Drug Design, Development and Therapy Dovepress

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Integrative Lipid Pseudotargeted Metabolomics and Amino Acids Targeted Metabolomics Unravel the Therapeutic Mechanism of Rhizoma Paridis Saponins on Experimental Colitis of Damp-Heat Type

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Purpose: Inflammatory bowel disease (IBD) is a serious disease that affects the metabolism and inflammatory responses of human beings. From the perspective of traditional Chinese medicine, damp-heat syndrome is one of the main syndromes of IBD. Rhizoma Paridis, also known as the root of Paris polyphylla, a well-known herbal medicine used in China, is used to treat IBD with damp-heat syndrome (IBD-DH). However, uncertainty still exists regarding the underlying mechanisms and the impact of Rhizoma Paridis on IBD-DH.

Methods: The rats in the model (DAT) and medication administration (Rhizoma Paridis total saponins (RPTS) and Pennogenin (PN)) groups were given a high temperature and high humidity environment, high fat and high sugar diet combined with 2,4,6-trinitrobenzene sulfonic acid (TNBS) to establish the model of experimental colitis of damp-heat type, and the normal control group (RNC) rats were given a normal diet at normal temperature and humidity. Damp-heat control group (DNC) was set with the same condition as DAT without TNBS. Hematoxylin-Eosin (HE) staining was used to observe the histopathological morphology of the rat colorectum. The expression of the metabolism-related genes (Phospholipase A2 (sPLA2, cPLA2), and phosphatidylethanolamine N-methyltransferase (PEMT)) was assessed by using real-time quantitative PCR analysis (RT–qPCR). And the levels of the metabolism-related proteins (sPLA2, cPLA2), S100A8/9, Arg-1, and cytokines were detected by enzyme-linked immunosorbent assay (ELISA) kit. To investigate lipids and amino acids which closely associated with the IBD and IBD-DH, lipid pseudotargeted metabolomics with UHPLC-TQ/MS analysis method, as well as targeted quantitative amino acid analysis were performed.

Results: Our data showed that RPTS (50 mg/kg) and PN (20 mg/kg) significantly ameliorated the severity of TNBS-induced colitis and downregulated the levels of circulating proinflammatory cytokines. Compared with RNC group, lipid pseudotargeted metabolomics demonstrated that glycerophospholipids, sphingolipids, carnitine, and glycerolipids were the four most perturbed lipid classes, and amino acids targeted metabolomics demonstrated that serine, N-acetylneuraminic acid, histidine, proline, taurine, and kynurenine changed significantly in DAT group . Correlation analyses showed tight associations between most of differential metabolites and proinflammatory cytokines. RPTS and PN both regulated glycerophospholipid metabolism and sphingolipid metabolism. However,

Received: 19 July 2024
Accepted: 14 October 2024
Published: 12 November 2024

both of them did not have a significant effect on amino acid modulation. RPTS and PN potentially regulated sPLA2, cPLA2, and PEMT.

Conclusion: These results showed that RPTS (50 mg/kg) and PN (20 mg/kg) effectively alleviated rats' colitis of damp-heat type, affected cytokines, and altered lipid metabolism without significant modulation on amino acid metabolism.

Keywords: inflammatory bowel disease, lipid pseudotargeted metabolomics, amino acids targeted metabolomics, Rhizoma Paridis saponins, cytokines

Introduction

The main forms of inflammatory bowel disease (IBD) are Crohn's disease (CD) and Ulcerative colitis (UC). These diseases are becoming more common and more prevalent all over the world.[1] However, the pathophysiology and etiology are still poorly understood. According to current theories, abnormalities in intestinal homeostasis originate from the interaction of genetic, environmental, and microbial factors, which weaken intestinal barrier function and produce a disorganized immune response.[2] This requires further research on IBD to identify not only therapeutic targets but also diagnostic and prognostic markers to improve disease management.

Metabolomics is a powerful tool for examining the pathophysiology of disease and locating potential biomarkers, utilizing biofluids (such as plasma and/or urine), tissues, or other biological extracts.[3] By measuring hundreds of metabolites in biological samples, metabolomics enables the identification of each patient's unique metabolic profile.[4] These profiles could advance metabolomic personalized therapy by enhancing our comprehension of this complicated disease and potentially enhancing the diagnosis and treatment of IBD.[5] Researchers use metabolomics to distinguish between CD and UC, as well as the many IBD subclassifications,[6] and explore metabolomic variations based on disease activity and relapse predictors.[7] Moreover, the link between IBD metabolome dynamics and treatment response has been further shown by recent studies, however, more research is still needed in this area.[8]

Researches have been done to identify biomarkers for IBD diagnosis and to demonstrate the illness mechanism, which showed that various lipid species, important substances for energy metabolism, and amino acids were noticeably altered in patients with IBD.[9–11] Studies show that the etiopathogenesis of IBD may be significantly influenced by changes in the lipid profile. Primary bile acid biosynthesis, arachidonic acid metabolism, sphingolipid metabolism, fatty acid elongation, and glycerophospholipid metabolism were all connected to IBD dysregulation, according to lipid profile research in individuals with IBD.[12,13] In the past few years, an increasing number of studies have employed lipidomics to identify particular biomarkers for different diseases by detecting changes in lipid species.[13] Also, studies find disruption of amino acid metabolism in IBD. Some studies have shown that leucine, lysine, and valine, as well as the semi-essential amino acids arginine and glutamine, and the non-essential amino acid serine, were all reduced in CD patients compared to control subjects[11] and UC patients had decreased amounts of creatine, proline, and tryptophan.[14] This irregularity in amino acid metabolism may have important effects on the prognosis of IBD.[15] Similar to this result, our previous studies have shown that a number of aromatic acid and lipid derivative metabolites may serve as potential biomarkers to distinguish IBD from healthy controls. When compared to healthy controls, phosphatidylcholine (PC), phosphatidylethanolamines (PE) are differential metabolites in our IBD patients, which mostly functioned as components of distinct glycerophospholipid metabolites. Aside from that, acylcarnitine can also distinguish between IBD and healthy controls.[16]

From the perspective of traditional Chinese medicine, the consensus from the Chinese Association of Traditional Chinese Medicine and the previous retrospective analysis showed that damp-heat syndrome is one of the main subtypes of active IBD.[17,18] Studies have shown that damp-heat syndrome could lead to disorders of lipid metabolism and amino acid metabolism.[16,19,20] Consistent with this, some studies have shown that there are differential metabolic pathways between patients in the active stage of UC with syndrome of damp-heat and syndrome of non-damp-heat, which are mainly concentrated in tryptophan metabolism, sphingolipid metabolism, glycerophospholipid metabolism, and pyrimidine metabolism.[21] In summary, we can conclude that both our previous studies[16] and other studies[21] have found that patients with damp-heat syndrome with IBD have shown metabolic changes clinically, but the specific mechanism is still unclear. Therefore, we aim to explore the metabolic characteristics of rats' experimental colitis of damp-heat type, so as to analyze the possible mechanism of such metabolic changes. Based on these results, we focused on lipids and amino

acids metabolism in experimental colitis of damp-heat type and therapeutic interventions, such as medical plants treatment.

At the same time, there is evidence that herbs and herbal formulations are effective in treating IBD.[22] Professor Xu Jingfan, the great Traditional Chinese Medicine (TCM) master, used Rhizoma Paridis to treat refractory IBD. The ZaoXiu (Chinese synonym of Rhizoma Paridis) formulation has been a representative prescription according to the collection and studies of his therapeutic experiences. Zaoxiu formulation extracts showed promising effects on protecting the animals from 2,4,6-trinitrobenzene sulfonic acid (TNBS) -induced colitis.[23] However, the specific mechanism and major pharmacological active ingredient still need further studies.

Rhizoma Paridis is a well-known Chinese herbal medicine, which has effects of clearing heat and detoxification and is often used to treat pustulosis, sore throat, snakebite envenoming, traumatic injury and other diseases.[24] What's more, it has been applied to treat IBD in recent years.[23] Rhizoma paridis total saponins (RPTS) are the main active components of Rhizoma Paridis, which have various pharmacological effects, such as anti-tumor, anti-bacterial, anti-viral, hemostatic and immune regulation.[24] The RPTS is a mixture of various saponins extracted from the Rhizoma Paridis, including a variety of saponins with different structures, among which the main saponins are pennogenin and diosgenin, while pennogenin (PN) includes Polyphyllin IV and Polyphyllin VII.[25]

Based on these background, we conducted the following study. The primary goal of this investigation was to identify significant metabolites related to TNBS-induced colitis with damp-heat type and explore the role of Rhizoma Paridis active substances in regulating its metabolic profiles. We used lipid pseudotargeted metabolomics and amino acids targeted metabolomics to observe changes of lipids and amino acids in rat model. Meanwhile, we described the dysregulated metabolic pathways and certain cytokine levels in the IBD rat model of damp-heat type in this study and explored the correlations between circulating metabolites and cytokines. Furthermore, we explored the mechanism of Rhizoma Paridis in treating experimental colitis of damp-heat type.

Materials and Methods

Chemicals and Reagents

5% TNBS (2,4,6-Trinitrobenzenesulfonic acid) (P2297-5X, 10 mL, SLCG2348, Sigma Corporation, USA); Rhizoma Paridis Total Saponins (RPTS) (MUST-20203001), Polyphyllin VI (PPVI) (MUST-21090811, ≥ 98%) and Polyphyllin VII (PPVII) (MUST-21040610, ≥ 97%) were purchased from Chengdu Manstead Biotechnology Co. Ltd, China; 5-ASA (RH110815, Shanghai Macklin Biochemical Co.,Ltd). RPTS, PPVI, PPVII, and 5-ASA were prepared into 0.5% carboxymethylcellulose sodium (CMC-Na) (C104987, Shanghai Aladdin Bio-Chem Technology Co., LTD) solution respectively when used. All reagents used in the HE staining were purchased from Zhuhai Baso Medical Device Co., Ltd. Methanol, acetonitrile, and formic acid were purchased from Fisher Scientific (USA). Ultra-pure water was generated employing a Milli-Q Integral Water Purification System from Merck Millipore (Merck Millipore, USA). All standards of metabolites were purchased from Sigma Corporation. All enzyme-linked immunosorbent assay (ELISA) kits were purchased from Jianglai Industrial Company, Shanghai, China.

Animal Experiments

Sprague-Dawley (SD) rats (male, 6–8 weeks, weighting 180–220 g) were obtained from SPF (Beijing) Biotechnology Co., Ltd. Animals were housed in cages, controlled temperature (22°C ± 2°C), humidity (45% ± 5%) and illumination (12 h light / dark cycle). Rats were given sterile food and water libitum, and were adapted to the facility for one week before experiments.

After one week of adaptive feeding, rats were randomly divided into 8 groups: control (RNC) (n=7), control with damp-heat type (DNC) (n=7), TNBS-treated with damp-heat type (DAT) (n=13), low RPTS dose-treated (50 mg/kg) (DRL) (n=9), high RPTS dose-treated (100 mg/kg) (DRH) (n=9), low PN dose-treated (20 mg/kg) (DPL) (n=9), high PN dose-treated (40 mg/kg) (DPH) (n=9), 5-ASA-treated group (100 mg/kg) (DAS) (n=9). RPTS and PN were suspended in 0.5% CMC-Na solution and supplemented to rats by gavage for 14 days. The experiments strictly followed the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, and the animal study was approved by the

Animal Welfare Committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School. Six samples were randomly selected from each group for the following experiments, including lipid pseudotargeted metabolomics and amino acids targeted metabolomics, ELISA, and RT–qPCR. Each repeat of the experiments was performed as a separate, independent experiment.

Establishment of Experimental Colitis Model with Damp-Heat Type

Before modeling experimental colitis, we need to construct the rat model of large intestine damp-heat type. Using the procedure described in the paper, except for the RNC group, the other 7 groups of rats were all housed in an environmental simulation room under an ambient temperature of 28°C ± 2°C at a relative humidity of 60–80% for 9 h every day for 14 days. The 7 groups living in the damp-heat area were given drinking water containing honey (200 g/L) and a high-sugar and high-fat diet (HSHFD) throughout the whole process.[26–29] From day 1 to day 14, RPTS, PN, and 5-ASA were administered by gavage once a day. The normal group was fed a normal diet and lived in an environment with a relative humidity of 45% ± 5% and an ambient temperature of 22°C ± 2°C.

The experimental colitis model was constructed under damp-heat environment. On the 11th day of feeding, experimental colitis model manipulation was performed. Rats were fasted for 24 h with freely drinking before modeling. Rats were anesthetized with isoflurane. The rats in RNC and DNC groups were given 1 mL of 0.9% saline by rectal administration, the other groups were given 1mL TNBS solution (100 mg/kg) (5% TNBS dissolved in 50% ethanol) by rectal administration. The mixed solution was slowly injected into 4–6 cm of the proximal end of the descending colon, and the rats were kept in the vertical position for 60s. The severity of colitis was recorded daily, including weight changes, diarrhea, and bloody stools. All rats were sacrificed on day 14 and the colon tissues and plasma were removed for further analysis.

Indicators Related to Colitis

Status checks on laboratory animals were done every day. The weight was recorded. Each lab animal was graded every day for the Disease Activity Index (DAI) including body weight loss, blood in the stool, and stool consistency. The rats were anesthetized and sacrificed at day 14, and the pertinent samples were gathered. The Colon length and Colon weight / length ratio were recorded. The gross morphological changes linked to TNBS-induced colitis were documented. For histology evaluation, segments of colonic tissues were fixed in 10% formalin. Based on the tissue damage seen under the optical microscope, hematoxylin and eosin (HE) staining was done to evaluate histopathological alterations. The evaluation scale was used to get the damage score.

Enzyme-Linked Immunosorbent Assay (ELISA)

According to the manufacturer's instructions, ELISA kits (Jianglaibio, Shanghai, China) were used to detect the serum levels of tumor necrosis factor (TNF)-α (JL13202-96T, 081901006132020826), interleukin (IL)-6 (JL20896-96T, 081901006208960826), interleukin (IL)-1B (JL20884-96T, 081901006208840826), interferon (IFN)-γ (JL13241-96T, 081901006132410826), IL-10 (JL13427-96T, 081901006134270826), IL-13 (JL20877-96T, 081901006208770826), IL-18 (JL20882-96T, 081901006208820826), IL-4 (JL20894-96T, 081901006208940826), S100A8 (JL15633-96T, 081901006156330826), S100A9 (JL24667-96T, 081901006246670826), sPLA2 (JL15703-96T, 081901006211580826), cPLA2 (JL15759-96T, 081901006157590826), Arg-1 (JL21104-96T, 081901006211040826) and TGF-β (JL13643-96T, 081901006136430826).

Real-Time Quantitative PCR Analysis (RT–qPCR)

With the use of Trizol (R401-01, Vazyme, China), total RNA from the colon was extracted, and an RT SuperMix kit (R323-01, Vazyme, China) was then utilized for the reverse transcription procedure. Real-time PCR analysis was performed using an Applied Biosystems™ QuantStudio™ (thermofisher) and a SYBR Green qPCR Master Mix kit (Q711-02, Vazyme, China). Table S1 in the supporting information lists the primer sequences. Using the comparative CT approach and GAPDH as a correction, the expressions of pertinent genes were measured and quantified.

Metabolomic

Plasma Sample Handling

A. Lipid Pseudotargeted Metabolomics

Plasma samples (100 μL) were homogenized in a 400 μL 75% methanol, including internal standard substances (Table S2). After homogenization, 1 mL MTBE was added. The mixture was vortexed for 2 min and stood at a low temperature for 1 h. The mixture homogenized in 250 μL water was vortexed for 2 min and stood at low temperature for 10 min, then centrifuged at 14,000 rpm at 4°C for 15 min. 400 μL of supernatant was absorbed and evaporated, then stored at −20°C. The extract samples were redissolved in 120 μL of Acetonitrile / isopropanol / water (65:30:5). Until complete dissolution, the supernatant was taken by oscillation and centrifugation for UHPLC-TQMS analysis.

The blank sample used in the experiment was acetonitrile / isopropanol / water (65:30:5). QC samples were prepared by mixing a portion of all actual samples and using the same data acquisition method as the actual samples. The pretreatment of QC samples and blank samples was the same as that of analyzed samples.

B. Amino Acids Targeted Metabolomics

A total of 35 amino acids were detected by targeted metabolomics analysis (Table S3). Plasma samples (100 μL) were homogenized in 400 μL 50% methylene acid water. The mixture was vortexed and mixed for 10 min, and ultrasound for 20 min. Then centrifuged at 20,000 rpm at 4°C for 10 min. The supernatant was removed, and the supernatant was taken by oscillation and centrifugation for UHPLC-TQMS analysis. All standards were purchased from Sigma Corporation. The standards were dissolved in methanol and diluted in 80% methanol to 1 ppb, 10 ppb, 50 ppb, 100 ppb, 500 ppb, 500 ppb, and 1000 ppb.

The blank sample was a 20% methanol/aqueous solution. QC samples were prepared by mixing a portion of all actual samples and using the same data acquisition method as the actual samples. The pretreatment of QC samples and blank samples was the same as that of analyzed samples.

Chromatography-Mass Spectrometry Acquisition Conditions

A. Lipid Pseudotargeted Metabolomics

Chromatography Analysis Conditions. The chromatography was performed on the BEH C8 column (2.1×100 mm × 1.7 μm, Waters, Milford, MA, United States). The flow rate was 0.2 mL/min. The injection volume was 5 μL. The mobile phase was composed of solvent A (water with 60% acetonitrile and 10 mm ammonium acetate) and solvent B (isopropanol: acetonitrile = 9:1 (v / v) and 10 mm ammonium acetate). The detailed LC method is summarized in (Table S4-1).

Mass Spectrometry Conditions. Under positive-ion mode (Table S4-2), AB 5500 (SCIEX, USA) was used. The Spray Voltage was +3.5 kV in positive ionization mode and −3.0 kV in negative ionization mode. The other mass spectrometry conditions under negative-ion mode are the same as positive-ion mode (Table S4-2). The Capillary temperature was maintained at 300°C. The Curtain Gas (CUR) was 35 Arb. The Collision Gas (CAD) was Medium. Ion Source Gas1 (GS1) and Ion Source Gas1 (GS2) was 60 Arb. Entrance Potential (EP) was 10. CXP was 13. The Mass Spectrometry conditions are the same as in positive-ion mode.

B. Amino Acids Targeted Metabolomics

Chromatography Analysis Conditions. The chromatography was performed on the Kinetex PFP C18 (250*4.6 mm, 5 μm). The flow rate was 0.50 mL / min. The injection volume was 2 μL. The mobile phase was composed of solvent A (water with 0.1% acetic acid) and solvent B (methyl alcohol with 0.1% acetic acid). The detailed LC method is summarized in (Table S5-1). Under negative-ion mode, the chromatographic conditions are the same as in positive-ion mode.

Mass Spectrometry Conditions. The Thermo TSQ Quautiva was used. The IS Pos: 3500.0 V, Neg: 2500.0 V.TEM was 550.0°C. The Sheath GAS was 40.0 psi. Aux GAS was 10 psi. The Ion Transfer TUBE Temp was 350°C. The vaporizer Tempwas 350°C (Table S5-2).

Data Acquisition Instructions

We use the DRMS / HRMS data quality online real-time monitoring software to realize the process monitoring of data quality.

Data Processing and Statistical Analysis

All measurement data are collected by Xcalibur data acquisition software. The "80% rule" was used as a pretreatment on the data to lessen the entry of missing values. For multi-dimensional statistical analysis using principal component analysis, partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA), the raw data were imported into the One-MAP / PTO software. With the variable importance projection (VIP) > 1.0 as the standard to identify potential difference variables. Two sample unpaired t-tests are used in metabolomics research to demonstrate which metabolites have the ability to distinguish between the various groups in the data set, $p < 0.05$ was used as a threshold. The fold change (FC) was calculated relative to a given reference sample based on averaged raw signal intensities and serves as a metric for the relative change in a given concentration of the metabolite in the various settings under examination. A criterion of FC > 3/2 or < 2/3 was established. The possible biomarkers were screened using the two-sample unpaired *t*-test, fold change (FC) analysis, and variable importance projection (VIP) from the peak intensity. Finally, it was decided that data with VIP > 1 and $p < 0.05$, FC > 1.5, or < 2/3 were differential metabolites. The One-MAP platform (www.5omics.com) was used to map the above already obtained metabolites, to metabolic pathways. One-step Metabolomics was used to analyze all the data. For lipidomics, we used the LIPEA website to get the already obtained qualitative annotation analysis, mapping onto the metabolic pathways.

Statistical Analysis

We used two software programs for statistical analysis: GraphPad Prism 8 and IBM SPSS Statistics software (version 22.0). The Shapiro–Wilk normality test was performed to determine the data distribution. To assess differences between the two groups, the Student's *t*-test was applied and to assess differences among multiple groups, a one-way ANOVA was applied for data following the normal distribution. Non-normally distributed continuous variables were compared by using the Kruskal–Wallis test. Using Pearson Correlation, the relationships between cytokines and metabolites were examined. $p < 0.05$ was considered significant and used for subsequent analysis. Related images were drawn by the bioinformatics platform (https://www.bioinformatics.com.cn/).

Results

Low-Dose RPTS Treatment and PN Treatment Ameliorated TNBS-Induced Colitis

The rat colitis model was induced by TNBS. The administration of TNBS dramatically reduced food intake, and loss of weight was seen. The animals of the DAT group, DRL group, and DPL group suffered a drastic weight loss compared with the RNC group (Figure 1a). While the RNC groups retained their normal structure, TNBS caused damage to the intestinal mucosa of the rats, including transmural involvement, ulcerations, edema, and bowel wall thickening. Colonic tissue responded better to the RPTS therapy (50 mg/kg), with improvements in the damage score, degree of damaged areas, and colon weight / length ratio, leading to an increase in colon length (Figure 1b and c). Compared with the DAT group, 50 mg/kg RPTS significantly reduced DAI score ($p < 0.05$), alleviated colonic shortening, and improved colonic mucosal bleeding (Figure 1d).

Histologically, the DAT group, in contrast to the RNC groups, displayed an intensive transmural interruption, significant ulceration, inflammation, edema, and huge infiltration of neutrophils, primarily in the mucosa (Figure 1f). Mild interstitial neutrophil and lymphocyte infiltration were observed in the DNC group when compared to the RNC group. The DAS, DRL, DRH, DPL, and DPH groups showed various degrees of colonic mucosal erosion remission, but discontinuous intestinal epithelial lesions with granulomatous hyperplasia, mild to moderate interstitial neutrophil and lymphocyte infiltration, and structural changes in the crypt were still visible. Compared with the RNC group and DNC group, the histology score was

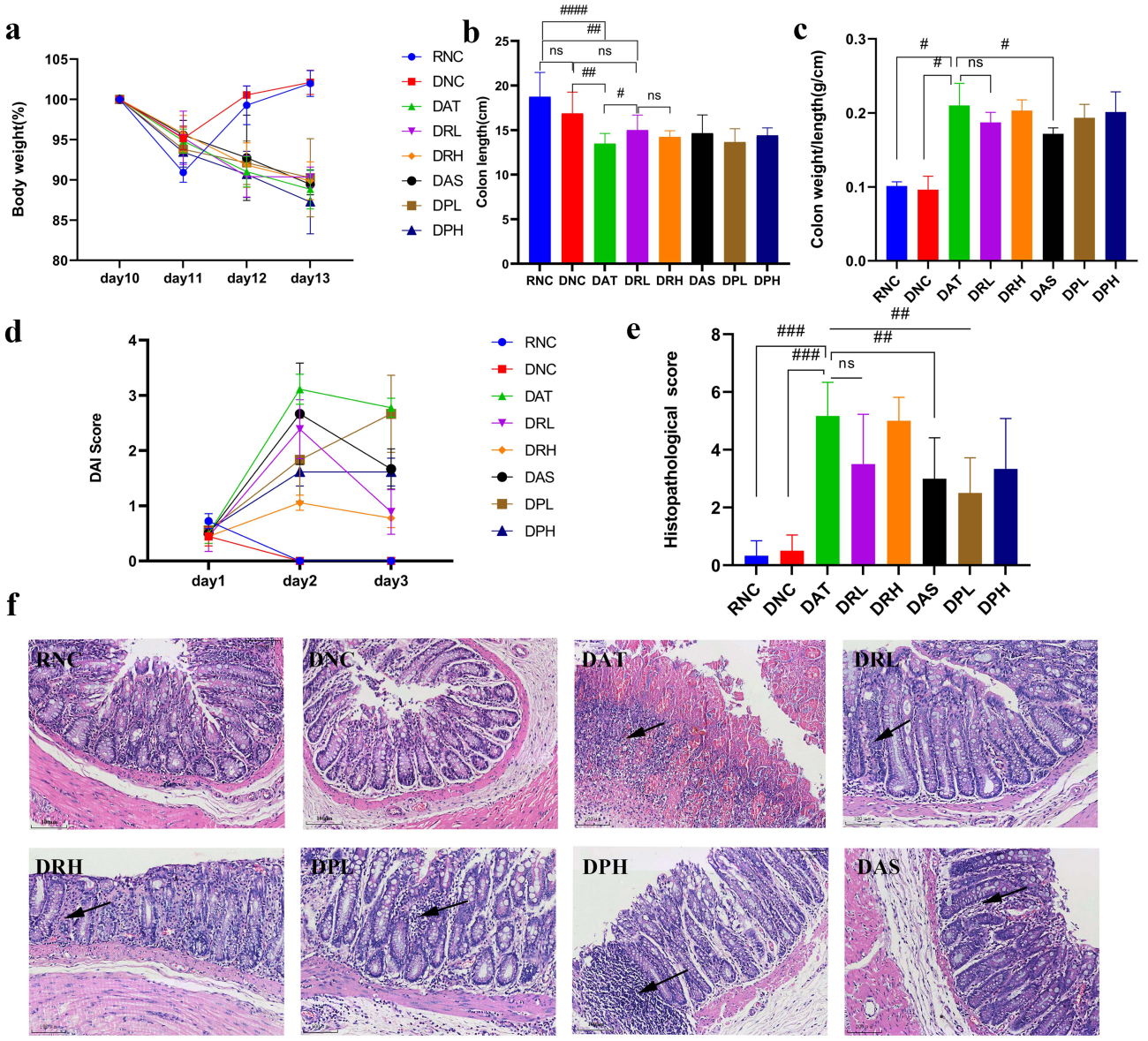

Figure 1 Effects of groups treated with high- and low-dose RPTS and PN on experimental colitis. (**a**) Body weight changes were recorded after TNBS treatment for 4 days; (**b**) Colon length values of the Rats; (**c**) Colon weight/length ratio of the Rats; (**d**) DAI; (**e**) Histopathology change reflected by histopathological scores (n = 6 per group). All data shown are representative of three independent experiments. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. (**f**) Representative histological colonic tissue sections from groups included in the colitis experiment; were stained with hematoxylin and eosin. Arrows indicate the location of the lesion. Colon HE staining (magnification, 200×).

significantly increased in the DAT group ($p < 0.001$). Compared with the DAT group, the histology score of the DPL and DAS groups decreased significantly ($p < 0.01$) (Figure 1e). DRL reduced histology scores, but the difference was not statistically significant.

In conclusion, low-dose RPTS and PN ameliorated the severity of TNBS-induced colitis. The therapeutic effect of low doses of RPTS and PN was more effective than that of high doses of RPTS and PN for the relief of colitis. Based on this, we continued to focus on the therapeutic effects of low-dose RPTS and PN in the treatment of TNBS-induced experimental colitis and investigate the mechanism of action.

Low-Dose RPTS and PN Regulated the Levels of S100A8/9, Arg-1 and Cytokines in TNBS-Induced Colitis

Abnormal activation of the immune response plays a direct role in the development and progression of IBD. Cytokines and cytokine-producing immune cells play a key role in the pathogenesis of IBD.[30] We studied the effects of RPTS and

PN on the levels of S100A8/9, Arg-1 and cytokines. Figure 2 illustrates the variations in TNF-α, IL-1B, IL-18, IFN-γ, IL-6, IL-4, TGF-β, IL-10, IL-13, S100A8, S100A9, and Arg-1 levels in the plasma of the rats in each group. In the DAT group, plasma levels of pro-inflammatory cytokines (TNF-α ($p < 0.01$), IL-1B ($p < 0.05$), IFN-γ ($p < 0.001$), and IL-6 ($p < 0.01$)) were significantly increased, and anti-inflammatory cytokines (IL-4 ($p < 0.01$), TGF-β ($p < 0.01$), IL-10 ($p < 0.01$), IL-13 ($p < 0.05$)) significantly decreased, and IL-18 did not change significantly. Meanwhile, the levels of S100A8 ($p < 0.001$), S100A9 ($p < 0.001$), and Arg-1 ($p < 0.01$) were significantly increased in the DAT group. Low-dose PN significantly decreased the levels of pro-inflammatory cytokines TNF-α ($p < 0.01$), IL-1B ($p < 0.01$), IFN-γ ($p < 0.001$) and IL-6 ($p < 0.01$) and elevated the levels of anti-inflammatory cytokines IL-4 ($p < 0.01$), TGF-β ($p < 0.001$), IL-10 ($p < 0.01$) and IL-13 ($p < 0.05$) while decreasing levels of S100A8, S100A9, and Arg-1 ($p < 0.01$). Compared with the DAT group, low-dose RPTS significantly reduced the levels of pro-inflammatory cytokines TNF-α ($p < 0.05$), IFN-γ ($p < 0.01$), and IL-1B ($p < 0.05$) and could downregulate the levels of IL-6, S100A8, S100A9, Arg-1, and increased the levels of IL-4, TGF-β, IL-10, IL-13, but the difference was not significant.

The Unbalance of Lipid Metabolism is Closely Associated with Experimental Colitis with Damp-Heat Type

As previously mentioned, patients with IBD have abnormal lipid metabolism profiles. To investigate the different lipids closely associated with the illness process in experimental colitis of damp-heat type and to get ready for the subsequent

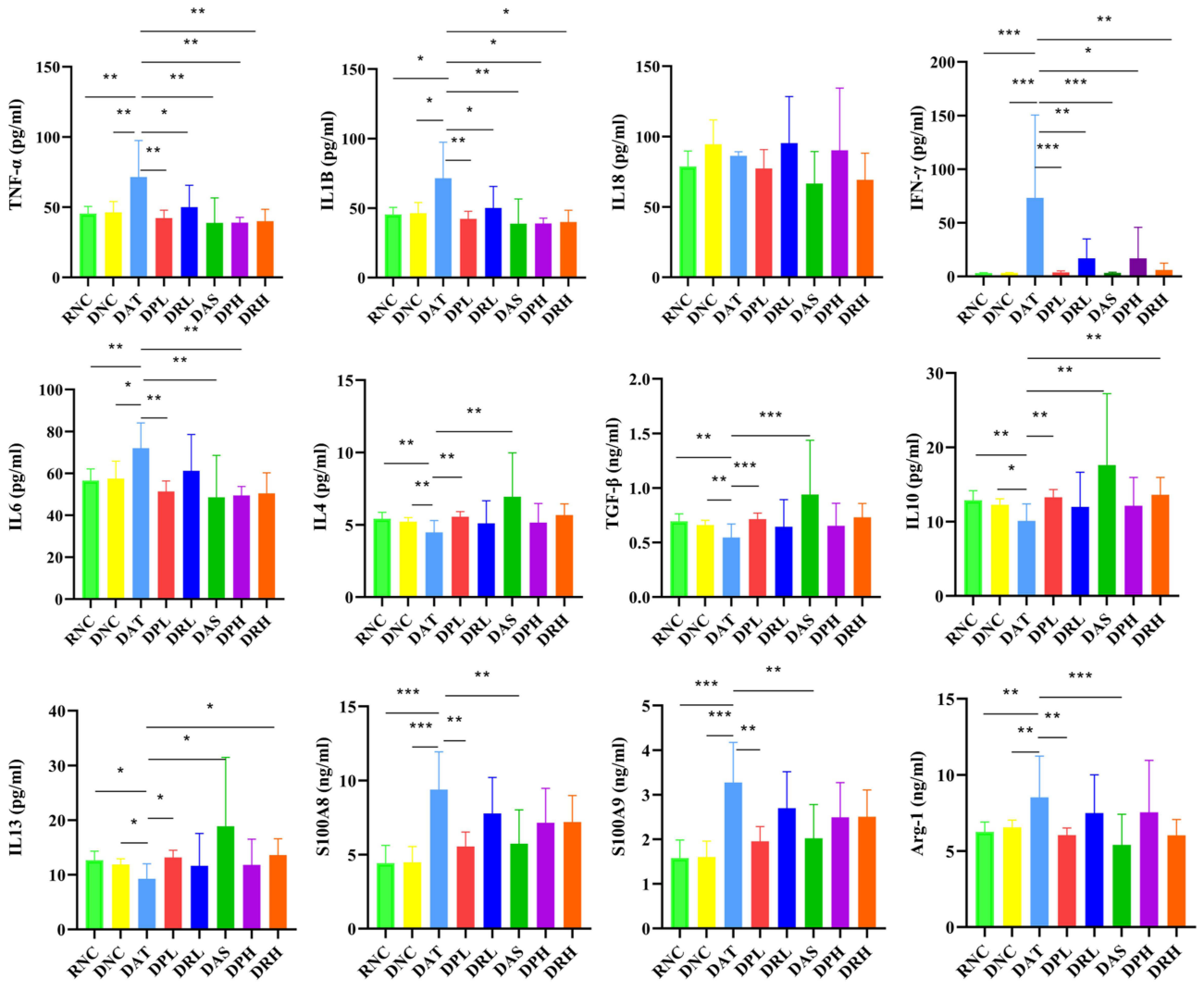

Figure 2 The levels of TNF-α, IL-1B, IL-18, IFN-γ, IL-6, IL-4, TGF-β, IL-10, IL-13, S100A8, S100A9, and Arg-1 in the plasma of the rats in each group (n = 6 per group). *$p < 0.05$, **$p < 0.01$, ***$p<0.001$.

study on the mechanism of the anti-IBD effect of RPTS and PN, lipid Pseudotargeted metabolomics technologies based on UHPLC-TQ/MS systems were undertaken. After data preprocessing and metabolite identification, we discovered 2696 lipids characteristics from the raw data recorded in positive- and negative-ionization modes, including 701 triacylglycerols (TGs), 254 Diacylglycerol (DGs), 25 Cardiolipin (CLs), 50 Lysophosphatidylcholine (LPCs), 37 Lysophosphatidylethanolamine(LPEs), 152 Phosphatidic acids (PAs), 261 phosphatidylcholines (PCs), 268 phosphatidylethanolamines (PEs), 109 Phosphatidylglycerols (PGs), 142 phosphoinositols (PIs), 114 Phosphatidylserine (PSs), 82 ceramides (Cers), 88 monohexosylceramide(Hexcer), 78 dihexosylceramide (Hex2cer), 80 Sphingomyelins (SMs), 31 Cholesterol ester (CEs), 27 other sterol lipids, 39 Acyl carnitine (CARs), 43 fatty acids (FAs), 83 unsaturated fatty acids (USFA) and others (Table 1). According to a thorough investigation, glycerophospholipids, which made up 42.95% of the overall lipid abundance, had the highest relative abundance when compared to sphingolipids, glycerolipids, and fatty Acyls. Glycerolipids had the second largest relative abundance, followed by sphingolipids and fatty Acyls (Figure 3).

To further analyze the effects of different factors on rats, we first performed a comparative analysis of RNC and DAT two groups. The RNC group and DAT group were distinct from each other using the PCA approach, which was focused on discriminating against the tested groups in an unsupervised pattern (Figure S1a and b). To demonstrate that the DAT group was significantly separated from the control group, OPLS-DA was a more effective application (Figure S1c–f). In the positive and negative modes, 245 and 49 important differential metabolites with significant effects on the modeling analysis were obtained. Furthermore, based on VIP > 1, $p < 0.05$, and FC > 1.5 or < 2/3, 294 differentiated metabolite

Table 1 Category of Detected Lipids in Lipidomic Profiles

Category	Lipids	Counts
Fatty Acyls (174)	CAR	39
	FA	43
	Prostaglandin	9
	Unsaturated fatty acids	83
Glycerolipids (955)	DG	254
	TG	701
Glycerophospholipids (1158)	CL	25
	LPC	50
	LPE	37
	PA	152
	PC	261
	PE	268
	PG	109
	PI	142
	PS	114
Sphingolipids (328)	Cer	82
	Hex2Cer	78
	HexCer	88
	SM	80

(*Continued*)

Table 1 (Continued).

Category	Lipids	Counts
Sterol Lipids (58)	CE	31
	Other Sterol Lipids	27
Prenol Lipids		2
Other lipids		21

ions were identified. 209 ions were upregulated and 85 ions were downregulated in the DAT group (Table S6). These metabolites are mainly composed of 4 major classes. Through cluster analysis (heatmap) (Figure S1g and h) and volcano plot (Figure S1i and j), we could observe a distinct metabolic profile between DAT and RNC in plasma samples. Compared with the RNC group, the rats in the DAT group showed predominantly glycerophospholipid metabolic changes accompanied by changes in glycerolipids and sphingolipids abundance, and these differential lipid abundance were markedly elevated. Using the same statistical approach, compared with the DNC group, we find that the plasma lipid profiles of rats in the DAT group (Table S7) still showed changes in glycerophospholipid metabolism, accompanied by changes in the abundance of glycerolipids and sphingolipids, and the abundance of these different lipids was significantly higher (Figure S2). However, the trends of changes in the types and abundance of differentiated lipids in the DAT and DNC groups were different from those in the DAT and RNC groups. In the same way, comparing the DNC group with the RNC group (Table S8), we found that the differential lipids in the DNC group were dominated by PC, PI, and TG, with a significant decrease in partial glycerophospholipid abundance compared with the RNC group, which was the main change of lipid metabolism in damp-heat type (Figure S3).

To better screen the specific lipids for experimental colitis with damp-heat type, we performed a comprehensive analysis of the three groups of differential metabolites. Analysis of variance (ANOVA) was performed on the screened metabolites from the three groups. We found that 146 differentiated metabolite ions of the DAT group were identified compared to RNC and DNC (Table S9). PCA and particularly OPSL-DA suggest differences in metabolite profiles of the three groups (Figure 4). A heatmap depicting the top 25 differentially abundant metabolites between DAT, DNC, and RNC (Figure 4g and h) Among them, glycerophospholipids (47.95%), sphingolipids (26%), carnitine (15%), and

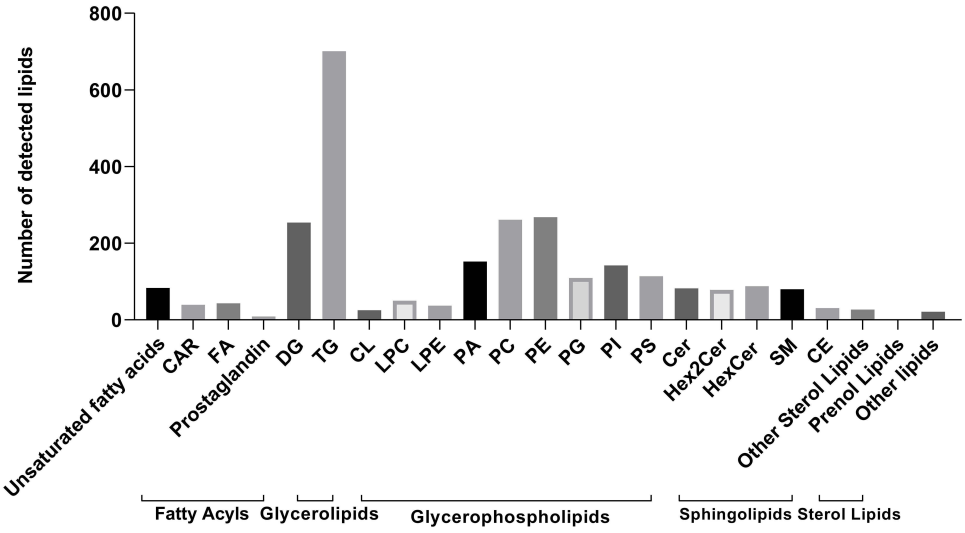

Figure 3 A number of detected lipids in lipidomic profiles.
Abbreviations: CARs, Carnitine; FA, fatty acid; TG, triacylglycerols; CL, Cardiolipin; DG, Diacylglycerol; PA, Phosphatidic acid; PC, Phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; Cer, ceramide; Hexcer, monohexosylceramide; Hex2cer, dihexosylceramide; SM, sphingomyelin; CE, Cholesterol ester.

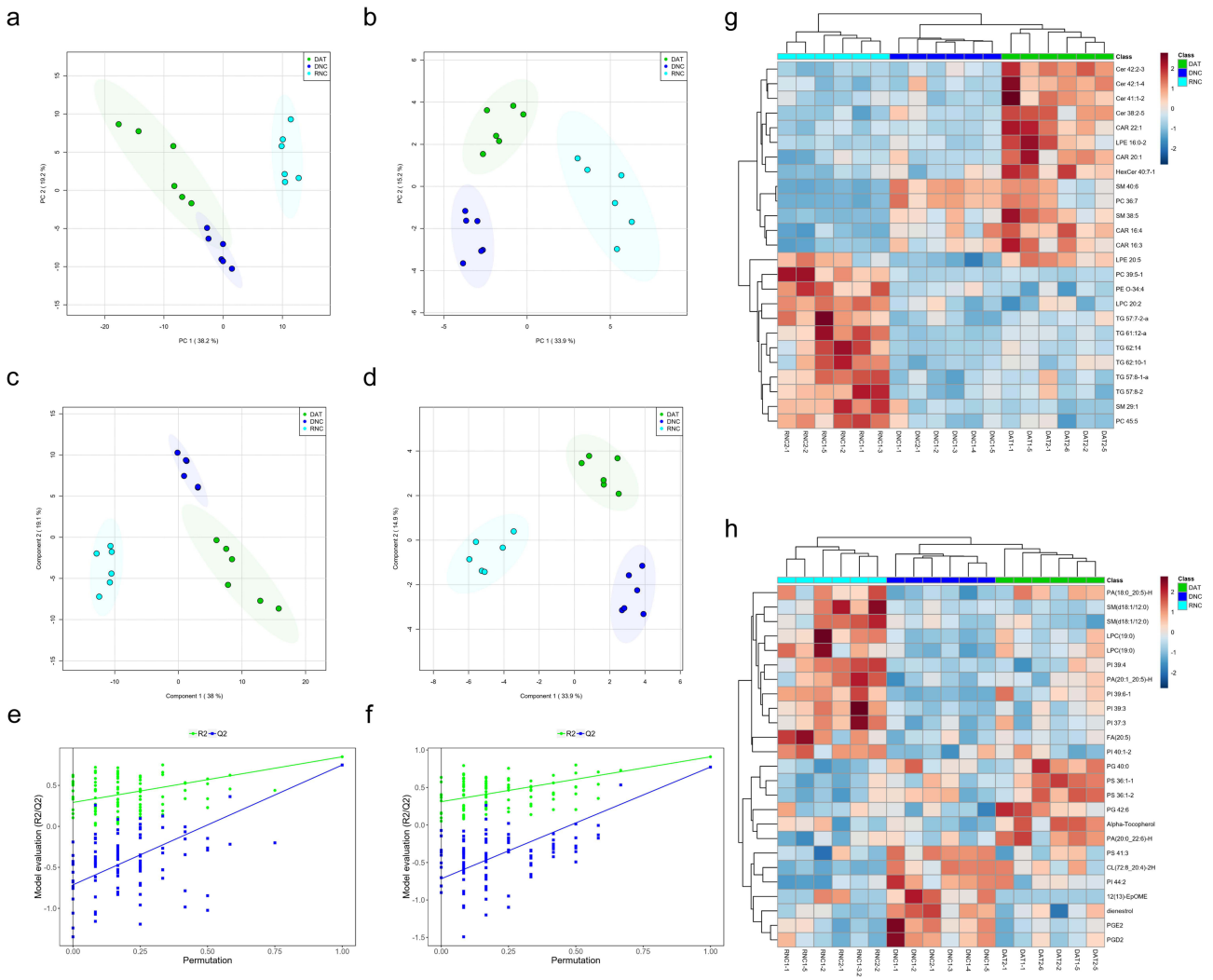

Figure 4 Metabolomics Profile of DAT versus DNC and RNC (n = 6 per group). (**a** and **b**) Scores scatter plot of PCA; (**c**–**f**) Scores scatter plot of OPLS-DA; (**g** and **h**) cluster analysis (heatmap). (**a**), (**c**), (**e**), and (**g**) Positive-ion mode. (**b**), (**d**), (**f**), and (**h**) Negative-ion mode.

glycerolipids (7.5%) were the four most perturbed lipid classes in DAT groups' plasma samples. LPC 11:0, PC 43:6, PE 32:4, PE 32:5, PI 35:4, and PI 44:2 were significantly downregulated and others were upregulated. These metabolites were specific targets of DAT (Table 2).

RPTS and PN Modulated Lipid Profile

The results of animal studies showed that low doses of RPTS and PN were more effective in relieving colitis. A lipidomic analysis was also performed to determine the lipid profile transformation in DRL and DPL in comparison with DAT.

First, we screened the differential metabolites between the DPL and DAT groups to explore the changes in lipid metabolic profiles after using PN. The treatment group and model group were distinct from each other using the PCA approach. Furthermore, based on VIP > 1, $p < 0.05$, and FC > 1.5 or < 2/3 in OPLS-DA, 68 differentiated metabolite ions were identified. 28 ions were upregulated and 40 ions were downregulated in the DPL group. Through cluster analysis, we could observe a distinct metabolic profile between DPL and DAT in plasma samples. PGE2, PGF2\PGD2, PGD2, 8(9)-EpETrE, 14.15-DiHETrE, TG 64:16-2-a, TG 61:7, Hex2Cer 39:2–1, Hex2Cer 37:1–5 and Dehydroepiandrosterone were upregulated. PG 40:7–2, PS 38:5–2, PS 35:2, PS 37:6, PE O-33:0, PE O-35:0, PE 34:0, CL (72:6_18:2) −2H, DG 40:1–8 and so on were downregulated. Compared with the DNC and RNC groups, it is also noteworthy that PE 34:0, PE

Table 2 Top 20 Specific Metabolites of DAT Selected Based on the P-values

NO.	Compounds	p.value
1	Cer 42:2–3	9.1694E-08
2	PI 39:6–1	0.00001
3	CAR 22:1	0.00005
4	CAR 16:4	0.00007
5	Cer 42:1–4	0.00012
6	CAR 16:3	0.00015
7	Cer 41:1–2	0.00015
8	CAR 20:1	0.0003
9	HexCer 40:7–1	0.0003
10	CAR 18:1	0.0003
11	LPE 16:0	0.00036
12	LPE 18:0	0.00048
13	CAR 20:2	0.0005
14	SM 40:0	0.00057
15	TG 56:4–1	0.0007
16	TG 57:9–2	0.0007
17	PC 43:6	0.0008
18	PE O-37:0	0.0008
19	PS 36:1–1	0.0010
20	CAR 18:0	0.0011

O-33:0, PE O-37:0, HexCer 36:2–6, HexCer 40:7–1, TG(e)54:2 were all significantly upregulated in DAT groups, which were then reversed by PN treatments.

At the same time, when compared to DAT, RPTS could regulate disorders of lipid metabolism. Among them, PGE2, PGD2, PGF2\PGD2, 8(9)-EpETrE, PS 44:8–1, PE 48:0, trenbolone, HexCer 38:3–7, HexCer 34:0–1, DG 37:2–7 and others were upregulated. PG 43:6, PI 32:0, PS 38:5–2, PE O-33:0, PE 34:1, LPE 20:5, DG 40:7–4, HexCer 39:1–4, and so on were downregulated. The considerable upregulation of PI 32:0, PG 42:6, PE 34:0, HexCer 40:7–1, LPE 20:3, LPE 22:5, PE 38:6, PE 40:8, and TG(e) 54:2 was reversed by RPTS treatments.

Both RPTS and PN significantly downregulated the contents of PE 34:0, PE O-33:0, and TG(e)54:2. However, PG 42:6, PI 32:0, LPE 20:3, LPE 22:5, PE 38:6, and PE 40:8 were only moderately downregulated by RPTS. This suggested that these lipids were specific targets of RPTS but not PN. On the other hand, Cer 38:2–5, HexCer 36:2–6, and PE O-37:0 were specific targets of PN. Since RPTS and PN had differing preferences for modifying lipid profiles, the underlying processes of effect on TNBS-induced experimental colitis were diverse (Table 3).

Table 3 RPTS and PN Modulate Lipid Metabolites

Lipid Metabolism	DRL vs DAT		DPL vs DAT	
	Trend	P	Trend	P
HexCer 40:7–1	↓	0.0267	↓	0.0463
PE 34:0	↓	0.0111	↓	0.0047
PE O-33:0	↓	0.0001	↓	0.0011
TG(e)54:2	↓	0.0253	↓	0.0073
LPE 20:3	↓	0.0134	–	–
LPE 22:5	↓	0.0338	–	–
PE 38:6	↓	0.0334	–	–
PE 40:8	↓	0.0233	–	–
PI 32:0	↓	0.0096	–	–
HexCer 36:2–6	–	–	↓	0.0422
PE O-37:0	–	–	↓	0.0193

Experimental Colitis with Damp-Heat Type is Closely Related to Amino Acid Disorder

In addition to the changes in lipid profile, the amino acid metabolic profile of IBD patients was also disturbed. Therefore, we conducted amino acids targeted metabolomics to determine the amino acid metabolism characteristics of experimental colitis with damp-heat type. To evaluate the repeatability and reliability of the method, QC samples were found clustered in the PCA scores plot. Then, the DAT and DNC groups were also well separated from the RNC group in the PCA and OPLS-DA analyses, with good model parameters respectively. A total of 35 amino acids were detected. It indicated that significant amino acid metabolic changes occurred in the DAT and RNC groups (Table S10). Compared with the RNC group, the levels of serine, histidine, N-acetylneuraminic acid, and sulfocysteine were found significantly increased in the DAT group ($p < 0.05$). Kynurenine and taurine were found significantly decreased in the DAT group ($p < 0.05$). Amino acid metabolism was also altered between incident DAT and DNC groups (Table S11). Proline and tryptophan were down-regulated and histidine and N-acetylneuraminic acid were up-regulated in the DAT, compared with the DNC ($p < 0.05$). In addition, there were also significant differences between DNC and RNC groups (Table S12). The levels of serine, N-acetylneuraminic acid, glycine, and sulfocysteine were found to increase in DNC, compared with the RNC ($p < 0.05$), while kynurenine was decreased ($p < 0.05$). Compared with the RNC group, the levels of N-acetylneuraminic acid were found to significantly increase both in the DAT and DNC groups ($p < 0.05$), and Kynurenine was found to significantly decrease ($p < 0.05$) (Table 4). Different from their effects on lipid metabolism, RPTS, and PN did not regulate abnormal amino acid metabolism effectively. The levels of serine and sulfocysteine were found to increase in the DPL group, compared with the DAT group ($p < 0.05$) (Table S13). Serine was found to increase in the DRL group, compared with the DAT group ($p < 0.05$), while arginine was decreased in the DRL group ($p < 0.05$) (Table S14). There was a tendency for taurine, tryptophan, and proline to elevate in the DPL group, but it was not statistically significant, whereas these amino acids were decreased in the DAT group. Furthermore, we found that the abundance of leucine in the DRL group tends to increase, but without significant difference. The lipids and amino acids involved in the findings are shown in Figure 5.

Regulation of Lipid-Related Metabolic Enzymes by RPTS and PN

We found that the main altered lipids in the DAT group were glycerophospholipids dominated by PE, PC, and LPE. Moreover, we found that RPTS and PN regulate disorders of glycerophospholipid metabolism. Thus, the therapeutic

Table 4 Comparison of Amino Acid Between Groups

Amino Acid	DAT VS RNC		DAT VS DNC		DNC VS RNC		DRL VS DAT		DPL VS DAT	
	Trend	P	Trend	P	Trend	P	Trend	P	Trend	P
Serine	↑	0.0003	/	/	↑	0.0001	↑	0.0432	↑	0.0111
Kynurenine	↓	0.0043	/	/	↓	0.0073	/	/	/	/
Histidine	↑	0.0142	↑	0.0013	/	/	/	/	/	/
N-acetylneuraminic acid	↑	0.0148	↑	0.0351	↑	0.0008	/	/	/	/
Taurine	↓	0.0152	/	/	/	/	/	/	/	/
Sulfocysteine	↑	0.0175	/	/	↑	0.0239	/	/	↑	0.0193
Proline	/	/	↓	0.0007	/	/	/	/	/	/
Tryptophan	/	/	↓	0.0235	/	/	/	/	/	/
Glycine	/	/	/	/	↑	0.0073	/	/	/	/
Arginine	/	/	/	/	/	/	↓	0.0013	/	/

effect of RPTS and PN on experimental colitis with damp-heat type might be related to its ability to regulate disorders of glycerophospholipid metabolism. In order to better understand how RPTS and PN work, we chose to look into the regulatory impact of RPTS and PN on glycerophospholipid metabolism-related enzymes, such as cytosolic phospholipase A2 (cPLA2), Secreted phospholipases A2 (sPLA2), phosphatidylethanolamine N-methyltransferase (PEMT). Compared with the RNC group, the colonic expression levels of sPLA2 and cPLA2 in the DAT group were significantly increased, and low-dose RPTS and PN treatment could lower the expression of sPLA2 and cPLA2 (Figure 6a and b). In addition, compared with the RNC group, the colonic expression of PEMT was markedly decreased in the DAT group, while low-dose RPTS and PN administration had an influence on the level of PEMT (Figure 6c). Meanwhile, the levels of sPLA2 and cPLA2 in the plasma were significantly increased in the DAT group. Low-dose PN significantly decreased the levels of sPLA2 and cPLA2 ($p < 0.05$). Compared with the DAT group, low-dose RPTS reduced the plasma levels of sPLA2 and cPLA2, but the differences were not statistically significant (Figure 6d and e).

Correlation Analysis of Serum Metabolites and Cytokines

In our study, DAT group-specific metabolites were correlated with cytokines (Figure 7). Among them, PE O-37:0 and Cer 41:1–2 were highly positively correlated with IL-1B, TNF-α and IL-6 ($r > 0.8$, $p < 0.001$). CAR 22:1, CAR 20:1, and LPE 18:0 were highly positively correlated with IFN-γ ($r > 0.8$, $p < 0.001$). CAR 22:1 and LPE 18:0 were moderately positively correlated with IL-1B, TNF-α, and IL-6 ($r > 0.5$, $p < 0.001$). CAR 16:3, Cer 42:1–4, Cer 42:2–3, HexCer 40:7–1, LPE 16:0, CAR 18:0, TG 56:4–1 and TG 57:9–2 were moderately positively correlated with IL-1B, TNF-α, IL-6 and IFN-γ ($r > 0.5$, $p < 0.05$). In addition, PE O-37:0 was highly negatively correlated with TGF-β, IL4, IL-10 and IL-13 ($|r| > 0.8$, $p < 0.001$). N-acetylneuraminic acid was moderately correlated with IL-1B, TNF-α, IL-6, and IFN-γ ($r > 0.5$, $p < 0.01$). Meanwhile, N-acetylneuraminic acid had a highly negative correlation for TGF-β, IL4, IL-10, and IL-13 ($|r| > 0.8$, $p < 0.001$). Histidine was moderately correlated with IL-1B, TNF-α, IL-6, and IFN-γ ($r > 0.5$, $p < 0.05$).

Discussion

IBD is a chronic inflammatory disease and is generally thought to be caused by a variety of factors, including genetics, the environment, and the gut microbiome etc. Nowadays, the incidence of IBD is on the rise in the worldwide scale, and some anti-inflammatory drugs and immunosuppressants have been used to treat IBD. However, current therapeutic strategies have inherent drawbacks and are unable to reach the ultimate goal with long-term remission. Many Chinese herbs have shown clinical efficacy against IBD and are expected to be candidates for the treatment of IBD. Among the

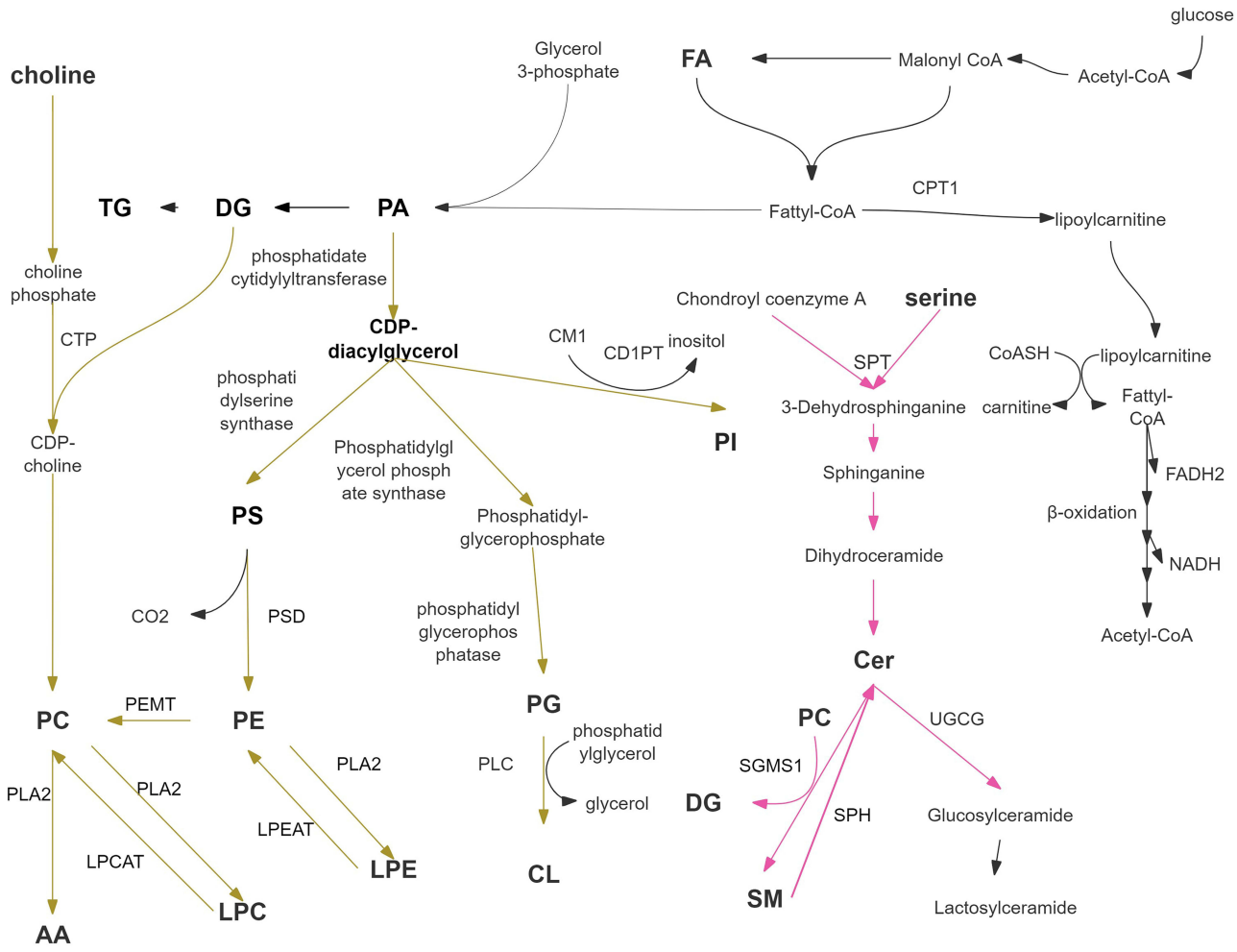

Figure 5 The lipids and amino acids involved in the findings are shown. Arrow color: pink, and brown indicated sphingolipid metabolism pathway and glycerophospholipid metabolism pathway, respectively. Metabolic pathways were mapped based on KEGG pathways.

herbs, Rhizoma Paridis has been reported to have antioxidant, anti-infection, anti-inflammatory, anti-allergy effects. To the best of our knowledge, the therapeutic effect of Rhizoma Paridis on IBD has not been reported. In this paper, the therapeutic effects of its main extracts, RPTS or PN on experimental colitis of damp-heat type was studied, and some new insights were provided for the basic research and clinical application of Rhizoma Paridis.

In the current study, we established TNBS-induced colitis with the damp-heat type and explored its lipid and amino acid metabolic profile, as well as demonstrated that both RPTS and PN improved the disease. Our data showed that low-dose RPTS and PN significantly downregulate circulating cytokines in experimental colitis with damp-heat type. Furthermore, we intensively assessed alterations in lipid and amino acid metabolism in rats with TNBS-induced colitis with damp-heat type by using integrative pseudotargeted lipidomic and amino acids targeted metabolomics analyses. Pseudotargeted lipidomic metabolomics revealed that glycerophospholipids, sphingolipids, carnitine, and glycerolipids were the four most perturbed lipid classes in plasma samples of DAT groups. Amino acids targeted metabolomics revealed that, in the DAT group, serine, N-acetylneuraminic acid, histidine, proline, taurine, and kynurenine changed significantly compared with the RNC group. Among these differential lipids and amino acids, some of Cer, SM, CAR, LPE, PE, TG, and N-acetylneuraminic acid were highly positively correlated with pro-inflammatory cytokines, suggesting that these metabolites are closely related to inflammation and immunity. Meanwhile, we assessed the effects of low dosages of RPTS and PN on the lipid metabolic spectrum and amino acid metabolism in plasma. Interestingly, the effects of RPTS and PN on the dysregulation of the lipid profile were surprisingly different, although RPTS and PN both

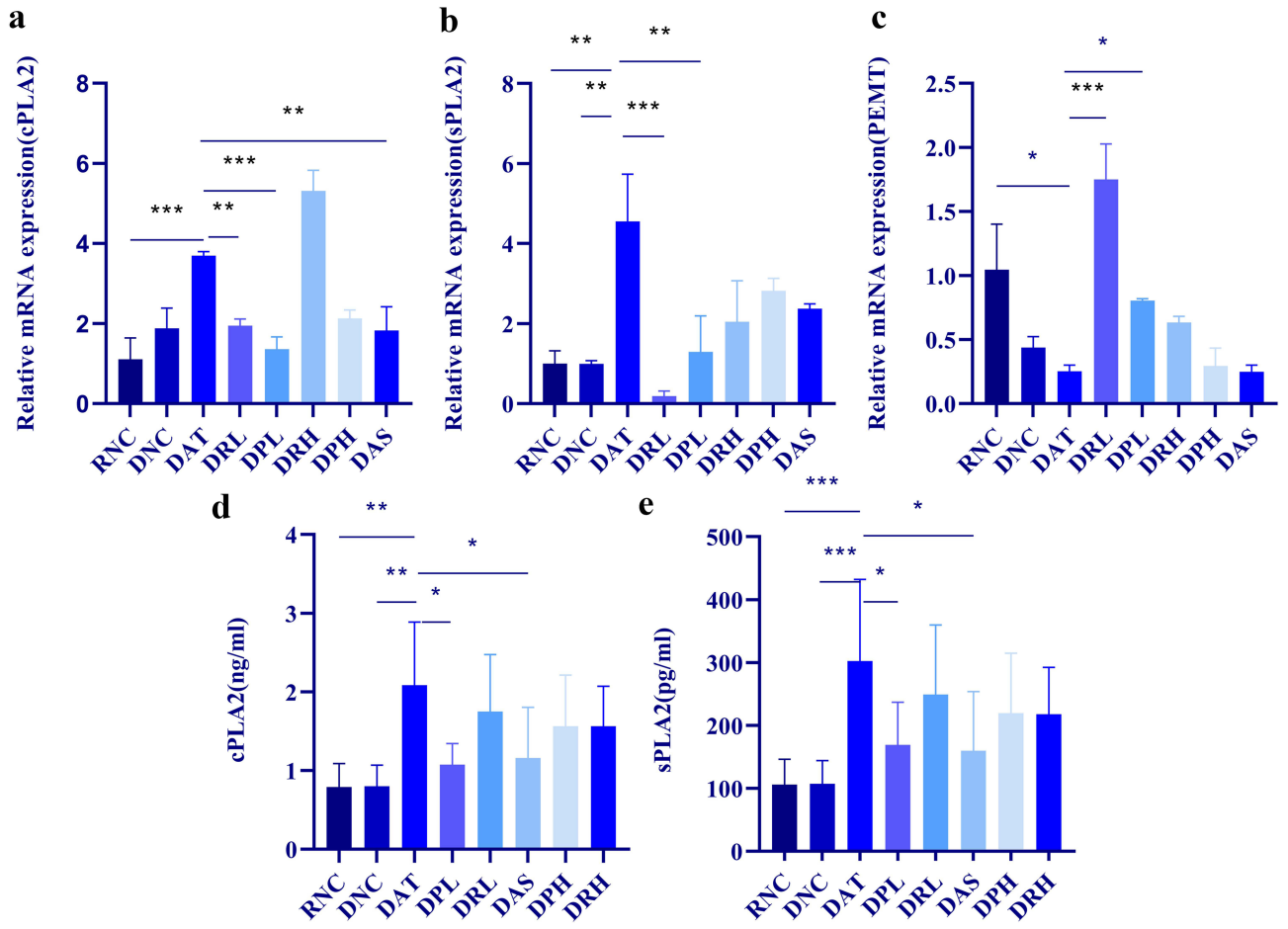

Figure 6 (**a**–**c**) The colonic expression of sPLA2, cPLA2, and PEMT mRNA in each group; (**d** and **e**) The levels of sPLA2 and cPLA2 in the plasma of rats in each group (n = 6 per group).*p < 0.05, **p < 0.01, ***p<0.001.

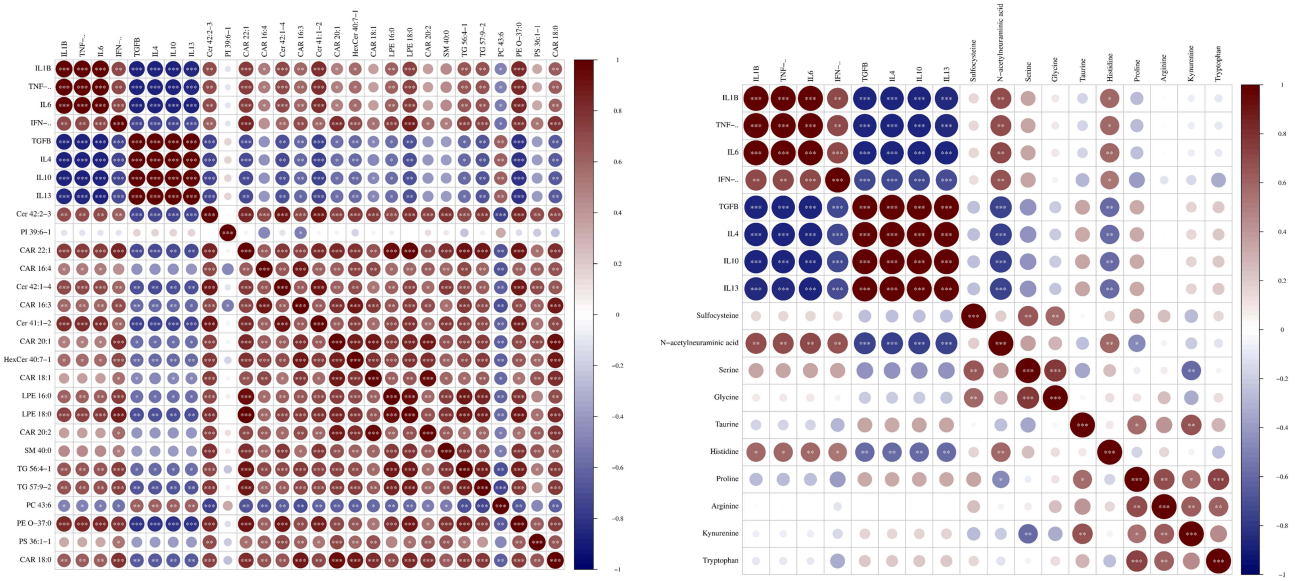

Figure 7 Correlation analysis of plasma metabolites and cytokines. Red represents a positive correlation. Blue represents a negative correlation. Circle node size and color depth are related to the correlation coefficient. p-values are marked in the figure (*p < 0.05, **p < 0.01, ***p<0.001).

regulate glycerophospholipid metabolism and sphingolipid metabolism, especially the abundance of PE and LPE. RPTS mainly regulates PE, PG, and PS, and significantly reduced TG accumulation. However, PN mainly regulated metabolites related to sphingolipid metabolism. These results revealed that, although both RPTS and PN affected TNBS-induced experimental colitis, their underlying mechanisms might be distinct. However, both of them did not have a significant effect on amino acid modulation.

Based on the results of drug efficacy of RPTS and PN on damp-heat type with experimental colitis in rats, we found that low doses of RPTS and PN were more effective in relieving colitis than high doses group. Low-dose RPTS could increase body weight, decrease DAI score, decrease histopathological score and significantly increase colon length. Low dose of PN could increase colon length, decrease colon weight / length ratio and significantly reduce the histopathologic score. However, the effect of high-dose RPTS and PN groups was poor. Studies have shown that low intake of Rhizoma Paridis may have therapeutic effects on disease, but long-term excessive intake could cause irreversible damage.[31,32] In order to explore the metabolic regulation of RPTS and PN on experimental colitis of damp-heat type, the study has focused on the effect of the low-dose group on the damp-heat type with experimental colitis.

Based on our previous clinical findings,[16] the IBD and IBD with damp-heat syndrome (IBD-DH) groups showed changes in plasma glycerophospholipid metabolic pathways, but the types of glycerophospholipid metabolites were different. However, there are few studies on the metabolic characteristics of IBD-DH patients and animal models. In this investigation, we described a comprehensive plasma lipidome of experimental colitis of damp-heat type. To be more specific, we showed that the plasma lipidomic profiles in the DAT group were significantly altered when compared to the RNC group. In the DAT group, there were noticeable variations in the glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, and carnitine metabolism. Similar to clinical study, in animal experiments, glycerophospholipids (PLs) metabolism disorder were also found in the DAT group. Glycerophospholipids serve as binding sites for both internal and extracellular proteins and are the primary elements of cell membranes.[33] PC, PE, PG, PI, and PS are the five groups that are divided based on polar headgroup.[34] Several interrelated enzyme pathways, including the Kennedy pathway (PC and PE synthesis), the cytidine diphosphate diacylglycerol (CDP-DAG) pathway (synthesis of PS and PI from PA), and the Lands cycle, are used to generate and modify PLs (FA removal and reattachment to PLs).[35] Previous studies have confirmed that IBD patients and experimental colitis animals both have altered glycerophospholipids.[3,13] In previous studies, PC and LPC were the metabolites that changed significantly. For instance, research has shown that disturbed PC catabolism and LPCs had considerably higher plasma abundance in the TNBS-induced acute colitis rats model.[3] In the DSS-induced colitis mice, Wang et al discovered altered LPC expressions, including decreased LPC (18:1) and LPC (18:2), and elevated LPC (18:0).[36] Reduced LPC (18:1) was also found in pediatric CD patients who had recently received a diagnosis.[37] Similarly, our data showed that some LPCs and PCs have risen and some have fallen in the DAT group. Moreover, PC is hydrolyzed by phospholipase (PLA) to create LysoPC (LPC).[38] LPC is mainly derived from the turnover of phosphatidylcholine (PC) in the circulation by phospholipase A2 (PLA2).[39] Changes in PC and LPC may be closely related to PLA2. Patients with IBD have significantly higher activity and expression levels of PLA2 in their inflamed intestinal regions.[40] Low-dose PN reduced sPLA2 and cPLA2 levels measured by ELISA in our study. We, therefore, hypothesized that PN is acting on PLA2 and thus regulating PC and LPC.

The most noticeable differences in PE and LPE were observed between the healthy control group and the IBD group.[41–43] These phenomena are consistent with what we have observed. In our results, significant changes in PE and LPE in the DAT group were found. Almost all cell membranes of organisms contain the two lipid groups, PE, and LPE, which are involved in cellular signaling, division, death, and inflammation. Mammalian cells can produce PE by four different processes, including the base-exchange pathway, the CDP-ethanolamine pathway, the PSD pathway, and the acylation of LPE.[44] We therefore speculated that elevated PE may be related to multiple pathways and other phospholipid transformations. PS is delivered to the mitochondria for decarboxylation into PE by PS decarboxylase (PSD). PS and PE levels are closely related to mitochondrial function.[44] Research has shown that UC patients had a considerably higher abundance of LPS and PS, and patients with CD had significantly greater concentrations of PSacyl (PSa) 40:3 and PSa 38:3 and also had a positive correlation with Crohn's Disease Activity Index.[45] Our data also showed that in the DAT group, the abundance of PS increased. Apart from PS, PC is closely related to PE. An essential enzyme in the formation

of hepatic phosphatidylcholine (PC) is phosphatidylethanolamine N-methyltransferase (PEMT). During three consecutive methylation processes, PEMT transforms phosphatidylethanolamine (PE) into PC.[46] Excessively high or low cellular PC / PE molar ratios influence the energy metabolism of different tissues, which have also been connected to the development of disease.[47] In addition, in CD patients significant changes were also found in PI and PA.[9,41] In addition to serving as a substrate for lipid kinases and phosphatases, which can produce phosphoinositide derivatives, phosphatidylinositol (PI) is a crucial structural phospholipid (PIP). According to in vivo studies, phospholipids play a protective role in barrier function and have a significant impact on cytokine release.[9] Our results showed a decline in the abundance of PI in DAT group. Both RPTS and PN regulate PE and LPE and PE-related glycerophospholipids. In addition to regulating PLA2, RPTS and PN may regulate other glycerophospholipid-related enzymes, such as PEMT.

Similar to PLs, sphingolipids (SLs) were significantly altered in the DAT group. Representative membrane lipids known as SLs are crucial for the maintenance and balance of the gastrointestinal (GI) immune system.[48] Studies have specifically examined the role of SLs in IBD. Ceramides increase in the current data in accordance with the UC condition, from remission to active inflammation,[42] its trends are consistent with the results of our study. Ceramides and their derivatives, including lactosylceramide, sphingomyelin, and sphingosine 1-phosphate, have been shown to change in IBD patients in several investigations.[13] Ceramide is regarded as the center of SL metabolism as well as the structural foundation of other SLs. Ceramides can be produced de novo, from the remedial pathway, or from the hydrolysis of sphingolipids (SM) or other complex SLs, which play a crucial role in inflammation, immunology, and inflammatory diseases.[49] Ceramides can also be produced by additional routes, which may involve certain bacteria and cytokines. The abundance of ceramide in macrophage cell lines was previously demonstrated to increase quickly in response to LPS, TNF, and IL-1.[50] In our research, there was a strong correlation between circulating levels of inflammatory cytokines such as IL-6, IL-1B, and TNF and Cer42:2:3, Cer41:1:2. Thus, it became crucial to concentrate on more complex issues, such as which specific SL(s), at what dose, and via what mechanism mediates cytokine signaling (s). Furthermore, the addition of ceramide may initiate PLC activation and subsequently potentiate the sequential PKC-MAPK cascade-cPLA2 pathway in order to cause arachidonic acid (AA) release and cPLA2 activation.[51] Ceramide 1-phosphate (Cer-1-P) activates cPLA2 possibly in response to IL-1B stimulation.[52] Ceramide was also discovered to mediate sPLA2 activation and the expression of TNF-induced COX-2.[53] We should also focus on the effect of ceramide on PLA2 in response to proinflammatory cytokine stimulation. Consistent with the change in Ceramides, LacCer may be a promising biomarker for IBD patients. On granulocytes, monocytes, and platelets, LacCer, also known as CD17, is expressed. It is involved in cell-cell communication, intracellular signaling, nitric oxide production, and phagocytosis. TNF-α, a proinflammatory cytokine that is a well-known target in the treatment of IBD, mediates LacCer production.[30] Moreover, LacCer causes the release of arachidonic acid (AA), an inflammatory mediator, and the activation of phospholipase A2 (PLA2).[54] In addition, SM and Hexcer also changed significantly in the DAT group. PN tends to regulate sphingolipids more than RPTS. We speculate that this tendency may be related to low-dose PN modulating pro-inflammatory factors better.

Unlike ceramide, which is highly correlated with IL-6, CAR22:1, and CAR20:1 are highly correlated with IFN. Total medium-chain acylcarnitines and long-chain carnitine esters were evidently present in larger amounts in UC patients than in control persons. Carnitine transports and carries long-chain fatty acids (LCFAs). As a result, the interaction of different lengths of fatty acid chains attached to acylcarnitines may play a role in the pathogenesis of UC.[55] Additionally, L-carnitine is crucial for energy metabolism, since it allows the transfer of activated long-chain fatty acids (LCFA) as carnitine esters through the inner mitochondrial membrane. Since the availability of acylcarnitines is the rate-limiting step in the -oxidation of fatty acids, this fact along with a higher abundance of acylcarnitine derivatives suggests an enhanced oxidation of fatty acids in IBD patients.[56] Indeed, higher energy demanded to mobilize immune cells to combat inflammation may be the cause of increased oxidation in IBD patients. The immunological response could become more pronounced and pro-inflammatory factors produced.[57] Consistent with this, we found that CARs were highly correlated with IFN. However, we did not find evidence for RPTS and PN in regulating CAR in the lipid profiles.

Our metabolomic profiling also discovered changes in certain amino acid metabolism in the DAT group. Of these, serine is closely related to lipid metabolism. Serine is involved in ceramide synthesis and PS synthesis. Several studies have shown that non-essentialamino acids (serine) were decreased in IBD subjects compared to control subjects.[11,58]

However, our results indicated an increase in serine in the DAT group compared to the RNC group, but no significant difference in serine of the DAT group compared to the DNC group. More experiments are needed to verify the exact mechanism. Additionally, tryptophan and its metabolites have been identified as significantly altered in the blood of patients with IBD compared to controls. A vital amino acid called tryptophan can be found in meals high in protein. The kynurenine pathway is responsible for the metabolism of more than 90% of the dietary tryptophan. The initial and rate-limiting step in converting TRP to KYN is carried out by the enzyme indoleamine 2.3-dioxygenase (IDO) 1. Blood levels of IBD patients have lower level of tryptophan.[59] Our results showed that tryptophan and kynurenine were significantly lower in the DAT group. Since we noted that kynurenine was also decreased in the DNC group, we speculate that the difference may be related to damp-heat type. Specific mechanisms need to be explored further.

Despite the large number of metabolites that have been discovered, there is still a significant gap between the identification of these substances and the understanding of how they specifically contribute to IBD. This research might reveal novel molecular pathways and biomarkers implicated in the etiology of IBD. Future research should incorporate the metabolomic profiles with additional findings obtained from other omics. Analytical procedure variations could potentially contribute to the conflict between studies. Future studies with larger sample sizes should further validate changes in the lipidomic profiles provided here when utilizing a quantitative targeted approach.

Conclusion

Low dose of RPTS and PN from Rhizoma Paridis could alleviate experimental colitis of damp-heat type. Lipidomic and targeted quantitative amino acid metabolomics revealed disorders of plasma lipid and amino acid metabolism in rats caused by combined modeling of damp-heat type and TNBS. Most of these differential metabolites were significantly associated with pro-inflammatory cytokines. Low doses of RPTS and PN could regulate glycerophospholipid metabolism. The mechanism may be related to reducing pro-inflammatory cytokines, increasing anti-inflammatory cytokines, and regulation of lipid metabolism-related enzymes, such as sPLA2, cPLA2, and PEMT. But it may not be related to amino acid metabolism.

Abbreviations

AA, Arachidonic acid; CARs, Carnitine; CD, Crohn's disease; CE, Cholesterol ester; Cer, ceramide; Cer-1-P, Ceramide 1-phosphate; CL, Cardiolipin; cPLA2, Cytosolic phospholipase A2; DAI, Disease Activity Index; DG, Diacylglycerol; ELISA, Enzyme-linked immunosorbent assay; FA, Fatty acid; FC, Fold change; HE, Hematoxylin-Eosin; Hexcer, Monohexosylceramide; Hex2cer, Dihexosylceramide; HSHFD, High-sugar and high-fat diet; IBD, Inflammatory bowel disease; IBD-DH, IBD with damp-heat syndrome; IDO, Indoleamine 2.3-dioxygenase; LCFAs, Long-chain fatty acids; LPC, Lysophosphatidylcholine; LPE, Lysophosphatidylethanolamine; OPLS-DA, Orthogonal partial least squares discriminant analysis; PA, Phosphatidic acid; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PEMT, Phosphatidylethanolamine N-methyltransferase; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; PIP, Phospholipid; PLs, Glycerophospholipids; PN, Pennogenin; PS, Phosphatidylserine; PSD, PS decarboxylase; RPTS, Rhizoma paridis total saponins; RT–qPCR, Real-time quantitative PCR analysis; SLs, Sphingolipids; SM, Sphingomyelin; sPLA2, Secreted phospholipases A2; TG, Triacylglycerols; TNBS, 2,4,6-trinitrobenzene sulfonic acid; UC, Ulcerative colitis; USFA, unsaturated fatty acids; VIP, Variable importance projection.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics Approval and Informed Consent

Animal care and experimentation were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, and the animal study was approved by the Animal Welfare Committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School (No.2021AE02022).

Acknowledgments

The authors appreciate Dalian Chem Data Solution Information Technology Co. Ltd. for the technical support provided in this study.

Funding

This research was funded by the National Natural Science Foundation of China (no.81873160) and Nanjing Drum Tower Hospital Senior Talents Projects.

Disclosure

Prof. Dr. Wu Jing reports grants from the National Natural Science Foundation of China and grants from Nanjing Drum Tower Hospital. The authors declare no other conflicts of financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Kaplan GG. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol*. 2015;12(12):720–727. doi:10.1038/nrgastro.2015.150
2. Ramos GP, Papadakis KA. Mechanisms of disease: inflammatory bowel diseases. *Mayo Clin Proc*. 2019;94(1):155–165. doi:10.1016/j.mayocp.2018.09.013
3. Zhang X, Choi FFK, Zhou Y, et al. Metabolite profiling of plasma and urine from rats with TNBS-induced acute colitis using UPLC-ESI-QTOF-MS-based metabonomics—a pilot study. *FEBS J*. 2012;279(13):2322–2338. doi:10.1111/j.1742-4658.2012.08612.x
4. Zhang X, Zhang J, Zhou Z, et al. Integrated network pharmacology, metabolomics, and transcriptomics of Huanglian-Hongqu herb pair in non-alcoholic fatty liver disease. *J Ethnopharmacol*. 2024;325:117828. doi:10.1016/j.jep.2024.117828
5. Aldars-García L, Gisbert JP, Chaparro M. Metabolomics insights into inflammatory bowel disease: a comprehensive review. *Pharmaceuticals*. 2021;14(11):1190. doi:10.3390/ph14111190
6. Williams HRT, Willsmore JD, Cox IJ, et al. Serum metabolic profiling in inflammatory bowel disease. *Dig Dis Sci*. 2012;57(8):2157–2165. doi:10.1007/s10620-012-2127-2
7. Balasubramanian K, Kumar S, Singh RR, et al. Metabolism of the colonic mucosa in patients with inflammatory bowel diseases: an in vitro proton magnetic resonance spectroscopy study. *Magn Reson Imaging*. 2009;27(1):79–86. doi:10.1016/j.mri.2008.05.014
8. Bjerrum JT, Steenholdt C, Ainsworth M, et al. Metabonomics uncovers a reversible proatherogenic lipid profile during infliximab therapy of inflammatory bowel disease. *BMC Med*. 2017;15(1):184. doi:10.1186/s12916-017-0949-7
9. Sewell GW, Hannun YA, Han X, et al. Lipidomic profiling in Crohn's disease: abnormalities in phosphatidylinositols, with preservation of ceramide, phosphatidylcholine and phosphatidylserine composition. *Int J Biochem Cell Biol*. 2012;44(11):1839–1846. doi:10.1016/j.biocel.2012.06.016
10. Masoodi M, Pearl DS, Eiden M, et al. Altered colonic mucosal Polyunsaturated Fatty Acid (PUFA) derived lipid mediators in ulcerative colitis: new insight into relationship with disease activity and pathophysiology. *PLoS One*. 2013;8(10):e76532. doi:10.1371/journal.pone.0076532
11. Scoville EA, Allaman MM, Brown CT, et al. Alterations in lipid, amino acid, and energy metabolism distinguish Crohn's disease from ulcerative colitis and control subjects by serum metabolomic profiling. *Metabolomics*. 2018;14(1):17. doi:10.1007/s11306-017-1311-y
12. Santoru ML, Piras C, Murgia F, et al. Metabolic alteration in plasma and biopsies from patients with IBD. *Inflamm Bowel Dis*. 2021;27(8):1335–1345. doi:10.1093/ibd/izab012
13. Guan S, Jia B, Chao K, et al. UPLC-QTOF-MS-based plasma lipidomic profiling reveals biomarkers for inflammatory bowel disease diagnosis. *J Proteome Res*. 2020;19(2):600–609. doi:10.1021/acs.jproteome.9b00440
14. Notararigo S, Martín-Pastor M, Viñuela-Roldán JE, Quiroga A, Dominguez-Munoz JE, Barreiro-de Acosta M. Targeted 1H NMR metabolomics and immunological phenotyping of human fresh blood and serum samples discriminate between healthy individuals and inflammatory bowel disease patients treated with anti-TNF. *J Mol Med*. 2021;99(9):1251–1264. doi:10.1007/s00109-021-02094-y
15. He F, Wu C, Li P, et al. Functions and signaling pathways of amino acids in intestinal inflammation. *Biomed Res Int*. 2018;2018:1–13. doi:10.1155/2018/9171905
16. Wu X, Liu K, Wu Q, et al. Biomarkers of metabolomics in inflammatory bowel disease and damp-heat syndrome: a preliminary study. *Evid Based Complement Alternat Med*. 2022;2022:3319646. doi:10.1155/2022/3319646
17. Wu J, Shan Z, Pena AS. Retrospective analysis of 115 cases of inflammatory bowel disease and their treatment with Chinese and western medicine. *Pract Clin J Integr Tradit Chin West Med*. 2004;2004(01):4–6.
18. Zhang S, Shen H, Zheng K, Bai Y. Expert consensus opinion on the diagnosis and treatment of ulcerative colitis in Chinese medicine (2017). *Chin J Trad Chin Med*. 2017;32(08):3585–3589.
19. Wenliang L. *Experimental Study on the Effect and Mechanism of the Method of Clearing Away Damp and Heat on the Epidemic Febrile Disease Syndrome of Damp and Heat*. Hubei University of Chinese Medicine; 2005.
20. Peng XT, Ma Q, Zhang XS, et al. Urine metabolomics in the rat model of damp-heat diarrhea. *Acta Lab Anim Sci Sin*. 2019;27(6):700–708. doi:10.3969/jissn1005-4847.2019.06.003
21. Ge CC, Lu Y, Shen H, Zhu L. Analysis of serum metabolomic characteristics of patients in the active stage of ulcerative colitis with syndrome of dampness-heat in large intestine. *J Beijing Univ Tradit Chin Med*. 2024;47(5):686–698.
22. Guo B, Bian Z, Qiu H, Wang Y, Wang Y. Biological and clinical implications of herbal medicine and natural products for the treatment of inflammatory bowel disease. *Ann N Y Acad Sci*. 2017;1401(1):37–48. doi:10.1111/nyas.13414

23. Wang M, Chen Y, Yang B, Wang Q, Zhou J, Wu J. Therapeutic effect of Zaoxiu formula on TNBS-induced inflammatory bowel disease in mice and its mechanisms. *Jiangsu J Tradit Chin Med*. 2015;47(09):80–83.
24. Jin TT, Liu FJ, Jiang Y, et al. Molecular-networking-guided discovery of species-specific markers for discriminating five medicinal Paris herbs. *Phytomedicine*. 2021;85:153542. doi:10.1016/j.phymed.2021.153542
25. Wang YY, Jiang Y, Yang CJ, et al. Research progress on chemical constituents, pharmacological activities, and clinical applications of Paris polyphylla var. yunnanensis. *Chin Tradit Herbal Drugs*. 2022;53(23):7633–7648.
26. Weng YJ, Zheng XB. Model of ulcerative colitis rats with damp-heat syndrome. *Lishizhen Med Mater Med Res*. 2011;22(10):2522–2525.
27. Xu Y, Gong Y-X, Tang Y-P, Liu S. Effect of herbal decoctionin the ulcerative colitis rat with dampness heat syndrome. *Chin J Integr Tradit West Med Dig*. 2020;28(07):485–488+493.
28. Zheng Y, Liang C, Li Z, et al. Study on the mechanism of Huangqin Decoction on rats with ulcerative colitis of damp-heat type base on mtDNA, TLR4, p-PI3K, p-Akt protein expression and microbiota. *J Ethnopharmacol*. 2022;295:115356. doi:10.1016/j.jep.2022.115356
29. Zhang Y, Yao W, Zhang W, et al. Yujin powder improves large intestine dampness-heat syndrome by regulating gut microbiota and serum metabolism. *Biomed Chromatogr*. 2023;37(11):e5719. doi:10.1002/bmc.5719
30. Friedrich M, Pohin M, Powrie F. Cytokine networks in the pathophysiology of inflammatory bowel disease. *Immunity*. 2019;50(4):992–1006. doi:10.1016/j.immuni.2019.03.017
31. Li Z, Fan Q, Chen M, et al. The interaction between polyphyllin I and SQLE protein induces hepatotoxicity through SREBP-2/HMGCR/SQLE/LSS pathway. *J Pharm Anal*. 2023;13(1):39–54. doi:10.1016/j.jpha.2022.11.005
32. Yang R, Wang Y, Shi M, et al. Antitumor activity in vitro and toxicity of the total saponins from Paris forrestii. *Chin J Clin Pharmacol*. 2018;34 (04):439–442.
33. Bochkov VN, Oskolkova OV, Birukov KG, Levonen AL, Binder CJ, Stöckl J. Generation and biological activities of oxidized phospholipids. *Antioxid Redox Signaling*. 2010;12(8):1009–1059. doi:10.1089/ars.2009.2597
34. O'Donnell VB, Rossjohn J, Wakelam MJO. Phospholipid signaling in innate immune cells. *J Clin Investig*. 2018;128(7):2670–2679. doi:10.1172/JCI97944
35. Lands WEM. Metabolism of glycerolipides; a comparison of lecithin and triglyceride synthesis. *J Biol Chem*. 1958;231(2):883–888. doi:10.1016/S0021-9258(18)70453-5
36. Wang R, Gu X, Dai W, et al. A lipidomics investigation into the intervention of celastrol in experimental colitis. *Mol Biosyst*. 2016;12 (5):1436–1444. doi:10.1039/c5mb00864f
37. Daniluk U, Daniluk J, Kucharski R, et al. Untargeted metabolomics and inflammatory markers profiling in children with Crohn's disease and ulcerative colitis—a preliminary study. *Inflammatory Bowel Dis*. 2019;25(7):1120–1128. doi:10.1093/ibd/izy402
38. Wymann MP, Schneiter R. Lipid signalling in disease. *Nat Rev Mol Cell Biol*. 2008;9(2):162–176. doi:10.1038/nrm2335
39. Law SH, Chan ML, Marathe GK, Parveen F, Chen CH, Ke LY. An updated review of lysophosphatidylcholine metabolism in human diseases. *IJMS*. 2019;20(5):1149. doi:10.3390/ijms20051149
40. Markovic M, Ben-Shabat S, Nagendra Manda J, et al. PLA2-triggered activation of cyclosporine-phospholipid prodrug as a drug targeting approach in inflammatory bowel disease therapy. *Pharmaceutics*. 2022;14(3):675. doi:10.3390/pharmaceutics14030675
41. Horta D, Moreno-Torres M, Ramírez-Lázaro MJ, et al. Analysis of the association between fatigue and the plasma lipidomic profile of inflammatory bowel disease patients. *J Proteome Res*. 2021;20(1):381–392. doi:10.1021/acs.jproteome.0c00462
42. Diab J, Hansen T, Goll R, et al. Lipidomics in ulcerative colitis reveal alteration in mucosal lipid composition associated with the disease state. *Inflammatory Bowel Dis*. 2019;25(11):1780–1787. doi:10.1093/ibd/izz098
43. Tefas C, Ciobanu L, Tanțău M, Moraru C, Socaciu C. The potential of metabolic and lipid profiling in inflammatory bowel diseases: a pilot study. *Bosn J Basic Med Sci*. 2019. doi:10.17305/bjbms.2019.4235
44. Vance JE. Historical perspective: phosphatidylserine and phosphatidylethanolamine from the 1800s to the present. *J Lipid Res*. 2018;59 (6):923–944. doi:10.1194/jlr.R084004
45. Iwatani S, Iijima H, Otake Y, et al. Novel mass spectrometry-based comprehensive lipidomic analysis of plasma from patients with inflammatory bowel disease. *J Gastroenterol Hepatol*. 2020;35(8):1355–1364. doi:10.1111/jgh.15067
46. Van Der Veen JN, Lingrell S, McCloskey N, et al. A role for phosphatidylcholine and phosphatidylethanolamine in hepatic insulin signaling. *FASEB j*. 2019;33(4):5045–5057. doi:10.1096/fj.201802117R
47. Van Der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim Biophys Acta Biomembr*. 2017;1859(9):1558–1572. doi:10.1016/j.bbamem.2017.04.006
48. Bryan PF, Karla C, Edgar Alejandro MT, Sara Elva EP, Gemma F, Luz C. Sphingolipids as mediators in the crosstalk between microbiota and intestinal cells: implications for inflammatory bowel disease. *Mediators Inflammation*. 2016;2016:1–11. doi:10.1155/2016/9890141
49. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature*. 2014;510(7503):58–67. doi:10.1038/nature13475
50. MacKichan ML, DeFranco AL. Role of ceramide in Lipopolysaccharide (LPS)-induced signaling. *J Biol Chem*. 1999;274(3):1767–1775. doi:10.1074/jbc.274.3.1767
51. Sato T, Kageura T, Hashizume T, Hayama M, Kitatani K, Akiba S. Stimulation by ceramide of phospholipase A2 activation through a mechanism related to the phospholipase C-initiated signaling pathway in rabbit platelets. *J Biochem*. 1999;125(1):96–102. doi:10.1093/oxfordjournals.jbchem.a022275
52. Pettus BJ, Bielawska A, Subramanian P, et al. Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A2. *J Biol Chem*. 2004;279 (12):11320–11326. doi:10.1074/jbc.M309262200
53. Kitatani K, Akiba S, Sato T. Ceramide-induced enhancement of secretory phospholipase A2 expression via generation of reactive oxygen species in tumor necrosis factor-α-stimulated mesangial cells. *Cell Signalling*. 2004;16(8):967–974. doi:10.1016/j.cellsig.2004.02.003
54. Nakamura H, Moriyama Y, Watanabe K, et al. Lactosylceramide-induced phosphorylation signaling to group IVA phospholipase A_2 via reactive oxygen species in tumor necrosis factor-α-treated cells. *J Cell Biochem*. 2017;118(12):4370–4382. doi:10.1002/jcb.26091
55. Bene J, Komlósi K, Havasi V, et al. Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis. *WJG*. 2006;12(1):110. doi:10.3748/wjg.v12.i1.110
56. Lai Y, Xue J, Liu CW, et al. Serum metabolomics identifies altered bioenergetics, signaling cascades in parallel with exposome markers in Crohn's disease. *Molecules*. 2019;24(3):449. doi:10.3390/molecules24030449

57. Lu K, Knutson CG, Wishnok JS, Fox JG, Tannenbaum SR. Serum metabolomics in a *Helicobacter hepaticus* mouse model of inflammatory bowel disease reveal important changes in the microbiome, serum peptides, and intermediary metabolism. *J Proteome Res*. 2012;11(10):4916–4926. doi:10.1021/pr300429x
58. Schicho R, Shaykhutdinov R, Ngo J, et al. Quantitative metabolomic profiling of serum, plasma, and urine by (1)H NMR spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. *J Proteome Res*. 2012;11(6):3344–3357. doi:10.1021/pr300139q
59. Kohashi M, Nishiumi S, Ooi M, et al. A novel gas chromatography mass spectrometry-based serum diagnostic and assessment approach to ulcerative colitis. *J Crohns Colitis*. 2014;8(9):1010–1021. doi:10.1016/j.crohns.2014.01.024