



Dietary inflammatory potential in relation to the gut microbiome: results from a cross-sectional study

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Abstract

Diet has direct and indirect effects on health through inflammation and the gut microbiome. We investigated total dietary inflammatory potential via the literature-derived index (Dietary Inflammatory Index (DII®)) with gut microbiota diversity, composition and function. In cancer-free patient volunteers initially approached at colonoscopy and healthy volunteers recruited from the medical centre community, we assessed 16S ribosomal DNA in all subjects who provided dietary assessments and stool samples (n 101) and the gut metagenome in a subset of patients with residual fasting blood samples (n 34). Associations of energy-adjusted DII scores with microbial diversity and composition were examined using linear regression, permutational multivariate ANOVA and linear discriminant analysis. Spearman correlation was used to evaluate associations of species and pathways with DII and circulating inflammatory markers. Across DII levels, α - and β -diversity did not significantly differ; however, *Ruminococcus torques*, *Eubacterium nodatum*, *Acidaminococcus intestini* and *Clostridium leptum* were more abundant in the most pro-inflammatory diet group, while *Akkermansia muciniphila* was enriched in the most anti-inflammatory diet group. With adjustment for age and BMI, *R. torques*, *E. nodatum* and *A. intestini* remained significantly associated with a more pro-inflammatory diet. In the metagenomic and fasting blood subset, *A. intestini* was correlated with circulating plasminogen activator inhibitor-1, a pro-inflammatory marker ($\rho = 0.40$), but no associations remained significant upon correction for multiple testing. An index reflecting overall inflammatory potential of the diet was associated with specific microbes, but not overall diversity of the gut microbiome in our study. Findings from this preliminary study warrant further research in larger samples and prospective cohorts.

Key words: Diet: Inflammation: Gut microbiota: Cross-sectional studies: Circulating markers

Diet is one of the most influential and accessible modulators of the gut microbiome, the human intestine's vast and diverse microbial ecosystem increasingly recognised as a key player

in the development of obesity, type 2 diabetes, CVD and cancer^(1–6). The composition and collective function of the gut community affects how and what the human host is able to

Abbreviations: AMPK, AMP-activated protein kinase; B-H, Benjamini–Hochberg; DII, dietary inflammatory index; LASSO, least absolute shrinkage and selection operator; LEfSe, linear discriminant analysis effect size; NCI, National Cancer Institute; PAI-1, plasminogen activator inhibitor-1; WGS, whole-genome shotgun.

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extract from the diet by providing the machinery that converts dietary content into biological signals with profound systemic effects on host health⁽⁷⁾.

Not unlike the complex and interactive nature of the microbiome, dietary habits are multidimensional with many inter-related components. Recently, Bowyer *et al.* and Maskarinec *et al.* demonstrated the utility of *a priori* dietary patterns or indices for capturing and controlling for variation in the gut microbiome due to the effects of participants' diets^(8–12). The Dietary Inflammatory Index (DII[®]) assesses the balance of pro- and anti-inflammatory dietary factors based on literature-derived associations between various dietary components and inflammatory biomarkers. This index, with its specific focus on inflammation, inherently differs from other index-based scores assessed in previous studies measuring adherence to established dietary guidelines or healthy eating patterns (e.g. the Healthy Eating Index or Mediterranean Diet Score)⁽¹³⁾.

Diet–microbiome interactions may be one of the most promising targets to reduce chronic inflammation, a key pathophysiological mechanism underlying diet's influence on multiple chronic diseases through the common mechanism of the NF- κ B pathway and a complex interaction of cellular, molecular and metabolic factors^(14–21). Given that diet can modulate inflammation through both pro-inflammatory and anti-inflammatory mechanisms, we hypothesised that the inflammatory potential of usual dietary habits would be associated with the overall composition and functional capacity of the gut microbiome. In the present study, we investigated total dietary inflammatory potential, as assessed by the literature-derived DII, in relation to gut microbiota diversity and composition. In a subset of individuals with fasting blood samples and metagenomic data, we further explored microbes and microbial gene pathways associated with DII score and circulating inflammatory markers.

Methods

Study population

The study population is composed of 101 cancer-free individuals, including patient volunteers initially approached in the colonoscopy clinic of MD Anderson Cancer Center (*n* 36) and community volunteers recruited from the medical centre community (*n* 65). Detailed study recruitment and eligibility criteria are provided in online Supplementary Fig. S1. Initially, from 2013 to 2016, a total of 132 patient volunteers with no history of cancer and 449 community volunteers expressed an interest in participating. Both subgroups were screened and interviewed by a clinical provider and/or trained study coordinator with regard to their medical history and medication use and asked to provide stool samples using the same collection protocol. Study ineligibility criteria included current smoker, antibiotic use within the past month, incident or prevalent cancer other than non-melanoma skin cancer, one or more chronic conditions that restricts dietary intake (e.g. coeliac disease), major intestinal surgery (e.g. gastric bypass), currently pregnant or lactating. BMI was calculated from measured weight (kg)/height (m)² and categorised based on WHO criteria⁽²²⁾. Study subjects were included in the current analysis if they completed the dietary

questionnaires, provided a stool sample and passed microbiome data quality filtering criteria. All procedures were reviewed and approved by The University of Texas MD Anderson Institutional Review Board. Informed consent was obtained from all research participants, and all methods were performed in accordance with relevant guidelines and regulations.

Dietary assessment

Participants completed dietary histories via one of two versions of the National Cancer Institute (NCI)-developed FFQ. For consistency with historic recruitment of patients in prior and ongoing MD Anderson studies, patient volunteers completed a modified version of the NCI Health Habits and History Questionnaire^(23,24), which queries the frequency of intake and portion size of 165 food and beverage items, including ethnic foods commonly consumed in the Texas region. Daily nutrient consumption was estimated using the US Department of Agriculture Food and Nutrient Database for Dietary Studies⁽²⁵⁾. Community volunteers completed the NCI Dietary History Questionnaire II (DHQ II), a more recent web-based adaptation of the NCI Health Habits and History Questionnaire⁽²⁵⁾, which queries 134 food items. NCI Dietary History Questionnaire II responses were processed using Diet*Calc software⁽²⁶⁾.

Energy-adjusted dietary inflammatory index score calculation

Food and nutrient intake derived from responses to FFQ was linked to the corresponding inflammatory effect scores designated in the DII to calculate the energy adjusted (E-DII) score for each participant⁽¹³⁾. The DII is a literature-derived, population-based dietary index designed to quantify the overall inflammatory potential of an individual's entire diet. The details of the development and scoring algorithm are described elsewhere⁽¹³⁾. In short, approximately 2000 primary research articles published through 2010 which investigated the effects of forty-five different food parameters (mostly macronutrients, micronutrients, some bioactive components and individual food items such as garlic and tea) on six inflammatory markers (i.e. IL-1 β , IL-4, IL-6, IL-10, TNF- α and C-reactive protein) were identified and scored to derive the component-specific inflammatory effect scores⁽¹³⁾. Thirty-one DII components (plus garlic in the NCI Health Habits and History Questionnaire) were used to calculate the instrument-specific, energy-adjusted E-DII score for analysis. Both FFQ lacked information on some less commonly consumed spices (ginger, saffron, turmeric, pepper, oregano, rosemary) and phenols/flavonoids (eugenol, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins, isoflavones). Food and nutrient consumption was first energy-adjusted per 4184 kJ (1000 kcal), and subsequently standardised for each component using mean and standard deviation data derived from a composite dietary database representing energy-adjusted intake from eleven populations around the world⁽¹³⁾. The energy-adjusted and standardised dietary intakes were then converted to centred proportions to account for skewness, multiplied by the inflammatory effect score for each available DII parameter and summed across all DII components to obtain the overall E-DII score⁽¹³⁾. Higher (i.e. more positive) E-DII scores represent



more pro-inflammatory diets, while lower (i.e. more negative) E-DII scores indicate more anti-inflammatory diets. The E-DII score has been construct validated and consistently associated with elevated inflammatory biomarker levels such as IL-6⁽²⁷⁾, TNF- α ⁽²⁷⁾ and C-reactive protein⁽²⁸⁾.

Stool and blood sample collection

All participants were provided the same stool sample collection kit with detailed instructions. Following defecation into a plastic 'toilet hat', gloved participants used a sterile BBL culture swab collection and transport system (Becton Dickinson) to collect a small portion of their sample. Samples were either Express (overnight or same day) shipped or brought to their next scheduled visit. All faecal samples were received within less than 48 h of collection, stored at -80°C and processed within 1 year of collection⁽²⁹⁾. Residual fasting blood samples drawn at the colonoscopy clinic visit were available in a subset of thirty-four patient volunteers with FFQ and stool samples.

Microbiome characterisation

Total genomic DNA was extracted from faecal samples using the MoBio PowerSoil DNA Isolation Kit following the manufacturer's instructions. The 16S rRNA gene was amplified using V4-targeted primers (GGACTACHVGGGTWTCTAAT and GTGCCAGCMGCCGCGGTAA)⁽³⁰⁾, and amplicons were sequenced using the MiSeq platform (Illumina). Raw FASTQ sequences were processed as previously described⁽³¹⁾. Following quality filtering and chimera removal, sequence reads were clustered into operational taxonomic units using UPARSE⁽³²⁾, with operational taxonomic units subsequently mapped to a V4-optimised version of the SILVA database (version 123) at 97% similarity level. The remaining samples were rarefied to 2742 reads/sample, and relative abundances were calculated (online Supplemental Table S1). Basic Local Alignment Search Tool was used to identify the likely species represented by each operational taxonomic units (OTU) centroid sequence⁽³³⁾.

Whole-genome shotgun (WGS) sequencing was performed to comprehensively assess microbial genomic DNA of faecal samples in a subset of thirty-four patient volunteers who also had FFQ and residual fasting blood samples. WGS data provide species-level taxonomy of the gut microbiome, as well as the metabolic or functional gene content pathways represented within the microbial community. Individual libraries constructed from extracted total gDNA for each sample were pooled and sequenced via HiSeq 2000 (Illumina) using the 1×100 bp paired-end read protocol. Pooling resulted in a sequencing depth >3 Gb/sample. Quality filtering, trimming, demultiplexing and read mapping were carried out by an in-house pipeline described previously.⁽³⁴⁾ To determine metabolic pathway content of the entire metagenome, reads aligning to known orthologues in the Kyoto Encyclopedia of Genes and Genomes database were tabulated and pathways constructed by calculating the minimum set through MinPath45⁽³⁵⁾.

Circulating inflammatory markers

Plasma leptin, TNF- α , IL-6, lipocalin 2, plasminogen activator inhibitor-1 (PAI-1), C-peptide and monocyte chemoattractant

protein-1 were assessed via multiplex assay (Millipore). All samples were run in duplicate with internal standards (pooled cancer case plasma), healthy control samples (normal plasma) and kit quality controls to assure plate to plate consistency.

Statistical analyses

The E-DII score was categorised according to instrument-specific tertiles. Characteristics of study participants were described with medians and standard errors of the mean for continuous variables and frequencies and percentages for categorical variables. We examined the difference of categorical variables across E-DII tertiles by the χ^2 test and the difference of continuous variables by the Kruskal–Wallis non-parametric test. We also examined the associations of these covariates with bacterial α - and β -diversity to assess potential confounders for inclusion in the adjusted models of E-DII and microbiota associations.

α -Diversity was assessed by observed OTU, Shannon diversity index, Chao1 index and Simpson's diversity index. Differences in α -diversity across E-DII tertiles among all subjects and by study subgroup were assessed via the Kruskal–Wallis non-parametric test. We also examined associations of the continuous E-DII score with α -diversity in each subgroup using linear regression models. None of the potential confounders examined, including age (continuous), sex (males and females), BMI (continuous), medication use (yes/no) and study subgroup (patient *v.* community volunteers), was associated with α -diversity or appreciably changed the model estimates and thus was not included in the final adjusted model.

Similarly, Bray–Curtis dissimilarity, Weighted Unifrac, Unweighted Unifrac and Jaccard (β -diversity metrics) between the highest *v.* the lowest E-DII tertile were assessed via permutational multivariate ANOVA among all the subjects and by study subgroup⁽³⁶⁾. While none of the measured covariates were clearly associated with β -diversity, we further examined whether BMI-status modified associations of β -diversity with E-DII in stratified analysis.

Differentially abundant bacterial taxa by E-DII tertiles were assessed using linear discriminant analysis effect size (LEfSe) under Galaxy environment⁽³⁷⁾, applying the one-against-all strategy with a logarithmic linear discriminant analysis score threshold of 3 and α of 0.1 for factorial Kruskal–Wallis test among classes. Analysis was restricted to bacteria present in $\geq 20\%$ of the study population. We further assessed the associations between differentially abundant candidate taxa identified from LEfSe and potential confounders as mentioned above. Given some taxa were associated with BMI, we subsequently evaluated associations of LEfSe-identified taxa in association with E-DII tertiles via a negative binomial regression model adjusted for age and BMI.

Due to the large number of low abundance species in metagenomic data, we employed the least absolute shrinkage and selection operator (LASSO) method to identify bacterial species and microbial gene content pathways associated with E-DII among thirty-four patient volunteers with residual fasting blood samples using the glmnet package in R⁽³⁸⁾. Spearman's rank correlation method was subsequently used to estimate the





correlations of selected species and functional pathways based on LASSO and 16S LefSe analyses with seven circulating inflammatory biomarkers including leptin, TNF- α , IL-6, lipocalin 2, PAI-1, C-peptide and chemoattractant protein-1. The Benjamini-Hochberg (B-H) method was used to adjust *P* values in the multiple correlation analyses while controlling for the expected false discovery rate at 0.05.

Sensitivity analysis was performed by rerunning all the analyses described above after removing one subject identified as an outlier based on his/her Shannon index value, defined as 1.5 interquartile range below the 25th or above the 75th percentile of the population's value. All analyses were performed in SAS, R, Galaxy⁽³⁷⁾, Agile Toolkit for Incisive Microbial Analyses⁽³⁹⁾. All tests were two-sided, with *P* values <0.05 considered statistically significant unless otherwise noted.

Results

Characteristics of participants

The median E-DII score was 1.79 (range -5.15 to -3.08) for patient volunteers and -0.85 (range -4.04 to 2.83) for community

volunteers. Subjects with higher E-DII scores (i.e. more pro-inflammatory diets) tended to have higher BMI (Table 1). However, there was no statistically significant difference across E-DII levels in the distribution of age, sex, history of precancerous colorectal polyps and medication use. When we examined these factors in relation to overall microbial diversity, none of the factors were strongly associated with α -diversity (all *P* \geq 0.15) or β -diversity (all rho \leq 0.05; data not shown).

Associations of energy-adjusted dietary inflammatory index score with microbial diversity

α -Diversity (within sample diversity), a measure of microbial richness and/or evenness, did not differ across E-DII levels among all participants in the crude analysis (Shannon index: *P* = 0.63 (Fig. 1(A)); other metrics (Table 1)). Null results were similarly consistent within study subgroup (Fig. 1(B) for Shannon Index; data not shown for other metrics). Null results also were observed when examining continuous associations of E-DII and α -diversity by subgroup (data not shown).

We examined several β -diversity (between sample diversity) metrics, representing different measures of dissimilarity or

Table 1. Characteristics of participants by energy-adjusted dietary inflammatory index (E-DII) score (*n* 101) (Medians and standard errors; numbers and percentages)

	Most anti-inflammatory diet				Most pro-inflammatory diet		<i>P</i> †
	Tertile 1*		Tertile 2*		Tertile 3*		
<i>n</i>	33		34		34		
	Median	SE	Median	SE	Median	SE	
Age (years)	36.0	2.49	45.0	2.44	41.5	2.47	0.78
BMI (kg/m ²)	23.10	1.04	28.6	1.52	30.2	1.09	0.001
Microbial α -diversity measures							
Shannon index	2.45	0.12	2.61	0.10	2.63	0.11	0.63
Observed OTU	70.58	4.18	76.29	4.24	80.12	4.83	0.41
Chao1 index	88.94	5.58	100.11	6.60	104.15	6.61	0.22
Simpson index	0.83	0.03	0.87	0.02	0.85	0.02	0.65
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Sex							
Male	9	27.27	11	32.35	15	44.12	0.33
Females	24	72.73	23	67.65	19	55.88	
Study subgroup							
Community volunteers	22	66.67	21	61.67	22	64.71	0.91
Patient volunteers	11	33.33	13	38.24	12	35.29	
History of precancerous colorectal polyps							
Yes	8	24.24	11	32.35	6	17.65	0.37
No	25	75.76	23	67.65	28	82.35	
Medication use for the following conditions							
Hyperlipidaemia							
Yes	3	9.09	6	17.65	6	17.65	0.53
No	30	90.91	28	82.35	28	82.35	
High blood sugar							
Yes	1	3.03	0	0	1	2.94	0.60
No	32	96.97	34	100	33	97.06	
Gastro-oesophageal reflux							
Yes	2	6.06	4	11.76	4	11.76	0.67
No	31	93.94	30	88.24	30	88.24	
Medication use for one or more of above conditions							
Yes	6	18.18	8	23.53	10	29.41	0.56
No	27	81.82	26	76.47	24	70.59	

OTU, operational taxonomic units.

* E-DII values across the three levels were calculated from FFQ-specific E-DII scores and categorised into tertiles based on the distributions in each study subgroup.

† The difference of categorical variable across E-DII tertiles was tested by the χ^2 test, and difference of continuous variables was tested using the Kruskal-Wallis non-parametric test as they are not normally distributed.

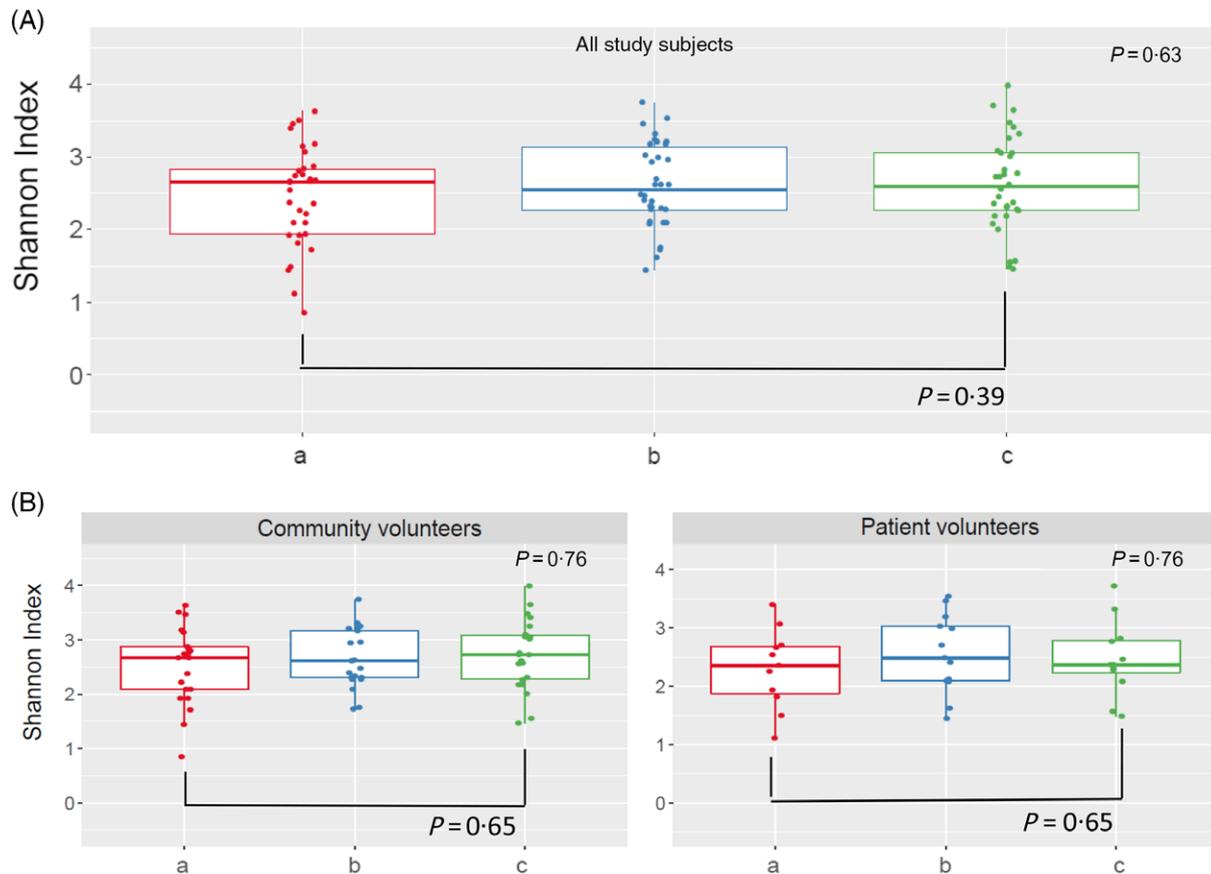


Fig. 1. Microbial α -diversity, as assessed by the Shannon index, across energy-adjusted dietary inflammatory index (E-DII) tertiles among (A) all study subjects (n 101) and (B) by study subgroup (n 65 community volunteers, n 36 patient volunteers). E-DII level: ■, a (most anti-inflammatory diet); ■, b; ■, c (most pro-inflammatory diet).

distance between groups by highest *v.* lowest E-DII level. We observed modest visual differences in overall community composition (Fig. 2 and online Supplementary Fig. S2). Although the results of Unweighted Unifrac reached statistical significance, E-DII clusters, comparing the highest (pro-inflammatory) *v.* lowest (anti-inflammatory) levels, were not overly distinct ($P=0.03$, $\rho=0.02$; online Supplementary Fig. S2). Similar results were observed when we examined E-DII and β -diversity associations stratified by BMI status.

Differentially abundant taxa by energy-adjusted dietary inflammatory index score

We identified several differentially abundant taxa in the LEfSe analysis across the three levels of E-DII score (Fig. 3). *Ruminococcus torques*, *Eubacterium nodatum*, *Acidaminococcus intestini* and *Clostridium leptum* were more abundant in subjects consuming the most pro-inflammatory diets, while *Akkermansia muciniphila* was enriched in subjects with the most anti-inflammatory diets. With adjustment for age and BMI in the negative binomial model, E-DII associations with these five taxa did not appreciably change compared with crude model (Table 2). *R. torques*, *E. nodatum* and *A. intestini* remained significantly associated with a more pro-inflammatory diet.

Associations of energy-adjusted dietary inflammatory index with microbial species, pathways and circulating inflammatory markers

Although the E-DII has been construct validated in several large studies^(40–42), no significant associations were observed with inflammatory markers among the subset of subjects with fasting blood samples available, but all were in the expected direction (n 34; online Supplementary Table S2). In the correlation analysis of microbial species and functional pathways with inflammatory biomarkers (n 34), *Luteimonas mephitis*, a low abundance species identified via LASSO based on its non-zero negative correlation with E-DII, was found to be inversely related to PAI-1 ($\rho=-0.40$, $P=0.02$; B-H adjusted $P=0.24$). *A. intestini*, the taxa associated with the most pro-inflammatory diet in the overall (16S) analysis, was positively related to PAI-1 ($\rho=0.40$, $P=0.02$; B-H adjusted $P=0.24$) in the WGS subset. Another LASSO-selected Lachnospiraceae bacterium strain associated with a more pro-inflammatory E-DII was also positively correlated with C-peptide ($\rho=0.41$, $P=0.02$; B-H adjusted $P=0.24$). No other taxa selected from LEfSe in the larger 16S analysis, or LASSO in the WGS subset analysis were associated with circulating markers (all P values >0.05 ; Fig. 4). Polyketide sugar unit biosynthesis was significantly positively correlated with E-DII ($\rho=0.32$, $P=0.03$; B-H adjusted $P=0.50$).



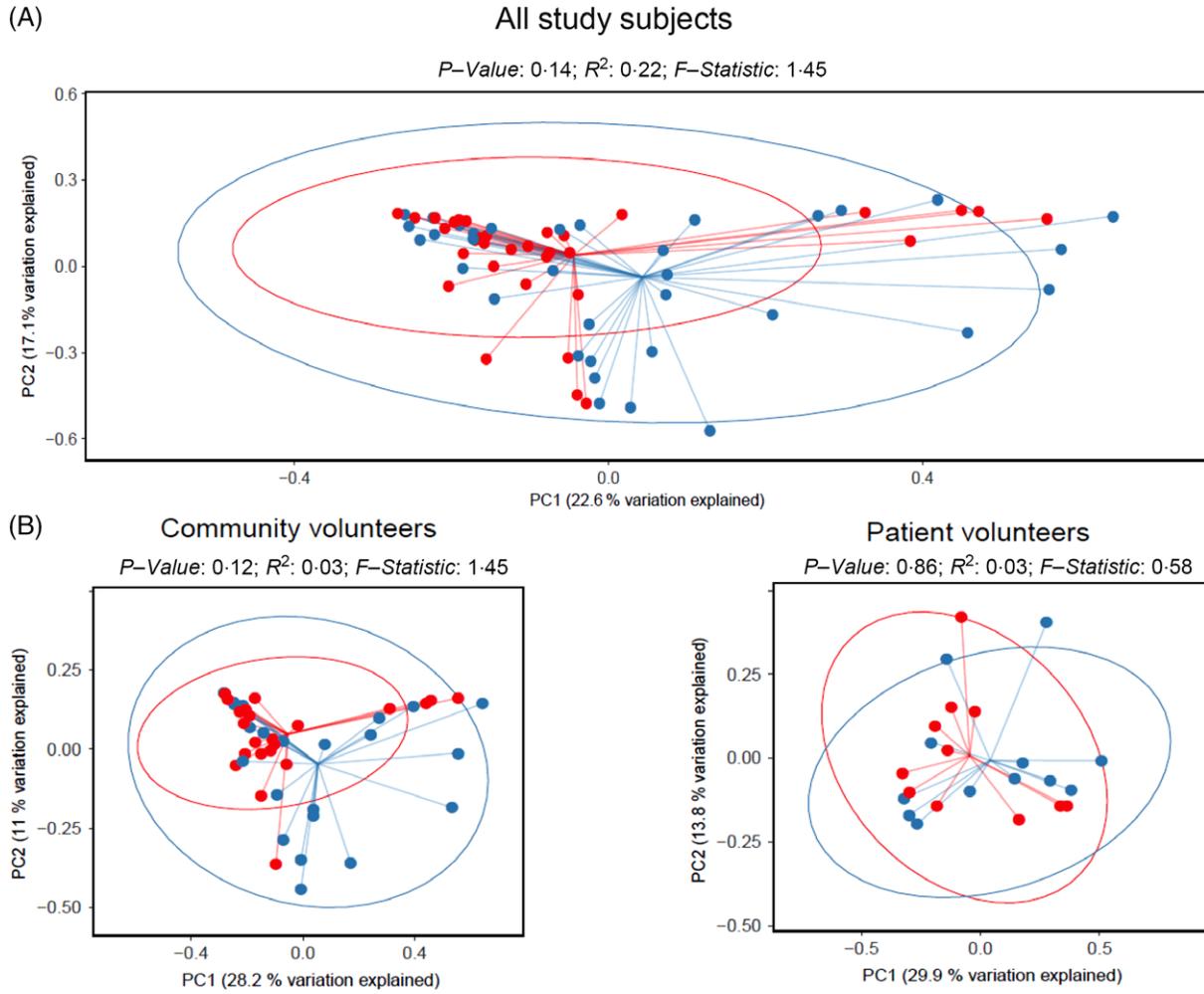


Fig. 2. Microbial community differences, as assessed by Bray–Curtis dissimilarity, between individuals with the most anti-inflammatory *v.* pro-inflammatory diet among (A) all study subjects and (B) by study subgroup. Energy-adjusted dietary inflammatory index (E-DII) level ●, a (most anti-inflammatory diet); ●, c (most pro-inflammatory diet).

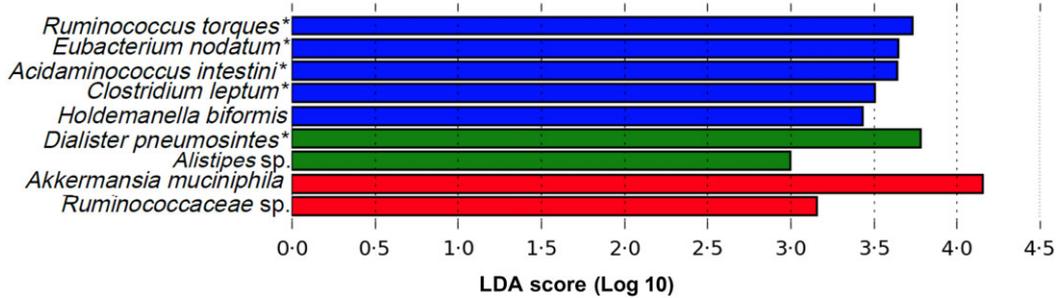


Fig. 3. Differentially abundant taxa across energy-adjusted dietary inflammatory index (E-DII) tertiles using the linear discriminant analysis (LDA) effect sizes approach among 101 subjects. ■, E-DII group 1 (most anti-inflammatory diet); ■, E-DII group 2; ■, E-DII group 1 (most pro-inflammatory diet).

Secondary bile acid biosynthesis was positively correlated with IL-6 ($\rho = 0.35$, $P = 0.04$; B-H adjusted $P = 0.50$), but appeared to be modestly inversely correlated with E-DII scores favouring a more anti-inflammatory diet. Mammalian AMP-activated protein kinase (AMPK) signalling and carbon metabolism pathways, which were each modestly correlated with E-DII

scores favouring a more anti-inflammatory diet, were inversely correlated with C-peptide ($\rho = -0.36$ for AMPK and $\rho = -0.40$ for carbon metabolism, respectively; both P values < 0.05 ; B-H adjusted $P \geq 0.50$; Fig. 5). Of note, none of these associations were statistically significant following correction for multiple testing.



Table 2. Crude and age- and BMI-adjusted associations of energy-adjusted dietary inflammatory index (E-DII) with five candidate taxa* (n 101) (e^{β} and 95 % confidence intervals)

	Most anti-inflammatory diet			Most pro-inflammatory diet		P*
	Tertile 1 (n 33)	Tertile 2 (n 34)		Tertile 3 (n 34)		
	e^{β} †	e^{β} †	95 % CI	e^{β} †	95 % CI	
<i>Ruminococcus torques</i>						
Crude model	1.00 (ref)	1.17	0.62, 2.21	3.16	1.68, 5.93	0.001
Age- and BMI-adjusted model	1.00 (ref)	1.12	0.55, 2.28	3.03	1.48, 6.20	0.002
<i>Acidaminococcus intestine</i>						
Crude model	1.00 (ref)	18.93	2.23, 160.93	257.21	30.90, 2140.72	<0.0001
Age- and BMI-adjusted model	1.00 (ref)	7.59	0.83, 69.58	127.96	9.85, 1661.54	0.0005
<i>Eubacterium nodatum</i>						
Crude model	1.00 (ref)	0.57	0.19, 1.70	2.10	0.86, 5.16	0.03
Age- and BMI-adjusted model	1.00 (ref)	0.43	0.13, 1.36	1.74	0.65, 4.67	0.018
<i>Clostridium leptum</i>						
Crude model	1.00 (ref)	0.59	0.17, 2.04	1.26	0.38, 4.20	0.48
Age- and BMI-adjusted model	1.00 (ref)	0.33	0.08, 1.34	1.24	0.36, 4.26	0.13
<i>Akkermansia muciniphila</i>						
Crude model	1.00 (ref)	0.74	0.18, 3.09	0.23	0.06, 0.97	0.16
Age- and BMI-adjusted model	1.00 (ref)	2.30	0.45, 11.65	0.59	0.10, 3.72	0.17

ref, Reference; LEfSe, linear discriminant analysis effect sizes.

* Computed for the association between E-DII and five LEfSe selected taxa; the significant P value indicates E-DII is significantly associated with abundance of the taxa.

† Negative binomial model with log link was used to estimate e^{β} and 95 % CI. Magnitude indicates how much more abundant the taxa (treated as count), for example, three times more abundant in the highest, as compared with the lowest E-DII tertile.

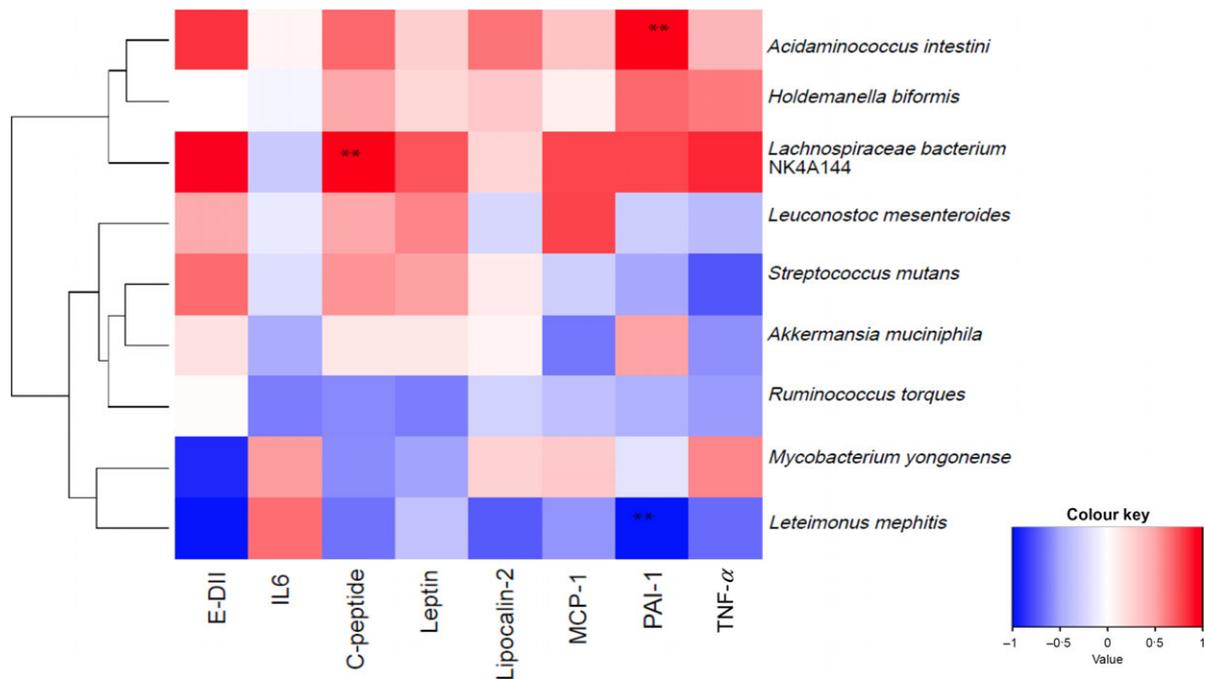


Fig. 4. Correlation heatmap of energy-adjusted dietary inflammatory index (E-DII)-associated species and circulating markers among thirty-four subjects with residual fasting blood samples and whole-genome shotgun (WGS) sequencing of the gut microbiome. *Acidaminococcus intestini*, *Akkermansia muciniphila*, *Ruminococcus torques* and *Holdemanella biformis* were selected as differentially abundant operational taxonomic units (OTU) in the 16S analyses, while other species were selected using the least absolute shrinkage and selection operator (LASSO) method based on non-zero estimates of correlation with E-DII. ** Statistically significant Spearman correlations ($P < 0.05$) before correction for multiple testing (none of the correlations was significant after Benjamini–Hochberg adjustment). MCP-1, monocyte chemo-attractant protein-1; PAI-1, plasminogen activator inhibitor-1.

Discussion

Dietary inflammatory potential as measured by the E-DII score was associated with differential composition of specific microbes, but not overall diversity of the gut microbiome in this cross-sectional sample of 101 cancer-free individuals. In the

overall analysis, *R. torques*, *E. nodatum*, *A. intestini* and *C. leptum* were more abundant in subjects consuming the most pro-inflammatory diets, while *A. muciniphila* was enriched in subjects with the most anti-inflammatory diets. In analysis adjusted for both age and BMI, *R. torques*, *E. nodatum* and

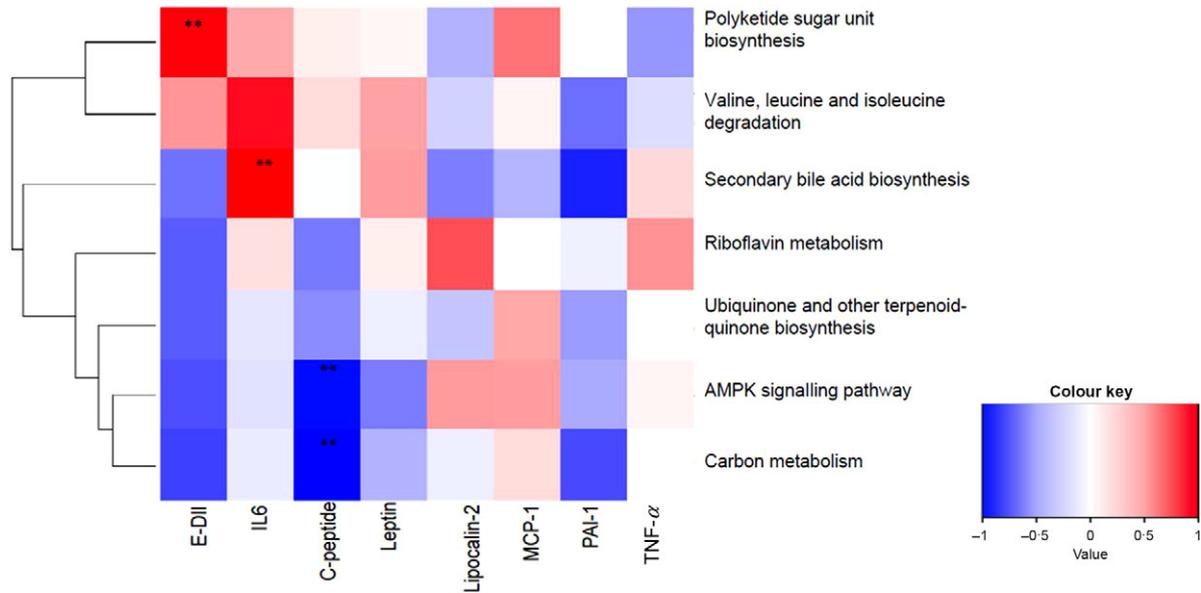


Fig. 5. Correlation heatmap of energy-adjusted dietary inflammatory index (E-DII)-associated pathways and circulating markers among thirty-four subjects with residual fasting blood samples and whole-genome shotgun (WGS) sequencing of the gut microbiome. A total of seven WGS characterised pathways were selected using the least absolute shrinkage and selection operator (LASSO) method based on non-zero estimates of correlation with E-DII. ** Statistically significant Spearman correlations ($P < 0.05$) before correction for multiple testing (none of the correlations was significant after Benjamini–Hochberg adjustment). MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.

A. intestini remained significantly associated with a more pro-inflammatory diet. In an exploratory subset of individuals with fasting blood samples and metagenomic characterisation of the gut microbiome, pathways reflecting the functional capacity of the gut microbiome to support AMPK signalling, carbon metabolism, polyketide and secondary bile acid biosynthesis were associated with the inflammatory potential of the diet and/or systemic inflammation in the host.

We found several differentially abundant microbes by E-DII level. Four bacteria: *R. torques*, *E. nodatum*, *A. intestini* and *C. leptum* were associated with the most pro-inflammatory diet, and *A. muciniphila* was enriched in participants with the most anti-inflammatory diet. *A. muciniphila* and *R. torques* have previously been highlighted in a randomised cross-over intervention trial of the fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet among twenty-seven irritable bowel syndrome patients and six healthy subjects⁽⁴³⁾. Results of this trial showed that low compared with moderate FODMAP intake was associated with a 5-fold reduction in *A. muciniphila* and a 1.5-fold increase in *R. torques*⁽⁴³⁾. FODMAP substrates are fermented by gut bacteria to SCFA with anti-carcinogenic and anti-inflammatory actions⁽¹⁶⁾. *A. muciniphila* is a producer of SCFA, primarily acetate and propionate, through mucin degradation. Mouse studies have demonstrated that a high-fat diet (i.e. pro-inflammatory diet) decreases the abundance of this species and support a causative role for this species in lowering adipose tissue inflammation and improving insulin sensitivity and lipid metabolism⁽⁴⁴⁾. However, the role of *A. muciniphila* in modulating inflammation in humans remains unclear. Two studies reported no association of *A. muciniphila* with systemic inflammatory markers, including high sensitivity-

C-reactive protein, IL-6 and lipopolysaccharides^(45,46). In a recently published randomised, double-blind, placebo-controlled pilot study of thirty-two overweight/obese insulin-resistant volunteers, a 3-month administration of pasteurised *A. muciniphila* was found to significantly decrease two enzyme activities that are thought to be involved in modulating inflammation, that is, dipeptidyl peptidase 4 and γ -glutamyltransferase⁽⁴⁷⁾. Previous human studies that investigated other *a priori* dietary patterns or indices (e.g. Healthy Eating Index, Mediterranean Diet Score and Healthy Food Diversity index) also showed that greater adherence to healthy diets was associated with increased abundance of other SCFA-producing microbes, such as *Faecalibacterium prausnitzii*^(8,10).

Less is known with regard to other potential inflammation-related taxa observed in our study and their interactions with the inflammatory potential of diet. *E. nodatum* is a periodontal pathogen⁽⁴⁸⁾ that has been linked to active rheumatoid arthritis and psoriatic arthritis characterised by chronic inflammation affecting joints and connective tissues⁽⁴⁹⁾. *C. leptum* is a carbohydrate-fermenting bacteria associated with Treg responses known to modulate intestinal and systemic inflammation in experimental models and found to be lower in patients with inflammatory bowel disease^(50–52). Although in our study higher *C. leptum* was observed in healthy individuals with a more pro-inflammatory diet, this cross-sectional association was attenuated with adjustment for age and BMI. *A. intestini*, previously recovered from various human clinical samples of hospitalised patients⁽⁵³⁾, appears to be responsive to supplementation with quercetin and partially hydrolysed guar gum in human faecal samples^(54,55) and is positively associated with LPS-stimulated TNF α production⁽⁵⁶⁾. This is generally consistent with our study finding

of higher *A. intestini* in the most pro-inflammatory diet group and its positive correlation with circulating PAI-1, a pro-inflammatory marker closely associated with obesity, type-2 diabetes and CVD risk⁽⁵⁷⁾. Conversely, *L. mephitis*, a species of Proteobacteria that reduces nitrite to nitrous oxide without production of nitrogen⁽⁵⁸⁾, was inversely correlated with E-DII and PAI-1 in our study.

Metagenomic pathways are expected to more closely reflect the functional nature of the gut microbiome as an interacting community of multiple microorganisms that supports (or hinders) the host. We found that the AMPK signalling pathway, a central regulator of cellular energy homeostasis and glucose and lipid metabolism⁽⁵⁹⁾, was inversely associated with E-DII and C-peptide. In both human and animal studies, activation of AMPK in adipose tissue has been linked to several anti-inflammatory dietary factors, such as *n*-3 PUFA, polyphenolic compounds and fibre^(60,61). However, there is little evidence to support our findings for microbial AMPK and carbon metabolism pathways with C-peptide, an insulin resistance marker⁽⁶²⁾. We also identified a positive association of secondary bile acids biosynthesis with IL-6. Primary bile acids are converted to secondary bile acids through microbial modifications in the gut and modulate signalling via the nuclear bile acid receptors, that is, farnesoid X receptor and G-protein-coupled bile acid receptor (TGR5). Farnesoid X receptor and TGR5 signalling influence many different metabolic processes in the host, including energy homeostasis, glucose homeostasis, obesity and inflammatory responses, involving IL-6 and TNF- α ^(63–65). The positive association between E-DII and polyketide biosynthesis is also interesting, as bacterially derived polyketides exhibit a number of bioactive properties that modulate antibacterial, antitumour and antiviral activities^(66–68). The direction of this relationship and some others (e.g. the suggestive inverse association of DII with secondary bile acids biosynthesis) are somewhat unexpected and may be chance findings due to very limited sample size (*n* 34). While these pathways reflect the presence of genes needed to perform a particular metabolic function, they do not necessarily reflect the gut-derived metabolites that may or may not be produced from interactions between microbes and host diet or other exposures (e.g. medications). Additionally, some pathological pathways may not be activated given the study exclusion criteria and focus on healthy individuals. Larger prospective studies are warranted to confirm or refute our findings, as this analysis was exploratory in nature and none of our findings were significant following adjustment for multiple testing.

The biological processes underlying diet–microbiome interactions that modulate inflammation are not fully known. With a diet enriched with more anti-inflammatory components such as fibre- and polyphenol-rich plant foods, saccharolytic fermentation of carbohydrates by gut microbes can produce SCFA (i.e. butyrate, acetate and propionate). SCFA promote anti-inflammatory responses in the host through a series of mechanisms, including intestinal homeostasis, genetic/epigenetic regulation and immunomodulatory signalling⁽¹⁶⁾. Conversely, a pro-inflammatory diet (e.g. high in fat, simple carbohydrates and meat but low in fibre-rich plant foods) creates a pro-inflammatory milieu of protein catabolites and deconjugated bile

acid residues, leading to increased inflammation-related phenotypes in the host⁽¹⁶⁾. These include impairment of the mucosal barrier and altered gut permeability and immune responses⁽¹⁸⁾. A local inflammatory environment further alters gut microbiota to affect systemic inflammation via adherence to the gut epithelium, passage through the gut barrier to enter systemic circulation, activation of an immune response through toll-like receptor binding and/or activation of regulatory T cells and through the synthesis and secretion of cytotoxic biomolecules or metabolites^(16,18,69). Additional human research is needed to elucidate the microbial pathways and metabolites that modulate diet-induced inflammation.

Interestingly, despite our findings for specific microbes and functional pathways, we did not observe significant or striking associations between gut microbial (α and β) diversity and E-DII score in either the crude or adjusted analyses. While this could be attributed to insufficient variation, sample size or residual confounding due to unmeasured factors, several studies comparing faecal microbiota diversity across participants following distinct diets (e.g. vegetarian, vegan and omnivore) also reported no, or only modest, differences in microbial diversity between diet groups^(70–74). Notably, the DII focuses on a balance of dietary components similar to other diet indices, but it may not be directly comparable to other studies due to its specific focus on inflammation. The Healthy Eating Index and Mediterranean Diet Score were significantly associated with various α -diversity metrics in an assessment of three different dietary indices based on FFQ data from 2070 members of the TwinsUK cohort⁽⁸⁾. One⁽⁹⁾ of three additional observational studies focused on increased adherence to the Mediterranean Diet Score or other diet quality indices reported a concurrent increase or difference in microbial α -diversity^(11,12). Similarly, a dietary intervention among overweight and obese subjects, as well as a companion observational study focused on healthy *v.* unhealthy dietary patterns, reported increased faecal microbial gene richness (total gene counts) among participants with healthier diets^(1,75).

Our study is novel in its specific focus on diet-related inflammation and its interaction with microbiome, a hypothesis rooted in biologically plausible relationships that are highly relevant to host health^(14,76). Using a construct-validated tool which converts major inflammation-related dietary factors commonly consumed by Americans to an overall interpretable diet score, the E-DII provides a comprehensive summary of the dietary inflammatory potential of participants' entire diets^(41–43). Although not all of the DII components were queried in our study, the majority of the missing dietary components, like spices, are typically consumed in very low amounts in the USA. As previously reported, the range of DII scores may rely more on the amount of foods actually consumed rather than on the number of available DII components⁽⁷⁷⁾. Furthermore, misclassification in dietary inflammatory potential due to the missing dietary factors would likely be non-differential, thus attenuating our results toward the null. Other limitations of nutritional epidemiological studies also apply here, as the FFQ is prone to response bias and measurement error. Importantly, other important factors or exposures that may affect the microbiota composition (e.g. medications) were carefully collected in personal interviews and medical



charts. However, we are still learning the complexity and breadth of factors that affect the microbiome or mask observed diet–microbiome associations, and our sample size limited our ability to explore multiple factors simultaneously. At the time of recruitment, we excluded individuals who reported antibiotic use in the past month; however, this may not have been sufficient for some individual's microbial communities to 'return to normal'. Recent studies demonstrate that the recovery time may vary by the type, dose and duration of antibiotic use^(78,79). Our inclusion of cancer-free patient and community volunteers was designed to maximise the variation needed to identify associations of diet with the microbiome. However, homogeneity due to exclusion criteria coupled with limitations in sample size could explain the low variation observed in α -diversity, β -diversity and other associations. Also, it may have been easier to detect differences had the range in DII scores gone towards their theoretical extremes (i.e. -9 to $+8$). We were particularly limited in our subset analysis of patients with residual fasting blood samples, for which we also conducted WGS sequencing of the gut microbiome. To address this, we used LASSO, which is an efficient method to select species and pathways given a small number of subjects and the large number of zero-inflated species revealed in WGS sequencing. Taken overall, the present study is of a preliminary and hypothesis-generating nature given the cross-sectional design with evaluation of diet, microbiome and blood markers at a single time point, as well as the lack of significant associations following adjustment for multiple comparisons, all of which prohibit causal and mechanistic inferences.

Dietary inflammatory potential was associated with differential composition of specific microbes but not overall gut microbiota diversity in this well-defined sample of 101 individuals. Our analysis highlighted several biologically plausible microbes potentially related to diet-induced inflammation. *R. torques*, *E. nodatum*, *A. intestini* and *C. leptum* were more abundant in subjects consuming the most pro-inflammatory diets, while *A. muciniphila* was enriched in subjects with the most anti-inflammatory diets. Correlations between E-DII, microbes, functional gene content pathways and inflammatory biomarkers in an exploratory subset further support the role of diet–microbiota interactions in modulating systemic inflammation in the host. Future prospective studies of dietary inflammatory potential and its interactions with the microbiome are warranted. It is increasingly important to understand how diet as a whole shapes the composition and function of the gut microbiome to modulate host inflammation, an important mechanism in the development of cancer and other major chronic diseases.

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J. Z. and C. R. D. designed the research and drafted the manuscript; J. Z. conducted the analysis; J. Z., K. L. H., P. W., S. M. S. and C. R. D. contributed to the design of the analysis and interpretation of results. K. L. H., J.-S. C. and P. W. provided statistical support. C. R. D., S. M. S. and J. F. P. designed and carried out the initial studies; N. S. and J. R. H. contributed to the scoring of the E-DII and helped with E-DII score interpretation; D. D. D., G. J. B. and K. L. H. were involved in the collection and processing of the data. S. H. and J. F. P. provided essential reagents or materials. All authors contributed to the critical revision and approval of the manuscript. C. R. D. had primary responsibility for final content.

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Supplementary material

For supplementary materials referred to in this article, please visit <https://doi.org/10.1017/S0007114520001853>

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