.5
Cholesterol	0.4
Cellulose	19.0
Mineral mix	35.0
Sodium chloride	4.0
Vitamin mix	10.0
Choline bitartrate	1.4
Antioxidant	0.03

Table 2
Top 13 ingredients in the REG and SAD in descending order of inclusion.

REG	SAD
Ground wheat	Bleached white flour
Ground corn	Casein
Ground oats	Sucrose
Wheat middlings	Maltodextrin
Fish meal	Hydrogenated vegetable shortening
Soybean meal	Mineral mix
Alfalfa meal	Corn starch
Corn gluten	Anhydrous milkfat
Soybean oil	Cottonseed oil
Dicalcium phosphate	Cellulose
Brewers dried yeast	Lard
Calcium carbonate	Soybean oil
Iodized salt	Beef tallow

1.3. Radiant heat paw withdrawal testing

A modified Hargreaves' method [40] was used to test thermal sensitivity. Rats were placed individually in transparent Plexiglas cubicles placed on an elevated glass table with a portable radiant heat source (IITC Inc.; Woodland Hills, CA) underneath. The heat source was focused on the ventral surface during testing. Both paws were tested and data presented represent an average of the two hind paws. The withdrawal latency was defined as the time to withdraw the hind paw from the heat source with a maximum of 40 s as a cut-off point.

1.4. Spontaneous pain: Rat Grimace Scale

To quantify spontaneous pain in rats, the Rat Grimace Scale [41] was used. Rats (two at a time) were placed in transparent Plexiglas cubicles ($21 \times 10.5 \times 9$ cm high) with a removable stainless steel wall to separate them. A digital video camera was placed on either side of the cubicle in order to capture a clear headshot. Rats were videotaped using high-resolution (408×3456, 16MP) video cameras (Polaroid iD975 Dual Shot) for 20 min. Spontaneous pain was measured every 4 weeks by blinded observers.

1.5. Open field

To measure locomotor activity, distance traveled was quantified using video tracking software (Stoelting ANY-maze Version 4.99). Rats were placed individually into custom made Plexiglas cubicles ($12'' W \times 12'' L \times 12'' H$) for 15 min. Open field testing was performed every 4 weeks.

1.6. Blood glucose

Blood was sampled via tail vein puncture and a standard glucometer (TRUEresult, NIPRO Diagnostics, Fort Lauderdale, FL) was used to measure blood glucose levels. Rats were fasted for 6 h prior to blood collection. Glucose levels were assessed at weeks 8, 12, 16 and 20. Blood samples were conducted at the same time for each time point to rule out time-of-day effects.

1.7. Dual energy X-ray absorptiometry (DXA)

At week 20, all rats underwent a DXA scan to assess fat, soft-lean tissue and bone in vivo. Rats were sent to the Small Animal Phenotyping Subcore (Nutrition and Obesity Research Center) at the University of Alabama at Birmingham where the Lunar PIXImus and Norland pDEXA Sabre small animal DXA was utilized. Animals were anesthetized (isoflurane) and placed prostrate in the imaging area with the front and back legs extended away from the body. Each scan lasted approximately five minutes.

1.8. Persistent inflammation

After 20 weeks of SAD exposure, mechanical sensitivities were tested and rats were injected with CFA (MP Biomedicals, Solon, OH; 100%, in a 150 μl injection volume) into the left hind paw. Rats were retested 24 h later to confirm the presence of mechanical allodynia and then on days 3, 5, 8, 10, 12, 15, 17, 19, 22, 26, 29, 31, 33, 36 and 40 following CFA injection. Groups were considered to have returned to their baseline level of sensitivity once the group mean was at least 90% of the pre-CFA withdrawal threshold.

1.9. Immunohistochemistry

After 26 weeks of diet exposure and full recovery from CFA-induced hypersensitivity (as assessed by von Frey mechanical sensitivity testing), rats were anesthetized with ketamine (80 mg/kg) and xylazine (5 mg/kg) for transcardial perfusion using phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Whole blood was taken directly from the heart for further analysis. Perfusion-fixed spinal cords were extracted and stored in 4% paraformaldehyde for 3 days. Tissue was then immersed in graded sucrose (5% for 24 h, 10% for 24 h, 15% for 24 h, and 20% for 24 h) and embedded in a 2:1 ratio of Tissue-Tek Optimal Cutting Temperature compound (Fisher Scientific) and 20% sucrose, and frozen. Spinal cord sections (30 μm; L4–L6) were prepared on the Leica CM1850 cryostat slicer (Leica Microsystem, Buffalo Grove, IL) and mounted on gelatin-coated slides for a subsection of rats (3–4/condition). Sections were blocked with 3% bovine serum albumin (BSA) and 10% serum (from the host of the secondary antibody) for 1 h. For antigen retrieval, sections were incubated in citrate buffer for 10 min at 37 °C followed by 20 min at room temperature. Tissue was then incubated with primary antibodies (rabbit anti-Iba-1, 019-19741-SAR 6502, Wako; 1:500; 24 h in 5% serum in 0.3% Triton X PBS with 3% BSA) and secondary antibodies (Alexa fluor 488 donkey anti-rabbit, 439378, Invitrogen; 1:1000; 1 h in 5% serum in 0.3% TritonX PBS with 3% BSA). Sections were coverslipped with ProLong Gold anti-fade mounting media (Invitrogen). Four representative images were captured on a Leica confocal microscope for each rat and quantified using ImageJ software. Images were converted to 8-bit and thresholds adjusted to display proper contrast. The region of interest was the dorsal horn of the spinal cord and number of particles was analyzed using a custom macro that identified cells within a specific range of area (microns^2). In each case,

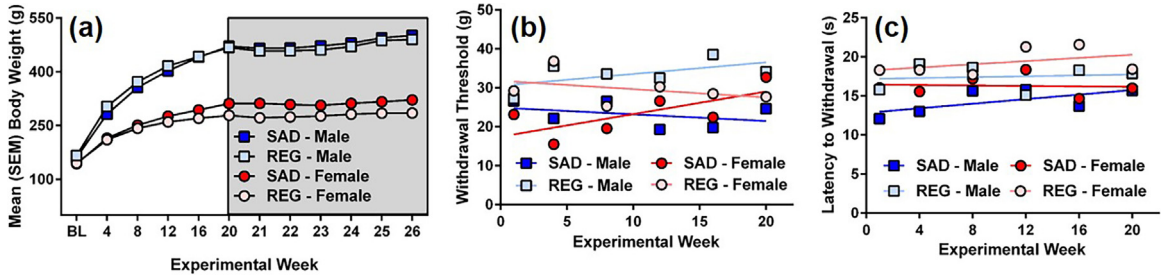

Fig. 1. Weight changes and behavioral sensitivity during diet. (a) Body weights (g) for male (squares) and female (circles) rats fed the SAD (dark shades) or REG diet (light shades) every 4 weeks. The shaded area defines the period after CFA injection and includes weekly measures. (b) Mechanical thresholds over the course of the 20-week exposure for SAD-fed male (dark blue squares) and female (dark red circles) and REG-fed male (light blue squares) and female (light red circles) rats expressed as 50% withdrawal threshold (in grams). (c) Radiant heat paw-withdrawal thresholds (20% full intensity, expressed as latency to withdraw from light source) for rats over the 20-week exposure period. All data are expressed as mean ± SEM.

number of Iba-1 positive cells was counted in the gray matter of the dorsal horn on the left and right sides (L4–L6 region).

1.10. Blood analyses

After sacrifice, cardiac blood was allowed to sit at room temperature for 20 min and then was spun at 4 °C for 20 min to isolate serum. All perfusions were carried out in the morning (0800–1200 h).

1.11. Serum leptin

Blood samples were sent to the Human Physiology Core (Diabetes Research Center) at the University of Alabama at Birmingham. Leptin concentrations were determined in duplicate using a Millipore rat leptin ELISA kit. The intra-assay coefficient of variation was 6.66% and the detection limit was 0.2 ng/ml.

1.12. Inflammatory cytokines

Blood samples were analyzed by the Human Physiology Core using a rat multiplex cytokine assay for interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-13 (IL-13), interferon gamma (IFN-γ), chemokine (C-X-C motif) ligand 1 (CXCL1), and tumor necrosis factor alpha (TNFα), as per manufacturer instructions (MesoScale Discovery).

1.13. Triglycerides

Blood samples were analyzed by the Human Physiology Core using the Sirrus Chemical Analyzer (Stanbio; Boerne, TX).

1.14. Statistical analysis

Data are shown as means ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was performed for body weight, blood glucose, Iba-1 positive cell counts and fat mass and a two-way ANOVA was performed for spontaneous pain and open field data. ANOVA results were followed up with appropriate post hoc tests. Linear regression was performed for the mechanical and thermal sensitivity data. The slopes of the 2 lines were compared with 0 (no change from baseline) and with each other. A repeated measures ANOVA was performed on allodynia measures following CFA using Sex as a variable. In addition, analysis of covariance (ANCOVA) was used to determine the effects of Diet (independent variable) and Fat Mass (covariate) on leptin levels in separate analyses. A multivariate ANOVA was used to analyze the cytokine levels.

2. Results

Due to our previous research demonstrating the existence of sex differences [10,11], all analyses were carried out with Sex as a variable. Where significant, or if there was a trend, each sex was further analyzed separately.

2.1. Body weight

Initially, the goal of this study was to determine whether this novel diet influences peripheral metabolic measures such as body weight. For the weeks prior to CFA induction, there were significant main effects of Sex ($F(1,31) = 499.616$, $p < 0.001$), and Time ($F(20,620) = 2475.088$, $p < 0.001$) on body weight. There were significant interactions of Time and Diet ($p < 0.001$), Time and Sex ($p < 0.001$), Diet and Sex ($p < 0.05$) and a three-way interaction of Time, Diet and Sex ($p < 0.001$) (Fig. 1a).

In females there was a significant main effect of Time ($F(26,390) = 356.568$, $p < 0.001$) and Diet ($F(1,15) = 9.497$, $p < 0.05$). There was a significant Time by Diet Interaction ($F(26,390) = 9.52$, $p < 0.05$). In males there was a significant main effect of Time ($F(26,390) = 1190.078$, $p < 0.001$), but no main effect of Diet ($p > 0.05$). There was a significant Time by Diet Interaction ($F(26,390) = 4.736$, $p < 0.05$). Diet consumption increased body weight in female rats, but had no significant effect on the male rats. These data support the notion that sex differences exist in the metabolism of diet and deposition of adipose tissue.

2.2. Sensitivity thresholds

No sex differences were found for mechanical (Fig. 1b) or thermal sensitivity (Fig. 1c). Following linear regression, SAD increased mechanical thresholds over 20 weeks of diet exposure ($F(1,19) = 11.64$, $p < 0.01$), relative to baseline thresholds. Thresholds for the REG group did not differ from zero ($p > 0.05$). The slopes for the diets were not significantly different from one another ($p > 0.05$). Thresholds for thermal sensitivity increased over 20 weeks for SAD ($F(1,19) = 7.742$, $p < 0.05$) and REG ($F(1,19) = 8.648$, $p < 0.01$). The slopes for the diets were not significantly different ($p > 0.05$).

2.3. Spontaneous pain

There were no differences in spontaneous pain in any group ($p > 0.05$; Fig. 2). Baseline images were lost due to technical malfunction.

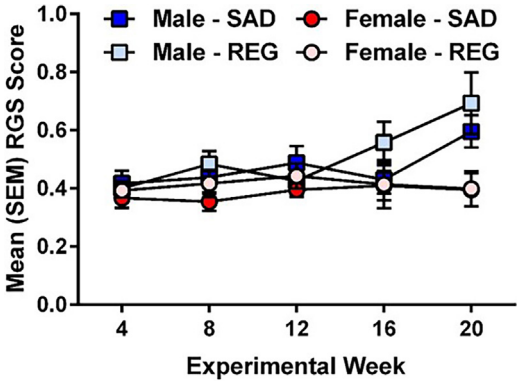

Fig. 2. Spontaneous pain at experimental weeks 4, 8, 12, 16 and 20 of diet consumption as measured by Rat Grimace Scale scores for female (red hues, circles) and male (blue hues, squares) rats fed the REG (light colors) or SAD (dark colors). All data are expressed as mean ± SEM.

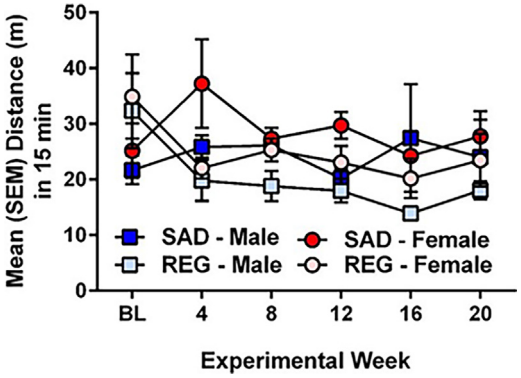

Fig. 3. Locomotor activity at baseline and weeks 4, 8, 12, 16 and 20 of diet consumption for female (red hues, circles) and male (blue hues, squares) rats fed the REG (light colors) or SAD (dark colors). All data are expressed as mean ± SEM distance traveled (m) in 15 min.

2.4. Open field

There was a Time by Diet interaction ($F(5,155) = 3.186$, $p < 0.01$) and a trend towards a main effect of Sex ($p = 0.06$). No other differences were found in locomotor activity (p's > 0.05; Fig. 3).

2.5. Blood glucose

A diet high in carbohydrates is expected to increase blood glucose levels acutely and can lead to the development of Type 2 Diabetes mellitus over time. Here, there was a significant main effect of Time ($F(3,90) = 29.349$, $p < 0.001$) and Diet ($F(1, 30) = 23.084$, $p < 0.001$) but there was no effect of Sex ($p > 0.05$). There was a significant Time by Sex by Diet interaction ($F(3, 90) = 5.713$, $p \leq 0.001$) (Fig. 4). Blood glucose levels were significantly elevated in SAD-fed rats at 8 weeks ($F(1,33) = 7.565$, $p < 0.05$), 12 weeks ($F(1,32) = 7.794$, $p < 0.01$), 16 weeks ($F(1,33) = 4.906$, $p < 0.05$) and 20 weeks ($F(1,33) = 25.297$, $p < 0.001$).

2.6. Immunohistochemistry

To explore a central effect of the SAD, we examined microglial immunoreactivity in the dorsal horn of the spinal cord at the level corresponding to our mechanical and thermal sensitivity testing. There was no difference in cell counts between ipsilateral and contralateral sides; therefore the data presented represent the average of the two sides. There was a main effect of Diet ($F(1,9) = 32.343$, $p < 0.001$) in which animals on the SAD had significantly more Iba-1

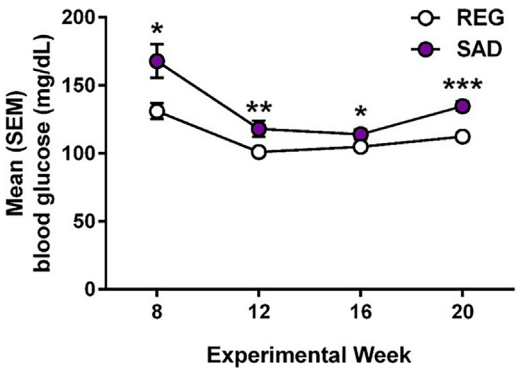

Fig. 4. Blood serum glucose levels (mg/dL) following 8, 12, 16 and 20 weeks of diet consumption for REG- (white) or SAD-fed (purple) rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. All data are expressed as mean ± SEM.

stained cells compared to REG animals (Fig. 5; REG, $n = 7$; SAD, $n = 6$; 3 males in each condition; 4 sections/animal). There was no significant effect of Sex ($p > 0.05$) or Sex by Diet interaction ($p > 0.05$). Thus, chronic consumption of the SAD resulted in elevated microglial recruitment and/or activation – evidence of an activated immune system.

2.7. Body composition and physiology

2.7.1. Body fat

At the time of the scan, body weight was analyzed. There was a significant main effect of Diet ($F(1,31) = 6.806$, $p < 0.05$) and Sex ($F(1,31) = 476.256$, $p < 0.001$). To account for different body weights between groups, fat and lean mass weights were converted to percent of total body mass for comparison. When percent fat mass was analyzed (Fig. 6a), there was a significant main effect of Sex ($F(1,31) = 7.868$, $p < 0.01$) and of Diet ($F(1,31) = 73.463$, $p < 0.001$). Similar effects were seen for percent lean mass [Sex ($F(1,31) = 8.176$, $p < 0.01$), Diet ($F(1,31) = 74.647$, $p < 0.001$)]. Following diet exposure, female rats that consumed the SAD had increased percent fat mass ($F(1,15) = 56.014$, $p < 0.001$) and lower percent lean mass ($F(1,15) = 58.081$, $p < 0.001$) compared to REG-fed females. Male rats on the SAD had increased percent fat mass compared to REG ($F(1,16) = 26.807$, $p < 0.001$) with a concomitant decrease in percent lean mass ($F(1,16) = 26.929$, $p < 0.001$). For both sexes, the SAD increased percent of body weight that was fat and decreased lean mass.

2.7.2. Serum leptin

There was no main effect of Sex when serum leptin levels were analyzed. However, due to the significant Sex effect when analyzing percent fat mass, we opted to consider each sex separate for this analysis. For both male ($F(1,16) = 7.21$, $p < 0.05$) and female ($F(1,15) = 5.776$, $p < 0.05$) rats there was a significant Diet effect. Rats that consumed the SAD had higher serum leptin levels (Fig. 6b). An analysis of covariance (ANCOVA) was conducted using fat mass as the covariate. For female rats, there was a main effect of Fat Mass ($F(1,14) = 10.934$, $p < 0.01$), but no effect of Diet ($p > 0.05$). For male rats, there was a similar main effect of Fat Mass ($F(1,15) = 16.467$, $p < 0.001$) and no effect of Diet ($p > 0.05$). Together, these data suggest that the difference in leptin could be accounted for by adipose tissue differences; greater fat mass resulted in greater circulating leptin.

2.7.3. Triglycerides

There was a significant main effect of Diet ($F(1,31) = 3.336$, $p < 0.05$) but no effect of Sex on triglycerides ($p > 0.05$, data not

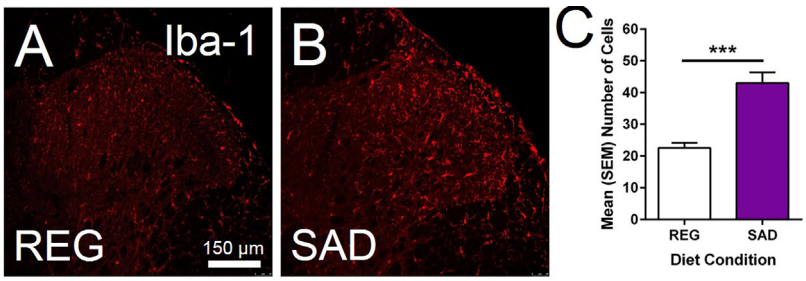

Fig. 5. Effects of the SAD on Iba-1 immunoreactivity in rat spinal cord dorsal horn. (a) Representative spinal dorsal horn image for REG-fed (b) and SAD-fed rats. (c) Quantification of number of cells in each representative slide ($n = 7$, REG; $n = 6$, SAD; 3 males in each condition; 4 sections/animal). ***$p < 0.001$. All data are expressed as mean ± SEM.

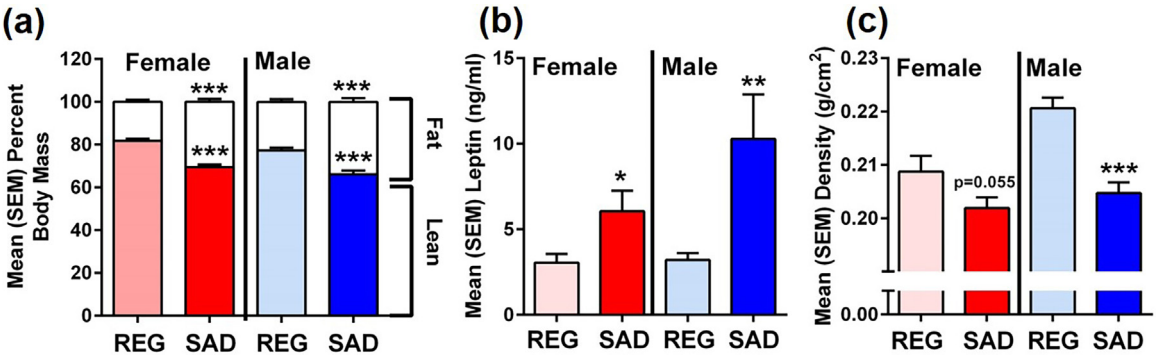

Fig. 6. Effects of the SAD on fat physiology and bone mineral density in rats. (a) Percent of body weight composed of fat (top, white portion) and lean mass (bottom, colored portion) in rats, measured by DXA. (b) Leptin levels in serum (ng/ml) following 26 weeks of diet exposure and recovery from inflammatory injury. (c) Bone mineral density (g/cm^2) following 20 weeks of diet exposure as measured by DXA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. All data are expressed as mean ± SEM. Female animals in shades of red, males in shades of blue with dark shades reflecting SAD-fed animals.

shown). The SAD increased triglyceride levels in both male and female rats.

2.7.4. Bone mineral density

There was a significant main effect of Sex ($F(1,31) = 8.184$, $p < 0.05$) for bone mineral density. Males that consumed the SAD had significantly lower bone mineral density ($F(1,16) = 23.661$, $p < 0.01$). There was no difference in bone mineral density in female rats, though there was a trend ($p = 0.055$) (Fig. 6c).

2.8. Inflammatory cytokines

Main effects of Sex were found for many cytokines (p's < 0.05), therefore data were analyzed by Sex (Table 3). Female rats on the SAD had significantly higher IL-10 ($F(1,15) = 7.596$, $p < 0.05$), IL-13 ($F(1,15) = 8.317$, $p < 0.05$), IL-4 ($F(1,15) = 6.013$, $p < 0.05$), TNF-α ($F(1,15) = 4.945$, $p < 0.05$), IFN-γ ($F(1,15) = 11.307$, $p < 0.01$) and IL-6 ($F(1,15) = 11.902$, $p < 0.01$) compared to REG-fed rats. Male rats on the SAD had significantly higher IL-1β ($F(1,16) = 11.597$, $p < 0.01$) compared to REG males.

2.9. Persistent inflammation

Following induction of persistent inflammation, we felt that it was important to examine recovery from injury as a diet-related outcome measure. Therefore, we measured mechanical sensitivity in rats following CFA. As above, animals were considered recovered when group thresholds were at least 90% of baseline mechanical thresholds. There was a significant main effect of Time ($F(15,300) = 11.248$, $p < 0.001$) and Diet ($F(1,20) = 5.388$, $p < 0.05$) and a Time by Diet interaction ($F(15,300) = 1.740$, $p < 0.05$). There was no main effect of Sex ($p > 0.05$) (Fig. 7a).

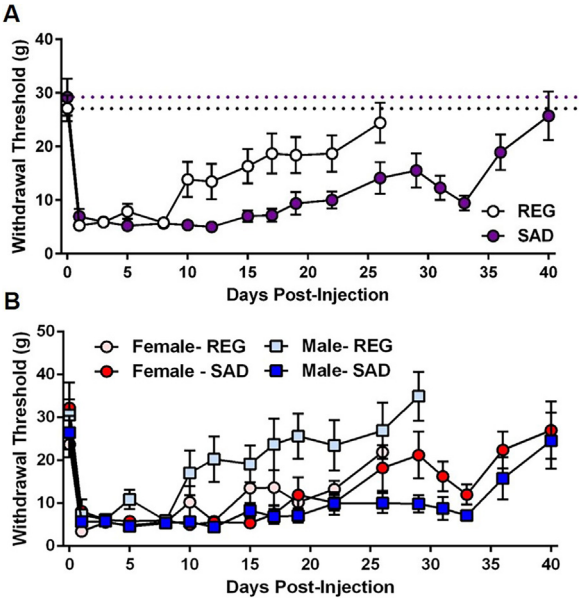

Fig. 7. Allodynia following CFA administration is prolonged with SAD consumption. (a) Allodynia following CFA administration as measured by 50% withdrawal threshold for REG-fed (white circles) and SAD-fed (purple circles) rats. Dotted lines represent the baseline sensitivity. Both groups were considered as returned to baseline sensitivity when group thresholds achieved 90% of pre-CFA thresholds. Both lines stop at the point of return to baseline sensitivity. (b) Allodynia following CFA administration separated by Sex and Diet measured by 50% withdrawal threshold for female (red hues, circles) and male (blue hues, squares) rats fed the REG (light colors) or SAD (dark colors). Groups were considered as returned to baseline sensitivity when group thresholds achieved 90% of pre-CFA thresholds. All lines stop at the point of return to baseline sensitivity. All data are expressed as mean ± SEM.

Table 3
Levels of pro- and anti-inflammatory mediators in serum (pg/ml) following 26 weeks of diet exposure. All data are expressed as mean ± SEM.

Analyte	Male			Female		
	REG	SAD	*p* value	REG	SAD	*p* value
IL-1β	86.34 ± 8.93	125.89 ± 7.42	**0.004**	83.72 ± 6.51	199.02 ± 88.62	**0.010**
IL-4	3.95 ± 0.35	3.96 ± 0.22	0.994	2.93 ± 0.11	3.57 ± 0.25	**0.027**
IL-5	34.19 ± 3.87	36.69 ± 3.28	0.629	39.27 ± 8.30	87.11 ± 47.96	0.314
IL-6	223.33 ± 14.99	256.11 ± 11.11	0.098	190.78 ± 8.53	289.88 ± 29.03	**0.004**
IL-10	18.18 ± 1.53	21.63 ± 1.39	0.114	15.90 ± 0.80	19.45 ± 1.03	**0.015**
IL-13	13.27 ± 0.69	13.97 ± 0.65	0.471	10.55 ± 0.52	13.55 ± 0.94	**0.011**
IFN-γ	9.93 ± 0.55	11.02 ± 0.68	0.229	8.23 ± 0.37	10.69 ± 0.66	**0.004**
TNF-α	5.12 ± 0.32	6.12 ± 0.43	0.079	4.42 ± 0.26	5.51 ± 0.43	**0.042**
CXCL1	369.78 ± 53.02	391.89 ± 41.56	0.747	320.89 ± 24.35	446.63 ± 66.13	0.081

Statistically significant results are shown in bold font.

Male and female rats that consumed the SAD demonstrated allodynia on days 1–36 and returned to 90% pre-CFA sensitivity by day 40. In contrast, male rats on the REG were allodynic on days 1–26 and returned to 90% pre-CFA sensitivity by day 29. Additionally, female rats on the REG were allodynic on days 1–22 and returned to 90% pre-CFA sensitivity by day 26, 14 days earlier than SAD-fed females (Fig. 7b). There was no main effect of Sex or Diet (p's > 0.05) in the contralateral paw (data not shown).

3. Discussion

Chronic pain as a possible outcome related to obesity/diet and the interaction is a growing concern worldwide including the United States. In fact, a population-based survey in the U.S. revealed that more than half of participants reporting chronic pain were overweight or obese [42]. In a large-scale study in Norway, both BMI and inflammatory cytokine levels were positively associated with pain severity [6] and BMI was a predictor of musculoskeletal complaints [43]. Furthermore, levels of C-reactive protein were positively associated with cold sensitivity in chronic pain-free control subjects [44], together suggesting that adipose tissue and/or systemic inflammation is a risk factor for chronic pain and pain sensitivity. Poor quality diet is a significant contributor to obesity and our previous studies in male mice reveal that poor quality diet can affect pain thresholds and recovery [34]. However, our previous work focused on median values of nutrients instead of a more translatable diet pattern and used only male animals. Therefore, we undertook to expand our findings to females and to another related species using a translatable diet. We utilized a Standard American Diet (SAD) to investigate how poor diet affects function and physiology in rats of both sexes in order to mimic the poor diet choices made by a number of Americans.

Over the course of 20 weeks, male and female rats gained a significant amount of weight. However, female rats on the SAD gained significantly more weight than their regular diet counterparts. In contrast, there was no difference in weight for males. As previously found in a different strain of rat, a high-fat diet resulted in greater group differences in weight for female animals [45]. This suggests that a poor quality diet has a greater impact on females over males in terms of weight gain. Female SAD-fed animals also had increased triglyceride levels, which were not seen to the same extent in males. Male and female SAD-fed animals had elevated percent body fat and decreased percent lean mass. However, only the female SAD-fed rats showed a significant weight gain, possibly due to the greater increase in percent fat mass (18.33% vs. 30.61% in females and 22.72% vs. 33.90% in males). The shift from lean to fat mass for males due to SAD consumption is likely the reason for the lack of difference in weights between the males of both groups. This is in line with our previous findings in male mice using the TWD [34]. Additionally, males consuming the SAD had lower bone mineral density compared to males on the REG. It should be noted that there was a reduction in bone mineral density in female SAD-fed rats that was non-significant. In sedentary obese children and adolescents, there is also a decrease in bone mineral density [46] and there are known links between bone mineral density, inflammation and diet [47].

The SAD is high in refined sugar and prolonged exposure to the diet could contribute to hyperglycemia and insulin resistance (and potentially type 2 diabetes mellitus). Not surprisingly, rats on the SAD had higher blood glucose levels compared to control animals. While not measured, it is likely that the SAD-fed animals had higher insulin levels, potentially contributing to the development of inflammatory metabolic disorders.

Adipose tissue is known to release leptin, an adipokine satiety signal, which directly activates the immune system [23,48] and contributes to the release of pro-inflammatory cytokines. All SAD-fed rats had increased leptin levels due to the increase in adipose tissue. As anticipated, pro-inflammatory cytokine levels were elevated in SAD animals. In other hands, following administration of CFA in rats there is a significant increase in IL-1β at 3 h post-CFA that steadily declines for the following 120 h with no changes in the non-inflamed paw. Taken together, this suggests that local cytokine levels peak shortly after injection of CFA and then steadily decrease over time with limited effects on circulating peripheral levels [49]. Therefore, because we assessed cytokine levels 40 days post-CFA and after hypersensitivity had abated, it is reasonable to assume that the diet exposure, and not CFA administration, led to the observed inflammatory state. This potential chronic state of inflammation could feasibly have resulted in the prolonged sensitivity following administration of CFA. One explanation we consider is that chronic inflammation may lead to sensitization of C fibers [50] and demyelination of nociceptive fibers [51], both contributing to prolonged hypersensitivity. Thus, the observed persistent inflammation was possibly due to immune system activation in the peripheral nervous system.

To investigate a possible central mechanism for our behavioral observations, we performed immunohistochemistry on the rat spinal cords, specifically looking at microglial activation. In addition to responding to injury or pathogens, glial cells in the central nervous system have a major role in chronic pain [7,8,14] and blocking their activity can reduce pain [9,11,12]. Interestingly, following a high-fat diet, glial cells are found in high levels in multiple brain regions [52–54] and their activation leads to the release of pro- and anti-inflammatory cytokines and subsequent inflammation in the central nervous system. As expected, animals that consumed the SAD had significantly more Iba-1 immunoreactivity, suggesting more microglial activation or recruitment in the dorsal horn compared to animals that were on the regular diet. In either case, it is clear that the immune system of the SAD-fed rats was more active. This diet-induced immune activation may play a role in the prolonged recovery that is seen in the SAD animals. There was no sex difference in the level of microglial activation, which is in line

with previous research in which we and others have shown similar microglial activation in male and female animals following injury [11,55].

Following CFA injection, mechanical thresholds for both male and female rats were significantly decreased. However, recovery time for SAD-fed rats was approximately double that of their REG-fed counterparts. Microglial activation is similar between male and female rats, but we have yet to explore intracellular pathway activation with respect to microglia. It has been reported that male and female mice use different cells within the immune system to mediate chronic pain; whereas males utilize microglia, females rely on T cells [11]. In addition, spinal toll-like receptor 4 (TLR4) contributes to pain in male but not female mice [10]. Our findings show that differential inflammatory mediator expressions were present in male and female rats due to the SAD. Females had increased levels of IL-1β, IL-4, IL-6, IL-10, IL-13, TNF-α, and IFN-γ whereas males had increased levels of IL-1β. Of note, IL-13, IL-4 and CXCL1 ($p = 0.081$) are often associated with T cell-related activity, while IL-1β, TNFα and IL-6 are related to both microglia and T cells. This result suggests that there may be two different sex-dependent cellular populations being activated by the SAD resulting in similar chronic systemic inflammation.

4. Conclusions

Taken together, our findings indicate that the SAD led to a slower recovery of CFA-induced hypersensitivity in male and female rats, perhaps due to prolonged immune system activation peripherally (elevated mediators) and centrally (increased Iba-1 immunoreactivity). It is known that an inflammatory challenge can result in nociceptive priming of the immune system [56–58] and persistent hypersensitivity following a future challenge. It is possible that the chronic pro-inflammatory state induced by the SAD resulted in a primed state that increased susceptibility to CFA-induced hypersensitivity. As such, it is further possible that the excess prevalence of chronic pain in obese individuals is due to an immune system in a state of chronic low-grade activation. Finally, sex differences exist in the effects of an American diet on physiology and immune system activation. Future studies directed at examining the underlying mechanisms of these effects should take sex into account.

Author contributions

RES and SKT designed the experiments, SKT tested the animals, SKT and TLQ performed the blood testing and specimen collection. RES, SKT and BAG designed the SAD with the assistance of Dr. Tina Herfel (Envigo). BAG oversaw the blood analysis. RMC and LM ran the immunohistochemistry, TLQ imaged the slides, TLQ and LJS performed quantification. RES and SKT wrote the manuscript.

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Ethical issues

None.

Conflict of interest

The authors report no real or perceived conflicts of interest.

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References

[1] McCarthy LH, Bigal ME, Katz M, Derby C, Lipton RB. Chronic pain and obesity in elderly people: results from the Einstein aging study. J Am Geriatr Soc 2009;57:115–9, http://dx.doi.org/10.1111/j.1532-5415.2008.02089.x. PMID: 19054178; PMCID: 2763486.

[2] Narouze S, Souzdalnitski D. Obesity and chronic pain: systematic review of prevalence and implications for pain practice. Reg Anesth Pain Med 2015;40:91–111, http://dx.doi.org/10.1097/AAP.0000000000000218. PMID: 25650632.

[3] Smuck M, Kao MC, Brar N, Martinez-Ith A, Choi J, Tomkins-Lane CC. Does physical activity influence the relationship between low back pain and obesity? Spine J 2014;14:209–16, http://dx.doi.org/10.1016/j.spinee.2013.11.010. PMID: 24239800.

[4] Stone AA, Broderick JE. Obesity and pain are associated in the United States. Obesity 2012;20:1491–5, http://dx.doi.org/10.1038/oby.2011.397. PMID: 22262163.

[5] Janke EA, Collins A, Kozak AT. Overview of the relationship between pain and obesity: what do we know? Where do we go next? J Rehabil Res Dev 2007;44:245–62. PMID: 17551876.

[6] Sibille KT, Steingrimsdottir OA, Fillingim RB, Stubhaug A, Schirmer H, Chen H, McEwen BS, Nielsen CS. Investigating the burden of chronic pain: an inflammatory and metabolic composite. Pain Res Manag 2016;2016:7657329, http://dx.doi.org/10.1155/2016/7657329. PMID: 27445627; PMCID: PMC4909918.

[7] Beggs S, Salter MW. Microglia-neuronal signalling in neuropathic pain hypersensitivity 2.0. Curr Opin Neurobiol 2010;20:474–80, http://dx.doi.org/10.1016/j.conb.2010.08.005. PMID: 20817512; PMCID: 3589562.

[8] Beggs S, Salter MW. The known knowns of microglia-neuronal signalling in neuropathic pain. Neurosci Lett 2013;557(Pt A):37–42, http://dx.doi.org/10.1016/j.neulet.2013.08.037. PMID: 23994389.

[9] Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217–31, http://dx.doi.org/10.1038/nri3621. PMID: 24577438.

[10] Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin JS, Ritchie J, Chanda ML, Graham AC, Topham L, Beggs S, Salter MW, Mogil JS. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neurosci 2011;31:15450–4, http://dx.doi.org/10.1523/JNEUROSCI.3859-11.2011. PMID: 22031891; PMCID: 3218430.

[11] Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji RR, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci 2015, http://dx.doi.org/10.1038/nn.4053. PMID: 26120961.

[12] Trang T, Beggs S, Salter MW. ATP receptors gate microglia signaling in neuropathic pain. Exp Neurol 2012;234:354–61, http://dx.doi.org/10.1016/j.expneurol.2011.11.012. PMID: 22116040; PMCID: 3748033.

[13] Watkins LR, Maier SF. Glia: a novel drug discovery target for clinical pain. Nat Rev Drug Discov 2003;2:973–85, http://dx.doi.org/10.1038/nrd1251. PMID: 14654796.

[14] Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. Trends Neurosci 2001;24:450–5. PMID: 11476884.

[15] Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 2001;276:16683–9, http://dx.doi.org/10.1074/jbc.M011695200. PMID: 11278967.

[16] Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, Tsukumo DM, Anhe G, Amaral ME, Takahashi HK, Curi R, Oliveira HC, Carvalheira JB, Bordin S, Saad MJ, Velloso LA. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci 2009;29:359–70, http://dx.doi.org/10.1523/JNEUROSCI.2760-08.2009. PMID: 19144836.

[17] Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie 2009;91:791–5, http://dx.doi.org/10.1016/j.biochi.2009.01.008. PMID: 19455748.

[18] Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes 2005;54:1–7. PMID: 15616004.

[19] Levitan EB, Cook NR, Stampfer MJ, Ridker PM, Rexrode KM, Buring JE, Manson JE, Liu S. Dietary glycemic index, dietary glycemic load, blood lipids, and C-reactive protein. Metabolism 2008;57:437–43, http://dx.doi.org/10.1016/j.metabol.2007.11.002. PMID: 18249220; PMCID: 2262400.

[20] Liu S, Manson JE, Buring JE, Stampfer MJ, Willett WC, Ridker PM. Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. Am J Clin Nutr 2002;75:492–8. PMID: 11864854.

[21] Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M, Fischer-Posovszky P, Barth TF, Dragun D, Skurk T, Hauner H, Bluher M, Unger T, Wolf AM, Knippschild U, Hombach V, Marx N. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. Arterioscler Thromb Vasc Biol 2008;28:1304–10, http://dx.doi.org/10.1161/ATVBAHA.108.165100. PMID: 18420999.

[22] Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH, Libby P. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ Res 2008;103:467–76, http://dx.doi.org/10.1161/CIRCRESAHA.108.177105. PMID: 18658050; PMCID: 2740384.

[23] Fernandez-Riejos P, Najib S, Santos-Alvarez J, Martin-Romero C, Perez-Perez A, Gonzalez-Yanes C, Sanchez-Margalet V. Role of leptin in the activation of immune cells. Mediators Inflamm 2010;2010:568343, http://dx.doi.org/10.1155/2010/568343. PMID: 20368778; PMCID: 2846344.

[24] Fisher G, Hyatt TC, Hunter GR, Oster RA, Desmond RA, Gower BA. Markers of inflammation and fat distribution following weight loss in African-American and white women. Obesity 2012;20:715–20, http://dx.doi.org/10.1038/oby.2011.85. PMID: 21527894; PMCID: 3687549.

[25] Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 2007;56:1010–3, http://dx.doi.org/10.2337/db06-1656. PMID: 17287468.

[26] Verdam FJ, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, Buurman WA, de Vos WM, Rensen SS. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity 2013;21:E607–15, http://dx.doi.org/10.1002/oby.20466. PMID: 23526699.

[27] Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. J Clin Investig 2003;112:1785–8, http://dx.doi.org/10.1172/JCI20514. PMID: 14679172; PMCID: 297006.

[28] Buckman LB, Hasty AH, Flaherty DK, Buckman CT, Thompson MM, Matlock BK, Weller K, Ellacott KL. Obesity induced by a high-fat diet is associated with increased immune cell entry into the central nervous system. Brain Behav Immun 2014;35:33–42, http://dx.doi.org/10.1016/j.bbi.2013.06.007. PMID: 23831150; PMCID: 3858467.

[29] De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 2005;146:4192–9, http://dx.doi.org/10.1210/en.2004-1520. PMID: 16002529.

[30] Maysami S, Haley MJ, Gorenkova N, Krishnan S, McColl BW, Lawrence CB. Prolonged diet-induced obesity in mice modifies the inflammatory response and leads to worse outcome after stroke. J Neuroinflammation 2015;12:140, http://dx.doi.org/10.1186/s12974-015-0359-8. PMID: 26239227; PMCID: 4524371.

[31] Pohl J, Luheshi GN, Woodside B. Effect of obesity on the acute inflammatory response in pregnant and cycling female rats. J Neuroendocrinol 2013;25:433–45, http://dx.doi.org/10.1111/jne.12023. PMID: 23331909.

[32] Pohl J, Sheppard M, Luheshi GN, Woodside B. Diet-induced weight gain produces a graded increase in behavioral responses to an acute immune challenge. Brain Behav Immun 2014;35:43–50, http://dx.doi.org/10.1016/j.bbi.2013.09.002. PMID: 24026015.

[33] Sampey BP, Vanhoose AM, Winfield HM, Freemerman AJ, Muehlbauer MJ, Fueger PT, Newgard CB, Makowski L. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. Obesity 2011;19:1109–17, http://dx.doi.org/10.1038/oby.2011.18. PMID: 21331068; PMCID: 3130193.

[34] Totsch SK, Waite ME, Tomkovich A, Quinn TL, Gower BA, Sorge RE. Total western diet alters mechanical and thermal sensitivity and prolongs hypersensitivity following Complete Freund's Adjuvant in mice. J Pain 2016;17:119–25, http://dx.doi.org/10.1016/j.jpain.2015.10.006. PMID: 26597348.

[35] Last AR, Wilson SA. Low-carbohydrate diets. Am Fam Physician 2006;73:1942–8. PMID: 16770923.

[36] Daniel CR, McCullough ML, Patel RC, Jacobs EJ, Flanders WD, Thun MJ, Calle EE. Dietary intake of omega-6 and omega-3 fatty acids and risk of colorectal cancer in a prospective cohort of U.S. men and women. Cancer Epidemiol Biomarkers Prev 2009;18:516–25, http://dx.doi.org/10.1158/1055-9965.EPI-08-0750. PMID: 19190143.

[37] Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT. Estimated intakes of trans fatty and other fatty acids in the US population. J Am Diet Assoc 1999;99:166–74, http://dx.doi.org/10.1016/S0002-8223(99)00041-3, quiz 75-6. PMID: 9972183.

[38] Hintze KJ, Benninghoff AD, Ward RE. Formulation of the Total Western Diet (TWD) as a basal diet for rodent cancer studies. J Agric Food Chem 2012;60:6736–42, http://dx.doi.org/10.1021/jf204509a. PMID: 22224871.

[39] Dixon WJ. Staircase bioassay: the up-and-down method. Neurosci Biobehav Rev 1991;15:47–50. PMID: 2052197.

[40] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88. PMID: 3340425.

[41] Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain 2011;7:55, http://dx.doi.org/10.1186/1744-8069-7-55. PMID: 21801409; PMCID: 3163602.

[42] Toblin RL, Mack KA, Perveen G, Paulozzi LJ. A population-based survey of chronic pain and its treatment with prescription drugs. Pain 2011;152:1249–55, http://dx.doi.org/10.1016/j.pain.2010.12.036. PMID: 21397401.

[43] Andorsen OF, Ahmed LA, Emaus N, Klouman E. A prospective cohort study on risk factors of musculoskeletal complaints (pain and/or stiffness) in a general population. The Tromso study. PLOS ONE 2017;12:e0181417, http://dx.doi.org/10.1371/journal.pone.0181417. PMID: 28727753; PMCID: PMC5519093.

[44] Schistad EI, Stubhaug A, Furberg AS, Engdahl BL, Nielsen CS. C-reactive protein and cold-pressor tolerance in the general population: the Tromso Study. Pain 2017;158:1280–8, http://dx.doi.org/10.1097/j.pain.0000000000000912. PMID: 28420008.

[45] Amengual-Cladera E, Llado I, Gianotti M, Proenza AM. Sex differences in the effect of high-fat diet feeding on rat white adipose tissue mitochondrial function and insulin sensitivity. Metabolism 2012;61:1108–17, http://dx.doi.org/10.1016/j.metabol.2011.12.016. PMID: 22401878.

[46] Junior IF, Cardoso JR, Christofaro DG, Codogno JS, de Moraes AC, Fernandes RA. The relationship between visceral fat thickness and bone mineral density in sedentary obese children and adolescents. BMC Pediatr 2013;13:37, http://dx.doi.org/10.1186/1471-2431-13-37. PMID: 23510224; PMCID: 3606829.

[47] Hardy R, Cooper MS. Bone loss in inflammatory disorders. J Endocrinol 2009;201:309–20, http://dx.doi.org/10.1677/JOE-08-0568. PMID: 19443863.

[48] Agrawal S, Gollapudi S, Su H, Gupta S. Leptin activates human B cells to secrete TNF-alpha, IL-6, and IL-10 via JAK2/STAT3 and p38MAPK/ERK1/2 signaling pathway. J Clin Immunol 2011;31:472–8, http://dx.doi.org/10.1007/s10875-010-9507-1. PMID: 21243519; PMCID: 3132280.

[49] Woolf CJ, Allchorne A, Safieh-Garabedian B, Poole S. Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha. Br J Pharmacol 1997;121:417–24, http://dx.doi.org/10.1038/sj.bjp.0701148. PMID: 9179382; PMCID: 1564704.

[50] Richter F, Natura G, Loser S, Schmidt K, Viisanen H, Schaible HG. Tumor necrosis factor causes persistent sensitization of joint nociceptors to mechanical stimuli in rats. Arthritis Rheum 2010;62:3806–14, http://dx.doi.org/10.1002/art.27715. PMID: 20722011.

[51] Stoll G, Jung S, Jander S, van der Meide P, Hartung HP. Tumor necrosis factor-alpha in immune-mediated demyelination and Wallerian degeneration of the rat peripheral nervous system. J Neuroimmunol 1993;45:175–82. PMID: 8331160.

[52] Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschop MH, Schwartz MW. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Investig 2012;122:153–62, http://dx.doi.org/10.1172/JCI59660. PMID: 22201683; PMCID: 3248304.

[53] Tran DQ, Tse EK, Kim MH, Belsham DD. Diet-induced cellular neuroinflammation in the hypothalamus: Mechanistic insights from investigation of neurons and microglia. Mol Cell Endocrinol 2016;438:18–26, http://dx.doi.org/10.1016/j.mce.2016.05.015. PMID: 27208620.

[54] Valdearcos M, Robblee MM, Benjamin DI, Nomura DK, Xu AW, Koliwad SK. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. Cell Rep 2014;9:2124–38, http://dx.doi.org/10.1016/j.celrep.2014.11.018. PMID: 25497089.

[55] Taves S, Berta T, Liu DL, Gan S, Chen G, Kim YH, Van de Ven T, Laufer S, Ji RR. Spinal inhibition of p38 MAP kinase reduces inflammatory and neuropathic pain in male but not female mice: sex-dependent microglial signaling in the spinal cord. Brain Behav Immun 2015, http://dx.doi.org/10.1016/j.bbi.2015.10.006. PMID: 26472019.

[56] Ferrari LF, Gear RW, Levine JD. Attenuation of activity in an endogenous analgesia circuit by ongoing pain in the rat. J Neurosci 2010;30:13699–706, http://dx.doi.org/10.1523/JNEUROSCI.2867-10.2010. PMID: 20943910; PMCID: 2970511.

[57] Kim JY, Tillu DV, Quinn TL, Mejia GL, Shy A, Asiedu MN, Murad E, Schumann AP, Totsch SK, Sorge RE, Mantyh PW, Dussor G, Price TJ. Spinal dopaminergic projections control the transition to pathological pain plasticity via a D1/D5-mediated mechanism. J Neurosci 2015;35:6307–17, http://dx.doi.org/10.1523/JNEUROSCI.3481-14.2015. PMID: 25904784.

[58] Reichling DB, Levine JD. Critical role of nociceptor plasticity in chronic pain. Trends Neurosci 2009;32:611–8, http://dx.doi.org/10.1016/j.tins.2009.07.007. PMID: 19781793; PMCID: 2787756.