Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), is a chronic disorder of the gastrointestinal tract. Considerable variation in the epidemiology of IBD has been observed around the world, with the highest incidence rates and prevalence in North America and Europe; notably, over 1.4 million people in the United States suffer from IBD (32, 41). Although the etiology of IBD is not fully understood, it is thought to involve a multifactorial interaction of inheritable components, environmental stress, microbial insults, and autoimmune events (24, 28). Currently, a number of experimental IBD models have been established, e.g., dextran sulfate sodium (DSS)- and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced IBD models, that closely resemble the clinical and morphological features of human UC and CD. Although none of these models precisely mimic the human disorders, they have proven to be useful for a more complete understanding of the mechanistic basis of this disease, identifying new targets for therapeutic intervention, and testing novel therapeutic agents (30, 46, 51).

Peroxisome proliferator-activated receptor-α (PPARα) is a nuclear hormone receptor that serves as a xenobiotic and lipid sensor to regulate energy combustion, hepatic steatosis, lipoprotein synthesis, inflammation, and liver cancer (42). Fibrates such as fenofibrate are PPARα agonists and have been used for several decades as hypolipidemic agents in the clinic. However, contradictory observations exist on the role of fibrates in host response to acute inflammation, with unclear mechanisms. The role of PPARα in colitis was assessed using fenofibrate and Ppara-null mice. Wild-type or Ppara-null mice were subjected to acute colitis under three different protocols, dextran sulfate sodium, trinitrobenzenesulfonic acid, and Salmonella Typhi.

Serum and colon lipidomics were analyzed to characterize the metabolic profiles by ultra-performance liquid chromatography-coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. Messenger RNAs of PPARα target genes and genes involved in inflammation were determined by quantitative PCR analysis. Fenofibrate treatment exacerbated inflammation and tissue injury in acute colitis, and this was dependent on PPARα activation. Lipidomics analysis revealed that bioactive sphingolipids, including sphingomyelins (SM) and ceramides, were significantly increased in the colitis group compared with the control group; this was further potentiated following fenofibrate treatment. In the colon, fenofibrate did not reduce the markedly increased expression of mRNA encoding TNFα found in the acute colitis model, while it decreased hydrolysis and increased synthesis of SM, upregulated RIPK3-dependent necrosis, and elevated mitochondrial fatty acid β-oxidation, which were possibly related to the exacerbated colitis.

**PPARα; lipidomics; fenofibrate; colitis; sphingomyelin**

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ample evidence emphasizes their role in the regulation of inflammatory responses (5, 7, 16, 49). In this study, ultra-performance liquid chromatography, coupled with electrospray ionization quadrupole time of flight mass spectrometry (UPLC-ESI-QTOFMS), was used to profile the lipidome of experimental colitis mice treated with fenofibrate. UPLC-ESI-QTOFMS is an analytical platform that has been employed for the detection and characterization of small organic molecules in biological systems (22, 25, 39, 40). With the aid of multivariate analysis methods, metabolite markers associated with colitis were identified, and the roles of fenofibrate on colitis were explored.

MATERIALS AND METHODS

Chemicals and reagents. Lipid markers such as N-palmitoyl-t-erythro-sphingosylphosphorylcholine (SM 16:0) and N-palmitoyl-t-erythro-sphingosine (C16 ceramide) were obtained from Avanti Polar Lipids (Alabaster, AL). All solvents and organic reagents were of the highest obtainable grade.

Mice and treatments. Wild-type (WT) and Ppara-null male mice, 6- to 8-wk-old, on the C57Bl6/N background, were fed either standard NIH31 diet or an NIH31 modified diet containing 0.1% fenofibrate ad libitum (Bioserv, Frenchtown, NJ) 3 days before the experiment and through the end of the study. Mice in the dextran sulfate sodium (DSS)-induced colitis groups received 3.0% DSS (MP Biomedicals, Solon, OH) in drinking water (wt/vol). For the TNBS-induced colitis model, a 2 mg of TNBS in 50% ethanol were administered intrarectally to anesthetized mice via a 50.8-mm round-bottom needle. For the Salmonella Typhi (S. Typhi) experiment, mice were dosed with streptomycin at 75 mg/kg body wt by gavage, and 24 h following antibiotic treatment, the mice were gavaged with S. Typhi. For all these colitis models, the presence of fenofibrate in the diet did not affect food or water intake compared with the control groups not administered the drug. The mice were killed 7 days after DSS administration, 3 days after TNBS administration, or 4 days after S. Typhi administration, all after 8 h of overnight fasting, and the colons were flushed and resected. Serum samples were collected by retro-orbital bleeding, and tissue samples were harvested and stored at −80°C before analysis. The National Cancer Institute or University of Michigan Animal Care and Use Committee approved all animal studies.

Colitis evaluation. Daily changes of body weights were recorded and diarrhea, rectal bleeding, and bloody stool were assessed daily and reported as a score from 0 to 4. For assessment of macroscopic colon damage, the colon was opened longitudinally, flushed with phosphate-buffered saline, and fixed in 10% buffered formalin. Colitis was measured by blinded analysis on a routine hematoxylin and eosin-stained section according to the morphological criteria described previously (13, 38).

Lipidomics. For serum lipidomics analysis 25 µl serum were extracted by 4-fold cold chloroform:methanol (2:1) solution containing 2 µM lysophosphatidylcholine (LPC; 17:0), phosphatidylcholine (PC; 17:0), sphingomyelin (SM; 17:0), and ceramide (CER; 17:0) from Avanti Polar Lipids, Alabaster, AL) as internal standards. The samples were vortexed for 30 s and then allowed to stand for 5 min at room temperature. The mixture was centrifuged at 13,000 rpm for 5 min, and then the lower organic phase was collected and evaporated at room temperature under vacuum, and the residue was dissolved in chloroform:methanol (1:1), followed by diluting with isopropanol:acetone/methanol:H2O (2:1:1) before UPLC-MS analysis. For tissue lipidomics analysis, ~50 mg accurately weighted tissues were homogenized with 700 µl methanol:H2O (4:3) solution and then extracted using 800 µl chloroform containing 2 µM LPC (17:0), PC (17:0), SM (17:0), and CER (17:0) as internal standards. The homogenate was incubated at 37°C for 20 min followed by centrifuged for 20 min at 13,000 rpm. The lower organic phase was transferred to a new tube and dried under vacuum. The residue was suspended with 100 µl chloroform: methanol (1:1) solution and then diluted with isopropanol:acetone/methanol:H2O (2:1:1) solution before injection.

For lipidomics discovery, samples were analyzed by UPLC-ESI-QTOFMS using a Waters Acuity CSH 1.7-µm C18 column (2.1 × 100 mm) under the following conditions: UPLC: A: acetonitrile/water (60/40); B: isopropanol/acetone (90/10), and both A and B contained 10 mM ammonium acetate and 0.1% formic acid. Gradient was initial 60% A to 57% A at 2 min, to 50% A at 2.1 min*, to 46% A at 12 min, to 30% A at 12.1 min*, to 1% A at 18 min before returning to initial conditions at 18.5 min with equilibration for 2 additional minutes (* indicates ballistic gradient). Flow rate was 0.4 ml/min. Column temperature was maintained at 55°C. MS was same conditions as above, except run time was 18 min.

Biomarkers were screened by analyzing ions in the loadings scatter plot, and metabolomics databases (METLIN and Madison Metabolomics Consortium Database) were searched to find potential candidates. To confirm the identities of the putative markers, the authentic standards were compared with the metabolites based on MS/MS fragmentation patterns and retention times. Concentrations of the metabolites were determined by multiple reaction-monitoring mass spectrometry based on standard curves using authentic standards. Quantification of lipid markers was performed by MRM and/or parent ion scanning using a Waters UPLC Acquity system coupled to a Waters Xevo TQ mass spectrometer. A Waters Acuity BEH C18 column (2.1 × 100 mm) was used. UPLC was A: water; B: acetonitrile/IPA (5/2), and both A and B contained 10 mM ammonium acetate and 0.1% formic acid. Gradient was initial 70% A for 1 min, then linear gradient to 50% A at 3 min, to 1% A at 8 min, held until 15 min, then returning to initial conditions over 1 min, and held for an additional 2 min for column equilibration. Flow rate was 0.4 ml/min. Column temperature was maintained at 50°C. Waters Xevo TQ was operated in MRM mode. The following instrument conditions were used: capillary, 2.2, kV, source temperature, 150°C, and desolvation gas flow, 850 l/h at 450°C. Total run time was 18 min. The cone voltage and collision energy for the MRM transitions were optimally determined for each transition by the instrument’s IntelliStart software.

Data processing and multivariate data analysis. Chromatographic and spectral data were deconvoluted by MarkerLynx software (Waters). A multivariate data matrix containing information on sample identity, ion identity (retention time and m/z), and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The intensity of each ion was calculated by normalizing the single ion counts vs. the total ion counts in the whole chromatogram. The data matrix was further exported into SIMCA-P software (Umetrics, Kinnelon, NJ) and transformed by mean-centering and pareto scaling, the technique that increases the importance of low abundance ions without significant amplification of noise. Statistical models including principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), were established to represent the major latent variables in the data matrix.

RNA analysis. RNA was extracted using TRIzol reagent (Invitrogen). Quantitative real-time PCR (qPCR) was performed using cDNA generated from 1 µg total RNA with the SuperScript II Reverse Transcriptase kit (Invitrogen). Primers were designed for qPCR using Primer Express software (Applied Biosystems, Foster City, CA), and sequences are available upon request. qPCRs were carried out using SYBR green PCR master mix (Applied Biosystems) in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Values were quantified using the comparative threshold cycle method, and samples were normalized to β-actin.

Western blot analysis. Protein expression of TNFα and IL-6 in colon was detected by Western blotting. Briefly, colon tissues were collected and lysed with 1× RIPA lysis buffer [50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1%
sodium dodecyl sulfate (SDS), 5 mM EDTA, 2 mM phenylmethyl-
sulfonyl fluoride (PMSF), 20 μg/ml aprotinin, 20 μg/ml leupeptin, 10
μg/ml pepstatin A, and 150 mM benzamidine] on ice for 10–15 min.
Supernatants were collected after centrifugation, and protein quanti-
fication was determined using the Pierce BCA Protein Assay Kit
(Thermo Scientific). The samples were resolved by SDS-polyacryl-
amide gel electrophoresis, transferred to polyvinylfluoride mem-
branes, and incubated with the appropriate primary and secondary
antibodies. Finally, the results were detected using the ECL Plus
Western blotting system kit (Amersham). Primary antibodies were
used in our study as follows: rabbit anti-TNFα (ab1739; Abcam) and
rabbit anti-IL-6 (ab6672; Abcam). All of the primary antibodies were
at a working dilution of 1:1,000. Secondary antibodies (1:5,000
dilution) were goat anti-rabbit IgG (sc-2004; Santa Cruz Biotechnol-
ogies). The results of immunoblotting were recorded with the Perfe-
tion 3490 photo gel imaging systems (Epson) and analyzed with
Image Pro PLUS. The expression level of target proteins was normal-
ized to GAPDH.

ELISA analysis. Colon IL-6 and TNFα levels were determined
using commercially available enzyme-linked immunosorbent assay
(ELISA) kit (JRDUN Biotechnology, Shanghai, China). Absorbance
values from each ELISA were normalized using a Pierce BCA Protein
Assay Kit (Thermo Scientific) respective to each sample, and the results
are expressed in units of nanograms per grams of tissue protein.

Fig. 1. Effect of fenofibrate on dextran sulfate sodium (DSS)-induced colitis in wild-type and Ppara-null mice (n = 4 for each group). A: fenofibrate-treated wild-type (WT) colitis group (WT-FF/DSS) had more significantly decreased body weight compared with the control diet-treated groups (***P < 0.01, comparing WT-Cont/Water and WT-Cont/DSS groups; **P < 0.01, comparing WT-Cont/DSS and WT-FF/DSS groups). B: fenofibrate-treated WT group had more significantly decreased body weight compared with the Ppara-null [knockout (KO)] groups (***P < 0.01 and *P < 0.05, comparing WT-FF/DSS and KO-FF/DSS groups). C: Comparison of the colon lengths of the 4 groups: control (WT-Cont/Water), colitis (WT-Cont/DSS), fenofibrate-treated colitis in WT mice (WT-FF/DSS), and fenofibrate-treated colitis in KO mice (KO-FF/DSS) (**P < 0.01 and *P < 0.05). D and E: rectal bleeding and diarrhea scores of the
4 groups. The scores were graded according to the disease activity index (DAI) criteria (***P < 0.01 and **P < 0.05, comparing WT-FF/DSS and KO-FF/DSS groups). F–I: representative images of hematoxylin and eosin-stained colon tissues from control, colitis, fenofibrate-treated colitis on WT mice, and fenofibrate-treated colitis on Ppara-null mice, respectively.
RESULTS

Effect of fenofibrate on colitis in WT and Ppara-null mice. Following DSS treatment mice had significantly decreased body weights and other signs of intestinal injury including rectal bleeding, diarrhea, shorter colon length, and inflammation and epithelial degeneration (13, 38). Upon treatment with a fenofibrate-supplemented diet, WT mice under the experimental colitis protocol displayed a more significant decrease in body weight (Fig. 1, A and B) and shorter colon lengths (Fig. 1C), as well as increased diarrhea and rectal bleeding scores compared with control-treated WT mice (Fig. 1, D and E). Histological analysis further revealed more severe inflammation with loss of crypts and surface epithelia in the fenofibrate-treated colitis group (Fig. 1H). However, fenofibrate-treated Ppara-null mice were less susceptible to colitis as they had higher body weights (Fig. 1B), longer colons (Fig. 1C), and milder colon inflammation (Fig. 1D) compared with fenofibrate-treated WT colitis mice. In addition fenofibrate treated mice had increased susceptibility to TNBS- (Fig. 2) and S. typhi-induced inflammation (Fig. 3), demonstrating that the effect was not limited to a single experimental colitis model. Histology further confirmed the presence of disease in these models and the exacerbation by fenofibrate treatment (Fig. 3, E and F). These results indicated that fenofibrate treatment exacerbates tissue injury and inflammation in mouse models of colitis, possibly dependent on PPARα activation.

Lipidomic analysis of experimental colitis induced by DSS. To discover biochemical markers for disease status and to gain mechanistic insights into the exacerbation of symptoms by fenofibrate in the DSS model of colitis, serum metabolic profiles of untreated WT mice, DSS-treated WT mice, fenofibrate/DSS-treated WT, and fenofibrate/DSS-treated Ppara-null mice were examined by establishing an unsupervised PCA model. Clear separation among these groups was observed based on the scores plot, with the cumulative R²X 0.658 for this model (Fig. 4A), indicating that these four groups have distinct lipidomics profiles. A similar clustering pattern of these groups was observed in extracts from colon samples (Fig. 4B). Consistent with tissue injury and inflammation (Fig. 1), both the colitis and fenofibrate-treated colitis groups were located in the same quadrant, whereas the fenofibrate-treated Ppara-null mice colitis group was more close to the control group.

OPLS-DA was then performed to maximize the difference of serum lipidomic profiles between control and colitis mice as well as to highlight potential markers that contribute to the clustering. The S-plot of OPLS-DA data revealed covariance and correlation between the variables and the model and decreases the risk of false positives in the selection of potential biomarkers (52). Using a statistically significant threshold of variable confidence ~0.75 in the S-plot, a number of ions were screened out as potential markers (Fig. 5), which were then identified by tandem mass spectrometry and retention time comparisons with authentic standards as some PC, LPC, and one SM (43). Among these lipids, and consistent with previous findings, LPC 18:0 increased whereas LPC 18:1 and LPC 18:2 decreased in the serum of mice with colitis (10). These trends were further confirmed in colons of the colitis mice in this study (data not shown).

Figure 2: Fenofibrate treatment worsened colitis phenotype on trinitrobenzenesulfonic acid (TNBS)-induced colitis model (n = 4 for each group). A: fenofibrate-treated colitis group had more significantly decreased body weight comparing to control diet-treated groups (**P < 0.01 and *P < 0.05, comparing control and control/TNBS groups; **P < 0.01 and *P < 0.05, comparing control/TNBS and FF/TNBS groups). B: fenofibrate-treated colitis group had shorter colon lengths comparing to other groups (**P < 0.01). C and D: scores of rectal bleeding and diarrhea, respectively (**P < 0.01 and *P < 0.05, comparing control/TNBS and FF/TNBS groups).
Quantitation of SM and CER in serum and colon. Among the above identified lipid metabolites, SM 16:0, a sphingomyelin metabolite was of great interest to examine further, since SM is one of the most important membrane sphingolipids and its hydrolysis by either acid sphingomyelinase or neutral sphingomyelinase (nSMase) generates CER (19, 50). Both SM and CER are bioactive sphingolipids implicated in modulating various cellular events, including proliferation, differentiation, apoptosis, and inflammation (16). SM concentrations are relatively low and CER are typically found at trace levels in tissues (18, 26), and thus they may be missed as metabolite markers using statistical methods. Therefore, to determine the level trends of SM and CER in various groups, a set of SM and CER metabolites were quantitated using UPLC-MS/MS. Serum and colon levels of SM and CER were increased in mice with colitis, and this was further potentiated in fenofibrate/DSS-treated mice. This further potentiation was not observed in fenofibrate/DSS-treated Ppara-null mice (Fig. 6). These results suggest that activation of PPARα by fenofibrate may alter levels of sphingolipids including SM and CER, which may be related to the exacerbated colitis after fenofibrate treatment.

Analysis of gene expression patterns and protein levels for IL-6 and TNFα (for DSS model). In both liver and colon of fenofibrate-treated colitis mice, the PPARα target gene mRNAs acyl-coenzyme A oxidase1 (Acox1), acyl-CoA thioesterase (Acot), and fatty acid transporter Cd36 were significantly increased compared with control diet-treated colitis mice, confirming that PPARα is activated by fenofibrate in liver (Fig. 7A) and colon (Fig. 7B) (42, 54). In colon, the mRNA encoded by genes involved in mitochondrial fatty acid β-oxidation (Fig. 7B), such as acetyl-CoA acyltransferase 1 (Acaal) and carnitine palmitoyltransferase 1 (Cpt1), were also upregulated, in-
indicating increased fatty acid β-oxidation in colon after fenofibrate treatment. Receptor-interacting protein kinase 3 (Ripk3) has emerged as a critical regulator of programmed necrosis/necroptosis to promote inflammation (33). In the colon, the expression of several genes in the RIPK3-dependent necrosis and inflammation pathway (Fig. 7C), including IL-1β (Il1b), Toll-like receptor 3 (Tlr3), and Toll-like receptor 4 (Tlr4) were induced, suggesting more severe colon damage following fenofibrate treatment. nSMase is regarded as a key enzyme in regulating activation of the sphingomyelin cycle and cell signaling (50). The mRNAs encoding those two nSMases, sphingomyelin phosphodiesterase 2 (SMPD2) and sphingomyelin phosphodiesterase 4 (SMPD4), were downregulated in the colon samples of DSS-treated WT mice, and further potentiated in fenofibrate/DSS-treated WT mice. Sphingomyelin synthase 2 (SGMS2), one of the terminal enzymes for SM de novo biosynthesis (27), was upregulated in DSS-treated WT mice (Fig. 8A). These observations support the findings of increased SM levels in serum and colon following colitis. Colon TNFα levels were dramatically upregulated in both DSS and fenofibrate/DSS-treated mice (Fig. 8B). To further confirm the pro-inflammatory cytokine upregulation in the IBD models, colon IL-6 and TNFα protein levels were measured (Fig. 9). The IBD groups had significantly increased IL-6 and TNFα protein levels compared with the control group, and according to ELISA analysis fenofibrate/DSS-treated WT mice had the highest IL-6 and TNFα protein levels.

**DISCUSSION**

In this study, fenofibrate was shown to exacerbate DSS-induced colitis. The fenofibrate effect was largely due to PPARα activation, since fenofibrate-treated Ppara-null mice were less susceptible to colitis compared with fenofibrate-treated WT mice. This phenotype was further confirmed using TNBS and *S. typhi*-induced colitis models. Lipidomics revealed SM 16:0 as a lipidomic marker for disease, suggesting the possible importance of bioactive sphingolipids in colitis.

![Fig. 4](http://ajpgi.physiology.org/)

**Fig. 4.** A: principal component analysis (PCA) of serum samples (with the cumulative R²X 0.658). B: partial least squares-discriminant analysis (PLS-DA) analysis of colon samples (with the cumulative R²X 0.555, R²Y 0.93, and Q² 0.634) from all the 4 examined groups, i.e., control (WT-CTR/Water), inflammatory bowel disease (IBD) (WT-CTR/DSS), fenofibrate-treated colitis [WT-FF/DSS on WT mice, and KO-FF/DSS on Ppara-null (KO) mice], clear separation among these groups was observed based on the scores plot.

![Fig. 5](http://ajpgi.physiology.org/)

**Fig. 5.** OPLS-DA analysis of control vs. colitis mouse serum. A: based on the score plots, distinct serum lipidomics profiles of control and colitis mice were shown; B: based on the S-plot the most significant ions that led to the separation between control and colitis groups were obtained and identified as follows: 1-PC 16:0/18:2, 2-PC 16:0/20:4, 3-PC 18:0/18:2, 4-LPC 18:0, 5-SM 16:0, 6-LPC 18:1, and 7-LPC 18:2. SM, sphingomyelin; PC, phosphatidylcholine; LPC, lysophosphatidylcholine.

**ROLE OF PPARα ACTIVATION IN EXPERIMENTAL COLITIS**

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Indeed, current evidence supports a role for sphingolipids including SM and CER in modulating inflammation (3, 16). In this study, increased SM and CER levels were observed in both serum and colon of DSS-treated mice. It was reported that the activity of a specific intestinal alkaline sphingomyelinase, which also hydrolyzes SM, was reduced in the rat colitis model as well as in UC and colon cancer patients (3, 47). Higher SM concentrations were also detected in ileum from patients suffering from CD (7). These findings of downregulated colon Smpd2 and Smpd4 mRNAs as well as upregulated Sgms2 mRNA suggest that increased SM levels are probably due to decreased hydrolysis and increased synthesis. CER, acting as a second messenger for intracellular signaling with the potential to control inflammatory responses (2), was suggested to induce arachidonic acid (AA) release and phospholipase A2 (PLA2) activation (44). The consequence of CER accumulation may be an increase of cell death and higher susceptibility to infection (49). Generation of CER is required for the production of matrix metalloproteinase-1 (MMP-1), which is believed to damage the colonic mucosa in patients with UC (31). Others demonstrated increased CER in colonic lipid extracts from DSS-treated mice in vivo (5), which was consistent with the present findings.

Higher SM and CER levels were observed in fenofibrate treated WT colitis mice but not in Ppara-null fenofibrate and DSS-treated mice, indicating that PPARα activation plays a role in exacerbating colitis. For example, in the serum of fenofibrate/DSS-treated mice, the SM 18:0 level is more than twice the control diet/DSS-treated WT mice. The reduced colon Smpd2 and Smpd4 mRNA expression levels observed in fenofibrate/DSS-treated WT mice indicated further downregulated SM hydrolysis, resulting in a much higher SM level in

![Fig. 6. Target quantitation of SM and ceramide (CER) by multiple reaction-monitoring mass spectrometry based on standard curves using authentic standards. A: serum SM and CER levels in the examined groups. B: colon SM and CER levels in the examined groups. Data were expressed as means ± SD. *P < 0.05 and **P < 0.01.](http://ajpgi.physiology.org/)

![Fig. 7. Quantitative real-time PCR analysis of liver and colon mRNA expression of genes in control diet or fenofibrate-treated colitis mice. A: expression of PPARα target gene mRNAs expressed in liver that are involved in fatty acid β-oxidation (Acox1 and Acot). B: Expression of PPARα target gene mRNAs expressed in colon that are involved in fatty acid β-oxidation (Acox1, Acaal, Cd36, and Cpt1). C: expression of mRNAs expressed in colon involved in the Ripk3 pathway (Ripk3, Il1b, Tlr3, and Tlr4). Data are expressed as means ± SD. *P < 0.05 and **P < 0.01, with respect to the control diet treated IBD group. Acox1, acyl-coenzyme A oxidase 1; Acot, acyl-CoA thioesterase; Acaal, acetyl-CoA acyltransferase 1; Cd36, fatty acid transporter; Cpt1, carnitine palmitoyltransferase 1; Ripk3, receptor-interacting protein kinase 3; Il1b, interleukin-1β; Tlr3, Toll-like receptor 3; Tlr4, Toll-like receptor 4.](http://ajpgi.physiology.org/)
this group. TNFα was increased in DSS-treated mice, indicating the occurrence of inflammation in the colon. Notably, TNFα signaling was reported to involve activation of a cell membrane nSMase (9), but this was blocked by Wy14643-activation of PPARα (12). This may partly explain the further downregulated nSMase levels after fenofibrate treatment. The increased CER levels in fenofibrate-treated WT colitis mice was coincident with the phenotype of exacerbated colitis for this group, although the specific mechanism for CER accumulation herein still awaits further study.

In colon, expression of Cpt1 mRNA was upregulated after fenofibrate treatment. CPT 1 catalyzes the transfer of fatty acid from CoA to carnitine, allowing the initial transport of fatty acids into mitochondria for β-oxidation (12). Fenofibrate administration was also reported to increase mitochondrial CPT I and II activities in rabbits (17). In an experimental colitis study, simultaneous decrease in the expression of carnitine transporters and the level of carnitine in colonocytes were observed, paralleling the impaired ability of colonocytes to oxidize butyrate (14). Therefore, abnormal oxidation status induced by fenofibrate treatment and upregulated mitochondrial fatty acid β-oxidation may also lead to excessive oxidation damage in colon and exacerbate colitis. Upregulation of the RIPK3-dependent necrosis and inflammation pathway by fenofibrate treatment suggests even more severe colon damage, although the mechanism remains unclear.

In conclusion, fenofibrate was found to exacerbate the pathological phenotype in acute models of colitis in a PPARα-dependent manner. Lipidomics further revealed the possible involvement of bioactive sphingolipids in colitis. Activation of PPARα by fenofibrate treatment may affect sphingolipid metabolism and upregulate mitochondrial fatty acid β-oxidation and RIPK3-dependent necrosis in colon, resulting in more severe tissue injury. While earlier studies revealed that activation of other nuclear receptors in the intestine such as the PPARγ (1), pregnane X receptor (11, 45), and vitamin D receptor (23) suppressed acute colitis, this is the first evidence that a nuclear receptor exacerbates the disease. The present study would suggest that patients with CD and UC should be carefully monitored when they are administered fibrate drugs for hyperlipidemia. This work further yields clues to pathways other than increased inflammation in the etiology of colitis that could lead to new therapeutic opportunities.

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AUTHOR CONTRIBUTIONS


