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Original article

Dietary inflammatory potential mediated gut microbiota and metabolite alterations in Crohn's disease: A fire-new perspective

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SUMMARY

Background & aims: Pro-inflammatory diet interacting with gut microbiome might trigger for Crohn's disease (CD). We aimed to investigate the relationship between dietary inflammatory potential and microflora/metabolites change and their link with CD.

Methods: The dietary inflammatory potential was assessed using a dietary inflammatory index (DII) based on the Food Frequency Questionnaire from 150 new-onset CD patients and 285 healthy controls (HCs). We selected 41 CD patients and 89 HCs who had not received medication for metagenomic and targeted metabolomic sequencing to profile their gut microbial composition as well as fecal and serum metabolites. DII scores were classified into quartiles to investigate associations among different variables.

Results: DII scores of CD patients were significantly higher than HCs (0.56 ± 1.20 vs 0.23 ± 1.02, $p = 0.017$). With adjustment for confounders, a higher DII score was significantly associated with higher risk of CD (OR: 1.420; 95% CI: 1.049, 1.923, $p = 0.023$). DII score also was positively correlated with disease activity ($p = 0.001$). *Morganella morganii* and *Veillonella parvula* were increased while *Coprococcus eutactus* was decreased in the pro-inflammatory diets group, as well as in CD. DII-related bacteria were associated with disease activity and inflammatory markers in CD patients. Among the metabolic change, pro-inflammatory diet induced metabolites change were largely involved in amino acid metabolic pathways that were also observed in CD.

Conclusions: Pro-inflammatory diet might be associated with increased risk and disease activity of CD. Diet with high DII potentially involves in CD by mediating alterations in gut microbiota and metabolites.

1. Introduction

Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) and its incidence is increasing globally, particularly in newly industrialized countries [1,2]. China is undergoing a transition to an urban and industrialized society, which is paralleling a staggering rise in CD [2]. It is believed that industrialization-and urbanization-induced dietary changes may also trigger CD. A recent high-quality meta-analysis showed that dietary factors including fish, vegetable, fruit, fibre, and dairy product intake were protective against CD, but higher intake of meat, meat products, fat and fatty acids consistently increased the risk of CD [3]. Diet plays a crucial role in the pathogenesis of CD by influencing the gut microbiome and its metabolites, the host barrier function, and mucosal immunity [4]. However, dietary intake involves complex interactions between different foods and

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Abbreviations

IBD	inflammatory bowel disease
CD	Crohn's disease
UC	ulcerative colitis
HCs	healthy controls
HC-pro	HCs in the most pro-inflammatory group excluding CD patients
HC-anti	HCs in the most anti-inflammatory group excluding CD patients
DII	dietary inflammatory index
FFQ	food frequency questionnaires
OR	odds ratios
BAs	bile acids
SCFAs	short-chain fatty acids
AAs	amino acids
CDAI	Crohn's disease activity index
CDEIS	Crohn's Disease Endoscopic Index of Severity
PUFAs	monounsaturated fatty acids
CA	cholic acid
BSCFAs	branched short-chain fatty acids
DCA	deoxycholic acid
LCA	lithocholic acid
BMI	body mass index
WBC	white blood cell
Hb	hemoglobin
HCT	hematocrit
PLT	platelet counts
CRP	C-reactive protein
ESR	erythrocyte sedimentation rate
ALB	albumin
FC	fecal calprotectin

nutrients. Hence, the relationship between dietary and CD might not be quite precisely captured because many studies only focused on single food or one of micronutrients [5]; therefore, a complete dietary pattern should be considered while examining the overall role of diet in CD.

Dietary patterns could be derived using reduced rank regression, principal component analysis, and index-based methods, such as Mediterranean diet score, and the Diet inflammatory index (DII) [6]. Recently, a review on index-based dietary patterns and IBD shown that Mediterranean diet was negatively associated with IBD risk and progression, whereas a diet with high inflammatory potential could increase risk and aggravate disease activity in CD [7]. Inflammatory potential of diet could be assessed by DII which design was based on a wider range of human populations, study designs, and nutritional assessment methods [8], and the DII has been widely used to examine the relationship between diet and chronic diseases risk, such as cancer, cardiovascular disease, IBD and age-related diseases [9]. Two large recent prospective cohort studies indicated an association between dietary patterns with a high inflammatory potential and an increased risk of CD [5,10]. In addition, several other case–control studies have suggested that the inflammatory potential of diet may be associated with the disease severity in patients with CD [11,12], but these studies were observational and did not explore the underlying mechanisms behind the inflammatory potential of diet in CD.

Diet plays a key role in defining the composition of the human gut microbiota and the consequent production of microbial metabolites [13]. For example, compared with European children, an increase in the concentration of *Bacteroidetes* and decrease in Firmicutes and *Enterobacteriaceae* concentration in rural African children has been observed; this variation is largely attributed to the differences between the dietary patterns of the two populations [14]. Additionally, diets rich in fat and sugar resulted in intestinal dysbiosis characterized by overabundance of proteobacteria and decrease in protective bacteria [15]. Symbiotic between gut microbiota and the host contributes to epithelial barrier function, which is critical to maintain intestinal immune homeostasis. Disorder in the composition of the gut microbiota and metabolites might disrupt epithelial barrier function and activate the mucosal immune system can resulting in contribute to the risk of developing CD [16,17].

A recent study suggested that the inflammatory potential of whole diet is associated with composition and functional of the gut microbiome [18]. However, the role of the dietary inflammatory potential on the composition and function of the intestinal microbiota in disease conditions has not been explored. Therefore, we hypothesized that the inflammatory potential of diet may be associated with CD risk and disease activity by modulating the composition and functional of the gut microbiome and metabolites. In this study, we investigate the association between dietary inflammatory potential and CD and explore the association of dietary inflammatory potential and gut microbiota/metabolites to shed light and facilitate in-depth novel insights on CD pathogenesis.

2. Materials and methods

2.1. Study population

To reduce the possibility of changes in dietary intake adjustment following CD diagnosis, we recruited 179 patients newly-diagnosed with CD with a disease course of <1 year visiting the Department of Gastroenterology of the First Affiliated Hospital of Sun Yat-sen University, Guangdong Province, China, from July 2019 to September 2021. However, only 150 eligible patients with CD were finally included in the study (Fig. 1). Three hundred healthy controls (HCs) were recruited at random by public advertising according to 1:2 (CD: HC); however, only age- and sex-matched HCs (n = 285) were eventually included because Food Frequency Questionnaire (FFQ) data were missing for 15 HCs. Diagnoses of CD was based on the clinical, colonoscopy, and imaging features, and histological

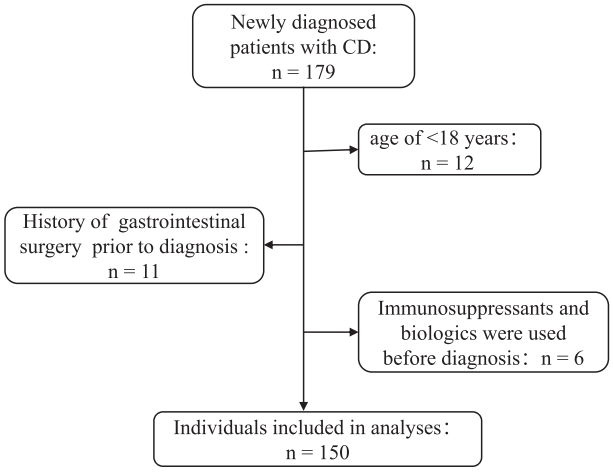

Fig. 1. Flow chart of eligible CD patients in the study.

examinations. Participants were excluded if they had a history of gastrointestinal surgery, other gastrointestinal illnesses, carcinoma, metabolic diseases, human immunodeficiency virus infection, hyperthyroidism, cardiac or hepatic disease, were pregnant or breastfeeding women, and had taken immunosuppressive drugs or biological agents. We also excluded participants with an implausible daily energy intake (<800 or >4000 kcal/day for men and <500 or >3500 kcal/day for women) based on FFQ. In addition, participants on special diets (ketogenic diet, vegan diet, exclusive enteral nutrition, and CD exclusion diet) were excluded. All the participants provided written informed consent. This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

2.2. Collection of clinical data from patients

The disease activity was determined using the Crohn's disease activity index (CDAI) score, and the Crohn's Disease Endoscopic Index of Severity (CDEIS) was used to assess the level of inflammation in the ileocolonic mucosa [19,20]. Disease activity was defined as CDAI $\geq$150 for patients with active CD and CDAI <150 for patients with inactive CD. Laboratory assessments included body mass index (BMI), smoking history, white blood cell (WBC) count, hemoglobin (Hb) level, hematocrit (HCT), platelet counts (PLT), C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR) and albumin (ALB). Only 54 of the 150 patients with CD were examined fecal calprotectin (FC).

2.3. Assessment of dietary intake and the inflammatory potential of diet

The information of the usual diet of the participants was collected using a 133 food item-validated FFQ [21]. The dietary habits of patients with CD one year prior to disease onset and those of HCs one year before the interview were collected. The consumption of nutrient components was converted based on the results of the questionnaire, and 30 parameters were used to calculate the DII score of diets, namely: energy, carbohydrate, protein, fat, fibre, cholesterol, vitamin A, riboflavin, thiamin, niacin, vitamin C, vitamin E, magnesium, iron, zinc, selenium, n-3 polyunsaturated fatty acids (n-3 PUFAs), n-6 PUFAs, saturated fat (SFA), monounsaturated fatty acids (MUPAs), PUFAs, folic acid, vitamin B6, vitamin B12, vitamin D, β-carotene, anthocyanidins, isoflavones, alcohol, and onions. Firstly, we calculate the z-score for the 30 food components by subtracting the “standard global mean” from the participants' reported intake for each item, and divided this value by “global standard deviation”, which we took from the paper [8]. All z-scores were then converted to percentiles to reduce the skewness. Each percentile score was doubled and then ‘1’ was subtracted to achieve a symmetrical distribution with values centered on 0 (null) and bounded between−1 (maximally anti-inflammatory) and +1 (maximally pro-inflammatory). We multiplied the centered percentile scores by the inflammatory effect score of each food parameter derived from the study of Shivappa et al. [8]. Finally, we summed the 30 food DII scores of each participant to construct their overall DII score. DII score above zero represented a pro-inflammatory diet whereas the score below zero represented an anti-inflammatory diet. To control the effect of total energy intake, DII per 1000 kcal was calculated to ensure that the DII scores were precise.

2.4. Sample processing

Subjects were selected only if they had not taken antibiotics, probiotics, or prebiotics within the previous three months prior to sample collection. After selection, stool and serum samples were collected from 41/150 patients with CD and 89/285 HCs. In addition, patients with CD had no history of medication, including corticosteroids, immunosuppressive drugs, and biological agents. Participants were asked to fast for at least 12 h before collecting the blood and feces. Stool samples were collected before breakfast and were immediately (within 2 h) transferred to the gastroenterology laboratory of the First Affiliated Hospital of Sun Yat-sen University. Samples were then divided into several 2 ml cryovials (0.5 g per tube). To avoid mixing with other substances, participants were asked to drain their feces into a simple clean collection bowl, and the stool was then transferred into a sterilized desiccative stool collection tube using a scoop. Blood was collected through peripheral veins prior to eating breakfast and centrifuged (3000 rpm, 15 min) at 4 °C, and the serum was immediately aliquoted into 0.2 ml microtubes. All biological samples were stored at −80 °C prior to analysis.

2.5. Metagenome profiling

Total microbial genomic DNA was extracted using the DNeasy Power Soil Kit (QIAGEN, Inc., Netherlands), according to the manufacturer's instructions. The Illumina TruSeq Nano DNA LT Library preparation kit was used to construct metagenome shotgun sequencing libraries with insert sizes of 400 bp, and these libraries were sequenced using the Illumina HiSeq X-ten platform (Illumina, USA) with the PE150 strategy at Personal Biotechnology Co., Ltd. (Shanghai, China). Sequencing adapters were removed from the raw sequencing reads using Cutadapt (v1.2.1). Low-quality reads were trimmed using a sliding-window algorithm. The reads were aligned to the host genome using Burrows–Wheeler alignment (BWA) tool (http://bio-bwa.sourceforge.net/) to remove host contamination. Quality-filtered reads were assembled de novo to construct the metagenome for each sample via Iterative De Bruijn graph Assembler for sequencing data with highly uneven depth (IDBA-UD). MetaGeneMark (http://exon.gatech.edu/GeneMark/metagenome) was used to predict coding regions (CDS) of metagenomic scaffolds longer than 300 bp. To obtain a non-redundant gene catalog, the CDS sequences of all samples were clustered using CD-HIT at 90% protein sequence identity. Soap.coverage (http://soap.genomics.org.cn/) was used to assess the abundance of the genes in each sample. After aligning the genes against the NCBI-NT database using BLASTN (e value < 0.001), the lowest common ancestor taxonomy of non-redundant genes was obtained. The functional profiles of the non-redundant genes were obtained by annotating against databases including GO, KEGG, EggNOG, and CAZy databases using double-index alignment of next-generation sequencing data (DIAMOND). Linear discriminant analysis effect size (LEfSe) was used to measure differentially abundant taxa and functions between groups using default parameters. Bray–Curtis distance metrics were employed to investigate beta diversity and were visualized using principal coordinate analysis (PCoA). Permutational multivariate analysis of variance (PERMANOVA) was used to assess differences in beta diversity among groups.

2.6. Targeted serum and feces metabolome profiling

The serum and fecal samples were prepared and derivatized according to the methodology described in previous studies [22]. Metabolites in all biological samples were quantitatively detected using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S; Waters Corp, Milford, MA, USA). MassLynx software (v4.1, Waters, Milford, MA, USA) was used to process the raw data files generated by UPLC-MS/MS, and the statistical data obtained using principal

component analysis (PCA), partial least square discriminant analysis (PLS-DA), orthogonal partial least square discriminant analysis (OPLS-DA), and univariate analysis were analyzed through the self-developed platform iMAP (v1.0, Metabo-Profile, Shanghai, China). A pathway analysis for metabolomic data was conducted using MetaboAnalyst (http://www.metaboanalyst.ca/) to predict the enriched pathways of the differential metabolites.

2.7. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA) and IBM SPSS Statistics version 26. The Pearson's chi-square test or Fisher's exact test was used for categorical variables, and the independent samples Student's t-test, Mann–Whitney U test, or Kruskal–Wallis rank-sum test were used for continuous variables. Continuous variables are presented as mean ± standard deviation (SD). A logistic regression was used to investigate the association between the inflammatory potential of the diet with CD and disease activity, and a Spearman's rank correlation was used to assess the correlations among the microbiota composition and metabolites, DII, and several inflammatory markers. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Baseline characteristics and dietary intake of the study population

A total of 285 HCs and 150 patients newly diagnosed with CD (with a disease course of less than one year) were enrolled in the study (Fig. 1). The characteristics of the HCs and patients with CD are presented in Table 1, and there were significant differences in the BMI and 14 of 30 nutrients ($p < 0.05$) between the HCs and patients with CD and age, gender, and smoking habits were not significantly different. Of the 150 patients with CD, 61 (40.67%) were in the inactive stage, and 89 (59.33%) had active CD based on CDAI scores. Patients with active disease were slightly younger, had a smaller BMI at the time of diagnosis, and had more structuring and/or penetrating and complicated perianal lesions than inactive patients (Table 2). As expected, the CDAI and CDEIS were significantly lower in patients with inactive disease ($p < 0.001$), and the levels of several inflammatory biomarkers were significantly lower than in those with active disease ($p < 0.001$; Table 2). In terms of dietary intake, 10/30 dietary components were higher whereas 2/30 components were lower in patients with inactive CD than in patients with active CD (Supplementary Table 1).

Table 1
Demographic characteristics and daily nutrient intake of HCs and CD patients.[a].

	HCs (n = 285)	CD (n = 150)	*p*-value
Age (18–60years)	33.48 ± 12.92	32.31 ± 10.05	0.330
Gender, n (%)			0.08
Female	120 (42.11)	52 (34.67)	
Male	165 (57.89)	98 (65.33)	
BMI (kg/m^2)	21.54 ± 2.82	19.21 ± 3.43	<0.0001
Smoking habit (%)			0.166
Yes	60 (21.05)	25 (16.67)	
No	225 (78.95)	125 (83.33)	
Energy intake (kcal/d)	2165 ± 660.08	2161 ± 644.22	0.981
Protein (g)	42.26 ± 10.28	41.29 ± 8.75	0.463
Carbohydrate (g)	127.41 ± 24.45	131.90 ± 20.33	0.042
Fibre (g)	5.88 ± 2.18	5.17 ± 1.85	<0.001
Fat (g)	36.59 ± 7.94	35.11 ± 7.01	0.056
Cholesterol (mg)	226.83 ± 113.68	217.81 ± 102.92	0.576
Vitamin A (ug)	797.40 ± 470.11	714.14 ± 379.83	0.026
Thiamin (mg)	0.52 ± 0.17	0.50 ± 0.16	0.085
Riboflavin (mg)	0.63 ± 0.18	0.58 ± 0.15	0.001
Niacin (mg)	12.92 ± 2.55	12.51 ± 2.47	0.016
Vitamin C (mg)	82.45 ± 47.08	70.34 ± 34.71	0.001
Vitamin E (mg)	14.71 ± 3.95	13.40 ± 3.71	<0.001
Magnesium (mg)	165.79 ± 34.53	154.92 ± 29.97	<0.001
Iron (mg)	12.43 ± 2.47	12.11 ± 2.63	0.033
Zinc (mg)	7.48 ± 1.29	7.40 ± 1.37	0.075
Selenium (ug)	30.93 ± 11.94	30.58 ± 10.58	0.978
Folic acid (ug)	227.58 ± 121.11	206.66 ± 127.50	0.011
β-carotent (ug)	2006.69 ± 1357.80	1729.56 ± 1058.16	0.007
Anthcoyanidin (mg)	3.00 ± 3.54	2.33 ± 2.31	0.006
Isoflavone (mg)	3.57 ± 3.43	3.65 ± 3.75	0.543
SFA (g)	10.88 ± 3.18	10.51 ± 2.91	0.328
MUFAs (g)	13.83 ± 3.40	13.40 ± 3.05	0.278
PUFAs (g)	8.83 ± 2.50	8.20 ± 3.05	0.063
n-6 PUFAs (g)	8.27 ± 2.39	7.66 ± 2.37	0.074
n-3 PUFAs (g)	0.65 ± 0.23	0.63 ± 0.17	0.521
Alcohol (g)	1.15 ± 3.88	0.68 ± 4.63	<0.001
Vitamin D (ug)	4.62 ± 3.86	4.28 ± 3.36	0.246
Vitamin B6 (mg)	1.06 ± 0.19	1.01 ± 0.21	<0.001
Vitamin B12 (ug)	2.94 ± 2.83	3.02 ± 3.35	0.579
Onion (g)	2.72 ± 8.65	4.08 ± 11.29	0.144
DII score	−0.55 ± 1.40	0.56 ± 1.20	<0.001

Abbreviations: HCs, Healthy controls; CD, Crohn's disease; BMI, Body mass index; DII, Dietary inflammatory index; SFA, Saturated fatty acid; MUFAs, Monounsaturated fatty acid; PUFAs, Polyunsaturated fatty acids.
[a] Data are presented as mean ± SDs, and DII and nutrition components per 1000 kcal are calculated.

3.2. Pro-inflammatory diet associated with the risk of CD and disease activity

The DII was calculated to assess the inflammatory potential of the diets of patients with CD and those of the HCs. The average DII score was higher in the patients with CD (0.56 ± 1.20; range: −1.72–4.49) than HCs (0.23 ± 1.02; range: −1.97–3.13) ($p = 0.017$; Fig. 2a), which implied that the diet of the patients with CD in the year before disease diagnosis had a pro-inflammatory potential. Regression models were developed to analyze the relationship between the DII score and occurrence of CD (OR: 1.317; 95% CI: 1.097, 1.581, $p = 0.003$) before and after adjusting for age, BMI, sex, smoking habits, and daily energy intake (OR: 1.420; 95% CI: 1.049, 1.923, $p = 0.023$), with the aim of determining whether a diet associated a high DII score may be a risk factor for developing CD.

The DII of patients with active disease was significantly higher than that of patients in a non-active stage (0.77 ± 1.25 vs. 0.25 ± 1.06, $p = 0.009$) (Fig. 2b). The linear regression showed a positive association between CDAI and the DII score in the crude model (β = 17.165; $p = 0.013$) and remained significant (β = 11.726; $p = 0.045$) after adjustment for age, sex, BMI, smoking habits, daily energy intake, disease location, disease behavior, perianal lesion, and treatment before diagnosis. These results indicated that a pro-inflammatory diet was associated with disease activity. In addition, CDAI and FC were positively correlated with DII (Fig. 2c, d), while no significant correlations were observed between the DII score and other inflammatory biomarkers, such as ESR, CRP, and PLT (Supplementary Fig. 1).

3.3. Gut microbial feature and functional pathways associated with the inflammatory potential of diet

To investigate the role of gut microbiota under the inflammatory potential of diet, we further analyzed 89 HCs and 41 patients with new-onset CD, who were not taking any medication. The 130 participants were divided into four groups based on the quartiles of their DII score (Fig. 3), and the most pro-inflammatory quartile (Quartile 4) included 40.63% of the patients with CD (13/41) whereas only 18.18% (6/41) of the patients with CD were in the most anti-inflammatory quartile (Quartile 1). We found no significant

Table 2
Demographic and clinical characteristics of active and inactive CD patient[a].

	Total (n = 150)	Active CD (N = 89)	Inactive CD (N = 61)	*p*-value
Age	32.31 ± 10.05	29.47 ± 9.17	36.46 ± 9.91	<0.0001
Sex (%)				
Female	52 (34.67)	32 (35.96)	20 (32.79)	0.412
Male	98 (65.33)	57 (60.04)	41 (67.21)	
BMI (kg/m^2)	19.21 ± 3.43	18.13 ± 2.90	20.80 ± 3.54	<0.0001
Smoking habit (%)				0.069
Yes	25 (16.67)	11 (12.36)	14 (22.95)	
No	125 (83.33)	78 (87.64)	47 (77.05)	
Ileum involving (%)				0.530
Yes	144 (96)	85 (95.51)	59 (96.72)	
No	6 (4)	4 (4.49)	2 (3.28)	
Disease behavior (%)				0.004
Non- Stenosis, non-penetrating	101 (67.33)	52 (58.43)	49 (80.33)	
Stenosis or/and penetrating	49 (32.67)	37 (41.53)	12 (19.67)	
Perianal lesion (%)				0.003
Yes	73 (48.67)	52 (58.43)	21 (34.43)	
No	77 (51.33)	37 (41.57)	40 (65.57)	
Treatment before diagnosis (%)				0.218
No	89 (59.33)	50 (56.18)	39 (63.93)	
5-ASA or/and corticosteroids	61 (40.67)	39 (43.82)	22 (36.07)	
WBC ($\times 10^9$/L)	7.34 ± 2.59	7.63 ± 2.79	6.92 ± 2.21	0.099
HB (g/L)	120.58 ± 24.06	110.51 ± 22.04	135.28 ± 18.83	<0.001
HCT	37.77 ± 6.38	35.12 ± 5.83	41.64 ± 5.06	<0.001
PLT ($\times 10^9$/L)	348 ± 141.63	397.53 ± 145.04	276.23 ± 100.39	<0.001
ALB (g/L)	38.68 ± 32.07	34.46 ± 5.86	44.83 ± 49.38	<0.001
ESR (mm/h)	43.29 ± 31.50	54.89 ± 31.17	26.36 ± 23.39	<0.001
CRP (mg/L)	25.77 ± 33.28	35.04 ± 34.75	12.23 ± 25.82	<0.001
CDAI	177.79 ± 101.58	240.91 ± 81.69	85.70 ± 36.16	<0.001
CDEIS	15.07 ± 8.19	17.71 ± 8.44	11.23 ± 6.05	<0.001
Energy intake (kcal/d)	2161 ± 644.22	2118.29 ± 640.15	2224.33 ± 650.24	0.261
DII	0.56 ± 1.20	0.77 ± 1.25	0.25 ± 1.06	0.009

Abbreviations: CD, Crohn's disease; BMI, Body mass index; WBC; White blood cell; Hb, Hemoglobin; HCT, Hematocrit; PLT, Platelet counts; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; ALB, albumin; CDAI, Crohn's disease activity index; CDEIS, Crohn's Disease Endoscopic Index of Severity; DII, Dietary inflammatory index.
[a] Data are given as N (%) of the total number or as mean ± SDs, and significance was set at $p < 0.05$ are shown.

differences between the subjects' baseline characteristics, but there were differences in the daily intakes of 18 nutrients components between the subjects in Quartile 1 and Quartile 4 (Table 3). Thereafter, we examined changes in microbiota of the participants in Quartile 1 and Quartile 4 to describe the relationship between dietary inflammatory potential and microbiota. There was no difference in the alpha diversity of microflora between the highest and lowest DII levels (p (Simpson, Shannon, Chao 1, and ACE) > 0.05, Supplementary Fig. 2a); however, the fecal microbial taxonomic profiles differed significantly between the two groups ($p = 0.019$, PERMANOVA, Supplementary Fig. 2b). Further, we evaluated the taxonomic signatures in the two groups using LEfSe analysis and found that Quartile 4 was characterized by a relative expansion of bacterial families *Morganellaceae* and *Enterobacteriaceae* compared with Quartile 1 (LDA ≥2, Supplementary Fig. 2c). At the species level, the abundance of 14 species included *Escherichia coli*, *Bacteroides thetaiotaomicron*, *Morganella morganii*, and other unidentified species, were increased in Quartile 4, whereas Quartile 1 was characterized by the enrichment of 19 species (such as *Eubacterium hallii*, *Bacteroides dorei*, and *Flavonifractor plautii*). In terms of the functional pathways, the KEGG database showed that carbohydrate metabolism-related microbial genes were the most abundant, followed by amino acid metabolism (Supplementary Fig. 2e), but no significant differences were observed between the two groups ($p > 0.05$, Supplementary Fig. 2d). The Quartile 4 group was associated with limonene and pinene degradation, and glycosphingolipid biosynthesis ganglio series (LDA ≥2, Supplementary Fig. 2f). To avoid the interference of disease status on the relationship between dietary inflammatory potential and microbiota or metabolites, we excluded patients with CD and again examined the profile and functions of the microbiota in Quartile 1 and Quartile 4 (HC-anti vs. HC-pro). There were no differences in alpha diversity and the overall community composition between HC-pro and HC-anti (Fig. 4a, b). Notably, LEfSe analysis showed that the abundances of the family *Morganellaceae,* genus *Morganella and Veillonella* were also higher in HC-pro than in HC-anti group (LDA ≥2, Fig. 4c). In addition, the abundance of 10 species, including *Veillonella parvula*, *M. morganii*, *Prevotella lascolaii* and others, were increased in HC-pro, while the abundance of *Coprococcus eutactus* and several other species were abundant in HC-anti. Similarly, function analysis found that the overall functional characteristics and several functional pathways were the same as those in Quartile 4 (Fig. 4d-f). In addition, abundance of microbial genes related immune system RIG-I-like-receptor (RLRs) signaling pathway was increased in HC-pro and genes of bacterial invasion of epithelial cells tended to increase compared with HC-anti (LDA <2, $p = 0.048$).

We also described the gut microbial features of CD patients and HCs to determine whether bacteria associated with the dietary inflammatory potential play a role in CD (Supplementary Fig. 3a–d). Interestingly, the microbiota associated with the inflammatory potential of the diet included *Morganellaceae*, *Morganella, Veillonella*, *M. morganii* and *V. parvula* which were elevated in CD similarly, while *C. eutactus* was enriched in HCs (LDA>3, $p < 0.01$). Spearman correlation analysis showed that *Morganella and M. morganii* were positively correlated with CDAI and ESR (rho ≥0.4, $p < 0.05$) and negatively associated with HCT, Hb and ALB (rho ≤ −0.4, $p < 0.05$) (Table 4), which suggests that bacteria associated with dietary inflammatory potential may play a role in patients with CD. We also used KEGG to analyze the gene function of bacteria (Supplementary Fig. 3e–g) and found that microbial genes associated with the bacterial invasion of epithelial cells,

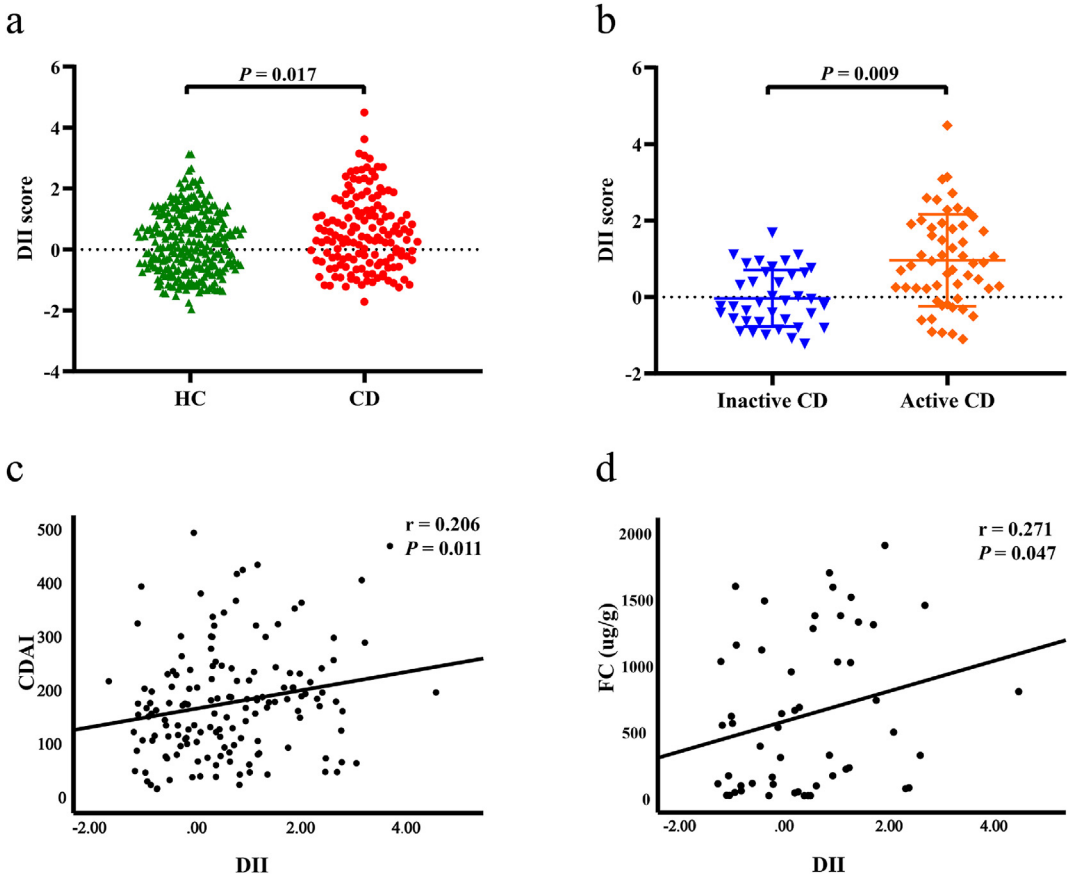

Fig. 2. Comparison of dietary inflammatory potential between CD patients and HCs and inflammatory potential of diet was associated with disease activity in CD patients. (a) Patients with Crohn's disease (CD) consume a diet with higher DII scores than healthy controls (HCs). (b) The DII of active CD patients was significantly higher than non-active CD patients. (c) Crohn's disease activity index (CDAI) score and DII are positively correlated (150 CD patients). (d) Calprotectin (FC) positively relates to DII (54 CD patients). Student's t-test and spearman's correlations were performed. $P < 0.05$ was considered statistically significant.

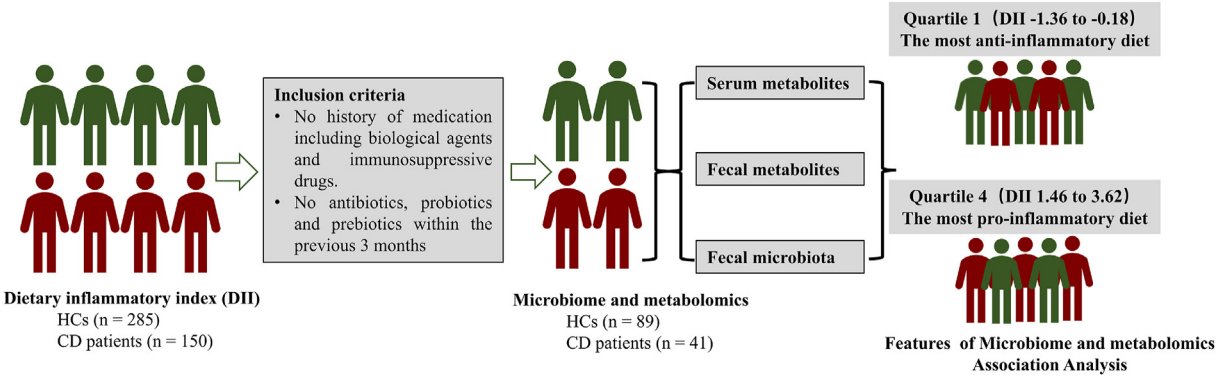

Fig. 3. Flow chart for investigating dietary inflammatory potential in relation to changes in intestinal microbiota and metabolites.

which were observed in HC-pro, were also significantly higher in the CD group than in HCs (LDA = 2.4, $p < 0.01$).

3.4. Fecal metabolite profile associated with the inflammatory potential of diet

To explore the correlation between the inflammatory potential of diet and its metabolites, we performed targeted quantitative metabolomic profiling of all stool samples. Compared to Quartile 1, Quartile 4 was characterized by significant differences in 15 metabolites mainly belonging to amino acids (33.3%), fatty acids (20%), and organic acids (20%), and these metabolites were mainly enriched in the amino acid (AAs) metabolism pathways (Supplementary Fig. 4a–c). After excluding patients with CD, univariate statistical analysis showed that eight metabolites, mainly including organic acids (50%), were still significantly altered in HC-pro compared to HC-anti (Fig. 5a, b). Six metabolites were remarkably increased in HC-pro, and a KEGG pathway analysis showed that these were involved in metabolic pathways associated with short chain fatty acids (SCFAs) biosynthesis and metabolism,

Table 3
Characteristics and daily nutrient intake of the participants based on quartiles of DII[a].

	Quartile 1 (n = 33) HC-anti (n = 27)	Quartile 2 (n = 32) NA	Quartile 3 (n = 33) NA	Quartile 4 (n = 32) HC-pro (n = 19)	*p*-value[b]
No. of CD patients (%)	6 (18.18)	10 (31.25)	12 (36.36)	13 (40.63)	0.207
Age	33.88 ± 12.96	34.19 ± 12.19	35.79 ± 10.46	33.81 ± 11.93	0.983
Female (%)	15 (45.45)	14 (43.75)	12 (36.36)	18 (56.25)	0.453
BMI (kg/m^2)	20.47 ± 2.24	19.84 ± 2.72	20.63 ± 3.06	20.39 ± 3.08	0.91
Smoking habit (%)					0.349
Yes	7 (21.21)	7 (21.88)	8 (24.24)	4 (12.50)	
Energy intake (kcal/d)	2108.9 ± 563.14	2157.90 ± 476.97	2019.78 ± 378.64	2096.34 ± 504.66	0.924
Protein (g)	47.27 ± 10.91	39.67 ± 6.74	39.61 ± 8.65	36.57 ± 8.48	<0.001
Carbohydrate (g)	120.99 ± 25.43	137.02 ± 18.10	133.00 ± 22.78	128.56 ± 16.58	0.160
Fibre (g)	6.92 ± 2.30	5.71 ± 1.97	4.75 ± 2.03	4.36 ± 1.66	<0.001
Fat (g)	37.34 ± 7.94	34.03 ± 6.49	35.16 ± 7.74	38.12 ± 6.14	0.661
Cholesterol (mg)	233.66 ± 103.73	184.27 ± 85.68	213.02 ± 117.73	210.87 ± 594.88	0.529
Vitamin A (ug)	929.94 ± 473.90	767.06 ± 469.11	590.47 ± 345.01	464.55 ± 257.52	<0.001
Thiamin (mg)	0.65 ± 0.21	0.522 ± 0.15	0.50 ± 0.15	0.48 ± 0.16	<0.001
Riboflavin (mg)	0.70 ± 0.17	0.57 ± 0.10	0.56 ± 0.16	0.52 ± 0.14	<0.001
Niacin (mg)	14.28 ± 2.17	12.89 ± 1.72	12.60 ± 2.51	11.71 ± 2.42	<0.001
Vitamin C (mg)	99.48 ± 50.18	77.84 ± 39.01	56.96 ± 29.23	45.44 ± 26.98	<0.001
Vitamin E (mg)	13.69 ± 3.19	11.48 ± 3.43	12.91 ± 3.56	14.93 ± 3.42	0.136
Magnesium (mg)	182.69 ± 32.30	159.43 ± 22.37	145.88 ± 26.63	137.25 ± 21.96	<0.001
Iron (mg)	12.71 ± 1.69	12.06 ± 1.30	11.56 ± 1.94	10.74 ± 1.26	<0.001
Zinc (mg)	8.23 ± 1.28	7.44 ± 0.86	7.10 ± 1.00	6.72 ± 1.17	<0.001
Selenium (ug)	34.29 ± 11.79	27.53 ± 8.26	29.22 ± 11.54	25.56 ± 7.29	0.001
Folic acid (ug)	217.47 ± 75.18	178.25 ± 61.49	175.61 ± 101.02	141.64 ± 47.70	<0.001
β-carotent (ug)	2276.14 ± 1281.67	1865.32 ± 1390.49	1352.49 ± 875.46	1051.26 ± 540.69	<0.001
Anthcoyanidin (mg)	4.77 ± 5.81	3.85 ± 5.17	2.29 ± 2.07	2.00 ± 2.30	0.003
Isoflavone (mg)	3.67 ± 2.64	2.99 ± 2.59	2.92 ± 3.15	2.58 ± 2.75	0.028
SFA (g)	11.64 ± 3.38	10.29 ± 2.41	10.56 ± 2.82	11.28 ± 2.44	0.636
MUFAs (g)	13.89 ± 3.70	12.71 ± 2.42	13.34 ± 3.01	14.07 ± 2.53	0.820
PUFAs (g)	8.14 ± 2.00	8.54 ± 2.22	8.28 ± 2.18	10.54 ± 2.46	<0.001
n-6 PUFAs (g)	7.56 ± 1.89	8.09 ± 2.09	7.81 ± 2.06	10.05 ± 2.35	<0.001
n-3 PUFAs (g)	0.64 ± 0.21	0.57 ± 0.16	0.58 ± 0.15	0.64 ± 0.13	0.495
Alcohol (g)	1.22 ± 3.04	0.30 ± 0.59	0.83 ± 2.26	1.19 ± 4.19	0.274
Vitamin D (ug)	5.10 ± 4.60	3.95 ± 3.58	4.07 ± 2.83	3.43 ± 2.01	0.185
Vitamin B6 (mg)	1.20 ± 0.18	1.03 ± 0.13	1.00 ± 0.17	0.95 ± 0.17	<0.001
Vitamin B12 (ug)	2.52 ± 1.93	1.86 ± 0.91	2.71 ± 3.09	1.92 ± 1.17	0.104
Onion (g)	0.722 ± 1.90	0.99 ± 3.18	1.00 ± 3.04	0.43 ± 1.20	0.471

[a] Data are presented as mean ± SDs, and nutrition components per 1000 kcal are calculated.
[b] Compared with Quartile 1 and Quartile 4, $p < 0.05$ was statistically significant.

including citrate cycle, pyruvate metabolism, propanoate metabolism, and amino acid metabolism (phenylalanine, tyrosine, glycine, serine, threonine, and cyanoamino acid) (Fig. 5c). It has been reported that *M. morganii*, which is associated with the inflammatory potential of diet, participates in phenylalanine metabolism [23].

We hypothesized that the effect of the dietary inflammatory potential on fecal metabolic profiles may have a role in CD. Hence, we analyzed the changes of fecal metabolite profiles of patients with CD relative to HCs. The results showed that 63 metabolites included AAs, fatty acids, bile acids (BAs), and SCFAs, were differentially abundant in CD compared with HC (Supplemental Fig. 5a). Furthermore, pathway analysis revealed that the metabolic pathways associated with the dietary inflammatory potential, such as phenylalanine metabolism and glycine, serine and threonine metabolism, were also highly enriched in CD (Supplemental Fig. 5b). Additionally, these differential metabolites were strongly associated with disease activity and inflammatory marks (Supplementary Fig. 6a). These findings imply that a high dietary inflammatory potential might be associated with CD via the regulation of certain crucial metabolic pathways, which leads to the disruption of metabolic homeostasis.

3.5. Serum metabolite profile associated with the inflammatory potential of diet

In the serum of participants in Quartile 1 and Quartile 4, 9 differential metabolites were mainly involved in certain AAs metabolic pathways (such as phenylalanine, tyrosine, and tryptophan), ubiquinone and other terpenoid-quinone biosynthesis, and aminoacyl-tRNA biosynthesis (Supplementary Fig. 4d–f). However, only six serum metabolites were reduced in HC-pro relative to HC-anti (Fig. 5d, e), and these metabolites were only related to aromatic AAs (phenylalanine, tyrosine, and tryptophan), and ubiquinone and terpenoid-quinone biosynthesis pathways (Fig. 5f). The pathway enrichment analysis showed that phenylalanine metabolism was also affected, but not significantly ($p = 0.07$), which implies that the dietary pro-inflammatory potential likely impacts the metabolic pathways of a few serum AAs. Compared to HCs, we found that most metabolites were significantly reduced in CD, and they mainly participated in numerous AA metabolic pathways (Supplemental Fig. 5c and d), while aromatic AA metabolic pathways were connected to the dietary inflammatory potential. Furthermore, the differential metabolites in these pathways, including tyrosine, tryptophan, and histidine, were correlated with disease activity (CDAI) and inflammatory marks levels (rho $\geq$0.4 or rho ≤ -0.4, all $p \leq 0.05$, Supplementary Fig. 6b). Therefore, these results suggest that the dietary inflammatory potential may mediate aromatic AAs synthesis and the metabolic disorders involved in CD.

3.6. Association between microbiome changes based on DII and metabolomic alteration in CD

To investigate the relationship between microbes associated with the inflammatory potential of diet and the differential metabolites in the feces and serum of CD patients, spearman

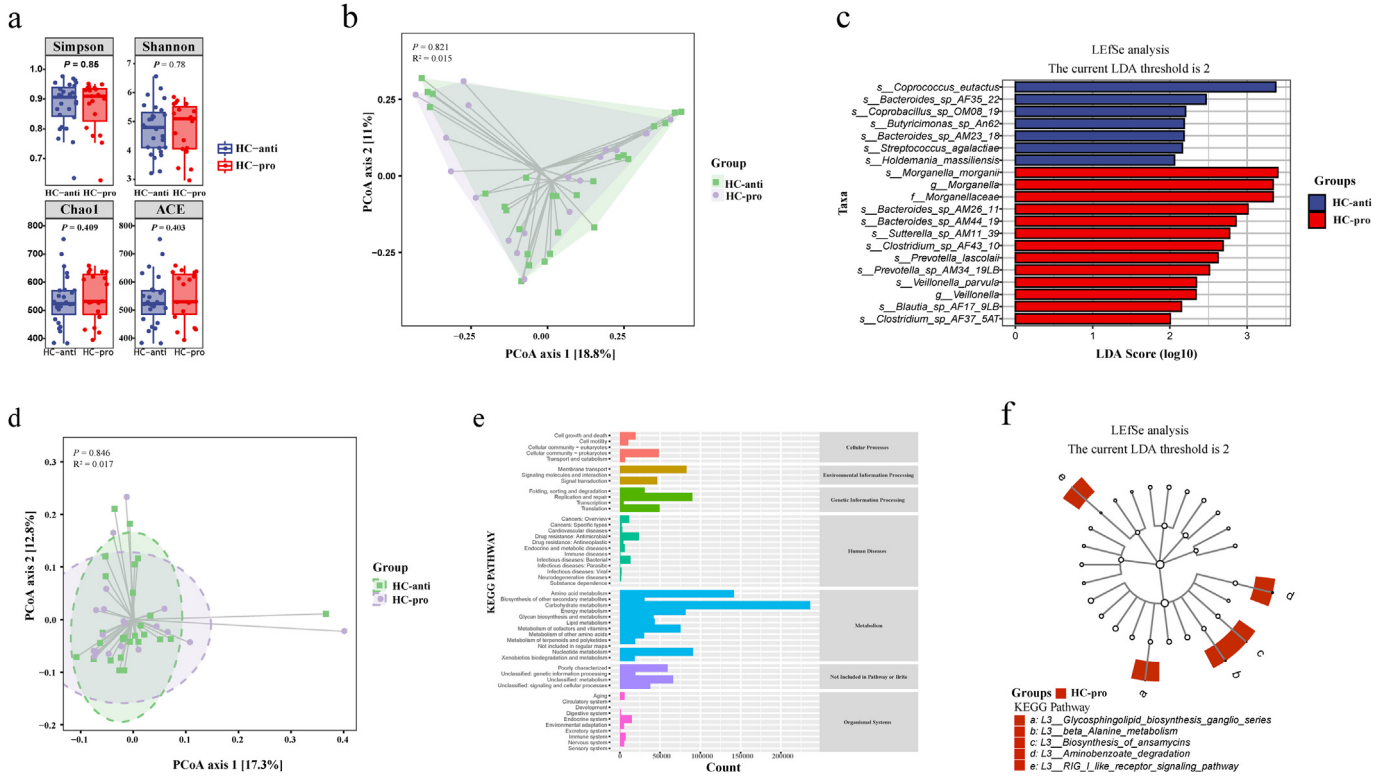

Fig. 4. Changes in gut microbial features and functional pathways in the most pro-inflammatory group without CD patients (HC-pro). (a) Microbial alpha diversity assessed by Shannon index, Simpson index, Chao1 and ACE in the most anti- and pro-inflammatory group (HC-anti vs HC-pro). (b) Beta diversity of gut microbiota calculated via PCoA across HC-anti and HC-pro samples via Bray–Curtis distance. P-value was calculated by PERMANOVA. (c) LEfSe identified the most differentially abundant taxons between HC-anti and HC-pro. (Red) taxa enriched in HC-pro; (blue) taxa enriched in HC-anti. Only taxa meeting an LDA significant threshold ≥2 and $p < 0.05$ are shown. (d) Variation of gut microbial function between HC-pro and HC-anti represented by PCoA plots based on Bray–Curtis distance. (e) Distribution of genes among the KEGG functional categories. (f) The functional taxon differentially represented in HC-pro and HC-anti. Functional taxon (red nodes) enriched in HC-pro. Letters identify the names of taxa that differ significantly between groups. Only pathways meeting an LDA significant threshold ≥2 and $p < 0.05$ are shown.

Table 4
Spearman correlation between bacteria associated with and indicators of disease activity[a].

Taxa	Indicators of disease activity	rho	*p*-value
Morganellaceae	CDEIS	0.3	0.03
Morganellaceae	ESR	0.4	0.02
Morganellaceae	Hb	−0.3	0.05
Morganellaceae	ALB	−0.4	0.02
Veillonella	CDEIS	0.3	0.04
Veillonella	CRP	0.3	0.04
Morganella	CDAI	0.4	<0.01
Morganella	ESR	0.4	0.02
Morganella	Hb	−0.4	0.01
Morganella	HCT	−0.4	0.01
Morganella	ALB	−0.4	0.01
Morganella morganii	CDAI	0.5	<0.01
Morganella morganii	CDEIS	0.3	0.03
Morganella morganii	ESR	0.4	0.02
Morganella morganii	Hb	−0.5	<0.01
Morganella morganii	HCT	−0.5	<0.01
Morganella morganii	ALB	−0.4	<0.01
Morganella morganii	CDEIS	0.3	0.05

Only |rho| ≥ 0.3 and $P \leq 0.05$ were shown in table. $P < 0.05$ was considered statistically significant. Abbreviations: Hb, Hemoglobin; HCT, Hematocrit; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; ALB, albumin; CDAI, Crohn's disease activity index; CDEIS, Crohn's Disease Endoscopic Index of Severity.

[a] Spearman correlation analysis was used for non-normally distributed data.

correlation coefficients were calculated for pairwise combinations of microbial abundances (from metagenomic data) and metabolites alterations were presented as visual heatmaps. Microbiota (*M. morganii*, *V. parvula* and *C. eutactus*) associated with DII score were correlated with serum and fecal differential metabolites in CD relative to HCs (Fig. 6a, b). With respect to fecal metabolites, *M. morganii* and *V. parvula* were positively correlated with AAs, unsaturated fatty acids (monounsaturated fatty acids and n-6 PUFAs), cholic acid (CA), metabolites in the phenylalanine metabolic pathway, branched short-chain fatty acids (BSCFAs), and some other compounds, but negatively correlated with SCFAs, deoxycholic acid (DCA), lithocholic acid (LCA) and other BAs (rho ≥0.4 or rho ≤ −0.4, $p < 0.05$). The correlation between *C. eutactus* and these metabolites was opposite to that of high DII-related microbiota. In serum, AAs, organic acids, BAs, SCFAs were inversely correlated with high DII-related bacteria but positively associated with *Coprococcus* eutactus (rho ≥0.2 or rho ≤ −0.2, $p < 0.05$). However, the correlation of microbiota with serum metabolites was weaker than that with fecal metabolites, showing that fecal metabolites act as a better microbial function readout than serum metabolites for evaluating the response to an inflammatory diet. These results illustrate that alterations in metabolites under a pro-inflammatory diet may mediate host–microbiota interactions in the pathogenesis of CD.

4. Discussion

In this study, diet with high DII score was associated with risk of CD, and the inflammatory potential of diet in the year prior to diagnosis was positively correlated with disease activity at the time of diagnosis, regardless of whether medication had been used

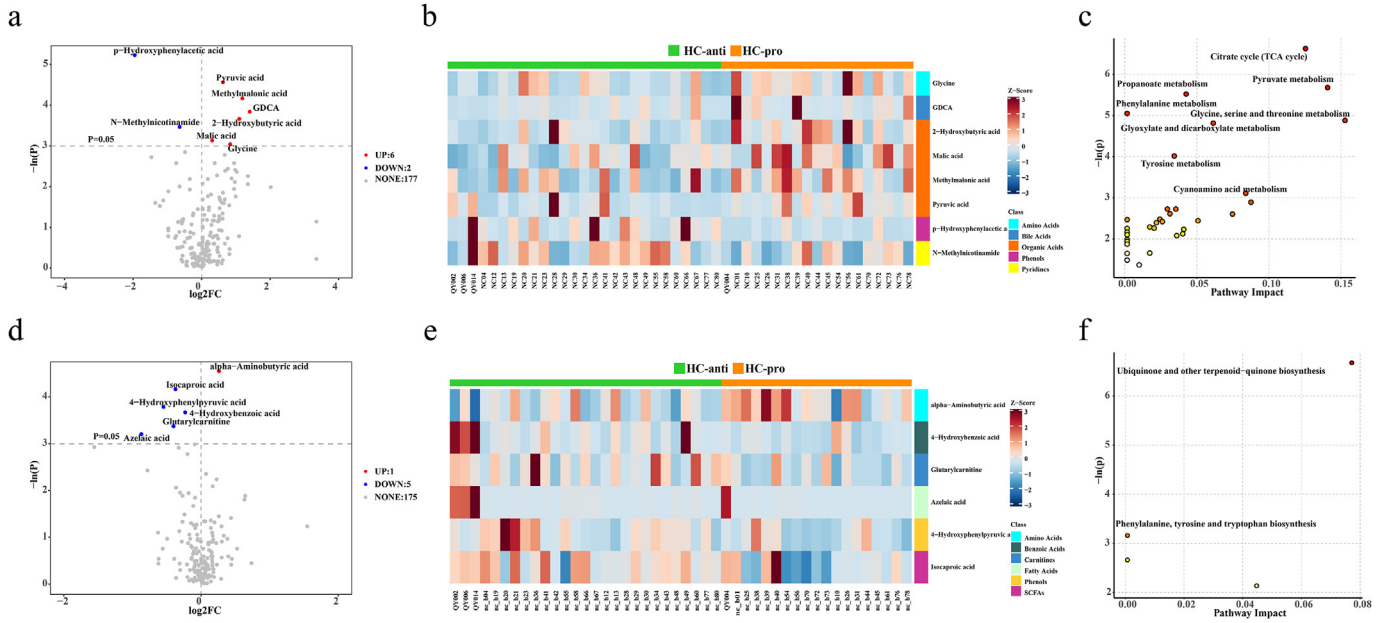

Fig. 5. Alteration of the metabolite and related to the pathway in the most pro-inflammatory group without CD patients (HC-pro). (a) Volcano plots showing the results of pairwise comparisons of fecal metabolites in HC-pro sample's group relative to HC-anti, metabolites (points with red highlight in the right) increased in HC-pro, metabolites (points with blue highlight in the left) decreased in HC-pro. (b) Heatmap shows the classification of fecal differential metabolites between HC-pro and HC-anti. (c) Enrichment analysis of fecal differential metabolites using KEGG is shown in bubble plot. In the bubble plot, Y-axis represents the negative logarithm of *p*-value obtained by pathway enrichment analysis, and only pathways with $p < 0.05$ will be marked with names. The X-axis indicates the degree of impact on the pathway, and the size of the circle is proportional to the pathway impact. (d) Serum differential metabolites are shown in the volcano plots. Metabolites (points with red highlight in the right) increased in HC-pro, metabolites (points with blue highlight in the left) decreased in HC-pro. (e) Heatmap showing the classification of serum differential metabolites between HC-pro and HC-anti. (f) Pathway enrichment analysis of serum differential metabolite is based on the KEGG database, and only pathways with $p < 0.05$ will be marked with names. Differential metabolites are analyzed by univariate statistical analysis. FC is fold change between groups. $p < 0.05$ and $|\log2FC| \geq 0$ is threshold value for differential metabolites).

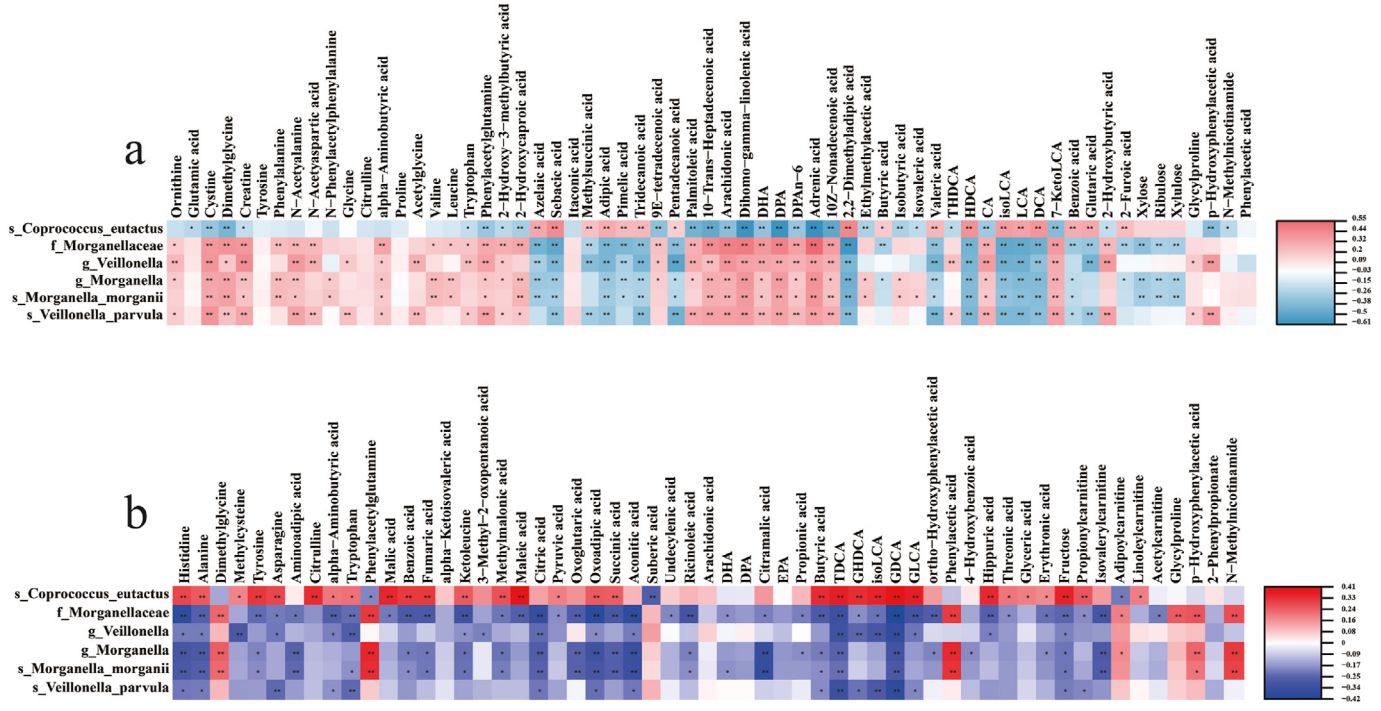

Fig. 6. Heatmap of spearman correlation between DII associated taxa and differential metabolites of CD relative to HCs. (a) DII associated taxa connect to fecal metabolites. $p < 0.05$ and rho ≥ 0.4 or rho ≤ -0.4 are Marked *, $p < 0.01$ and rho ≥ 0.4 or rho ≤ -0.4 are marked **. (b) DII associated taxa relate to serum metabolites. $p < 0.05$ and rho ≥ 0.2 or rho ≤ -0.2 are marked *, $p < 0.01$ and rho ≥ 0.2 or rho ≤ -0.2 are Marked **.

before diagnosis. Furthermore, the metagenomic analyses revealed that diets with high DII scores correlated to the presence of specific microbes, but not the overall diversity of the gut microbiome. *M. morganii* and *V. parvula* were more abundant in the most pro-inflammatory diets group, whereas *C. eutactus* was enriched in the most anti-inflammatory diets group. The relative abundance of those bacteria associated with the dietary inflammatory potential was also significantly altered in CD patients and were correlated with disease activity and inflammatory markers. In metabolic profile, alterations of AAs metabolic pathways in feces and serum of the most pro-inflammatory diets group were also observed in CD group. Additionally, interactions of metabolites and microbiota associated with the dietary inflammatory potential may be involved in the pathogenesis of CD.

Long-term diet has been reported to be an important external factor in IBD etiology. As early as 2016, Shivappa et al. explored the relationship between DII scores and UC, but not CD [24]. In this study, we observed that a diet with high inflammatory potential was associated with a higher risk of CD in new-onset patients, which is consistent with previous reports from well-established disease cohorts [5,10]. However, in terms of disease activity, Mirmiran et al. determined no association between the inflammatory potential of the diet and disease activity in patients with CD [25]. However, some other studies have confirmed that disease activity is higher in patients with CD consuming a diet of higher inflammatory potential [11,12]. We also observed that the DII score was positively associated with CD disease activity. Diet with high inflammatory potential affects the occurrence and development of CD may be related to increased intake of pro-inflammatory dietary components and decreased anti-inflammatory dietary components, which may disorder the gut microbiota, disrupt epithelial barrier function and intestinal immune homeostasis, and then trigger or aggravate intestinal inflammation [4,13].

The overall inflammatory potential of the diet was associated with gut microbiota [18]. We found that the alpha diversity and beta diversity of fecal microbiota were not related to inflammatory potential of diet, but several differentially abundant microbes associated with DII levels. *M. morganii* and *V. parvula* were positively associated with DII, and *C. eutactus* was negatively associated with DII, whereas bacteria positively and negatively correlated with the DII were enrichedand significantly decreased in CD, respectively. Diets with high pro-inflammatory potential contain relatively little dietary fibre. An animal experiment has shown that increased dietary fibre intake was beneficial to increase the relative abundance of *C. eutactus* in the colon [26]. *C. eutactus* is characterized by its butyrate-producing ability [27], and butyrate exerts an anti-inflammatory effect by regulating the immune system, increasing the acetylation of histones, decreasing the activation of nuclear factor-κB, and enhancing the epithelial barrier via stimulating the mucus production from epithelial cells and the rearrangement of tight junction proteins [28]. Hence, a decrease of *C. eutactus* resulted in the decrease of butyric acid, which then presumably promotes intestinal inflammation. Although the role of *C. eutactus* in CD has not been reported to date, some studies have suggested that the genus *Coprococcus* is significantly decreased in CD patients [29,30]. The decrease of *Coprococcus* is not conducive to the response of CD patients to immunomodulators or biologics indicating it may be more related to the pathogenesis of CD [31].

Among the high DII-associated taxa, *Veillonella* and *V. parvula* are opportunistic pathogens and have been reported in many bacterial infection-related diseases [32]. The genus *Veillonella* is usually proposed to be significantly increase in CD compared with HCs [33]. Lipopolysaccharide (LPS) from *Veillonella* and *V. parvula* can stimulate tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in human peripheral blood mononuclear cells in a dose-dependent manner [34]. TNF-α and IL-6 are the most common pro-inflammatory cytokines and could exert pro-inflammatory functions via several paths in CD [35]. In addition, several studies have suggested that *Veillonella* is negatively correlated with secondary BAs, such as DCA and LCA [36], as observed in this study. DCA and LCA are potent agonists for several BA receptors, and they exert anti-inflammatory effects in the colon. LCA can also regulate adaptive immune responses to regulate inflammation [37]. Therefore, the enrichment of *Veillonella* and *V. parvula* in CD patients may facilitate intestinal inflammation. The class *Morganellaceae*, *Morganella* and *M. morganii* belong to the *Enterobacteriaceae* family which were also significantly enriched in the CD group in our study. *M. morganii* has been recognized as an increasingly important pathogen [38]. *M. morganii* can convert the aromatic amino acid L-phenylalanine into phenethylamine (a potentially toxic metabolite) and histamine [39]. It has shown that histamine decarboxylases (specifically from *M. morganii*) are enriched in CD patients, and the rate of histamine secretion was increased in CD, while increased levels are associated with mucosal inflammatory disorder and disease activity [39,40]. In addition, *M. morganii* can stimulate monocyte-derived dendritic cells and Th17 cells, leading to enhance pro-inflammatory cytokine secretion and provoking inflammation [41]. Although it is known that *M. morganii* is enriched in CD patients, little is known about its role in mediating inflammation, and more mechanistic studies are thus needed to explore its role in CD.

The metagenomic pathways investigated here indicated that RLRs and the bacterial invasion of epithelial cell signaling pathways are positively associated with the inflammatory potential of diet, and it has been shown that the two signaling pathways are likely correlated with CD. The RLRs signaling pathway is a vital immunoregulatory pathway, and a few studies have suggested a link between RLRs signaling pathway and CD [42]. A western diet is connected with increased susceptibility to adherent-invasive *E*scherichia coli infection, which implied that invasion of epithelial cells is associated with a higher inflammatory potential [43]. Increased *E. coli* in the gut is associated with CD pathogenesis, indicating potential correlation of bacterial invasion of epithelial cells and CD dysbiosis.

The dietary inflammatory potential can also impact serum and fecal metabolic profiles. In this study, the DII-associated fecal metabolites were found to be involved in eight metabolic pathways, including SCFA-related metabolism (citrate cycle, pyruvate, and propanoate) and AAs metabolism (phenylalanine, tyrosine, glycine, serine, threonine, and cyanoamino acid), whereas DII-associated serum metabolites were only involved in phenylalanine, tyrosine, tryptophan, ubiquinone, and other terpenoid-quinone biosynthesis and metabolism. Metabolic pathways perturbed by inflammatory pathways of diet have been highlighted in CD [44,45]. We also observed alterations in these metabolic pathways in CD patients. Pyruvate is the vital intermediate in the synthesis of SCFAs, and disorders of pyruvate metabolism can result in the disruption of SCFAs synthesis and metabolism [46]. Abnormal pyruvate metabolism has been described in CD; however, the mechanism has not been investigated [47]. Alterations of phenylalanine and tryptophan metabolism in CD pathology has been known for years [23]. Increased serum tryptophan metabolism has been confirmed to be inversely correlated with disease activity and tryptophan levels in CD [48]. Tryptophan is metabolized by the gut microbiota into a range of indole metabolites, some of which can act as aryl hydrocarbon receptors (AhRs). AhR is an important transcription factor responsible for numerous developmental and tissue-dependent effects on T cell immunity, and it exerts protective and anti-inflammatory effects in the gut through IL-22 [16]. Phenylethylamine from phenylalanine metabolism has also been repeatedly

reported to be elevated in the stools of patients with CD, but the specific mechanisms of these metabolites require further exploration.

Finally, we found that DII-related bacteria were associated with differential metabolites in patients with CD compared to HCs. These bacteria were related to serum metabolites to a certain extent, but the correlation was weaker than that of fecal metabolites. For fecal metabolites, high DII-related bacteria were positively associated with elevated metabolites (such as AAs, n-6PUFAs, CA, and BSCFAs) and negatively associated with decreased metabolites (such as SCFAs, LCA, and DCA) in patients with CD, while the opposite correlation was found between *C. eutactus* and these metabolites. Studies suggested that stool samples of CD patients consistently contain higher levels of AAs, n-6 PUFAs, CA, BSCFAs, and they could compromise intestinal stability and aggravate inflammation of the intestinal tract [16,36,49–51]. SCFAs, LCA and DCA could promote anti-inflammatory responses in the host through various mechanisms, but their levels were reduced by varying degrees in CD patients [16,36]. Therefore, diets with a high inflammatory potential may induce gut microbiota and metabolic disorders, and the interaction between microbiota and metabolites would further deteriorate intestinal homeostasis, which appears to increase the risk of intestinal inflammation.

The field of IBD is now taking the first steps toward disease prediction and prevention [52]. Diet habit is an important and readily modifiable risk factor [53]. Our study found that adherence to a diet with high inflammatory potential may be associated with an increased risk of CD. Furthermore, we observed that the alterations in the microbiome profile in healthy subjects that consumed the most pro-inflammatory diets were similar to those observed in CD, which suggests that evaluating diet inflammatory potential and conducting microbiome profiling may be potential indicators of the risk of CD or susceptibility to it. Therefore, larger and prospective cohort studies are required to explore whether HCs on an inflammatory diet develop certain inflammation-related diseases, including CD, and whether CD can be prevented by decreasing the inflammatory potential of the diet. Our study provides scientific evidence for preventing and predicting the occurrence of CD through accurate diet management. Moreover, there is an urgent need to clarify how the dietary inflammatory potential affects the composition and function of the gut microbiome to modulate host inflammation, as this might provide a new strategy for diet- and metabiotic-based therapy for patients with CD.

This is the first study to use metagenomics and metabolomics to explore the role of the inflammatory potential of diet in CD. Nevertheless, this study has certain limitations. First, although a validated FFQ was applied to assess the diet, there is still a possibility of measurement errors and misclassification by the study participants. Second, the DII only comprises food parameters, whereas food additive use is not included. It has been reported that food additives can modify the gut microbiota and health status [54]. Third, the potential pathways identified from fecal and serum metabolomics need to be confirmed in animal or in vitro studies because of the inherent limitations of observational studies and correlative evidence. Furthermore, the study included people of Chinese ethnicity, and other genetic or environmental backgrounds may mask or change the associations if the results of this study are generalized to other ethnicities or regions.

5. Conclusions

The results of this study indicate that consuming a diet that has a high DII score may not only be a risk factor for CD, but it may also be associated with increased CD activity. A correlation analysis between the inflammatory potential of diet, microbes, metabolites, and their related pathways showed that a pro-inflammatory diet casued microbes, characterized by the increase of bacteria *M. morganii* and *V. parvula*, and decrease of *C. eutactus* which associated with differential metabolites mostly involved in AAs metabolism might be risk of CD. Future studies are necessary to clarify the microbial mechanism of dietary inflammatory potential in CD pathogenesis.

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Author contributions

RF and MHC designed the study and obtained funding; ZYT, XJZ, MZ, XZL, RQM collected the data; ZYT, SYZ, ZQZ, SN, and HJR analyzed the data; ZYT, XJZ, SXH, YJZ, CT, RF and ZRZ interpreted the results, wrote and edited the manuscript; and all authors: read and approved the final manuscript.

Data and code availability

Raw sequences have been deposited on NCBI public repository (Bioproject #PRJNA793776).

Conflict of Interest

All authors declared to have no conflict of interests in this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.04.014.

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