ORIGINAL RESEARCH—CLINICAL

Metabolomic Signatures Highlight Fiber-Degrading Bacteroides Species in Mediterranean Diet Response Among Ulcerative Colitis

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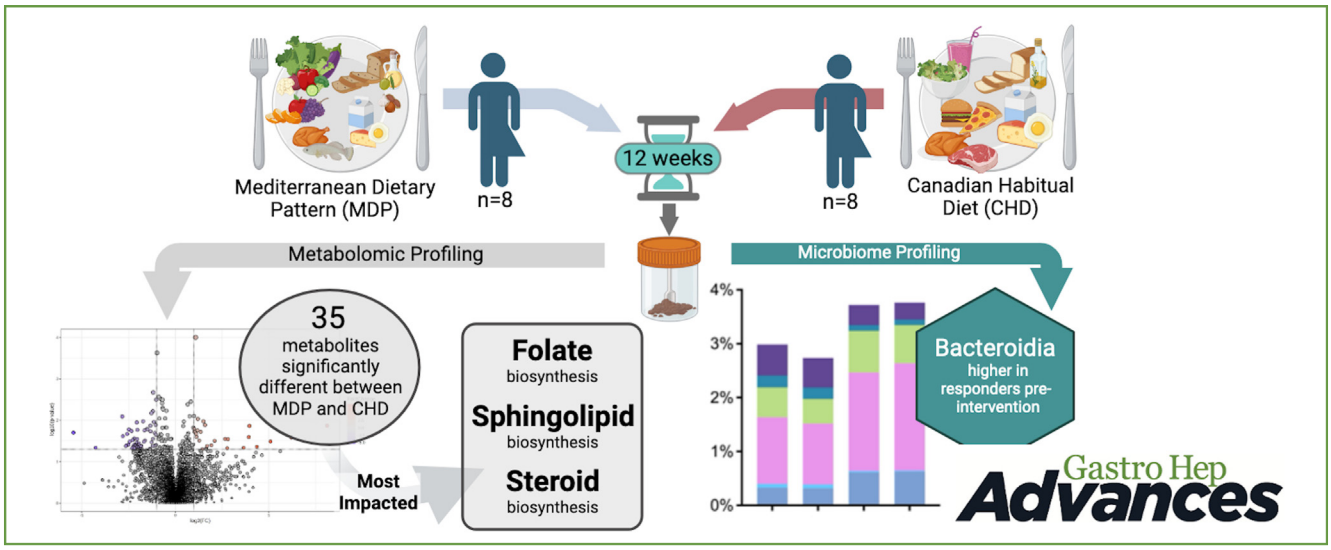

BACKGROUND AND AIMS: The Mediterranean diet pattern (MDP) is associated with health-associated gut microbes and metabolites. However, the impact of the MDP on the fecal metabolome in ulcerative colitis (UC) remains unclear. We characterized the fecal metabolome of patients with UC with high adherence to the MDP compared to the Canadian habitual diet (CHD). Furthermore, we explored potential differences in the fecal metabolome between dietary responders and non-responders to the MDP. **METHODS:** Utilizing untargeted metabolomics on a subset of fecal samples obtained from a randomized controlled trial, adult patients with quiescent UC underwent a 12-week intervention following either the MDP (n = 8) or CHD (n = 8). Liquid chromatography-tandem mass spectrometry was employed to profile endogenous fecal metabolites, while 16S amplicon sequencing was utilized to profile the fecal microbiota. **RESULTS:** A total of 701 human metabolites were detected, with 35 exhibiting significant differential expression between the MDP and CHD groups. Noteworthy, folate biosynthesis, sphingolipid biosynthesis, and steroid biosynthesis were identified as major pathways affected. Moreover, microbial analysis showed that individuals with increased levels of the class Bacteroidia (*Bacteroides vulgatus* [*B. vulgatus*], *B. uniformis, and B. acidifaciens*) in their stool at baseline were more likely to respond to the MDP. **CONCLUSION:** High adherence to an MDP is associated with beneficial metabolite changes associated with reducing inflammation in UC. In addition, fiber-degrading microbes abundant before dietary intervention played a role in the responsiveness to the MDP. This work lays the groundwork for developing a metabolic signature associated with the MDP to develop personalized nutrition strategies for UC prevention and treatment. ClinicalTrials.gov Number: NCT03053713.

***Both authors contributed equally.**

Abbreviations used in this paper: **ASV, amplicon sequence variant; CHD, Canadian habitual diet; CRF, corticotrophin-releasing factor; ESI, electrospray ionization; FC, fold change; FCP, fecal calprotectin; IBD, inflammatory bowel disease; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDP, Mediterranean diet pattern; NS, nonresponders; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; PMS, partial Mayo score; RCT, randomized control trial; RS, responders; S1P, sphingosine-1-phosphate; SCCAI, Simple Clinical Colitis Activity Index; UC, ulcerative colitis; VIP, variable importance in projection; WGCNA, weighted gene correlation network analysis.**

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Introduction

Inflammatory bowel disease (IBD) is a global health issue. The chronic relapsing and remitting pattern of IBD, including ulcerative colitis (UC) and Crohn's disease, imposes a lifelong burden, including metabolic and neuropsychiatric comorbidities and extraintestinal manifestations, leading to challenges in disease management.[1,2] The financial burden of these diseases is immense and has been ranked as one of the fifth most costly gastrointestinal conditions in annual health-care expenditures.[3] This is concerning since recent trends show a steady increase in the incidence of IBD in India, China, Africa, and South America.[4]

The modern Western diet, characterized by higher consumption of animal and dairy products, refined sugars, processed foods, and a reduction in plant-based fibers, is frequently implicated as an environmental risk factor for IBD.[5] Processed foods in Western diets often contain additives, emulsifiers, and bulking agents, which are not typically present in traditional diets.[6] This diet significantly differs from the traditional diets of earlier generations, which were locally sourced and consumed shortly after harvest. Another major difference is the addition of xenobiotics, mainly derived from environmental pollution or pesticides.[7] Diet should be included as a tool to optimize the treatment of IBD, especially considering that it is a major predictor of the gut microbiome. The gut microbiome is critical in modulating immune responses and metabolic functions and maintaining intestinal barrier integrity. The Westernized microbiome is distinguished by the decline of Volatile and/or Associated Negatively with Industrialized Societies of Humans taxa, commonly found in populations adhering to traditional lifestyles, whereas the rise of Bloom or Selected in Societies of Urbanization/Modernization taxa are prevalent in modern, industrialized populations.[8,9] In IBD, evidence supports a lack of diversity and general instability in the bacteriome, often with a dysbiosis characterized by the relative depletion of *Faecalbacterium praustnitzii* and *Roseburia hominis* and the enrichment of *Escherichia coli*, *Ruminococcus torques*, and *R. gnavus*.[10] The metabolomic profile is marked by diminished concentrations of short-chain fatty acids like butyrate, alterations of amino acids—particularly branched-chain amino acids—and alterations in fatty acid esters (acylcarnitines). Additionally, there are variations in the profiles of secondary bile acids.[10–12] Despite these advances, discrepancies among various studies have made it challenging to validate microbiome and metabolome signatures across diverse populations, diminishing their diagnostic value in IBD. Therefore, it is crucial to understand the complex interactions between diet, the gut microbiome, the metabolome, and the host immune system to effectively utilize diet as a therapeutic tool for managing IBD.

One dietary approach gaining attention in IBD is the Mediterranean diet pattern (MDP).[13–15] The diet is characterized by increased consumption of legumes, whole grains, vegetables, fruits, nuts, and seeds, and low intake of red meat with olive oil as the main culinary fat.[16] The synergy of the high levels of dietary fiber, phytochemicals, and the fat blend (high monounsaturated fat) confer beneficial anti-inflammatory and antioxidant properties.[17] Observational studies demonstrate that adherence to an MDP is associated with reductions in all-cause mortality[18] and lower levels of fecal calprotectin (FCP).[13,19–21] Results from clinical trials suggest that the MDP is associated with improvements in biomarkers of inflammation (C-reactive protein and FCP, reduction of disease activity indices [partial Mayo score]), alterations in the bacteriome and improved quality of life.[12,14,15,22,23] Given the benefits of the MDP to overall health and the tolerability of the diet in IBD, the American Gastroenterology Association recommends that all patients with IBD follow an MDP.[24]

In this study, we characterized the fecal metabolome in patients with quiescent UC following an MDP compared to those following the CHD. Additionally, we aimed to determine whether baseline gut microbiota could predict the response level to an MDP.

Materials and Methods

Study Design and Sample Collection

The present study is a post hoc exploratory analysis based on data obtained from a 12-week randomized control trial (RCT) (intention to treat analyses) that investigated whether the MDP vs CHD would affect disease activity, inflammation, and the gut microbiome in patients with mild, quiescent UC.[22] Briefly, following informed consent, participants were randomized to either an MDP or CHD. The participants randomized to the MDP received a series of one-on-one online coaching sessions from a Master's trained registered dietitian (RD; NH) with expertise in nutrition and IBD. The participants were counseled on how to adapt their diet based on the Mediterranean Diet Pyramid.[16] The CHD participants met with the dietitian at a similar intervention intensity to the MDP and received a nutritional analysis comparing their intakes to the dietary reference intakes; however, they were not provided tailored dietary advice. The Mediterranean Diet Serving Score (MDSS) assessed adherence to the MDP.[25] The MDSS score ranges from 0 to 24 points, with greater than 13.5 points indicating adherence to the MDP. Moreover, the ASA24-Canada 2016[26] data were used to validate the MDSS and enhance compliance. Comprehensive information on participant recruitment, the dietary intervention, collection of dietary data, baseline and week-12 diet details, and MDP dietary adherence scores are described elsewhere.[27]

Untargeted metabolomics was completed on a subset of the fecal samples MDP (n = 8) or CHD (n = 8). As this was an exploratory analysis, we chose to examine samples in the MDP group that were most adherent to the MDP (median MDSS = 21), whereas those with lowest adherence scores to the MDSS scores (median 13 points) were chosen from the CHD group (Table 1).

Table 1. Demographic Description of the Cohort of the Participants at Week 12 (n = 16)

Factors	Canadian habitual diet (n = 8) median (IQR)	MDP (n = 8) median (IQR)
Demographics		
Male, n (%)	3 (38)	2 (25)
Age (y)	42 (34–55)	56 (35–64)
Body Mass Index (kg/m^2)	24 (22–28)	22 (20–29)
Disease activity		
Partial Mayo score	0 (0–1)	0 (0)
SCCAI	1 (0–1)	0 (0–1)
Δ in fecal calprotectin over 12 wk (mcg/g)[a]	293 ([–19]–458) (n = 7)	3 ([–95]–75)
Diet and quality of life		
Healthy eating index	55 (51–62)	85 (83–91)
MDSS	13 (8–15)	21 (16–23)
SIBDQ	62 (58–64)	63 (54–65)
Medications[b]		
No medications, n (%)	0	1 (13)
5-ASA, n (%)	8 (100)	6 (75)
Immunomodulator, n (%)	0	1 (13)
Biologic, n (%)	0	1 (13)

5-ASA, 5-aminosalicylic acids; IQR, interquartile range; MDP, Mediterranean Diet Pattern; SCCAI, Simple Clinical Colitis Activity Index; SIBDQ, Short Inflammatory Bowel Disease Questionnaire.
[a]Δ in fecal calprotectin = change from baseline to week 13.
[b]Participants were presribed one or more therapy.

To identify which patients responded more effectively to the MDP intervention, we subset the microbiome and metabolome data from the MDP group for more refined analysis. To distinguish responders (RS) from nonresponders (NS), we defined RS as those achieving or sustaining remission, characterized by a 50% reduction in FCP from baseline to week 12, or sustained remission (FCP <50) from baseline to week 12 accompanied by a decrease or no change in disease activity according to the Simple Clinical Colitis Activity Index.[28]

Metabolomics Analysis by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry

Fecal samples from week 12 (final samples) were analyzed by Agilent 1290 UPLC attached to a Q-TOF (Agilent 6530B) (Agilent, CA, USA) with dual Agilent Jetstream electrospray source. Analyte separation was achieved using a Waters BEH-C18 column (2.1 × 100 mm and 1.7 um; Milford, MA, USA). For dual-ion data acquisition, a gradient-elution of 0.05% aqueous formic acid with 5-mM ammonium formate (Solvent A) and acetonitrile with 0.05% formic acid (Solvent B) was used. The linear gradient of positive electrospray ionization (ESI+) mode was described as follows: 0 minutes: 4% B; 2 minutes: 4% B; 8 minutes: 40% B; and 16 minutes: 96% B; 24 minutes: 100% B; 38 minutes: 100% B; 39 minutes: 4% and negative (ESI-) mode was: 0 minutes: 4% B; 2 minutes: 4% B; 8 minutes: 40%; 16 minutes: 90%; 24 minutes: 100%; 38 minutes: 100%; 39 minutes: 4% B. For MS analysis, the scan range was set to full MS—we selected ion monitoring with a scan range between 85–950 m/z in ESI+ and 85–1050 m/z in ESI-. The Jetstream electrospray source was run in both ESI+ and ESI- ionization mode with a sheath gas temperature of 325 °C, capillary voltage is set as 3000 volts for ESI+ and 4000 volts for ESI-, nozzle voltage was set as 1000 volts in ESI+ and 2000 volts in ESI-, the fragmentor voltage was 120 volts for both ion modes. Mass correction was done automatically through MassHunter software using the standard Agilent reference masses of 121.050873 and 922.009798 in ESI+ and 112.985587 and 1033.988109 in ESI-.

Data Processing and Annotation

Data (ESI+ and ESI-) were preprocessed with the online XCMS (https://xcmsonline.scripps.edu/) platform for peak annotation. The parameters were selected as: 10 ppm, mzdiff = 0.01, retention time correction method = obiwarp with all other parameters set as default. The data matrix containing variables (retention time, m/z, peak intensity) was obtained for further annotation against the human metabolome database.[29]

Pathway Enrichment Analysis

For pathway analysis, we utilized the human and prokaryote Kyoto Encyclopedia of Genes and Genomes (KEGG) database integrated within MetaboanalystR 4.0[30] to map metabolites from both host and microbial communities. For pathways showing statistically significant differences between groups, the annotated compounds within these pathways were extracted and visualized using GraphPad Prism v10.0 (GraphPad Software, Boston, Massachusetts, USA, www.graphpad.com)

Weighted Gene Coexpression Analysis (Weighted Correlation Network Analysis [WGCNA])

Correlation analysis of metabolites was performed using WGCNA,[31] with a soft thresholding power range from 1 to 20. The scale-free topology model fit (R-2) of 0.6 was achieved at a power of 14. The features were clustered into modules by hierarchical clustering with Euclidean distance and a minimum module size of 10. Metabolites within each cluster were then analyzed for KEGG pathway enrichment using MetaboanalystR 4.0.[30]

Fecal Microbiome Analysis

The sequencing and bioinformatics analyses were previously described.[22] Briefly, fecal DNA was extracted using the QIAamp PowerFecal Pro DNA kit (Qiagen, #51804). Sequencing was performed by the Gut4Health Microbiome Core Facility (BC Children's Hospital Research Institute, Vancouver, BC), targeting the V4 hypervariable region of the 16S rRNA gene. Amplification was conducted on the Illumina MiSeq platform using the following primers: Forward 5′-AGTCAGTCAGCCGGACTACNVGGGTWTCTAAT and Reverse 5′-AGTCAGTCAGCCGGACTACNVGGGTWTCTAAT, which included the Illumina adapter overhang.

After sequencing, analyses were carried out using the QIIME 2 platform (version 2021.4).[32] Demultiplexed reads from 2 MiSeq runs were imported into QIIME 2, and primers were removed using the q2-cutadapt plugin.[33] Quality control involved filtering, dereplication, chimera removal, denoising,

and merging paired-end reads for each run separately using the DADA2 plugin with default parameters.[34] The resulting amplicon sequence variant (ASV) tables were merged for downstream analysis. A phylogenetic tree was constructed using the SATé-enabled phylogenetic placement technique via the q2-fragment-insertion plugin,[35] utilizing a backbone tree based on the Greengenes reference database (version 13.8).[36]

For taxonomic classification, a classifier trained on the full length of the 16S region was applied, incorporating environment-specific abundance weights tailored to human fecal samples from the *readytowear* tool.[37] This weighted approach enhanced classification accuracy compared to standard Naive Bayes methods.[38] Alpha diversity metrics were calculated, including ASV richness, Shannon's diversity index, and Faith's phylogenetic diversity.[32] Beta diversity was assessed using compositional tensor factorization to account for interindividual variation over time. ANOVA was applied to the compositional tensor factorization results to assess differences between dietary groups.[38]

To account for the longitudinal design of the study (baseline and week 12 fecal samples, with subject-level variation), the BIRDMAn tool [https://birdman.readthedocs.io/en/stable/index.html] was employed to rank ASVs most associated with each group. These rankings were visualized using Qurro,[39] and the top 10 ASV differentials for each group were exported into R[40] via the *qiime2R* package [https://github.com/jbisanz/qiime2R] for additional custom visualization and statistical analysis. BugBase [https://doi.org/10.1101/133462] was used to predict high-level phenotypes, assessing the proportions of Gram-positive, Gram-negative, aerobic, anaerobic, facultative anaerobic, biofilm-forming, and mobile element-containing bacteria.

Statistical Analysis

Samples were normalized by median to correct for systematic differences, and then logarithmic transformation (log10) was applied to normalize for data distribution. Pareto scaling was applied to mitigate the influence of differing scales among features. Next, annotated metabolites were analyzed using a significance threshold of fold change (FC) > 2 and P value $<.05$. Unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) were used to visualize data variances by R 4.2.0 and ggplot2. PLS-DA cross-validation showed the top three components. Scores were plotted for each pairwise comparison and statistical differences were determined by the Wilcoxon rank-sum test using MetaboanalystR 4.0.[30]

Results

In liquid chromatography-mass spectrometry, ESI is used to detect and analyze metabolites. There are two common modes, positive (ESI+) and negative (ESI-), with each mode able to detect different metabolites based on their chemical properties. A total of 4279 peaks were detected in the (ESI+) mode and 7841 peaks in the ESI negative (ESI-) (Table A1). In ESI+, 242 features were upregulated (FC >2), and 279 (FC <0.5) were downregulated in the MDP compared to control. Statistical analyses using a t-test (FC >2 and FC <0.5, P $<.05$) identified 156 significant features (19 upregulated and 45 downregulated features in the MDP) (Table A2). In the ESI- mode, 324 features were upregulated, and 208 were downregulated in the MDP. T-test identified 72 significant features, with 41 upregulated and 31 downregulated features in MDP based on FC and t-test criteria (Table A3).

While PCA did not show clear separation between the 2 groups, PLS-DA revealed clear stratifications between groups (Figure 1A and B). A volcano plot (FC >2, $P < .05$) revealed 27 upregulated peaks and 59 downregulated peaks (Table A4, Figure 1C). The top 50 variable peaks are shown in Figure 1D and E shows an upregulation of organic acids while nucleosides, nucleotides, and analogu are among the downregulated molecules. Metabolites with a variable importance in projection (VIP) score cutoff above 1.25 are shown in Figure A1.

Identification of Metabolites Associated with Consumption of the MDP

A detailed analysis using the human KEGG database revealed a total of 701 human metabolites. Of these, 35 showed significant differential expression between the MDP and CHD groups (Figure 2B). The top 25 differential pathways are shown in Figure 2A, with the two six showing significance (false discovery rate <0.05). By segregating the upregulated compounds (FC >2) and downregulated compounds (FC <0.5) into distinct subsets regardless of the P value, notable findings emerged. The upregulated and downregulated compounds exhibited significant coverage in distinct pathways as shown in Figure 2C.

Sphingosine-1-phosphate (S1P) mediates the inflammatory response by binding to a family of S1P receptors, which are involved in various cellular processes, including lymphocyte trafficking, endothelial barrier function, and cascade of inflammatory responses.[41,42] It is worth noting, compounds such as 4-hydroxy sphinganine, phytosphingosine, sphingosine, L-threo-sphingosine, and dihydrosphingosine, and 7-hexadecenoic acid were significantly downregulated in the MDP as shown in Figure 2D. The decreased S1P pathway parallels the observed reduction in stress steroid hormones including cortisol, 21-deoxycortisol, corticosterone, and aldosterone in MDP group, while 11-dehydrocorticosterone was upregulated (Figure 2E). Steroid hormones, in particular cortisol, are produced locally in the intestinal mucosal tissues in response to immune cell activation and in turn, contribute to balancing these immune responses to avoid tissue damage.[43]

Folate biosynthesis is the top enriched pathway across all detected metabolites (Figure 2F). While folate itself did not significantly differ ($P = .3861$), downstream metabolites like sepiapterin ($P = .0064$) and 7,8-dihydroneopterin ($P = .0064$) showed significant increases in those following the MDP. These results are relevant not only for inflammatory conditions but also for the gut–brain–microbiome axis.[44] Figure 2C shows an upregulation in tyrosine and tryptophan metabolism. However, serotonin, kynurenine, and

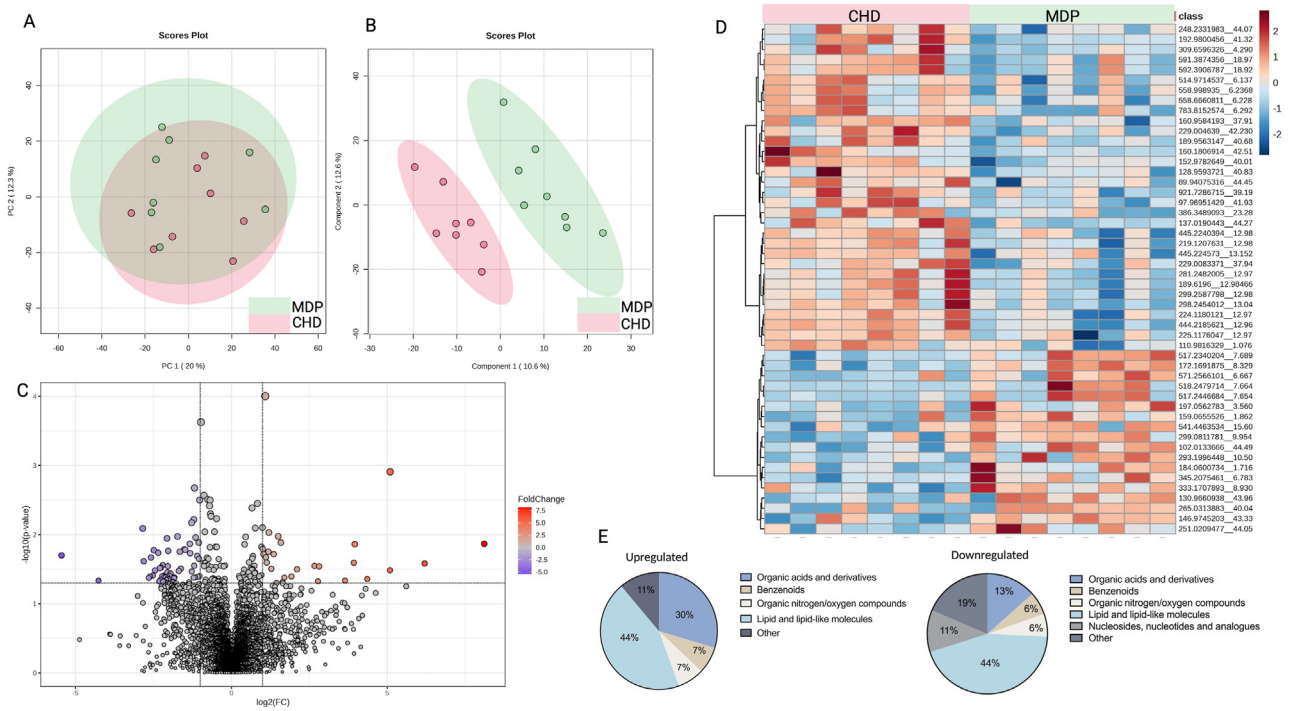

Figure 1. MDP dietary intervention alters the fecal metabolome. PCA (A) and pl-SDA (B) analysis of the metabolites from patients with either CHD or MDP. (C) Volcano plot demonstrates altered peak features between MDP and CHD ($P < .05$, FC >2). (D) Top 50 significantly altered peak features between CHD and MDP group. (E) Chemical classification of upregulated and downregulated metabolites in the MDP group.

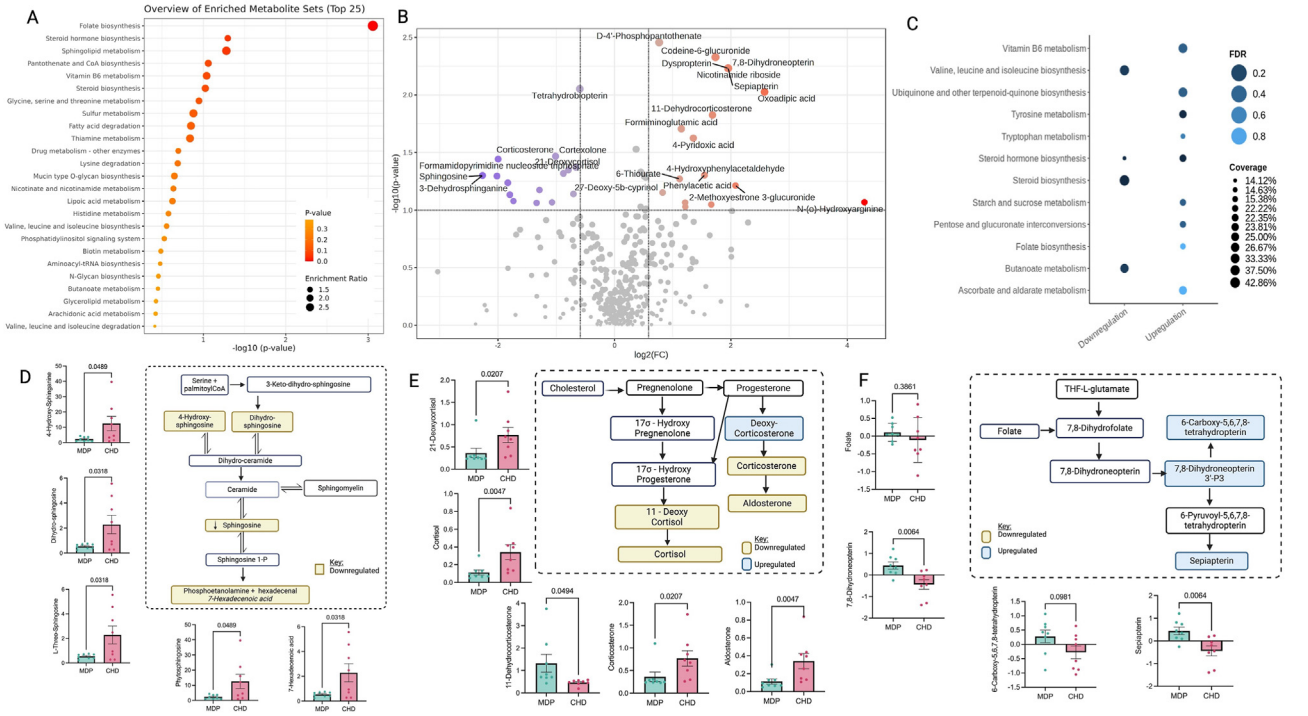

Figure 2. Pathway analysis of metabolites altered in patients receiving the MDP intervention. (A) Overview of enriched metabolite pathways. (B) Volcano plot depicts the significantly altered host metabolites ($P < .05$). (C) Bubble plot illustrating the regulation of metabolic pathways. The x-axis represents the direction of regulation (downregulation or upregulation), while the y-axis lists the various metabolic pathways. The bubble size corresponds to the coverage (%), and the color gradient indicates the false discovery rate with darker shades representing lower values. MDP decreased the major metabolites in sphingosine biosynthesis (D), steroid hormone biosynthesis (E) and increased folate biosynthesis (F).

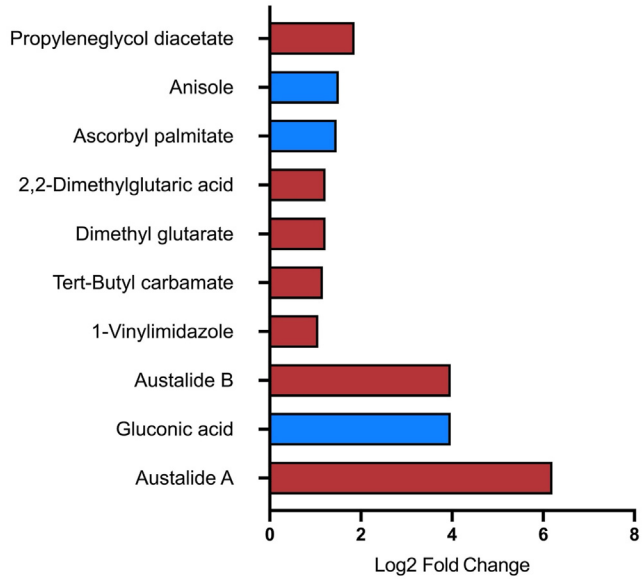

Figure 3. Environmental chemicals or food additives that are upregulated with consumption of the MDP. Log2fold change of environmental chemicals are shown in red, and food additives are shown in blue.

dopamine did not show significant differences across dietary patterns.

Additionally, we observed environmental chemicals and food additives in our cohort. Figure 3 highlights the top 10 metabolites (Log2 FC >1, $P < .05$). Among these compounds, several are commonly found in various food products and environmental sources. The potential health effects of these specific compounds remain unclear, and there are limited reports of their detection in human tissue.

Differences in Metabolites Associated with Consumption of the Mediterranean Diet Pattern Between Responders and Non-Responders

Table A5 show the characteristics of the responders (RS) and non-responders (NS) to the MDP. The PCA analysis (Figure 4A) demonstrates a clear separation between the two groups, while PLS-DA reveals an even more distinct differentiation (Figure 4D), indicating a distinct metabolic profile in the context of MDP treatment. Further investigation into RS and NS to the MDP intervention revealed differences in 109 annotated metabolites (Table A4), with 19 unique peaks identified in the human KEGG database (Figure 4B). Upregulated metabolites are shown in Figure 4C. VIP scores for the top 15 metabolites contributing to the variation in metabolic profiles between NS and RS samples are shown in Figure 4E. The metabolites that meet the VIP score cutoff above 1.25 are shown in Figure A2.

Further WGCNA was analyses identified 28 modules, grouped into 3 clusters (Figure 5A and B). Cluster 1 increased in RS treatment compared to NS, cluster 2 decreased, and cluster 3 remained unchanged (Figure 5C). Metabolites from clusters 1 and 2 were separately analyzed for pathway enrichment. Significantly upregulated and downregulated pathways (false discovery rate <.05) are shown in Figure 5E. The chemical superclasses of upregulated and downregulated metabolites are shown in Figure 5D.

Non-responders to the MDP Have Less Bacteroidia in Their Fecal Microbiome

We investigated the microbial differences between RS and NS to the MDP. Our findings indicate that individuals with reduced levels of the class Bacteroidia in their stool at baseline were less likely to respond positively to the MDP, as illustrated in Figure 6A. Moreover, we focused on individual species to investigate variations within these microbial groups at baseline between RS and NS. By filtering out taxa with extensive zero values across samples, we identified 6 relevant taxa for comparison (Figure 6B). Among these taxa, known fiber degraders like *Bacteroides acidifaciens*, *B. vulgatus*, *B. uniformis*, were observed in higher abundance levels in patients who responded favorably to the dietary intervention before the treatment.

Discussion

To our knowledge, this is the first published report to profile the fecal metabolome from a UC RCT following an MDP. We uncovered metabolites linked to 3 crucial pathways—sphingosine, steroid, and folate biosynthesis—which correlated with high adherence to an MDP. Increasing observational data from population-based research has revealed significant alterations in IBD patients' gut microbial structure and metabolic profiles.[45,46] Recently, a study in a pediatric cohort with UC found alterations in sphingolipid and folate biosynthesis metabolic pathways,[46] and a large observational cohort observed a marked increase in the fecal levels of sphingolipids, including several sphingomyelins and ceramides.[45] It is important to note that these studies, being observational in nature, did not control for dietary variables or patterns, unlike our study, which accounted for these factors. Metabolomic studies in IBD reveal alterations in tryptophan metabolism among patients.[47] Although we initially detected a signal in our dataset, further investigation did not uncover significant differences between our groups.

We noted a significant decrease in fecal sphingolipids (4-hydroxy sphinganine, phytosphingosine, sphingosine, L-threo-sphingosine, and dihydro-sphingosine, and 7-hexadecenoic acid) in the MDP. Sphingolipids are integral components of the intestinal cell membrane, synthesized either through the de novo condensation of serine with palmitoyl-CoA or via the absorption of endogenous and dietary sphingolipids.[48] S1P serves as a central player in orchestrating the inflammatory cascade. It achieves this by binding to a family of S1P receptors, which govern diverse cellular processes, including lymphocyte trafficking,

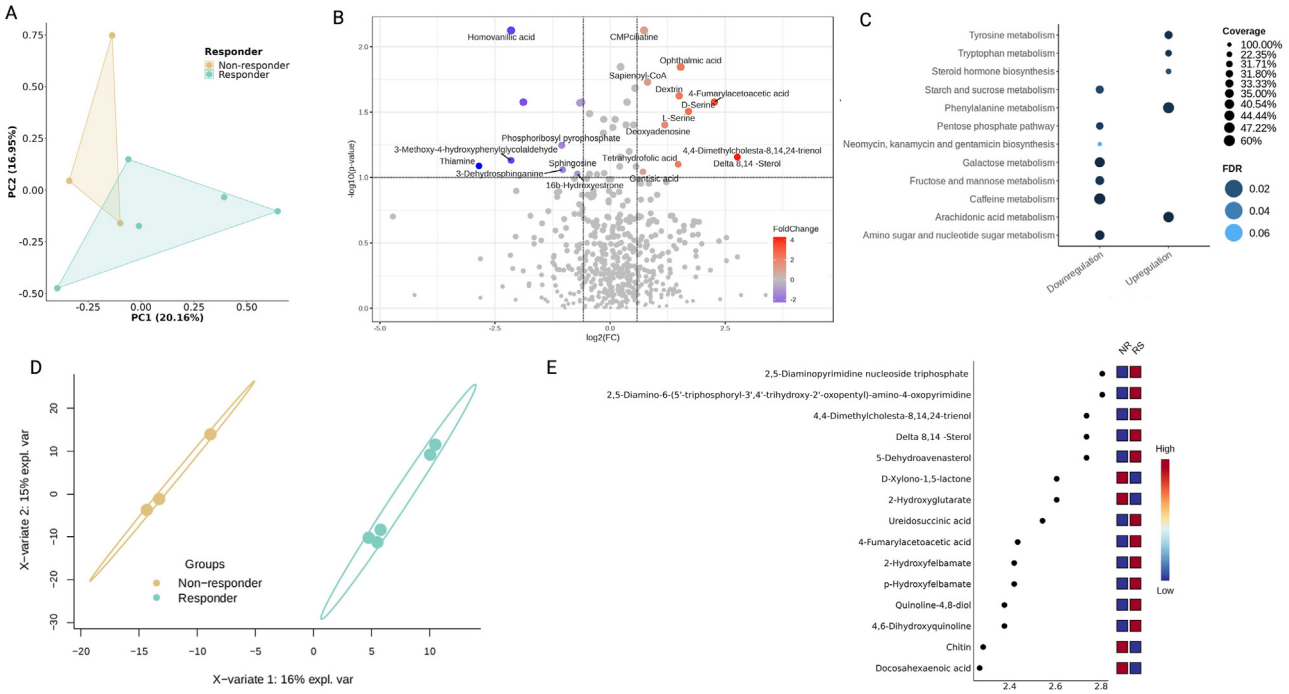

Figure 4. Patients who respond to the MDP have a distinct fecal metabolomic profile. (A) PCA plot demonstrates a clear separation between RS and NS. (B) Volcano plot highlights the statistically significant metabolites between the two groups. (C) Pathway enrichment analysis of the differentially expressed metabolites, showing the biochemical pathways that influence RS and NS. (D) PLS-DA analysis further confirms the separation of the RS and NS groups based on their metabolic profiles. (E) VIP plot from the PLS-DA analysis identified the top 15 important metabolites driving the group separation.

endothelial barrier function, and the initiation of inflammatory responses.[41,48] The upregulation of the S1P signaling pathway is implicated in the pathophysiology of IBD, contributing to both inflammation and barrier dysfunction.[49] In the context of UC treatment, S1P inhibitors may be used in select cases to impede the trafficking of lymphocytes from lymph nodes, diminishing the pool of inflammatory cells available to migrate to the intestinal mucosa and facilitating gut inflammation resolution.[49] We hypothesize that the MDP could potentially participate in S1P inhibition, and may be one mechanism of how the diet exerts its anti-inflammatory effects. Remarkably, the MDP responders experienced an additional downregulation of metabolites of the S1P pathway (ie, 3-dihydrosphinganine and sphingosine).

Stress hormones, cortisol and corticosterone, have been associated with intestinal barrier dysfunction and can trigger flares in IBD.[50–52] Stress directly activates the hypothalamus to release corticotrophin-releasing factor (CRF). This induces the anterior pituitary gland to secrete adrenocorticotrophic hormone, further stimulating the adrenal cortex to secrete cortisol.[53] CRF can induce mast cell degranulation and increase mucosal permeability.[54] In our study, participants adhering to the MDP had a reduction in corticosterone and cortisol levels. The reduction in metabolites associated with steroid biosynthesis suggests the MDP modulates cortisol, supporting its anti-inflammatory effects. Notably, corticosterone and its downstream inactive metabolite, 11-dehydrocorticosterone, exhibited additional reductions in patients who responded positively to the MDP.

Patients with IBD are at a higher risk of folic acid deficiency, as they frequently avoid food products, such as fresh fruits and vegetables, which are the main sources of folic acid. Additionally, the use of sulfasalazine by patients may result in folic acid deficiency.[55] On the contrary, high levels of folate reduce the risk of IBD and colon cancer.[56,57] The increase in folate biosynthesis may be linked to increased consumption of folate-rich foods in the MDP, measured by increased consumption of vegetables, fruit, and legumes.[22,27] Folate plays a crucial role in DNA methylation, red blood cell formation, and reduction in cancer risk.[58,59] One of the key downstream metabolites of the folate pathway that upregulated in the MDP was sepiapterin. In a rodent model of colitis, sepiapterin administration significantly reduced the number of infiltrating inflammatory macrophages and neutrophils and the expression of proinflammatory cytokines interleukin-1β, interleuckin-6, and interleuckin-17A.[60] Additionally, sepiapterin treatment was associated with a decrease in the number of tumors. The anti-inflammatory and antitumor effects of sepiapterin must be further investigated in humans.

We saw several unexpected metabolites upregulated in the stool of the MDP group, which included pesticides (tert-butyl carbamate), herbicides (dimethyl glutarate, 2,2-dimethylglutaric acid), environmental chemicals (mycotoxins; Austalide A and Austalide A, propyleneglycol

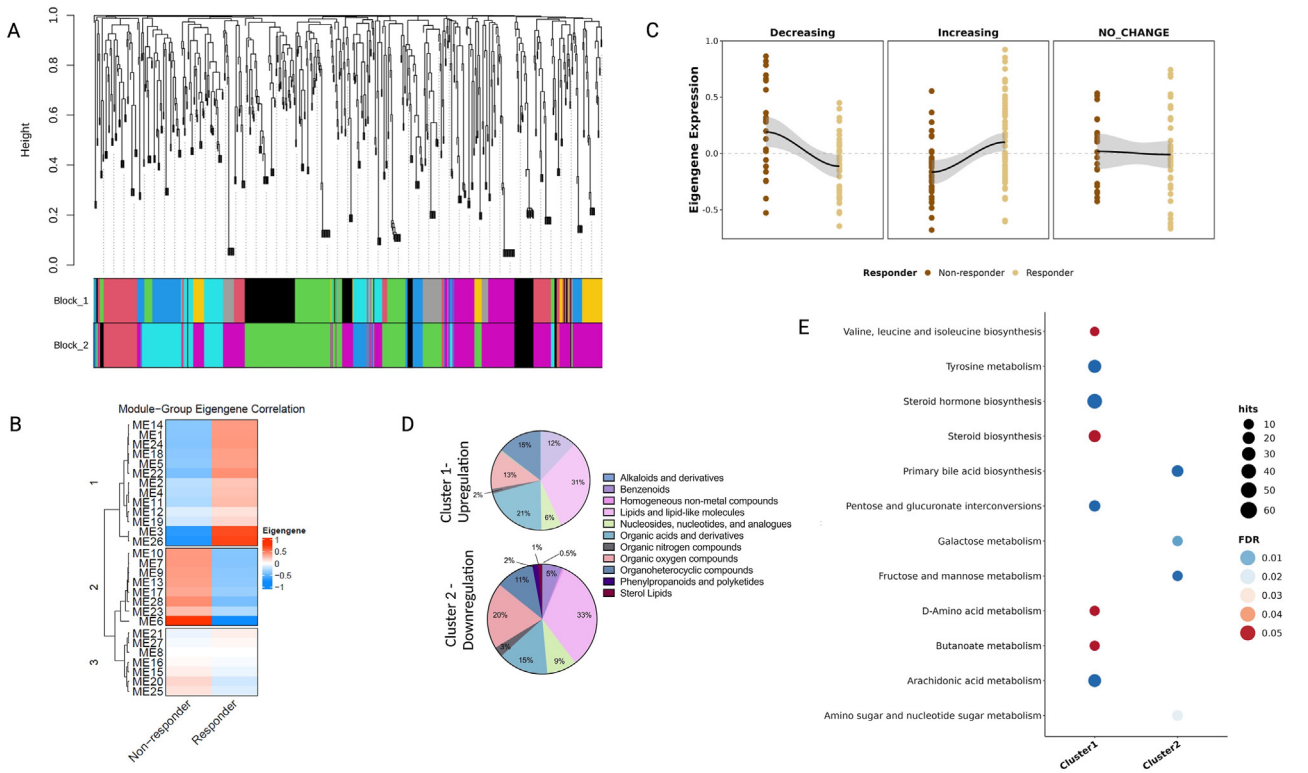

Figure 5. WGCNA analysis of metabolic differences between RS and NS following the MDP. (A) WGCNA dendrogram classifies the metabolites into distinct modules based on their expression patterns. (B) Eigengene network analysis illustrates the correlation of each module with the RS and NS groups, with modules categorized into 3 clusters based on their module alteration patterns between RS and NS, summarized in (C). (D) Chemical categorization of upregulated metabolites from cluster 1 and downregulated metabolites from cluster 2. (E) Pathway enrichment analysis showing the biochemical pathways associated with the upregulated and downregulated metabolites from cluster 1 and 2, respectively.

diacetate, N-vinylimidazole) and food additives and flavorings (ascorbyl palmitate, anisole, gluconic acid). It is important to consider that while the MDP is generally associated with positive health outcomes, these metabolites indicate that even healthy dietary patterns can introduce certain chemicals into the body. A recent study that compared urinary pesticide metabolite levels between conventional and organic foods revealed statistically significant disruptions in metabolic pathways associated with inflammation, oxidative stress, and the requirements of xenobiotic detoxification.[61] The consumption of pesticides has been linked to increased levels of oxidative stress and inflammation, potentially contributing to various diseases, including cancer.[61] Despite the growing body of literature

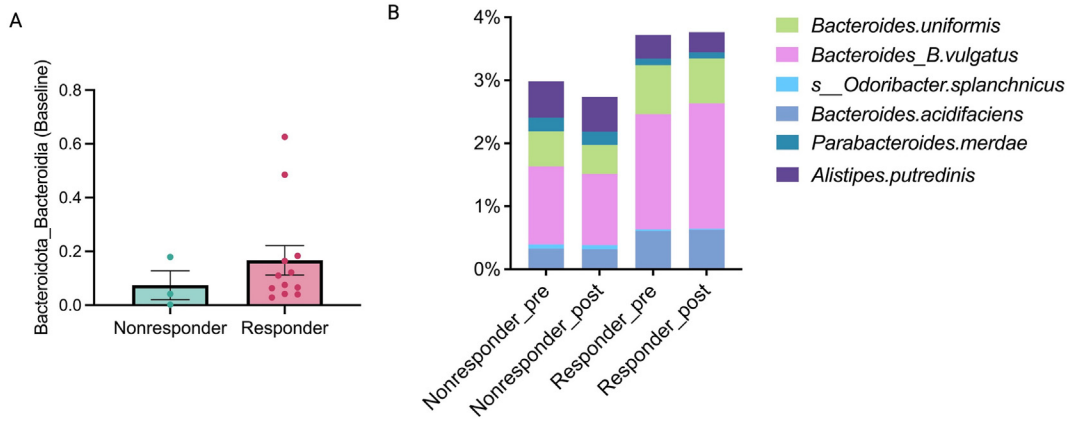

Figure 6. Microbiome composition as a predictor of MDP responsiveness. (A) Comparison of relative abundance of Bacteroidia class between RS and NS at baseline. (B) Relative abundance analysis of all species and genus within the Bacteroidia class.

about the adverse health consequences of pesticide and food additive exposure in recent years, studies focusing on human exposure through the environment and dietary intake remain limited.

There is considerable inter-individual variation in response to nutritional interventions. Therefore, some interventions may benefit certain individuals or population subgroups more than others, depending on their genotype, phenotype, and environment.[62] Here, we identify microbiome-derived biomarkers (taxon) that differ between RS and NS. At the class level, we observed a reduction in Bacteroides. At the genus and species level, responders appeared to have an increased abundance of *Bacteroides vulgatus* (*B. vulgatus*), *B. uniformis, and B. acidifaciens*. *B. vulgatus*, *B. uniformis* and *B. acidifaciens*, all of which have been shown to have a protective effect on colitis, including alleviating the colitis phenotype, reducing inflammatory response, and improving intestinal barrier function in animal models.[63–65] Bacteroidetes (in particular, *B. acidifaciens*) have an important role in gut microbiota by producing high levels of short-chain fatty acids, including acetate, propionate, or butyrate. Many experimental animal data suggest that their high levels can discontinue the progress of several inflammatory, autoimmune and allergic diseases.[66] NS had an increased abundance of *Parabacteroides merdea* and *Ordoribacter splanchnicus*. On the contrary, *Parabacteroides merdae* was increased in human patients with hypertension and polycystic ovary syndrome[67] *Ordoribacter splanchnicus* has been identified as an opportunistic pathogen with potential significance for diagnosing and treating UC.[68] Although these findings need to be confirmed in a larger sample, these microbes provide a potential hypothesis as to why individuals respond or do not respond effectively to the MDP.

Our study has several strengths. Firstly, we employed a nontargeted approach to provide a comprehensive and detailed view of the fecal metabolome. This methodology enabled us to illustrate the relative contribution of each pathway to the overall metabolic alterations. Despite the small sample size, our findings are biologically plausible, and the identified biological pathways and functions align with those described in previous studies. As a hypothesis-generating study, the sample size restricts our ability to adjust for all potential confounders. such as medication use variations, other chronic comorbidities, and different environmental influences, whose impacts on metabolic profiles remain unknown. Furthermore, the cross-sectional design prevented us from observing longitudinal changes in metabolomic profiles and limited us to reporting associations between diet and omics measurements at a single time point. Future studies with larger sample sizes and a longitudinal approach will be necessary to identify alterations in UC more definitively.

In conclusion, this study offers valuable insights into an adult UC population's gut microbiome and fecal metabolome that responds to an MDP. We identified several key metabolites and pathways associated with the diet's anti-inflammatory effects. Future research involving larger, longitudinal studies is essential to further validate these results and explore the therapeutic potential of dietary interventions in managing IBD.

Supplementary Materials

Material associated with this article can be found in the online version at doi: https://doi.org/10.1016/j.gastha.2024.100606.

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Authors' Contributions:
Natasha Haskey: Study design, investigation, data analysis, recruitment, writing – original draft. Deanna L Gibson: Study design, recruitment, data analysis, writing – final draft, review, supervision, resources. Jiayu Ye: Data analysis, writing – final draft, review. Jessica Josephson: Data analysis, writing – final draft, review. Sanjoy Ghosh: Data analysis. Maitreyi Raman: Writing – final draft, review, supervision, resources.

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Ethical Statement:
The study was approved by the University of British Columbia Clinical Research Ethics Board (H16-03300) and the University of Alberta Clinical Research Ethics Board (Pro00106271) and registered with clinicaltrials.gov with identifier NCT03053713. All participants signed an informed letter of consent. The first participant was registered in the trial on April 4, 2017.

Data Transparency Statement:
To access additional files associated with the randomized control trial, please see the data repository at Haskey, Natasha (2024), "Dietary compliance with the Mediterranean diet pattern in a randomized clinical trial of patients with quiescent Ulcerative Colitis", Mendeley Data, V3, https://doi.org/10.17632/2ffyvrdd97.3.

Reporting Guidelines:
Consolidated Standards of Reporting Trials for randomized controlled trials and Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.