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High-Fat Diet-Induced Obesity Exacerbates Inflammatory Bowel Disease in Genetically Susceptible *Mdr1a*$^{-/-}$ Male Mice[1-3]

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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-*Abcb1a*^{tm1Bor}N7 (*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 ($R^2 = 0.5$; $P < 0.0001$) and mesenteric lymph nodes ($R^2 = 0.4$; $P < 0.0001$). 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)[6] 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 animal models of IBD as well as patients with Crohn's disease (CD) and ulcerative colitis (5–10). Treatment to prevent inflammation or inhibit expression of genes involved in inflammation has been shown to improve insulin sensitivity in obese animals (4,11). In addition, epidemiological evidence indicates that increased fat 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.

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

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[3] Supplemental Figures 1 and 2 and Table 1 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[6] Abbreviations used: CD, Crohn's disease; DSS, dextran sodium sulfate; HFD, high-fat diet; IBD, inflammatory bowel disease; LFD, low-fat diet; MDR, multidrug resistance protein; MLN, mesenteric lymph node; PGP, P-glycoprotein; WT, wild type.
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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$^{-/-}$ 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-*Abcb1a*^{tm1Bor}N7 (*Mdr1a*$^{-/-}$) 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-3, pneumonia virus of mice, epizootic diarrhea of infant mice, Theiler's murine encephalomyelitis virus, lymphocytic choriomeningitis virus, ectromelia, Sendai virus, sialodacryoadenitis 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 Humilin (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 CO_2 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 changes 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$^{-/-}$ *and WT mice.* Dietary treatment was given to mice at 6 wk old, long before spontaneous IBD develops in *Mdr1a*$^{-/-}$ mice (7). Both WT and *Mdr1a*$^{-/-}$ 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 steatitis scoring criteria[1]

Severity score	Criteria
0	None, normal
1	Minimal, few interstitial cells in scattered foci
2	Mild, increased numbers of cells, clusters of 5–9 cells
3	Moderate, multifocal to coalescing foci with numerous cells >10 in foci
4	Severe, abundant cells in coalescing foci with disruption of normal tissue architecture

[1] Inflammation was characterized by types and numbers of inflammatory cells, distribution in the tissues, and severity.

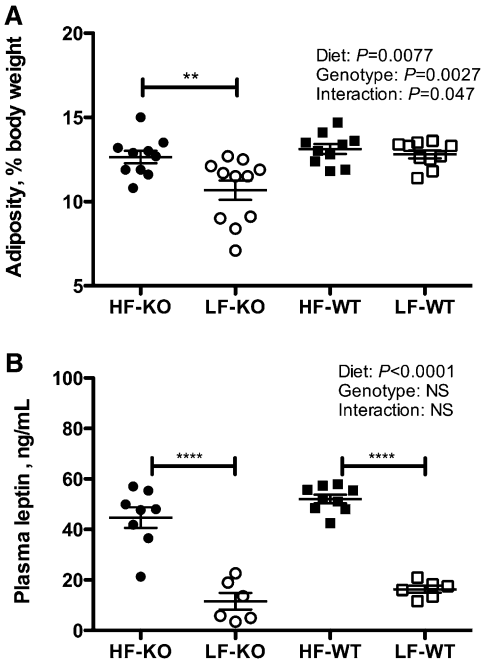

FIGURE 1 Adiposity (*A*) and plasma leptin concentrations (*B*) in *Mdr1a*$^{-/-}$ and WT mice fed an HFD or LFD for 12 wk beginning at 6 wk of age. Horizontal bars represent means ± SEMs, n = 10–11. Asterisks show significant differences between mice fed the HFD compared with the LFD of the same genotype: $^{**}P < 0.01$, $^{****}P < 0.0001$. HFD, high-fat diet; HF-KO, *Mdr1a*$^{-/-}$ mice fed the high-fat diet; HF-WT, WT mice fed the high-fat diet; LFD, low-fat diet; LF-KO, *Mdr1a*$^{-/-}$ mice fed the low-fat diet; LF-WT, WT mice fed the low-fat diet; WT, wild type.

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*$^{-/-}$ mice had greater adiposity compared with the LFD-fed *Mdr1a*$^{-/-}$ mice (**Fig. 1***A*), 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. 1*B*).

The HFD resulted in insulin resistance and fatty liver in Mdr1a$^{-/-}$ *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. 2***A*,*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. 2*C*).

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. Thus, we determined TGs and total cholesterol from terminal plasma samples as well as liver homogenates. Both genotypes and diets affected plasma TG and cholesterol concentrations to varying degrees (**Fig. 3***A*,*B*). Plasma TG concentrations were higher in mice fed the LFD compared with those fed the HFD within the same genotype (Fig. 3*A*) 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. 3*B*). Genotypes and diets also significantly influenced total TG and cholesterol concentrations in liver (Fig. 3*C*,*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*$^{-/-}$ mice (Fig. 3*D*).

The HFD increased severity of IBD in Mdr1a$^{-/-}$ *mice.* Beginning at 7 wk after diet initiation, diarrhea, presumably associated with IBD, was observed in all cages housing *Mdr1a*$^{-/-}$ mice fed the HFD. One of 10 *Mdr1a*$^{-/-}$ 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

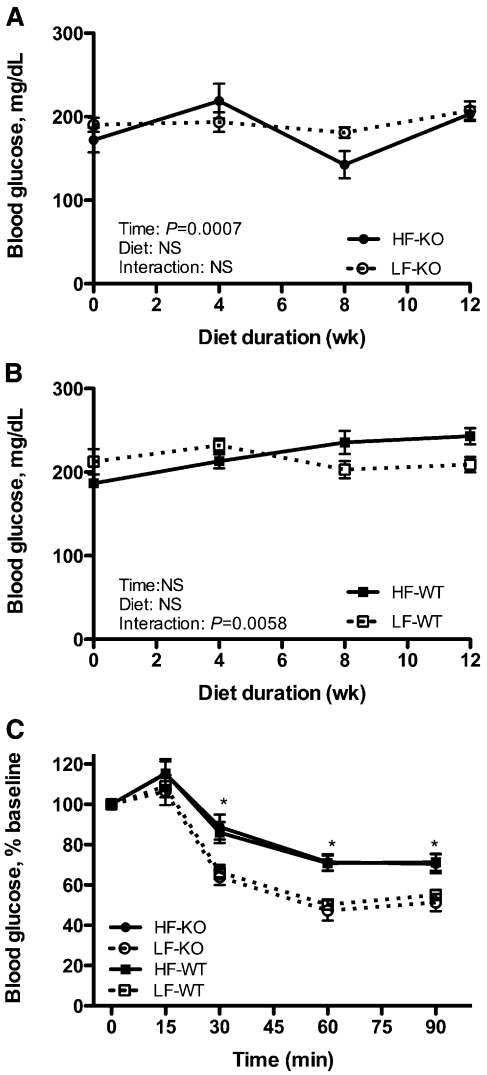

FIGURE 2 Feed-deprived blood glucose concentrations of *Mdr1a*$^{-/-}$ (*A*) and WT (*B*) mice at 4-wk intervals during the 12-wk experimental period and insulin tolerance test (*C*) performed at 10 wk post diet initiation. Values are means ± SEMs, n = 10–11. Asterisk shows significant differences between mice fed the HFD compared with the LFD of the same genotype, $P < 0.05$. HFD, high-fat diet; HF-KO, *Mdr1a*$^{-/-}$ mice fed the high-fat diet; HF-WT, WT mice fed the high-fat diet; LFD, low-fat diet; LF-KO, *Mdr1a*$^{-/-}$ mice fed the low-fat diet; LF-WT, WT mice fed the low-fat diet; WT, wild type.

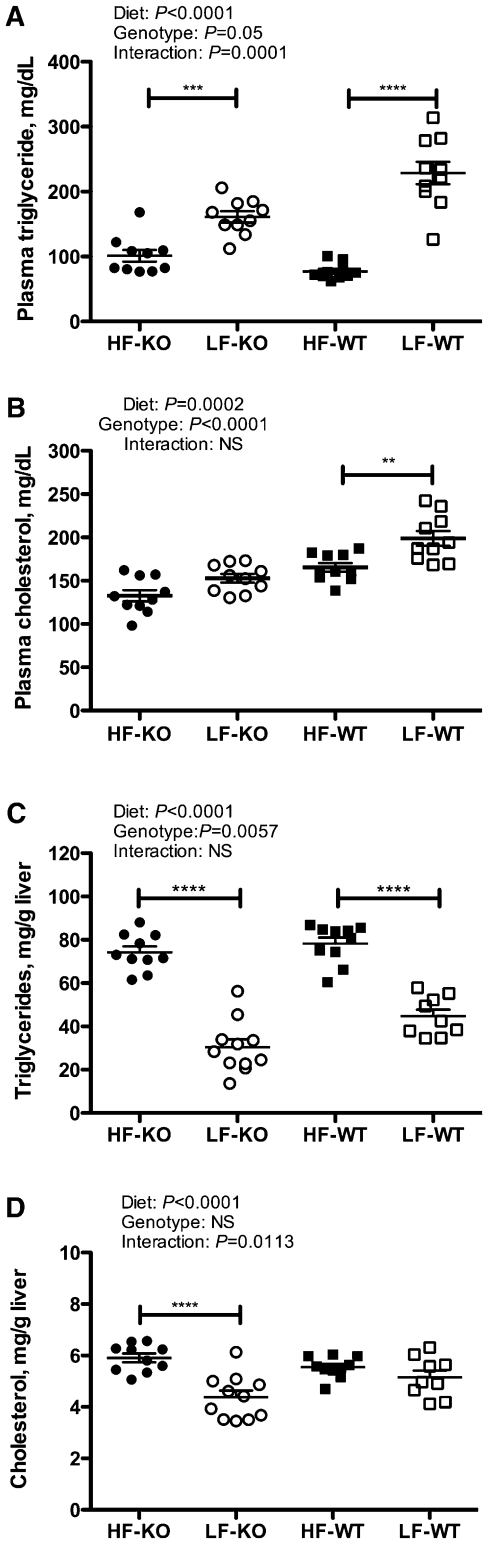

FIGURE 3 Total TG (*A,C*) and cholesterol (*B,D*) concentrations in plasma and liver. Horizontal bars represent means ± SEMs, n = 10–11. Asterisks show significant differences between mice fed the HFD and the LFD of the same genotype: $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. HFD, high-fat diet; HF-KO, *Mdr1a*$^{-/-}$ mice fed the high-fat diet; HF-WT, WT mice fed the high-fat diet; LFD, low-fat diet; LF-KO, *Mdr1a*$^{-/-}$ mice fed the low-fat diet; LF-WT, WT mice fed the low-fat diet; WT, wild type.

9 *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. 4***A*). Cryptitis and crypt abscesses were also present (Fig. 4*B*). Both genotype and diet influenced the severity of IBD (Fig. 4*C*). 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. 5*A*). In comparison, inflammation in mesenteric fat near the duodenum (Fig. 5*B*) 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. 1) and had more severe inflammation in mesenteric fat and MLN compared with mice fed the LFD (Fig. 5*C,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. 5*E,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.

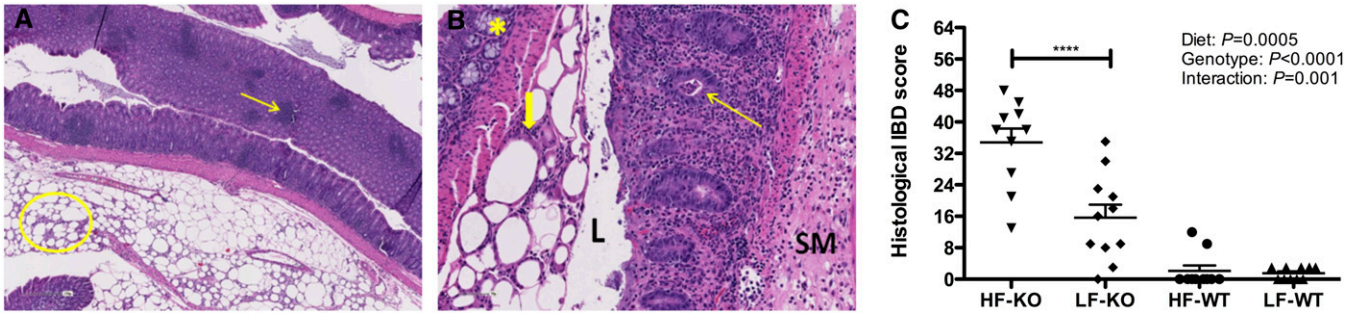

FIGURE 4 Representative H&E-stained Swiss roll histological sections from a *Mdr1a*$^{-/-}$ mouse fed the HFD [50× (A) and 200× (B)] and IBD scores (*C*) determined from H&E-stained cecum and colon sections. (*A*) The proximal colon is markedly inflamed with a thickened mucosa and numerous intramucosal lymphoid aggregates (arrow). Note the adjacent mesenteric fat has multifocal to coalescing inflammation (circle). (*B*) Section of proximal colon adjacent to neighboring mesentery. This orientation where a lumen (L) of one section of proximal colon mucosa is adjacent to the mesentery of mid colon segment is a result of the Swiss roll preparation where loops of bowel are in close proximity. The mucosa is markedly inflamed with crypt loss and replacement by inflammatory cells and loss of goblet cells (in contrast to adjacent bowel, asterisk, where goblet cells are clearly seen as light blue staining large cells lining crypts). Additionally, there is a crypt abscess (arrow) and expansion of the submucosa (SM) with inflammatory cells and edema. Note the adjacent mesenteric fat is hypercellular with abundant mononuclear inflammatory cells (thick arrow). Asterisks show significant differences between mice fed the HFD vs. LFD of the same genotype: ****$P < 0.0001$. H&E, hematoxylin and eosin; HFD, high-fat diet; HF-KO, *Mdr1a*$^{-/-}$ mice fed the high-fat diet; HF-WT, WT mice fed the high-fat diet; IBD, inflammatory bowel disease; LFD, low-fat diet; LF-KO, *Mdr1a*$^{-/-}$ mice fed the low-fat diet; HFD-induced LF-WT, WT mice fed the low-fat diet; WT, wild type.

To test our hypothesis, we chose an animal model that has features resembling CD: *Mdr1a*$^{-/-}$ mice (7). Similar to CD patients, *Mdr1a*$^{-/-}$ 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*$^{-/-}$ 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

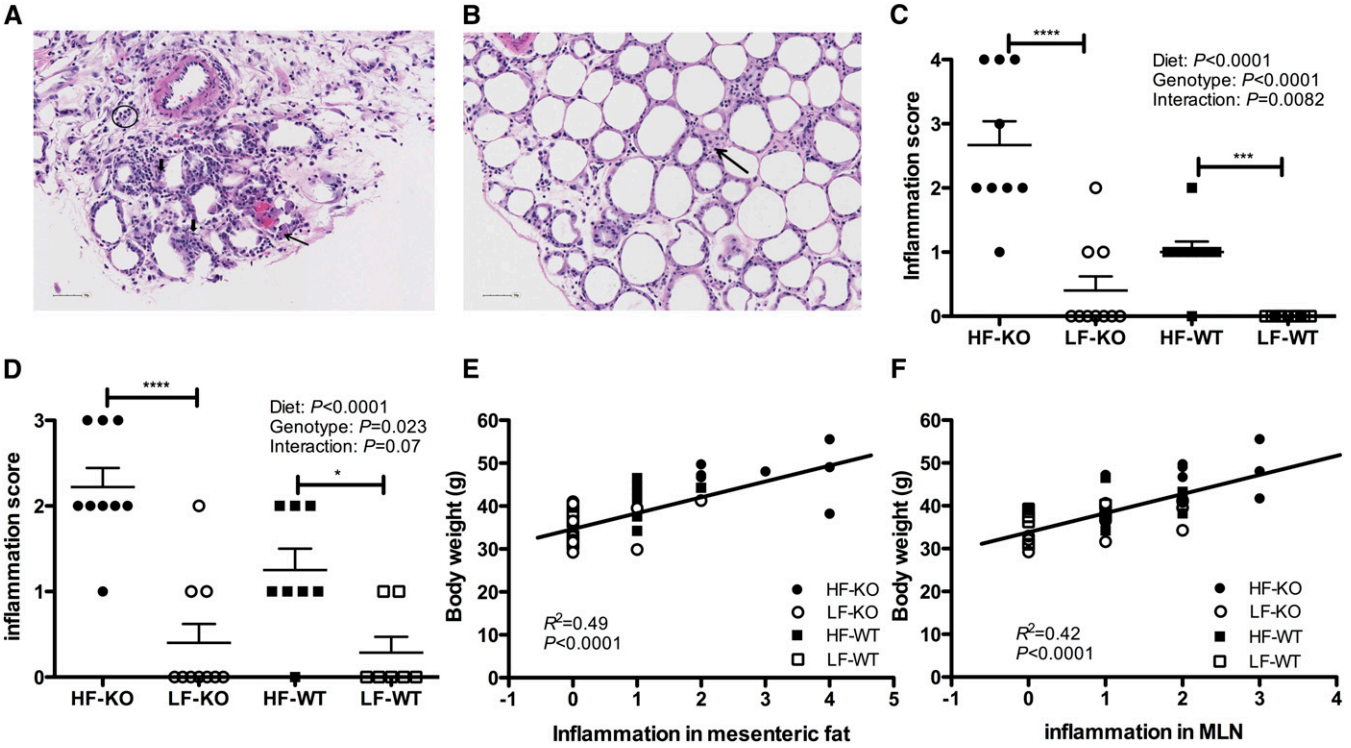

FIGURE 5 Representative H&E-stained mesenteric fat near proximal colon (*A*) and duodenum (*B*). Original magnification, 200×. Inflammation score of mesenteric fat (*C*) and MLN (*D*). Mesenteric fat and MLN sections were stained with H&E and graded for severity of inflammation. Body weight was positively correlated with mesenteric steatitis (*E*) and lymphadenitis (*F*). H&E, hematoxylin and eosin; HF-KO, *Mdr1a*$^{-/-}$ mice fed the high-fat diet; HF-WT, WT mice fed the high-fat diet; LF-KO, *Mdr1a*$^{-/-}$ mice fed the low-fat diet; LF-WT, WT mice fed the low-fat diet; MLN, mesenteric lymph node; WT, wild type.

observation: total TG and cholesterol concentrations in liver were higher in HFD-fed mice (Fig. 3*C*,*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, as a HFD can influence microbiota, which in turn can affect host metabolism as well as gut integrity (44). We did not examine changes in microbiota in response to the HFD or their influence on intestinal epithelial cell integrity, as this was beyond the scope of this study. However, assuming that the HFD would cause similar changes in microbiota in *Mdr1a*$^{-/-}$ and WT mice housed and raised in the same facility, alterations in microbiota do not appear to be sufficient to cause IBD; the HFD induced weight gain and metabolic changes in both WT and *Mdr1a*$^{-/-}$ mice, but IBD developed only in the *Mdr1a*$^{-/-}$ mice. This is not surprising considering that complex interactions between host genes and microbiota exist. For example, although the relative abundance of bacterial phyla that are often linked to metabolic phenotypes (44) may be similar between strains of mice raised in the same facility, specific bacterial species may differ depending on genotypes of mice (45). Moreover, the same strain of mice had critical differences in metabolic as well as immune phenotypes when they were raised in different facilities (45,46). Thus, further studies are warranted to determine the differences and similarities of microbiota in *Mdr1a*$^{-/-}$ and WT mice to elucidate the contribution of microbiota in the development and progression of IBD in response to a HFD.

Foucaud-Vignault et al. (47) reported that another PGP mutant strain, *Mdr1ab*$^{-/-}$ mice (lacking both *Mdr1a* and *1b*), develop obesity and abnormal glucose metabolism with age compared with WT mice when fed either a LFD or HFD. The authors suggest that PGP is involved in lipid metabolism and thus, the lack of PGP may have caused these phenotypic changes. In contrast, our results show that *Mdr1a*$^{-/-}$ mice do not exhibit consistent differences in lipid metabolism compared with WT mice fed either diet and do not develop obesity unless fed a HFD. It is not clear whether the discrepancy is due to the additional deletion of *Mdr1b* in *Mdr1ab*$^{-/-}$ mice or their longer study duration (36 vs. 18 wk). If *Mdr1a*$^{-/-}$ mice are also predisposed to develop obesity as are *Mdr1ab*$^{-/-}$ mice, it is possible that the obesity phenotype may have been noticed if our study was carried out beyond 18 wk of age, based on the Foucaud-Vignault (47) study. In that study, significantly different weight gains were noted when *Mdr1ab*$^{-/-}$ mice were older than 19 wk compared with WT mice. Although not stated in the Foucaud-Vignault (47) report, *Mdr1ab*$^{-/-}$ did not appear to develop IBD, as this would cause weight loss in affected animals. Additionally, we have not observed IBD in *Mdr1ab*$^{-/-}$ even when they were infected with *H. bilis* (L Maggio-Price, E Kelly, R Ho, unpublished observation). Thus, it is likely that the phenotypes of *Mdr1a*$^{-/-}$ might be quite different from *Mdr1ab*$^{-/-}$ mice in many respects, including predisposition to develop obesity and IBD.

Obesity and inflammation in regional adipose tissue 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.

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J.P., T.B., and L.M.-P. designed the research; J.P., Y.F., 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

1. Chandalia M, Abate N. Metabolic complications of obesity: inflated or inflamed? J Diabetes Complications. 2007;21:128–36.
2. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112:1821–30.
3. Weisberg SPMD, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112:1796–808.
4. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science. 2001;293:1673–7.
5. Burich A, Hershberg R, Waggie K, Zeng W, Brabb T, Westrich G, Viney JL, Maggio-Price L. Helicobacter-induced inflammatory bowel disease in IL-10- and T cell-deficient mice. Am J Physiol Gastrointest Liver Physiol. 2001;281:G764–78.
6. Maggio-Price L, Shows D, Waggie K, Burich A, Zeng W, Escobar S, Morrissey P, Viney JL. Helicobacter bilis infection accelerates and H. hepaticus infection delays the development of colitis in multiple drug resistance-deficient (mdr1a−/−) mice. Am J Pathol. 2002;160:739–51.
7. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J Immunol. 1998;161:5733–44.
8. Siegmund B, Lehr HA, Fantuzzi G. Leptin: a pivotal mediator of intestinal inflammation in mice. Gastroenterology. 2002;122:2011–25.
9. Peyrin-Biroulet L, Chamaillard M, Gonzalez F, Beclin E, Decourcelle C, Antunes L, Gay J, Neut C, Colombel J, Desreumaux P. Mesenteric fat in Crohn's disease: a pathgenetic hallmark or an innocent bystander? Gut. 2007;56:577–83.
10. Solem CA, Loftus EV Jr, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. Inflamm Bowel Dis. 2005;11:707–12.
11. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, et al. Prevention of fat-induced insulin resistance by salicylate. J Clin Invest. 2001;108:437–46.
12. Reif S, Klein I, Lubin F, Farbstein M, Hallak A, Gilat T. Pre-illness dietary factors in inflammatory bowel disease. Gut. 1997;40:754–60.
13. Geerling BJ, Dagnelie PC, Badart-Smook A, Russel MG, Stockbrugger RW, Brummer RJ. Diet as a risk factor for the development of ulcerative colitis. Am J Gastroenterol. 2000;95:1008–13.
14. Andersen V, Olsen A, Carbonnel F, Tjonneland A, Vogel U. Diet and risk of inflammatory bowel disease. Dig Liver Dis. 2012;44:185–94.
15. Gentschew L, Ferguson LR. Role of nutrition and microbiota in susceptibility to inflammatory bowel diseases. Mol Nutr Food Res. 2012;56:524–35.
16. Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H, Eckel RH. The metabolic syndrome. Endocr Rev. 2008;29:777–822.
17. Rimm AA, Hartz AJ, Fischer ME. A weight shape index for assessing risk of disease in 44,820 women. J Clin Epidemiol. 1988;41:459–65.
18. Fink C, Karagiannides I, Bakirtzi K, Pothoulakis C. Adipose tissue and inflammatory bowel disease pathogenesis. Inflamm Bowel Dis. 2012;18:1550–7.
19. Schinkel AH, Mol CA, Wagenaar E, van Deemter L, Smit JJ, Borst P. Multidrug resistance and the role of P-glycoprotein knockout mice. Eur J Cancer. 1995;31A:1295–8.
20. Maggio-Price L, Bielefeldt-Ohmann H, Treuting P, Iritani BM, Zeng W, Nicks A, Tsang M, Shows D, Morrissey P, Viney JL. Dual infection with Helicobacter bilis and Helicobacter hepaticus in p-glycoprotein-deficient mdr1a−/− mice results in colitis that progresses to dysplasia. Am J Pathol. 2005;166:1793–806.
21. Torrence AE, Brabb T, Viney JL, Bielefeldt-Ohmann H, Treuting P, Seamons A, Drivdahl R, Zeng W, Maggio-Price L. Serum biomarkers in a mouse model of bacterial-induced inflammatory bowel disease. Inflamm Bowel Dis. 2008;14:480–90.
22. Haluzik M, Colombo C, Gavrilova O, Chua S, Wolf N, Chen M, Stannard B, Dietz KR, Le Roith D, Reitman ML. Genetic background (C57BL/6J versus FVB/N) strongly influences the severity of diabetes and insulin resistance in ob/ob mice. Endocrinology. 2004;145:3258–64.
23. Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, Cushman SW, Periwal V. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLOS Comput Biol. 2009;5:e1000324.
24. Martin TL, Alquier T, Asakura K, Furukawa N, Preitner F, Kahn BB. Diet-induced obesity alters AMP kinase activity in hypothalamus and skeletal muscle. J Biol Chem. 2006;281:18933–41.
25. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr. 1997;127:S838–41.
26. Moolenbeek C, Ruitenberg EJ. The "Swiss roll": a simple technique for histological studies of the rodent intestine. Lab Anim. 1981;15:57–9.
27. Paik J, Fierce Y, Drivdahl R, Treuting PM, Seamons A, Brabb T, Maggio-Price L. Effects of murine norovirus infection on a mouse model of diet-induced obesity and insulin resistance. Comp Med. 2010;60:189–95.
28. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995;269:540–3.
29. Jia H, Lubetkin EI. Trends in quality-adjusted life-years lost contributed by smoking and obesity. Am J Prev Med. 2010;38:138–44.
30. Malnick SD, Knobler H. The medical complications of obesity. QJM. 2006;99:565–79.
31. Hass DJ, Brensinger CM, Lewis JD, Lichtenstein GR. The impact of increased body mass index on the clinical course of Crohn's disease. Clin Gastroenterol Hepatol. 2006;4:482–8.
32. Blain A, Cattan S, Beaugerie L, Carbonnel F, Gendre JP, Cosnes J. Crohn's disease clinical course and severity in obese patients. Clin Nutr. 2002;21:51–7.
33. Boudina S, Sena S, Sloan C, Tebbi A, Han YH, O'Neill BT, Cooksey RC, Jones D, Holland WL, McClain DA, et al. Early mitochondrial adaptations in skeletal muscle to diet-induced obesity are strain dependent and determine oxidative stress and energy expenditure but not insulin sensitivity. Endocrinology. 2012;153:2677–88.
34. Shah NR, Braverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. PLoS ONE. 2012;7:e33308.
35. Hoda MR, Scharl M, Keely SJ, McCole DF, Barrett KE. Apical leptin induces chloride secretion by intestinal epithelial cells and in a rat model of acute chemotherapy-induced colitis. Am J Physiol Gastrointest Liver Physiol. 2010;298:G714–21.
36. Kirk EA, Moe GL, Caldwell MT, Lernmark JA, Wilson DL, LeBoeuf RC. Hyper- and hypo-responsiveness to dietary fat and cholesterol among inbred mice: searching for level and variability genes. J Lipid Res. 1995;36:1522–32.
37. Biddinger SB, Almind K, Miyazaki M, Kokkotou E, Ntambi JM, Kahn CR. Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearoyl-CoA desaturase 1, and the development of the metabolic syndrome. Diabetes. 2005;54:1314–23.
38. Mayes P. Lipid transport and storage. In: Murray R, Granner D, Mayes P, Rodwell V, editors. Harper's biochemistry. 22nd ed. Norwalk (CT): Appleton & Lange; 1990. p. 234–48.
39. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007;117:175–84.
40. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes. 2007;56:16–23.
41. Guri AJ, Bassaganya-Riera J. Systemic effects of white adipose tissue dysregulation and obesity-related inflammation. Obesity (Silver Spring). 2011;19:689–700.
42. Teixeira LG, Leonel AJ, Aguilar EC, Batista NV, Alves AC, Coimbra CC, Ferreira AV, de Faria AM, Cara DC, Alvarez Leite JI. The combination of high-fat diet-induced obesity and chronic ulcerative colitis reciprocally exacerbates adipose tissue and colon inflammation. Lipids Health Dis. 2011;10:204.
43. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol. 2011;106:563–73.
44. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest. 2011;121:2126–32.

45. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010;328:228–31.
46. Letran SE, Lee SJ, Atif SM, Flores-Langarica A, Uematsu S, Akira S, Cunningham AF, McSorley SJ. TLR5-deficient mice lack basal inflammatory and metabolic defects but exhibit impaired CD4 T cell responses to a flagellated pathogen. J Immunol. 2011;186:5406–12.
47. Foucaud-Vignault M, Soayfane Z, Menez C, Bertrand-Michel J, Martin PG, Guillou H, Collet X, Lespine A. P-glycoprotein dysfunction contributes to hepatic steatosis and obesity in mice. PLoS ONE. 2011;6:e23614.
48. Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermudez-Humaran LG, Smirnova N, Berge M, Sulpice T, et al. Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol Med. 2011;3:559–72.
49. Lam YY, Ha CW, Campbell CR, Mitchell AJ, Dinudom A, Oscarsson J, Cook DI, Hunt NH, Caterson ID, Holmes AJ, et al. Increased gut permeability and microbiota change associate with mesenteric fat inflammation and metabolic dysfunction in diet-induced obese mice. PLoS ONE. 2012;7:e34233.
50. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW, Jr. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest. 2006;116:115–24.
51. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259:87–91.