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Original Article

A Mediterranean Diet Pattern Improves Intestinal Inflammation Concomitant with Reshaping of the Bacteriome in Ulcerative Colitis: A Randomised Controlled Trial

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Abstract

Background and Aims: Dietary patterns are important in managing ulcerative colitis [UC], given their influence on gut microbiome-host symbiosis and inflammation. We investigated whether the Mediterranean Diet Pattern [MDP] vs the Canadian Habitual Diet Pattern [CHD] would affect disease activity, inflammation, and the gut microbiome in patients with quiescent UC.

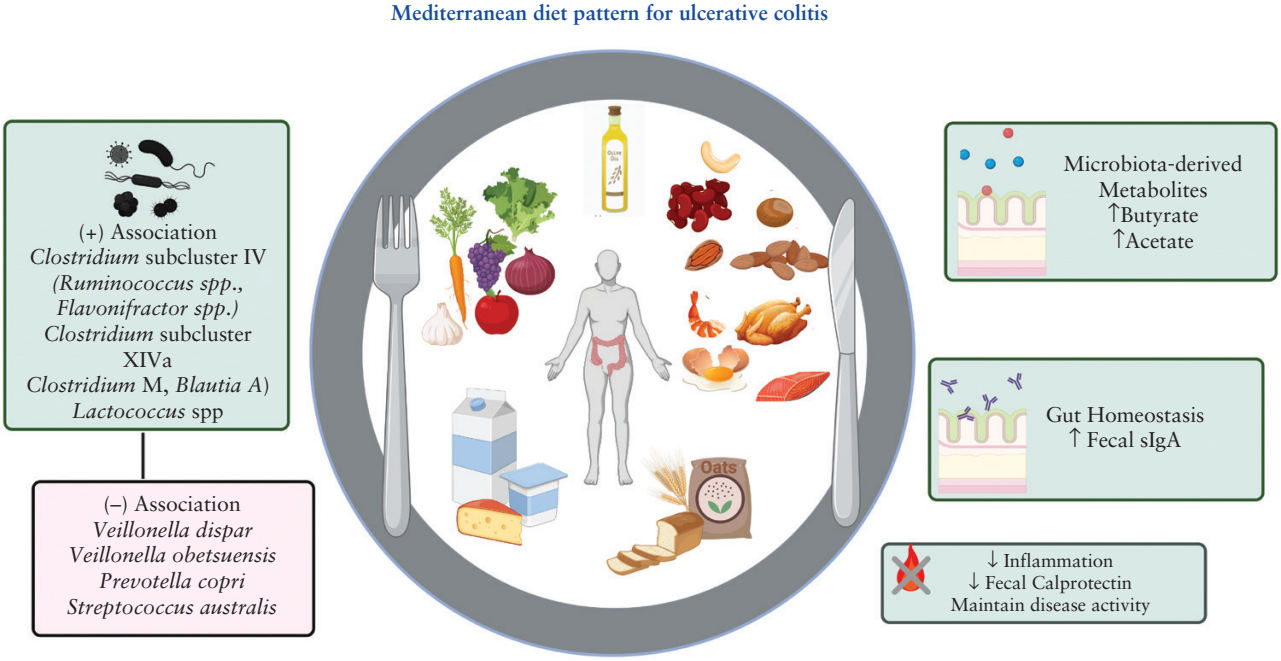
Methods: We performed a prospective, randomised, controlled trial in adults [65% female; median age 47 years] with quiescent UC in an outpatient setting from 2017 to 2021. Participants were randomised to an MDP [n = 15] or CHD [n = 13] for 12 weeks. Disease activity [Simple Clinical Colitis Activity Index] and faecal calprotectin [FC] were measured at baseline and week 12. Stool samples were analysed by 16S rRNA gene amplicon sequencing.

Results: The diet was well tolerated by the MDP group. At week 12, 75% [9/12] of participants in the CHD had an FC >100 μg/g, vs 20% [3/15] of participants in the MDP group. The MDP group had higher levels of total faecal short chain fatty acids [SCFAs] [p = 0.01], acetic acid [p = 0.03], and butyric acid [p = 0.03] compared with the CHD. Furthermore, the MDP induced alterations in microbial species associated with a protective role in colitis [*Alistipes finegoldii* and *Flavonifractor plautii*], as well as the production of SCFAs [*Ruminococcus bromii]*.

Conclusions: An MDP induces gut microbiome alterations associated with the maintenance of clinical remission and reduced FC in patients with quiescent UC. The data support that the MDP is a sustainable diet pattern that could be recommended as a maintenance diet and adjunctive therapy for UC patients in clinical remission. ClinicalTrials.gov no: NCT0305371

Key Words: Mediterranean diet; ulcerative colitis; inflammation; microbiome

Graphical Abstract



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1. Introduction

Ulcerative colitis [UC] is a chronic inflammatory bowel disease [IBD] with debilitating symptoms, including bloody diarrhoea, abdominal pain, cramping, tenesmus, and fatigue.[1] Patients live with considerable symptom burden, increased risk of disability, and lower quality of life [QoL] despite medical treatment.[2] With the pathogenesis of diseases such as IBD linked to alterations in the gut microbiome [dysbiosis],[3] the prospect of reshaping or promoting eubiosis towards health-promoting microbes through diet is of interest to patients. In IBD, although dysbiosis has never converged to a true fingerprint, evidence supports a lack of diversity and a general instability, often with a dysbiosis characterised by the relative depletion of *Faecalbacterium praustnitzii* and *Roseburia hominis* and the enrichment of *Escherichia coli*, *Ruminococcus torques*, and *R. gnavus*.[4] Dysbiosis could have functional significance in disease manifestation—one example of this is that *F. praustnitzii* and *R. hominis* produce the beneficial metabolite butyrate, with the microbes and this metabolite being conspicuously reduced in IBD.[5]

The complexity of diet in human health cannot be overemphasized. Dietary patterns consist of multiple dietary characteristics [foods or nutrients] with various combinations of foods and beverages and considerations of quantity and frequency of consumption.[6] Evaluating dietary patterns considers the potential for synergistic nutrient effects between components in the diet. Several prospective cohort studies describe the relationship between food groups or nutrients and IBD; however, few have examined dietary patterns.[7] The Mediterranean Diet Pattern [MDP] is characterised by increased consumption of legumes, whole grains, vegetables, fruits, nuts, seeds, and olive oil, moderate consumption of fish, poultry, and dairy foods, and low consumption of processed foods and red meat.[8] The synergy of the high levels of dietary fibre, the phytochemicals, and the fat blend [high monounsaturated fat] confer beneficial anti-inflammatory and antioxidant properties.[9] Recently, a Mediterranean-like diet was associated with an increased abundance of fibre-degrading bacteria, including *Ruminococcus sp.* and *Faecalibacterium sp.*, along with lower levels of faecal calprotectin [FC] in asymptomatic first-degree relatives of patients with CD.[10] A 6-week MDP pilot intervention study in CD patients observed an increase in Bacteroidetes and the *Clostridium* clusters *IV* and *XIVa* and a decrease in Proteobacteria and Bacillaceae.[11] IBD patients adherent to the MDP for 6 months had reduced FC and CRP, as well as improved QoL.[12] In contrast, a diet that lacks fibre, with increased animal protein, n-6 polyunsaturated fatty acids, and ultra-processed foods, is linked to the onset and progression of IBD.[13]

Given the mounting evidence for the role of diet and gut microbial composition and function in IBD development and exacerbation, a richer understanding of the role of diet in disease pathogenesis in UC is needed. The primary aim of this study was to investigate the efficacy of the MDP compared with a Canadian Habitual Diet Pattern [CHD] on UC disease activity, inflammation, and the gut microbiome.

2. Materials and Methods

2.1. Participants

Details on the recruitment of participants, the dietary intervention, the collection of dietary data, baseline and week 12 diet information, and dietary adherence scores have been previously described in detail.[14]

Briefly, using an allocation ratio of 1:1 in blocks of two, participants were randomly assigned to follow the MDP or CHD for 12 weeks. The MDP group received a series of one-on-one coaching sessions from a dietitian on adapting their diet based on the Mediterranean Diet Pyramid.[8] The CHD group followed their usual diet and were asked not to change their diet. Adult participants [18–65 years] diagnosed with mild-moderate UC in remission [Partial Mayo score

0–2] were recruited through outpatient gastroenterology clinics from April 2017 to September 2021. Included were those on a stable dose of UC therapy [no dosage adjustments for 2 months before starting the study for participants prescribed oral 5-aminosalicylates, thiopurines, or methotrexate or if taking biologics [infliximab, adalimumab], for 3 months before starting the study. A total of 80 patients with IBD were screened for their eligibility, with a final study sample of 28 participants [MDP, $n = 15$ vs CHD, $n = 13$]. The physician completed a physical examination and partial Mayo score at baseline to assess disease status. Nutrition and medical history, blood work, and stool collection were completed at baseline and Week 12. Disease activity was assessed at baseline and Week 12 through the Simple Clinical Colitis Activity Index [SCCAI] and Partial Mayo Score [PMS].[15]

2.2. Serum and faecal biomarkers

Serum biomarkers were based upon routine laboratory tests ordered by the gastroenterologist at baseline and Week 12, including a complete blood count [CBC], C-reactive protein [CRP], albumin, vitamin B12, and vitamin D. Stool was collected and stored as previously described.[16]

2.3. Short chain fatty acid analyses

Direct-injection gas chromatography was used to quantify SCFAs acetic, propionic, butyric, valeric, iso-butyric, and iso-valeric acid from faecal samples. Briefly, 50 mg of homogenised faecal samples were mixed in isopropyl alcohol containing 2-ethyl butyric acid [0.01% *v/v*] used as an internal standard, and subsequently homogenised for 13 min at 30 Hz with metal beads. Homogenates were centrifuged, and the supernatant was removed. The supernatant was injected into a Trace 1300 Gas Chromatograph, equipped with a flame ionisation detector, with AI1310 autosampler [Thermo Scientific, Walkham, MA, USA] in splitless mode. A fused silica FAMEWAX [Restekas, Bellefonte, PA, USA] column 30 m × 0.32 mm i.d. coated with 0.25 µm film thickness was used. Helium was supplied as the carrier gas at a flow rate of 1.8 ml/min. The initial oven temperature was 80°C, maintained for 5 min, raised to 90°C at 5°C/min, then increased to 105°C at 0.9°C/min, and finally increased to 240 °C at 20°C/min and held for 5 min. The flame-ionisation detector and the injection port temperature were 240°C and 230°C, respectively. The flow rates of hydrogen, air, and nitrogen as make-up gas were 30, 300, and 20 ml/min, respectively. Data were analysed with Chromeleon 7 software [Bannockburn, IL, USA]. Standard volatile acid mix [Sigma Aldrich] was used to determine the retention times of acids. Peaks were analysed on the software, and the area under the peaks was calculated. An aliquot of 50 mg of homogenised stool was freeze-dried to measure the dry weight, and measurements are expressed as mass % [g of SCFA per g of dry-weight stool].

2.4. Determination of faecal secretory immunoglobulin A [sIgA] concentration

A faecal suspension of: 0.5 mg faeces; 1:10, v/v faeces in extraction buffer (0.01 M phosphate-buffered saline [PBS] [pH 7.4) with 0.5% Tween was vortexed for 5 min, then centrifuged at 10 000 x *g* for 10 min. A portion of the supernatant [up to 1.0 ml] was transferred to a sterile Eppendorf tube containing 20 µL of protease inhibitor cocktail [Protease Inhibitor Cocktail, VWR Life Science Amresco] and stored at – 20°C until analysis. A commercially available ELISA kit was used to determine faecal sIgA concentrations [RedDot Biotech, Kelowna BC, Canada; catalogue number: RD-slgA-Hu], following the manufacturer's instructions.

2.5. Determination of faecal calprotectin concentration

A faecal suspension of: 0.5 mg faeces; 1:10, v/v faeces in extraction buffer (0.01 M phosphate-buffered saline [PBS] [pH 7.4]) with 0.5% Tween was vortexed for 5 min, then centrifuged at 10 000 x *g* for 10 min. A portion of the supernatant [up to 1.0 ml] was transferred to a sterile Eppendorf tube containing 20 µL of protease inhibitor cocktail [Protease Inhibitor Cocktail, VWR Life Science Amresco] and stored at – 20°C until analysis. Faecal calprotectin was analysed by Eve Technologies [evetechnologies.com; Calgary, AB, Canada] using the human faecal calprotectin ELISA kit [catalogue # MBS584845]. FP response was defined as a 50% in FC levels from baseline, maintenance of response defined as no change in FC, and loss of response defined as an increase in baseline values by 50%.

2.6. Bioinformatic analysis

Whole faecal DNA was extracted using the QIAamp Powerfaecal Pro DNA kit [Qiagen; #51804]. Samples were sequenced by the Gut4Health Microbiome Core Facility [Child and Family Research Institute, Vancouver, BC] for 16S sequencing. The V4 hypervariable region of the 16S rRNA gene was amplified using the Illumina MiSeq platform using the following primers: Forward 5'AGTCAGT CAGCCGGACTACNVGGGTWTCTAAT and Reverse: 5' AGTCAGTCAGCCGGACTACNVGGGTWTCTAAT attached to the Illumina adapter overhang. The samples were sequenced by the Gut4Health Microbiome Core Facility [BC Children's Hospital Research Institute, Vancouver, BC] using the Illumina MiSeq platform. Post-sequencing analyses were performed using the QIIME 2 platform [version 2021.4].[17] Demultiplexed reads from two MiSeq runs were imported into the QIIME 2 environment, and primers were removed using the q2-cutadapt plugin.[18] Quality control entailed filtering, dereplication, chimera removal, denoising, and merging paired-end reads on each run separately using the DADA2 plugin with default settings.[19] The resulting amplicon sequence variant [ASV] tables were merged for downstream analysis. A phylogenetic tree was constructed using a SATé-enabled phylogenetic placement [SEPP] technique as implemented in the q2-fragment-insertion plugin[20] using a backbone tree built from the Greengenes reference database [version 13.8].[21] For taxonomic classification, we trained a classifier on the entire length of the 16S region and incorporated environment-specific abundances weights specific to human faecal samples, acquired from the *readytowear*.[22] This weighted bespoke approach for taxonomic classification has significantly improved accuracy over standard Naive Bayes classification methods.[22] Alpha-diversity metrics were calculated [ASV richness, Shannon's diversity index, and Faith's phylogenetic diversity].[17] For beta-diversity, we used the compositional tensor factorisation [CTF] method to account for inter-individual variation across time.[23] An analysis of variance [ANOVA] was run on the CTF to determine the differences between diet groups. To account for the longitudinal nature of our data [baseline and Week 12 faecal samples and subject-level variation], we used the tool BIRDMAn to obtain ASV rankings most associated with each group [https://

birdman.readthedocs.io/en/stable/index.html]. These differential ranks were visualised using Qurro[24] and the differentials for the top 10 ASVs related to each group were exported into R[25] using the *qiime2R* package [https://github.com/jbisanz/qiime2R] for further custom visualisation and statistical analysis. To predict high-level phenotypes, BugBase [https://doi.org/10.1101/133462] was used to determine the proportion of Gram-positive, Gram-negative, aerobic, anaerobic, facultative anaerobic, biofilm-forming, and mobile element-containing bacteria.

2.7. Statistical methods

Continuous data are presented as the median and interquartile range [IQR] and categorical data are presented as absolute value and percentage. The D'Agostino and Pearson test assessed normality of the data. The Wilcoxon matched-pairs signed-rank test was used for paired data to assess pre- and post-dietary intervention. The Mann–Whitney U test was used for the comparison between groups. Categorical variables were compared using Fisher's exact test; p-values of <0.05 were considered statistically significant. The statistical package GraphPad Prism Version 9.3.0 [Graph Pad Software, San Diego, CA, USA] and R[25] were used for the analyses and figures.

2.8. Outcomes

The primary outcome was to assess whether the MDP intervention could reduce the SCCAI score by Week 12 from baseline. A reduction of SCCAI >1.5 was considered clinically significant.[26] Secondary outcomes were to assess: reduction in FC levels [cut-off value of 100 ug/g or 50% change to discriminate inflammatory status from non-inflammatory status]; and changes in the microbiome.

2.9. Ethics 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 [NCT03053713]. The manuscript was prepared according to the Consolidated Standards of Reporting Trials Statement [http://www.consort-statement.org]. All participants signed an informed letter of consent. All authors had access to the study data and reviewed and approved the final manuscript.

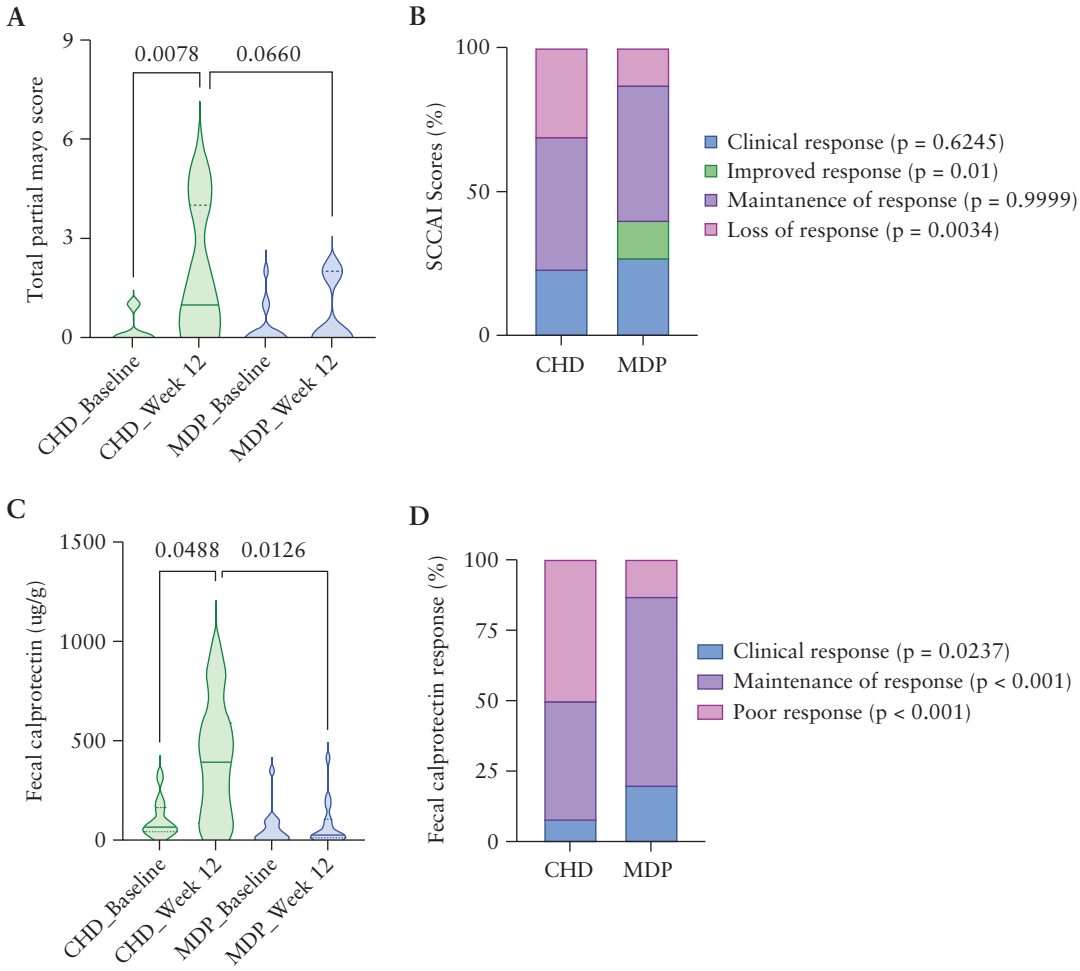

Figure 1. The MDP reduces faecal calprotectin and disease activity in UC patients. [A] Partial Mayo Score at baseline and week 12. [B] Disease activity response rates measured by the SCCAI at Week 12. [C] Faecal calprotectin values [ug/g] at baseline and week 12. [D] Faecal calprotectin response by diet, with response defined as a 50% in faecal calprotectin levels from baseline, maintenance of response defined as no change, and loss of response defined as an increase in baseline values. The Wilcoxon matched-pairs signed-rank test and Mann–Whitney test were performed for [B] and [F]. Fisher's exact test was performed for [C], p <0.05. MDP, Mediterranean Diet Pattern; UC, ulcerative colitis; SCCAI, Simple Clinical Colitis Activity Index

3. Results

3.1. The MDP reduces clinical symptoms and reduces inflammation

A total of 96% [27/28] of participants entered the study in remission [PMS of 0], with one participant having mild disease [PMS = 2] [Figure 1A]. At week 12, the PMS score of the CHD increased significantly [$p = 0.008$] with almost half [6/13, 46%] with mild and moderate disease versus 33% [5/15] with mild disease in the MDP [Supplementary Table 1]. A total of 40% of the MDP saw marginal improvements in SCCAI scores, 4/15 [27%] experienced a clinical response [SCCAI decreased by 1.5 points], and 2/15 [13%] showed an improvement [SCCAI decreased at least 1 point] [Figure 1B] In the CHD, 3/13 [23%] experienced a clinical response. A similar number of participants were in remission at Week 12 [no change in SCCAI] in the MDP and CHD [7/15, 47%, and 6/13, 46%, respectively]. Two participants [13%] in the MDP and 4/13 [31%] in the CHD experienced a loss of response [increase in SCCAI of 1 point or greater] [$p = 0.003$].

We next examined subclinical markers of inflammation, C-reactive protein [CRP] and FC [Supplementary Table 2]. FC is a useful clinical marker in predicting impending relapse and directly measures intestinal inflammation with high sensitivity.[27] Most participants in both treatment groups had an FC <100 µg/g [20/27, 71%] at baseline. At week 12, 75% [9/12] of participants in the CHD had an FC >100 µg/g, versus 20% [3/15] of participants in the MDP group. No significant differences were observed in FC between the MDP pre-and post-intervention [$p = 0.375$]; however, there was a significant increase in FC in the CHD pre-and post-intervention [$p = 0.0488$] [Figure 1C]. Three participants [20%] in the MDP demonstrated a 50% reduction in FC, 67% maintained FC, and two [13%] participants had an increase in FC [Figure 1D]. One participant [8%] in the CHD saw a reduction of FC by 50%, but half of the CHD group experienced an increase of greater than 50% in their FC. At week 12, 87% of participants in the MDP had an FC less than 100 µg/g, versus 25% in the CHD. In summary, these data show that the MDP is well tolerated, reduces bowel symptoms, and has anti-inflammatory effects, compared with participants' habitual diets.

3.2. The MDP promotes faecal sIgA production

The sIgA has been identified to play an important role in mucosal immunity. One potential mechanism is the capacity to bind pathogenic bacteria and their virulence factors, ultimately influencing the microbiota composition.[28] We found a significant increase in faecal sIgA in the MDP diet intervention from baseline to week 12 [$p = 0.0040$; Figure 2]. Faecal sIgA concentrations were not affected by the CHD pre- and post-diet intervention [$p = 0.0803$].

3.3. The MDP is positively associated with microbes that produce potentially beneficial metabolites

The stool was analysed by 16S ribosomal RNA sequencing at baseline and week 12 of the diet intervention. Alpha diversity did not significantly change over time [Supplementary Figure 3A–C]. For beta-diversity, we observed a significant shift along PC [Axis 2] in microbial composition between the MDP and CHD, which accounted for 35% of the variation among taxonomic ranks [Figure 3A]. To determine the taxa that changed the most between the MPD and CHD, the ratio

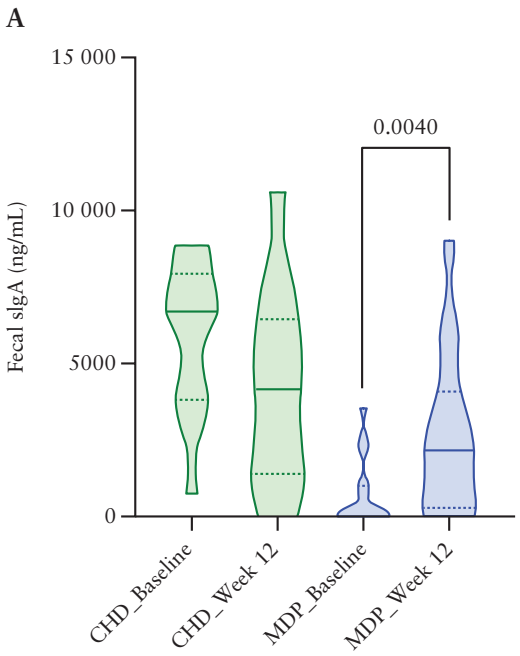

Figure 2. Faecal secretory IgA levels at baseline and Week 12. Faecal secretory IgA levels at baseline and week 12. The Wilcoxon matched-pairs signed-rank test and Mann–Whitney test were performed, $p < 0.05$.

of taxa [log-ratios] using differential ranking was calculated [Figure 3B]. Of the top 10 taxa most positively associated with the MDP, 9/10 taxa belonged to the phylum Firmicutes, class *Clostridia,* and largely *Clostridium* subclusters IV [*Ruminococcus* spp., *Flavonifractor* spp.], subcluster XIVa [*Clostridium* M, *Blautia A*], and *Lactococcus* spp. One taxon belonged to the phylum Bacteroidota, genus *Alistipes*. Several of these species have been shown in the literature to have a protective role in rodent models of colitis [*Alistipes finegoldii* and *Flavonifractor plautii*].[29,30] In contrast, others are linked to the degradation of compounds found in food substrates that regulate host health [*Clostridium boltae*, *Ruminococcus bromii*, *Blautia A* spp., and *Lactococcus lactis*].[31–34] Among the top-ranked taxa most negatively associated with the MDP, most taxa belonged to the phylum Firmicutes, followed by Actinobacteriota and Bacteriodota. *Bifidobacterium*, *An144*, *GCA-900066135*, *Veillonella*, *Blautia*, *Streptococcus*, and *Massiloclostridium* were the dominant genera. The predicted genera negatively associated with the MDP are opportunistic pathogens [*Veillonella dispar*, *Veillonella obetsuensis*, *Prevotella copri*, and *Streptococcus australis*].[35–37] *Prevotella copri* correlates with autoimmune diseases such as rheumatoid arthritis and CD.[38] In summary, these analyses of the bacteriome demonstrate that the MDP is associated with reshaping the gut microbiome.

3.4. The MDP is negatively associated with microbes predicted to carry pathobiont traits

In further support of our findings that the predicted taxa negatively associated with the MDP have the potential to be opportunistic pathogens [*V. dispar*, *V. obetsuensis*, *P. copri*, and *S. australis*], we saw a predicted decrease in the relative abundances of potentially pathogenic microbes and microbes containing mobile elements [Figure 4] at week 12 in the CHD.

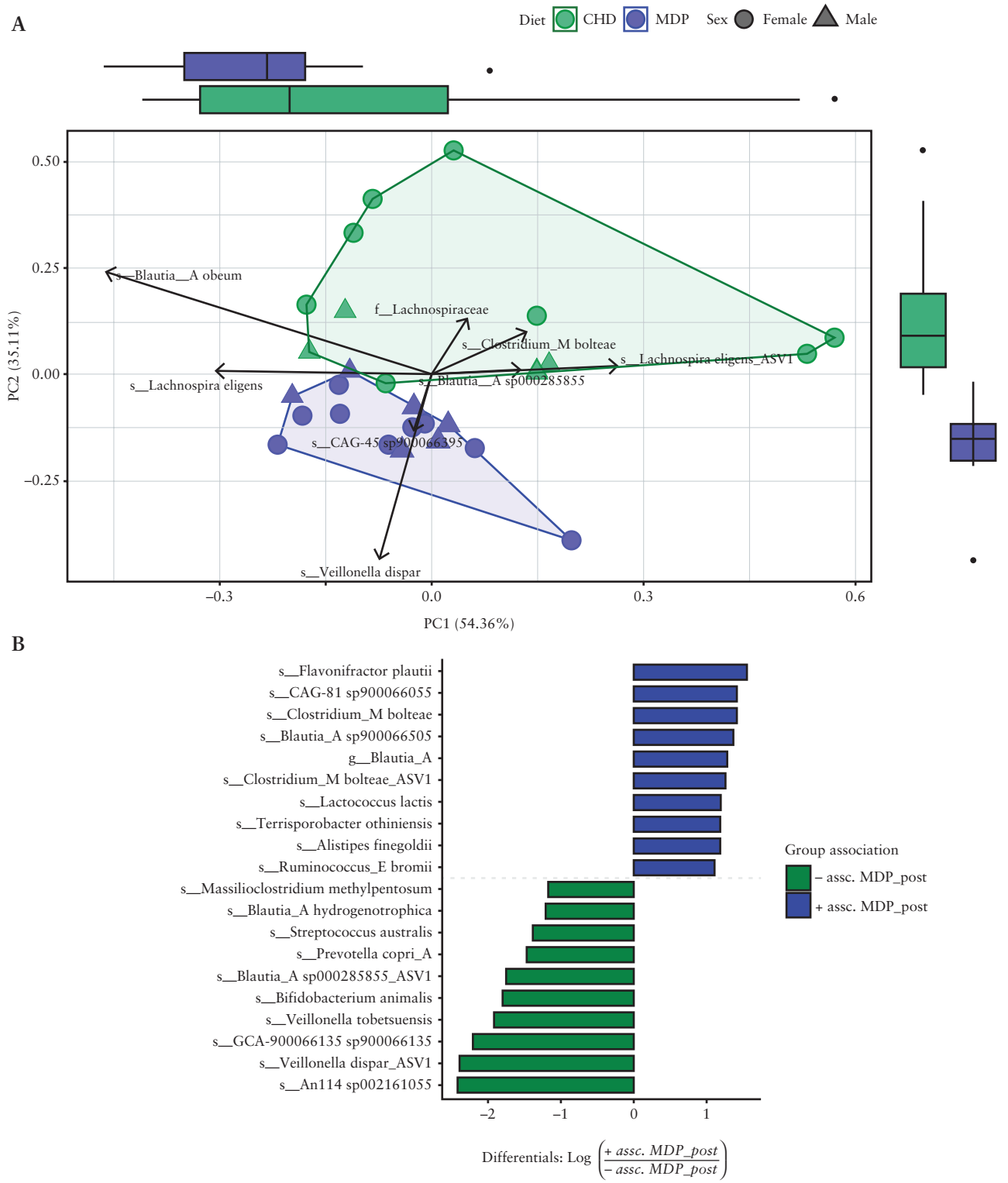

Figure 3. The MDP reshapes the bacteriome in UC patients. [A] The compositional tensor factorization biplot demonstrates a significant separation of communities by diet over time. [B] The differential ranks generated by BIRDMAn highlight the taxa positively and negatively associated with the MDP compared with the CHD at week 12 [$p = 0.00018$]. MDP, Mediterranean Diet Pattern; UC, ulcerative colitis; CHD, Canadian Habitual Diet Pattern.

Finally, we saw a decrease in microbes that form biofilms in the MDP at Week 12 [Figure 4]. There were no differences between aerobic, anaerobic, facultative anaerobic, and stress-tolerant bacteria [Figure 4]. In summary, the MDP has predicted microbial genetic traits associated with a decrease in virulence.

3.5. The MDP increases faecal SCFA production

SCFA are produced by microbes, have important immunomodulatory properties, and promote gut homeostasis.[39] Patients with IBD have been shown to have lower levels of SCFAs compared with healthy controls and a reduced abundance of SCFA-producing bacteria, including

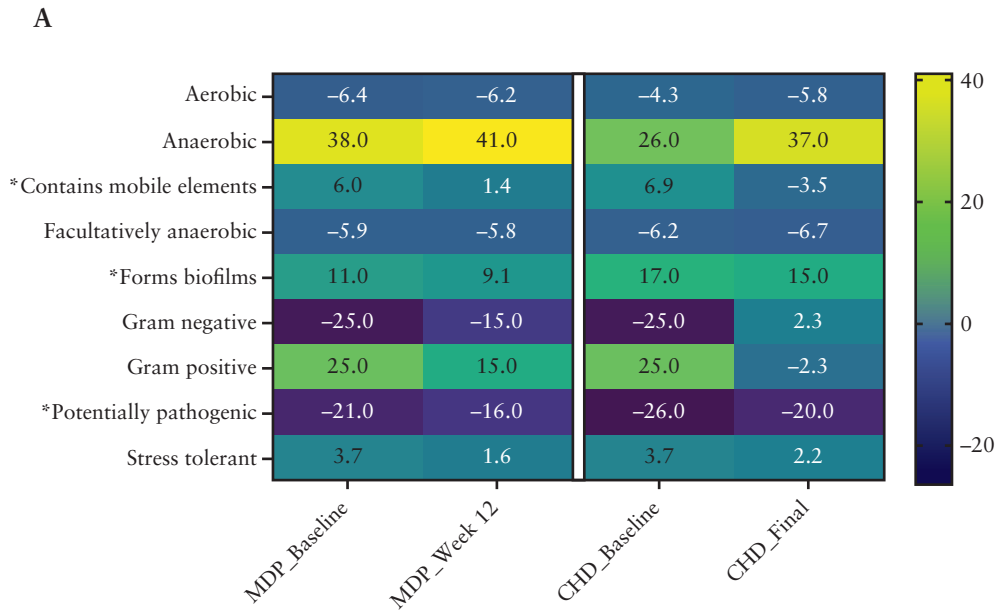

Figure 4. The MDP is negatively associated with microbes carrying pathobiont traits. [A] A heatmap of predicted bacterial genera at baseline to Week 12 in the MDP and CHD; *indicates microbes carry potentially pathobiont traits. MDP, Mediterranean Diet Pattern; CHD, Canadian Habitual Diet Pattern.

F. prausnitzii and *Roseburia intestinalis*.[39] Higher levels of faecal concentrations of total SCFAs [$p = 0.0129$] [Figure 5A], butyric acid [$p = 0.0287$] Figure 5B], acetic acid [$p = 0.0325$] [Figure 5C], and valeric acid [$p = 0.0083$] [Figure 5D] were seen in the MDP compared with the CHD at week 12. Acetic acid and valeric acid concentrations in the CHD dropped significantly [$p = 0.0398$ and 0.0215, respectively]. These results suggest that the increased production of SCFAs may contribute to the anti-inflammatory effects seen in the MDP, notably butyrate's role in reducing FC levels.[40]

4. Discussion

This intervention study examined the influence of the MDP in UC, on inflammatory biomarkers, SCFAs, and the gut microbiome composition. The MDP was associated with reductions in FC and beneficial changes to the microbiome.

A consistent body of evidence supports that higher adherence to the MDP is associated with improved health outcomes in IBD,[41,42] including decreased inflammation[12,43,44] and lower risk of developing IBD.[45] The results demonstrate that the MDP improved the faecal inflammatory biomarker FC, improved/maintained disease activity, altered the composition of the gut bacteriome, and increased faecal SCFA concentrations, relative to the CHD. Furthermore, 20% of participants following an MDP achieved an FC response, with 67% maintaining the FC at week 12, whereas 75% of the CHD experienced an increase in FC. Previous reports suggest that a high FC [>100 ug/g] has an 80% chance of clinical relapse within 6 months.[46] To support the FC results, we saw that the MDP improved disease activity in 6/15 participants [by SCCAI] and 10/15 participants remained in remission [PMS], in contrast to the CHD where 3/13 participants noted an improvement in disease activity [SCCAI] with 6/13 deemed having mild or moderate disease activity.

Microbe-derived metabolites are widely known to mediate several physiological processes, including strengthening the gut barrier and having immunomodulatory benefits.[47] Several lines of evidence show that patients with IBD have reduced levels of SCFAs and that a Western Diet Pattern decreases protective SCFA-producing bacteria, which promotes inflammatory responses in the gut.[48] Here, we show significant differences between the MDP and CHD in total faecal SCFAs, butyrate, and acetate concentrations at week 12. We observed a positive association in the MDP with *R. bromii,* which are known producers of butyrate derived from foods high in resistant starch [eg, legumes, oats, whole grains].[49] The microbiota also metabolises other dietary substrates such as catechins, which are polyphenol compounds found in many plant-based foods such as grapes, berries, apricots, nuts, olives, and red wine, and metabolise quercetin and produce butyrate.[29,50] In the MDP group, we observed a positive association with the catechin metaboliser *F. plautii*, shown to be protective against murine colitis through the expansion of Treg cells.[29] Similarly olive oil, rich in polyphenol compounds and a critical dietary fat component of the MDP, has been shown to increase butyrate concentrations.[51] *L. lactis,* a lactic acid-producing bacterium [LAB] found in fermented dairy products and vegetables and known to have health benefits,[32] was also positively associated with the MDP. Our findings demonstrate that food components commonly consumed in an MDP are positively associated with many of those beneficial bacteria.

The bacteriome of the CHD had significantly lower levels of SCFAs compared with the MDP. We observed several negative associations with the MDP with predicted species such as *V. dispar*, *V. obetsuensis*, *and S. australis*. Previous studies have linked these microbes with opportunistic infections.[35–37] *P. copri* was negatively associated with the MDP in our cohort. Interestingly, *P. copri* thrives in the pro-inflammatory colitic microbiome.[38] In support of these findings, the MDP is negatively associated with microbes that have predicted pathogenic traits. Although this study cannot ascribe a causative association to changes in the microbiome, these data, combined with the lack of clinical improvement in the CHD,

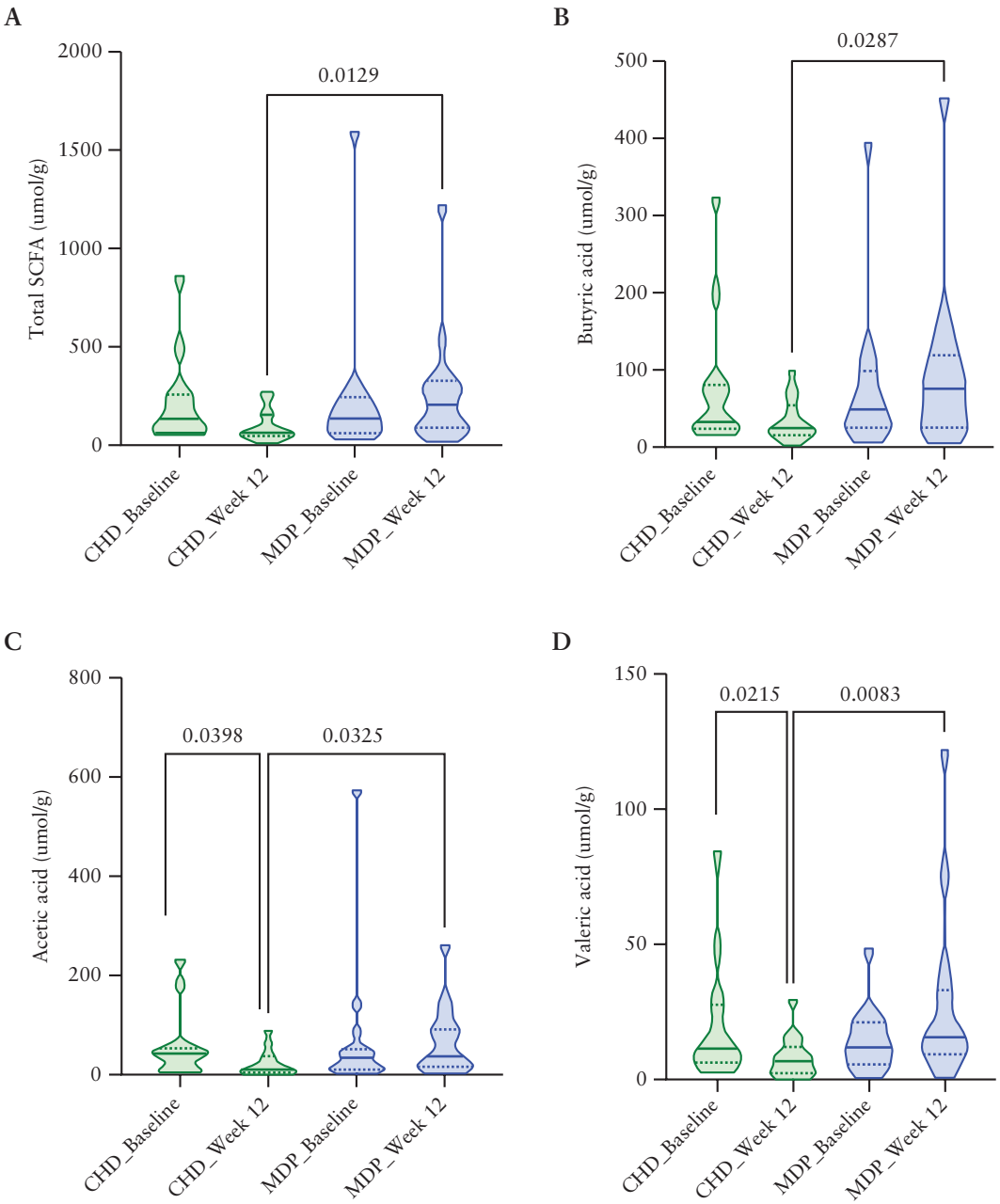

Figure 5. The MDP increases faecal short chain fatty acid [SCFA] production in UC patients. Faecal SCFAs measured at baseline and week 12 [umol/g]. [A] Total SCFAs. [B] Butyric acid. [C] Acetic acid. [D] Valeric acid. Wilcoxon matched-pairs signed-rank test and Mann–Whitney test, $p < 0.05$. UC, ulcerative colitis.

suggest that the CHD leads to a microbiome with pathobionts with the potential to promote inflammatory responses in the gut. Indeed, the CHD group did increase their FC increase over time.

Secretory IgA plays an integral role in maintaining mucosal homeostasis, primarily through interactions with the intestinal microbiota and its ability to bind disease-inducing bacteria and thus prevent microbial pathogens from gaining access to the intestinal epithelium.[52] This study confirmed that whereas sIgA levels remained consistent in CHD, we saw a significant increase in faecal sIgA in the MDP. The fact that a negative association was found between opportunistic pathobionts and the MDP could indicate a role for sIgA in pathogen exclusion. However, the exact mechanism needs to be elucidated in further studies.

There are several strengths to this intervention study, including: 1] its longitudinal design, which is essential in understanding the variation of the microbiome within individual subjects[53]; 2] the use of validated questionnaires; and 3] the use of a registered dietitian-scientist with expertise in IBD and evidence-based nutrition. The limitations of this research include a small sample size, although it was sufficiently powered to detect some changes in relative abundance in bacterial composition and inflammation. Endoscopic analysis, the gold standard to assess mucosal healing, was not used here; however, we did include FC, an objective stool marker

that has been shown to correlate well with subclinical intestinal inflammation and to predict future relapses in IBD.[46] Last, two participants in the CHD's background diet were following a Mediterranean-like diet, which is a confounding effect that may have contributed to the improvement in the SCCAI [clinical response]. Further studies are needed to define the extent of endoscopic and histological healing expected from the MDP. In conclusion, the MDP is well tolerated and is a reasonable, healthy eating pattern that practitioners can recommend to patients with UC in remission to prevent relapses, in addition to their standard medical therapy.

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Conflict of Interest

The authors declare no competing interests.

Author Contributions

NH and DLG designed the research and conducted the study; SS, KJ, and LD identified and recruited the participants and provided expertise and advice on inclusion/exclusion criteria; NH and RS screened patients and obtained consent; NH, RS, JY, and ME analysed the data; NH wrote the original draft of the manuscript; DLG critically revised the manuscript; KJ, SS, RS, JY, ME, and LD reviewed and edited the manuscript; DLG supervised and provided resources and funding for this project.

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Data Availability Statement

To access the additional files accompanying this article, please see the data repository at Haskey, Natasha (2023), "Dietary compliance with the Mediterranean diet pattern in a randomized clinical trial of patients with quiescent Ulcerative Colitis ", Mendeley Data, V2, doi:10.17632/2ffyvrdd97.2.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

References

1. Magro F, Gionchetti P, Eliakim R, *et al*. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis* 2017;**11**. doi:10.1093/ecco-jcc/jjx008.
2. Shafer LA, Walker JR, Restall G, *et al*. Association between IBD disability and reduced work productivity [presenteeism]: a population-based study in Manitoba, Canada. *Inflamm Bowel Dis* 2019;**25**. doi:10.1093/ibd/izy236.
3. Aldars-García L, Chaparro M, Gisbert JP. Systematic review: The gut microbiome and its potential clinical application in inflammatory bowel disease. *Microorganisms* 2021;**9**:977. doi:10.3390/microorganisms9050977.
4. Lloyd-Price J, Arze C, Ananthakrishnan AN, *et al*. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019. doi:10.1038/s41586-019-1237-9.
5. Lucas López R, Grande Burgos MJ, Gálvez A, Pérez Pulido R. The human gastrointestinal tract and oral microbiota in inflammatory bowel disease: a state of the science review. *APMIS* 2017;**125**. doi:10.1111/apm.12609.
6. Kant AK. Dietary patterns: Biomarkers and chronic disease risk. *Appl Physiol Nutr Metab* 2010;**35**:199–206. doi:10.1139/H10-005.
7. Tian Z, Zhuang X, Zhao M, *et al*. Index-based dietary patterns and inflammatory bowel disease: a systematic review of observational studies. *Adv Nutr* 2021. doi:10.1093/advances/nmab069.
8. Bach-Faig A, Berry EM, Lairon D, *et al*.; Mediterranean Diet Foundation Expert Group. Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr* 2011;**14**:2274–84. doi:10.1017/S1368980011002515.
9. Martinez-Gonzalez MA, Martin-Calvo N. Mediterranean diet and life expectancy; beyond olive oil, fruits, and vegetables. *Curr Opin Clin Nutr Metab Care* 2016. doi:10.1097/MCO.0000000000000316.
10. Turpin W, Dong M, Sasson G, *et al*.; Crohn's and Colitis Canada [CCC] Genetic, Environmental, Microbial [GEM] Project Research Consortium. Mediterranean-like dietary pattern associations with gut microbiome composition and subclinical gastrointestinal inflammation. *Gastroenterology*. 2022;**163**:685—98. doi:10.1053/j.gastro.2022.05.037
11. Marlow G, Ellett S, Ferguson IR, *et al*. Transcriptomics to study the effect of a Mediterranean-inspired diet on inflammation in Crohn's disease patients. *Hum Genomics* 2013;**7**:24. doi:10.1186/1479-7364-7-24.
12. Chicco F, Magrì S, Cingolani A, *et al*. Multidimensional impact of Mediterranean Diet on IBD patients. *Inflamm Bowel Dis* 2021;**27**. doi:10.1093/ibd/izaa097.
13. Rizzello F, Spisni E, Giovanardi E, *et al*. Implications of the westernized diet in the onset and progression of IBD. *Nutrients* 2019;**11**:1033. doi:10.3390/nu11051033.
14. Haskey N, Shim RCK, Davidson-Hunt A, *et al*. Dietary adherence to the Mediterranean diet pattern in a randomized clinical trial of patients with quiescent ulcerative colitis. *Front Nutr* 2022;**9**. doi:10.3389/fnut.2022.1080156.
15. Walmsley RS, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. *Gut* 1998;**43**:29–32. doi:10.1136/gut.43.1.29.
16. Gorzelak MA, Gill SK, Tasnim N, Ahmadi-Vand Z, Jay M, Gibson DL. Methods for improving human gut microbiome data by reducing variability through sample processing and storage of stool. *PLoS One* 2015;**10**:e0134802e0134802. doi:10.1371/journal.pone.0134802.
17. Bolyen E, Rideout JR, Dillon MR, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;**37**:852–7. doi:10.1038/s41587-019-0209-9.
18. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 2011;**17**:10. doi:10.14806/ej.17.1.200.
19. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016. doi:10.1038/nmeth.3869.

20. Janssen S, McDonald D, Gonzalez A, *et al*. Phylogenetic placement of exact amplicon sequences improves associations with clinical information. *mSystems* 2018. doi:10.1128/msystems.00021-18.
21. McDonald D, Price MN, Goodrich J, *et al*. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 2012;**6**. doi:10.1038/ismej.2011.139.
22. Kaehler BD, Bokulich NA, McDonald D, Knight R, Caporaso JG, Huttley GA. Species abundance information improves sequence taxonomy classification accuracy. *Nat Commun* 2019;**10**. doi:10.1038/s41467-019-12669-6.
23. Martino C, Shenhav L, Marotz CA, *et al*. Context-aware dimensionality reduction deconvolutes gut microbial community dynamics. *Nat Biotechnol* 2021;**39**. doi:10.1038/s41587-020-0660-7.
24. Fedarko MW, Martino C, Morton JT, *et al*. Visualizing 'omic feature rankings and log-ratios using Qurro. *NAR Genom Bioinform* 2020;**2**. doi:10.1093/nargab/lqaa023.
25. R Development Core Team R. *R: A Language and Environment for Statistical Computing*. 2019. doi:10.1007/978-3-540-74686-7
26. Higgins PDR, Schwartz M, Mapili J, Krokos I, Leung J, Zimmermann EM. Patient defined dichotomous end points for remission and clinical improvement in ulcerative colitis. *Gut* 2005;**54**:782–8. doi:10.1136/gut.2004.056358.
27. Gisbert JP, Bermejo F, Pérez-Calle JL, *et al*. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009;**15**:1190–8. doi:10.1002/ibd.20933.
28. Peterson DA, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe* 2007;**2**:328–39. doi:10.1016/j.chom.2007.09.013.
29. Ogita T, Yamamoto Y, Mikami A, Shigemori S, Sato T, Shimosato T. Oral administration of Flavonifractor plautii strongly suppresses Th2 immune responses in mice. *Front Immunol* 2020;**11**. doi:10.3389/fimmu.2020.00379.
30. Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A. The genus *Alistipes*: gut bacteria with emerging implications to inflammation, cancer, and mental health. *Front Immunol* 2020;**11**. doi:10.3389/fimmu.2020.00906.
31. Liu X, Mao B, Gu J, *et al*. Blautia—a new functional genus with potential probiotic properties? *Gut Microbes* 2021;**13**. doi:10.1080/19490976.2021.1875796.
32. Song AAL, In LLA, Lim SHE, Rahim RA. A review on Lactococcus lactis: From food to factory. *Microb Cell Fact* 2017;**16**. doi:10.1186/s12934-017-0669-x.
33. Collins MD, Lawson PA, Willems A, *et al*. The phylogeny of the genus Clostridium: Proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* 1994;**44**:812–26. doi:10.1099/00207713-44-4-812.
34. Ze X, Duncan SH, Louis P, Flint HJ. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *ISME J* 2012;**6**:1535–43. doi:10.1038/ismej.2012.4.
35. Mashima I, Kamaguchi A, Miyakawa H, Nakazawa F. Veillonella tobetsuensis sp. nov., an anaerobic, Gram-negative coccus isolated from human tongue biofilms. *Int J Syst Evol Microbiol*. 2013;**63**[PART4]. doi:10.1099/ijs.0.042515-0.
36. Cobo F, Pérez-Carrasco V, García-Salcedo JA, Navarro-Marí JM. Bacteremia caused by Veillonella dispar in an oncological patient. *Anaerobe* 2020;**66**:102285. doi:10.1016/j.anaerobe.2020.102285.
37. Willcox MDP, Zhu H, Knox KW. Streptococcus australis sp. nov., a novel oral streptococcus. *Int J Syst Evol Microbiol* 2001;**51**. doi:10.1099/00207713-51-4-1277.
38. He Q, Gao Y, Jie Z, *et al*. Two distinct metacommunities characterize the gut microbiota in Crohn's disease patients. *GigaScience* 2017;**6**. doi:10.1093/gigascience/gix050.
39. Parada Venegas D, de la Fuente MK, Landskron G, *et al*. Short chain fatty acids [SCFAs]-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol* 2019;**10**. doi:10.3389/fimmu.2019.00277.
40. Vernero M, de Blasio F, Ribaldone DG, *et al*. The usefulness of microencapsulated sodium butyrate add-on therapy in maintaining remission in patients with ulcerative colitis: A prospective observational study. *J Clin Med* 2020;**9**:3941. doi:10.3390/jcm9123941.
41. Strisciuglio C, Cenni S, Serra MR, *et al*. Effectiveness of mediterranean diet's adherence in children with inflammatory bowel diseases. *Nutrients* 2020;**12**:3206. doi:10.3390/nu12103206.
42. Fiorindi C, Dinu M, Gavazzi E, *et al*. Adherence to mediterranean diet in patients with inflammatory bowel disease. *Clin Nutr ESPEN 2021*. doi:10.1016/j.clnesp.2021.09.726.
43. Sasson AN, Ingram RJM, Zhang Z, *et al*. The role of precision nutrition in the modulation of microbial composition and function in people with inflammatory bowel disease. *Lancet Gastroenterol Hepatol* 2021. doi:10.1016/S2468-1253[21]00097-2.
44. Taylor L, Almutairdi A, Shommu N, *et al*. Cross-sectional analysis of overall dietary intake and mediterranean dietary pattern in patients with crohn's disease. *Nutrients* 2018;**10**:1761. doi:10.3390/nu10111761.
45. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol* 2011;**106**:563–73. doi:10.1038/ajg.2011.44.
46. Kwapisz L, Gregor J, Chande N, Yan B, Ponich T, Mosli M. The utility of faecal calprotectin in predicting the need for escalation of therapy in inflammatory bowel disease. *Scand J Gastroenterol* 2017;**52**:846–50. doi:10.1080/00365521.2017.1315740.
47. Venegas DP, de La Fuente MK, Landskron G, *et al*. Short chain fatty acids [SCFAs]mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol* 2019. doi:10.3389/fimmu.2019.00277.
48. Agus A, Denizot J, Thévenot J, *et al*. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. *Sci Rep* 2016;**6**. doi:10.1038/srep19032.
49. McOrist AL, Miller RB, Bird AR, *et al*. Faecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J Nutr* 2011;**141**:883–9. doi:10.3945/jn.110.128504.
50. Tomás-Barberán FA, Selma M, Espín JC. Interactions of gut microbiota with dietary polyphenols and consequences to human health. *Curr Opin Clin Nutr Metab Care* 2016;**19**. doi:10.1097/MCO.0000000000000314.
51. Farràs M, Martinez-Gili L, Portune K, *et al*. Modulation of the gut microbiota by olive oil phenolic compounds: Implications for lipid metabolism, immune system, and obesity. *Nutrients* 2020;**12**:2200. doi:10.3390/nu12082200.
52. Mantis NJ, Rol N, Corthésy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol* 2011;**4**. doi:10.1038/mi.2011.41.
53. Wagner BD, Grunwald GK, Zerbe GO, *et al*. On the use of diversity measures in longitudinal sequencing studies of microbial communities. *Front Microbiol* 2018. doi:10.3389/fmicb.2018.01037