Maternal High-Fat Diet Consumption Enhances Offspring Susceptibility to DSS-Induced Colitis in Mice

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Objective: Maternal high-fat diet (HFD) may alter the offspring intestinal immune system, thereby enhancing susceptibility toward inflammatory bowel disease. The objective of the current study was to investigate the impact of maternal HFD on offspring intestinal health using a mouse model of dextran sulfate sodium (DSS)-induced colitis.

Methods: Dams were provided with either HFD (60%) or control diet. After weaning, female offspring from both groups were kept on 45% HFD. At 14 weeks of age, offspring were subjected to 2.5% DSS in drinking water for 5 days, followed by 5 days of recovery.

Results: Offspring from maternal HFD had higher body weight gain before DSS induction and had higher liver and fat weights with increased adipocyte size at necropsy. When subjected to DSS treatment, HFD offspring had accelerated body weight loss and exaggerated disease activity index. HFD offspring had an elevated histopathological score and interleukin (IL)-1β, IL-6, and IL-17 expression with upregulated NF-κB signaling. Maternal HFD resulted in enhanced neutrophil infiltration associated with elevated expression of monocyte chemoattractant protein-1. Furthermore, maternal HFD suppressed AMP-activated protein kinase activity and decreased sirtuin 1 and p53 protein contents in offspring gut.

Conclusions: Maternal HFD consumption predisposes offspring to a higher susceptibility to develop inflammatory bowel disease.

Introduction

According to the latest National Health and Nutrition Examination Survey (2009-2010), 31.9% of nonpregnant women 20 to 39 years of age have obesity, and another one-third have overweight (1). Accompanying the rapidly increasing prevalence of obesity, the incidence of inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis, has rapidly increased in Europe and North America since the last decade of the 20th century. More alarming, the incidence of IBD is rising quickly in young children in the United States, having doubled from 1991 to 2002 (2). Around 1.5 million people suffer from IBD in the United States, and it is becoming increasingly common in the regions where people are adopting a Western lifestyle that includes less physical activity, more stress, and a diet rich in fat (3). IBD has resulted in huge medical costs in the United States and around the world (4). The etiological factors of IBD mainly comprise genetics, gut microbes, and environmental factors (4). Consumption of a diet rich in saturated fat has been shown to increase incidence of colitis through promoting sulphite-reducing pathobiont in interleukin (IL)-10 deficient mice, which are genetically susceptible to IBD (5). Epidemiological studies further link high-fat diet (HFD) exposure during infancy to the incidence of IBD in adults (6).

Maternal HFD intake negatively affects fetal development, which exerts lasting effects on the health of offspring, predisposing them to chronic diseases such as diabetes, coronary heart diseases, and hypertension (7,8). However, knowledge regarding the impact of maternal HFD or obesity on intestinal development and health of the progeny is limited (8), and the impact of maternal HFD on the incidence of and susceptibility to IBD in offspring has only been sparsely tested (9). Jacobson and colleagues demonstrated that maternal high linoleic acid (18:2n-6) intake enhanced 2,4-dinitrobenzene sulfonic acid-induced colitis symptoms in nursing offspring (9). In our previous studies, we have found that maternal obesogenic diet induced chronic gut inflammation in offspring (10,11) and impaired gut barrier function of offspring in nonobese diabetic mice (10). Recently, Gruber and colleagues reported that maternal HFD in combination with postnatal HFD accelerated ileitis onset in...
the distal ileum of offspring TNF$^{DARE/WT}$ mice, a genetically susceptible model for CD-like ileitis (12). In the current study, we examined the impact of maternal HFD on the IBD susceptibility of offspring using a dextran sulfate sodium (DSS)-induced colitis mouse model.

### Methods

#### Antibodies and chemicals

Antibodies against phospho-/total AMP-activated protein kinase (AMPK), phospho-/total-ERK1/2, IL-6, IκBα, phospho-/total-p38, phospho-/total-p65, p53, and SIRT1 were from Cell Signaling Technology (Beverly, Massachusetts). Anti-Ly-6B.2 monoclonal and anti-β-actin antibodies were purchased from Bio-Rad Laboratories Inc. (Hercules, California) and Sigma (Saint Louis, Missouri), respectively. IRDye 680 goat anti-mouse and IRDye 800CW goat anti-rabbit secondary antibodies were purchased from Li-Cor Biosciences (Lincoln, Nebraska). The VECTASTAIN Elite® ABC and DAB peroxidase (HRP) substrate kits were purchased from Vector Laboratories Inc. (Burlingame, California). TRIzol® Reagent was purchased from Sigma. DNase I and RNeasy Mini Kit were purchased from Qiagen (Valencia, California), and iScript™ cDNA synthesis kit was purchased from Bio-Rad Laboratories Inc. Colitis grade DSS (MW = 36,000-50,000) was purchased from MP Biomedicals (Santa Ana, California).

#### Animal care and experimental design

**Maternal diet and treatment.** Adult (~4 months old) female C57BL/6J mice (purchased from Jackson Laboratory [Bar Harbor, Maine] and inbred in our facility) that had been fed regular chow diet were randomized into two groups: maternal control diet group (CON) and maternal HFD group. Both of the groups were fed with control diet (10% energy from fat, D12450H; Research Diets Inc., New Brunswick, New Jersey; Supporting Information Tables S1-S2) up to mating with males of similar age. Mating was confirmed by the presence of vaginal plug. Mice were then fed either CON or 60% HFD (60% energy from fat, D12492; Research Diets Inc.; Supporting Information Tables S1-S2) diets during gestation and lactation (Figure 1A). The 60% HFD is commonly used to induce obesity during a short duration (13). At birth, the litter sizes were balanced to six pups. Mice were weaned at 3 weeks of age, and nine female offspring per treatment were randomly selected for further studies. Here, we used female offspring mice because they were more vulnerable to programmed changes than males (14) and to avoid the confounding effects of sex.

**Offspring diets and treatment.** After weaning, female offspring of both CON- and HFD-fed dams were fed the 45% HFD (45% energy from fat, D12451; Research Diets Inc.; Supporting Information Tables S1-S2) diet ad libitum for ~13 weeks, until necropsy (Figure 1A). The 45% HFD was used to mimic the effect of a typical diet of Western societies over a longer period (13). The body weights of offspring mice of both groups were recorded weekly.

**DSS treatment of offspring**

At 14 weeks of age, offspring mice from both CON- and HFD-fed dams were subjected to 2.5% (W/V) DSS water challenge for 5 days followed by 5 days of recovery (Figure 1B). Mice were monitored daily during DSS induction and the recovery stage for body weight loss, fecal consistency, and blood in the stool. Mice were then sacrificed and tissues were collected for histological and biochemical analyses. All mice were housed in a temperature-controlled room with a 12-hour light and 12-hour dark cycle and had free access to food and drinking water. Experiments were conducted per the animal procedure (BAF # 04341) approved by the Washington State University Animal Care and Use Committee.
Assessment of symptoms and colitis score
The disease activity index (DAI) score was assessed according to the combined scores of weight loss compared to initial weight, stool consistency, and bleeding in the stool using criteria detailed in Supporting Information Table S3 (15). The scores were recorded daily during the DSS treatment and recovery stages. The DAI score was the summation of three individual scores (body weight loss, stool consistency, and bleeding).

Tissue collection and fixation
Mice were anesthetized with CO2 inhalation, which was followed by cervical dislocation. Organs, including heart, spleen, liver, and cecum (containing lumen content), were collected and weighed. Subcutaneous fat (only lingual fat pad), visceral fat (gonadal fat collected around the ovaries), and brown adipose tissue (BAT, collected from the interscapular area) were weighed. A 5-mm segment of distal colon at a constant location and the visceral adipose tissues were fixed in freshly prepared 4% (w/v) paraformaldehyde (pH 7.0), processed, and embedded in paraffin. The remaining colon tissue containing both inflamed and noninflamed area was rinsed in phosphate-buffered saline, frozen in liquid nitrogen, and stored at −80°C for biochemical analysis.

Histological evaluation of colonic ulceration and adipocyte quantification
Paraffin-embedded tissues were sectioned at 5 μm thickness, deparaffinized, and subjected to hematoxylin and eosin staining. Histological examination and imaging were done under a Lecia DM2000 LED light microscope (Chicago, Illinois). At least one image was obtained per section, and nine sections per animal at constant intervals were used for microscopic examination.

The pathological score of the distal colon was evaluated and recorded blindly using previously published score criteria (15), which was the sum of the scores of crypt damage (none, basal 1/3, basal 2/3, and only surface epithelium intact), severity of inflammation (none, slight, moderate, and severe), and depth of injury (none, mucosal, mucosal and submucosal, and transmural). Paraffin-embedded adipose tissues were sectioned and processed as previously described (16). ImageJ 1.30v software (National Institute of Health, Bethesda, Maryland) was used to measure the area and diameter of adipocytes by drawing a horizontal edge-to-edge line in the middle of the adipocyte. Nine sections per animal at constant intervals were used and all the adipocytes per image were quantified.

Immunohistochemical analyses
Immunohistochemical analyses were carried out as previously described (17). Briefly, colonic tissue sections were deparaffinized and hydrated, followed by antigen retrieval, blocking, and overnight incubation with anti-Ly-6B.2 antibody. Signals were visualized using the VECTASTAIN ABC and DAB kits and hematoxylin counterstaining. Images were taken using the Lecia DM2000 LED light microscope (Chicago, Illinois). Neutrophil infiltration scores were assessed blindly using the criteria described previously (18). Briefly, the scores for the depth of neutrophil infiltration (scored as 0 to 3) and staining intensity (0 to 4) were recorded individually. The summation of both scores resulted in the total quantified score ranging from 0 to a maximum of 7 per distal colonic section. Nine sections per animal at constant intervals were used for microscopic examination and score assessment.

Immunoblotting analyses
Immunoblotting analyses were performed as previously described (19). Band density was quantified using the Odyssey® Infrared Imaging System and Image Studio™ Lite software (Li-Cor Biosciences, Lincoln, Nebraska) and normalized to the β-actin content.

Quantitative reverse transcriptase-polymerase chain reaction analyses
Total RNA was extracted from the powdered tissue with TRIzol Reagent. RNA was treated with DNase I and then purified with RNeasy Mini Kit. cDNA was synthesized with the iScript cDNA synthesis kit. Quantitative reverse transcriptase-polymerase chain reaction was performed per a published method (20). 18s was used as the reference gene. Primer sequences used in the study are listed in Supporting Information Table S4.

Statistical analysis
Data were analyzed as a complete randomized design using General Linear Model of Statistical Analysis System (2000). Data were expressed as means ± standard error of mean (SEM). Student’s t test was used for calculating significance. A significant difference was considered as P ≤ 0.05.

Results
Maternal HFD affects organ weight and visceral adipocyte size in offspring
The offspring from the maternal HFD group had a significantly higher liver and lower spleen weight per percentage of body weight than those from the maternal CON group (Figure 2B), though their body weights were not different at necropsy (Figure 2A). Similarly, the maternal HFD enhanced visceral fat and total fat mass, including subcutaneous fat, brown adipose tissue, and visceral fat (Figure 2C), as well as visceral adipocyte diameter and area (Figure 2D-2F).

Maternal HFD affects body weight loss and disease activity index in DSS-induced colitis
Although body weight in offspring from the maternal HFD group was significantly higher than that of the maternal CON group before DSS induction (Figure 3A), DSS treatment resulted in a greater loss of body weight in maternal HFD offspring at the recovery stage (Figure 3B). Colitis induction was further confirmed by diarrhea and blood in the stool in addition to body weight loss, as shown by the DAI score (Figure 3C). The DAI score increased during DSS treatment for both groups and was greater in maternal HFD offspring during the recovery stage (Figure 3C). Furthermore, the colonic length was shorter (4.33 ± 0.14 cm) in maternal HFD-fed offspring than the maternal CON group (4.64 ± 0.17 cm), though the difference was not significant.
Maternal HFD enhances histological distortion and inflammation in the colon of offspring mice subjected to DSS induction

Histological evaluation revealed that DSS treatment caused injuries to the distal colonic tissues, which resulted in the loss of crypt architecture (Figure 4A). The colon in maternal HFD offspring was more susceptible to DSS challenge with a trend of a higher histopathological score ($P \leq 0.10$) than those from CON offspring (Figure 4A-4B), indicating aggravated ulceration in the distal colon.

To gain more insight into the altered histopathology, we further analyzed the expression of proinflammatory cytokines and associated inflammatory signaling. Maternal HFD elevated IL-1$\beta$, IL-6, and IL-17 gene expression (Figure 4C) and increased IL-6 protein in offspring gut (Figure 4F). NF-$k$B (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling was exaggerated in the colonic tissues, as indicated by enhanced phosphorylation of p65 (Figure 4D-4E) and decreased I$\kappa$B$\alpha$ (inhibitor $k$B alpha, which sequesters and inactivates NF-$k$B Rel/p65 in cytoplasm) content (Figure 4D, 4F). In addition, maternal HFD resulted in more extensive and severe neutrophil infiltration in the colonic tissue of offspring mice (Figure 5A-5B), associated with elevated expression of monocyte chemoattractant protein-1 (MCP-1; Figure 5C). Accordingly, we observed decreased p38 phosphorylation (Figure 5D-5E) and enhanced ERK1/2 phosphorylation (Figure 5D, 5F).
in maternal HFD offspring. These findings collectively suggest that maternal HFD rendered offspring mice to be more susceptible to inflammation and colitis induced by DSS.

**Maternal HFD adversely affects AMP-activated protein kinase activity in offspring mice with DSS-induced colitis**

Maternal HFD reduced AMPK phosphorylation (Figure 6A-6B) in offspring colonic tissues. Consistently, both AMPK interacting proteins, SIRT1 and p53, were decreased (Figure 6C) in offspring mice from HFD-fed dams.

**Discussion**

A high-fat diet is a part of the Western lifestyle and has been proposed to increase the risk of IBD (3). The rapid increase of IBD incidence in young children (2) suggests the potential role of maternal obesogenic diet in the development of this disease, but direct evidence remains missing. In the current study, we assessed the impact of maternal HFD on the incidence of IBD in offspring using a DSS-induced colitis mouse model. We found that maternal HFD significantly aggravated the symptoms of acute colitis in the female offspring.

In this study, we initiated HFD (60%) dietary treatments after mating and maintained the diet during gestation and lactation in order to avoid maternal obesity that could induce gestational diabetes and other physiological changes in addition to the effect of HFD (21). All offspring were fed the obesogenic diet after weaning to mimic the typical diet of Western societies. The maternal HFD offspring were heavier at weaning, and body weight was higher in offspring of the maternal HFD compared to CON group from 7 weeks until DSS induction. The higher weight at weaning in the HFD-fed-group offspring was likely due to the maternal HFD (60%) diet consumption during...
gestation and lactation. Our findings are consistent with previous investigations that maternal high linoleic acid (18:2n-6) intake resulted in a higher body weight in the nursing rats (9), and maternal HFD consumption enhanced body weight gain and adiposity in the offspring mice (22,23). During the recovery period (post DSS induction), however, the body weight loss was higher in offspring of the maternal HFD group, suggesting more severe colitis in these mice compared to offspring of the maternal CON group. Consistently, during the course of recovery, a higher DAI score and a higher histopathological score was detected in the maternal HFD-fed group, showing more severe colitis and slower recovery of the offspring of HFD-fed dams. DSS, administered to mice in the drinking water, triggers intestinal inflammation by binding to medium-chain-length fatty acids in the mouse colon, resulting in the disruption of the colonic epithelial barrier (24) and eliciting inflammation (25).

Activated immune system produces proinflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α that further amplify the NF-κB inflammatory cascade (26). IL-17, in return, mediates neutrophil infiltration and upregulation of TNF-α and IL-6, which are the key mediators in chronic inflammation of ulcerative colitis (26,27). Consistent with previous reports and the observed more severe histopathological damage (9), maternal HFD enhanced IL-1β, IL-6, and IL-17 production with amplified NF-κB signaling in the offspring. These findings are aligned with earlier observations that maternal obesity predisposes offspring gut to inflammation in sheep (11) and nonobese diabetic mice (10), as well as in young rats (9).

In IBD, excessive activation and infiltration of neutrophils in colonic lesions result in epithelial cell necrosis, mucosal injury, and...
exaggerated colitis symptoms (28). High-density neutrophil flux across epithelial monolayers from basolateral to apical surface disrupts epithelium (29) and weakens the epithelial barrier, which worsens inflammation and IBD symptoms (28). Maternal HFD exacerbated mucosal neutrophil infiltration in offspring subjected to DSS treatment, which could contribute to the enhanced IL-17, the more severe tissue damage, and the elevated histopathological score in DSS-induced colitis. Consistent with enhanced neutrophil infiltration, MCP-1, the main chemokine regulating migration and infiltration of neutrophils (30), was increased in offspring gut from HFD-fed dams. These data were supported by a recent publication in which maternal HFD was found to promote an early onset of ileitis in a genetically driven CD mouse model, with enhanced neutrophil infiltration (12). Rapid apoptosis and removal of neutrophils are vital in the resolution of inflammation to avoid undesirable tissue damage. In human neutrophils, lipopolysaccharide- or TNFα-stimulated ERK activation contributes to the delayed apoptosis (31), while activation of p38 MAPK induced neutrophil apoptosis in vitro (32). Accordingly, the enhanced ERK1/2 phosphorylation and decreased p38 activation were detected in the offspring gut from HFD-fed dams, indicating that maternal HFD might delay neutrophil apoptosis in the inflamed colonic tissues.

AMPK is the central regulator of cellular energy balance (22). AMPK mediates inflammatory responses such as IL-6 expression (33), and a reduced AMPK protein level results in impaired epithelial barrier function (34). The reduced AMPK level due to maternal HFD in offspring with DSS-induced colitis suggested that AMPK signaling might be important in the pathophysiological effects of maternal HFD on inflammation and colitis in offspring gut. AMPK inhibits inflammation by inducing the expression or activation of a number of mediators such as SIRT1, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), p53, and Forkhead box O factors (35,36). AMPK activates SIRT1 deacetylase via increasing cellular NAD+ levels (35), while SIRT1 stimulates LKB1 activity, which subsequently activates AMPK (37). SIRT1 deacetylates the RelA/p65 and inhibits NF-κB signaling (36). Thus, the reduction in AMPK and SIRT1 might contribute to the enhanced activation of NF-κB signaling in HFD offspring gut. In this sense, AICAR-induced AMPK activation downregulates the innate and adaptive immune responses of TNBS-induced colitis (38). AMPK also activates p53, a tumor suppressor that mediates growth arrest or induces apoptosis (39). Mice deficient in p53 developed tumors upon DSS treatment even without azoxymethane injection (40), suggesting a vital protective role of p53 in IBD pathogenesis. Consistent with decreased AMPK activation, p53 protein was decreased in offspring colon of HFD-fed dams, which likely contributes to the colitis induction in the maternal HFD offspring.

In conclusion, maternal HFD exposure accelerated offspring body weight gain but enhanced DSS-induced body weight loss and colitis symptoms by activating NF-κB signaling and stimulating IL-1β, IL-6, and IL-17 expression as well as neutrophil induction in colonic tissues. Furthermore, maternal HFD exposure suppressed AMPK activity and downregulated SIRT1 and p53, which further aggravated DSS colitis in the offspring. Collectively, this study suggests that maternal HFD consumption predisposes offspring mice to IBD and related inflammatory gut diseases.

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