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The impact of diet and ethnicity on Gut Microbiota Variation in Irritable Bowel Syndrome: A Multi-centre Study

Running Title: Gut Microbiota and low FODMAP diet in IBS

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Sanjiv Mahadeva is a member of the Editorial Board of the Journal of Gastroenterology and Hepatology. To minimise bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication.

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Abstract

Background: The gut microbiota in irritable bowel syndrome (IBS) is known to vary with diet.

Aims: i) To analyse the gut microbiota composition of IBS patients from a multi-ethnic population and ii) explore the impact of a low FODMAP diet on gastrointestinal symptoms and gut microbiota composition amongst IBS patients.

Methods: A multi-centre study of multi-ethnic Asian patients with IBS was conducted in two phases: i) an initial cross-sectional gut microbiota composition study of IBS patients and healthy controls, followed by ii) a single-arm 6-week dietary interventional study of the IBS patients alone, exploring clinical and gut microbiota changes.

Results: A total of 34 adult IBS patients (IBS sub-types of IBS-D 44.1%, IBS-C 32.4% and IBS-M 23.5%) and 15 healthy controls were recruited. A greater abundance of *Parabacteroides species* with lower levels of bacterial fermenters and short-chain fatty acids producers were found amongst IBS patients compared with healthy controls. Age and ethnicity were found to be associated with gut microbiota composition. Following a low FODMAP dietary intervention, symptom and quality of life improvement were observed in 24 (70.6%) IBS patients. Symptom improvement was associated with adherence to the low FODMAP diet (46.7% poor adherence vs 92.9% good adherence, p=0.014), and gut microbiota patterns, particularly with a greater abundance of *Bifidobacterium longum*, *Anaerotignum propionicum* and *Blautia species* at baseline.

Conclusion: Gut microbiota variation in multi-ethnic IBS patients may be related to dietary intake and may be helpful to identify patients who are likely to respond to a low FODMAP diet.

Keywords: irritable bowel syndrome, gut microbiota, ethnicity, diet, the low FODMAP diet

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic disorder of the gut-brain axis characterised by abdominal pain or discomfort, associated with an alteration in stool consistency. Its' pathophysiology is recognised to be multi-factorial, with gut dysbiosis playing a major role in pathophysiology[1]. Cross-sectional studies amongst adults with IBS, compared to healthy controls, have demonstrated an increase in *Enterobacteriaceae* and *Lactobacillaceae* species and a reduction in *Faecalibacterium* and *Bifidobacterium* species [2]. A recent systematic review of case-control studies found that the diversity of gut microbiota amongst IBS adults compared to controls was reduced or unchanged [2]. However, the majority of studies were conducted on Western IBS patients, all were single-center studies, and there had been no dietary assessment.

IBS symptoms are recognised to be triggered by certain foods, and a diet low in fermentable oligo-, di- and monosaccharides and polyols (FODMAP) has become increasingly popular as a major form of therapy for this condition [3]. The lowered intake of small, indigestible and fermentable carbohydrates reduces intestinal osmolarity and gas production, which improves IBS symptoms [4]. An updated systematic review and meta-analysis of 12 randomised controlled trials in IBS concluded that the low FODMAP diet resulted in a moderate to high efficacy in reducing GI symptoms [5]. In a sub-analysis, the systematic review additionally observed a potential alteration in gut microbiota following a low FODMAP diet, but concluded that the marked heterogeneity in microbiological analytical measures and outcome measures precluded any firm conclusions. Notably, all studies on the low FODMAP diet and associated gut microbial analyses were conducted in Western IBS patients.

Dietary differences between Western and Asian populations are well recognised, which can influence gut microbiota composition [6]. Essentially an Asian diet has a higher carbohydrate and fiber composition, whereas a Western diet is enriched with total fats and animal proteins [7]. Studies have now shown that Asian food, in contrast to a Western diet, is associated with an

increase in beneficial bacterial abundances such as *Firmicutes* and a reduction of non-beneficial abundance, such as *Bacteroidetes* species [8-10]. Furthermore, gut microbiota is known to vary amongst different ethnic groups, despite having a similar type of diet [11].

The Malaysian population of IBS adults offers a unique opportunity to study the interaction between gut microbiota, diet, and ethnicity. The three major ethnic groups in this population, i.e. ethnic Malays, Chinese and Indians, have typically varied dietary habits due to cultural differences between these ethnic groups. We therefore aimed to i) analyse the gut microbiota composition of IBS patients from the three major ethnic groups in Malaysia, together with a detailed dietary assessment compared with healthy controls; and ii) explore the impact of a low FODMAP diet on GI symptoms and gut microbiota composition amongst IBS patients only.

METHODOLOGY

Study design

A multi-centre, study of IBS adult subjects attending the Gastroenterology Clinics of three major institutions in this country (University Malaya Medical Centre, National University Hospital Malaysia and Hospital Universiti Sains Malaysia) was conducted. The study consisted of two phases – i) an initial cross-sectional gut microbiota composition study of IBS patients and healthy controls (age and ethnicity matched), followed by ii) a single-arm diet interventional study of the IBS patients alone, exploring clinical and gut microbiota changes. Adults aged > 18 years fulfilling the locally validated enhanced Asian Rome III criteria for IBS [12] from the three institutions were invited to participate in the study. The following were exclusion criteria for the study: use of antibiotics or probiotics within two months of enrolment, use of laxatives or anti-diarrheal agents, dietary management of IBS within 12 months of the study, pregnancy, previous gastrointestinal surgery and presence of diabetes mellitus. Ethics

approval by the Institutional Review Boards (IRB) of all three institutions and written informed consent forms from all the participants were obtained prior to the conduct of the study.

Study procedure

Following eligibility and obtaining consent, all subjects (IBS and healthy controls) underwent a brief interview to collect demographic, clinical, dietary data and a Hydrogen Breath Test to exclude small intestinal bacterial overgrowth (SIBO). Any subjects found to have SIBO were excluded from further evaluation at this stage. Stool samples were collected from IBS and controls before the dietary intervention, and IBS subjects alone following the dietary intervention. IBS symptoms were assessed before and after the dietary intervention using the IBS severity scoring system (IBS-SSS) [13]. The IBS-SSS allows for a range of symptom severity from mild (score 75 – 175), moderate (175 – 300) and severe (> 300). The health-related quality of life (HRQOL) was additionally measured pre & post dietary intervention, using the locally validated EQ-5D instrument.[14] The EQ-5D comprises five questions on mobility, self-care, pain, usual activities and psychological status with three possible answers for each item (1 = no problems, 2 = moderate problems, 3 = severe problems). An overall utility score (mean) is calculated based on these domains, with a range score from 0 (worse health scenario) to a maximum of 1.0 (best health scenario).

Dietary data collection & low FODMAP diet intervention

The dietary intake of the participants was assessed using a three-day food record. It was a self-record method where the participants were asked to record their food and beverage intake in the food diaries with the actual portion consumed [15]. The three- day food records were provided during the baseline visit. At baseline, the participants were instructed by the dietitian to record

the detailed three-day dietary intake for two weekdays or working days and one-weekend (time, place, type of food, food preparation, portion intake) in a printed three- day food record. In the food record, participants were explained with illustrated food and drink images as well as food portion size references. Instructions, guidelines, and examples for the food record were provided in the leaflet in English and Malay. The references for the dietary assessment were mainly based on the "Nutrient Composition of Malaysia Food" and the "Atlas of Food Exchanges & Portion Size" [16, 17]. The participant's dietary intake was collected at baseline and after completion of the dietary intervention.

The dietary intervention involved a strict elimination of all foods containing a high FODMAP content, ie local food items which were high in fructans (e.g., wheat products, onions), Galacto-Oligosaccharides (e.g., legumes), polyols (e.g., pear, sugar-free gums), lactose (e.g., mammalian milk), and excess fructose (e.g., honey). This dietary advice was in accordance with standard instructions for low FODMAP restrictions in a South East Asian diet [18]. The duration of dietary intervention was planned for six weeks. Throughout the study, adherence to the diet was encouraged by regular telephone consultations by the dietitians.

Gut microbiota detection

Stool samples were collected from healthy controls, and from IBS patients before & after dietary intervention. The stool samples were stored in sterile containers and kept frozen at -80°C prior to analysis. Nucleic acid extraction was performed on ~200mg stool using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The purity and concentration of DNA were checked on Nanodrop and Qubit, respectively. The 16S amplicon sequencing was conducted using the Illumina Novaseq platform (IIlumina Inc., San Diego, USA) by targeting the v3-4 region (341-

F: 5'- CCTAYGGGRBGCASCAG-3'; 806R: 5'- GGACTACNNGGGTATCTAAT-3') of the 16SrRNA gene. A total of 4230470 raw reads were obtained and the sequences were deposited in the GenBank under BioProject ID PRJNA789106. The data were trimmed and quality filtered using dada2 R package [19] using the following conditions: maxN=0, maxEE=c(2,2), truncQ=2. The chimeric sequences were removed using the "consensus" method implemented under the "removeBimeraDenovo" command. The processed dataset consisted of 1170049 sequences or an average of 14445 sequences per sample. The sequence data was then exported to PAPRICA v.0.7.0 software [20, 21] for phylogenetic placement. The software "grouped" the sequences based on their position in the "guide tree", instead of the % sequence homology cutoff or single nucleotide variants used in OTUs or ASVs approaches. From PAPRICA, a total of 648 phylogenetic edges were resolved. However, after removing edges with a count <10 and presence in <5% of the samples, the final dataset contained 188 edges.

Statistical Analysis

The sample size was estimated at 15 subjects per ethnic group, with a 3:1 ratio for healthy controls, based on a previous Asian mono-ethnic gut microbiota study amongst IBS subjects [22]. For clinical data analysis, continuous and categorical variables were compared between IBS subjects and healthy controls using the Mann-Whitney U test or Chi-square/ Mc Nemar test where appropriate. For dietary data, the three-day food records were analyzed using a diet analysis software program (Nutritionist Pro, version 7.4). For gut microbiota analysis, The final dataset was exported to the phyloseq R package for processing [23]. The alpha diversity was derived using Shannon, Simpson and Pielou's evenness indices while the beta diversity was evaluated using Aitchison's Distance. Community comparison was conducted using Permutational and Multivariate Analysis of Variance (PERMANOVA). Principal Component Analysis (PCA) was conducted using mixOmics R package [24] to visualise the distribution of

the data points. Differentially abundant taxa were identified with linear discriminant analysis (LDA) effect size (LEfSe) analysis using the MicrobiomeMarker R package. A taxon is considered significant if the LDA value is > 2 and the Kruskal-Wallis P value is < 0.05.

RESULTS

Baseline characteristics

Between October 2018 and June 2021, a total of 46 adult IBS patients were screened from the three institutions, five patients dropped out after the screening, eight failed screening due to SIBO positive, and 34/46 (73.9%) were finally recruited for the study (Figure 1). In addition, a further 15 age and gender matched healthy adults were recruited as controls. Table 1 highlights the demographic and clinical data of the IBS subjects. Essentially their mean age was 48.9 ± 17.2 years, 57.5 % were females and Malay ethnicity (58.8%) was predominant. Most patients had moderate IBS severity (mean IBSS score 209 ± 119), with a disease duration of approximately four years. The proportion of IBS sub-types was as follows: IBS-D 44.1%, IBS-C 32.4% and IBS-M 23.5%.

Prior to dietary intervention, comparisons of food components were made between the three ethnic groups. No significant differences in sugars, fats and carbohydrates consumption were observed between the three ethnic groups (Table 2). However, there was a greater consumption of proteins amongst ethnic Chinese, whilst the diet of ethnic Indians had a greater quantity of fibre compared with the other ethnic groups (Table 2).

Gut Microbial Composition Between Healthy Control and IBS patients

The phylum and genus distributions of the microbial composition in healthy controls and IBS patients were provided in Supplementary Figure 1. No significant difference in alpha diversity and beta diversity was detected between the two groups (Table 3, Supplementary Figure 2).

However, age and ethnicity were found to be significantly associated with microbial composition. Specifically, different ethnic groups harbour distinct microbial composition, while age is correlated with the centroid of the data clouds inferred from microbial abundance. Despite the lack of overall microbial differences, the IBS group was found to harbour a significantly high abundance of *Parabacteroides sp. CT06* while elevated bacterial fermenters such as *Succinivibrio dextrinosolvens* and *Intestinibaculum porci* as well as Short-chain fatty acids (SCFAs) producer *Faecalibacterium prausnitzii* were detected in the healthy cohort (Figure 2).

Clinical impact of the low FODMAP diet

Clinical data collection was complete for all 34 IBS patients, whilst complete dietary data were available in 29 subjects. At the end of the six week study period, the mean IBS-SSS reduced significantly from 209 ± 119 to 136 ± 95 (mean difference 73 ± 98, $p < 0.001$) (Figure 3). Correspondingly, the health-related quality of life of IBS subjects showed a significant increase during the same period (EQ 5D VAS pre-intervention=63.1 ± 22, post-intervention 73.6 ± 20.1, p=0.001). Based on a criteria of IBS-SSS score reduction, it was observed that 24 (70.6%) IBS patients (IBS-D n=8, IBS-C n=10, IBS-M n=7) had an improvement in symptoms, whilst 10 (29.4%) did not have any improvement in symptoms. Amongst the symptom domains of the IBS-SSS, the most remarkable improvements were in "frequency of abdominal pain" (69%), "abdominal distension" (58.6%) and "bowel habit satisfaction" (58.6%).

Based on the dietary diaries of 29 IBS subjects, trained dietitians calculated the changes in food groups and energy levels pre and post low FODMAP diet intervention (Table 4). Although there was an expected reduction in fructose, sucrose, maltose, etc, the mean total carbohydrate content was not significantly different at the end, suggesting poor adherence to the low FODMAP diet.

We subsequently defined poor adherence to the low FODMAP diet based as "no reduction of carbohydrate content" at the end of the study period. Based on this definition of poor adherence, we observed that 15/ 29 (51.7%) of IBS subjects had poor adherence to the low FODMAP diet. It was observed that symptom improvement was significantly lower amongst IBS subjects with poor adherence compared to those with good adherence to the low FODMAP diet (46.7% poor adherence vs 92.9% good adherence, p=0.014) (Figure 4).

Gut microbial composition before and after low FODMAP intervention, and between responders and non-responders

A total of 24 individuals provided complete pre- and post-intervention stool samples. However, no significant difference in alpha and beta diversity was observed before and after FODMAP intervention (P > 0.05) (Supplementary Table 1 and Supplementary Figure 3). Interestingly however, among the 24 individuals, the responders showed marginal but insignificant higher baseline alpha diversity (Figure 5). We followed-up with the LEfSe analysis to identify the taxa that were differentially abundant between the responders and non-responders. At baseline, the responders showed a higher level of bacteria from family Lachnospiraceae such as *Bifidobacterium longum*, *Anaerotignum propionicum* and *Blautia sp. SC05B48*. The higher prevalence of Lachnospiraceae persisted after the FODMAP intervention. In contrast, the non-responder exhibited a higher level of mainly bacteria from Alphaproteobacteria such as *Micavibrio aeruginosavorus* and *Fluviicola taffensis* after the intervention (Figure 6 and Figure 7).

DISCUSSION

This study amongst Asian IBS subjects has demonstrated several interesting observations relating to dietary behaviour and gut microbiota composition. Although no significant

difference in alpha and beta diversity was observed between IBS patients and healthy controls, IBS patients were found to have a greater abundance of *Parabacteroides species* compared to non-IBS adults. Furthermore, a greater level of bacterial fermenters and short-chain fatty acids producers, such as *Faecalibacterium prausnitzii* were detected in healthy controls compared to IBS patients. These observations are fairly consistent with the published literature. In a systematic review of gut microbiota studies in IBS, Pittayanon et al highlighted that almost 50% of published studies similarly reported no difference in alpha and beta diversity between IBS patients and healthy controls [2]. In the same systematic review of 24 studies, Bacteroides species were found to be more abundant in IBS patients, whilst Bifidobacterium genus and *Faecalibacterium prausnitzii* were decreased compared to healthy controls [2].

The observations in the first component of our study support the hypothesis that certain pathogenic bacterial species, such as Bacteroides species, may have a causal association with IBS, whilst a lack of more "protective" organisms like *Faecalibacterium prausnitzii* may predispose individuals to developing IBS [2]. However, differences in the gut microbiota may equally result from a variation in diet. In a recent exploratory study, Choi et al demonstrated an increased alpha diversity and abundance of Faecalibacterium following a high fibre diet for three weeks [25] In our study, we were able to demonstrate a higher intake of dietary fibre amongst ethnic Indians compared to ethnic Chinese and Malays. Age and ethnicity were found to be associated with gut microbiota composition, but the sample size was too small to specifically identify which ethnic group had a greater diversity of microbiota.

In the second component of our study, a low FODMAP diet intervention over six weeks resulted in improvement in symptom and quality of life in 70.6% of IBS subjects. We observed that poor adherence to the low FODMAP diet was prevalent amongst 51.7% of IBS subjects, despite regular monitoring by our dietitians. This poor adherence to the low FODMAP diet has been reported before in Asian IBS patients [26]. Adherence to the low FODMAP diet directly

influences its' efficacy on IBS symptom improvement [5], and this was clearly observed amongst our patients (92.9% vs 46.7% symptom improvement between those with good vs poor adherence). However, even amongst IBS patients with a reported poor adherence, 46.7% still had symptom improvement, suggesting that factors other than adherence may have influenced a dietary intervention response.

Several recent studies have explored the potential role of using gut microbial composition to predict the response of IBS patients to a low FODMAP diet. In an exploratory study, Valeur et al had reported that IBS patients with significant symptom response to a low FODMAP diet had a higher level of *Bacteroides fragilis*, *Acinetobacter*, *Ruminiclostridium*, *Streptococcus*, and *Eubacterium* [27] A recent systematic review and meta-analysis exploring gut microbial changes after a low FODMAP diet highlighted that five out of eight studies reported a microbial change, with predominant reductions in bifidobacteria and actinobacteria organisms [5]. In our study, we have identified a higher baseline alpha diversity of gut microbiota, with a greater abundance of *Bifidobacterium longum*, *Anaerotignum propionicum* and *Blautia species* at baseline amongst IBS patients who responded to a low FODMAP diet. Our findings appear to concur with other investigators who have suggested a distinct microbial pattern amongst IBS patients who respond to a low FODMAP diet.

This study was of an exploratory nature and had several limitations. The study sample size was comparable to other studies involving resource-demanding procedures such as dietary-counselling, stool collection and microbiota analyses. However, it was insufficient to explore gut microbial variation in detail between the three major ethnic groups. Dietary intervention was not conducted in a randomised controlled manner, but the clinical efficacy of a low FODMAP diet in IBS is well established [5] and was not the main objective of this study. From a dietary and gut microbiota perspective however, this study has several advantages. Firstly, it is the only multi-centre study exploring dietary and gut microbiota factors in IBS to date.

Secondly, ours is the only multi-ethnic study amongst IBS subjects. Dietary data were collected via detailed 3-day records and SIBO was excluded by hydrogen breath testing in all subjects.

In conclusion, this multi-centre, gut microbiota study has highlighted a greater abundance of *Parabacteroides species* with lower levels of bacterial fermenters and short-chain fatty acids producers, such as *Faecalibacterium prausnitzii,* amongst IBS patients compared with healthy controls. Following a low FODMAP dietary intervention, symptom and quality of life improvement were observed in 70.6% of IBS subjects. A distinct microbial pattern was observed amongst symptom responders, suggesting that gut microbiota patterns may be useful in determining response to a low FODMAP diet in IBS patients.

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STATEMENT OF INTEREST

Authors have no conflict of interest to disclose.

TABLES

Table 1. Demographics and clinical characteristics of recruited participants

Characteristics	Control, n=15	IBS Patients, n=34	p-value
Age (years)	41.5 ± 14.2	48.9 ± 17.2	.120
Gender (%)			
Male	6 (40.0)	14 (42.4)	.875
Female	9 (60.0)	19 (57.6)	
Race (%)			.608
Chinese	2 (13.3)	8 (23.5)	
Malay	11 (73.3)	20 (58.8)	
Indian	2 (13.3)	6 (17.6)	
Height (cm)	154.8 ± 25.3	160.4 ± 8.2	.411
Weight (kg)	75.2 ± 26.5	61.6 ± 12.4	.075
BMI (kg per m^2)	20.6 ± 11.7	23.9 ± 4.4	.306
Duration of Disease (year)	-	3.8 ± 4.1	
IBS-Type (%)			
IBS-D	-	15 (44.1)	
IBS-C	-	11 (32.4)	
IBS-M	-	8 (23.5)	

Table 2. Dietary differences among different races of IBS patients

Diet content	Mean ± SD value			p-value
	Chinese	Malay	Indian	
Protein (g)	75.1 ± 22.1	52.5 ± 14.8	51.5 ± 21.9	0.015
Carbohydrates (g)	181.3 ± 67.8	192.7 ± 50.3	170.5 ± 8.9	0.642
Fat (g)	55.6 ± 11.4	41.3 ± 17.6	48.2 ± 19.7	0.139
Dietary Fibre (g)	3.2 ± 1.4	2.3 ± .4	4.4 ± 1.7	0.013
Sugar (g)	22.5 ± 17.3	24.2 ± 22.5	21.3 ± 10.8	0.948

Table 3. PERMANOVA analysis on the microbial compositions

PERMANOVA			
Univariate	F model	R2	P-value
Gender	1030	0.834	0.838
Race	1590.4	1.308	**0.012**
BMI	1230	0.99	0.493
Age	1275	0.61	**0.025**
Group	1106	0.897	0.712
Multivariate	F model	R2	P-value
+Race	1.4	0.051	**0.006**
+Age	1.112	0.604	**0.008**
+Group	1.043	0.189	0.328

Note: F model = Total sum dissimilarity between groups, R2 = total explained variation.

Table 4 Dietary data Pre and Post diet (n=29)

	Pre-intervention	Post-intervention	P-value
Energy (Kcal)	1418.11 ± 338.62	1448.13 ± 363.75	0.650
Protein (g)	58.06 ± 20.93	59.06 ± 18.82	0.746
Carbohydrate (g)	191.27 ± 52.71	193.68 ± 53.83	0.785
Fat (g)	46.96 ± 18.24	47.60 ±16.02	0.864
Dietary Fibre (g)	2.98 ± 1.61	3.26 ± 2.64	0.588
Total Sugar (g)	25.04 ± 19.79	25.30 ± 15.21	0.935
Glucose (g)	1.32 ± 1.51	1.01 ± 1.89	0.413
Galactose (g)	0.01 ± 0.04	0.02 ± 0.05	0.575
Fructose (g)	2.27 ± 5.06	0.93 ± 1.75	0.184
Sucrose (g)	11.5 ± 14.88	9.34 ± 12.61	0.389
Lactose (g)	0.05 ± 0.18	0.29 ± 1.21	0.223
Maltose (g)	0.24 ± 0.06	0.08 ± 0.36	0.430

FIGURE LEGENDS

Figure 1. Flow chart of the study.

Figure 2. Differentially abundant taxa between healthy controls and IBS patients identified using LEfSe analysis. A) Barplot B) Cladogram.

Figure 3. Boxplot illustrating reduction in IBS-SSS after low FODMAP diet for all IBS patients

Figure 4. The association of IBS-SSS symptoms improvement and the adherence to low FODMAP diet in IBS patients performed using chi-squared tests

Figure 5. The alpha and beta diversity of the responders and non-responders. A) Aitchison-based PCA before intervention, B) Aitchison-based PCA after intervention, C) The comparison of alpha diversity indices (e.g. Shannon diversity, Pielou's evenness and Simpson diversity indices) between responders and non-responders.

Figure 6. Differentially abundant taxa between responders and non-responders identified using LEfSe analysis. A) Barplot before intervention, B) Cladogram before intervention, C) Barplot after intervention, D) Cladogram after intervention

Supplementary Figure 1. The phylum and genus distribution of the bacterial taxa in healthy controls and IBS patients

Supplementary Figure 2. The alpha and beta diversity of the healthy controls and IBS patients. A) Aitchison-based PCA overlaid with healthy controls and IBS patients, B) Aitchison-based PCA overlaid with "Race", C) The comparison of alpha diversity indices (e.g. Shannon diversity, Pielou's evenness and Simpson diversity indices) between healthy controls and IBS patients.

Supplementary Figure 3. The alpha and beta diversity before and after FODMAP intervention. A) Aitchison-based PCA of the faecal microbial composition pre- and post-

intervention, B) Paired Shannon Diversity Index, C) Paired Pielou's Evenness Index, D)

Paired Simpson Diversity Index