High-Fat Diet-Induced Obesity Exacerbates Inflammatory Bowel Disease in Genetically Susceptible Mdr1a−/− Male Mice

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Abstract

Obesity is a chronic inflammatory disease and a risk factor for disorders such as heart disease, diabetes, and cancer. A high-fat diet (HFD), a risk factor for obesity, has also been associated with inflammatory bowel disease (IBD). A proinflammatory state characterized by systemic and local increases in cytokine and chemokine levels are noted in both obesity and IBD, but it is unclear whether obesity is a risk factor for IBD. To examine any association between obesity and IBD, we chose FVB.129P2-Abcb1a1/−/−m1Bor (Mdr1a−/−) mice, because this strain develops IBD spontaneously with age without a chemical or bacterial "trigger." In addition, its background strain, FVB, has been used for diet-induced obesity studies. Mdr1a−/− mice and wild-type (WT) mice were fed a HFD (~60% calories from fat) or a low-fat diet (LFD; ~11% calories from fat) for 12 wk. Obesity phenotypes examined included body weight measurements, glucose metabolism changes, and adiposity at termination of the study. IBD was determined by clinical signs, necropsy, and histopathology. We found that compared with those fed the LFD, both the Mdr1a−/− and WT mice fed the HFD had greater weight gains and elevated plasma leptin concentrations (P < 0.0001). When all mice were analyzed, weight gain was also associated with inflammation in mesenteric fat (R² = 0.5; P < 0.0001) and mesenteric lymph nodes (R² = 0.4; P < 0.0001). In contrast, the HFD was not associated with IBD in WT mice, whereas it exacerbated spontaneous IBD in Mdr1a−/− mice (P = 0.012; Fisher's exact test). Although a HFD and obesity were not associated with IBD in WT mice, our studies suggest that they are likely risk factors for IBD in a genetically susceptible host, such as Mdr1a−/− mice. J. Nutr. doi: 10.3945/jn.113.174615.

Introduction

Obesity/insulin resistance and inflammatory bowel disease (IBD) share common characteristics, most notably including chronic inflammation (1,2). Obese animals and humans have increased levels of both systemic (serum IL-6 and TNFα) and localized (liver and adipose) inflammatory cytokines and chemokines (2–4), similar to increases in proinflammatory molecules in peripheral adipose tissue, increasing adiposity centrally while losing it peripherally (9). Adipose tissue manifests proinflammatory transformation during both obesity and IBD, suggesting a possible link between the two (18). To explore this association, we examined whether an obesity-inducing high-fat diet (HFD) could also accelerate IBD development and progression using a genetically susceptible mouse model of IBD, Mdr1a−/− mice.

Mdr1a−/− mice were originally generated to study the involvement of P-glycoprotein [PGP; also known as multidrug resistance protein (MDR)] in drug metabolism (19). It was later discovered that these mice spontaneously develop IBD (7). The onset of IBD in this model is associated with the presence of commensal bacteria and thus can be prevented by treatment with broad-spectrum antibiotics in mice housed in specific pathogen-free facilities (7,20). Mdr1a−/− mice can also be induced to develop IBD quickly if they are infected with certain bacteria, such as Helicobacter spp. (6,20). Interestingly, Mdr1a−/− mice consumption, a risk factor for developing obesity, is associated with elevated risk for developing IBD, although conflicting results are also found (12–15).

Central obesity (apple-shaped body contour) compared with peripheral obesity (pear-shaped body contour) is a stronger predictor of metabolic syndrome and insulin resistance (16,17). Interestingly, patients with CD often manifest redistribution of adipose tissue, increasing adiposity centrally while losing it peripherally (9). Adipose tissue manifests proinflammatory transformation during both obesity and IBD, suggesting a possible link between the two (18). To explore this association, we examined whether an obesity-inducing high-fat diet (HFD) could also accelerate IBD development and progression using a genetically susceptible mouse model of IBD, Mdr1a−/− mice.
have a more inflammation-prone “environment,” i.e., they have higher inflammatory cytokines and chemokines in circulation and in colonic tissues compared with wild-type (WT) controls even without exposure to disease-inducing bacteria (20,21).

Mdr1a<sup>-/-</sup> mice are on a FVB background, a strain that has been used in diet-induced obesity studies (22–24), and thus can be used to dissect the associations between diet-induced obesity and IBD. Our studies were designed to address whether diet-induced obesity increases the risk of spontaneous IBD in this murine model.

Methods

**Mice and diets.** FVB/NTac (WT) and FVB.129P2-Abcb1atm1Bor<sup>+/+</sup>N7 (Mdr1a<sup>-/-</sup>) mice were originally purchased from Taconic and were maintained in a specific pathogen-free facility at the University of Washington campus by homozygous breeding. Infectious agents excluded from our facility include mouse hepatitis virus, mouse parvovirus, minute virus of mice, reovirus-1, pneumonia virus of mice, epizootic diarrhea of infant mice, Theiler’s murine encephalomyelitis virus, lymphocytic choriomeningitis virus, ectromelia, Sendai virus, staldacryoadenitis virus, rat parvoviruses, Mycoplasma pulmonis, pinworms, and fur mites. Mice were housed in a room that is free of Helicobacter to avoid bacterially induced IBD. Mice were confirmed to be Helicobacter-negative by PCR (5) from feces collected at necropsy. Only male mice were used for our studies to reduce variability in weight gain and glucose metabolism due to gender. Ten male mice of each genotype were fed either a HFD or low-fat diet (LFD) (Supplemental Table 1) for 12 wk beginning at 6 wk of age. The diets were formulated based on the AIN93M diet (25) but modified to contain different amounts of fat and were manufactured by Test Diet. The HFD (5A3N) contained ~60% fat by calories (9.6% calories from protein, 58.9% calories from fat, and 31.4% calories from carbohydrate) and the LFD (AIN93M) contained ~11% fat by calories (13.7% calories from protein, 10.8% calories from fat, and 75.5% calories from carbohydrate). Instead of using regular rodent chow as a low-fat control diet, we used a purified AIN93M diet as a control to reduce other variables (fiber, phytochemicals, etc.) and closely matched the micronutrient concentrations of the 2 diets.

To monitor weight gain, all mice were weighed weekly at the same time in the morning. Food intake was estimated from 1 wk of daily (morning) measurements of food left in the hopper in cages with 3–5 mice beginning at 4 wk after diet initiation.

Animals were treated in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH and the study protocol was reviewed and approved by the University of Washington’s Institutional Animal Care and Use Committee.

**Feed-deprived glucose and insulin tolerance test.** To determine changes in glucose metabolism due to obesity, feed-deprived glucose concentrations (6-h deprivation) were measured at the start of the diet and at 4, 8, and 12 wk following diet initiation. Blood glucose concentrations were measured from tail blood using the OneTouch Ultra glucometer (LifeScan). Changes in insulin sensitivity were determined by i.p. insulin tolerance test following 16-h feed deprivation at 10 wk after diet initiation. Blood glucose was measured at time zero, just prior to an i.p. injection of insulin [1 U/kg, regular Humulin (Eli Lilly) in sterile saline] and at 15, 30, 60, and 90 min after the injection.

**Biochemical assays.** Leptin, cholesterol, and TGs were measured from plasma samples obtained using terminal blood sampling. Leptin concentrations were measured using a Mouse Leptin ELISA kit (Millipore). Cholesterol concentrations were determined using a colorimetric assay (Cholesterol Phenol reagent, Diagnostic Chemicals Limited) with cholesterol standards (Pointe Scientific). TG concentrations were colorimetrically determined following the removal of free glycerol (Trig/GB, Roche).

**Tissue collection.** At 12 wk post diet initiation, feed-deprived (6 h) glucose concentrations were measured and mice were then killed by CO₂ asphyxiation. Blood (cardiac puncture), liver, adipose tissue, and mesenteric lymph nodes (MLNs) were harvested. Four major adipose depots (epididymal, mesenteric, retroperitoneal, and inguinal fat pads) were individually weighed and portions fixed in formalin for histological analyses. The colon and cecum were processed as previously described (5,6,26) and a fecal sample was taken from the cecum for Helicobacter PCR (to confirm negativity).

**Adiposity measurement.** Adiposity was defined as the [total weight of 4 adipose depots (mesenteric, epididymal, retroperitoneal and inguinal fat pads) divided by body weight at the time of necropsy] × 100. This is an indirect measure of percent body fat, which in our experience correlates well with body composition analysis performed by NMR (total body fat measured from dissected fat pads vs. total fat weight by NMR; J. Paik and Y. Fierce, unpublished observation).

**Histopathology.** Cecum, colon, mesenteric fat, and MLN were processed routinely and stained with hematoxylin and eosin through the Histology and Image Core at the University of Washington. Sections were graded by a board-certified veterinary pathologist (P.M.T.) unaware of the treatment and genotype of animals as previously reported with a slight modification (5,6). Inflammation in mesenteric fat (Table 1) and lymphadenitis and reactive hyperplasia in MLN (27) were analyzed using hematoxylin and eosin staining.

**Statistics.** Two-way ANOVA was used to test effects of genotypes, diets, and their interactions for all analyses with the exceptions of i.p. insulin tolerance test and percentage weight gain. For these latter 2 analyses, a t test was used to compare differences in glucose concentrations in response to insulin injection (percent baseline glucose) or differences in weight gain (percent weight gain) between mice fed the HFD and LFD of the same genotype at specified time points. Transformation of glucose concentrations to percent of their baseline values enables us to directly compare sensitivity with insulin at each time point between the groups with varying baseline glucose concentrations. Post hoc tests were carried out between the HFD and LFD-fed groups of the same genotype using Bonferroni corrections for multiple comparisons when significant differences were found with an initial ANOVA test. Association between weight and inflammation in MLN and mesenteric fat was analyzed using linear regression analysis. A Fisher’s exact test was used to determine significant differences in incidence. Prism statistical software (Graphpad) was used for all analyses. *P* < 0.05 was used to determine significance.

**Results**

The HFD induced obesity in both Mdr1a<sup>-/-</sup> and WT mice. Dietary treatment was given to mice at 6 wk old, long before spontaneous IBD develops in Mdr1a<sup>-/-</sup> mice (7). Both WT and Mdr1a<sup>-/-</sup> mice gained weight more rapidly, when fed the HFD compared with those fed the LFD (Supplemental Fig. 1), although they consumed similar quantities of food (~3 g/d). Differences in weight gain between the mice fed the HFD and

### TABLE 1 Mesenteric stenosis scoring criteria

<table>
<thead>
<tr>
<th>Severity score</th>
<th>Criteria</th>
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<tr>
<td>0</td>
<td>None, normal</td>
</tr>
<tr>
<td>1</td>
<td>Minimal, few interstitial cells in scattered foci</td>
</tr>
<tr>
<td>2</td>
<td>Mild, increased numbers of cells, clusters of 5–9 cells</td>
</tr>
<tr>
<td>3</td>
<td>Moderate, multifocal to coalescing foci with numerous cells &gt;10 in foci</td>
</tr>
<tr>
<td>4</td>
<td>Severe, abundant cells in coalescing foci with disruption of normal tissue architecture</td>
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*Inflammation was characterized by types and numbers of inflammatory cells, distribution in the tissues, and severity.*
LFD were seen starting at 1 wk post diet initiation and remained such for the entire study period (Supplemental Fig. 1). The rapid weight gain was likely due to the higher caloric content in the HFD (22.4 MJ/kg diet) compared with the LFD (16.0 MJ/kg diet).

HFD-fed Mdr1a<sup>−/−</sup> mice had greater adiposity compared with the LFD-fed Mdr1a<sup>−/−</sup> mice (Fig. 1A), whereas adiposity did not significantly differ between the HFD-fed and LFD-fed WT mice. Concentrations of circulating leptin, an adipokine known to increase in obesity (28), were significantly higher in the HFD-fed mice compared with mice of the same genotype consuming the LFD (Fig. 1B).

The HFD resulted in insulin resistance and fatty liver in Mdr1a<sup>−/−</sup> and WT mice. Feed-deprived blood glucose concentrations between mice fed the HFD and LFD did not significantly differ at any time point tested during the study in both genotypes (Fig. 2A,B). However, HFD-fed mice had a slower blood glucose reduction 30, 60, and 90 min after i.p. insulin injection (P < 0.05; HFD vs. LFD of the same genotype), suggesting increased insulin resistance in HFD-fed mice compared with LFD-fed mice of both genotypes (Fig. 2C).

At the termination of the study, we noticed that the livers of mice fed the HFD were mildly enlarged with rounded edges and a pale yellow reticulated pattern suggestive of a fatty liver. Both genotypes and diets affected plasma TG and cholesterol concentrations to varying degrees (Fig. 3A,B). Plasma TG concentrations were higher in mice fed the LFD compared with those fed the HFD within the same genotype (Fig. 3A) and this effect appeared to be more pronounced in WT mice. Plasma cholesterol concentrations were also elevated in LFD-fed mice compared with HFD-fed mice of the same genotype but were significantly different only in WT mice (Fig. 3B). Genotypes and diets also significantly influenced total TG and cholesterol concentrations in liver (Fig. 3C,D). However, the increase in total TG concentrations was found in HFD-fed mice compared with LFD-fed mice within the same genotype, whereas the HFD increased total cholesterol concentrations only in Mdr1a<sup>−/−</sup> mice (Fig. 3D).

The HFD increased severity of IBD in Mdr1a<sup>−/−</sup> mice. Beginning at 7 wk after diet initiation, diarrhea, presumably associated with IBD, was observed in all cages housing Mdr1a<sup>−/−</sup> mice fed the HFD. One of 10 Mdr1a<sup>−/−</sup> mice fed the HFD also showed dramatic weight loss toward the end of the 12-wk diet period and had severe pan-colitis at necropsy. Although the other
FIGURE 3 Total TG (A,C) and cholesterol (B,D) concentrations in plasma and liver. Horizontal bars represent means ± SEMs, n = 10–11. Asterisks show significant differences between mice fed the HFD and the LFD of the same genotype: **P < 0.01, ***P < 0.001, ****P < 0.0001.

3 Mdr1a−/− in the same diet group did not lose weight, 100% (10/10) of Mdr1a−/− mice fed the HFD had gross evidence of moderate to severe IBD involving both proximal and distal colon at necropsy performed after 12 wk of the diet; colons were diffusely thickened and opaque and lacked formed fecal pellets in the distal portion (Supplemental Fig. 2). In contrast, 45% (5/11) of Mdr1a−/− mice fed the LFD appeared to have mild IBD involving only proximal colon with formed fecal pellets in the distal portion. Thus, by gross examination, the incidence of IBD was higher in Mdr1a−/− mice fed the HFD compared with the Mdr1a−/− mice fed the low-fat diet (P = 0.012; Fisher’s exact test). We did not observe any gross or histologic abnormalities in colons of WT mice regardless of diet (0/20).

Histopathology was performed to determine the severity of IBD and to confirm our gross findings. Histologically, Mdr1a−/− mice fed the HFD had markedly thickened mucosa with elongated crypts, loss of goblet cells and prominent intramucosal lymphocytic accumulations (Fig. 4A). Cryptitis and crypt abscesses were also present (Fig. 4B). Both genotype and diet influenced the severity of IBD (Fig. 4C). The mean IBD score was significantly higher in Mdr1a−/− mice fed the HFD compared with those fed the LFD, whereas no differences were found in WT mice fed the 2 diets. This finding is in agreement with our observations at gross examination that the HFD induced more severe IBD in Mdr1a−/− mice during the 12-wk diet period compared with the LFD.

The HFD was associated with inflammation in mesenteric adipose tissue. Because obesity-associated adipose expansion is known to induce chronic inflammatory responses in visceral adipose tissues and HFD-induced obesity in our mice, we analyzed mesenteric fat and MLN for inflammation (Fig. 5). Mesenteric fat associated with small intestine was sampled for this purpose, as mesenteric fat associated with large bowel may also be inflamed due to a regional effect of active colitis. Hematoxylin and eosin-stained tissue sections from mice fed the HFD showed that the adipocytes in mesenteric fat near the proximal colon were surrounded by large pink cells with abundant, occasionally foamy cytoplasm (macrophages, thin arrow), lymphocytes, and plasma cells (circle) and fewer small bright pink cells with segmented nuclei (neutrophils, fat, thick arrows) (Fig. 5A). In comparison, inflammation in mesenteric fat near the duodenum (Fig. 5B) was principally composed of foamy macrophages (arrow) with fewer lymphocytes, plasma cells, and rare neutrophils. Both WT and Mdr1a−/− mice fed the HFD gained more weight (Supplemental Fig. 2) and had more severe inflammation in mesenteric fat and MLN compared with mice fed the LFD (Fig. 5C,D). Interestingly, increased body weight was significantly associated with more severe inflammation in mesenteric fat and MLN when data from all mice were included for regression analysis (Fig. 5E,F).

Discussion

As obesity increases, it has become a greater contributor to morbidity than smoking in the U.S. population (29). Obesity is also a risk factor for chronic diseases such as cardiovascular disease, diabetes, osteoarthritis, and some types of cancers (30). Inflammation plays a common and critical role in these chronic disease conditions, including obesity (30). Interestingly, higher BMI has been associated with shorter time to first surgery in CD patients (31), and obese patients have a greater tendency to develop active disease and require hospitalization (32). Hence, we hypothesized that local and systemic, low-grade, chronic inflammation found in obesity may adversely influence IBD progression in susceptible individuals.
To test our hypothesis, we chose an animal model that has features resembling CD: Mdr1a<sup>2/2</sup> mice (7). Similar to CD patients, Mdr1a<sup>2/2</sup> mice are immune competent but spontaneously develop IBD with age (7). In addition, its background strain, FVB, has been shown to develop a varying degree of obesity and metabolic abnormalities when fed a HFD (23,24,33). The HFD-induced obesity in both WT and Mdr1a<sup>2/2</sup> mice and, as expected, leptin concentrations were also elevated in these mice (Supplemental Fig. 1; Fig. 1). Leptin is an adipokine that increases with expanding fat deposition in adipose tissues and is a good indicator of body fat mass, a better predictor than BMI in humans (34). Leptin also has been shown to have a proinflammatory role in animal models of colitis (8,35). Thus, the HFD appears to result in a systemic increase in the proinflammatory molecule, leptin, by inducing obesity.

HFD-fed mice developed fatty liver by the end of the study period regardless of genotype. Liver lipid analysis confirmed this...
observation: total TG and cholesterol concentrations in liver were higher in HFD-fed mice (Fig. 3C,D). In contrast, plasma TG concentrations were higher in LFD-fed mice in both genotypes, which has been documented in other strains of mice (36,37). We postulate that this increase in plasma TGs in LFD-fed mice is likely due to the higher carbohydrate content in the LFD compared with the HFD (75.5 vs. 31.4% by calories), which would promote TG formation in liver for secretion into blood as VLDL (38). However, it is unclear why the HFD decreased circulating concentrations of cholesterol in both genotypes of mice.

In addition to an increase in systemic inflammatory markers, obesity is also associated with adipose tissue inflammation, which in turn contributes to metabolic abnormalities (39–41). One of the manifestations of CD is known as “creeping fat,” where fat wraps around inflamed bowel, which is also associated with transmural inflammation and perivascular infiltrations of macrophages (18). It is unclear whether this creeping fat contributes to IBD or is a consequence of IBD. We found that increased body weight was associated with inflammation in mesenteric fat (Fig. 5) in both WT and Mdr1a−/− mice. Interestingly, increased body weight was also associated with inflammation in MLNs as well as mesenteric fat. Thus, it appears that the diet-induced obesity likely elicits inflammatory responses locally and systemically in both WT and Mdr1a−/− mice. However, only Mdr1a−/− mice, genetically susceptible to disease, developed significant IBD. These data would suggest that the HFD-induced obesity itself does not cause IBD but may be a risk factor for disease in susceptible individuals.

Other animal models are consistent with this notion. Using a Dextran sodium sulfate (DSS) colitis model in C57BL/6 mice, Teixeira et al. (42) suggested that a HFD-induced obesity exacerbated IBD and increased immune cell infiltration in adipose tissues. However, because DSS induces acute and severe IBD, mice fed a HFD lost considerable amounts of weight and did not recover fully by the end of the study. Despite the loss of adipose tissue during DSS treatment, HFD-fed mice developed more severe colitis that was associated with increased macrophage infiltration in adipose tissues. Because 2 cycles of DSS treatment (wk 1 and 4) were performed during the study period (8 wk) and mice lost weight during the treatment, it is hard to determine whether significant obesity would have been achieved before the DSS treatment. If animals did not gain significant weight, then one might suggest that a HFD itself may contribute to exacerbation of IBD in this model.

Epidemiologic studies suggest that a HFD may be a risk factor for the development of IBD (43). In addition, type of fat may influence IBD differentially due to its effects on inflammatory processes (43). Because a HFD is a cause of obesity in our animal model, we are not able to separate the contribution of obesity from the HFD in the development or exacerbation of IBD. Pair-feeding studies (HFD-fed mice provided with a diet equal in calories to LFD-fed mice) could be conducted to decipher the potential contribution of a HFD compared with obesity to development of IBD.

Changes in microbiota in response to an HFD may play a role in IBD pathogenesis, and animal models may be useful to dissect any association. It has been shown that a HFD and/or obesity increase intestinal permeability and bacterial translocation from the intestinal lumen to mesenteric fat in mice as well as induce changes in the microbiota (48,49). HFD and obesity are also associated with increased inflammation in adipose tissue demonstrated by increased macrophage infiltration and increased inflammatory cytokines such as TNFα (3,50,51). We found that HFD-induced obesity significantly increased the severity of IBD in the genetically susceptible Mdr1a−/− mouse model. The fact that obesity and increased inflammation occurred in both WT and Mdr1a−/−, yet IBD only developed and exacerbated in Mdr1a−/−, suggests that additional genetic factor(s) are required before obesity becomes a risk factor for IBD. For Mdr1a−/− mice, this susceptibility is likely due to “leaky gut” (7), where a HFD-induced obesity or HFD itself further compromised the intestinal barrier, resulting in increased bacterial load and ensuing inflammation in both the intestine as well as the adjacent adipose tissue, mesenteric fat.

Acknowledgments
J.P., T.B., and L.M.-P. designed the research; J.P., Y.E., and P.M.T. conducted the research; J.P., P.M.T., T.B., and L.M.-P.
analyzed the data; J.P., Y.F., P.M.T., T.B., and L.M.-P wrote the paper; and J.P. had primary responsibility for the final content. All authors read and approved the final manuscript.

**Literature Cited**

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**Obesity and inflammatory bowel disease**

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