

The Relationships among Diet, Bacterial Taxa, Fecal Short Chain Fatty Acids (SCFA) and Symptoms among
Healthy Women and Women with Irritable Bowel Syndrome

Angelita G. Utleg

A dissertation

submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Washington

2024

Reading Committee:

Margaret Heitkemper, Chair

Hilaire Thompson

Robert Burr

Program Authorized to Offer Degree:

Nursing

©Copyright 2024
Angelita G. Utleg

University of Washington

Abstract

A Metabolomics-based Biomarker for Carbohydrate Intake: An Approach towards a Personalized Dietary Intervention for the Management of Irritable Bowel Syndrome Symptoms and Severity

Angelita G. Utleg

Chair of the Supervisory Committee

Margaret Heitkemper

Department of Biobehavioral Nursing & Health Informatics

Irritable bowel syndrome (IBS) is the most prevalent gastrointestinal (GI) disorder in western developed countries exerting a tremendous economic, social, and emotional burden. IBS, a disorder of gut-brain interaction (DGBI), characterized by abdominal pain and altered bowel habits, is one of the top 10 reasons for primary care consultations. IBS affects nearly 35 million Americans (10% to 15% adults), incurring \$20 billion in direct and indirect expenditures. Sex is a major risk factor for IBS; women experience more visceral pain and hypersensitivities than men. Two-thirds of the IBS population in the US are women, the majority in their reproductive years, and tend to report psychological comorbidities (anxiety and depression). Most of the IBS population seek multiple, conventional treatments to manage their IBS symptoms. There is increasing evidence for the effectiveness of a high fiber diet in reducing IBS symptoms, especially constipation. How a high dietary fiber (DF) intake affects IBS symptoms at a molecular level is not clear. Several studies have shown strong, empirical evidence that diet modifies the gut microbiome, however additional clinical research is warranted to confirm these laboratory findings. This study employed secondary analysis for the purpose of exploring the relationships between fecal short chain fatty acids (SCFA) and IBS clinical symptoms (i.e., constipation, diarrhea, abdominal pain), stool microbiome (based on 16S ribosomal DNA data), and DF intake, as well as in comparison group of healthy controls (HC). The results add to the evidence base for higher fiber intake as an effective

therapeutic intervention for the management of IBS symptoms, strengthen current evidence of the presence of gut dysbiosis in IBS, and could further advance nursing science and symptom management. The long-term goal of this line of research was to fill the gaps as described above. The specific aims of this research study included: Aim1) To compare fecal SCFA concentrations (millimolar) between IBS and HC groups. Aim 2) Separately within each IBS bowel pattern subgroup (IBS-C, IBS-D) and HC, determine the associations between (2a) SCFAs and GI symptoms, (2b) SCFAs (millimole) and DF (soluble and insoluble in grams) intake, (2c) DF (soluble and insoluble in grams) and GI symptoms (diarrhea, constipation, pain) and Aim 3) To explore the relationships of fecal SCFA metabolites with fecal bacteria taxa at the genus level in IBS bowel pattern subgroups (IBS-D and IBS-C) and HC groups. Hypotheses tested included: 1) IBS cohorts regardless of bowel group will have reduced fecal SCFA compared to HC (Aim 1); 2) lower total SCFA will be associated with higher abdominal pain severity (Aim 2a); 3) higher total SCFA will be associated with diarrhea, while lower total SCFA will be associated with constipation (Aim 2a). It was also expected that a DF intake above 25g/day will have higher SCFA fecal concentration as compared to <25g/day (Aim 2b). Also, a high and rapidly fermentable and soluble DF intake will be positively correlated with fecal SCFA concentrations and IBS abdominal pain severity (Aim 2c). Higher intake of insoluble and slowly fermentable DF intake will be associated with lower abdominal pain (Aim 2c). Aim 3 is exploratory.

Given the nature of this study, causality cannot be established; however, this study provides some degree of evidence that gut dysbiosis exists in women with IBS in this sample. Current knowledge claims that humans and the gut microbes are co-dependent with each other and alteration in gut microbiome has been associated with several inflammatory disorders including IBS. Confirmation of microbiome signature in IBS; however, is still ongoing and this observational study helps affirm some of these biological signatures. Specifically, this study found positive associations of high fecal butyrate concentration and reduced GI symptoms and non-GI symptoms in women with IBS. This finding is important because IBS is a gut-brain interaction disorder and currently, there is limited research in investigating the links of psychological markers and the gut microbiome in IBS. This study

supports the idea that gut commensals are indispensable to health and since diet modulates the gut microbiome, the development of evidence-based personalized dietary interventions is key in managing IBS symptoms. The generated fecal metabolomics data provided valuable information about mechanisms in which the microbiome influences clinical symptoms of IBS.

DEDICATION

To my mother, Magdalena, I love you with all my heart. Thank you for being there for me. To friends and family, thank you for your encouragement and support. To Blessed Sacrament Church, thank you for the safe space to write and inspiration to bear fruit. To my Blessed Mother, thank you for your spiritual love, prayers, and protection. To my King, Jesus Christ, glory and praise forever! "Man cannot be a measure of all things because there is a bigger Truth to which all things can be measured." I truly believe that God is the driver of all things. I thank God for giving me the courage, hope, and purpose to accept the many challenges that brought me to study nursing and the gut microbiome. I thank God for His blessings, love, and mercy.

ACKNOWLEDGEMENTS

I would like to thank the University of Washington, the Coast Salish Peoples of this land-- past, present, and future--the land which touches the shared waters of all tribes and bands within the Suquamish, Tulalip and Muckleshoot Nations. I am very grateful to my Advisor, Dr. Heitkemper, Chair of the Supervisory Committee, Professor, Biobehavioral Nursing and Health Informatics, Adjunct Professor, Division of Gastroenterology, School of Medicine, Affiliate Professor, Center for Ecogenetics and Environmental Health, Director for Center for Research on Management of Sleep Disturbances. I am also grateful to my Co-Advisor, Dr. Hilaire Thompson, Professor, Biobehavioral Nursing & Health Informatics, Associate Vice Provost for Academic Personnel, Office of Academic Personnel Co-Director, Interdisciplinary Nurse Scientist Training in Multilevel Approaches: Biology to Society (T32NR016913), Co-Director, ITHS TL1 Multidisciplinary Translational Research Training Program (TL1TR002318), Core Faculty, Harborview Injury Prevention and Research Center, Adjunct Professor, Biomedical Informatics and Medical Education, School of Medicine.

I would like to thank Ernie Tolentino for preparing the fecal samples. I am thankful for my advisors, Dr. Heitkemper & Dr. Thompson, Dr. Robert Burr, Dr. Kevin Cain, and the Office of Nursing Research team for their time and support for the F31 submission process. I am thankful for Dr. Gibbons and his colleague, Dr. Christian Diener at the Institute for Systems Biology for their time and introducing me with their SCFA metabolic flux computational platform, MICOM. I would like to thank Dr. Julia Cui and her Toxicology laboratory team, most especially Joe Lim for helping me learn bioinformatics/compositional pathway analysis for my independent research under the supervision of Dr. Cui. I would like to thank Dr. Nagana, Dr. Raftery, and Vadim Pascua at the UW Northwest Metabolomics Center. I would like to thank my GSR, Dr. Min Li. I would like to thank the Microbial Interactions & the Microbiome Center club and the UW Biostatistics Department. I would like to thank Betsy Mau for her support and the UW School of Nursing. The study was supported in part by the National Institute of Health NIH National Institute of Nursing Research (NINR), NIH, Grant Nos. 1R01NR014479, T32NR016913, F31 NR018794-01A1, King County Nurses Association Scholarship, and the Soule Chair in Nursing, RO-1 funding.

TABLE OF CONTENTS

LIST OF TABLES.....	x
LIST OF FIGURES.....	xi
LIST OF ABBREVIATIONS.....	xii
CHAPTER 1: BACKGROUND & LITERATURE REVIEW.....	1
I. Introduction	
1. Problem Statement	
2. Frameworks to inform symptoms management of IBS	
3. An ecological view of microbial resiliency to stress in IBS	
4. Gastrointestinal Tract	
5. Definition of Dietary Fiber (DF)	
a. Dietary Fiber Intake	
b. Physiological Properties of Fibers	
6. Gut Dysbiosis in the Context of IBS	
7. Dietary Fiber and Microbiota	
8. DF Improves GI Function	
9. IBS and Gut Microbiota	
10. Microbes and Metabolic Potential	
11. Short Chain Fatty Acids (SCFA)	
a. SCFA as primary colonic energy source	
b. SCFA Transport	
c. SCFA & Inflammation	
12. Diet Modulates the Microbiome	
13. SCFA & Inflammation	
CHAPTER 2: LITERATURE REVIEW ON NEXT GENERATION SEQUENCING METABOLOMICS.....	39
A. Scientific Approach to Study the Gut Microbiome	
a. Microbiomics: 16S ribosomal gene sequencing	
b. Metabolomics: Targeted vs. Non-targeted Metabolomics	
c. Fecal metabolome	

CHAPTER 3: METHODS.....	45
A. Study Design	
B. Study Population	
C. Clinical Measures	
a. 3-day Dietary Record	
b. Fecal Sample Collection	
D. MICOM/Flux Balance Analysis	
E. Compositional Analysis16S rDNA amplicon from MAP study	
F. SCFA Extraction Procedures/Metabolomics	
G. Data Analyses	
H. Statistical Power	
I. MetaboAnalyst 5.0	
CHAPTER 4 & CHAPTER 5: RESULTS & DISCUSSIONS	
A. AIM1	
a. Results.....	52
b. Discussion.....	62
B. AIM2a	
a. Results.....	84
b. Discussion.....	103
C. AIM2b	
a. Results.....	115
b. Discussion.....	124
D. AIM2c	
a. Results.....	131
b. Discussion.....	153
E. AIM3	
a. Results.....	162
b. Discussion.....	205
CHAPTER 6: IMPLICATION TO NURSING SCIENCE.....	218
REFERENCES.....	219
SUPPLEMENTAL.....	283

LIST OF TABLES

Introduction

Table 1: Four categories of DF according to solubility, viscosity, and fermentability

Table 2: Exemplars of Primary and Secondary Degradable Complex Carbohydrates and DF

AIM1

Table 1. Mean and Standard Deviation for the Absolute Concentration of Fecal SCFA (micromole)

Table 2. Ranges for the Measured Fecal SCFA (micromole)

Table 3. Raw p-value for the Individual SCFA (micromole)

Table 4. FDR Adjusted p-value for the Individual SCFA (micromole)

Table 5. Tukey Multiple Comparisons of Means Using ANOVA: 95% Family-Wise Confidence Level

Table 6. Proportions of SCFA (micromole)

Table 7. FDR adjusted p-value for the Proportions of SCFA derived from the Absolute Concentration

Table 8. Ranges for the Relative Concentration of SCFA Normalized with Fecal Dry Weight (FDW)

Table 9. Mean and Standard Deviation for the Relative Concentration of Fecal SCFA

Table 10. Raw p-values for the Individual Relative Concentration of FDW Normalized SCFA Comparisons

Table 11. FDR Adjusted p-value for the Relative Concentration of SCFA Comparisons

Table 12. Mean and Standard Deviation for the Relative Concentration of Other Metabolites

Table 13. FDR Adjusted p-value for the Relative Concentration of Other Metabolites Comparisons

Table 14. Tukey Multiple Comparisons of Means Using ANOVA: 95% Family-Wise Confidence Level

AIM2a

Table 1. Univariate Analysis of Metabolites (Absolute Concentration) and GI Symptoms in Healthy Control (HC) Women

Table 2. Adjusted p-value for Univariate Analysis of Metabolites and GI Symptoms in HC Women

Table3. Univariate Analysis of Metabolites (Absolute Concentration) and GI Symptoms in Women with IBS

Table4. Adjusted p-value for the Univariate Analysis of Metabolites and GI Symptoms in Women with IBS

Table5. Univariate Analysis of Metabolites (Absolute Concentration) and GI Symptoms in Women with IBS-C

Table6. Adjusted p-value for the Univariate Analysis of Metabolites and GI Symptoms in Women with IBS-C

Table 7. Univariate Analysis of Metabolites and GI Symptoms in Women with IBS-D

Table8. Adjusted p-value for the Univariate Analysis of Metabolites and GI Symptoms in Women with IBS-D

Table 9. Univariate Analysis of Metabolites and GI Symptoms in All Women (N=60)

Table 10. FDR-Adjusted p-value for All Analysis for AIM2a (N=60)

Table 11. GI Symptoms of High SCFA (Mean $\pm$ SD) Producers and Low SCFA Producers

Table 12. P-values Comparing GI Symptoms of High SCFA Producers and Low SCFA Producers

Table 13. Non-GI Symptoms Scores (Mean $\pm$ SD) of High SCFA Producers and Low SCFA Producers

Table 14. P-values Comparing non-GI Symptoms of High SCFA Producers and Low SCFA Producers

Table 15. GI symptoms of High Butyric Acid (Mean $\pm$ SD) Producers and Low Butyric Acid Producers

Table 16. P-value comparing GI symptoms of High Butyric Acid Producers and Low Butyric Acid Producers

Table 17. Baseline GI and non-GI Self-Reported Symptoms in Butyric Acid Producers

Table 18. P-value for Mean Differences in GI and Non-GI Symptoms in Butyric Acid Producers

AIM2b

Table 1. The Associations of Metabolites (Absolute Concentration) and Dietary Fiber (High vs. Low) in HC, IBS, and Subtypes of IBS

Table 2 The Associations of SCFA (Absolute Concentration) and Dietary Fiber in All Women

Table 3. The Associations of Metabolites (Absolute Concentration) and Dietary Fat (High vs. Low) in HC, IBS, and Subtypes of IBS

Table 4. The Associations of Metabolites (Absolute Concentration) and Dietary Fat Intake (High vs. Low) in All Women

Table 5. Adjusted P-Values for Metabolites Comparison under Dietary Fiber Intake (High vs. Low) in All Women

Table 6. Adjusted p-values for Metabolites under Dietary Fat intake (High vs. Low) in All Women

Table 7. Baseline Characteristics of Women (High SCFA Producers vs. Low SCFA Producers)

Table 8. P-values for Dietary Lactose & Fat Intake (High SCFA Producers vs. Low SCFA Producers)

AIM2c

Table 1. Education and Income for the 4 Groups.

Table 2. Baseline Characteristics of Fecal Donors: IBS (n=35) and HC (n=25)

Table 3. Comparison of 3-day Food Record Dietary intake in IBS and HC

Table 4. Baseline Characteristics and Dietary Nutrients Intake of HC and IBS Subtypes

Table 5 Comparison of 3-day Food Record Dietary intake in IBS-C, IBS-D, and HC Women

Table 6. Comparison of Diet Intake by Race, Education and Income on Dietary Intake in All Women (n=60)

Table 7. The Influence of Race, Education and Income on Dietary Intake in All Women

Table 8. The Influence of Race, Education, and Income on GI Symptoms in All Women

Table 9. The Influence of Race, Education, and Income on GI Symptoms in All Women

Table 10: Univariate Analysis between Diet and GI Symptoms in HC Women

Table 11: FDR Adjusted p-value for Diet and GI Symptoms in HC Women

Table 12: GI Symptoms under DF Intake (High vs Low) in HC Women

Table 13: Univariate Analysis between Diet and GI Symptoms in IBS-C Women

Table 14. FDR Adjusted P-value for Diet and GI Symptoms in IBS-C Women

Table 15. GI Symptoms under DF Intake (High vs Low) in IBS-C Women

Table 16. Univariate Analysis between Diet and GI Symptoms in IBS-D Women

Table 17. FDR Adjusted P-value for Diet and GI Symptoms in IBS-D Women

Table 18. GI Symptoms under DF Intake (High vs Low) in IBS-D Women

Table 19. Univariate Analysis between Diet and GI Symptoms in IBS Women

Table 20. FDR Adjusted P-value for Diet and GI Symptoms in IBS Women

Table 21. GI Symptoms under DF Intake (High vs Low) in IBS Women

Table 22. Univariate Analysis between Diet and GI Symptoms in All Women

Table 23. FDR Adjusted P-value for Diet and GI Symptoms in All Women

Table 24. GI Symptoms under DF Intake (High vs Low) in All Women

AIM3

Table 1. Ranges for Microbial Diversity indices and Other Microbial Measures

Table 2. Tukey Multiple Comparisons of Means for Microbial Indices Using ANOVA: 95% Family-Wise Confidence Level

Table 3. Bacteria Taxa Relative Abundances Derived from 16S Data from the MAP Study

Table 4. FDR Adjusted P-value for the 3 Groups Comparisons (Bacteria profile)

Table 5. Tukey Multiple Comparisons of Means Using ANOVA: 95% Family-Wise Confidence Level

LIST OF FIGURES

Introduction

Figure 1. Biopsychosocial Model of Irritable Bowel Syndrome

Figure 2. Personalized Dietary Intervention for the management of IBS symptoms

Figure 3. Theoretical Model of IBS symptom experience

Figure 4. Theoretical conceptual framework informing the relationships among dietary fiber intake, SCFA, gut microbial composition, and IBS symptoms

Figure 5. Theoretical framework for targeting the gut microbiome to alleviate the IBS symptom experience

Figure 6. The society to cell resilience framework (Szanton & Gill 2010)

Figure 7. Dietary reference intake for Fiber (Gropper & Smith, 2012 p. 120)

Figure 8. Different types of dietary fiber under the umbrella of carbohydrates

Figure 9. Fermentation of dietary fiber by the gut microbiome exert beneficial effect on gut function. High fiber intake helps normalize bowel patterns (Adapted from Cummings 1984).

Figure 10. SCFA metabolic pathways

AIM1

Figure 1a. Linear relationship of fecal dry weight (FDW) and measured fecal SCFA.

Figure 1b. Boxplot of fecal dry weight (FDW) in IBS and HC.

Figure 2. Metabolites in IBS vs HC women.

Figure 3a. Metabolites in IBS subtypes vs HC women.

Figure 3b. Metabolites in IBS subtypes vs HC women.

AIM2a

Figure 1. The relationship of butyric acid and abdominal pain.

Figure 2. The relationship of butyric acid with GI and non-GI symptoms.

Figure 3a-c. The relationship of total sc-SCFA, total bc-SCFA, and total SCFA with self-reported GI symptoms.

AIM2b

Figure 1. The relationship of dietary intake and SCFA

AIM2c

Figure 1: All Cohorts Boxplots: The Influence of Education on Dietary Intake

Figure 2. All Cohorts Boxplots: The Influence of Income on Dietary Intake

Figure 3. The dietary intake preferences by race in all women

Figure 4. All Cohorts: The Influence of Race on GI Symptoms from All Cohorts

Figure 5. The Influence of Education level on GI Symptoms from All Cohorts

Figure 6. The Influence of Income level on GI Symptoms from All Cohorts

Figure 7. The relationship of DF and GI symptoms.

Figure 8. The relationship of diet and GI symptoms.

AIM3

Figure 1. Two Clusters of SCFA Producers (High & Low)

Figure 2. Boxplots of SCFA-producing bacteria

Figure 3. Q-q plot of Two SCFA Producing Bacteria

Figure 4a. Boxplot of microbial alpha diversity indices for IBS and HC women.

Figure 4b. Histograms of microbial alpha diversity indices for IBS and HC women.

Figure 5a-c. Relative abundance of bacteria in HC vs. IBS

Figure 6. Relative abundance of bacteria in HC, IBS-C, and IBS-D

Figure 7a. The relationship of SCFA and age.

Figure 7b. The relationship of microbial diversity indices and age.

Figure 8. The relationship of butyric acid (absolute and relative concentration) and age.

Figure 9. The relationship of the absolute concentration of SCFA and weight.

Figure 10. Relationship of individual SCFA and microbial diversity indices.

Figure 11. Relationship of individual SCFA and specific SCFA-producing bacteria.

Figure 12a. Relationship of GI Symptom (percent days with constipation and abdominal pain) with bacteria

Figure 12b. Relationship of GI Symptom (percent days with constipation) and microbe.

Figure 12c. Relationship of GI symptom (abdominal pain, percent days diarrhea) and microbe.

Figure 13a. Linear relationship of metabolites (absolute concentration).

Figure 13b. Linear relationship of tryptophan and microbial tryptophan-metabolizers

Figure 13c. Linear relationship of tryptophan and abdominal pain in IBS (n=35).

Figure 14a-d. Relationship of microbiota genera and its metabolic function.

Figure 15. Relationship of two bacteria genera.

Figure 16a. Relationship of specific microbe and microbial diversity indices.

Figure 16b. Relationship of microbe diversity index and individual microbe

Figure 16c. Relationship of microbe diversity index and individual microbe

Figure 16d. Relationship of microbe diversity index and individual microbe

Figure 16e. Relationship of microbe diversity index and individual microbe

Figure 17. Relationship of caloric intake and SCFA.

Figure 18. Relationship of caloric intake and microbial diversity indices

Figure 19. Relationship of lactose intake and SCFA

Figure 20a. Relationship of microbial diversity index and dietary intake.

Figure 20b. Relationships of dietary fat and dietary fiber with total SCFA and microbe.

Figure 21. Relationship of carbohydrate -fermenting microbe and carbohydrate.

Figure 22a. Boxplots illustrating the Relative Abundance of Bacteria under high fiber diet (>25g/day)

Figure 22b. Boxplots illustrating the Relative Abundance of Bacteria under high fiber diet (>25g/day)

Figure 23. Relationship of the 7-SCFA Producers and Microbial Diversity Indices.

Figure 24. Relationship of the Total SCFA and Microbial Diversity Indices

Figure 25. Heatmap of the metabolites (relative abundance) identified via NMR

Figure 26. Principal component analysis from MetaboAnalyst 5.0 revealed separations of the metabolites (relative abundance) in the three groups of women.

Figure 27. Principal component analysis of HC vs. IBS women.

Figure 28. Metabolic biomarkers (absolute concentration) in IBS vs. HC women.

Figure 29. Principal component analysis of absolute concentration of metabolites.

Figure 30. Principal component analysis of metabolites (relative concentration) in IBS and HC women.

Succinic acid was found to be almost 3-fold higher in HC women compared to IBS.

Figure31. Principal component analysis from MetaboAnalyst 5.0 revealed separations of the metabolites (relative abundance) in the three groups of women.

Figure 32. The 10 most abundant taxa of the gut bacteria composition in IBS-S, IBS-D, and HC women.

Figure 33. Box plots of predicted SCFA metabolites in the context of a Western diet intake and providing that every person consumed the same nutrients.

LIST OF ABBREVIATIONS

NMR: nuclear magnetic resonance

DF: dietary fiber

ANS: autonomic nervous system

CNS: central nervous system

ENS: enteric nervous system

IBS: Irritable bowel syndrome

SCFA: short chain fatty acids

GI: gastrointestinal

HC: healthy controls

IBS-D: IBS-Diarrhea

IBS-C: IBS-Constipation

DGBI: disorders of gut-brain interaction

FGID: functional gastrointestinal disorder

HPA: hypothalamic-pituitary-adrenal axis

FMT: fecal microbiota transplantation

CBT: cognitive behavioral therapy

SCT: social cognitive theory

GHT: gut Hypnotherapy

5HT: 4-hydroxytryptamine or serotonin

FODMAP: fermentable oligosaccharides, disaccharides, monosaccharides and polyols

RET: rearranged during transfection kinase

PXR: pregnane X receptor

AHR: aryl hydrocarbon receptor

FXR: farnesoid X receptor

VDR: vitamin D receptor

PPAR: peroxisome proliferator activated receptor

CHAPTER 1. LITERATURE REVIEW

Problem Statement

Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal disorder (FGID) in western developed countries exerting a tremendous economic, social, and emotional burden (Heitkemper et al., 2002). All FGIDs are now called disorders of gut-brain interaction (DGBI) (Sperber et al., 2021). IBS is a complex, multifactorial, gastrointestinal (GI) disorder characterized with heterogeneous symptoms that include complex abdominal symptoms (i.e., abdominal pain, abdominal distension, altered GI motility and hypersensitivity) and altered bowel habits, as well as non-GI symptoms such as fibromyalgia, fatigue, lower back pain, pelvic pain, muscle pain, tension headache, sleep disturbance, depression, anxiety, stress-reaction disorders (Spiegel et al., 2008, 2011; Sperber & Dekel (2010); Choung et al., 2010; Donachie et al., 2017; Heitkemper et al., 2002, 2003, 2004; Tavel (2015); Simren et al., 2018). As cited by Heitkemper and colleagues (2002) persons with IBS oftentimes report low quality of life and reduced active daily living activities (Heitkemper et al., 2002).

IBS is a common DGBI with a global prevalence of 5-10% where roughly 70% of individuals with IBS are women (Donnachie et al., 2017; Windgassen et al., 2017, El-Salhy et al., 2017; Midenfjord et al., 2019; Barandouzi et al. 2021). Prevalence varies depending on the diagnostic criteria used to phenotype the disorder, study populations, data collection methodology, the feasibility to access healthcare services, differences in diet, and the presence of cultural sensitivity (Sperber et al., 2021; Kim & Kim 2018). A recent, multinational study conducted by groups of international investigators from 33 countries enrolled 73,076 participants and reported a global prevalence of IBS from 1.5 to 10.1% under Rome III, and 1.5% to 4.1 % under Rome IV criteria with USA having IBS rate of 5.3% under Rome IV criteria (Sperber et al., 2021). Sperber and colleagues also found that women had higher rates of IBS than men under either Rome III or Rome IV (Odd Ratio of 1.7; CI: 1.6-1.7). Currently IBS affects 25 to 45 million in the US (<https://aboutibs.org/what-is-ibs/facts-about-ibs/>) and nearly two billion people in the European Union (Harper et al., 2018). As cited by Kinsinger (2017), there is approximately 10-15% adults in the US have IBS, incurring

\$20 billion in direct and indirect expenditures (Kinsinger, 2017). IBS is one of the top 10 reasons for GI consultations in the US, incurring a total direct cost of more than \$6,000 per IBS patient per year (Buono et al., 2017). One study reported an annual national healthcare cost of 1.66 billion in the US (Staudacher et al., 2018).

Although a high proportion of patients with IBS report food as a primary trigger for their symptoms (Simren et al., 2001; Monsbakken et al., 2005; Halpert et al., 2007; Böhn et al., 2013; Hayes et al., 2014), the complex, multifactorial nature of IBS symptoms can be attributed to its physiological and psychological components that are often intertwined. Such physiological components include GI motor function dysregulation or GI motility disorder (Kellow & Phillips 1987; Chey et al., 2001), brain-gut interaction pathway alteration (Mayer & Tillisch (2011; Camilleri & Lorenzo 2012), altered neuro-endocrine pathways such as hypothalamic–pituitary–adrenal (HPA), (Chang et al., 2009); Kennedy et al., 2014), as well as 5-hydroxytryptamine (5HT) biosynthesis, distribution and signaling) (Faure et al., 2010; Mangel et al., 1999; Prather et al., 2000; Kilkens et al., 2004), alteration in autonomic nervous system (ANS) action (parasympathetic innervation) and/or altered ANS balance on GI systems (Jarrett et al., 2003), altered metabolic gene expression, altered immune-metabolic clock (Öhman & Simrén (2010), reduced gut integrity/increased gut permeability (Zhou et al., 2009), and baseline low-grade inflammation. Psychological factors include perceived or actual stress, emotional instability, childhood traumas and early life adversities; such factors may affect physiological stress response and the body's response to future stressors (e.g., response to infection later in life may not be optimal as a result of the earlier experience); this new state of responding to future stressors is driven by the new functioning of the central nervous system (CNS) and stressor-induced biological changes or epigenetics changes (White 2010).

Other pathophysiological components of IBS include alterations in the gut microbial composition and function (gut dysbiosis) that directly or indirectly influence the regulation of SCFA metabolism, bile acid metabolism, and tryptophan metabolism (Kassinen et al., 2007; Posserud et al., 2007). The gut microbiome (microbes and its metabolites) influences the nervous, endocrine, and immune systems. Investigators in the field have reported that SCFA has been found to bind to receptors in the CNS in animal models (Schroeder &

Bäckhed 2016) Genapathy et al., 2013). In fact, the G-protein coupled receptor, (GPCR) such as GPCR41 (now called FFAR3) is known to have higher affinity for butyrate and has been localized in human brain endothelial cells (Hoyles et al., 2018; O'Riordan et al., 2022). Moreover, studies in rats have shown that the 3 major SCFA can cross the blood brain barrier and human cerebrospinal fluid has been reported to contain 0-171 uM acetate, 0-5 uM propionate, and 0-2.8 uM for butyrate (cited by O'Riordan et al., 2022). As cited by O'Riordan and colleagues, the Human Metabolome Database also reported human brain tissue to contain butyrate (17 pmol/mg brain) and propionate (18.8 pmol/mg brain). The presence of increased expressions of GPCRs in the brain such as GPR109A, an anti-inflammatory receptor (also known as hydroxycarboxylic acid receptor 2 or niacin receptor1) preferentially binds to beta-hydroxybutyrate, but also bind to vitamin B3 or nicotinic acid/niacin (Wakade et al., 2014). Thus, the importance of SCFA in modulating brain function either directly or indirectly (via regulating 5-HT release) holds important clinical implication in managing IBS symptoms. Evidence exist that support SCFA (especially butyrate) in regulating serotonin release (Reigstad et al., 2015; Fukumoto et al. 2003; Yano et al., 2015; Lukić et al., 2022) and thereby its end product--melatonin (Baumann et al., 2020; Jin et al., 2016; Anderson et al., 2019; Ahmadi et al., 2024) including the release of dopamine (Laurent et.al; 2013). Thus, dysregulation of SCFA metabolism can result in altered SCFA signaling pathways in CNS, in ENS, and in epithelial cells (mediated by 5-HT signaling pathways both in neuronal and GI mucosal lumen) can exacerbate the attributes of DGBI.

Non-diagnostic symptoms of IBS that are commonly reported include: heartburn, dyspepsia, early satiety, difficulty swallowing, postprandial fullness, incomplete evacuation, nausea, and bloating. Many of these are reported at higher rates in women as compared to men (Kinsinger, 2017; Elsenbruch & Enck 2017(Simren et al., 2017; Drossman et al., 2016.). Many patients with reported moderate to severe IBS symptoms have comorbid psychological factors (e.g., stress, depression and anxiety symptoms) (Kinsinger, 2017; Elsenbruch et al., 2017; Stasi et al., 2013; Donnachie, 2017). Investigators in United Kingdom reported that 44% of IBS patients (n =107) have mental health disorders of which 23% reported some form of adult or childhood abuse (Guthrie et al., 2003). Researchers in Sweden reported a high GI and non-GI symptom burden (i.e., anxiety, depression, fatigue, visceral hypersensitivity) and low quality of life in IBS patients with

psychological distress where 45% of IBS patients have anxiety disorder (n=769) while roughly 26% have depression (Midenfjord et al., 2019). A systematic review and meta-analysis reported that 38% of IBS patients (~n= 64,000) have self-reported sleep disturbances (i.e., sleep fragmentation, poor sleep quality, reduced sleep duration) (Wang & Duan 2018; cited by Yan et al., 2021). A recent study conducted by Ballou and colleagues (2018) compared quality of sleep in several types of GI disorders (i.e., celiac disease (n=40), GERD (n=29), IBD (n=44), IBS (n=48), functional dyspepsia (n=10). They found that persons with IBS had the highest frequency of reported poor sleep quality (72%) compared to healthy controls (HC) (n=41, 39%) or the rest of the GI disorders (54% to 61%) and had the highest frequency of reported insomnia compared to HC (18%) and the rest of the GI disorders (27% to 37%). The IBS group also reported the highest percentage (40%) of sleep medication use compared to HC (15%) and to other GI disorders (13%-32%). Most importantly, Ballou et al. (2018) reported IBS group had the highest rate of circadian shifts and symptom severity. It is now universally accepted that the gut microbiome impacts our mental health in terms of moods, thinking process, and memory retention (Jenkins et al., 2016; ICox & Weiner 2018)

The disruption of biological, internal time keeping system that is established to detect environmental changes (i.e., high fat diet feeding times, sleep-wake cycle disruption as in jetlag or exposure to constant light, high gut pH, high body temperature), can alter gene expression pattern of the host and alter the gut microbial composition and their activities (Liang & Fitzgerald 2017). The gut microbiome has been reported to display circadian oscillation (i.e., feeding-fasting cycle, bacterial migration or niche localization, timely expression of genes involved in the production of gut hormones including the metabolic genes for degrading fiber), hence it can be inferred that bacterial composition, abundances, and function varies during different times of day, an adaptation to maximize the use of resources (Zhang, Sehgal, et al., 2023; Frazier & Chang 2021).

Furthermore, literature reports that SCFAs such as butyrate modulate the expression of the host's circadian clock genes expressed in the brain and in peripheral tissues (Tahara et al., 2018), influence sleep duration and body composition (Matenchuk et al., 2020;). Hence, the gut microbiome provides stability (synchronicity of gut-brain rhythmic processes) at times of rapid environmental changes (Zhang, Sehgal et al., 2023); other microbial circadian rhythm mediators that can influence the host's own circadian rhythm include tryptophan,

bile acids, and hydrogen sulfide (Liang & Fitzgerald 2017; Zhang, Sehgal et al., 2023) regulating the host's oscillating transcriptome and epigenome (Liang & Fitzgerald 2017). In turn, the host's metabolism (i.e., antimicrobial peptide, glucocorticoid hormones, mucus secretion, immune cells secretory molecules) can also influence the gut microbiome circadian rhythm, regulating its feeding activities and metabolic gene expressions (Frazier & Chang 2021; Liang & Fitzgerald 2017). The master clock that resides in the suprachiasmatic nucleus of the hypothalamus is innate and intrinsic and should function even without the presence of light (Adlanmerini & Lazar 2023). The master clock needs to work in concert with the peripheral clocks (immune cells, adipocytes, gut, liver, heart, kidney, retina, muscles) to regulate the oscillating behavior of the gut microbiome and the host. Therefore, the bidirectional relationship of the host and gut microbiome governs the temporal concentration of microbial metabolites that critically impact the host's biological and physiological processes.

Gender (being female) is a major risk factor for the development of IBS (Heitkemper & Chang 2009; Zia & Heitkemper, 2022); women experience more visceral pain and hypersensitivities than men; this may be due in part to specific stress response mechanism involving the hypothalamic-pituitary-adrenal axis (HPA), the autonomic nervous system (ANS) tone, and the role of gonadal hormones. Two-thirds of the IBS population in the US are women and majority of them are in their reproductive years, many have comorbid conditions such as anxiety and depression. Most of the IBS population seek multiple, conventional treatments to manage their IBS symptoms and more women reported increased used of healthcare services than men, therefore, targeting this population is key to reducing healthcare cost.

There is no consistent biomarker for IBS (Boeckxstaens et al., 2016). As such, IBS is diagnosed using a symptom-based approach with specific criteria known as the ROME criteria (Drossman et.al, 2018; Simren et al., 2017). These symptom-based criteria were developed by an international group of experts specializing in gastroenterology. The first set of ROME criteria was released in 1989 and the most current version (ROME IV criteria) was published in 2016. The ROME IV criteria requires that a person report recurrent abdominal pain with altered bowel habits on average of at least one day a week in the last three months to be diagnosed with

IBS (Simren et. al, 2017). According to Rome IV criteria, IBS is classified as a chronic Disorder of Gut-Brain Interaction (DGBI) (Schmulson & Drossman 2017).

Although all patients with IBS experience abdominal pain, IBS is further classified into four sub-categories based on stool frequency and consistency as defined using the Bristol Stool Form (BSF) scale: IBS-Diarrhea (IBS-D), IBS-Constipation (IBS-C), IBS-Mixed (IBS-M), IBS-Unclassified (IBS-U) (Simren, et al., 2017). IBS-Diarrhea subtype is the most predominant subtype with a prevalence of 40-60% incidence in women (Donnachie et al., 2017; Windgassen et al., 2017). However, Sperber and colleagues reported a higher global distribution of IBS-C than the IBS-D subtype (Sperber et al., 2021).

Currently, there is no single treatment that is effective in treating all IBS symptoms. Due to its complex and multi-factorial pathophysiology, IBS symptom management includes both pharmacological and non-pharmacological therapeutic interventions. Therapeutic modalities available for IBS include fecal microbiota transplantation (FMT); cognitive behavioral therapy (CBT); hypnotherapy; multicomponent psychological therapy--via internet or telephone and psychotherapy delivered face-to-face; psychologically based guided self-help (GSH) programs with the use of websites, workbooks, and computer programs; psychological medications (SSRI, TCA) and GI medications (i.e., alosetron, tegaserod) and other exercise-based relaxation techniques. The principles of psychological interventions stem from Bandura's Social Learning Theory, currently called the Social Cognitive Theory (SCT). The cognitive behavioral framework assumes that an individual's emotions and behaviors affect his/her IBS symptoms perceptions and appraisals. Maladaptive cognitions/central emotional processing can be targeted by psychological therapies such as CBT, Gut Hypnotherapy (GHT) and other relaxation techniques to reduce the psychological factors (catastrophizing tendency, anxiety and depression, somatizations, fatigue) associated with IBS. Literature have reported the short-term effectiveness of CBT and GHT in targeting the HPA-axis and CNS processing most especially in women with IBS who are found to report more visceral pain and hypersensitivities along with other somatic symptoms than men with IBS because sex differences exist in ANS regulation of the GI tract (Lackner et al., 2008; Ballou & Keefer, L. 2017, Heitkemper et al., 2002). GHT has been proven to be very effective (54-84%) in targeting the altered brain gut interaction component and its associated somatic comorbidities including symptoms of increased GI motility

and hypersensitivity. Although these studies were not sufficiently powered, a high successful rate of 84% (up to 95% for those under the age of 50, mostly women) was reported for severe, refractory IBS (Gonsalkorale et al., 2003; Whorwell et al., 1984, 1987; Schaefer et al., 2014). However, a systematic review (SR) that included 7 RCTS (N=374 patients) on the effectiveness of GHT in the treatment of IBS noted that these findings were fraught with considerable bias and its long-term effectiveness is still lacking (Lee et al., 2014). However, the most recent SR review that included 41 RCTs (4072 participants) reported the long-term effectiveness of CBT & GHT on IBS symptoms but also fraught with high risk of bias for all studies (Black et al., (2020). Furthermore, therapies such as in-person CBT may not be available. In recent decades several IBS drugs that affect motility have been removed from the market by the FDA due to adverse effects.

While these conventional medical treatments and stress relaxation techniques can be 50-60% effective (Heitkemper et al., 2002; Ford et al., 2018; Black et al., 2020), these interventions are expensive and had adverse side effects (cardiovascular ischemic events for alosetron and ischemic colitis for Tegaserod as listed under by the drug name FDA labeling). None are universally effective for the bowel and pain symptoms associated with IBS. Although existing evidence have cited serotonergic drugs such as alosetron (5-HT₃ antagonist) (Mangel & Northcutt 1999; Gershon 2013) to be effective in the treatment of IBS-D, while tegaserod (5-HT₄ agonist/5-HT_{2B} antagonist) is effective for the IBS-C phenotype; however, these drugs are under intense FDA regulation because of major side effects listed on the FDA labeling information. Biosynthesis of serotonin both occurs in neuronal cells of the CNS and ENS, as well as in the gut mucosa enterochromaffin cells (also called enteroendocrine cells) where most of the body's serotonin (~95%) is produced (Gershon & Tack, 2007). Serotonin receptors such as 5-HT₃ receptors are localized in the GI mucosa while the 5-HT₄ receptors are localized in the myenteric plexus (Gershon, 2004). Serotonin is known to stimulate neuronal and bone growth, and thus clinical providers must be cautious in prescribing these drugs (Sanger et al. 2008; Gershon 2013). Tegaserod was withdrawn from the market due to reported ischemic cardiovascular events from 13 patients out of 11,600 treated patients (Sanger 2008), but currently placed back in the market. The use of a non-absorbable antibiotic such as rifaximin to control overgrowth of commensal bacteria is chosen by most clinicians because it is well-tolerated and it is rarely caused C-difficile-associated

colitis, hence has a better safety profile than most antibiotics. As an environmental consequence, excessive use of antibiotics promotes the growth of *C. difficile* over the growth of other bacterial commensals (Karupiah & Pomianowski, (2017). Rifaximin is more suited for IBS-D patients and although it has been reported to change the microbiota structure, it has also been documented to improve IBS symptoms (Lembo et al., 2016). To date, there are newer drugs on the market available to treat IBS symptoms. For example, *tenapanor* and *plecanatide* are being tailored for IBS-C phenotype, while the other drug, *nerva* is tailored to target the dysfunctional brain-gut communication aspect of IBS. In addition, RET kinase inhibitors (target nicotinic cholinergic neurons) have been suggested to treat IBS-D phenotype (Schenck et al., 2018; Russell et al., 2019). RET stands for rearranged during transfection kinase, a neuronal growth receptor tyrosine kinase (Schenck et al., 2018).

The lack of success of most pharmacologic therapies has encouraged clinicians and patients to look for other approaches to reduce symptoms. To find other approaches for symptom management, the gut microbiome was targeted as the mechanistic link between diet and IBS symptoms (El-Salhy et al., 2017). The use of dietary interventions (psyllium fiber, prebiotics, probiotics, and low-fermented oligosaccharides disaccharides monosaccharides and polyols (FODMAP) or low-FODMAPs diet) have shown promising results in managing the IBS symptom burden (Schumann et al., 201; Halmos et al., 2014; Böhn et al., 2015; Staudacher et al., 2014; Staudacher et al., 2017; Van Lanen et al., 2021; Zhang & Duan et al., 2022; McFarland & Karakan, 2021; Ford et al., 2017). There are several probiotics that have been proven to be very successful in lowering IBS symptoms; *Bifidobacteria* has been found to be inversely related to abdominal pain (Jalanka-Tuovinen et al., 2011). Many persons with IBS believe that they may be sensitive to gluten and thus avoid foods with high gluten content and may also avoid lactose-containing products to reduce their debilitating self-reported GI symptoms. At the same time, intake of dietary fiber has been encouraged and while its effect on constipation is well known, less is known about the relationship of dietary fiber with gut microbiota characteristics such as their metabolites.

The current study was designed to test for differences in fiber intake between individuals with IBS and healthy controls and to look at the microbiome characteristics with a particular focus on SCFA.

Frameworks to inform symptoms management of IBS

IBS was first conceptualized with biopsychosocial model that includes the alteration of the gut-brain-microbiota axis and neuro-endocrine-immune circuits due to changes in internal (i.e., genetics, circadian

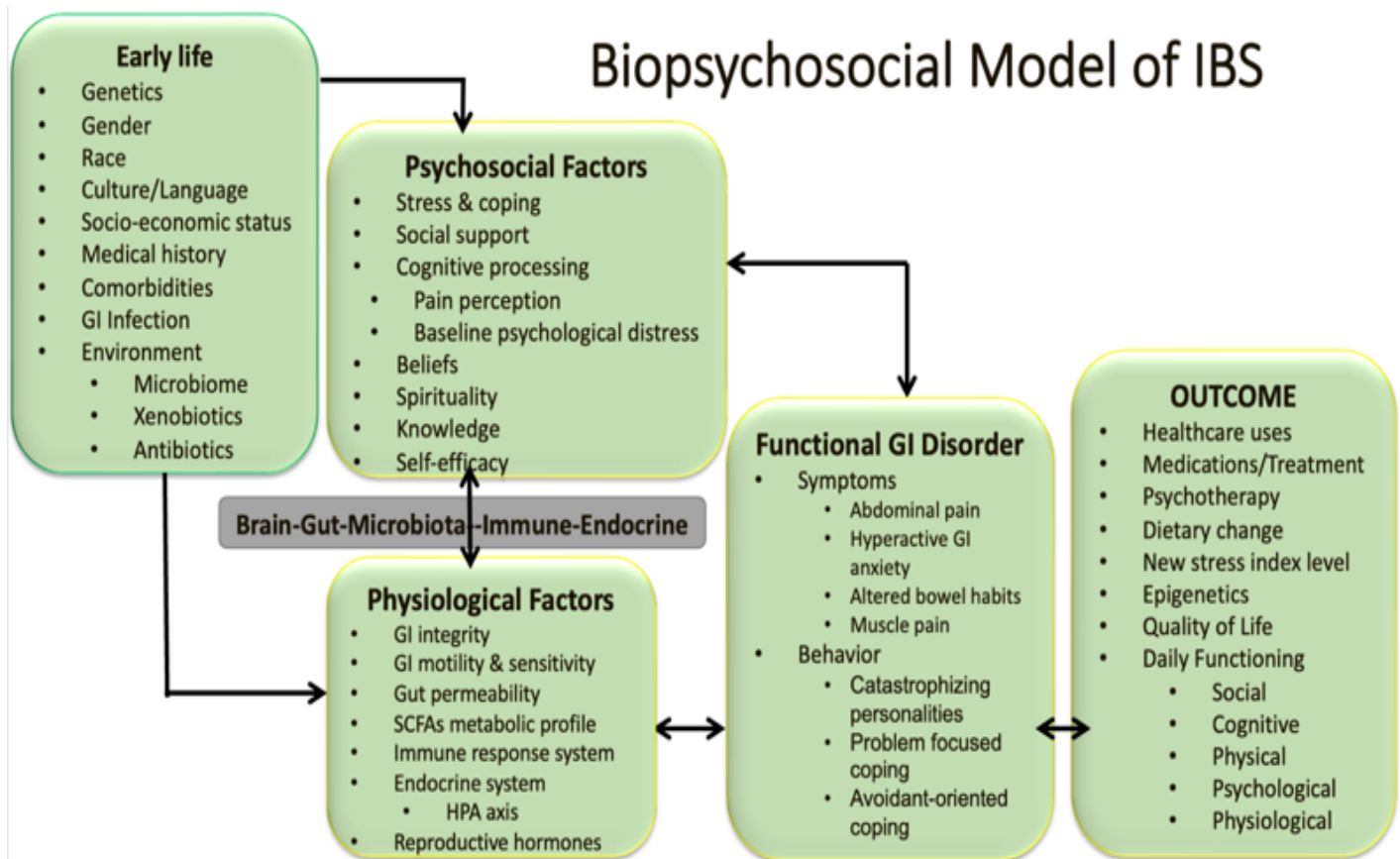


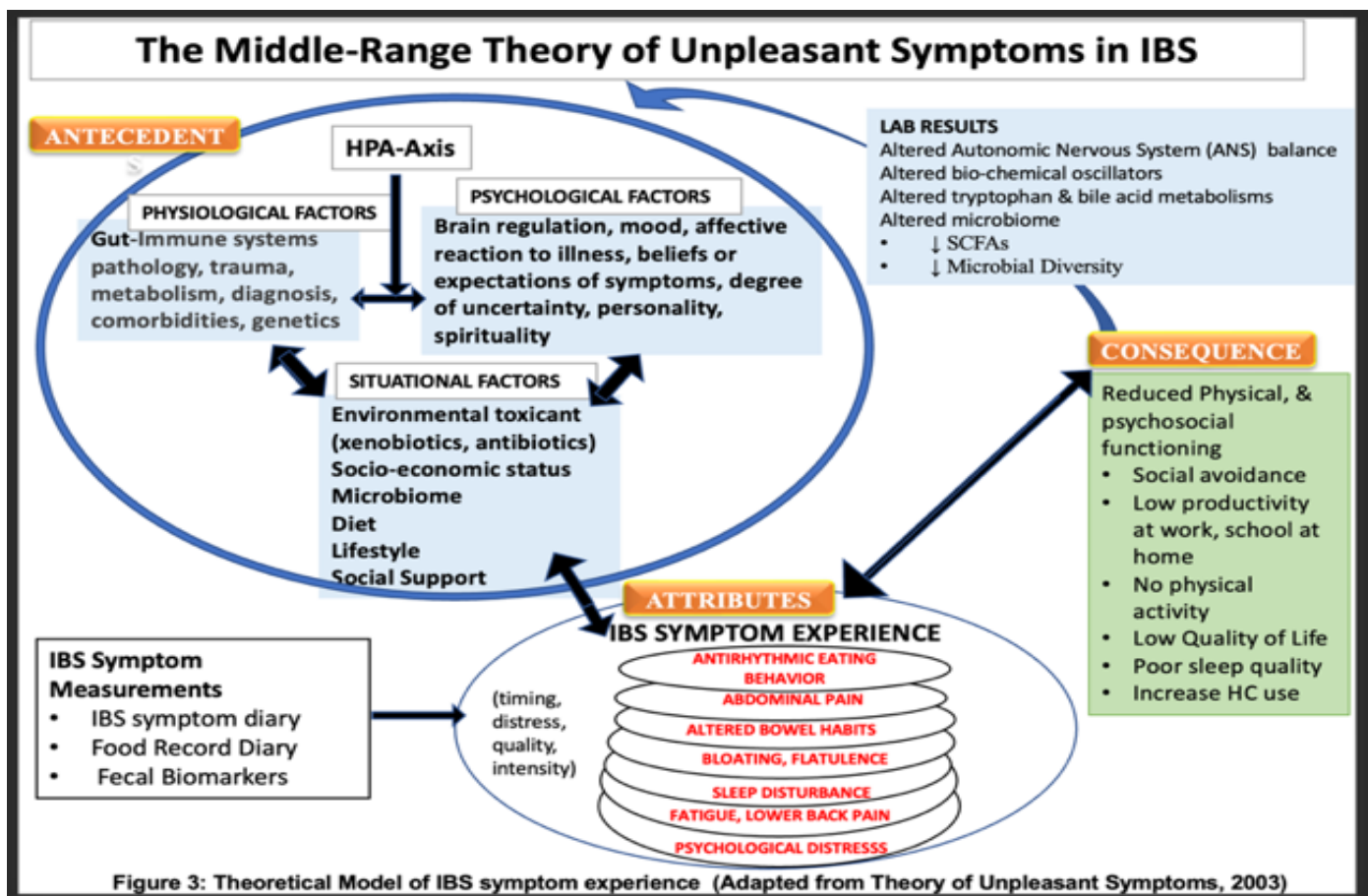
Figure 1. Biopsychosocial Model of Irritable Bowel Syndrome (Adapted from Tanaka et al, 2011; Drossman & Thompson 1992).

clocks, aging) and external factors (i.e., environmental stressors) as illustrated in

Figure 2. Adapted from NIH Symptom Science Model (NIH-SSM)

Figure 1. The Biopsychosocial Framework was originally designed by Engel in 1981 and adapted by Drossman and Thompson in 1992, modified by Levy et al., in 2006 and later Tanaka et al. in 2011. The model as depicted in Figure 1, incorporates biological biomarkers as well as psychosocial factors (early childhood adverse events, coping, social supports). Recent studies implicate the gut microbiome in IBS symptom generation. The microbiome is composed of microbiota and its metabolic byproducts. The microbiome can serve as the bidirectional bridge or the “communication pathway” that connects the brain and the gut,

continuously influencing both the host's physiological and psychosocial responses in the presence of external or environmental factors (i.e., stress, xenobiotics, food) throughout life. It is also worth noting that the host's biological master clock resides in the brain suprachiasmatic nucleus (SCN) of the hypothalamus and interacts with the gut microbiome (Sato et al., 2004; Mukherji et al. 2013; Lopez et al., 2021; Adlanmerini & Lazar 2023). The microbiota can interact directly with the CNS via the vagus nerve or indirectly with their metabolic products (i.e., neuropeptides, hormones, SCFA). The concept of severity in IBS is defined by Drossman as a "biopsychosocial composite of patient-reported GI and extraintestinal symptoms," and posits that the CNS provides a greater contribution than the visceral counterpart of the GI system (Drossman et. all, 2011).). The current study approach was also informed by the NIH-Symptom Science Model as shown in Figure 2. (Cashion et al., 2016).

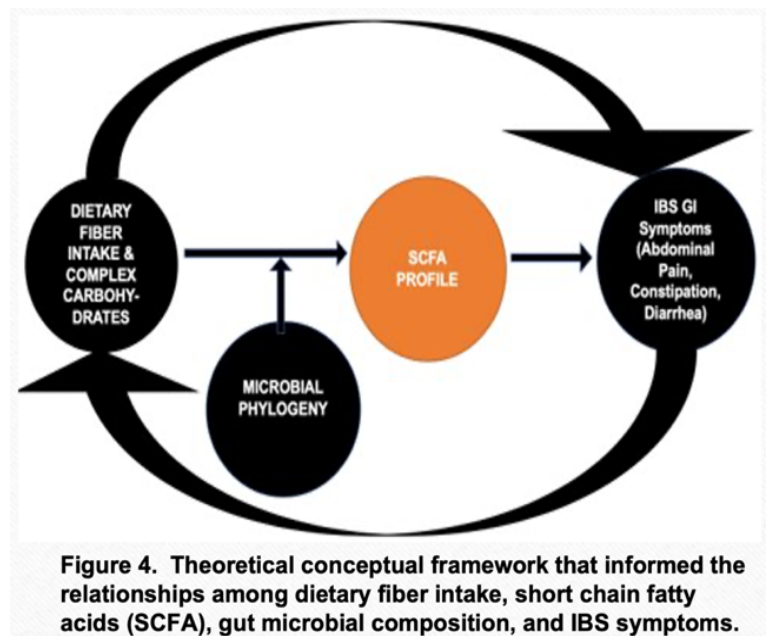


The middle-range theory of unpleasant symptoms in persons with IBS (Figure 3) provides a framework for explaining/contextualizing the IBS symptom experience. The bidirectional interaction between the gut microbiota and the brain are linked via the psychological and physiological factors that are strongly influenced by the HPA axis (Vodička et al., 2018). Animal studies have demonstrated that the gut microbiota is required for the development of the HPA axis (Sudo et al 2004; Moya-Pérez et al., 2017). There is mounting evidence about the role of the HPA axis in the stress response of persons with IBS and the influence of psychosocial factors that exacerbate this pathway resulting in reduced psychological threshold level in persons with IBS (Chang, 2011). Baseline characteristics that determine vulnerability to stress in patients with IBS include genetics responsible for proper regulation of the HPA pathway during a challenging situation, proper functioning of the brain-gut communication pathways (i.e., the ANS, the enteric nervous system (ENS), the neuroendocrine, and the immune system), as well as gonadal hormones. Under chronic stress, these biological pathways are altered and contribute to pathobiology of IBS. Constant stimulation of the HPA axis due to life stressors (physiological and/or psychological) can exacerbate GI symptoms in IBS (FitzGerald et al., (2009). In mammals, the HPA axis responds to stress by releasing corticotropin-releasing hormone (CRH) from the hypothalamus, which in turn triggers the release of ACTH from the anterior pituitary gland driving the release of catecholamines from the adrenal medulla and cortisol from the adrenal cortex (Thau et al., 2019; Fitzgerald et al., 2009). Fitzgerald and colleagues (2009) have shown evidence of HPA dysregulation in IBS; these investigators found blunted ACTH and cortisol responses to a protocol stressor (lumbar puncture). It is known that cortisol increases gut permeability with a concomitant increase in the release of proinflammatory cytokines (TNF- α , IL1 β , IL6), hence, the HPA axis stress response has the potential to alter the gut microbiota landscape (cited by Evans et.al, 2013). It has been shown that the stress response of the HPA axis and leakiness of the gut epithelial membrane can be attenuated through the administration of probiotics (Ait-Belgnaoui et. al 2012). In pre-clinical and clinical studies in patients with psychiatric and neurological conditions, there is evidence that having an unstable gut microbiota can alter brain-gut-axis (BGA) communication and that supplementation with probiotics can improve memory function and slow down

neurodegeneration (Mitra et al. 2023; Kesika et al, 2021). The gut bacteria through their metabolites use the vagus nerve as the major communication pathway to influence the person's neurochemistry and psycho-social behavior (Heijtz et al., 2011). SCFA are one group of metabolites that participate in this communication. (Filosa et al., 2018).

The key processes or pathways (mutable causes) to target in creating a change in IBS outcomes are categorized as either psychological or situational factors. The baseline characteristics listed under the physiological factors (non-mutable causes) are hard to change. Both mutable and non-mutable causes are predictors/moderators for IBS symptoms. Of interest to the current project is the mutable factor dietary fiber (DF) intake.

Figure 4 illustrates the positive clinical implication of increased complex carbohydrates intake in modulating the gut microbiome and subsequently positively impact the IBS symptom experience. The gut bacteria relative abundance



was chosen to act as predictor of IBS GI symptoms because certain bacteria are needed to specifically ferment DF from the diet to produce SCFA. SCFA can act directly and indirectly with the Brain-Gut-Immune system to impact the IBS symptom experience. Hence these metabolites serve as mechanisms in explaining the benefits of high DF intake on the health of a person with IBS. Dietary intervention may prove to be the best therapeutic strategy for alleviating the IBS symptoms. Several clinical trials have found that increasing DF intake reduces global IBS symptoms (Romano et al., 2013; Kamiya et al., 2014), reduces mean transit time (Romano et al., 2013; Polymeros et al., 2014; Prior, A., & Whorwell, 1987; Cann et al., 1984; Arffmann et al., 1985), and reduces abdominal pain (Romano et al., 2013; Rees et al., 2005; Manning et al., 1977). The production of SCFA depends on the abundance and diversity of gut bacteria, the substrates source and gut transit time (Wong et al., 2006). High DF can slow transit time and improve bowel patterns (both constipation and

diarrhea) in persons with IBS (Manning et al.,1997), as well as accelerate slow transit time in persons with non-specific diarrhea (Harvey et al., 1973) and in healthy adults (Payler et al., 1975). A moderately powered RCT, double-blind study demonstrated that high DF intake reduces mean transit time (MTT) in adults with IBS-C and normalized bowel habits in children with IBS (Prior, A., & Whorwell, 1987). MTT is highly correlated with fecal form (Davies et.al, 1986). Slowly fermented, long-chain fibers such as partially hydrolyzed guar gum (PHGG) improve bowel habits and reduce abdominal pain in adults (N=188; 70% women) with IBS (Parisi et al., 2002)

Figure 5 illustrates the 5 major domains (i.e., Brain-gut axis, altered bowel habits, GI hypersensitivity and gut dysbiosis) of IBS symptom experience that can be altered by DF modification intervention.

An ecological view of microbial resiliency to stress in IBS

Understanding the factors that contribute to impaired Brain-Gut-Microbiome axis (BGMA) functioning can add insights about the mechanisms underlying IBS pathology and treatment. The concept of gut dysbiosis can be viewed and analyzed from the perspective of a human health ecology framework. Environmental stimuli such as diet and environmental toxins associated with the “chosen diet” can change health-disease status within an individual’s lifetime that can be passed on to future generations via the process of environmental epigenetics (Guthman & Mansfield, 2013). Such “chosen diet” can perturb the individual’s gut microbiome signatures along with the biochemical pathways that it regulates, hence influencing the human’s biological cells, genes, and the host’s metabolome. Changes to the gut microbiome can occur rapidly depending on the type of interactions that the host (the individual) immune system exerts on its own resident—the gut bacteria. Such alterations of the gut bacteria diversity (composition and abundance) can have profound impact on the host physical and psycho-social functioning. To understand the mechanisms to

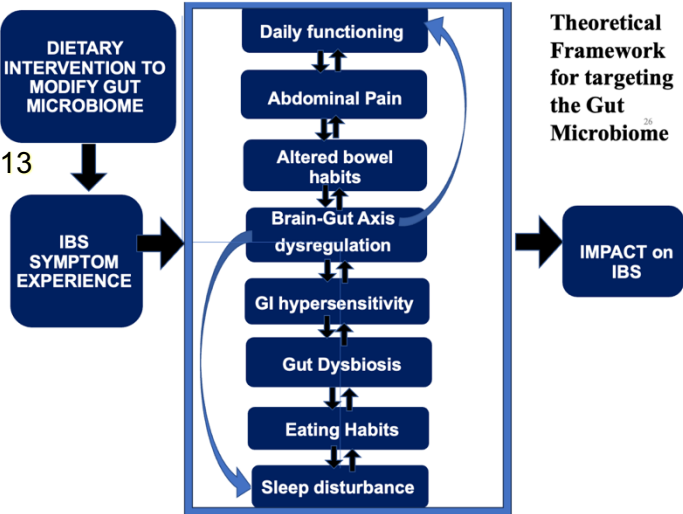


Figure 5. Theoretical framework for targeting the gut microbiome to alleviate the IBS symptom experience

which the microbiome impacts the host's life performance, it is important to apply the Society-to-Cell (STC) or (Cell to Society) framework for an ecological view of the processes that lead to gut dysbiosis. As illustrated in Figure 6, the STC is multilevel socio-ecological framework will be used to understand the factors that influence a person's behavior observed at six different levels of interactions (Szanton & Gill 2010). The individual is an

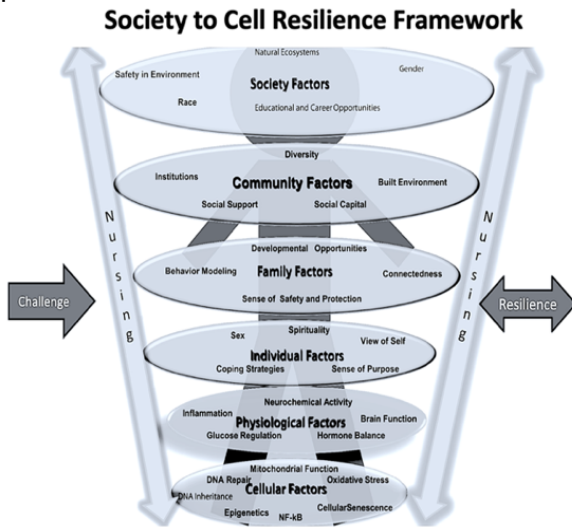


Figure 6. The society to cell resilience framework (Szanton & Gill 2010)

comprised of interacting physiological networks (i.e., the ENS, vagal nerves, endocrine systems, and the circulatory systems) including the digesta that is made of digested and undigested dietary components.

Studying the interactions of these gut microbes with their community and its ecosystem will help elucidate important mechanisms underlying the pathological behavior within a “community” or the gut microbes themselves.

The stability of the gut microbiota depends on the type of interactions with its own niche; negative interaction can lead to negative adaptation and beneficial bacteria may be eliminated while the harmful bacteria are selected to survive; hence gut dysbiosis ensues. Although gut dysbiosis can be explained at any level, the three most important levels of interactions start at the cellular level, then to the physiological level, and then to individual level. Higher evidence-based tools exist to measure gut dysbiosis operating at these levels. Such tools include the next generation sequencing to identify key players of the microbial community imbalance and the application of "omics" technology in identifying biomarkers as evidence of gut dysbiosis (metabolomics, transcriptomics, genomics). Other physiological tools that support gut dysbiosis is the application of gut permeability measurement technique (i.e., lactulose-mannitol test) used to measure

absorption rates and urinary excretion. Also, the use of Smart-Pill motility testing system can yield measurements of colonic transit times, colonic pH, colonic pressure, and temperature to describe GI motility status. Lastly, the trans-epithelial electrical resistance measurement (TEER) can be used to test the integrity of the gut measures the net flux of all ions across the epithelium and reflects the contribution of the paracellular resistance that reflects the resistance of tight junctions, and the transcellular resistance that reflects the resistance to ions of the apical and basolateral membranes and the subepithelial resistance; hence, an increased permeability signifies reduced TEER—a strong marker of gut dysbiosis. Gut dysbiosis is known to promote inflammation, therefore, measurements of serum cytokines (TNF- α , IFN- γ , IL13, IL-1 β ; regulators of both the transcellular and paracellular pathways) including proteases, and chemokines, as well as IgA secretion level (known to coat bacteria) provide evidence of increased growth of pro-inflammatory bacteria. Gut dysbiosis affects health and thus this bottom-up approach/view of the STC model enables the nurse scientist to gather a stronger evidence-base data to help support his/her nursing intervention and implementation to alleviate the disordered GI symptoms experienced by individuals with IBS.

It may be very difficult to contextualize the adaptability and resiliency of the gut microbiota, but this is key concept to be considered to reduce the harmful consequences of gut dysbiosis. There are ongoing studies that have focused on gut microbes where molecular components acquired from the environment (bacteria-to-bacteria or host-bacteria interactions) adjust the dynamic gut environment. Bacteria are versatile in acquiring genetic elements that allow them to adjust, i.e., change their metabolic capability in response to varying environmental stimuli or exposures to improve their fitness¹⁵ Such transformational processes under these selective pressures drives the changes that are observed in the gut microbiota landscape. Whether the direction proceeds to be beneficial or not, depends on the ultimate behavior at the individual level (i.e., eating habits, sleeping habits, overuse of over-the-counter drugs such as NSAIDS, ethanol, nicotine, including antibiotics) as observed through the lens of the STC ecological model. To be fully operational, the gut microbes must be able to recognize and communicate with other microbial organisms. For example, quorum sensing is used by bacteria to coordinate their behaviors allowing them to regulate biological/metabolic processes for their own survival. Other defining attributes of a resilient gut microbiota involve flexibility to adapt positively to

the changing environment without compromising its normal behavior, i.e., able to maintain the ability to degrade or metabolize harmful environmental toxins/medications; must be equipped with a repertoire of enzymatic machineries for degradation of various types of nutrients (the B-Glucuronidase (GUS) enzymes, must encode different types of import and export proteins necessary for absorption of nutrients and other metabolic products present in their environment, and must have the machinery to be able to oxidize these nutrients/metabolites for energy. For these attributes to be present, the individual must have established a stable gut microbiota early in life, usually by age three. It is known that the immune system is trained by the gut microbiota and its metabolites and thus a proper functioning of the immune system (i.e., expressions of TLRs precedes the evolution of a resilient gut microbiota community. A supportive gut environment (i.e., in the context of a complex carbohydrate dietary intake, having a high fiber diet instead of a high fat diet) is needed for the proper functioning of the gut microbes. In the presence of challenging stressors, ecological stability is achieved when these bacteria acquire new mechanisms to buffer such challenges and still function normally. On the other hand, when the gut bacteria fail to adapt to the new perturbed environment, a “disease state” is most likely to develop. In summary, an individual’s core bacteria’s health and well-being are influenced by environmental determinants of health such as diet, its preparation, nutritional contents, and chemical contaminants.

Gastrointestinal Tract

The contribution of the gut microbiome to health relies on the structure and function of the gastrointestinal (GI) tract. The human gut is colonized with 100 trillion of commensal and symbiotic bacteria known to produce important metabolites, vitamins (i.e., B vitamins, biotin), amino acids, hormones, neurotransmitters, bacteriocins, bile acids, choline, monoamines, indoles, and other bioactive molecules that benefit the host (Rivière et. al. 2016; Schroeder & Bäckhed 2016; Bourassa et.al., 2016). The gut is the largest body surface area that is exposed to environmental toxins from different sources: food, xenobiotics, chemicals, and air and water pollutants. The GI tract is considered the largest endocrine organ of the body (Ciorba 2018; Cummings, & Macfarlane 1991). The small intestine receives 8-9 liters of fluid and electrolytes and secretes about one liter a day (Ciorba 2018). The

large intestine is about 100-150 cm long and its large surface area ($\sim 1300 \text{ cm}^2$) facilitates efficient absorption of fluids and electrolytes to prevent diarrhea. Besides being the major site for water and salt absorption, it is also a major site for bacterial fermentation (Ciorba 2018; Cummings, & Macfarlane 1991). The gut is innervated by both the enteric nervous system or ENS (intrinsic neuronal pathways) and the CNS (extrinsic neuronal pathways) (Ciorba 2018). The ENS contains sensory or afferent neurons that sense changes in the environment (pH, osmolality, wall stretch/mechanical distension or tension) and secretomotor or efferent neurons that regulates motility, secretion, absorption, and immune functions of the gut by innervating the different cells (i.e., enteroendocrine cells such as enterochromaffin cells; goblet cells, Paneth cells, etc.) of the GI tract (Ciorba 2018). Parasympathetic stimulation via the cholinergic receptors (e.g., nicotinic, muscarinic) can stimulate and inhibit GI functions. The sympathetic stimulation of the CNS via the nicotinic, cholinergic receptors (responsive to agonist, nicotine) predominantly inhibits GI secretion and motility (Mandl & Kiss 2007; Ciorba 2018), while the ANS activation (parasympathetic component) on muscarinic cholinergic receptors (responsive to agonist, muscarine) such as the M3 GPCRs promote intestinal motility and increase gastric acid production (Tobin et al., 2009; Tanahashi et al., 2021; Uwada et al., 2023). Although the CNS can regulate function of the ENS and different intestinal cell types, the release of neuropeptides from the entero-endocrine cells of the GI tract can regulate the motor activity (i.e., intestinal peristalsis) of the entire GI tract without the input of the CNS (Ciorba 2018). The rhythmic contractions of the GI smooth muscle layers are modulated by nerves and hormones (i.e., neurotransmitters, neuropeptides) manufactured by both the host and by the gut commensal bacteria; however, other specialized cells (i.e., interstitial cells of Cajal) within the smooth muscle layers of the intestine can generate electrical rhythms or slow waves at a frequency of 12-20 spikes/min for the maintenance of normal GI tract motility (Ciorba 2018). Gut hormones such as motilin, ghrelin, gastrin, cholecystokinin, serotonin have been documented to generate peristaltic or segmental contractions of the GI tract and that melatonin produced by the neuroendocrine cells in the gut mucosa has been implicated to regulate the myoelectric rhythm (Konturek et al., 2011)

The GI tract is also considered an active immune organ (Wang et.al, 2017; Rea et.al., 2017). and at the same time, it is under constant immunological surveillance and expresses toll-like receptors (TLRs) that

identifies pathogenic bacteria necessary for innate and adaptive inflammatory response (Ganapathy; et.al, 2013). The mucosal surface layer of the gut is composed of enterocytes with their own innate and adaptive immune pattern recognition receptors, secretory cells (goblet cells, Paneth cells, neuroendocrine cells) and gut-associated lymphoid tissue (Wang et al., 2017; Rea et al., 2017). In addition, to these immune factors, tight junctions between the epithelial cells provide protection against pathogenic bacteria.

A healthy gut is essential for health and well-being and depends on the richness, diversity, and stability of a “normal” gut microbiota. Increased richness and diversity suggest higher and improved functional potential of the microbiota (Belkaid & Hand 2014). Microbial activities which are beneficial to the host are the result of positive, microbial interactions. The metabolites (i.e., SCFA, bile acids, tryptophan, vitamins, neurotransmitters) produced by the gut microbes help maintain gut homeostasis. The human gut is predominantly occupied by the phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes*, roughly about 195 bacterial strains representing 101 species (Collins, 2014).

Definition of Dietary Fiber (DF)

To date, there is not one universal definition of DF in the literature and the scientific community continues to debate what constitutes DF. Much of the debate is due to the lack of a single method that identifies all the low molecular weight (soluble DF) and high molecular weight (insoluble DF) forms of DF (Macagnan et al., 2016). Early definitions were based on the enzyme digestibility of DF, while the later definitions involved the addition of positive physiological effects on human health. In 1976, Trowell defined DF as “consisting of the plant polysaccharides and lignin which are resistant to hydrolysis by human digestive enzymes” (Trowell, H. 1978).

The Codex Committee on Nutrition and Foods for Special Dietary Use (CCNFSDU) is a part of World Health Organization/Food and Agricultural Organization (WHO-FAO) established in 1963 to set guidelines in food labeling, safety and quality of international food standards. In 2009, the CCNFSDU issued a global definition of DF as “carbohydrate polymers with ten or more monomeric units which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and must have beneficial physiological properties when

consumed” (Gordon, 2007; Phillips & Cui, 2011). Physiological effects listed by Codex include decreased intestinal transit time and increased stool bulk; fermentable by colonic microflora; reduced blood total and/or LDL cholesterol levels; and reduced post-prandial blood glucose and/or insulin levels.

In the US, the FDA nutrition labeling regulation (21 CFR101.9) codify DF contents to be validated via the method proposed by the Association of Official Analytical Chemists for food labeling and regulatory purposes. Additional definitions of DF have been put forth by other professional groups. In 2001, the Institute of Medicine (IOM) defined DF as “consisting of nondigestible carbohydrates and lignin that are intrinsic and intact in plants,” and additionally defined functional fiber as “consisting of isolate, nondigestible carbohydrates that have beneficial physiological effects in humans” (Gordon, 2007). The American Association of Cereal Chemists (AACC) International defined DF as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine and include polysaccharides, oligosaccharides, lignin, and associated plants substances and must promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and /or blood glucose attenuation” (Gordon 2007, Cho & Almeida, 2012; Phillips &Cui 2011, Jones 2014, Macagnan et.al, 2016).

In 2006, the WHO-FAO Scientific Update on Carbohydrates in Human Nutrition defined DF as “consisting of intrinsic plant cell wall polysaccharides and that DF should only be associated with fruits, vegetables, and whole grains.” The definition of DF also varies by country. For example, in the United Kingdom, DF is defined as non-digestible carbohydrates that include resistant starches, resistant oligosaccharides with three or more monomeric units, non-starch polysaccharides (non-alpha-glucan polysaccharides) such as cellulose, and non-cellulosic polysaccharides such as pectin, glucans, arabinogalactans, arabinoxylans, gums, mucilage, inulin, guar, chitin, and lignin. In Australia and New Zealand, DF is the fraction of the edible part of plants or their extracts, or synthetic analogues that are resistant to digestion and absorption in the small intestine with complete or partial fermentation in the large intestine that must come with one or more beneficial physiological effects” (Cho & Almeida 2012). Furthermore, Macagnan defined DF as source of bioactive compounds having low molecular weight carbohydrates such as fructo-

oligosaccharides (FOS), galacto-oligosaccharides (GOS) along with DF-associated non-extractable and extractable polyphenols that can potentiate the overall physiological effect of DF (Macagnan et al., 2016). DF has protective effects, specifically anti-inflammatory effects, at the physiological and biochemical levels to protect against the development of chronic metabolic disorders associated with chronic inflammation and immune dysfunction. As such DF may stop the progression of these chronic, metabolic-associated syndromes.

In summary, DF are rich in phytochemicals, antioxidants, vitamins and minerals that positively impact the gut epithelial cell metabolism and modulate brain function (Filosa et al., 2018); hence, the overall health of a person consuming high DF intake. To have nutritional relevance, all these definitions make sure that a new, functional DF that is being marketed to the public must be authenticated by current analytical chemistry methods to validate that it is composed of complex sugar moieties that are specifically metabolized by the gut microbiota in the colon to produce metabolites that confers health benefits to the consumer.

Dietary Fiber Intake

DF intake recommendations depend on age, sex and pregnancy/lactation status of the individual. The UK Food Standards Agency recommends 18 grams or more per day, while the US-FDA recommends 28 gram/2000 Calorie intake. The current recommended dietary allowances for DF are listed in Figure 7 (Gropper & Smith 2012). A more in-depth dietary reference intakes for macronutrients and micronutrients has been reported (Trumbo et. al., 2002). On average, Americans consume roughly about 15g/day with the lowest quintiles ingest below one-half of the daily recommended levels (Jones, 2014). In the National Health and Nutrition Examination Survey (NHANES) in 2013-2018 of 14,600 adults only 5% of men and 9% women in the US population adhered to this recommendation (<https://nutrition.org/most-americans-are-not-getting-enough-fiber-in-our-diets/>). Despite awareness of its positive benefits, DF intake by Americans remains low.

Physiological Properties of Fibers

DF comes in different varieties, the natural forms that are found in fruits, vegetables, and grains as well as the so called, “functional fibers” that have been isolated, extracted, and manufactured by food industries.

These carbohydrates include nondigestible oligosaccharides (inulin, oligofructose, FOS, GOS), and non-starch polysaccharides (cellulose, hemicellulose, pectins, hydrocolloids or gums, mucillages, and β -glucans).

Early experimental studies with animals including humans found that the functionality of DF is based on its physical, structural, and chemical properties, i.e., solubility and fermentability, in the gut. Experts in the field further categorize these non-digestible carbohydrates or resistant starches according to their solubility, fermentability, and viscosity. The

four subcategories are: 1) soluble, viscous/gel-forming, readily fermented (i.e., β -glucans from oats and barley, raw guar gum/PHGG) known to have prebiotic effect, glycemic control and lipid-lowering properties, as well as fecal bulking capacity by stimulating microbial growth; 2) soluble, viscous/gel forming, nonfermented (i.e., psyllium/Ispaghula husk) have stool-normalizing effect/laxation effect as well as increasing the consistency of loose stools, improving glucose control and lowering blood triglycerides; 3) soluble, non-viscous, readily fermented (i.e., inulin, wheat dextrin, OS, RS) also have prebiotic effects; and 4) insoluble, poorly fermented (large coarse wheat bran, cellulose, lignin) are resistant to microflora fermentation and increase fecal bulking. Soluble and viscous fiber are known to delay gastric emptying, are easily dissolved in aqueous environment and readily fermented by the gut microbes in the colon. Insoluble fiber does not easily dissolve in water and can be degraded by only certain bacteria in the colon and usually contributes to fecal bulk.

A balance of DF types is important to maintain health (Baxter et al., 2019). Table 1 provides examples of food sources and the benefits of each DF category (<https://lpi.oregonstate.edu/mic/other-nutrients/fiber>). A systematic review of clinical trials (36 studies; 25 included concurrent controls where 11 studies included only healthy participants that were sequentially designed) recommended that the effective dose for wheat bran is

Table 4.2 Recommended Fiber Intakes

Population Group	Age (years)	Total Fiber (g)
Men	19 to 50	38
	≥ 51	31
Women	19 to 50	25
	≥ 51	21
Children	1 to 3	19
	4 to 8	25
Girls	9 to 18	26
Boys	9 to 13	31
	14 to 18	38

Figure 7. Dietary reference intake for Fiber (Gropper & Smith, 2012 p. 120)

22g/day or with the use of dietary supplement i.e., psyllium is 7g/day (McRorie et al., 2020).

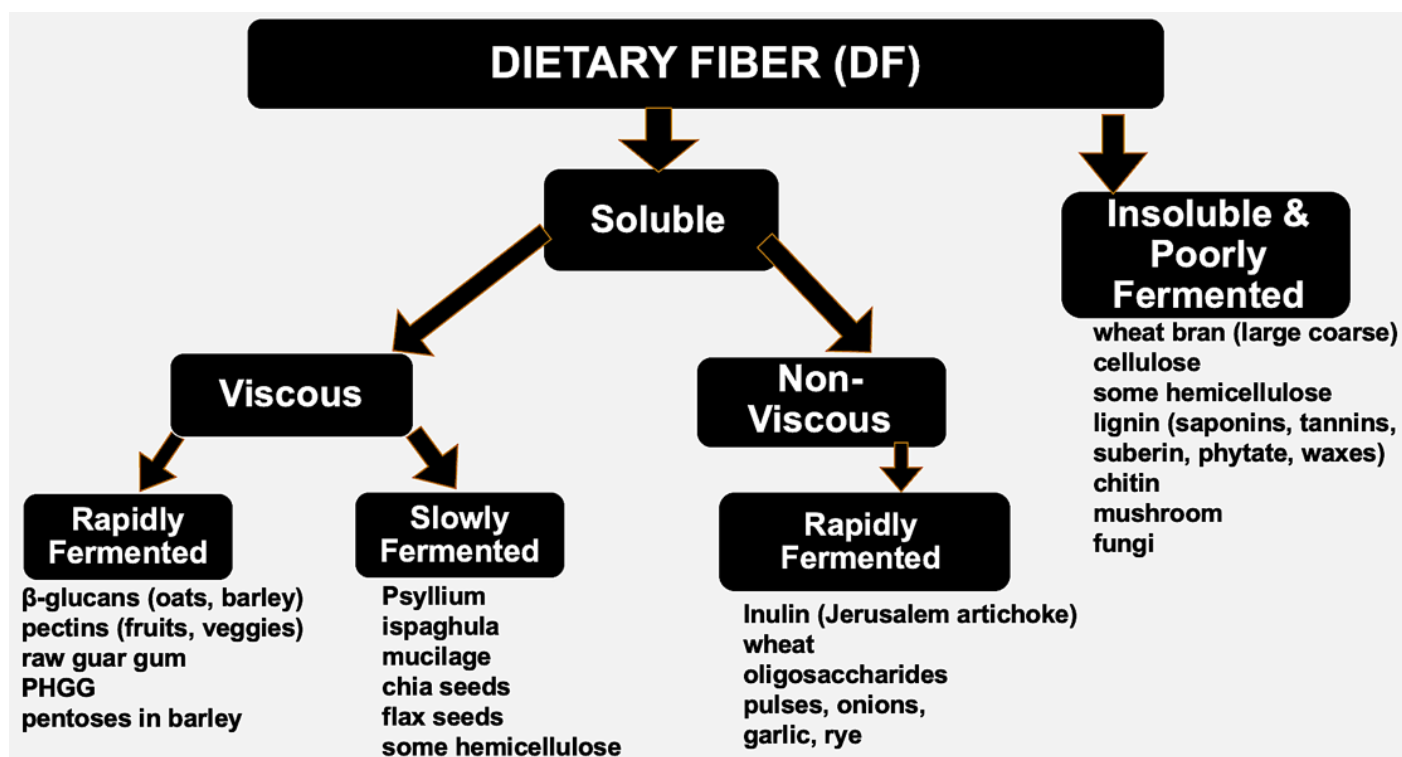


Figure 8. Different types of dietary fiber under the umbrella of carbohydrates (Spiller, 2001; Oregon State University website on Fiber. <https://lpi.oregonstate.edu/mic/other-nutrients/fiber>).

Table 1: Four Categories of DF according to Solubility, Viscosity, and Fermentability

Dietary Fiber	Examples	Food Source	Role
<u>soluble</u> <u>viscous</u> <u>rapidly fermented</u>	β-glucan pectin PHGG pentoses galactauronic acids	Oats, apples, barley, guar gum Plantago ovata husk and seed, and vegetables	have prebiotic effects, improve glycemic control and blood cholesterol, and serve as fecal-bulking agents
<u>soluble</u> <u>viscous</u> <u>slowly fermented</u>	psyllium ispaghula mucilage some hemicellulose arabinose, xylose	chia seeds flax seeds okra aloe vera Chinese yams Licorice roots seaweeds, green algae	normalize stool number/consistency, improve glucose control, and lower blood triglycerides
<u>soluble</u> <u>non-viscous</u> <u>rapidly fermented</u>	Inulin dextrin Oligosaccharides fructose galactose mannose arabinogalactans	artichokes wheat dextrin pulses, legumes red onions, shallots garlic, leeks, rye, barley chicory root, root vegetables	predominantly prebiotics

<u>insoluble, poorly fermented</u>	cellulose some hemicellulose lignin (saponins, tannins, suberin, phytate, waxes) chitin N- glucosamine Amino sugars	wheat bran (large coarse) mushroom fungi, algae, crustaceans leafy vegetables, peas whole seeds, grains	add to stool bulk
--	---	--	-------------------

Gut Dysbiosis in the Context of IBS

The literature has defined gut dysbiosis as a consistent perturbation of the normal GI bacterial composition and its associated metabolites (Ha, et al.,2014). Factors that can contribute to dysbiosis include diet, lifestyle and behaviors (i.e., alcohol consumption, smoking, medications), exposure to environmental toxicants, age, current physiological status, and the host's genomic landscape especially the DNA architecture of genes involved in the regulation of the innate and adaptive immune responses.

Gut dysbiosis can be defined as a biomarker for a disease where a consistent change in microbiome profiles driven by an imbalance of pro-inflammatory and anti-inflammatory bacteria and their metabolites. Dysbiosis results in a higher metabolic state or threshold. When a bacterial community structure is altered in the context of low DF intake and high saturated fat diet, the microbiome energy state has shifted from its lowest level into an altered and higher energy state which may predispose a person to increased risk of disease (i.e., metabolic disorders, diabetes, heart disease, cancer, and other inflammation-related disorders such as inflammatory bowel disease (IBD), arthritis and other autoimmune diseases, neurological disease such as Parkinson's and Alzheimer's). Dysbiosis may lead to growth and colonization of pathogenic bacteria in the gut and reduced butyrate-producing bacteria. This can lead to compromised gut barrier, immune dysregulation, and inflammatory disorders.

Dietary fiber influences GI function

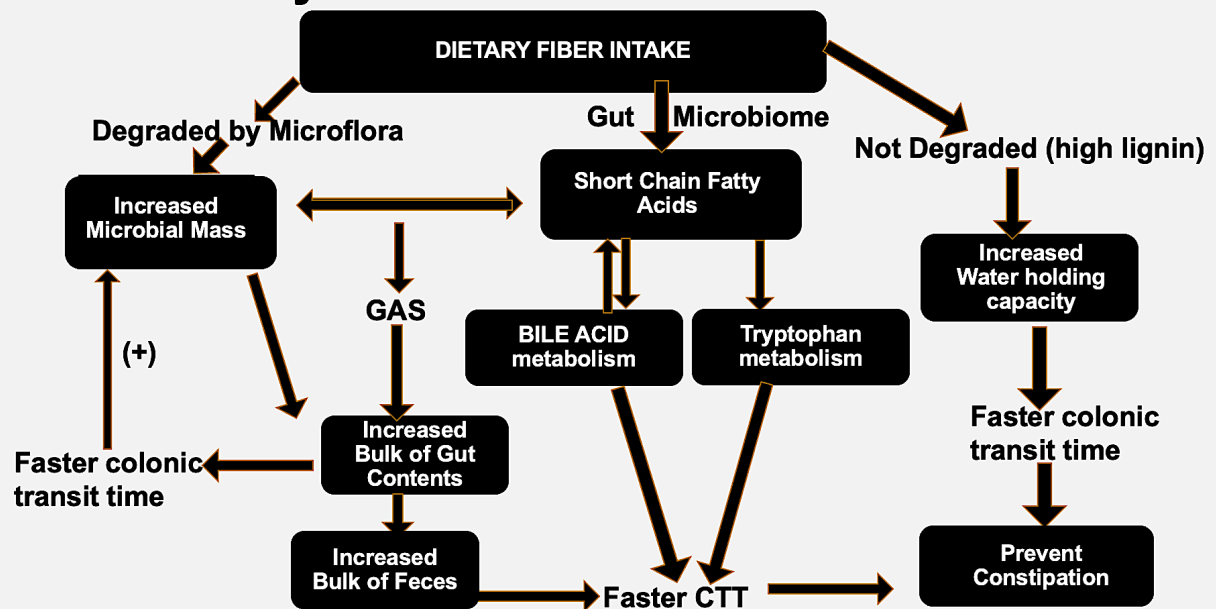


Figure 9. Fermentation of dietary fiber by the gut microbiome exert beneficial effect on gut function. High fiber intake helps normalize bowel patterns (Adapted from Cummings 1984).

Diet plays a major role in the maintenance of a stable microbial community structure (Duncan et al., 2007; Ha et al. (2014). Figure 7 illustrates a proposed pathway in which high DF intake can normalize bowel movements, relieve GI symptoms, and increase fecal bulking in IBS patients. Low DF and high saturated fat intake tend to increase the secretion of excess bile acids resulting in the production of sulfated compounds that induce colonic cell toxicity. The gut microbiota is equipped with enzymes (i.e., bile salt hydrolases [BSH]) that deconjugate the primary bile acids resulting in the release of taurine, a sulfated amino acid. Taurine stimulates the growth of pro-inflammatory microbes such as the sulfate-reducing bacteria (SRBs) that exploit the anerobic respiratory process (also known as dissimilatory sulfate reduction) with a concomitant release of hydrogen sulfides. As supported by both *in vitro* and *in vivo* studies, these sulfides are cytotoxic to colonocytes (Ha et al., 2014; Carbonero et al., 2012; McGar et al., 2005). As a result, these hydrogen sulfides metabolites induce morphological and biochemical changes to the gut epithelia (i.e., increased intestinal permeability due to reduced tight junction (TJ) proteins resulting in reduced intestinal TJ function (Yan & Ajuwon, (2017); lower gut TEER allows bacteria and/or its components, and other toxins to enter into the circulation, and subsequently result to a sustained low-grade chronic inflammation that ultimately drives the development of inflammation-

related diseases (Cockburn & Koropatkin, 2016; David et al., 2014). Butyrate reduces gut inflammation by enhancing GI barrier function, stimulating mucin production, and by enhancing the expression of TJ proteins and antimicrobial peptides (Tappenden et al., 2010).

A high fat diet also results in the reduction of butyrate-producing bacteria and production of SCFAs. As a result, the colonocytes are starved slowing down important cellular and molecular processes (i.e., production of ATP, electrolyte absorption, nutrient transport, detoxification reactions, synthesis of important gut neuropeptides and hormones, generation of action potential) (Scheppach et al., 1991; Scheppach, 1994). It can be inferred that such a disruption between the interaction between the GI tract and the gut microbiota and its associated metabolites may lead to development of IBS GI symptoms and other extraintestinal disorders.

Increased gut permeability has been associated with low-grade intestinal inflammation in persons with IBS and such phenotype is hypothesized to be linked to abdominal pain (Goo et al., 2012). Urinary proteome analysis of IBS-D cohorts done by Goo and colleagues reported that Niemann-Pick type C2 (NPC2) protein is elevated in IBS most especially in IBS-D with high pain group (Goo et al., 2012). NPC2 plays a role in the regulation of intracellular free cholesterol homeostasis and the expression of cellular reverse cholesterol transporters. Such efflux transporters are activated during high fat/high protein intake. High dietary fat intake has been associated with higher production of branched chain fatty acids (BCFA) and toxic compounds (i.e., nitroso-amines, secondary bile acid) (Korpela, 2018). High production of SCFA in the colon lowers the gut pH, rendering the bile acids and its toxic derivatives (i.e., lithocholic acid, deoxycholic acid) less soluble (less hydrophobic) and prevents their absorption back via the entero-hepatic circulation; at higher pH, these bile acids are excreted and are known to be highly present in the IBS-D phenotype (Min et al., 2022; Wong et al., 2012; Kamp et al., 2021). Wei et al., 2020). Bajor et al., 2015).

Dietary Fiber and Microbiota

Intake of DF and other microbiota accessible carbohydrates (25-60 grams/day) help maintain the integrity of the GI mucosa by modulating the gut microbiota composition and their metabolic activities (Martínez et al., 2010; Abell et al., 2008; Moschen et al., 2012). DF plays a major role in the maintenance of a stable

microbial community. High DF intake results in higher diversity (alpha diversity in terms of richness and evenness) in microbial community of the colon (De Filippo et al., 2010; Simpson et al., 2015). There are about 500-1000 bacterial species in humans and there are about 60,000 carbohydrate-degrading enzymes in the gut, but humans only have 17 glycoside hydrolases and lack polysaccharide lyases. Humans can only make enzymes that cleave the alpha-1,4-glucose linkages of carbohydrates and do not have the capacity to express a diverse array of glycoside hydrolases to degrade complex carbohydrates and DF, thereby, they are not absorbed in the upper GI tract and are degraded and fermented by the colon microbiome.

There is well-established evidence for the presence of beneficial colonic bacteria, and many have been marketed as probiotics. For example, *Akkermansia*, *Bifidobacteria*, and *Lactobacillus* are already being marketed as 'good' probiotics to improve the microbiome profile of individuals at risk for gut dysbiosis (Pattanik et al., 2022; Hidalgo-Cantabrana et al., 2018; Li et al., 2020; Maguire & Maguire, 2019; Lyte 2011). Furthermore, various strains of *Propionibacterium* have been found to induce apoptosis in colon cancer cells and other cancer cell lines, but not in normal cells; its cytotoxic potential is suggested mainly due to its three-fold (propionate: acetic) production capabilities (Jan et.al 2002).

DF Improves GI Function

DF has also been shown to directly and indirectly stimulate GI motility via its fermentation products modulated by the gut microbiome (Cummings 2001; Bleser 2006; El-Salhy et al., 2017; Bosaeus 2004). Several randomized clinical trials (RCT) provide evidence for the effectiveness of daily high DF intake in reducing constipation in the adult population of men and women as well as children with chronic constipation (See APPENDIX A for the list of studies). (Duncan et al., 2018; Hongisto, et al., 2006; Prior & Whorwell 1987; Reese et al., 2005; Bijkerk et al., 2009; Arffmann et al., 1985; Harvey et. al. 1973; Manning et al., 1979; Ritchie & Truelove 1979; Fowlie et al., 1992; Ornstein et. al. 1981; Cummings 1993; Romano et. al. 2013; Aller et al., 2004; Parisi et al., 2002; Parisi et al., 2005).

Based on DF type, there are differences in effectiveness of DF in constipation management. (NEED A NEWER REFERENCE) A recent review by McRorie et al., reported that psyllium is 3.4 times more effective than insoluble wheat bran for increasing stool output; however, both coarse wheat bran and psyllium increase stool

water content (McRorie et al., 2020). On the other hand, finely ground wheat bran decreases stool water content (McRorie et al., 2020). An earlier systematic review by Ford et al. looked at the effect of three different treatments (fiber, anti-spasmodics, and peppermint oil) in the treatment of IBS (Ford et al. 2008). The fiber treatment included 9 studies; authors reported that ispaghula husk (ISPH) is effective in alleviating constipation (Ford et al., 2008). In addition, soluble fiber also improves global GI symptoms (i.e., improved stool frequency, stool consistency and reduced straining) (Suarez & Ford, 2011).

The effectiveness of DF in alleviating constipation depends on its particle size, viscosity, solubility, digestibility, fermentability, and adsorptive or entrapment capacity and/or water-holding capacity. Greater particle size reduces the rate of fiber being digested in the upper GI tract, making it more likely to reach the colon intact which can physically stimulate colonic motility. Having a high degree of polymerization is known to trap water which can soften stools, also the trapping of minerals and gases also increases fecal bulk.

IBS and Gut Microbiota

Phenotypic characteristics such as the person's microbiota profile may be an important factor in the selection of optimal DF components. From the literature, it is known that specific bacterial taxa produce SCFA in the presence of fermentable DF. Members of the Phyla *Bacteroidetes* and *Actinobacteria* play important roles in the breakdown of insoluble non-starch polysaccharides that are categorized into 4 different types (i.e., Resistant Starch 1, (RS1 to RS4)). Some members of the genera *Bifidobacterium*, *Bacteroides*, *Clostridium* and several members of the Family *Lachnospiraceae* and *Ruminococcaceae* degrade RSs and are called primary degraders. In addition, they establish a positive environment (e.g., provision of easily accessible fermentable substrates such as oligosaccharides and other microbiota accessible carbohydrates) for other types of bacteria known as cross-feeders (sometimes referred to as butyrogenic or secondary bacteria). For example, it is known that the partnership of *Ruminococcus bromii* and *Eubacterium rectali* increase the production of butyrate in the gut in the presence of RS2, RS3, and inulin (Baxter et al., 2019). Different types of fermentable fibers yield different concentrations of SCFA; pectin, oats, wheat bran, sugarbeet and sugarbean

fibers can yield high concentrations of SCFA (Topping & Clifton 2001; Cherbut 2003; Bourassa et al., 2016). Ten grams of fermented carbohydrate yields approximately 50-75 mmol SCFA (Cummings & Macfarlane 1991). A list of exemplars of primary and secondary degraders is shown in Table 2 below.

Table 2: Exemplars of Primary and Secondary Degraders of Complex Carbohydrates and DF

Phyla	Genera	METABOLITE PRODUCTION				Metabolite Consumption
		Acetate	Propionate	Butyrate	Other	
<i>Verrucomicrobia</i>	<i>Akkermansia</i>	X	X			
<i>Bacteroidetes</i>	<i>Bacteroides</i>	XX	XX	XX	↑H ₂ , ↑formate	succinate
<i>Actinobacteria</i>	<i>Bifidobacteria</i>	XX			lactate, ethanol, succinate	lactate, ethanol, succinate
<i>Firmicutes</i>	<i>Blautia</i>	XX		X	succinate, H ₂	succinate, H ₂
<i>Firmicutes</i>	<i>Clostridium</i>	XX	XX	XX	H ₂ , ↑formate	
<i>Firmicutes</i>	<i>Coprococcus</i>	XXX		XXX	↑H ₂ , formate, succinate	lactate, formate
<i>Proteobacteria</i>	<i>Escherichia</i>	XXX			↑H ₂ , ↑formate, ethanol, succinate	H ₂ S
<i>Firmicutes</i>	<i>Eubacteria</i>		X	XX		
<i>Firmicutes</i>	<i>Faecalibacterium</i>			X	succinate, ethanol	propionate
<i>Firmicutes</i>	<i>Lactobacillus</i>	XXX	X	X	↑lactate succinate, ethanol,	
<i>Firmicutes</i>	<i>Megasphaera</i>	XX	X	XXX	↑H ₂ , H ₂ S, ethanol Isovalerate	lactate
<i>Archea</i>	<i>Methanosarcina</i> , <i>Methanobrevibacter</i>	X				acetate, ↑H ₂
<i>Bacteroidetes</i>	<i>Prevotella</i>	X			lactate, succinate, isovalerate, formate, ethanol	
<i>Firmicutes</i>	<i>Roseburia</i>		X	XX		
<i>Firmicutes</i>	<i>Ruminococcus</i>	XX	X		formate lactate, ethanol, H ₂	formate lactate, ethanol, H ₂
<i>Firmicutes</i>	<i>Veillonella</i>	XX	X		ethanol, ↑H ₂ , H ₂ S	

Microbes and Metabolic Potential

Microbes carry different numbers of genes encoding for hydrogenases, dehydrogenase, carboxylases, transferases, glycosidases, and glucuronidases that are necessary for ongoing anaerobic fermentation of non-digestible carbohydrates entering the gut. According to the Human Microbiome Project, more than 70% of hydrogen-encoding sequences belonged to Firmicutes and Bacteroides; both phyla represent about 85% of the gut microbial population (Smith et al., 2019). There are several metabolic pathways in which the main three, straight-chain short chain fatty acids (sc-SCFA) are generated. Such pathways include the glycolysis (acetic, butyric, propionic), the pentose pathway (mainly acetic), the acrylic pathway (mainly for propionic acid).

Metabolic hydrogen is largely produced during the formation of acetate and butyrate via the glycolysis pathway. Excess amount of hydrogen, formic acids, methanol, ethanol and methyl amines (i.e., choline, carnitine, betaine (trimethylglycine), trimethylamine-N-oxide or TMAO, and phosphatidylcholine are metabolized by the methanogens (*Archaea* phyla) and other hydrogen-consuming bacteria.

Hydrogen sinks (i.e., H₂, formate, NADH, lactate) reduces the TCA intermediates (oxaloacetate to malate, fumarate to succinate) used to produce propionic acids in the so-called, randomizing pathway (Ungerfeld et al., 2020; Gottschalk 1986; Russel & Wallace 1997), Henderson 1980). Propionic acid can also be formed from the oxidation of valine, isoleucine, methionine, threonine, odd-chain fatty acids, thiamine, and odd chain of cholesterol moieties (Scholem & Brown 1983). Louis & Flint (2018) delineated very detailed pathways that the gut microbiome used to produce SCFA.

Lactate can be derived from the reduction of pyruvate by lactate dehydrogenase (host's RBC, WBC) with a concomitant regeneration of NAD⁺. On the other hand, the lactate producers and utilizers in the gut can also convert lactate into SCFA. Of importance is that there is a regeneration of reduced flavoproteins when lactate is converted to propionic acid. The conversion of acetate to butyrate is special because not only are two molecules of NAD⁺ produced, the reduced form of ferredoxin is also generated (an important re-dox cofactors in regeneration of antioxidant enzymes), as well as proton gradient generation with concomitant ATP production via the electron transport chain of phosphorylation. Bacteria (i.e., methanogens, propionic/succinate producers, other hydrogenotrophs) that are equipped with higher number of genes encoding for hydrogenases ensure continued carbohydrate fermentation by regenerating the oxidized forms of reduced metabolic cofactors. During the production of SCFA, the hydrogen-producing bacteria are known to shift their activity away from the randomizing pathway (uses formic, lactate, ethanol) towards acetate production and saccharolytic fermentation.

Short Chain Fatty Acids (SCFA)

It is important to reiterate that SCFA are generated via the anaerobic bacterial fermentation of DF. Low quality of diet intake translates to low microbial diversity and composition, and in turn, low microbial diversity

translates to low levels of SCFA. Low SCFA have been shown to be associated with several health disorders and diseases such as IBD, IBS, colon cancer, obesity and diabetes, metabolic syndrome, non-alcoholic fatty acid disease, heart disease, and neurological disorders such as dementia (van der Hee & Wells 2021; Maguire & Maguire 2019; Sekirov et al., 2010).

Although extensive work has been done to establish causal relationship between diet and the gut microbiome in animals and a few human studies; substantial work is still needed to support a causal relationship between diet and gut microbiome. The microbial metabolic actions on dietary intake provide one potential mechanism. Microbial metabolites such as SCFA are organic, volatile, carboxylic acids with fewer than 8 carbons, existing in straight-, and branched-chain conformations (Skoglund 2016; Ríos-Covián et al., 2016). The intake of more than 30 grams of DF has been shown to increase SCFA production. Skoglund 2016; den Besten 2013; Maskarinec et. al. 2019). Roughly 95% of the total SCFAs are absorbed (60-70% oxidized by colonocytes) and 5% are excreted in the feces (Makki et al., 2018; Tappenden et al., 2010; Baxter et al., 2019).

SCFA as Primary Colonic Energy Source

SCFA are an important energy source for colonocytes and are known to facilitate the absorption of sodium and water (Scheppach 1994; Scheppach et. al. 1991; Kamath et. al. 1988). Greater than 90% of SCFA exist in their anionic forms and are absorbed via the use of antiporters and symporters located in the apical and basolateral membranes of the colonic lumen. The nonionic, protonated form can passively diffuse through the lipid membrane (Gibson & Roberfroid, 1999). Thus, the rate of absorption for these unprotonated SCFA are slower than the protonated ones. Healthy individuals with a high DF diet have high amount of acetate in their feces as confirmed by gas liquid chromatography and pH fecal measurements (Yangilar 2013). The rate of microbial SCFA production, most especially acetate, influences GI motility and CTT. (Müller et al., 2020). Rapidly fermented and soluble DF such as pectin and lactulose are known to significantly increase the microbial production of acetate (Wolever et. al. 1997). Kamath et al., found that SCFA-induced ileal rhythmic contractions after administering boluses of SCFA mixtures (66% acetate, 24% propionate, 10% butyrate;

approximated total of 100 mM SCFA) to healthy subjects. Healthy volunteers (N=18, both genders included) treated with SCFA infusions had higher amplitude of discrete phasic waves and prolonged propagated contractions but produced little contractile activity when normal saline or air were administered. The reported calculated motility index remained unchanged, and the authors hypothesized that mechanoreceptors and neuropeptides could play a role in the generations of these discrete colonic rhythmic contractions (Kamath et. al. 1988). It is possible that the SCFA may have stimulated the interstitial cells of Cajal, but the amount infused may not have been high enough to produce a greater action potential and hence, a low motility index was observed.

SCFA metabolism depends on the healthy metabolic state of a person and/or cells (i.e., presence of inflammation, starvation, old age) including micronutrients status (i.e., adequate vitamin B₅ a precursor of coenzyme A is necessary for oxidation of butyrate which is metabolized into acetyl-CoA, a key driver for important cellular processes) (Yoshi et. al. 2019; Donohoe et. al. 2012) including the status of internal biological clocks (i.e., Period1, Period2, Bmal1, CLOCK, Cytochrome1, Cytochrome2, REV-ERB α (Nr1d1) and REV-ERB β (Nr1d2), ROR α and ROR γ (Retinoic acid receptor-related orphan receptor, DBP and Nfil3/E4BP4, nuclear receptor corepressor (NCoR), Npas2) which when perturbed or depleted could result in alterations or constitutive expression of SCFA under constant feeding (Tahara et al., 2018; Adlanmerini & Lazar (2023). Butyrate is known to restore disturbances of these timekeepers (Tahara et al, 2018), and thus has the potential to rescue metabolic dysfunctions such as seen in obesity/diabetes and non-alcoholic fatty liver disease (Zhou et al., 2018; Baumann et al., 2020; Amiri et al., 2022).

As a signaling molecule, butyrate mechanism of action is mostly mediated via the GPCRs. By inhibiting or stimulating these receptors, butyrate can either be inflammatory or anti-inflammatory; this dual role of butyrate is dictated by the specific GPCR and the cells' microenvironment. Butyrate's anti-inflammatory effect on the colon is achieved via modulation of IL-8 and MIP-2 (macrophage inflammatory protein-2) activities and by inhibiting the degradation of inhibitor *i-kappaB α* (Gibson et al., 1995; Ohno et al., Usami et al., (2008). GPCRs are ubiquitously expressed in many tissues and circulating cells, however, specific ones such as the GPCR41 (FFAR3) and GPCR43 (FFAR2) are highly expressed in adipocytes and macrophages (Ohira et al.,

2014). FFAR2 is predominantly expressed in immune cells, while FFAR3 is predominantly found in adipocytes. It is important to note that pertussis toxin is known to block GPCR41 and inhibit the anti-inflammatory effect of butyrate (Ohira et al., 2014). Having high butyrate level can interfere with pertussis toxin and prevent neutrophil-associated injury and multiple organ dysfunction and resolve inflammation via HDAC inhibition or via the activation of the caspase cascade; butyrate (5 mM) is known to induce neutrophil apoptosis *in vitro* (Aoyama et al., 2010). Another mechanism for butyrate to regulate the fate of a cell is by acting as an HDAC inhibitor; butyrate prevents the activities of histone deacetylases and promotes hyperacetylation of genes. Butyric acids can potentially stop the progression of cancerous cells (Edelman et al., 2003), prevent lipolysis in inflamed obese adipocytes by blocking the transcription of hormone sensitive lipases and adipose tissue lipases (Ohira et al., 2014). Furthermore, butyrate is known to suppress the proinflammatory pathways involved in the formation of “inflamed macrophages” or inflammasomes (Feng et al., 2018, Swanson et al., 2019) in the context of inflamed adipocytes or endothelial cells (Pierantonelli, & Svegliati-Baroni, 2019); however, it is also known to activate inflammasome formation in the context of infection (Tsugawa et al., 2020). As a HDAC inhibitor, butyrate can downregulate the expression of genes (i.e. iNOS, JAK/STAT and the MAPK signaling pathways (Stemplej et al., 2007). In the presence of Warburg effect (aerobic glycolysis), butyrate is not metabolized; however, it serves as an HDAC inhibitor in the nucleus, (Donohoe et. al. 2012), thereby preventing cell cycle division and functioning as a tumor suppressor. HDAC inhibitors are also known to be regulated by biological peripheral clocks.

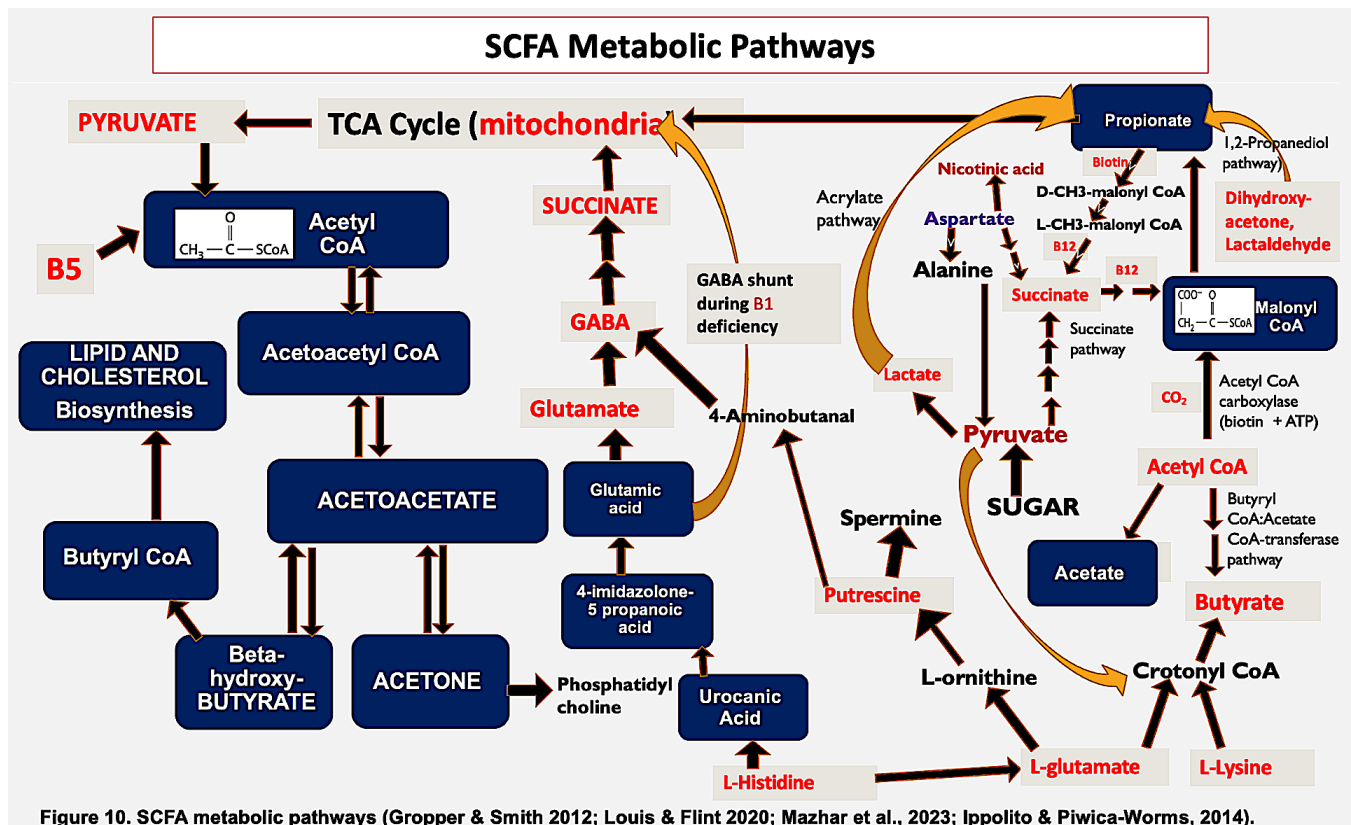


Figure 10. SCFA metabolic pathways (Gropper & Smith 2012; Louis & Flint 2020; Mazhar et al., 2023; Ippolito & Piwica-Worms, 2014).

Anaerobic bacterial fermentation of carbohydrates and DF produces CO_2 , H_2 , and volatile, weak organic carboxylic acids such as SCFA that have a pKa of 4.8 (Duncan et al., 2009). In the GI tract, the metabolic pathways that produce SCFA are mainly the ζ (glycolysis) and the pentose-phosphate pathways (Korpela, 2018). Butyrate is mainly produced from the glycolysis (Embden-Meyerhof-Parnas pathway). Figure 10 illustrates specific metabolites and necessary vitamins or essential cofactors such as vitamin B's and biotin that are necessary in the production of SCFA and its related metabolites.

All fecal SCFA originate from bacterial activities (Pituch et al., 2013). Under high DF intake ($\sim 30\text{g-}50\text{ gram/day}$), the combined normal concentration of fecal SCFA (acetate, propionate, butyrate) is approximately 50-150 mM (200-600 mmol) in the colon; 20-70 mmol/L produced in the distal colon and 70-140 mmol/L in the proximal colon (Cummings & Macfarlane 1991; Cook & Sellin 1998; Wolever et al., 1997; Mahadeven & Zieve 1969; Soergel 1994; Ruppert et al. 1980). In humans, the concentration of butyrate in peripheral circulation is estimated to be 1-4 micromole/L, while the portal circulation is estimated to have about 450 micromole/L total SCFAs. Literature reports that plasma butyrate can be increased by the butyrate prodrug, tributyrin (Miyoshi et al. 2011; Conley et al., 1998; Edelman et al., 2003).

SCFA Transport

SCFA are absorbed via passive diffusion as well as by enhanced uptake by these colonic transporters: HCO₃/SCFA exchanger, H⁺/MCT1, Na⁺/SMCT cotransporter (Miyauchi et al., 2004; Von Engelhardt et al., 1993, O'riordan et al., 2021). It is important to note that the SMCT (gene SLC548) is suggested to function as tumor suppressor and is upregulated in the presence of SCFA, preferring butyrate before propionate and lactate before acetate, but silenced in colon cancer (Li et. al. cited by Miyauchi et al., 2014). Studies by Jan et al., and others provided evidence of the cytotoxic potential of propionate via apoptosis and of butyrate by cell cycle arrest in colonic cancer cell lines (Jan et al., 2002).

Under normal conditions, the fermentation of unabsorbed carbohydrates such as DF provides the major source of SCFA, while the fermentation of protein (derived from plants and animals, bacterial polysaccharides, and the host's endogenous mucosal proteins) yield $\leq 5\%$ of branched chain fatty acids (BCFA) (Ríos-Covián et al., 2016; Korpela, 2018). Examples of BCFA such as isobutyric, isovaleric, and 2-methylbutyric acids are mainly from the fermentation of BCFA (i.e., valine, leucine, and isoleucine) mostly carried out by *Bacteroides* and *Clostridia* genera.

Literature reports that isovaleric has been shown to display significant diurnal oscillation (concentration varies with time); other high oscillating metabolites include serotonin, lysine, xylose, ergothioneine, glucose, and perhaps biotin (Liang et al., 2017). Thus, DF provides the major source of SCFA. SCFA promote gut health, while the BCFA are associated with adverse gut functioning (Ha et al., 2014; Scott et al., 2013). The fermentation of carbohydrates is shown to reduce the production of BCFA by 40-60% (Smith & Macfarlane 1997). Smith and Macfarlane reported that an average of 13 grams of protein sources are found in the colon and that high proteolytic fermentation products stimulate the growth of *Prevotella* and *Bacteroides* genera. In literature, butyrate decreases GI motility, while propionate decreases hepatic cholesterol synthesis. As a primary energy source of the colonocytes, butyrate is rapidly absorbed and oxidized while most of the acetate and propionate enter the blood via the entero-hepatic circulation and influence carbohydrate and lipid metabolism in the peripheral tissues (Cook & Sellin 1998; Wolever et.al, 1997; Mahadeven & Zieve 1969; Soergel 1994; Ruppin et. al. 1980; Pouteau, et al., 2003; Bergman 1990; Pomare et al., 1985). Butyrate

modulates colonic activity by increasing colonic blood flow (Scheppach 1994) and reducing colonic contractility (Bajka et al., 2010). Infusion studies have shown that acetate absorption is much faster than propionate and can increase the host acetic acid pool in the blood and serve as energy source for peripheral tissues (Wolever et al., 1997; Pouteau et al., 2003). It is worth noting that high levels of propionic and butyric acids in the blood signifies metabolic disorder because the sources are from the catabolism of amino acids (Pouteau et al., 2003; Gropper & Smith 2012; Wolever et al., 1997; Macfarlane et al., 1992). As reported by Macfarlane and colleagues (1992), fermentation of protein or amino acids in the colon contributes to up to 20% of total SCFA (11 mmol/kg) of the total SCFA; on the average, 4.6 mmol/kg is produced in the proximal colon and 6.3 mmol/kg from distal colon. Macfarlane and colleagues (1992) reported that the gut microbiome produces approximately 11 mmol of BCFA per day/12g protein (measurements derived from patients undergoing emergency surgery). BCFA production is depressed in the presence of carbohydrates in the colon.

Diet Modulates the Gut Microbiome

Through the production of SCFA, fermentable DF reduces colonic pH (Duncan et al., 2009). Colonic pH of 5.5 is known to promote the growth of *Firmicutes*, while colonic pH > 6.5 increases the abundance of gram negative, bile-resistant bacteria that can increase exopolysaccharide production and possess high BSH activity (Duncan et al., 2009; Elamin et al., 2013). High BSH activity results in deconjugation of primary bile acids into free bile acids that are less soluble and more likely to precipitate at intestinal pH facilitating excretion in the feces. Decreased formation of micelles reduces the absorption of dietary lipids. As a result, the available bile acid pool is reduced; this promotes the *de novo* synthesis of cholesterol. Acetate along with lactate lowers luminal colonic pH and prevents the growth of pathogenic *Clostridia* and *Enterobacteriaceae* (Duncan et al., 2009; Baxter et al., 2019). Increased growth of pro-inflammatory bacteria enhances gut permeability by lowering gut TEER. Polymeros et al., 2014; Prior, A., & Whorwell, 1987; Cann et al., 1984; Arffmann et al. 1985; Manning et al. 1977). Whether diet-linked changes in fecal SCFA profile accounts for the initiation and maintenance of IBS symptoms is unknown.

SCFA & Inflammation

High DF intake promotes the growth of healthy gut microflora, increases stool output, reduces secondary bile acids concentration in the colon, lowers ammonia concentration, and reduces oxidative stress in the colon (Zimmermann et al., 2020). SCFA have been linked to suppression of adiposity by regulating the satiety hormone, leptin via the free fatty acid receptor Ffar3/GPR41 and subsequently affect the secretion of GLP-1 and PYY from the enteroendocrine L cells of the gut epithelium via the Ffar2/GPR43 activation pathways (Hume et al., 2017). Moreover, SCFA can cross the blood-brain barrier and affect the energy homeostasis by binding to receptors in the brain (Makki et al., 2018; Hume et al., 2017). Reduced DF intake can result in increased gut permeability and intestinal inflammation. It is worth reiterating that intestinal pH greater than 6.5 not only increase the abundance of pathogenic, proteolytic, enterobacteria, BCFA-producers which are normally inhibited below pH of 6.5, a more alkaline pH also reduces the abundance of important SCFA-producing bacteria (Duncan et. al. 2009). Also, higher colonic pH enables the bile-resistant bacteria that are enriched with genes that encode for multidrug efflux pump systems or multidrug resistance transporters (Duncan et al., 2009; Genapathy et al., 2013; Zeng et al., 2019; Bustos et al., 2012; Tremaroli & Bäckhed 2012; Klassen & Cui 2015; Donaldson et.al 2016; Fiorucci & Distrutti 2015; Duszka, & Wahli (2018). Such efflux transporters are activated during high fat/high protein intake.

The Western diet constitutes high energy-dense food and stimulates bile acid secretion from the gallbladder to promote lipid absorption. The human bile acid pool is approximately 2.5 to 5 grams and recycles at least twice per meal (Gropper & Smith 2012). Other experts report that the BA pool can be recycled up to 15 times/day and about 5% is lost in the feces (Chiang (2013; Bustos et al., 2012). BA can act as a signaling molecule and activate pancreatic lipase release. Bile acids have detergent properties and are toxic to the gut microbiota and to the host by disrupting cellular membranes and induce oxidative damage that results in cell death. Naturally, the gut anaerobes evolved to metabolize these bile acids to reduce their toxicity; however, such biotransformation activities can also alter the host homeostasis (i.e., energy metabolism and immune system functioning). Furthermore, increased microbial beta-glucuronidase and beta-glucosidase activities can lead to a higher production of secondary bile acids which are more hydrophobic and more potent antimicrobial

agents than its conjugated form and have the potential to alter the gut microbial structure. The increased hydrophobicity of bile acids occurs when the pH is more alkaline; the production of SCFA and its related products (i.e., lactic acid, formic acids) lower the colonic pH and facilitate the excretions of these toxic secondary bile acid compounds instead of being absorbed. Both primary and secondary bile acids can act as selective pressures to eliminate microbes without detoxification systems.

The *Enterococci* are usually more resistant to bile acids and are less sensitive to their toxicity as compared to the gram-positive bacteria. Although, BSH activities has been demonstrated by bacterial genera belonging to *Lactobacillus*, *Bifidobacterium*, *Clostridium*, and *Listeria*, these bacteria may not be as resistant to the accumulation of bile acids and its bio-transformed moieties, and thus may be not as resilient and as a result these reduce gut microbial diversity. In contrast, high BSH activity favors the growth of pro-inflammatory, proteolytic microbes such as the *Enterococci* (Bustos et al., 2012). A review done by Bustos et.al reported that *Lactobacilli* are mostly responsible for BSH activity in the gut that results in the deconjugation of primary bile acid into free bile acids that are less soluble and more likely to precipitate at intestinal pH, facilitating excretion in the feces. Lower formation of micelles reduces the absorption of dietary lipids and results in reduced bile acid pool which consequently promotes *de novo* synthesis of cholesterol in the liver. Depending on the microbial landscape and the type of diet intake (i.e., high DF/low fat vs. low DF/high fat), altered bile acid metabolism can prove to be toxic or beneficial to the host. For example, the sustained presence and abundance of beneficial bacteria can enhance the production of protective bile acids such as ursodeoxycholic acids (UDCA). In summary, a high fat diet can increase the production of BCFA that are harmful to the host due to microbial proteolytic activities. Also, it is worth noting that the mucus-degrading bacteria are also activated when the supply of carbohydrates is scarce during starvation or fasting state.

As mentioned earlier, high fat diet intake can lead to gut dysbiosis that predisposes one's risks to developing metabolic disorders. Zeng et al., (2019) delineated the pathways where SCFA and bile acids can interact; these two signaling molecules seem to regulate each other, but also exert opposing effects on the host physiology. Bile acids are known to dissipate the pH gradient while SCFA are known to create a pH gradient. High BA environment promotes the growth of pathogenic bacteria belonging to genera *Clostridium*

(i.e. Cluster XVI), *Desulfomonas*, *Desulfobacter*, *Desulfobulbus*, *Desulfomaculum* and reduced *Bifidobacterium*, and *Lactobacillus*. High SCFA promote the growth of butyrogenic microbes (i.e., *Faecalibacteria*, *Eubacteria*, *Prevotella*, *Roseburia*, *Clostridium XIVa*, *Clostridium IV*). Although most secondary bile acids are excreted, high secondary BA promotes carcinogenesis by modulating intracellular signaling (i.e., induction of cyclooxygenase-2, secretion of matrix metalloproteinase-2, activation of plasma membrane muscarinic and epidermal growth factor receptors). In summary, secondary acids stimulate the production of superoxide radicals that are cytotoxic and genotoxic. SCFA, especially butyric acids regulate T-cell differentiation, influencing both the Th1/T effector T-cells and the regulatory T-cells (Tregs) maintaining intestinal homeostasis. As an immunomodulator, SCFA affects the activity of the immune cells via inhibiting the nuclear factor- κ B (NF- κ B) pathway and the migration of neutrophils preventing the release of pro-inflammatory cytokines and the formation of reactive oxygen species (ROS) (Sivaprakasam et al., 2016). *In vitro* studies have shown that SCFA increase the expression of IL-10 and suggested possible mechanisms (e.g., STAT3/mTOR signaling pathway activation, or by acting as an HDAC (histone deacetylase) inhibitor or through the activation of G-protein coupled receptors (GPRs)); butyrate stimulates the production of IL-10 in naïve and differentiated Th1 cells (Sun et al., 2018). In summary, SCFA such as butyrate can reduce disease risk to inflammation-related disorders/diseases by upregulating the expressions of predominantly anti-inflammatory molecules such as IL-10 and thereby preventing the formation of inflammasomes. Formation of inflammasomes put the host into a high oxidative stress state and can lead to the development of metabolic disorders including the initiation of tumorigenesis. At the genomic level, differences in genetic variants of IL-10 gene can also influence the host's inflammatory response to diseases. Single nucleotide polymorphisms (SNPs) of IL-10 are linked to GI inflammation. The GG allele is associated with high production of IL-10, individuals with the AA allele are low producers of IL-10 and those carrying the AG allele are intermediate producers of IL-10 (Gonsalkorale et al., 2003). In summary, the gut microbiome has the potential to influence the IL-10 gene architecture of the host via epigenetic mechanism.

CHAPTER 2: RESESARCH PROPOSAL

Microbiomics

On average, a human being produces about 128 grams/day of wet fecal matter; the dry component consists of 25%-54% bacterial biomass, epithelial cells, undigested food residues, large molecules (fiber, protein, mucopolysaccharides) and metabolites (Tebani et al., (2018).

Microbiomics. The gut microbiome can be explored using sequencing tools such as the next generation sequencing (NGS) technique to identify and classify bacterial species without prior cultivation steps. The 16S ribosomal DNA isolated from total bacterial DNA obtained from fecal sample are amplified for bacterial phylotyping via sequencing platforms (i.e., MiSeq (Illumina), 454 GS (Roche), Ion Torrent PGM, HiSeq, AB SOLID 5500,); each platform uses different sequence analysis tools and phylogenetic assemblers or annotation softwares to predict the phylogenetic assignments and their performance differs (i.e., length of reads, high or low throughput, sequencing fidelity/indel errors). The MiSeq takes the longest time to run but gives the lowest error. It is noteworthy that there are several pitfalls to 16S ribosomal RNA gene sequencing and one of them is that there is primer bias because there is no universal primer; the most abundant bacteria DNA isolated are well represented and the grouping of different species in clusters with more than one genomovar (DNA group) posit nomenclature problem. Furthermore, some genera are not discriminated well from others and are not as sensitive to detect species level. Also, species with multiple operons of 16S ribosomal RNA gene are overestimated.

Approximately one-third of total human microbiota are common to most people, while two-thirds are specific for each person suggesting that there may be a “core microbiota” that serves as an indicator of health status (Rojo et al., 2017). Thus far, 14 out of 664 genera are listed as core microbiota. Increased diversity and higher richness in the gut defines a normal, healthy microbial ecosystem (Rojo et al., 2017). While the 16S technique allows the uncovering of microbial structures that are not cultivable in laboratory, it cannot determine which species are actively producing metabolites and therefore altered taxonomic diversity does not infer altered microbial functions because functional redundancy exists within the gut microbial ecosystem. To measure the alteration of gene content and expression levels of SCFA-related genes (i.e., hydrolases,

glycosylates, peptidases, SCFA transporters), a whole genome shotgun metagenomics sequencing (WGS) can be utilized to pin down the true genetic potential of the host-gut microbiome metagenome and may provide greater information than the one obtained with the 16S rRNA gene sequencing method (Rojo et al., 2017).

Since dominant abundance does not necessarily mean increased expression or functionality, another omics method is needed to decipher the potential activity of the microbes and such approach includes metabolomics profiling.

Metabolomics

Metabolomics is a systems biology approach that can be used to measure what the bacteria are doing in terms of their fermentation products--metabolites. It is a science for investigating the whole metabolome of a given biological or ecological system that accounts for the interactions of genes and the environment (i.e., diet, lifestyle, microbiome) (Tebani et al., (2018). The metabolome are the metabolic/biochemical consequences of dynamic biological interactions made at the cellular and molecular level under specific conditions; these molecules may serve as predictors of disease risk. The fecal metabolome is composed of thousands of small molecules (sugars, organic acids, amino acids) (Karu et al., 2018). The emergence of systems biology approach has revolutionized biology and allowed biologists to study and measure multiple genes and their products at one point in time (Ideker et al., 2001). Systems biology approaches include genomics, transcriptomics, epigenomics, proteomics, lipidomics, and metabolomics. Metabolomics allows for the study of interactions of key metabolites and metabolic pathways in the context of environmental perturbations (Tebani et al., 2018). Metabolome is defined as "the terminal downstream product of the genome; a result of complex interactions between genome, transcriptome, proteome, and the environment" (Roberts et al., 2012). As such, these metabolites may serve as predictors of IBS symptoms. While the identification and characterization of thousands of biomolecules is efficient, the accumulation of enormous data requires sophisticated computational and analytical tools for data integration and interpretation (Ideker et al., 2001). As early as the 1960s, the advent of high-throughput metabolomics measurements tools (i.e., high field NMR, high resolution spectroscopy, NMR, LC-MS, GC-MS) equipped with visualization technologies and statistical analysis allowed

the detection of metabolites from more than one biological pathways. Since then, metabolomics has become one of the driving tools used to understand systems biology and for the discovery of biomarkers including modifications of metabolism, predictions of therapies and monitoring the progression of diseases (Pinu et al., 2019). To date, all metabolites of the human metabolome remain to be discovered; only >2000 have been identified (500 named) and there may be as many as 20,000 aqueous metabolites and 200,000 lipid metabolites (Raftery, 2016; <https://nwmetabolomics.org/services/assays-fees/>).

Fecal metabolites can be analyzed globally using an untargeted metabolomics approach to identify thousands of metabolites including novel ones that are not listed in available metabolomics databases. Such an approach is useful in carrying out exploratory analysis with the advantage of discovering new metabolites, and metabolic information pathways; however, data analysis can be time intensive. Furthermore, analyzing fecal metabolites via an untargeted approach requires a standardized protocol that maximizes extraction of all types of metabolites such as lipids and polar molecules with varying sizes; this procedure may require multiple different extraction solvents, and other fractionation methods. Untargeted metabolomics completely relies on sample integrity and generation of artifacts during sample handling and processing (i.e., methanol/solvent derived artifacts during the extraction process or during storage) may lead to false interpretations of data (Sauerschnig, 2018). On the other hand, a targeted metabolomics approach is used to identify specific metabolites that are of interest to answer specific research questions; either confirmatory or exploratory. A targeted approach almost always requires the measurement of metabolites of interest that are normalized with a reference or an internal standard to obtain reliable and reproducible data (Lu et al., 2017)

Fecal metabolome

There are several analytical tools that can be used for the detection of fecal metabolites. A human being produces about 128 grams/day of wet fecal matter; the dry component consists of 25%-54% bacterial biomass, epithelial cells, undigested food residues, large molecules (fiber, protein, mucopolysaccharides) and thousands of small molecules (sugars, organic acids, amino acids) known as the fecal metabolome (Karu, Naama, et al. 2018). ¹H-NMR is the most currently used platform for metabolite profiling. It provides structure

information and has high reproducibility and is less destructive when compared to other metabolomics identification platforms. NMR platform was chosen to identify the SCFA for this observational study.

Fecal Metabolome Background & Measurements

The majority of fecal metabolome is made up of non-volatile organic compounds, while the volatile organic compounds (VOC) represent only a fraction. Currently, about 300 unique VOCs have been identified (Karu et al., 2018). Due to differences in physical and chemical properties of metabolites, there is no single tool to measure all metabolites and the application of multiple measurement tools may be needed to capture all fecal metabolites, especially those with known important functions. High throughput analytical tools such as NMR and mass spectrometry (MS) are used for identification and quantification of unknown and known metabolites. These tools are often paired with efficient separation methods such as High-Performance Liquid Chromatography (HPLC) and/or Ultra-Performance Liquid Chromatography (UPLC) to improve resolution, sensitivity, and efficiency of detection. There are several types of MS analyzers, each comes with their own specifications. When a sample goes through a single ionization in an MS instrument, it is called a single MS configuration, while analyzers that ionize the sample in series yields tandem MS configuration or often called MS/MS. The MS/MS technique provides a deeper resolution of the fragmented compounds from the first ionization site, and thus, able to discriminate multiple isomers of a given compound (Faintuch & Faintuch 2019). The different MS platforms include the following: quadrupole (Q), linear ion trap (LIT), quadrupole ion trap (QIT), time of flight (TOF), Fourier transform ion cyclotron resonance (FTICR) and Orbitrap (Rafferty, 2016). Q, LIT, and QIT platforms offer the highest sensitivity, while TOF, FTICR and Orbitrap analyzers provide the highest resolutions of mass spectras or peaks as displayed by the instruments. The instruments used for tandem MS/MS include the triple-quadrupole ion trap (QTrap), triple quadrupole (TQ), quadrupole-TOF (Q-TOF), and linear quadrupole ion trap-Orbitrap or LTQ-Orbitrap (Rafferty, 2016). The high sensitivity and selectivity features of TQ and Qtrap MS analyzers are most suitable for targeted metabolomics, whereas the high-resolution power of T-TOF, LTQ-Orbitrap and FTICR analyzers are most suited for global/untargeted metabolomics. Other MS analyzers use ambient ionization methods and can be applied without the use of

additional chromatography separation steps and these techniques include DESI-MS, DART-MS, EESI-MS and thirty others (Raftery 2016). The principle behind NMR technology is that it uses interaction of spin active nuclei (^1H , ^{13}C , ^{31}P) with electromagnetic fields, yielding structural, chemical, and molecular environment information. While the spectra are analyzed with m/z isotopic modeling, m/z fragmentation pattern, or m/z retention time for MS platform, NMR is analyzed based on chemical shift (Faintuch & Faintuch 2019).

The various MS platforms can be packaged with biological computational/statistical tools such as principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) to analyze differentially altered metabolites that are derived from various human samples (i.e., feces, plasma, cerebrospinal fluid). A poor experimental design would show confounding between batch and treatment variable when running treatment and control fecal samples at separate times. Therefore, current protocols recommend that the metabolites should be studied together and the use of multivariate statistics such as PCA offers the best description of the data because it provides a graphical overview of the data, as well as the ability to visualize trends and identify clusters/groupings, and detect outliers (Chong et al., 2019).

Batch effects are unwanted sources of variation caused by different processing time, reagents, personnel, and machines. PCA is used to assess quality issues and the impact of batch effects (e.g., treatment and control processed at two different times are found to be clustered in 4 separate groups such as Treatment Batch (TB)1, TB2, CB1, CB2) instead of having all the treatment samples from batch1 and batch2 clustering into one group while the control batch1 and batch2 clustering as another group. Moreover, PCA is often used as a dimension reduction step and gives scores (summary of observation) and loadings (summary of variable space); loading scores can reveal which original variable contributes the most separation across the samples; however group separations of metabolite clusters are not always observed, and performing a linear discrimination analyses (LDA) may be necessary to maximize the SSB/SSW ratio (the F statistic used to reject null hypothesis) in order to obtain the best separation. The combination of PCA and LDA analyses is defined as the partial least squares discrimination analyses (PLS-DA) which is a multivariate analysis that can identify significant patterns of metabolites. Such patterns of expressions can be best visualized using heatmap which

provides “a visually intuitive overview of large “omic” data sets with or without the use of hierarchical clustering (Chong et al., 2019). The MetaboAnalyst5.0 software can generate a heatmap with dendrograms and the researcher can investigate the patterns of SCFA concentration changes across different groups.

Measurement Variation

There are several factors that can influence variability in microbiome-related metabolites. The reproducibility of fecal metabolomics data obtained from MS can be difficult. In addition to systematic errors that are inherent from similar instruments and environments (including the technician), other sources of variability include the following variables: 1) Fecal sample collection and storage, 2) Sample preparation before identification procedure, 3) Sample preparation before identification procedure. Optimal extraction of metabolites is carried out within an hour of stool collection. As described previously, stool sample collected within 24-hour is recommended to remain on ice if processing can be done within that period, otherwise, it is recommended to be homogenized, aliquoted to prevent multiple thawing of the sample, and stored at -80°C. Gratton et al., recommend that extracted, “ready to inject” processed sample should be stored in the freezer if sample analysis cannot be done on the same day. Again, it is recommended for these samples to be aliquoted in batches when researcher is planning on doing the analysis using different platforms (i.e., GC-MS, LC-MS, NMR) to obtain a more enriched coverage of the fecal metabolome. The transport of these extracted metabolites should be kept at -80°C to prevent unnecessary chemical and enzymatic changes leading to alteration of metabolites. Gratton et al., have demonstrated that extracted materials that are thawed three times has resulted in increased identification of phenylalanine and decreased N6-acetyllysine compared to control that was frozen only once (Gratton et al., 2016). Based on Karu et al., review on fecal metabolomics, differences in metabolic profiling could also be attributed from employing non-standardized sample extraction procedures and solvents that can modify the metabolites and add to metabolite variation upon analysis. Some solvents can increase artifacts (i.e., addition of methyl or methoxy groups) resulting in increased intensity of some metabolites and masking others that are not abundantly represented (Karu et al., 2018). Consistency of Fecal sample & Contaminants, 4) Standardized Sample Preparation Protocol, 5) Timing of fecal collection.

CHAPTER 3: METHODS

Study Design

This research project is a secondary, observational, case-control study. The goal is to describe the relationships of SCFA and the previously collected data (16S rDNA, diet record, IBS GI symptoms diary) from a NIH-funded study, Microbiome and Abdominal Pain (MAP), NRO14479 (Heitkemper, PI). Fecal samples from 30 women with IBS and 30 healthy control (HC) women were randomly placed into four batches (15 samples/batch) using a computer-generated program from a box of aliquoted and numerically labeled fecal samples stored at -80°C. These fecal subsamples were used to quantify SCFA metabolites using NMR platform at Dr. Raftery's laboratory located at the UW-Northwest Metabolomic Research Center (NWMC) in Seattle. We hypothesized that fecal SCFA and butyrate-producing bacterial genera would be associated with abdominal pain severity scores and stool consistency (diarrhea, constipation) measures. Before doing the metabolomics work, an "in silico" experiment was performed with 40 of the 60 samples. We expected there would be differences in SCFA fluxes between IBS and HC given the 16S data (bacteria community) and the western diet environment. Next, a new compositional analysis of the 16S rDNA sequencing data from MAP study was done prior to SCFA extractions.

Study Population

Fecal samples were drawn from the MAP study (NR014479), an exclusively female cohort. Participants were self-referred in response to community advertisements and notices in gastroenterology practices and screened by telephone for study eligibility. IBS patients and HCs were recruited in the Pacific Northwest from 2014 to 2016. All IBS participants met ROME III criteria. Under Rome III, a person must be experiencing chronic abdominal pain or discomfort at least 3 days per month in the last 3 months with onset of symptoms at least 6 months before diagnosis with two or more of the following: 1) improvement with defecation, 2) change in stool frequency and/or 3) change in form or appearance of the stool. Women with IBS and healthy women were matched on age, race, gender, and BMI (Body Mass Index). Inclusion criteria: 1) Participants were >18 years of age (range: 18-50), 2) proficient in English language, 3) completed consent

forms, 4) completed 3-day food diary, 4) completed 28-day IBS symptom diary, and 5) completed stool diary using the Bristol Stool Form (BSF) scale. Exclusion criteria. 1) diagnosis of celiac disease and other history of GI diseases/disorders, 2) on dietary restriction, 3) use of over-the-counter probiotics, laxatives, GI medications, 4) history of acute gastroenteritis, 5) use of antibiotics within the past two months, 6) history of other medical comorbidities such as heart diseases, cancer, severe psychological and neurological disorders, 7) enrolled in other GI research studies involving psychological treatments and dietary intervention programs. Women with IBS had to report at least 25% of days with moderate to severe symptoms on a 2-week symptom diary prior to study entry.

The Daily Diary

The 28-day IBS Symptom diary was used to look at the relationship between GI symptoms and SCFA. Twenty-six symptoms were recorded each evening for 4 weeks. The mean of the daily scores on 0 to 4 scale (0: not present, 1: mild, 2: moderate, 3: severe, 4: very severe) was used for the analysis that included abdominal pain, stomach pain, flatulence, bloating. Stool consistency ratings range from 1 to 5 (where 1: very hard to 5: very loose) to determine the presence of constipation and diarrhea. The MAP collaborators used the 16S data to compute diversity measures (Shannon and operational taxonomic units or OTUs Relative abundance). IBS Distress score (Severity) is the measurement concept. There are 26 items under the subheadings of 1) GI Pain: abdominal pain, stomach pain, abdominal distension, bloating and intestinal gas; 2) Stool Characteristics: diarrhea, constipation, urgency; 3) Psychological items: anger, anxiety, depressed or blue, stressed, fatigue/tiredness, and sleepiness during the day; 4) Somatic symptoms: backache, headache, joint pain, muscle pain; and 5) Stool Consistency: rated on a 5-point scale: very hard, hard, formed, loose, or watery. Symptoms were rated from 0 (not present) to 4 (extreme). Daily GI Distress Score calculated based on the most severe GI symptom on any given day and the advantage of this reporting is to decrease retrospective bias associated with recalled symptoms.

The 3-day Dietary Record

The 3-day food record was completed during the stool collection period. Food details included cooking methods, ingredients, brands of foods, food serving size, and estimated calorie content were analyzed by Nutrition Center at the FHCRC. Food intake was considered standard, personalized diet by the participants.

Fecal Sample

Of the 127 women who completed the MAP study, 60 samples were used for this project. Study participants collected stool samples during the early follicular phase of their menstrual cycle. They were provided with plastic catch containers (Stool collection kit, purchased from Eagle Biosciences, SKU: CAL35-C50) for stool collection. Each fecal sample collected was stored in the study participant's freezer for 1 to 2 days before delivering them to the laboratory. Each fecal sample was thawed, aliquoted, and stored at -80°C. Extracted DNA from each of the aliquoted samples were sent for 16S rRNA gene sequencing at Tor Savidge Laboratory in Texas Children's Medical Center. Reverse and forward V4 primers were used to amplify the variable region of V4 using the Illumina MiSeq sequencer platform. There are nine primers that can be used to sequence the variable regions of the ribosomal RNA gene (Abellan-Schneyder et al., 2021). Unused fecal sample aliquots were used for metabolomics study.

MICOM/ Flux Balance Analysis

Preliminary metabolic modeling of the human gut microbiome (also called Flux Balance analysis) was carried out on 40 samples (25 IBS, and 15 HC) by Dr. Diener & Dr. Gibbons at the Institute for Systems Biology. This method uses individual-specific 16S abundance profiles as input to initialize the in-silico gut bacterial community structure and a standard Western diet as the dietary input across all individuals. To model conditions within the human gut, the algorithm depletes all the nutrients that are usually absorbed in the small intestine by 90%. The MICOM framework uses constraint-based flux-balance analysis to identify a unique steady-state solution by maximizing total community biomass; however, integrates individual species-level

biomass maximizations through a computationally tractable heuristic approach (i.e., an L2 penalty). These in silico experiments identified gut metabolic features unique to IBS and HC phenotypes. Significant differences in metabolic fluxes across models can be analyzed to draw inferences about gut ecosystem functions. A comparative statistical analysis was done based on the average Western diet and providing that every person consumed the same nutrients. MICOM analysis reference can be found in this webpage:

<https://journals.asm.org/doi/10.1128/msystems.00606-19?permanently=true> and <https://github.com/micom-dev/q2-micom/blob/master/docs/README.md>.

Compositional Analysis 16S rDNA amplicon from MAP study

Characterization of the microbiota profile was done using the next generation sequencing technique (Savidge laboratory). Phylogenetic analysis on the 16S rDNA sequence was completed to determine which bacteria are enriched in women with IBS and in HC women. Statistical analysis of microbiota composition was analyzed previously by the MAP study. In 2017, Relative Abundance was defined as $100 * (\text{count for bacteria taxa}) / (\text{sum of counts for all taxa for one fecal donor})$. In June of 2020, the sequencing data was again re-analyzed for new bacterial composition. The next generation sequencing data (amplicon data of 16S rDNA variable region of V4) was generated from the MiSeq Illumina platform and converted into FASTQ files, a text-based sequencing data file format with quality scores. These FASTQ files containing raw sequencing data were obtained from the Savidge laboratory.

Amplicon data of 16S rDNA variable region of V4 was generated from MiSeq platform with paired-end sequencing protocol (effective read length: 300 bp for each V4 primer). The pair-end sequencing protocol uses two steps PCR: first PCR amplifies the 16S region of the rDNA and the second PCR applies the illumine specific adaptors to amplicon fragments that allow multiple DNA fragments to be sequenced simultaneously. MISEQ is the longest running instrument (analysis takes 27 hour), gives high sequencing depths (60 Megabase/hr) but gives the lowest error when compared to other NGS instruments such as the Roche 454 GS Junior (longest read, moderate error, lowest throughput) and the Ion Torrent PGM (shortest read, worst

performance, high throughput). Manifest file of the FASTQ files was created, repeated sequencing data per sample were concatenated. The Virtual analysis machine Oracle was uploaded into a personal computer to run the data where QIIME2 Core-2020.2 microbiome analysis pipeline was installed; qiime2 plugins (i.e. DADA2, FastTree) were uploaded used to demultiplexing, artifacts generation, and for visualization of data (<https://docs.qiime2.org/2023.2/>). The manifest data was used as an input into the virtual analysis machine and the output was a demultiplexed form of the manifest data. Absolute file path was created to save all the data. SILVA database (release 132) was used for taxonomy assignment of amplicon sequence variants (ASVs) from qiime2-DADA2 output. The 10 most abundant taxa of the gut bacteria composition in all 3 cohorts based on un-normalized 16S ribosomal gene sequencing data.

SCFA Extraction Procedures/Metabolomics

Fifteen randomized fecal samples were processed per batch per day; all 60 samples were processed in the same week and end products were placed at -80°C. Feces were resuspended in millwater (avoiding all particulate material such as fiber and gel-like material) and transferred into an Eppendorf tube. The tubes were dried down using a speed vacuum under the hood for 3 hours at room temperature. Fecal dry weight was measured. Approximately 300 mg (100 count) microbeads were added into each tube. To each tube, 400 microliter milliQ water was added, the caps sealed with parafilm and vigorously vortexed for 30 seconds at room temperature. Next, the tubes were placed in a homogenizer (Next Advance Bullet Blender) for 5 minutes at 4 °C; this homogenization step was carried out twice. The samples were then placed on ice. 800ul of absolute methanol was added to each tube and vortexed at maximum speed for 60 seconds at room temperature. The tubes were then placed at -20 °C for 30 minutes. Following this step, tubes were centrifuged at 14,000 rpm for 30 minutes at 4 °C. Supernatant was collected and transferred to a 2-ml Eppendorf tube and dried down using a speed vacuum for 6 hours at room temperature. The dried materials were stored at -80 °C prior to NMR analysis. After two weeks, the lyophilized/dried samples were resuspended with a 600 microliter Deuterium buffer containing 25 micromolar TSP as an internal standard. Each tube was vortexed for 30 seconds at room temperature and 500 microliters of resuspended material (fecal extract) was transferred to a

5mM NMR tube. Each fecal extract was injected and analyzed using NMR (298 K, Bruker Avance III 800 MHz) spectrometer equipped with a cryogenically cooled probe and z-gradients suitable for inverse detection; this machine is capable to routinely run water suppression experiments (per conversation with a Bruker specialist). Quantitation analysis was performed based on TSP-d4 reference.

Data Analyses

The associations between 2 variables from the 4 different domains of interests (diet, individual SCFA, GI symptoms, and bacteria genera relative abundance) were compared among IBS versus HC groups and analyzed in a univariate fashion using R software (version 3.61). Then, Pearson exploratory bivariate correlational analyses of variables from these domains (diet, 16S rDNA sequence bacterial taxa data, individual SCFA, and GI symptoms) were performed using R software. Lastly, multivariate analyses were done to study the association between an absolute concentration of a metabolite and high fiber intake (>25g fiber/day) adjusting for other covariates (i.e., (fat, some bacteria genera, BMI, IBS status); log transformation was done to satisfy model assumption of the generalized, linear multi-regression model. Statistical analysis of fecal metabolomics data and graphical representation were performed in R software (version 3.6.3).

Statistical Power

The participants in this project were subsampled from MAP study (NR014479), with a logistically constrained total sample size of 60 ($n[\text{IBS-C}] = 10$, $n[\text{IBS-D}] = 25$, and $n[\text{HC}] = 25$). Consistent with the specific aims, power disclosure analyses were performed for 3 basic analysis types: 1) a set of three 2-group comparisons: ($n=25$ vs $n=35$); ($n=25$ vs $n=25$) and ($n=25$ vs $n=10$) adjusting for 3 multiple comparisons, 2) a correlation across the whole sample ($n=60$), and 3) a correlation across the union of the two IBS subsets ($n=30$). The goals of the power analyses were to compute the smallest effect size that would have good power, based on the sample size, the significance threshold $\alpha = 0.05$ and the statistical power = 0.80. Due to limited resources, the total sample number was 60: 25 HC, 10 IBS-C and 25 IBS-D. For analyses involving all 60 subjects there will be 80% power for detecting a moderate to large effect size: standardized effect size for

IBS versus HC of 0.75, and a correlation of 0.36. However, subgroup analyses will only have 80% power if the effect sizes are quite large: standardized effect size of 1.05 for IBS-D vs IBS-C, and correlation 0.70 within each of the IBS-C and IBS-D groups. Nevertheless, these subgroup analyses may provide useful data by showing trends that could justify doing a larger study to validate the results. For further visualization of the data i.e., generating heatmaps, obtaining functional data/generating relevant biological pathways and generating biomarker discovery (uses PCA and PLS-DA statistical analyses), the whole fecal metabolites obtained from the NMR results were submitted in the MetaboAnalyst webpage: <https://www.metaboanalyst.ca>; the package has a built-in R package that annotate and interpreted the submitted metabolomics data to identify important nodes/hubs that are associated with SCFA metabolism (Chong, Wishart, Xia, 2019). Data transformation: Log transformation (base 10). For data scaling Pareto Scaling: (mean-centered and divided by the square root of the standard deviation of each variable), was completed.

MetaboAnalyst R Analysis for the 9 Metabolites (Absolute concentration in Micromole)

Log normalization of the NMR data was performed for all visualization (i.e., heatmaps, enrichment ratios of metabolites, biomarker discovery, pathway analysis). Pathway enrichment analysis was done to identify the most relevant pathways involved HC and IBS conditions; helps visualize the differences in SCFA metabolism in IBS and HC. An impact pathway score analysis of 0.5 is considered biologically meaningful.

CHAPTER 4 & 5: RESULTS & DISCUSSIONS

AIM1 RESULT: SCFA Concentrations between IBS and HC Women

It was hypothesized that IBS cohorts regardless of bowel group will have reduced fecal SCFA compared to HC and that lower total SCFA will be associated with higher abdominal pain severity. As shown in Figure 1, the average fecal dry weight for HC women was 116.8 mg (sd±30.3), IBS-C women was 119.2 mg (sd±28.6), IBS-D women was 116.9 mg (sd±0.3). It was also expected that total and individual fecal SCFA will not correspond to the production of the actual colonic acid SFCA fermentation in the gut. Note: The measured fecal SCFA via NMR platform yielded a thousand-fold lower when compared to reported fecal SCFA measured from healthy humans at time of death. Univariate analyses were performed to identify key metabolites at a significant level ($p<0.05$) to discriminate between groups. Given the method used for the identification of these metabolites, the concentration yielded a thousand-fold less than the actual colonic SCFA production in humans.

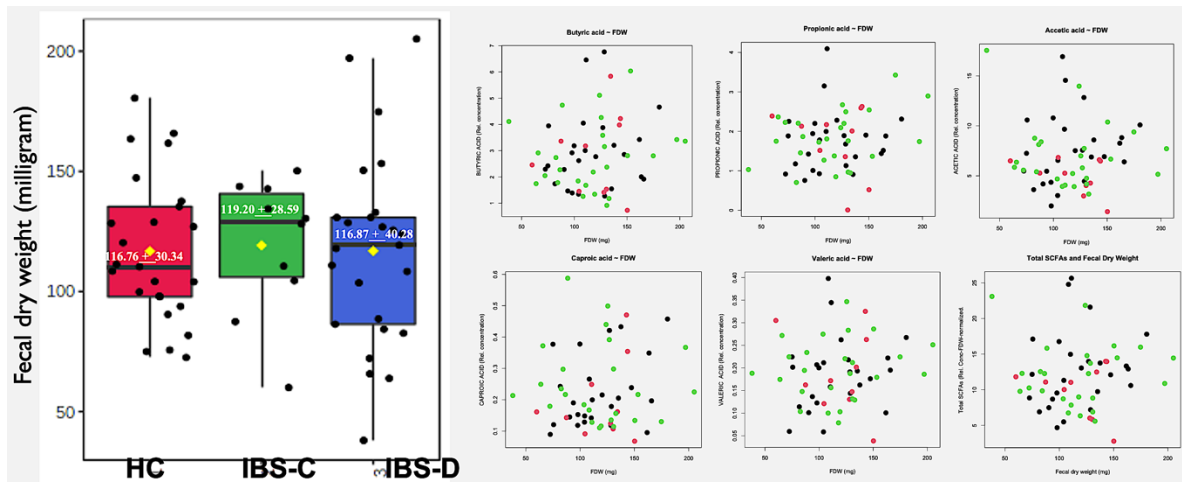


Figure 1a. Linear relationship of fecal dry weight (FDW) and measured fecal SCFA.

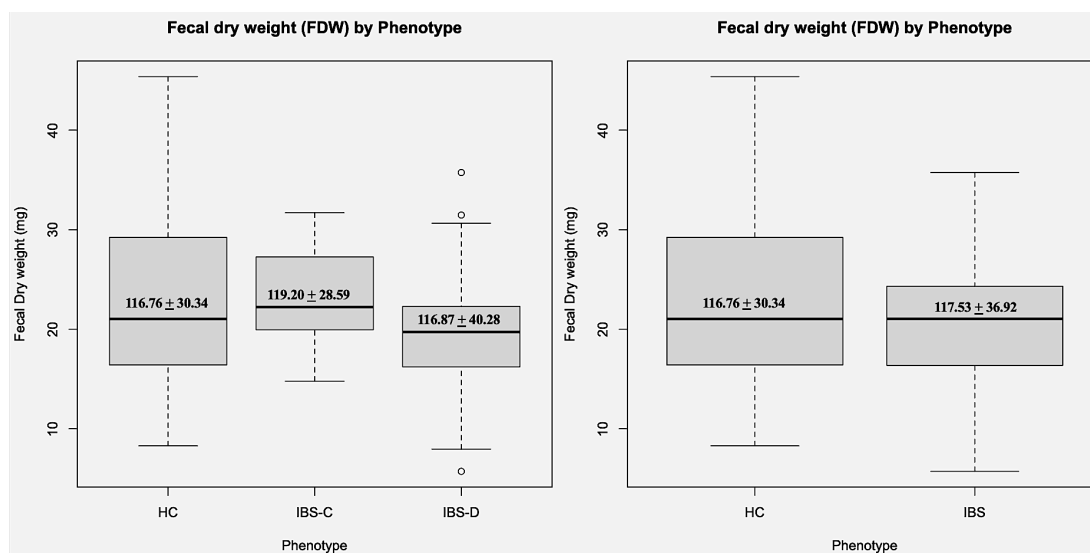


Figure 1b. Boxplot of fecal dry weight (FDW) in IBS and HC.

Table 1. Mean and Standard Deviation for the Absolute Concentration of Fecal SCFA (micromole)

Metabolites	HC n = 25) Mean $\pm$ SD	IBS (n = 35) Mean $\pm$ SD	IBS-C (n = 10) Mean $\pm$ SD	IBS-D (n = 25) Mean $\pm$ SD
Acetic acid	248.95 $\pm$ 133.42	196.23 $\pm$ 104.88	154.58 $\pm$ 63.64	212.89 $\pm$ 114.24
Propionic acid	93.8 $\pm$ 46.1	100.48 $\pm$ 59.2	97.71 $\pm$ 44.87	101.609 $\pm$ 64.85
Butyric acid	138.64 $\pm$ 83.35	133 $\pm$ 87.9	134.55 $\pm$ 92.86	132.38 $\pm$ 87.81
Caproic acid	19.01 $\pm$ 12.87	18.87 $\pm$ 12.65	16.66 $\pm$ 13.75	19.75 $\pm$ 12.37
Valeric acid	21.94 $\pm$ 11.19	22.44 $\pm$ 12.04	29.39 $\pm$ 12.01	22.86 $\pm$ 12.27
Lactic acid	47.48 $\pm$ 50.30	47.13 $\pm$ 43.51	39.82 $\pm$ 25.46	50.05 $\pm$ 49.07
Formic acid	1.42 $\pm$ 1.97	10.91 $\pm$ 4.65	0.67 $\pm$ 0.31	0.97 $\pm$ 0.60
sc-SCFA	481.38 $\pm$ 248.6	429.72 $\pm$ 226.36	386.83 $\pm$ 185.09	446.87 $\pm$ 242.21
bc-SCFA	40.96 $\pm$ 21.98	41.31 $\pm$ 22.94	38.05 $\pm$ 25.35	42.61 $\pm$ 22.33
totalSCFA	522.34 $\pm$ 265.26	471.02 $\pm$ 244.99	424.88 $\pm$ 207.88	489.48 $\pm$ 259.97

Table 2. Ranges for the Measured Fecal SCFA (micromole)

Metabolites	HC (n=25)	IBS-C (n=10)	IBS-D (n= 25)
Acetic acid	52.87 - 496.15	57.67 - 254.97	101.02 - 467.70
Propionic acid	30.28 - 206.88	35.55 - 171.72	17.82 - 272.20
Butyric acid	53.94 - 344.33	43.40 - 310.97	44.07 - 366.86
Caproic acid	4.52 - 57.54	6.65 - 46.79	5.65 - 50.42
Valeric acid	4.25 - 47.53	5.71 - 45.71	7.05 - 50.75
Lactic acid	11.97 - 257.89	14.49 - 97.98	10.53 - 212.96
Formic acid	0.36 - 10.30	0.35 - 1.42	0.35 - 2.61
sc-SCFA	17.14 - 275.21	17.83 - 105.65	19.01 - 251.23
bc-SCFA	0.00 - 100.00	46.00 - 101.89	0.00 - 54.46
Total SCFA	0.00 - 100.00	46.71 - 103.26	0.93 - 56.04

Table 3. Raw p-value for the Individual SCFA (micromole)

Metabolites	Healthy (n = 25) Vs. IBS (n = 35)	Healthy (n = 25) Vs. IBS-C (n = 10)	Healthy (n = 25) Vs. IBS-D (n = 25)	IBS-C (n = 10) Vs. IBS-D (n = 25)
	Raw p	Raw p	Raw p	Raw p
Acetic acid	0.060	0.01	0.16	0.04
Propionic acid	0.31	0.41	0.31	0.42
Butyric acid	0.40	0.45	0.4	0.48
Caproic acid	0.48	0.33	0.4	0.28
Valeric acid	0.43	0.06	0.39	0.09
Lactic acid	0.48	0.28	0.43	0.22
Formic acid	0.10	0.049	0.14	0.04
sc-SCFA	0.21	0.12	0.31	0.22
bc-SCFA	0.48	0.38	0.4	0.32
sc-SCFA+ bc-SCFA	0.23	0.14	0.33	0.23

Table 4. FDR Adjusted p-value for the Individual SCFA (micromole)

Metabolites	Healthy (n=25) Vs. IBS (n=35)	Healthy (n=25) Vs. IBS-C (n=10)	Healthy (n=25) Vs. IBS-D (n=25)	IBS-C (n=10) Vs. IBS-D (n=25)
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid	0.08	0.04	0.16	0.08
Propionic acid	0.42	0.42	0.42	0.42
Butyric acid	0.48	0.48	0.48	0.48
Caproic acid	0.48	0.48	0.48	0.48
Valeric acid	0.43	0.18	0.43	0.18
Lactic acid	0.48	0.48	0.48	0.48
Formic acid	0.13	0.098	0.14	0.098
Sc-SCFA	0.29	0.29	0.31	0.29
bc-SCFA	0.48	0.48	0.48	0.48
sc-SCFA+ bc-SCFA	0.31	0.31	0.33	0.31

The mean absolute concentration of sc-SCFA was higher in HC group and the mean bc-SCFA was higher in women with IBS these differences were not significant. The mean tryptophan level was higher in the IBS-C

group as compared to HC (p = 0.049). The IBS-D group had significantly higher mean formic acid compared to IBS-C subtype (p = 0.04). Table 1B shows the IBS-D cohort had higher mean lactic acid compared to IBS-C or HC. Table 11 lists the summary t-test results of the four group comparisons. After FDR adjustment for multiple comparison, the current study found significantly lower acetic acid in IBS-C compared to HC (p = 0.04) and only marginally lower compared to IBS-D (p = 0.08).

Table 5. Tukey Multiple Comparisons of Means (Absolute Concentration) Using ANOVA: 95% Family-Wise Confidence Level

	HC -IBS-C			HC-IBS-D			IBS-C-IBS-D			F-stat
Metabolites (micromole)	Diff	CI	p-adj.	Diff	CI	p-adj.	Diff	CI	p-adj.	
Acetic acid	-94.37	-199.49, 10.75	0.09	-36.05	-115.52, 43.41	0.52	58.32	-46.80, 163.44	0.38	2.38
Propionic acid	3.91	-45.27, 53.09	0.98	7.80	-29.38, 44.98	0.87	3.89	-45.29, 53.07	0.98	0.13
Butyric acid	-4.09	-82.24, 74.06	0.99	-6.26	-65.33, 52.82	0.96	-2.17	-80.32, 75.99	1.00	0.03
Caproic acid	-2.35	13.88, 9.18	0.88	0.74	-7.98, 9.45	0.98	3.09	-8.44, 14.62	0.80	0.21
Valeric acid	-0.55	-11.17, 10.06	0.99	0.91	-7.11, 8.94	0.96	1.47	-9.14, 12.08	0.94	0.07
Lactic acid	-7.65	-49.71, 34.40	0.98	10.23	-29.22, 34.37	0.98	10.23	31.83, 52.28	0.83	0.17
Formic acid	-0.75	-1.96, 0.457	0.30	-0.45	-1.37, 0.46	0.46	0.30	-0.91, 1.51	0.82	1.35

Table 6. Proportions of SCFA (micromole)

Metabolites	Mean ± SD (Absolute concentration in micromole)				Healthy (n=25) Vs. IBS (n=35)	Healthy (n=25) Vs. IBS- C (n=10)	Healthy (n=25) Vs. IBS- D (n=25))	IBS-C (n=10) Vs. IBS- D (n=25)
	Healthy (n =25)	IBS (N=35)	IBS-C (n= 10)	IBS-D (n= 25)	p-value	p-value	p-value	p-value
Acetic acid/Total	0.47 ± 0.06	0.43 ± 0.09	0.38 ± 0.08	0.44 ± 0.08	0.02	0.005	0.07	0.04
Propionic acid/Total	0.19 ± 0.04	0.22 ± 0.05	0.23 ± 0.02	0.21 ± 0.06	0.008	0.002	0.09	0.09
Butyric acid/Total	0.26 ± 0.05	0.27 ± 0.07	0.30 ± 0.08	0.26 ± 0.06	0.26	0.087	0.5	0.09
Caproic acid/Total	0.04 ± 0.18	0.04 ± 0.016	0.04 ± 0.01	0.04 ± 0.02	0.5	0.5	0.5	0.5

Valeric acid/Total	$\frac{0.04}{+0.012}$	$\frac{0.05}{0.014}$	$\frac{0.05}{+0.01}$	$\frac{0.05}{0.01}$	0.0004	0.0008\	0.5	0.013
sc-SCFA /sc+bc	0.92 ± 0.03	0.91 ± 0.03	0.91 ± 0.02	0.91 ± 0.03	0.20	0.14	0.13	0.5
bc-SCFA /sc+bc	0.08 ± 0.03	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.03	0.10	0.14	0.13	0.5

Table 7. FDR adjusted p-value for the Proportions of SCFA (micromole)

Metabolites	Healthy (n=25) Vs. IBS-(n=35)	Healthy (n=25) Vs. IBS-C (n=10)	Healthy (n=25) Vs. IBS-D (n=25))	IBS-C (n=10) Vs. IBS-D (n=25)
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid/Total	0.11	0.02	0.50	0.18
Propionic acid/Total	0.06	0.01	0.50	0.20
Butyric acid/Total	0.48	0.21	0.50	0.20
Caproic acid/Total	0.50	0.50	0.50	0.50
Valeric acid/Total	0.01	0.01	0.50	0.18
sc-SCFA /sc+bc	0.46	0.21	0.5	0.5
bc-SCFA /sc+bc	0.28	0.21	0.5	0.5

Table 8. Ranges for the Relative Concentration of SCFA Normalized with Fecal Dry Weight (FDW)

Metabolites	HC (n=25)	IBS-C (n=10)	IBS-D n= 25)
Acetic acid	2.00 - 16.92	1.42 - 6.84	3.18 - 17.55
Propionic acid	0.76 - 4.09	0.52 - 2.63	0.70 - 3.43
Butyric acid	1.27 - 6.76	0.73 - 5.84	0.91 - 6.04
Caproic acid	0.09 - 0.46	0.07 - 0.47	0.11 - 0.59
Valeric acid	0.06 - 0.40	0.04 - 0.32	0.08 - 0.35
Lactic acid	0.28 - 5.86	0.24 - 2.31	0.36 - 5.59
Formic acid	0.00 - 0.15	0.00 - 0.02	0.00 - 0.04
sc-SCFA	1.78 - 9.34	0.35 - 1.90	0.37 - 2.54
bc-SCFA	0.25 - 1.89	0.65 - 3.91	1.00 - 6.08
Total SCFA	2.22 - 10.23	1.00 - 5.81	1.37 - 8.61

Table 9. Mean and Standard Deviation for the Relative Concentration of Fecal SCFA

Metabolites Mean $\pm$ SD	HC n= 25) Mean $\pm$ SD	IBS (n=35) Mean $\pm$ SD	IBS-C (n= 10) Mean $\pm$ SD	IBS-D n= 25) Mean $\pm$ SD
Acetic acid	7.714 ± 3.580	6.387 ± 3.048	4.969 ± 1.790	6.954 ± 3.285
Propionic acid	1.752 ± 0.748	1.802 ± 0.738	1.732 ± 0.884	1.829 ± 0.690
Butyric acid	2.936 ± 1.443	2.795 ± 1.374	2.816 ± 1.594	2.786 ± 1.312

Caproic acid	0.224 ± 0.113	0.231 ± 0.130	0.193 ± 0.129	0.246 ± 0.130
Valeric acid	0.187 ± 0.079	0.193 ± 0.073	0.187 ± 0.089	0.196 ± 0.068
Formic acid	0.0205 ± 0.029	0.0137 ± 0.010	0.0097 ± 0.005	0.0153 ± 0.012
sc-SCFA	12.40 ± 5.42	10.98 ± 4.23	9.52 ± 3.61	11.57 ± 4.39
bc-SCFA	0.411 ± 0.162	0.424 ± 0.184	0.380 ± 0.205	0.442 ± 0.176
Total SCFA	12.81 ± 5.51	11.41 ± 4.34	9.90 ± 3.78	12.01 ± 4.47

Table 10. Raw p-value for the Individual Relative Concentration of FDW Normalized SCFA Comparisons

Metabolites	Healthy (n=25) Vs. IBS (n=35)	Healthy (n=25) Vs. IBS-C (n=10)	Healthy (n=25) Vs. IBS-D (n=25)	IBS-C (n=10) Vs. IBS-D (n=25)
	Raw p	Raw p	Raw p	Raw p
Acetic Acid	0.072	0.007	0.22	0.024
Propionic Acid	0.40	0.48	0.35	0.38
Butyric Acid	0.41	0.42	0.35	0.48
Caproic Acid	0.413	0.261	0.26	0.151
Valeric Acid	0.38	0.50	0.34	0.39
Lactic Acid	0.465	0.315	0.385	0.215
Formic Acid	0.14	0.05	0.21	0.042

Table 11. FDR Adjusted p-value for the Relative Concentration of SCFA Comparisons

Metabolites	Healthy (n=25) Vs. IBS (n=35)	Healthy (n=25) Vs. IBS-C (n=10)	Healthy (n=25) Vs. IBS-D (n=25)	IBS-C (n=10) Vs. IBS-D (n=25)
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic Acid	0.10	0.03	0.22	0.048
Propionic Acid	0.48	0.48	0.48	0.48
Butyric Acid	0.48	0.48	0.48	0.48
Caproic Acid	0.41	0.35	0.35	0.35
Valeric Acid	0.50	0.50	0.50	0.50
Lactic Acid	0.46	0.46	0.46	0.46
Formic Acid	0.10	0.11	0.21	0.11

Table 12. Mean and Standard Deviation for the Relative Concentration of Other Metabolites

Metabolites	Mean ± SD (Relative Concentration in Micromole) FECAL DRY WEIGHT NORMALIZED				Healthy (n=25) Vs. IBS (n=35)	Healthy (n=25) Vs. IBS- D(n=25)	Healthy (n=25) Vs. IBS- C(n=10)	IBS-C (n=10) Vs. IBS- D (n=25)
	Healthy (n =25)	IBS (N=35)	IBS-C (n= 10)	IBS-D (n= 25)	p-value	p-value	p-value	p-value

2-Aminobutyric acid	0.067 ± 0.041	0.079 ± 0.073	0.043 ± 0.014	0.094 ± 0.082	0.21	0.08	0.02	0.007
Tryptophan	0.0300 ± 0.0124	0.034 ± 0.012	0.0324 ± 0.0102	0.0351 ± 0.0129	0.11	0.08	0.28	0.26
Aspartic acid	0.210 ± 0.138	0.268 ± 0.109	0.285 ± 0.129	0.261 ± 0.102	0.047	0.08	0.08	0.31
Fumaric acid	0.0131 ± 0.007	0.012 ± 0.008	0.014 ± 0.011	0.011 ± 0.006	0.31	0.16	0.40	0.22
Urocanic acid	0.15 ± 0.057	0.14 ± 0.061	0.118 ± 0.043	0.152 ± 0.065	0.26	0.45	0.053	0.05
UDP-glucuronic acid	0.008 ± 0.0074	0.0157 ± 0.0263	0.006 ± 0.0022	0.0196 ± 0.0304	0.06	0.04	0.112	0.03
Malonic acid	0.1856 ± 0.1032	0.2051 ± 0.1505	0.1407 ± 0.0786	0.2309 ± 0.1653	0.28	0.13	0.10	0.03
Putrescine	0.278 ± 0.204	0.249 ± 0.114	0.209 ± 0.078	0.266 ± 0.123	0.26	0.39	0.09	0.07
Succinic acid	1.354 ± 3.261	0.625 ± 1.260	0.195 ± 0.153	0.797 ± 1.461	0.15	0.22	0.06	0.04
Nicotinic acid	0.0154 ± 0.0048	0.0144 ± 0.0051	0.0125 ± 0.0045	0.0151 ± 0.0052	0.21	0.42	0.06	0.09
3-Methyl-Oxovaleric Acid	0.0735 ± 0.0403	0.0638 ± 0.0301	0.0569 ± 0.0278	0.0666 ± 0.0311	0.16	0.25	0.10	0.19
3-HydroxyPhenyl acetic acid	0.0448 ± 0.0345	0.0531 ± 0.0312	0.0520 ± 0.0392	0.0535 ± 0.0284	0.18	0.17	0.31	0.45
Choline	0.098 ± 0.130	0.069 ± 0.0429	0.0476 ± 0.0183	0.078 ± 0.047	0.15	0.23	0.04	0.01
Glutamic acid	2.889 ± 1.134	2.567 ± 0.711	2.282 ± 0.717	2.681 ± 0.690	0.11	0.219	0.05	0.08
Pyroglutamic acid	0.0511 ± 0.029	0.0502 ± 0.0305	0.0321 ± 0.0070	0.0575 ± 0.0332	0.46	0.24	0.007	0.003
D-galactose	0.033 ± 0.0276	0.0519 ± 0.1011	0.0215 ± 0.0085	0.0641 ± 0.118	0.15	0.11	0.05	0.05
D-xylose	0.115 ± 0.0994	0.113 ± 0.126	0.0718 ± 0.020	0.130 ± 0.146	0.47	0.33	0.03	0.04
Valine	1.065 ± 0.467	1.047 ± 0.487	0.942 ± 0.429	1.089 ± 0.511	0.44	0.43	0.24	0.20
Alanine	2.7390 ± 1.4128	2.4207 ± 1.1587	2.0765 ± 0.9436	2.5584 ± 1.2243	0.18	0.32	0.07	0.12
Proline	0.160 ± 0.0752	0.173 ± 0.105	0.120 ± 0.0421	0.194 ± 0.1151	0.29	0.11	0.04	0.01
Threonine	0.156 ± 0.0577	0.188 ± 0.0673	0.176 ± 0.0659	0.193 ± 0.0685	0.03	0.025	0.21	0.25
Uracil	0.214 ± 0.0902	0.1818 ± 0.0776	0.156 ± 0.0601	0.192 ± 0.0825	0.08	0.19	0.03	0.09
Hypoxanthine	0.1242 ± 0.0533	0.1140 ± 0.0479	0.1217 ± 0.0491	0.1109 ± 0.0480	0.23	0.182	0.45	0.28
Dihydroxyacetone	0.021 ± 0.023	0.020 ± 0.017	0.017 ± 0.019	0.015 ± 0.017	0.43	0.15	0.30	0.39
Sarcosine	0.0801 ± 0.0852	0.0908 ± 0.0962	0.0642 ± 0.0372	0.1014 ± 0.1104	0.33	0.24	0.23	0.09
Glycine	0.0941 ± 0.0410	0.0919 ± 0.0526	0.0789 ± 0.0229	0.0971 ± 0.0601	0.43	0.42	0.10	0.11

Dimethylglycine	0.0439 ± 0.0361	0.0601 ± 0.0803	0.0493 ± 0.0354	0.0645 ± 0.0927	0.15	0.16	0.35	0.25
Glutamine	0.4410 ± 0.2194	0.4083 ± 0.1092	0.4041 ± 0.1071	0.4099 ± 0.1121	0.25	0.27	0.26	0.44
Tyrosine	0.3527 ± 0.1572	0.3855 ± 0.1507	0.3633 ± 0.1288	0.3944 ± 0.1601	0.21	0.18	0.42	0.28
Methionine	0.2101 ± 0.0794	0.2332 ± 0.0888	0.2259 ± 0.0825	0.2362 ± 0.0927	0.15	0.15	0.31	0.38
Leucine	1.7199 ± 0.7812	1.7722 ± 0.7337	1.5899 ± 0.6384	1.8452 ± 0.7684	0.40	0.29	0.31	0.17
Lysine	0.6260 ± 0.3137	0.6272 ± 0.3126	0.5493 ± 0.2191	0.6584 ± 0.3419	0.49	0.37	0.22	0.15
Phenylalanine	0.9939 ± 0.3862	1.0065 ± 0.3827	0.9620 ± 0.3507	1.0243 ± 0.4003	0.45	0.39	0.41	0.33
Glucose	0.5290 ± 0.9442	0.3785 ± 0.4468	0.2832 ± 0.1546	0.4166 ± 0.5182	0.23	0.30	0.12	0.13

Table 13. FDR Adjusted p-value for the Relative Concentration of Other Metabolites Comparisons

Metabolites	Healthy (n=25) Vs. IBS-(n=35)	Healthy (n=25) Vs. IBS-D (n=25)	Healthy (n=25) Vs. IBS-C (n=10)	IBS-C (n=10) Vs. IBS-D (n=25)
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid_adjusted	0.10	0.22	0.03	0.048
Propionic acid_adjusted	0.48	0.48	0.48	0.48
Butyric acid_adjusted	0.48	0.48	0.48	0.48
Caproic acid_adjusted	0.41	0.35	0.35	0.35
Valeric acid_adjusted	0.50	0.50	0.50	0.50
Lactic acid_adjusted	0.46	0.46	0.46	0.46
Formic acid_adjusted	0.18	0.21	0.11	0.11
2-Aminobutyric acid_adjusted	0.21	0.03	0.10	0.03
Tryptophan_adjusted	0.22	0.22	0.28	0.28
Aspartic acid_adjusted	0.11	0.11	0.11	0.31
Fumaric acid_adjusted	0.38	0.38	0.41	0.38
Urocanic acid_adjusted	0.35	0.45	0.10	0.10
UDPglucuronic Acid_adjusted	0.08	0.08	0.13	0.08
Malonic acid_adjusted	0.28	0.17	0.17	0.11
Putrescine_adjusted	0.35	0.40	0.18	0.18
Succinic acid_adjusted	0.20	0.22	0.11	0.11
Nicotinic acid_adjusted	0.30	0.42	0.17	0.17
3-Methyl-2-oxovaleric acid_adjusted	0.25	0.25	0.25	0.25
3-hydroxyPhenylacetic acid_adjusted	0.35	0.35	0.42	0.46
Choline_adjusted	0.20	0.24	0.09	0.04

Glutamic acid_adjusted	0.15	0.22	0.15	0.15
Pyroglutamic acid_adjusted	0.45	0.32	0.01	0.01
Dgalactose_adjusted	0.15	0.14	0.11	0.11
Dxylose_adjusted	0.47	0.45	0.08	0.08
Valine_adjusted	0.44	0.44	0.41	0.41
Alanine_adjusted	0.24	0.32	0.24	0.24
Proline_adjusted	0.29	0.15	0.08	0.04
Threonine_adjusted	0.06	0.06	0.25	0.25
Uracil_adjusted	0.12	0.19	0.11	0.12
Hypoxanthine_adjusted	0.38	0.38	0.45	0.38
Dihydroxyacetone_adjusted	0.43	0.43	0.43	0.43
Sarcosine_adjusted	0.33	0.31	0.31	0.31
Glycine_adjusted	0.43	0.43	0.23	0.23
Dimethylglycine_adjusted	0.31	0.31	0.35	0.33
Glutamine_adjusted	0.36	0.36	0.36	0.44
Tyrosine_adjusted	0.38	0.38	0.42	0.28
Methionine_adjusted	0.30	0.30	0.38	0.38
Leucine_adjusted	0.40	0.40	0.40	0.17
Lysine_adjusted	0.49	0.49	0.43	0.15
Phenylalanine_adjusted	0.45	0.45	0.45	0.33
Glucose_adjusted	0.30	0.30	0.27	0.14

Table 14. Tukey Multiple Comparisons of Means (Relative Concentration) Using ANOVA: 95% Family-Wise Confidence Level

	HC -IBS-C			HC-IBSD			IBS-C-IBS-D			F-statistics
Metabolites (FDW-normalized)	Diff	CI	p-adj.	Diff	CI	p-adj.	Diff	CI	p-adj.	
Acetic acid	-2.74	-5.65, 0.17	0.068	-0.76	-2.96, 1.44	0.69	1.98	-0.93, 4.90	0.24	2.58
Propionic acid	-0.02	-0.69, 0.65	1.00	0.08	-0.43, 0.59	0.93	0.10	-0.58, 0.77	.94	0.093
Butyric acid	-0.12	-1.39, 1.15	0.97	-0.15	-1.11, 0.81	0.93	-0.03	1.30, 1.24	0.10	0.07
Caproic acid	-0.031	-0.14, 0.079	0.78	0.02	-0.061, 0.106	0.80	0.054	-0.057, 0.16	0.48	0.70
Valeric acid	-0.0001	-0.069, 0.069	0.99	0.01	-0.043, 0.06	0.91	0.009	-0.060, 0.08	0.95	0.10

Lactic acid	-0.14	-1.05, 0.77	0.93	0.09	-0.60, 0.78	0.95	0.23	-0.68, 1.14	0.82	0.19
Formic acid	-0.011	-0.029, 0.0077	0.34	- 0.005	-0.019, 0.0088	0.65	0.00 6	-0.013, 0.024	0.74	1.07
Tryptophan	0.002	-0.009, 0.013	0.87	0.005	-0.003, 0.013	0.32	0.00 3	-0.008, 0.014	0.82	1.06
2-Aminobutyric acid	-0.02	-0.077, 0.03	0.55	0.03	-0.014, 0.068	0.25	0.05	-0.0032, 104	0.07	2.89
Aspartic acid	0.08	-0.035, 0.186	0.24	0.05	-0.033, 0.134	0.32	-0.03	-0.14, 0.086	0.85	1.75
Fumaric acid	0.001	- 0.0057, 0.007	0.96	- 0.002	-0.007, 0.003	0.65	- 0.00 3	-0.009, 0.004	0.60	0.63
Urocanic acid	-0.03	-0.085, 0.021	0.32	0.002	-0.038, 0.042	0.99	0.03	-0.019, 0.087	0.28	1.31
UDP-glucuronic acid	-0.002	-0.020, 0.016	0.96	0.01	-0.002, 0.025	0.11	0.01	-0.005, 0.032	0.18	2.67
Malonic acid	-0.05	-0.162, 0.072	0.63	0.05	-0.043, 0.13	0.44	0.09	-0.03, 0.21	0.16	1.88
Putrescine	-0.07	-0.212, 0.0721	0.47	-0.01	-0.120, 0.094	0.95	0.06	-0.085, 0.199	0.60	0.71
Succinic acid	-1.16	-3.25, 0.93	0.38	-0.56	-2.14, 1.02	0.67	0.60	-1.49, 2.69	0.77	0.96
Nicotinic acid	-0.003	- 0.0073, 0.0016	0.27	- 0.000 3	-0.004, 0.003	0.97	0.00 3	-0.002, 0.007	0.35	1.29
3-Methyl-Oxovaleric acid	-0.02	-0.048, 0.015	0.41	- 0.007	-0.031, 0.017	0.76	0.01	-0.022, 0.041	0.74	0.84
3-HydroxyPhenylacetic acid	0.007	-0.022, 0.037	0.83	0.009	-0.0137, 0.031	0.62	0.00 1	-0.028, 0.031	0.99	0.47
Choline	-0.05	-0.13, 0.03	0.30	-0.02	-0.081, 0.041	0.71	0.03	-0.051, 0.11	0.64	1.16
Glutamic acid	-0.61	-1.42, 0.21	0.18	-0.21	-0.83, 0.41	0.70	0.40	-0.42, 1.22	0.47	1.61
Pyroglutamic acid	-0.02	-0.045, 0.0069	0.19	0.006	-0.013, 0.03	0.71	0.03	-0.0005, 0.051	0.06	2.79
D-galactose	-0.01	-0.082, 0.059	0.92	0.03	-0.022, 0.09	0.92	0.04	-0.028, 0.113	0.32	1.47
D-xylose	-0.04	-0.147, 0.061	0.58	0.02	-0.063, 0.094	0.89	0.06	-0.045, 0.162	0.37	0.92
Valine	-0.12	-0.56, 0.31	0.78	0.02	-0.30, 0.35	0.98	0.15	-0.29, 0.58	0.70	0.34
Alanine	-0.66	-1.81, 0.48	0.35	-0.18	-1.04, 0.68	0.87	0.48	-0.66, 1.63	0.57	0.97

Proline	-0.04	-0.121, 0.042	0.47	0.034	0.028, 0.096	0.39	0.07	-0.0082, 0.155	0.09	2.49
Threonine	0.02	-0.038, 0.08	0.70	0.04	-0.0063, 0.08	0.11	0.02	-0.040, 0.075	0.74	2.12
Uracil	-0.06	-0.13, 0.017	0.16	-0.02	-0.078, 0.035	0.62	0.04	-0.039, 0.11	0.49	1.74
Hypoxanthine	-0.003	-0.048, 0.043	0.99	-0.01	0.048, 0.021	0.62	- 0.01 1	-0.056, 0.035	0.84	0.46
Dihydroxyacetone	-0.003	-0.021, 0.015	0.90	- 0.005	-0.019, 0.0081	0.61	- 0.00 2	-0.020, 0.016	0.96	0.46
Sarcosine	-0.02	-0.098, 0.067	0.89	0.023	-0.041, 0.084	0.69	0.04	-0.041, 0.084	0.53	0.69
Glycine	-0.02	-0.059, 0.028	0.67	0.003	-0.030, 0.036	0.97	0.02	-0.025, 0.062	0.57	0.53
Dimethylglycine	0.005	-0.054, 0.065	0.97	0.021	-0.024, 0.066	0.52	0.02	-0.044, 0.075	0.81	0.63
Glutamine	-0.04	-0.19, 0.11	0.82	-0.03	-0.14, 0.082	0.79	0.00 6	-0.14, 0.15	0.99	0.29
Tyrosine	0.01	-0.13, 0.15	0.98	0.04	-0.06, 0.15	0.61	0.03 1	-0.108, 0.17	0.85	0.47
Methionine	0.02	-0.061, 0.093	0.88	0.03	-0.032, 0.084	0.53	0.01	-0.067, 0.088	0.94	0.59
Leucine	-0.13	-0.81, 0.55	0.89	0.13	-0.39, 0.64	0.83	0.26	-0.42, 0.93	0.64	0.44
Lysine	-0.08	-0.36, 0.21	0.79	0.03	-0.18, 0.25	0.93	0.11	-0.17, 0.391	0.62	0.43
Phenylalanine	-0.03	-0.38, 0.32	0.97	0.03	-0.23, 0.29	0.96	0.06	-0.29, 0.41	0.90	0.10
Glucose	-0.25	-0.88, 0.39	0.62	-0.11	-0.59, 0.37	0.84	0.13	-0.50, 0.77	0.87	0.47

AIM1 DISCUSSION

IBS is the most prevalent gastrointestinal (GI) disorder in western developed countries exerting a tremendous economic, social, and emotional burden (Heitkemper et. al., 2002). IBS provides the best context to study the impact of the gut microbiome on GI symptoms and the impact of GI symptoms on the stability of the gut microbial composition and its function. Metabolomics of fecal sample to characterize the SCFA metabolomic profile in women with IBS and healthy controls was done in search for mechanistic link that would

explain the notion that high dietary fiber intake is associated to health and well-being of persons with IBS, and more importantly whether SCFA metabolism is altered in IBS in reference to healthy controls.

The goal of Aim1 was to look for differences of fecal SCFA profile in women with IBS as a composite compared to healthy controls, as well as to investigate whether the two subtypes of IBS differ from each other and to HC women based on their fecal SCFA profile. This observational study is a pilot study that leverages the data obtained by the MAP study (Microbiome & Abdominal Pain, NR014479). Leveraging the MAP study 16S ribosomal gene sequencing data (bacteria composition data), metabolomics was done to complement the results; observing the functionality of the identified bacteria in terms of the metabolites they produce is vital to determine gut dysbiosis.

Other studies have measured fecal SCFA in individuals with IBS compared to healthy people and several have compared IBS subgroups (Sun et al., (2019). However, the results have been conflicting, partly due to differences in measurements tools, storage and processing considerations, and the delivery of different intervention protocols (Sun et al., (2019). In addition, there are several different ways of analyzing and reporting metabolomics data. As reported by several investigators, the concentration of SCFA were reported using absolute concentration (Mars et al., 2020; Born et al., 2000; Gargari et al. ,2016), as well as ratios and percentages (Born et al., (2000), Kopechny et al., 2002), Valeur et al., 2010).

In the current study, the absolute concentration was quantified based on the internal standard, TSP (25 micromolar); this is particularly meaningful in terms of clinical relevance. The relative concentrations of metabolites are included because they were also normalized with the sample fecal dry weight (FDW) in addition to the TSP internal standard and perhaps provide a “better story.” In the current study, FDW was found to be highly correlated with measured fecal SCFA levels. However, the mean FDWs for all three groups were not statistically different. The IBS-D and healthy controls were similar (117 mg) in the number of bacterial cells (dry weight), while the IBS-C was slightly higher (119 mg). While the number of bacterial cells (dry weight) of specific bacteria genera determined the SCFA measured concentration, the 16S data confirmed that the number of bacteria cells was not the main driver for the differences in SCFA concentrations found between IBS and healthy women. For example, healthy women had higher relative DNA abundances for several

bacteria taxa that were positively correlated with fecal SCFA concentrations (see Figure: High and Low SCFA Producers, Bar graphs with CIs). Thus, it may be that the difference in SCFA found between groups were due to differences in the fermentation patterns of specific and enriched gut bacteria.

Since nuclear magnetic resonance spectroscopy (NMRS) can be used for targeted and non-targeted metabolomics, it was expected that other metabolites would be identified using this platform. In this project, the protocol to extract SCFA was designed for targeted metabolomics and optimized for the extraction of the three major SCFA (acetate, propionate, butyrate). The comparison of SCFA among healthy women (n=25) and IBS (n=35) or healthy women versus IBS-D (n=25) met the 80% power analysis requirement; however, the comparison between healthy women and IBS-C or the subgroup comparison (IBS-C vs. IBS-D) did not. Both the proportion of SCFA and absolute levels were examined. As such, the results were discussed with respect to patterns of associations among the identified metabolites and IBS status in all women.

Bacterial metabolites that are derived from fermentation of unavailable carbohydrates (not metabolized by the host) play a positive role in gut physiology, particularly, the three major SCFA. Altered SCFA metabolism could prove to be the important trigger for IBS symptom generation, exacerbation, and its maintenance (persistent symptoms unrelieved by any of the existing conventional therapy). Inspired from the idea of finding the real “trigger” for the disorders experienced by people with IBS and its relation to the symptom based-diagnostic criteria (Rome criteria), it was hypothesized that IBS women, regardless of bowel group, would have reduced fecal SCFA compared to healthy women. Stool consistency (bowel pattern) assessed by the Bristol Stool Scale is considered as the surrogate marker for colonic transit time (CTT) by the scientific community. Since stool consistency is closely related to transit time, it was expected that higher total SCFA would be associated with diarrhea, while lower total SCFA would be associated with constipation. Furthermore, it was expected that lower total SCFA would be associated with higher abdominal pain severity score.

As hypothesized, the results showed that the IBS women, regardless of bowel pattern predominance, had lower concentrations of total fecal SCFA as compared to healthy women, albeit not significant (after adjustment for multiple comparisons). Since the metabolites showed non-uniform distribution, adjustment using

the Benjamini-Hochberg False discovery Rate (FDR) was used for multiple adjustment comparisons since ANOVA requires uniform distribution of data (Fitzgerald et al. 2009). Following FDR adjustment of p-values obtained from the univariate analyses, the fecal acetic acid concentration (absolute and relative concentrations) of the IBS-C group was significantly lower compared to healthy individuals. Furthermore, as predicted, stool consistency was strongly associated with measured fecal SCFA levels; constipated stools from IBS-C women correlated with lower fecal SCFA concentrations, while more watery type stools from IBS-D women correlated with higher fecal SCFA concentrations. In summary, the fecal SCFA metabolic profiling using NMR platform did not significantly differentiate IBS as a composite from healthy women after adjusting for multiple comparisons (FDR adj. $p = 0.1$); however, this adjusted p-value may be clinically significant considering the heterogeneity found in human samples.

The study found similarities and differences in SCFA metabolites among the IBS subgroups. Although not significant, propionic acid was slightly higher in each IBS subtype, while butyric acid was slightly higher in healthy women compared to each IBS subtype. The IBS-C group had higher butyrate levels than the IBS-D group; however, the range for butyrate was wider in IBS-D compared to IBS-C or healthy women and the IBS-C group had the narrowest range for butyrate. Butyrate is the most important and the most studied SCFA. Depending on the microenvironment, butyrate can be both inflammatory and anti-inflammatory. Taken together from the literature (over a century of continued research) regarding the gut microbiome's role in maintaining body homeostasis, it could be surmised that the physiological role of butyrate in preventing inflammation/oxidative stress in the colon and maintaining gut integrity (reduced gut permeability, normal growth of colonic epithelial cells, cell cycle arrest for abnormal colonic cells) is ensured when the right substrate (i.e., high fiber, high carbohydrates with low saturated fat diet) is available for fermentation (Postler & Ghosh, 2017; Herter and Kendall, 1910, Metchnikoff, 1908; Bach et al., 2018; Lous & Flint 2017). Diet intake of at least 30g/day by a healthy person (without other health comorbidities) should be sufficient for optimal production of SCFA and prevent gut dysbiosis.

Valeric and caproic acids were higher in IBS women than healthy women; however, IBS-C had the lowest of the branched chain (bc)-SCFA compared to IBS-D or healthy women. Trends in lower fat and higher

fiber intakes by the IBS-C group could have contributed to the observed lower bc-SCFA in IBS-C. Under high fiber intake, the growth and activities of the saccharolytic bacteria are favored over the proteolytic bacteria (Simpson et al., 2015).

In this study, body weight was associated with both the gut microbiome and metabolome. SCFA are known to regulate satiety and influence endocrine function by stimulating leptin production via the GPR 41 receptor (also called FFAR3) in adipocytes. As reported by other investigators, high fecal acetate is associated with obesity (Wolever et. al, 1997). The body weight of healthy women and IBS-C women were approximately similar (136 pounds and 132 pounds, respectively) while the weight of IBS-D women was higher (160 pounds). It can be conjectured that women with IBS-D have reduced capacity to absorb SCFA due to faster or reduced transit time, or perhaps they are unable to metabolize SCFA and as such, this altered microbiome may be causing the rapid transit time. Building on the first hypothesis, a percentage of women with IBS have altered bile acid absorption and bile acids and its salt conjugates increase colonic pH creating a low pH gradient. Because of this environment, there is reduced production of nonionic/protonated forms of SCFA. Since the nonionic form (the protonated species) are the majority species of SCFA being rapidly taken up by the colonocytes via passive diffusion this leaves the ionic forms to be transported via facilitated active transport processes (monocarboxylate transporters facilitated by sodium or hydrogen). Thus, the absorption rate of these anions is lower than the protonated SCFA. Furthermore, differences in host genetics (including its epigenome) could play a role in the slow absorption of SCFA. Another possible theory would be that IBS-D women have higher SCFA production rates than the IBS-C women and the over-saturation of SCFA transporters is causing SCFA buildup in the colonic lumen, hence higher SCFA in the feces (primary contribution is due to transit time). A review by Vahor and colleagues (2020) reported that IBS-D phenotype is found to have higher serotonin level (lower serotonin uptake transporters or SERT expressions) than persons with IBS-C. Several researchers have suggested that SCFA stimulate the release of serotonin which is known to cause an increase in intestinal motility and strong evidence of this observation was seen in rat studies by Fukumoto and colleagues (Dimidi et al., 2017; Vahor et al., 2020; Tanaka 2010; Faure et al., 2010; Fukumoto et al., 2003). Thus, the higher amount of SCFA in IBS-D could be attributed from having higher ranges of

tryptophan concentrations compared to the other groups. In summary, there are several mechanisms that could account for the observations in this study.

High Lactate in IBS-D. The presence of higher amount of lactic acid found in IBS-D samples could reflect a lower abundance of lactic acid-utilizing and acetic-consuming bacteria. Glucose fermentation results in lactate formation which is an intermediate for SCFA production. Lactate, if not immediately absorbed (due to pathology status) is readily converted to other metabolites by lactate-consuming bacteria and does not usually accumulate in the healthy gut. Literature reports that total L- lactate in feces is around 1.5 mM and the neurotoxic form, D-lactate is not usually detectable by any lab measures without the presence of extreme gut dysbiosis as seen in GI disorders (e.g., short bowel syndrome, ulcerative colitis). High D-lactic acid levels (>3mM) is known to cause neurological disturbances in mammals (i.e., depression), CNS dysfunction including ataxia and coma, as well as diarrhea (Abeysekara et al., 2007). Unlike D-lactic, L-lactic is metabolized by neurons and astrocytes having no mitochondria are net producers of L-lactic acid form. The presence of anaerobic, lactate-utilizing bacteria (i.e., *Eubacterium halii*, *Anaerostipes caccae*, *Veillonella*, *Megasphaera*, *Clostridia Cluster XIVa* that include *Eubacterium rectale*, *Roseburia intestinalis*, other *Eubacterium* species, *Coprococcus*) prevents the accumulation of lactate to an abnormal level (>5 mM) in the gut or in the feces (Duncan et.al. 2004), however, the overgrowth of lactate-producing bacteria alters the normal lactate fecal concentration. In published literature, healthy individuals do not accumulate lactate, but this is seen in individuals with ulcerative colitis (Gibson et.al. 1996) and with persons with short bowel syndrome or had gut resection procedures (cited by Duncan et al., 2004). Mayeur et. al. (2013) reported that the total amount of D-lactate ranged from 2 to 110 millimolar (mM) and the L-lactate ranged from 2 to 80 mM in people with short bowel syndrome. Building on the observation of high lactate level in persons with IBS-D, this suggests that there may be a high aerobic glycolysis (phenomenon of Warburg effect) happening in the colon; high lactic acid is an attribute of ATP depletion. Aerobic glycolysis produces less ATP compared to oxidative phosphorylation of carbohydrates/substrates. The reduced ATP production could be a result of high catabolic reactions happening (e.g., reduced level of hypoxanthine due to increased activity of xanthine oxidase). Mars and colleagues (2020) found an increased expression of xanthine oxidase and hypoxanthine phosphoribosyl

transferase (XPRT); both enzymes are important in hypoxanthine metabolism. XPRT is purine salvage enzyme that liberates xanthine from xanthosine-monophosphate as an early step in purine salvage pathway (Mars et al., 2020). It has been reported that bacteria genera such as *Barnesiella* and *Prevotella* were found to be associated with hypoxanthine, a host-microbial co-metabolite, known to regulate the production of hydrogen peroxide (H_2O_2) and superoxide anions in IBS (as cited by Xiao et al., 2021; Zhao et al., 2013). In this study, *Barnesiella* is reduced in IBS-D and *Prevotella* is significantly reduced in IBS. Hypoxanthine has been implicated as a source of energy post injury or hypoxia (Lee et al., 2018 and 2019; Xiao et al., 2021). Note: the *de novo* synthesis of a purine molecule is an energy consuming process that requires 5 ATP/molecule. It was suggested that the purine salvage pathway offers an alternative energy-conserving pathway for the host (Xiao et al., 2021). Moreover, a study has shown that lactate is upregulated during inflammation (Manoharan et al. (2021). The high lactate level in several of the IBS-D women could be a physiological response to inflammation. Mature white blood cells normally lack or have only a few mitochondria and thus prefer aerobic metabolism resulting in the conversion of pyruvate to lactate (Champe et al., 2005; Kempner, 1939; Kramer et al., 2014), There is increased expression of the enzyme lactate dehydrogenase during times of physiologic demand (e.g., inflammation) and the use of lactate as energy can reduce the negative effects of reactive oxygen species (ROS), a normal product of cell metabolism (Bolisetty & James 2013; Glancy et al., 2021). Also, it is possible that the saturation of the sodium and/ hydrogen coupled monocarboxylate transporters could explain the accumulation of lactate.

Several microbiome differences were found across IBS and healthy control groups that may account for the differences in SCFA levels. In the IBS group the lower fecal SCFA could reflect a reduction in the number of fecal SCFA-producing bacteria taxa, while in the healthy control group there may be a higher proportion of SCFA-producing bacteria consortia. In IBS subgroup comparison, IBS-D may have more unavailable carbohydrate in the colon, hence higher fermentation equates to more fecal SCFA and more SCFA excreted in the feces, and this could be a normal process. Healthy control women had higher acetate-producing bacteria like *Bifidobacteria* (contributes up to 60-70% of acetate) and *Lactobacillus* (a lactic acid bacteria). Furthermore, sulfate-reducing bacteria such as *Desulfovibrio* that are also higher in IBS-C consume hydrogen

to reduce CO₂ to methane, but lower relative abundance in methanogens such as *Methanobrevibacter*. The formation of methane depletes the necessary one carbon material (formic acid) needed to form the major SCFA; without the methanogens, methane and hydrogen gas accumulate instead of being formed into SCFA intermediates. This rationale is reflected in IBS-C group having lower amount of total SCFA due to the presence or reduced quantity of specific bacteria (i.e., *Methanobrevibacter*). Several *Clostridia* genera such as *Clostridium IV*, *Clostridium XIVa*, *Clostridium XVIII* were also found to be higher in IBS-C group compared to healthy control or IBS-D subjects; however, the difference was not significant. These *Clostridia* genera are known as acetic and lactic acid consumers and as cited by Guo et al. (2020) these three taxa are reduced in intestinal failure. Interestingly, it is worth noting that IBS-D and HC women had similar and higher amount of *Clostridium XI* and *Clostridium sensui stricto* compared to IBS-C group; *Clostridium XI* clusters (where *C. difficile* belongs; bile acid resistant, produce toxic secondary bile acids) was significantly higher in HC and IBS-D women compared to IBS-C women while *Clostridium sensu stricto* (known as acetic acid producer; Li et al. 2023) was significantly higher in IBS-D than in IBS-C women. Both *Clostridia XIVb* and *Clostridium XVIII* were found to be significantly higher in IBS-D compared to HC women. IBS-D women certainly do not have intestinal failure, however, these microbiome markers reported by these investigators in the field does have merits when considering IBS symptom trajectory.

Furthermore, the reduced amount of acetic acid in IBS-C could mean that the SCFA are efficiently absorbed and utilized by colonocytes or being rapidly converted to other forms of metabolites (i.e., acetoacetate, propionic acids). Also, the high mass of bacteria associated with constipation could also deplete the SCFA and its related metabolites; this depletion of SCFA to support the growth of bacteria suggests less energy for the colonocytes and can induce oxidative stress without the introduction of additional unabsorbed carbohydrates. In healthy control women, higher levels of acetic acid-producing bacteria matched their higher amount of fecal acetate level. It is also possible that their gut microbiota harbored higher numbers of SCFA-metabolizing genes when compared to IBS-C. On the other hand, the IBS-C women did not have a consortium of high-acetate producing bacteria, and in fact, IBS-C women had lower acetate-producing bacteria (i.e.,

Bifidobacteria, *Coprococcus*, *Lachnospiricea*), and higher lactate-utilizing bacteria (i.e., *Eubacteria*) compared to healthy women.

The gut microbiome and metabolome may influence energy metabolism in patients with IBS. In line with the microbiome findings done by Parthasarathy et al., (2016), this study revealed a significantly higher amount of *Bacteroides* in women with IBS-C. Higher relative abundance of *Bacteroides* could disrupt the normal serotogenic pathways in IBS-C. Preclinical studies have shown that low levels of *Bacteroides* is associated with obesity. For example, leptin gene deficient mice (ob/ob mice) have at least a 50% reduction in their *Bacteroides* community. In the current study, the IBS-C women were leaner and younger than healthy controls or IBS-D. Furthermore, the IBS-C group had higher succinate-utilizing bacteria (e.g., *Phascolarctobacterium*) than healthy control women. This propionic acid-producing bacteria (Rachek et.al, 2021) was significantly higher in IBS-C women who consumed a high fiber diet when compared to healthy controls in the same diet category. Hence, differences in the bacterial community may contribute differently to host SCFA signature and should be examined in future work.

To date, published data comparing fecal SCFA concentration levels collected from normal bowel (healthy donors) and disordered bowel (IBS donors) have been contradictory. Earlier investigations comparing IBS (subgroup or as a composite) and healthy controls found statistically significant differences in the three major SCFA (Kopechny et.al. (2002); Tana et.al., (2010); Ahmed et.al. (2013); Chassard et.al (2012), Farup et al. (2016); Treem et al. (1996) and Mars et.al 2020. Still other investigators found non-significant differences in the major fecal SCFA between IBS and HC women ((Zhang et al., 2019; Gargari & Cremon et.al., (2018); Lee et. al. (2020). However, Zhang et.al (2019) found significantly higher caproic acid in IBS-D (n=30, Rome IV) compared to healthy controls (n=15) and a higher trend in butyrate in IBS-D. Other investigators only compared IBS subgroups without healthy controls. Statistically significant subgroup differences were found in few studies (Ringel-Kulka et al., 2015; Mortensen et.al., 1987; Valeur et.al., 2016).

Consistent with the result of the current study, an observational study in the US by Ringel-Kulka et.al. (2015) found lower total SCFA (although non-significant) in IBS women (n=114; Rome III) compared to healthy

controls (n=33). In the IBS group the lower fecal SCFA could reflect a reduction in lower number of fecal SCFA-producing bacteria taxa while, in the healthy control group there may be higher proportion of SCFA-producing bacteria consortia. Furthermore, healthy control women had higher acetate-producing bacteria like *Bifidobacteria* (contributes up to 60-70% of acetate) and *Lactobacillus*, a lactic-acid producing bacteria. Ringel-Kulka and colleagues also found differences when subgroups were compared. Consistent with the current findings (post multiple comparison with FDR adjustment), Ringel-Kulka found significantly lower fecal acetic acid in IBS-C compared to IBS-D and healthy controls. A similar result was found in an older study by Treem and colleagues (1996). In that study using gas chromatography (GC) age-matched 10 IBS-D and 10 healthy controls were found to have lower acetate and propionic acid levels, but higher butyrate concentrations (including calculated butyrate proportion) in IBS-D (Rome II) than healthy controls. In addition to quantified baseline SCFA concentration, this study was the first to look at fermentation patterns of the bacteria collected from IBS patients using five different types of carbohydrates (i.e., lactulose, pectin, potato starch, and hemicellulose) for 24 hours using an anaerobic fermentation system. They noted that net SCFA production rates occurred after the first 6 hours of carbohydrate fermentation because the production significantly decreased at 16-hour timepoint. IBS-D fecal culture showed lower total SCFA than healthy controls; a lower percentage of acetate and a higher proportion of n-butyrate observed in IBS-D group. In both groups, lactulose fermentation resulted in the highest rate of SCFA production, followed by citrus pectin, then potato starch, and lastly hemicellulose. The healthy control cohort generated a higher concentration of total SCFA, acetate, and propionate with all substrates tested at all time points. The fecal sample analyzed for IBS-D subjects were pooled from two different weeks of fecal collection in which only formed stool was homogenized within 2 hours of collection; this would make it comparable to the consistency of the stool collected from the healthy controls. Although Treem and colleagues (1996) did not enumerate or identify bacteria, this study has its own merit leveraging on the carbohydrate fermentation experiments done by earlier investigators Mortensen and colleagues (1987) with fecal samples from healthy volunteers and among historical contributors (i.e., Williams and Olmsted, 1936, Burkitt et. al, 1972, Trowell & Southgate et. al., 1976) as listed by Cummings and Macfarlane (1991). A Scandinavian study by Mortensen et. al., (1987) using an RCT double-blind crossover-

design (18 IBS and 9 healthy controls) found IBS persons had lower fecal SCFA measured by GLC with steam distillation than the healthy controls. Mortensen's group also dichotomized fecal SCFA concentration into high vs. low. Within the IBS group treated with placebo, they showed that IBS patients categorized in the low SCFA had lower fecal mean mass and longer transit time compared to IBS patients categorized in the high SCFA group. The interpretation of this is that lower fecal mass leads to lower SCFA in IBS-C.

Another observational study done in Norway carried out by Valeur et al., (2010) enrolled 35 IBS (Rome II) and 15 healthy controls; Valeur's study reported both absolute and relative concentration of fecal SCFA. Valeur found lower percentage of acetic acid, but higher percentage of n-butyric acid in IBS compared to healthy controls using GLC identification technique. A more recent exploratory study done by Valeur (2016) only enrolled IBS subjects (34 IBS-D, 10 IBS-C, and 19 IBS-M; Rome III) and found that the baseline SCFA levels in IBS-D were higher compared to IBS-C patients using GLC platform. Their results followed the same trend post intervention with low FODMAP diet. These results were validated in their carbohydrate fermentation study. It is worth noting that they found higher protein fermentation products (bc-SCFA measured as the sum of isovaleric and iso-butyrate) post low FODMAP diet intervention.

Another observational study carried out by investigators from Norway (Farup et al., 2016) found significantly lower proportion of butyrate in IBS (n=25; 9 IBS-C, 8 IBS-D, 7 IBS-M, 1 IBS-U), diagnosed with Rome III compared to healthy controls (n=25) using GLC. Consistent with the current study, Farup and colleagues (2016) reported both absolute (millimole/L) and relative concentration (% or proportion); they found higher mean propionic acid in IBS and the mean butyric acid (molar %) was statistically lower in IBS than the healthy control group. Farup and colleagues measured the ratio of propionic acid to butyric acid and the difference of propionic and butyric to differentiate IBS from healthy control groups. The difference of propionic and butyric gave the largest magnitude of effect size in their group and subgroup comparisons. Inconsistent with the current finding, they found a higher mean concentration of acetic acid compared to healthy control and since acetic acid makes up about 60-70%, IBS cohorts ended up having higher total SCFA. Similarly, another older observational study that included 26 IBS (11 IBS-C, 8 IBS-D, 7 IBS-M; Rome II criteria) in Japan measured fecal SCFA by HPLC found significantly higher levels of total fecal SCFA in IBS than healthy

subjects (acetic acid and propionic acid); butyric acid to a lesser extent) compared with healthy controls (n=26) (Tana, 2010). Both Farup et al (2016) and Tana et al., (2010) analyzed the subtypes of IBS as a group and this strategy may not be the best way to look at differences between IBS and healthy controls. Consistent with Farup and colleagues (2016) findings, another case study done by Ahmed et.al (2013) in the United Kingdom enrolled IBS-D patients diagnosed with Manning criteria (before Rome criteria era) used GC-MS analytical to measure fecal SCFA, reported that IBS-D patients had significantly higher total SCFA and each individual SCFA compared to healthy controls (n=109).

There may be clinical significance related to the proportions of each metabolite. Reported portions of fecal SCFA vary in observational studies (including those baseline data gathered by clinical trials) due to differences in personal dietary intake by the subjects. The three SCFA in this study were all linearly correlated with each other, although each exerts a different effect on the host by binding to different SCFA receptors or co-receptors found in intestinal epithelial cells, adipose cells, and immune cells. These three major SCFA regulate inflammatory processes differently and differentially modulate the immune system.

Except for Farup and colleagues (2016), most of the observational studies to date have found reduced acetic acid in IBS relative to healthy control subjects, while the reported proportion of propionic and butyric in IBS-D was not consistent. These findings suggest that the microbial community may not be stable in IBS-D most especially when symptoms are also changing. It can be speculated that IBS subjects may be experimenting with fad or alternative diets (i.e., Mediterranean diet, Atkins diet, Paleo diet, Gluten-free diet, Dash diet, Macrobiotic diet, the Keto diet, Acid-Alkaline diet), hence their microbiome are also changing with these dietary changes. This speculation is supported by current literature (Mardinoglu et al., 2018; David et al., 2014; Zhao et al., (2018); Makki et al., 2018) where a drastic change in diet rapidly changed the gut microbiome within 24 hours, and the microbial ecosystem may take a week to stabilize (Mardinoglu et al., (2018). Another possible reason as to why the IBS-D group had higher total SCFA could be related to the fecal collection time. Studies have implicated that bacterial fermentation activity is influenced by time; most bacteria were reported to be more active in the late afternoon or at night (Kaczmarek et al., (2017), Besides the timing

of feeding/eating time, the microbiome, under the influence of circadian clocks, produce varying amount of SCFA throughout the day (Thais et al., 2014).

There are a few reports examining the fecal metabolome in concert with the microbiome measures. One recent RCT, multi-centered, double-blind, placebo-controlled cross-over design longitudinal study in Italy was done by Gargari et al., (2018). They enrolled 40 IBS (14 IBS-C, 12 IBS-D, 11 IBS-U, 3 IBS-M; Rome III) and examined five fecal samples (collected every 4 weeks) and analyzed metabolites using UPLC-HR-MS. Inconsistent with the current result, they found higher valeric acid in IBS-D as compared to IBS-C and lower isovaleric acid in IBS-D compared to healthy controls. The current study found higher valeric acid in IBS-C compared to IBS-D or healthy controls. However, consistent with the current findings and with exception of *Bacteroides*, Gargari et al. (2018) also found *Prevotella* to be higher in healthy controls and an enrichment of number of *Clostridiales* clusters in IBS-C compared to IBS-D subjects and IBS-D with *Parabacteroides* species. An RCT in Australia by Halmos et al., (2015) enrolled 30 IBS (Rome III) and healthy controls (n=8) and reported higher baseline fecal caproic acid in IBS of all subtypes (n=27) compared to healthy controls, while the current study found higher caproic acid only in IBS-D compared to IBS-C or healthy controls. This difference could be attributed to small sample size or differences in dietary preferences.

In the current study, the IBS-D group had higher fecal levels of phenylalanine, tryptophan, caproic acid, leucine, valine, and methionine and a higher Simpson index. These findings are consistent with an observational study done in Beijing by Zhu et al. (2019) who enrolled 15 IBS-D (Rome III) and 15 healthy control subjects, reported 31 fecal metabolites that were differentially expressed between the groups. However, the finding of higher Simpson index needs to be cautiously interpreted; a higher Simpson's index translates to lower microbial diversity. Consistent with the current findings, Zhu et al. also showed lower microbial diversity index for IBS-D as was demonstrated from the boxplot presented (Zhu et al., 2019). These investigators also showed that the microbial richness (ACE index) in IBS-D was higher than healthy controls; this means that certain bacteria OTUs predominated over other bacteria. However, richness does not necessarily mean higher diversity and it could mean the opposite. For example, intake of an anti-depressant drug such as fluoxetine could enrich one type of bacteria at the expense of another such as *Akkermansia*.

Similarly, the consistent intake of proton inhibitors selects the growth of bacteria that thrives in higher pH while it reduces the abundance of other commensals that would prefer to thrive in a more acidic environment. Overall, the protein fermentation products reported by Zhu and colleagues (2019) followed the same trend as the current study.

Another study done by Zhang et al., (2019) in Beijing enrolled 30 IBS-D (22 males, 8 females, Rome IV) patients and age- and sex- matched healthy controls (n=15) found no significant differences in total and individual SCFA measured via GC-MS. Consistent to the current findings in this study, Zhang and colleagues found that butyrate and total SCFA were slightly higher in healthy controls compared to IBS-D subjects. These investigators did not report propionic acid because it was mentioned that propionate was only detected in 4 patients and 4 healthy controls and acetic acid was not detected in five of the healthy controls. This problem of detection was probably due to the destructive nature of GLC method. The other metabolites identified were run using LC-MS.

In the US, a recent prospective observational, longitudinal study done by Mars et al. (2020) integrated multi-omics measurements, enrolled 51 IBS (22 IBS-C, 29 IBS-D, Rome III) and gender, age- BMI- matched healthy controls (n=24) reported that IBS-C subjects had significantly lower total SCFA and the 3 major SCFA compared to healthy controls via NMR. Consistent with the current findings, Mars and colleagues also found significant difference in acetate in IBS-C compared to healthy controls and IBS-C had higher alpha diversity compared to IBS-D subjects (Mars et.al. 2021). Mars' group reported higher variability in microbial composition over time in patients with IBS-C compared to healthy controls and IBS-D. However, they did not report on the variability of metabolite profile over time. This seems to be an enigma because constipation is usually associated with higher microbial diversity index and IBS-D is usually associated with more unstable bacterial profile.

Mars et al. also reported that mucosal biopsy samples had significantly reduced acetate (measured via GLC) in IBS-C compared to healthy controls. Using another metabolomics tool (LC-MS), they found significantly higher fecal tryptophan in IBS-D compared to healthy controls; similar trend was observed in the current study. In line with the current results, Mars group found lower uracil in IBS-C compared to healthy

controls; IBS-D followed similar trend but was not significant. This study also found a significantly lower fecal lysine and hypoxanthine in IBS-C and reduced amount of fecal hypoxanthine in IBS-D (Mars et al., 2020). The current study also followed the same trend for both IBS-C and IBS-D, although not significant (see Figure 2c). and metagenomics data showed higher modules of the purine salvage enzyme, XPRT and xanthine oxidase which indicates higher gut microbial catabolism of purine in IBS. Mars and colleagues also found a higher abundance of metagenomic Kegg Orthologs (KO) modules for alcohol dehydrogenase in addition to xanthine oxidase module in IBS-D during flares; this indicates increased generation of reactive oxygen or nitrogen species during flares in IBS-D. On the other hand, IBS-C had a higher abundance of metagenomic KO modules involved in dissimilatory nitrogen reduction pathway; this indicates higher generation of nitrite, nitric oxide, and ammonia by the gut bacteria and could translate to high oxidative stress in IBS-C. Mars et. al. also identified the bacteria involved in the dissimilatory pathways in IBS and reported more than 20 *Lactobacillus* species that are associated with flares (severe pain) in IBS-D. Also, during flares the group found high tryptamine, cholic acid, chenodeoxycholic acid in both IBS subtypes relative to baseline levels without flares. Moreover, KEGG pathway enrichment analysis showed the presence of inflammation related pathways in IBS (prostaglandin D2 synthesis). These investigators also conducted epigenetics study which showed differentially methylated genes associated with epithelial electrolyte transports, aquaporins, and voltage-gated potassium channel in IBS-D. Taken together, these data highlighted the presence of altered microbiome in concert with the host's altered epigenome involve in epithelial transports, absorption, and metabolism.

An interesting observational study from Ireland by Jefferey et al., (2020) enrolled 80 IBS patients (diagnosed under Rome IV criteria; all subtypes: 21 IBS-D, 30 IBS-C, 29 IBS-M) with age-matched healthy controls (age: 16-70, n=80) reported significantly higher level of amino acids, glycine and ribonucleotides in IBS compared to healthy controls via GC and LC-MS metabolic platforms. They also noted significantly lower fecal bacterial alpha diversity in IBS patients compared to healthy controls. In addition, they found that a high percentage (74%) of IBS-D patients were positive for bile acid malabsorption (bile acid retention score via SeHCAT assay) and that urine metabolomics were associated with altered fatty acid oxidation (fatty acid oxidation defects) in IBS. In summary, fecal metabolites showed alteration in amino acid metabolism,

overrepresentation of glycine metabolic pathways, and higher ribonucleotide biosynthesis in IBS compared to healthy controls. In this same study, Jefferey's group also reported different group of *Bacterioides* predominates in IBS with concomitant reduction in several of *Bacteroidetes* species, lower *Eubacteria*, lower *Paraprevotella* and certain species of *Ruminococcus* but higher in *R. gnavus*, *R. torques* and *Lachnospiraceae*. It was reported that IBS subjects consumed significantly less dietary fiber and had higher glycemic index dietary food intake compared to healthy controls. The network analyses showed that the metabolism of protein is very different in the context of IBS relative to healthy controls. Furthermore, fecal microbiome showed clusters of associated microbes and metabolites associated with tryptophan metabolism, while the urine metabolome clustered with the utilization of proton pump inhibitors. Consistent with the current study findings, these investigators reported that phase 2 metabolism pathways (high in glucuronic acid and glycine and its related metabolites) are upregulated in IBS-D; however, their metagenomic analyses showed lower galactose degradation, lower sulfate reduction and assimilation, lower cysteine biosynthesis, but higher starch degradation pathways in IBS. Although SCFA are not identified in Jefferey's group, the amino acid metabolites they identified follow similar trend with the current study; NMR proved to be better in identifying SCFA and at the same time was able to identify amino acids.

Another observational study done in Korea by Lee et al. (2020) used an NMR approach to examine the metabolic profile of persons with IBS-D (n=29; Rome IV) as compared to healthy controls (n=33). They compared a group of healthy controls who had taken a laxative to those who had not and found similar metabolic markers suggesting no differences based on CTT. Consistent with the current findings, Lee's group found a trend with higher levels of amino acids (i.e., proline, threonine, tryptophan, methionine, phenylalanine, tyrosine, valine, leucine, lysine, glycine) and other metabolites (i.e., caproic, valeric, lactate, malonic acid, xylose, sarcosine, D-galactose) in IBS-D compared to healthy controls. Unlike the findings of the current study, Lee's group found higher levels of metabolites (i.e., hypoxanthine, putrescine, urocanic acid, glutamine, formic acid, fumarate, methyl succinate, uracil, choline, glucose, acetate, butyrate) in IBS-D compared to healthy controls. It could be surmised that the higher concentration of these metabolites could reflect a higher energy

and metabolic activity (altered TCA pathway) in IBS and/or it could be a result of cultural diet (Korean diet as a confounder) influencing the microbial activities.

Contradictory results were found in a larger observational study done in China carried out by Han et al., (2022) that enrolled all subtypes of IBS (Rome IV). Subjects were labelled as “discovery cohort” that comprised 330 men and women (214 IBS-D, 24 IBS-C, 19 IBS-M, 7 IBS-U, 66 HC) and “validation cohort” that comprised of 101 men and women (57 IBS-D, 7 IBS-C, 13 IBS-M and 9 IBS-U, 15 HC). Q-TOF/MS metabolomics platform with metagenomic DNA sequencing predicted microbial functions and associated metabolic pathways; however, this group did not find associations between the fecal metabolome and IBS subtype. Furthermore, this group was unable to discriminate IBS from healthy controls via PLS-DA using the fecal metabolome data. Interestingly, they reported that total fecal bile acids were associated with lower bacterial alpha diversity and reported fatty acid dysregulation in IBS. Leveraging on the same sample subjects, Fang et al., (2020) reported higher levels of fecal and serum tryptophan in IBS compared to healthy controls. Inconsistent with current findings, Fang’s group reported higher concentrations of amino acids (i.e., isoleucine, threonine, tyrosine, lysine) in healthy controls compared to IBS. Similarly, Fang and colleagues also found higher number of *Clostridium* in IBS-C and identified *Fusobacterium* in both IBS subtype; however, found higher counts of *Fusobacterium* in IBS-D instead of IBS-C. Although the enrollment of a large cohort was very impressive, the investigators were not able to detect the volatile SCFA partly because the QTOF-MS may not be as sensitive and the diet intake among the subjects may be quite similar.

There are several important limitations to this study that should be highlighted. The SCFA metabolomic profile can be confounded by several factors that were not measured in the MAP study such as gut transit time (proxy for duration of fermentation has been reported to be longer in women than men) including the time of day of fecal collection because the gut microbiome is known to display diurnal changes and thus the concentrations of SCFA vary over the course of the day. Because this was a cross-sectional study, microbial day-to-day variability cannot be assessed, and thus may have influenced the interpretations of results. Other confounding factors include the processing methods used for extraction and analytical tools used for quantification. For example, fecal samples were aliquoted according to the regions of the frozen feces instead

of having the fecal sample homogenized first in solution before preparing the material for a long-time storage at minus 80°C. Also, the amount of material entering the colon from exogenous (fermentable dietary materials) and endogenous materials (host's mucopolysaccharides, enzymes) contributes to production of SCFA. Moreover, the quantity of SCFA produced also depends ultimately on the physical and chemical properties of the fermentable substrate; these features also influence transit time, and the microbiota profile. Differences in the gut microbiome (the bacteria plus the DNA) may play a key role because different bacteria taxa can have different genetic composition and different level of metabolic capacities (i.e., number of genes for polysaccharides degrading enzymes) that influence colonic SCFA productions.

With respect to aim 1, the FDW of IBS-C sample who consumed a higher fiber diet was higher than the healthy control group (116 mg vs. 143 mg). This could indicate that more bacterial biomass should be expected in IBS-C women who consume a high fiber intake. However, when the relative concentrations of the measured fecal SCFA were normalized by FDW, only IBS-C showed a positive trend while the IBS-D and healthy control groups did not. Slower transit time in IBS-C may have meant that the microbes had more time to efficiently extract all the energy from diet and more time for cell division, hence, increased bacterial biomass in the feces. There are mounting evidence from the literature suggesting that the gut microbiome of healthy women with constipation are more diverse than healthy individuals with soft stools (De la Cuesta-Zuluaga et al., 2018).

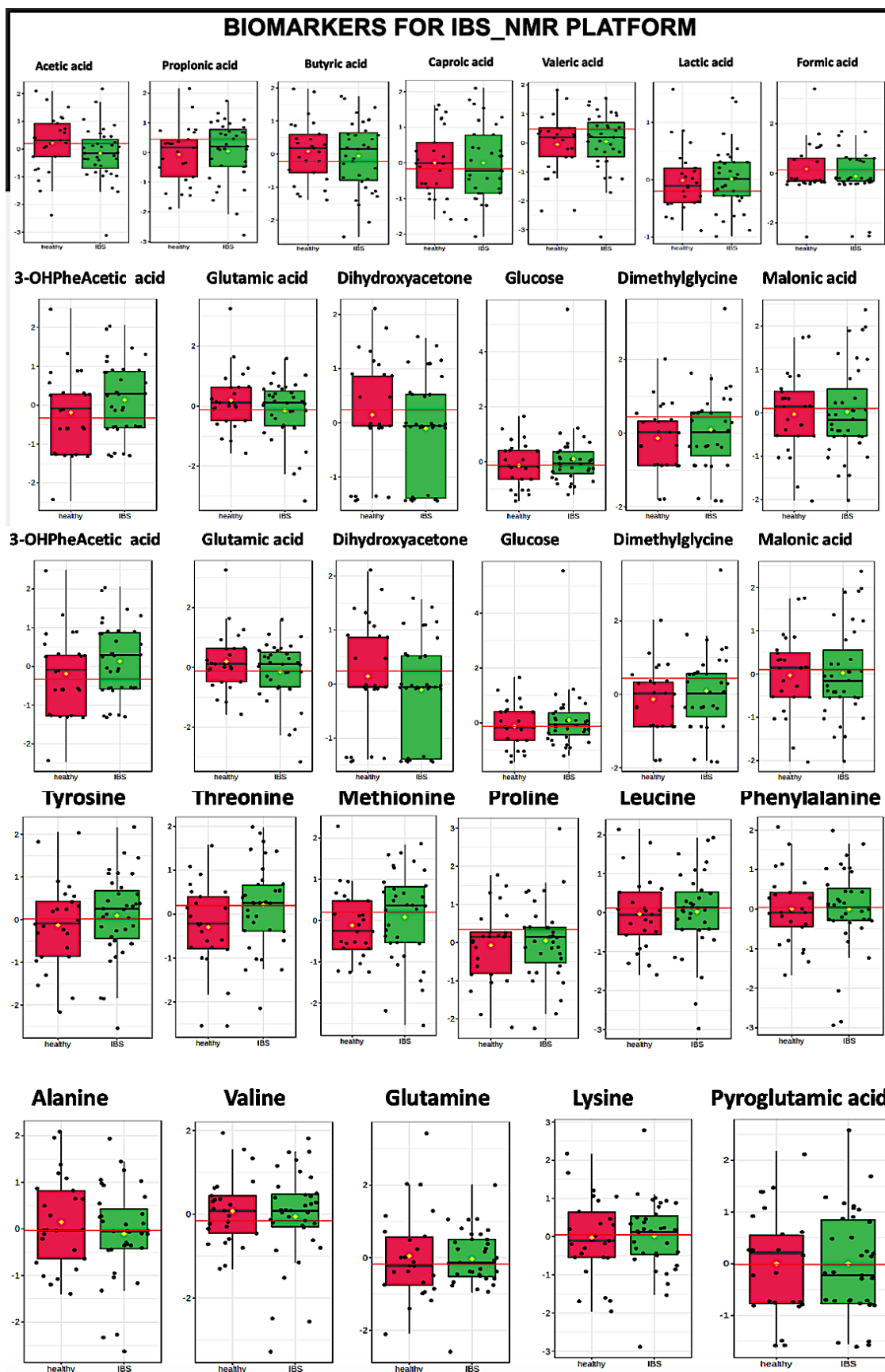


Figure 2. Metabolites in IBS vs HC women. Boxplot center represents median, box represents IQR.

BIOMARKERS FOR IBS_NMR PLATFORM

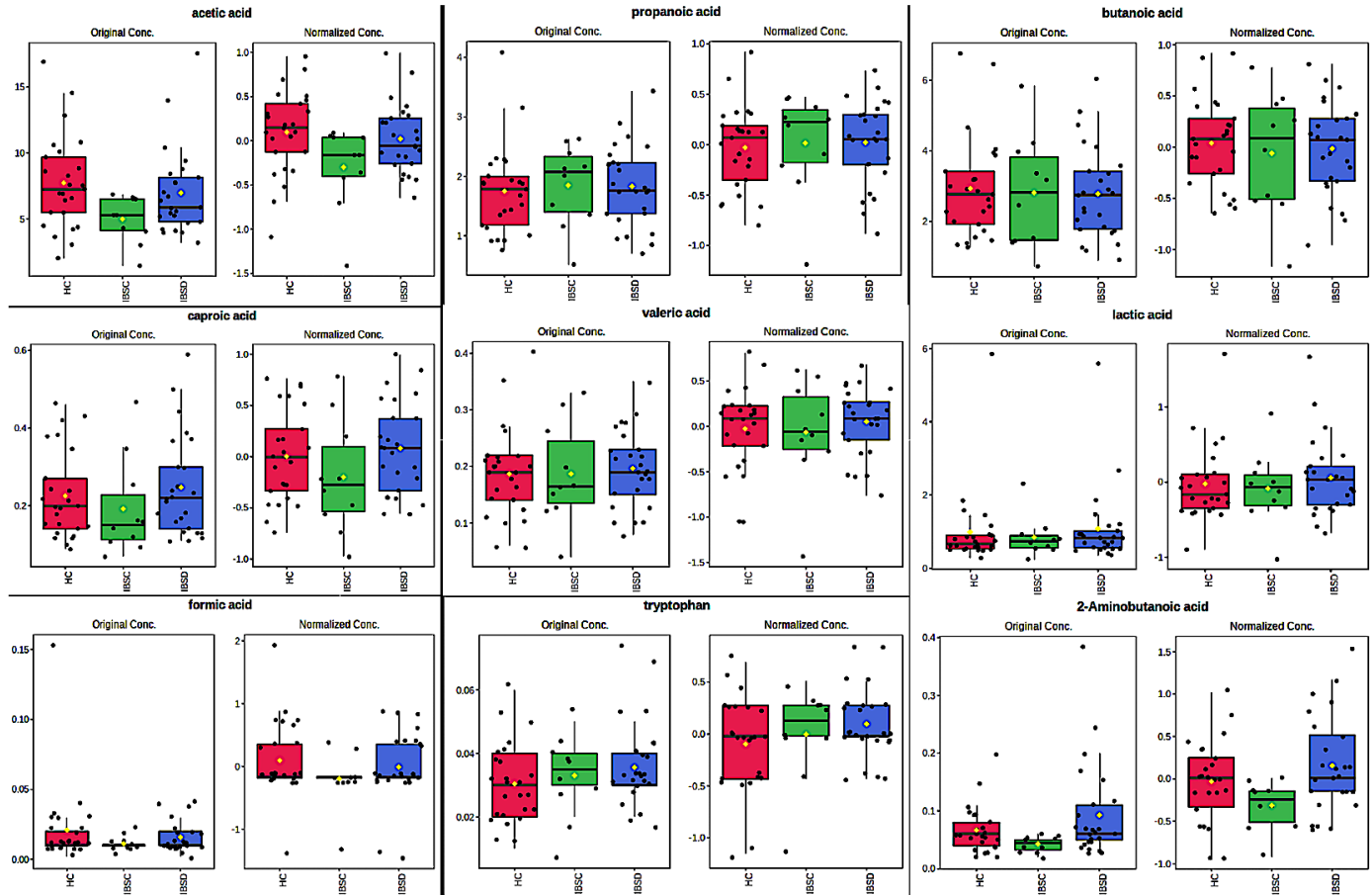


Figure 3a. Metabolites in IBS subtypes vs HC women.

BIOMARKERS FOR IBS_NMR PLATFORM

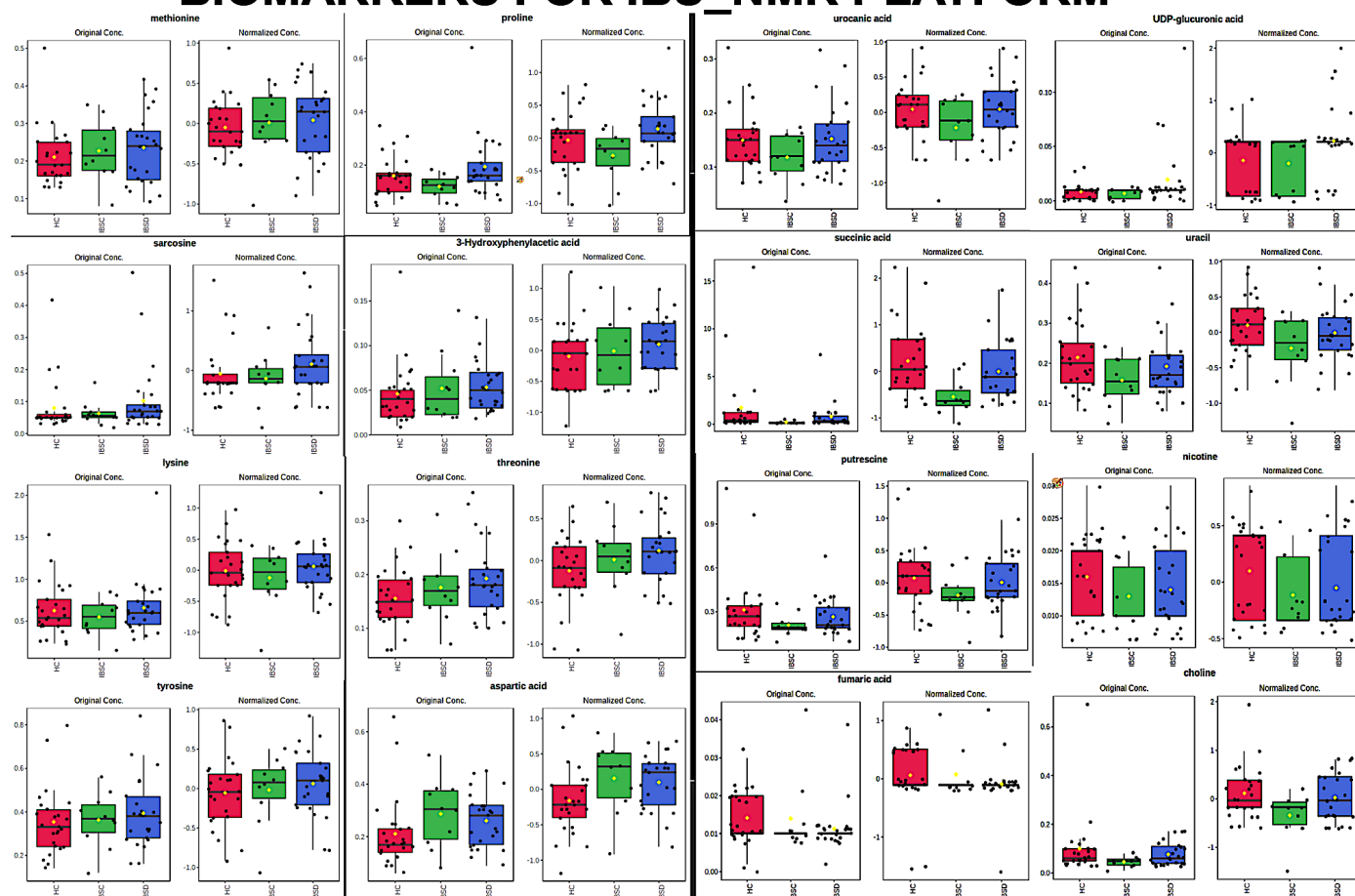


Figure 3b. Metabolites in IBS subtypes vs HC women.

BIOMARKERS FOR IBS_NMR PLATFORM

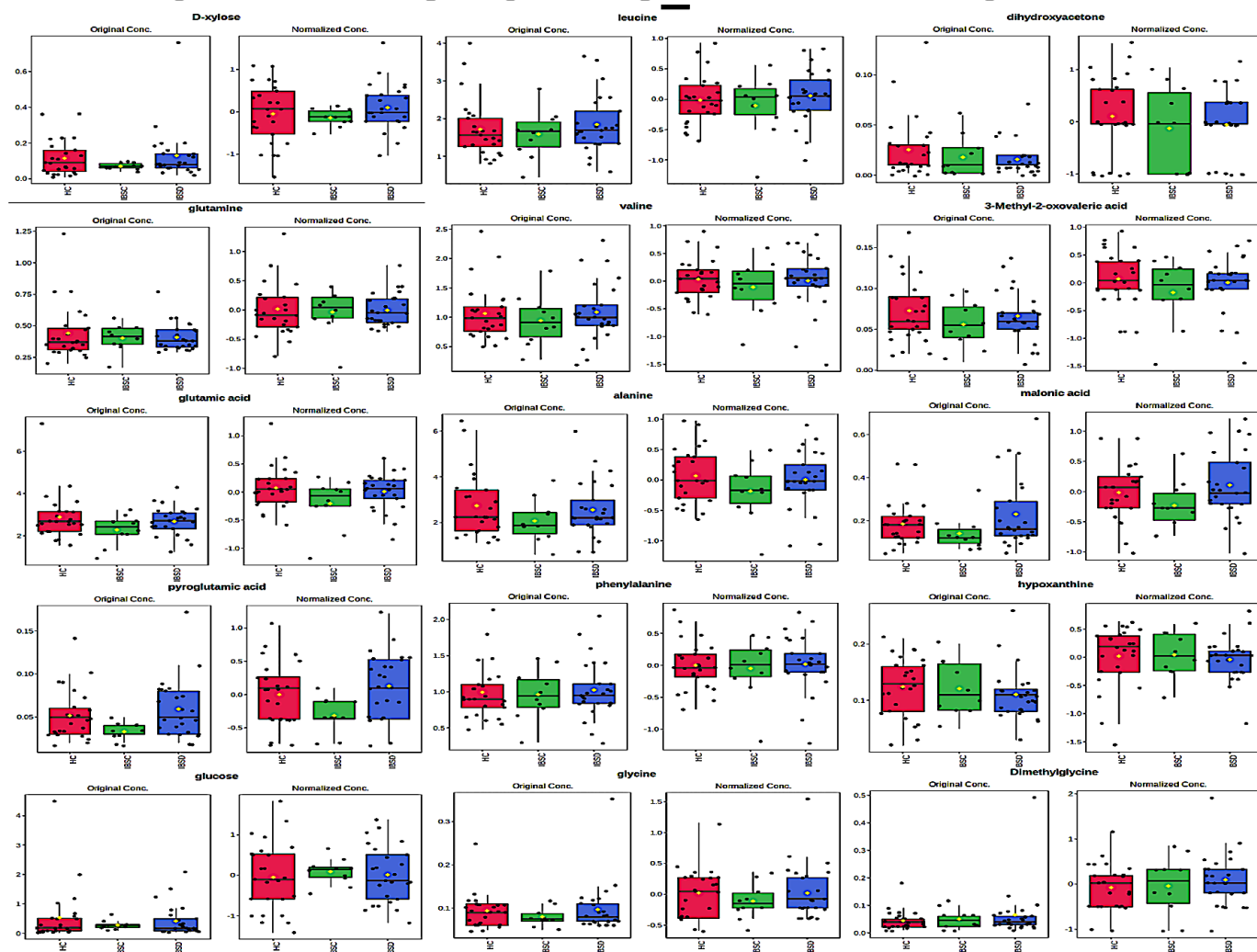


Figure 3c. Metabolites in IBS subtypes vs HC women.

AIM2a RESULT: Relationships between SCFA and GI Symptoms

In this study, it was hypothesized that higher total SCFA will be associated with diarrhea, while lower total SCFA will be associated with constipation. The associations between symptoms (dependent variable) and SCFA (independent variable) were analyzed in various ways: 1) Visualizations of the relationships using biplot with R version 3.6.1 software, 2) categorization of the independent variable (SCFA levels), 3) Univariate Analysis using R package (linear regression), and 4) Pearson correlation. Scatter plots demonstrate the best evidence for the inter- and intra- individual associations of metabolites and GI symptoms for all women.

Table1. Univariate Analysis of Metabolites (Absolute Concentration) and GI Symptoms in HC Women

Variables	HC (n=25)															
	Abdominal pain		Constipation		Percent days with Constip.		Diarrhea		Percent days with diarrhea		Stomach pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Acetic acid	0.13	0.54	-0.38	0.06	-0.26	0.20	0.54	0.01	0.35	0.08	0.16	0.43	-0.04	0.84	0.07	0.74
Propionic acid	0.17	0.43	-0.17	0.42	-0.32	0.12	0.28	0.17	0.19	0.19	0.20	0.33	0.07	0.74	0.22	0.30
Butyric acid	-0.08	0.69	-0.30	0.15	-0.08	0.70	0.41	0.04	0.19	0.36	0.06	0.79	-0.05	0.81	0.04	0.83
sc-SCFA	0.07	0.73	-0.33	0.10	-0.23	0.27	0.48	0.02	0.30	0.14	0.11	0.61	-0.03	0.90	0.09	0.66
Caproic acid	-0.08	0.93	-0.30	0.14	-0.08	0.75	0.41	0.89	0.19	0.75	0.06	0.85	-0.05	0.29	0.04	0.72
Valeric acid	0.20	0.35	-0.16	0.46	-0.21	0.32	0.36	0.08	0.22	0.30	0.20	0.35	0.09	0.68	0.31	0.13
bc-SCFA	0.09	0.67	-0.26	0.22	-0.07	0.76	0.17	0.43	0.07	0.74	0.08	0.71	0.17	0.41	0.20	0.33
sc-SCFA + bc-SCFA	0.07	0.72	-0.34	0.10	-0.22	0.29	0.46	0.02	0.29	0.16	0.11	0.61	-0.01	0.96	0.10	0.62
Formic acid	0.07	0.72	0.16	0.44	-0.06	0.76	0.54	0.00	0.41	0.04	0.21	0.31	-0.02	0.91	0.23	0.27
Lactic acid	0.25	0.23	-0.13	0.53	-0.10	0.63	0.69	0.00	0.45	0.03	0.32	0.11	-0.06	0.79	0.19	0.37

Table2. Adjusted p-values for Univariate Analysis of Metabolites and GI Symptoms in HC Women

Variables	HC FDR Adjusted P-values							
	Abdominal Pain	Constipation	Percent Constipation	Diarrhea	Percent Diarrhea	Stomach Pain	Flatulence	Bloating
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid	0.72	0.22	0.40	0.05	0.22	0.70	0.84	0.84
Propionic acid	0.49	0.49	0.49	0.49	0.49	0.49	0.74	0.49
Butyric acid	0.83	0.60	0.83	0.35	0.83	0.83	0.83	0.83
sc-SCFA	0.84	0.37	0.54	0.13	0.37	0.84	0.90	0.84
Caproic acid	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Valeric acid	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
bc-SCFA	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
sc-SCFA + bc-SCFA	0.83	0.41	0.58	0.17	0.43	0.83	0.96	0.83
Formic acid	0.87	0.70	0.87	0.04	0.18	0.61	0.91	0.61
Lactic acid	0.46	0.71	0.72	0.00	0.10	0.30	0.79	0.60

In HC women, fecal acetic acid level was negatively associated with constipation ($p = 0.06$, $r = -0.38$; FDR adjusted $p = 0.047$), but positively associated with diarrhea ($p = 0.006$, $r = 0.54$) and positively associated with percent days with diarrhea ($p = 0.08$, $r = 0.35$). Butyric acid was positively associated with diarrhea ($p = 0.06$, $r = 0.41$). Formic acid was positively associated with diarrhea ($p = 0.005$, $r = 0.54$; FDR adjusted $p = 0.04$) and percent days with diarrhea ($p = 0.04$, $r = 0.41$; FDR adjusted $p = 0.18$). Lactic acid was positively associated with diarrhea ($p = 0.0001$, $r = 0.69$; FDR adjusted $p = 0.001$) and percent days with diarrhea ($p = 0.03$, $r = 0.45$; FDR adjusted $p = 0.10$). Valeric acid was positively correlated with diarrhea ($p = 0.08$, $r = 0.36$). Total sc-SCFA was positively correlated with diarrhea ($p = 0.016$, $r = 0.48$; FDR adjusted $p = 0.13$) and total SCFA was significantly, positively correlated with diarrhea ($p = 0.021$, $r = 0.46$; FDR adjusted $p = 0.17$).

Table3. Univariate Analysis of Metabolites (Absolute Concentration) and GI Symptoms in Women with IBS

Variable s	I BS-C + IBS-D (n=35)															
	Abdominal Pain		Constipati on		Percent days with Constipati on.		Diarrhea		Percent days with Diarrhea		Stomach Pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Acetic acid	-0.04	0.82	-0.14	0.42	-0.21	0.22	0.19	0.29	0.11	0.51	-0.13	0.44	-0.08	0.65	-0.16	0.36
Propionic acid	-0.27	0.11	-0.06	0.71	-0.12	0.51	0.17	0.34	-0.03	0.88	-0.34	0.04	0.11	0.53	-0.06	0.73
Butyric acid	-0.32	0.06	-0.12	0.50	-0.12	0.50	0.03	0.87	-0.09	0.61	-0.34	0.05	-0.17	0.31	-0.16	0.36
Sc-SCFA	-0.21	0.22	-0.13	0.47	-0.17	0.32	0.14	0.42	0.01	0.95	-0.28	0.10	-0.08	0.66	-0.15	0.39
Caproic acid	-0.23	0.18	-0.05	0.77	-0.15	0.39	-0.03	0.86	0.08	0.64	-0.18	0.29	-0.11	0.54	-0.01	0.97
Valeric acid	-0.32	0.06	-0.10	0.55	-0.17	0.33	0.08	0.64	-0.02	0.93	-0.26	0.13	0.01	0.97	-0.07	0.69
bc-SCFA	-0.30	0.08	-0.08	0.64	-0.17	0.33	0.03	0.88	0.04	0.83	-0.24	0.17	-0.06	0.75	-0.04	0.82
sc-SCFA + bc-SCFA	-0.20	0.33	0.19	0.38	-0.07	0.72	0.16	0.44	-0.18	0.38	-0.27	0.19	-0.08	0.71	-0.11	0.61
Formic acid	-0.05	0.83	0.06	0.79	-0.03	0.90	0.24	0.24	-0.11	0.59	0.17	0.40	0.02	0.91	-0.03	0.89
Lactic acid	0.03	0.87	-0.20	0.34	-0.12	0.56	-0.20	0.34	-0.23	0.28	0.12	0.56	-0.30	0.15	-0.04	0.86

Table4. Adjusted P=values for the Univariate Analysis of Metabolites and GI Symptoms in Women with IBS

Variables	IBS-C + IBS-D FDR Adjusted P-values							
	Abdominal Pain	Constipati on	Percent days with Constipati on	Diarrhea	Percent days with Diarrhea	Stomach Pain	Flatulence	Bloating
	FDR adj. pv	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid	0.82	0.68	0.68	0.68	0.68	0.68	0.74	0.68
Propionic acid	0.46	0.83	0.83	0.83	0.88	0.34	0.83	0.83
Butyric acid	0.24	0.66	0.66	0.87	0.69	0.24	0.66	0.66
sc-SCFA	0.62	0.62	0.62	0.62	0.95	0.62	0.76	0.62
Caproic acid	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Valeric acid	0.47	0.92	0.88	0.92	0.97	0.53	0.97	0.92
bc-SCFA	0.67	0.88	0.87	0.88	0.88	0.69	0.88	0.88
sc-SCFA+ bc-SCFA	0.63	0.63	0.63	0.63	0.94	0.63	0.76	0.63
Formic acid	0.77	0.77	0.65	0.65	0.65	0.65	0.77	0.77
Lactic acid	0.86	0.86	0.86	0.86	0.97	0.86	0.86	0.86

Table5. Univariate Analysis of Metabolites (Absolute Concentration) and GI Symptoms in Women with IBS-C

Variables	IBS-C (n=10)															
	Abdominal Pain		Constipation		Percent Days with Constipation		Diarrhea		Percent days with Diarrhea		Stomach Pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Acetic acid	-0.26	0.47	-0.55	0.10	-0.26	0.48	-0.07	0.85	0.34	0.33	-0.32	0.36	0.01	0.97	-0.10	0.79
Propionic acid	-0.47	0.17	-0.62	0.06	-0.27	0.46	-0.30	0.40	-0.02	0.96	-0.44	0.21	-0.21	0.56	-0.25	0.49
Butyric acid	-0.55	0.10	-0.57	0.09	-0.29	0.41	-0.22	0.55	-0.10	0.78	-0.49	0.15	-0.25	0.48	-0.19	0.61
sc-SCFA	-0.48	0.16	-0.63	0.05	-0.30	0.40	-0.21	0.57	0.06	0.86	-0.46	0.18	-0.17	0.63	-0.19	0.61
Caproic acid	-0.51	0.13	-0.41	0.23	-0.19	0.59	-0.30	0.39	0.02	0.95	-0.41	0.23	-0.29	0.42	-0.12	0.74
Valeric acid	-0.34	0.34	-0.46	0.19	-0.26	0.46	-0.17	0.64	0.08	0.83	-0.23	0.53	-0.06	0.86	0.04	0.92
bc-SCFA	-0.44	0.21	-0.44	0.20	-0.23	0.52	-0.24	0.50	0.05	0.89	-0.33	0.35	-0.18	0.61	-0.05	0.90
sc-SCFA + bc-SCFA	-0.48	0.16	-0.61	0.06	-0.29	0.41	-0.21	0.56	0.06	0.87	-0.45	0.19	-0.18	0.63	-0.17	0.64
Formic acid	0.32	0.36	0.22	0.54	-0.06	0.86	-0.04	0.91	0.40	0.25	0.08	0.82	0.08	0.82	0.19	0.60
Lactic acid	0.11	0.75	-0.11	0.75	-0.07	0.84	0.01	0.98	0.41	0.24	-0.11	0.76	0.04	0.91	-0.02	0.96

Table6. Adjusted P-values for the Univariate Analysis of Metabolites and GI Symptoms in Women with IBS-C

Variables	IBS-C FDR Adjusted P-values							
	Abdominal Pain	Constipation	Percent days with Constip.	Diarrhea	Percent days with Diarrhea	Stomach Pain	Flatulence	Bloating
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid	0.76	0.76	0.76	0.97	0.76	0.76	0.97	0.97
Propionic acid	0.56	0.46	0.65	0.65	0.96	0.56	0.65	0.65
Butyric acid	0.40	0.40	0.70	0.70	0.78	0.41	0.70	0.70
sc-SCFA	0.48	0.43	0.73	0.73	0.86	0.48	0.73	0.73
Caproic acid	0.63	0.63	0.79	0.68	0.95	0.63	0.68	0.85
Valeric acid	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
bc-SCFA	0.81	0.81	0.81	0.81	0.90	0.81	0.81	0.90
sc-SCFA + bc-SCFA	0.51	0.49	0.73	0.73	0.87	0.51	0.73	0.73
Formic acid	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Lactic acid	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98

IBS-C. In women with IBS-C, propionic acid was negatively correlated with constipation ($p = 0.06$, $r = -0.62$). Butyric acid was negatively correlated with abdominal pain ($p = 0.099$, $r = -0.55$) and

negatively correlated with constipation ($p = 0.085$, $r = -0.57$). Total sc-SCFA was associated with constipation ($p = 0.05$, $r = -0.62$) and total SCFA (sc- SCFA and bc-SCFA) was negatively correlated with constipation ($p = 0.06$, $r = -0.61$).

IBS-D. In women with IBS-D, valeric acid was negatively correlated with abdominal pain ($p = 0.08$, $r = -0.36$). Propionic acid was negatively correlated with stomach pain ($p = 0.099$, $r = -0.34$).

All Cohort. In all women ($N=60$), acetic acid was negatively associated with percent days with constipation ($p = 0.04$, $r = -0.27$), negatively associated with flatulence and bloating ($p = 0.09$, $r = -0.22$). Total sc-SCFA were negatively associated with stomach pain ($p = 0.099$, $r = -0.21$).

Table 7. Univariate Analysis of Metabolites and GI Symptoms in Women with IBS-D

Variables	IBS-DIARRHEA (n=25)															
	Abdominal Pain		Constipation		Percent days with Constipation		Diarrhea		Percent days with Diarrhea		Stomach Pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Acetic acid	-0.09	0.68	0.20	0.35	0.05	0.83	0.09	0.67	-0.23	0.28	-0.16	0.44	-0.16	0.43	-0.11	0.59
Propionic acid	-0.24	0.24	0.17	0.42	-0.12	0.58	0.29	0.16	-0.10	0.64	-0.34	0.10	0.19	0.37	-0.01	0.97
Butyric acid	-0.23	0.27	0.11	0.61	-0.16	0.43	0.13	0.53	-0.15	0.46	-0.29	0.16	-0.15	0.48	-0.16	0.45
sc-SCFA	-0.19	0.36	0.18	0.40	-0.07	0.74	0.17	0.42	-0.19	0.37	-0.27	0.19	-0.08	0.70	-0.11	0.59
Caproic acid	-0.16	0.45	0.30	0.14	-0.05	0.82	0.03	0.88	-0.02	0.91	-0.12	0.57	-0.07	0.76	0.07	0.73
Valeric acid	-0.36	0.08	0.12	0.56	-0.18	0.40	0.14	0.51	-0.15	0.48	-0.29	0.16	0.02	0.94	-0.09	0.66
bc-SCFA	-0.20	0.33	0.19	0.38	-0.07	0.72	0.16	0.44	-0.18	0.38	-0.27	0.19	-0.08	0.71	-0.11	0.61
sc-SCFA+bc-SCFA	-0.20	0.33	0.19	0.38	-0.07	0.72	0.16	0.44	-0.18	0.38	-0.27	0.19	-0.08	0.71	-0.11	0.61
Formic acid	-0.05	0.83	0.06	0.79	-0.03	0.90	0.24	0.24	-0.11	0.59	0.17	0.40	0.02	0.91	-0.03	0.89
Lactic acid	0.03	0.87	-		-		-		-		-		-		-	
	0.03	0.87	0.20	0.34	-0.12	0.56	0.20	0.34	-0.23	0.28	0.12	0.56	-0.30	0.15	-0.04	0.86

Table8. Adjusted P-values for the Univariate Analysis of Metabolites and GI Symptoms in Women with IBS-D

Variables	IBS-DIARRHEA FDR ADJUSTED P-VALUES							
	Abdominal Pain	Constipation	Percent days with Constipation	Diarrhea	Percent days with Diarrhea	Stomach Pain	Flatulence	Bloating
	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p	FDR adj. p
Acetic acid	0.77	0.77	0.83	0.77	0.77	0.77	0.77	0.77
Propionic acid	0.64	0.67	0.73	0.64	0.73	0.64	0.67	0.97
Butyric acid	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
sc-SCFA	0.68	0.68	0.74	0.68	0.68	0.68	0.74	0.74
Caproic acid	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Valeric acid	0.62	0.74	0.74	0.74	0.74	0.65	0.94	0.75
bc-SCFA	0.71	0.71	0.72	0.71	0.71	0.71	0.72	0.72
sc-SCFA+ bc-SCFA	0.71	0.71	0.72	0.71	0.71	0.71	0.72	0.72
Formic acid	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Lactic acid	0.87	0.68	0.75	0.68	0.68	0.75	0.68	0.87

Table 9. Univariate Analysis of Metabolites and GI Symptoms in All Women (N=60)

Variable s	ALL COHORTS (N=60)																
	Abdominal Pain		Constipation n		Percent days with Constipation		Diarrhea		Percent days with Diarrhea		Stomach Pain		Flatulence		Bloating		
	R	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	
Acetic acid	-0.19	0.14	-0.21	0.11	-0.27	0.04	-	0.02	0.90	0.02	0.85	-0.22	0.09	-0.20	0.12	-0.22	0.09
Propionic acid	-0.07	0.60	-0.02	0.90	-0.15	0.26	0.16	0.23	0.07	0.58	-0.13	0.31	0.11	0.39	0.01	0.95	
Butyric acid	-0.17	0.20	-0.10	0.43	-0.11	0.40	0.01	0.91	-0.03	0.84	-0.20	0.13	-0.12	0.37	-0.11	0.39	
sc-SCFA	-0.18	0.18	-0.15	0.26	-0.21	0.11	0.03	0.80	0.02	0.88	-0.21	0.10	-0.12	0.36	-0.15	0.25	
Caproic Acid	-0.10	0.44	-0.05	0.73	-0.07	0.57	-	0.02	0.86	0.03	0.84	-0.09	0.47	-0.02	0.86	0.00	0.97
Valeric Acid	-0.12	0.37	-0.06	0.64	-0.17	0.20	0.08	0.54	0.05	0.70	-0.11	0.40	0.03	0.81	-0.02	0.90	
sc-SCFA+ bc-SCFA	-0.17	0.19	-0.14	0.27	-0.21	0.11	0.03	0.80	0.02	0.87	-0.21	0.11	-0.11	0.40	-0.14	0.28	
Formic acid	-0.15	0.26	-0.12	0.37	-0.14	0.29	-	0.02	0.88	0.07	0.62	-0.11	0.42	-0.14	0.30	-0.13	0.33
Lactic acid	0.03	0.80	-0.12	0.35	-0.12	0.35	-	0.02	0.91	0.11	0.41	0.05	0.70	-0.11	0.41	-0.02	0.85

Table 10. FDR-Adjusted p-value for All Analyses for AIM2a (N=60)

Variables	acetic	propionic	butyric	sc-SCFA	caproic	valeric	bc-SCFA	Total SCFA	formic	lactic
Abdominal pain_HC	0.81	0.75	0.85	0.85	0.97	0.87	0.97	0.83	0.91	0.96
Constipation_HC	0.54	0.75	0.73	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Percent days constipation_HC	0.73	0.75	0.85	0.71	0.97	0.87	0.97	0.75	0.91	0.98
Diarrhea_HC	0.23	0.75	0.73	0.65	0.97	0.87	0.97	0.64	0.20	0.0056
Percent days diarrhea_HC	0.54	0.75	0.76	0.69	0.97	0.87	0.97	0.64	0.89	0.51

Stomach pain_HC	0.76	0.75	0.88	0.83	0.97	0.87	0.97	0.82	0.91	0.96
Flatulence_HC	0.90	0.84	0.88	0.92	0.97	0.87	0.97	0.96	0.91	0.98
Bloating_HC	0.90	0.75	0.88	0.83	0.97	0.87	0.97	0.82	0.91	0.96
Abdominal pain_IBS	0.90	0.75	0.73	0.71	0.97	0.87	0.97	0.64	0.91	0.98
Constipation_IBS	0.76	0.84	0.77	0.75	0.97	0.87	0.97	0.76	0.91	0.96
Percent days constipation_IBS	0.74	0.77	0.77	0.71	0.97	0.87	0.97	0.75	0.91	0.96
Diarrhea_IBS	0.76	0.75	0.89	0.71	0.97	0.87	0.97	0.75	0.91	0.98
Percent days diarrhea_IBS	0.79	0.97	0.79	0.95	0.97	0.87	0.97	0.96	0.91	0.98
Stomach pain_IBS	0.76	0.75	0.73	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Flatulence_IBS	0.87	0.77	0.77	0.83	0.97	0.97	0.97	0.83	0.91	0.96
Bloating_IBS	0.76	0.84	0.77	0.71	0.97	0.87	0.97	0.75	0.91	0.98
Abdominal pain_IBS-C	0.76	0.75	0.73	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Constipation_IBS-C	0.54	0.75	0.73	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Percent days constipation_IBS-C	0.76	0.76	0.77	0.71	0.97	0.87	0.97	0.75	0.91	0.98
Diarrhea_IBS-C	0.90	0.75	0.78	0.83	0.97	0.87	0.97	0.82	0.91	0.98
Percent days diarrhea_IBS-C	0.76	0.97	0.88	0.92	0.97	0.96	0.97	0.91	0.91	0.96
Stomach pain_IBS-C	0.76	0.74	0.73	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Flatulence_IBS-C	0.97	0.77	0.77	0.83	0.97	0.96	0.97	0.82	0.91	0.98
Bloating_IBS-C	0.90	0.77	0.79	0.83	0.97	0.96	0.97	0.82	0.91	0.98
Abdominal pain_IBS-D	0.87	0.75	0.77	0.71	0.97	0.87	0.97	0.75	0.91	0.98
Constipation_IBS-D	0.76	0.75	0.79	0.71	0.97	0.87	0.97	0.75	0.91	0.96
Percent days constipation_IBS-D	0.90	0.77	0.77	0.85	0.97	0.87	0.97	0.83	0.91	0.98
Diarrhea_IBS-D	0.87	0.75	0.78	0.70	0.97	0.87	0.97	0.75	0.91	0.96
Percent days diarrhea_IBS-D	0.76	0.80	0.77	0.71	0.97	0.87	0.97	0.75	0.91	0.96
Stomach pain_IBS-D	0.76	0.75	0.72	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Flatulence_IBS-D	0.76	0.75	0.77	0.85	0.97	0.96	0.97	0.83	0.91	0.96
Bloating_IBS-D	0.84	0.97	0.77	0.83	0.97	0.87	0.97	0.82	0.91	0.98
Abdominal pain_ALL	0.55	0.77	0.77	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Constipation_ALL	0.54	0.97	0.77	0.71	0.97	0.87	0.97	0.75	0.91	0.96
Percent days constipation_ALL	0.54	0.75	0.77	0.69	0.97	0.87	0.97	0.64	0.91	0.96
Diarrhea_ALL	0.93	0.75	0.91	0.89	0.97	0.87	0.97	0.89	0.91	0.98
Percent days diarrhea_ALL	0.90	0.77	0.88	0.92	0.97	0.87	0.97	0.91	0.91	0.96
Stomach pain_ALL	0.54	0.75	0.73	0.69	0.97	0.87	0.97	0.64	0.91	0.98
Flatulence_ALL	0.54	0.75	0.77	0.71	0.97	0.96	0.98	0.75	0.91	0.96
Bloating_ALL	0.53	0.97	0.76	0.71	0.97	0.96	0.98	0.76	0.91	0.98

Pearson correlation analysis (using R package) revealed that acetic acid was negatively correlated with percent days constipation in all women ($r = -0.27$, 95% CI: -0.49, -0.02). Normal fecal total SCFA was reported

to be between 400-600 millimolar (Skugland, 2016; den Besten et al. 2013), and thus it was fitting to look at the trend for women who produced high amount of fecal SCFA (>600 to 1100 millimole) vs. low amount of fecal total SCFA low (150 to <600 micromole). When categorizing women with high and low SCFA profile, women with lower total SCFA (low SCFA producers) were associated with higher mean abdominal pain severity scores compared to high SCFA producer women.

HC. Interestingly, the high SCFA producer HC women (mean = 783 micromole) had significantly higher abdominal pain associated with total SCFA ($p = 0.000056^{***}$) than the low SCFA producer HC women (mean = 348 micromole). Significant difference was also found in abdominal pain score in high propionic acid producer HC women (>100-300 micromole, mean=137 micromole, n=10) compared to low propionic acid producer HC women (35 to <100mM, mean = 65 micromole, n=15); higher fecal propionic acid concentration was associated with higher abdominal pain score ($p = 0.00016^{***}$) in HC. HC with high fecal butyric acid (>30-50 micromole, mean = 180 micromole, n=16) reported significantly higher abdominal pain score ($p = 0.0002^{***}$) than women with low butyric acid (4 mM-<30 micromole, mean = 65 micromole, n=9). High valeric acid HC women (30-60 micromole) was also positively associated with higher abdominal pain score ($p = 0.0005^{***}$) than with low valeric acid HC women (4-30 micromole). Fecal caproic acid (4-30 micromole vs >30-60 micromole) followed the same trend but to a lesser degree ($p = 0.004^{**}$). Fecal lactic acid also followed the same trend with abdominal pain; higher lactic acid (60-260 micromole vs. 10-<60 micromole) was significantly associated with higher abdominal pain score ($p = 0.04^{*}$) in HC women. Lastly, in HC women, formic acid (0.3-1.9 micromole vs. 2-10 micromole) was only weakly associated with higher abdominal pain ($p = 0.06$). In HC women, the mean percent days with diarrhea was not statistically different ($p = 0.08$) with the high SCFA producers compared to the low SCFA producers. Similarly, the mean diarrhea events followed the same trend ($p = 0.07$). the mean diarrhea events were about the same in women who produced high and low butyric acid ($p = 0.10$). The mean lactic acid was more than two times higher (74 micromole vs. 30 micromole) in high SCFA producer HC women than in low SCFA producer HC women. There was no difference in percent days constipation between the two groups (high vs low SCFA producer HC women). However, constipation events were reported lower in high SCFA producer HC women but did not reach statistical significance ($p = 0.08$).

IBS-D. High SCFA producer IBS-D women (mean = 827 micromole, n = 8) reported significantly lower stomach pain (p = 0.03) than the low SCFA producer IBS-D women (mean = 331 micromole, n = 17). IBS-D women with higher fecal propionic acid (>100-300 micromole, mean = 169 micromole, n = 10) reported significantly lower stomach pain (p = 0.03), and lower abdominal pain (p = 0.06) than IBS-D women with less fecal propionic acid (30mM -100 micromole, mean = 63 micromole, n = 15). IBS-D women who produced less fecal valeric acids (10- <60 micromole, mean = 17 micromole, n = 19) reported lower abdominal pain score (p = 0.04) than women who have higher fecal valeric acids (>60-260 micromole, mean = 41 micromole, n = 8). IBS-D women who had higher amount of fecal butyric acid (>100-375 micromole, mean = 205 micromole, n = 12) reported higher diarrhea events (p = 0.05) than IBS-D women who had lower fecal butyric acids (40 -100 micromole, mean = 65 micromole, n=13). Percent days diarrhea (67% vs 51%) was higher (p = 0.06) in low acetic acid producer IBS-D women (50-300 mM, mean = 170, n = 21) than high acetic acid producer IBS-D (>300 – 500 mM, mean = 440 mM, n = 4). Similarly, percent days with diarrhea (68% vs 48%, p = 0.04) and diarrhea events (p= 0.08) were reported more in IBS-D women with lower amount of lactic acid (10 – 60 micromole, mean = 34 micromole, n = 21) than in IBS-D women with higher fecal lactic acid (>60 – 260 micromoles, mean =135 micromole, n = 4). Note: Majority of lactic acid is converted to acetic acid. IBS-D women with high acetic acid (300-600 micromole) reported less diarrhea events compared to IBS-D women with low acetic acid (50-<300 micromoles).

Table 11. GI Symptoms of High SCFA (Mean± Sd) Producers and Low SCFA Producers

Variable s	ALL women (N=60)		Healthy Women (n=25)		IBS combined (n=35)		IBS-C (n=10)		IBS-D (n=25)	
	High SCFA (n=21)	Low SCFA (n=39)	High SCFA (n=10)	Low SCFA (n=15)	High SCFA (n=11)	Low SCFA (n=24)	High SCFA (n=3)	Low SCFA (n=7)	High SCFA (n=8)	Low SCFA (n=17)
Abdominal pain	0.55 ± 0.58	0.87 ± 0.78	0.05 ± 0.04	0.04 ± 0.05	1.38 ± 0.54	1.02 ± 0.42	0.58 ± 0.26	0.56 ± 0.24	1.18 ± 0.35	1.44 ± 0.54
Stomach pain	0.35 +0.40	0.66 +0.65	0.03 ± 0.04	0.02 ± 0.03	0.64 ± 0.65	1.06 ± 0.51	0.46 ± 0.25	0.94 ± 0.52	0.70 ± 0.38	1.11 ± 0.52
Bloating	0.44 ± 0.56	0.71 +0.80	0.05 ± 0.07	0.06 ± 0.07	0.79 ± 0.59	1.12 ± 0.78	0.58 ± 0.26	1.25 ± 0.85	0.60 ± 0.54	1.10 ± 0.77
Constipation	0.33 ± 0.48	0.50 +0.72	0.01 ± 0.03	0.04 ± 0.06	0.62 ± 0.52	0.78 ± 0.80	0.99± 0.02	1.54 ± 0.93	0.48 ± 0.55	0.47± 0.49
Diarrhea	0.32 ± 0.46	0.29 +0.32	0.04 ± 0.06	0.01 ± 0.03	0.57 ± 0.52	0.56 ± 0.30	0.08 ± 0.11	0.24 ± 0.35	0.76 ± 0.49	0.56 ± 0.23

Table 12. P-values Comparing GI Symptoms of High SCFA Producers and Low SCFA Producers

GI Symptoms	ALL Women High vs. Low	Healthy Women High vs. Low	IBS-C & IBS-D combined High vs. Low	IBS-C High vs. Low	IBS-D High vs. Low
Abdominal pain	0.046	0.39	0.002	0.06	0.096
Stomach pain	0.002	0.39	0.0003	0.09	0.03
Bloating	0.03	0.37	0.03	0.44	0.06
Constipation	0.08	0.08	0.25	0.13	0.50
Diarrhea	0.40	0.10	0.48	0.20	0.15

Table 13. Non-GI Symptoms Scores (mean+ SD) of High SCFA Producers and Low SCFA Producers

Variables	ALL women (N=60)		Healthy Women (n=25)		IBS combined (n=35)		IBS-C (n=10)		IBS-D (n=25)	
	High SCFA (n=21)	Low SCFA (n=39)	High SCFA (n=10)	Low SCFA (n=15)	High SCFA (n=11)	Low SCFA (n=24)	High SCFA (n=3)	Low SCFA (n=7)	High SCFA (n=8)	Low SCFA (n=17)
Muscle pain	0.21 ± 0.29	0.39 ± 0.55	0.11 ± 0.17	0.05 ± 0.11	0.31 ± 0.36	0.60 ± 0.61	0.11 ± 0.09	0.75 ± 0.54	0.38 ± 0.39	0.54 ± 0.64
Anxiety	0.40 +0.58	0.67 +0.60	0.09 ± 0.14	0.02 ± 0.03	0.68 ± 0.68	0.97 ± 0.55	0.17 ± 0.16	0.99 ± 0.52	0.88 ± 0.71	0.97 ± 0.58
Stress	0.53 ± 0.55	0.73 +0.64	0.21 ± 0.21	0.23 ± 0.20	0.81 ± 0.61	1.05 ± 0.62	0.48 ± 0.52	1.4 ± 0.61	0.94 ± 0.63	0.90 ± 0.58
Depressed	0.26 ± 0.52	0.38 +0.56	0.05 ± 0.08	0.07 ± 0.09	0.46 ± 0.67	0.57 ± 0.50	0.08± 0.05	0.80 ± 0.60	0.60 ± 0.75	0.49± 0.46

Table 14. P-values Comparing non-GI Symptoms of High SCFA Producers and Low SCFA Producers

Non-GI symptoms	ALL Women High vs. Low	Healthy Women High vs. Low	IBS-C + IBS-D High vs. Low	IBS-C High vs. Low	IBS-D High vs. Low
Muscle pain	0.01	0.18	0.05	0.046	0.24
Anxiety	0.03	0.15	0.12	0.03	0.38
Stress	0.06	0.39	0.15	0.07	0.44
Depressed	0.18	0.26	0.32	0.048	0.35

Table 15. GI symptoms of High Butyric Acid (Mean+ sd) Producers and Low Butyric Acid Producers

Non-GI Symptoms	Healthy Women (n=25)		IBS combined (n=35)		IBS-C (n=10)		IBS-D (n=25)	
	High Butyric (n=16) Mean: 180	Low Butyric (n=9) Mean: 65.3	High Butyric (n=11) Mean: 206	Low Butyric (n=24) Mean: 64	High Butyric (n=5) Mean: 206.5	Low Butyric (n=5) Mean: 62.6	High Butyric (n=12) Mean: 205.5	Low Butyric (n=13) Mean: 65

	Fiber: 23.5 Fat: 72.4 Weight: 134 Age: 30	Fiber:23.9 Fat: 74.4 Weight:140 Age:30	Fiber:22g Fat: 82 Weight: 146 Age: 31 FDW:148	Fiber: 20 g Fat: 71 Weight:141 Age:25 FDW:101	Fiber: 26g Fat: 69g Weight:134 Age:28 FDW: 124	Fiber: 21g Fat: 79g Weight:130 Age:24 FDW: 128	Fiber :20.5 g Fat: 87 g Weight: 150.5 Age: 32 FDW: 143.6	Fiber: 19.2 g Fat: 68g Weight: 145 Age:26 FDW:92.2
Abdominal pain	0.05 ± 0.04	0.03 ± 0.04	1.15 ± 0.56	1.37 ± 0.48	0.71 ± 0.26	1.38 ± 0.62	1.34 ± 0.56	1.37 ± 0.45
Stomach pain	0.03 ± 0.04	0.008 ± 0.016	0.80 ± 0.50	1.06 ± 0.49	0.49 ± 0.24	1.11 ± 0.51	0.92 ± 0.53	1.04 ± 0.50
Flatulence	0.17 ± 0.27	0.14 ± 0.15	0.79 ± 0.50	1.80 ± 0.62	0.74 ± 0.24	1.20 ± 0.66	1.18 ± 0.44	1.17 ± 0.64
Bloating	0.06 ± 0.08	0.04 ± 0.04	0.94 ± 0.71	1.10 ± 0.76	0.95 ± 0.62	1.58 ± 0.71	0.93 ± 0.78	0.91 ± 0.71
Constipation	0.03 ± 0.06	0.03 ± 0.05	0.66 ± 0.56	0.80 ± 0.85	0.76 ± 0.32	1.98 ± 0.63	0.61 ± 0.64	0.35 ± 0.30
Diarrhea	0.03 ± 0.05	0.01 ± 0.03	0.55 ± 0.45	0.45 ± 0.30	0.085 ± 0.088	0.30 + 0.41	0.75 ± 0.39	0.51 ± 0.24
Percent days constipation	14.04 ± 13.87	27.23 ± 29.11	29.05 ± 26.47	38.64 ± 33.67	60.92 ± 18.34	81.34 ± 24.38	15.77 ± 15.59	22.21 ± 18.87
Percent days diarrhea	23.01 ± 17.51	12.88 ± 11.16	51.74 ± 30.30	50.30 ± 24.34	14.59 ± 6.60	18.71 ± 12.46	51.49 ± 12.38	67.26 ± 17.52
Depressed	0.05 ± 0.08	0.07 ± 0.10	0.52 ± 0.65	0.55 ± 0.47	0.27 ± 0.41	0.84 ± 0.63	0.62 ± 0.71	0.44 ± 0.36
Hard to Concentrate	0.09 ± 0.14	0.11 ± 0.16	0.80 ± 0.59	0.81 ± 0.59	0.73 ± 0.46	1.21 ± 0.75	0.84 ± 0.65	0.65 ± 0.47
Stress	0.21 ± 0.19	0.25 ± 0.23	0.88 ± 0.57	1.06 ± 0.67	0.61 ± 0.44	1.64 ± 0.54	0.99 ± 0.60	0.84 ± 0.58
Muscle pain	0.11 ± 0.16	0.01 ± 0.03	0.40 ± 0.52	0.61 ± 0.58	0.34 ± 0.50	0.78 ± 0.54	0.43 ± 0.54	0.55 ± 0.60
Anxiety	0.12 ± 0.18	0.18 ± 0.24	0.82 ± 0.71	0.94 ± 0.49	0.46 ± 0.58	1.02 ± 0.50	0.97 ± 0.73	0.91 ± 0.51
Daily interference	1.03 ± 0.05	NA	1.39 ± 0.41	1.65 ± 0.38	1.10 ± 0.08	1.81 ± 0.25	1.51 ± 0.43	1.59 ± 0.41
Cramps	0.07 ± 0.09	0.04 ± 0.05	0.28 ± 0.30	0.47 ± 0.38	0.09 ± 0.12	0.61 ± 0.32	0.35 ± 0.32	0.42 ± 0.40
Fatigue	0.37 ± 0.35	0.32 ± 0.28	1.13 ± 0.72	1.41 ± 0.68	0.55 ± 0.41	1.77 ± 0.59	1.37 ± 0.69	1.27 ± 0.69
Urgency	0.02 ± 0.03	0.02 ± 0.04	0.42 ± 0.32	0.65 ± 0.56	0.55 ± 0.69	0.11 ± 0.16	0.55 ± 0.28	0.63 ± 0.49

IBS-C high butyric producer was driven by higher intake of insoluble fiber (p=0.07). HC women and IBS-D high butyric acid produce was driven by higher intake of soluble fiber (p=0.09) and fat intake (p=0.02); both groups had approximately similar dietary intake for these two types of nutrients.

IBS Low Butyric Acid Producer. In the IBS group, the low butyric producer had significantly higher *Alistipes* (0.002), *Bacteroides* (p=0.01), *Odoribacter* (p=0.04), *Turicibacter* (p=0.05), *Veillonella*, (p =0.06), *Bilophila* (p=0.09), *Bifidobacteria* (p=0.30), *E. Shigella* (p=0.13), *Phascolarctobacteria* (p=0.15), *Lactococcus* (p=0.18), *Clostridia IV* (p=0.20). However, the low butyric producer also had significantly lower *Roseburia* (p =0.01), *Dorea* (p=0.01), *Blautia* (p=0.02), *Lactobacillus* (p=0.16), *Lachnospiraceae insertia sedis* (p=0.13); *Butyricicoccus* (p=0.15), *Prevotella* (p=0.20), *Collinsella* (p=0.10), *Butyricimonas* (p=0.10), *Gemmiger* (p=0.07), lower total bacteria (p=0.20),

IBS High Butyric Acid Producer. *Paraprevotella* and *Finegoldia* were found only in the High butyric IBS group and was not identified in the Low butyric IBS group. Three IBS-D women were detected to have genera *Finegoldia* (belonging to class *Clostridia*. *Finegoldia* is known to cause inflammation (Neuman et al., 2020) while only one HC woman had *Finegoldia* and IBS-C women had no *Finegoldia* (at least not detected).

Table 16. P-values comparing GI symptoms of High Butyric Acid Producers and Low Butyric Acid Producers

GI Symptoms	HC High vs. Low	IBS-C & IBS-D High vs. Low	IBS-C High vs. Low	IBS-D High vs. Low
Abdominal pain	0.08	0.12	0.044	0.43
Stomach pain	0.03	0.07	0.035	0.29
Flatulence	0.37	0.25	0.11	0.48
Bloating	0.15	0.26	0.10	0.47
Constipation	0.41	0.27	0.009	0.11
Diarrhea	0.10	0.22	0.15	0.049
Percent days constipation	0.12	0.18	0.10	0.19
Percent days diarrhea	0.06	0.44	0.27	0.06
Depressed	0.32	0.43	0.08	0.22
Hard to Concentrate	0.42	0.50	0.14	0.22
Stress	0.33	0.20	0.015	0.13
Muscle pain	0.02	0.13	0.12	0.30
Anxiety	0.31	0.29	0.09	0.41
Daily interference	NA	0.03	0.002	0.34
Cramps	0.15	0.05	0.01	0.33
Fatigue	0.38	0.13	0.01	0.37
Urgency	0.39	0.08	0.09	0.31

IBS women with high butyric acid level had significantly lower daily activity interference (p = 0.03) compared to the low butyric acid producer IBS women. High butyric acid producer IBS-C women had

significantly lower abdominal pain, stomach pain, less events of constipation reported, and less stress when compared to Low butyric acid producer IBS-C women. HC women with high butyric acid level significantly reported higher muscle pain compared to the low butyric acid producer HC women,

Table 17. Baseline GI and non-GI Self-Reported Symptoms in Butyric Acid Producers

Variables	ALL women (N=60) Mean $\pm$ SD	
	High Butyric acid Producer Mean = 193.23, SD = 75.07 Range: 108.30- 366.86 micromole Mean Age: 31 Mean Body Weight: 140 Mean FDW: 133 mg (n =33)	Low Butyric acid Producer Mean = 64.60, SD = 12.21 Range: 43.40 - 89.60 micromole Mean Age: 27 Mean Body Weight: 140 Mean FDW: 98 mg (n =27)
Abdominal pain	0.62 $\pm$ 0.69	0.92 $\pm$ 0.75
Stomach pain	0.43 $\pm$ 0.53	0.71 $\pm$ 0.64
Flatulence	0.62 $\pm$ 0.57	0.83 $\pm$ 0.71
Bloating	0.51 $\pm$ 0.67	0.74 $\pm$ 0.80
Constipation	0.35 $\pm$ 0.51	0.55 $\pm$ 0.78
Diarrhea	0.30 $\pm$ 0.42	0.30 $\pm$ 0.32
Percent days constipation	21.77 $\pm$ 22.33	34.83 $\pm$ 32.12
Percent days diarrhea	37.81 $\pm$ 28.56	37.83 $\pm$ 27.37
Depressed	0.29 $\pm$ 0.52	0.39 $\pm$ 0.44
Hard to Concentrate	0.46 $\pm$ 0.56	0.57 $\pm$ 0.59
Stress	0.56 $\pm$ 0.54	0.79 $\pm$ 0.68
Muscle pain	0.26 $\pm$ 0.41	0.41 $\pm$ 0.55
Anxiety	0.48 $\pm$ 0.63	0.68 $\pm$ 0.56
Daily activity interference	1.32 $\pm$ 0.39	1.61 $\pm$ 0.40
Cramps	0.18 $\pm$ 0.24	0.33 $\pm$ 0.37
Fatigue	0.76 $\pm$ 0.68	1.05 $\pm$ 0.78
Urgency	0.23 $\pm$ 0.31	0.44 $\pm$ 0.55

Table 18. P-value for Mean Differences in GI and Non-GI Symptoms in Butyric Acid Producers

Self-Reported Clinical Symptoms	P-values
Abdominal pain	0.06
Stomach pain	0.04
Flatulence	0.11
Bloating	0.12
Constipation	0.14
Diarrhea	0.48
Percent days Constipation	0.04
Percent days Diarrhea	0.50
Urgency	0.04

Stress	0.08
Muscle pain	0.12
Anxiety	0.10
Daily activity interference	0.004**
Cramps	0.04
Fatigue	0.07
Sleep Quality	0.03
Depressed	0.23
Hard to Concentrate	0.22

Body weights were similar in both High and Low Butyric acid Producers.

Note for All Women: High Butyric Acid Producers (HBAP) had significantly higher tryptophan ($p=0.00001$) and the relative abundance of *Bifidobacteria* ($p = 9.600182e-33$), *Dorea* ($p = 0.009$), *Prevotella* ($p = 0.01$), *Paraprevotella* ($p = 0.01$), *Roseburia* ($p = 0.04$), and *Blautia* ($p = 0.04$) were, significantly higher compared to the Low Butyric Acid Producers (LBAP). Interestingly, *Bacteroides* was significantly lower in the HBAP compared to the LBAP. The more pathogenic bacteria genera, *Alistipes* was found to be significantly lower ($p = 0.002$) in the HBAP compared to the LBAP. *Turicibacter*, a tryptophan consumer was also lower ($p = 0.10$) in the HBAP than LBAP. *Dialister* and *Bilophila*, both belonging in the *Bacteroidota* phyla were also lower ($p = 0.05$) in the HBAP than the LBAP.

HBAP. Although not significant *Lactobacillus* ($p = 0.08$), *Coprococcus* ($p = 0.10$) and *Lachnospiraceae insertia sedis* ($p = 0.14$) had higher relative abundance in the HBAP than the LBAP. The relative abundance of *Clostridia IV* ($p = 0.05$), *Fusobacteria* ($p = 0.15$), *Phascolarctobacterium* ($p=0.05$), and *Veillonella* ($p = 0.08$) were also lower ($p=0.08$) in the HBAP compared to the LBAP.

LBAP. Although not significant, *Akkermansia*, *Ruminococcus*, *Ruminococcus2*, *Parabacteroides*, *Faecalibacteria*, *Lactococcus* were higher in the LBAP than HBAP.

Shared bacteria included: *Eubacteria*, *Oscillobacter*, *Unc.Lachno*, *Clostridia XIVa*, *Clostridia XIVb*, *Clostridia XVIII*, *Eggerthella*, *Butyricicoccus*, *Enterococcus*, *Unc. Firmicutes*. Microbial indices (Shannon and Inverse Simpson) for the HBAP were slightly higher but not significantly different compared to LBAP.

Relative Concentration High Butyric Producers. Women with HBAP derived from the relative concentration of butyrate (normalized with fecal dry weight) showed that HBAP was associated with

significantly lower cramps ($p=0.01$), intestinal gas ($p=0.02$), nausea ($p=0.02$), flatulence ($p=0.02$), stomach pain ($p=0.04$), and daily activity interference ($p=0.02$). Although it is not significant, the HBAP also had lower abdominal pain ($p=0.13$), anxiety ($p=0.14$), bloating ($p=0.09$), constipation ($p=0.14$), fatigue ($p=0.10$), headache ($p=0.09$), joint pain ($p=0.12$), panic feeling ($p=0.15$), sleepiness ($p=0.09$), urgency ($p=0.09$), stressed ($p=0.19$), and is associated with feeling refreshed ($p=0.06$) after a night sleep, improved quality of sleep ($p=0.15$), and feeling healthy ($p=0.09$),

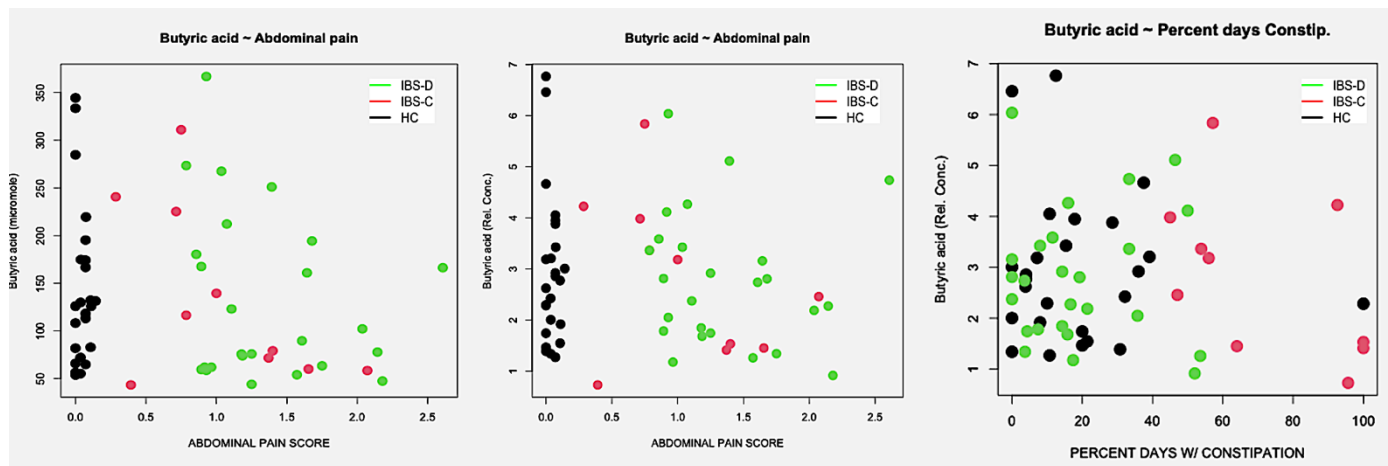


Figure 1. The relationship of butyric acid and abdominal pain.

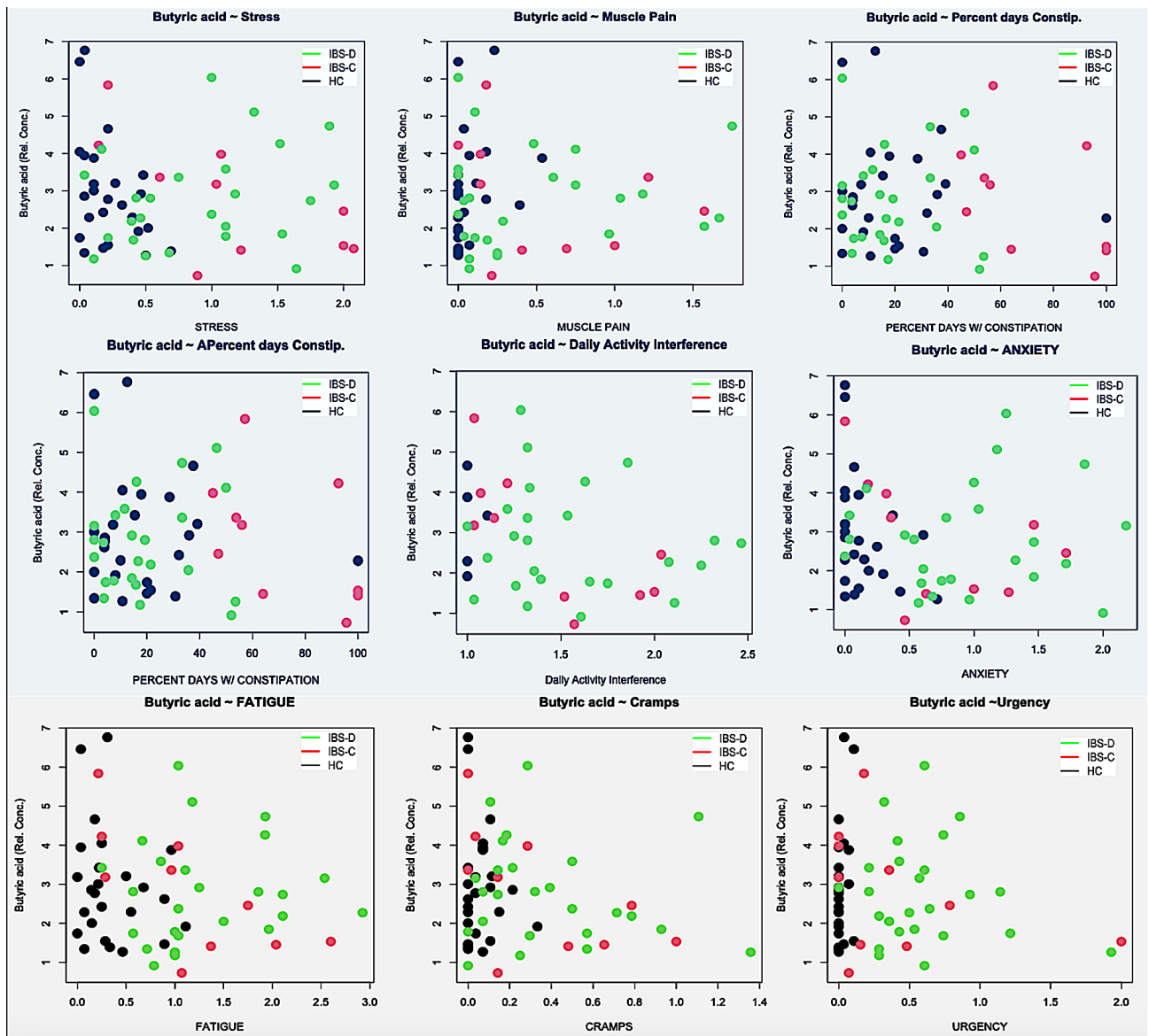


Figure 2. The relationship of butyric acid with GI and non-GI symptoms.

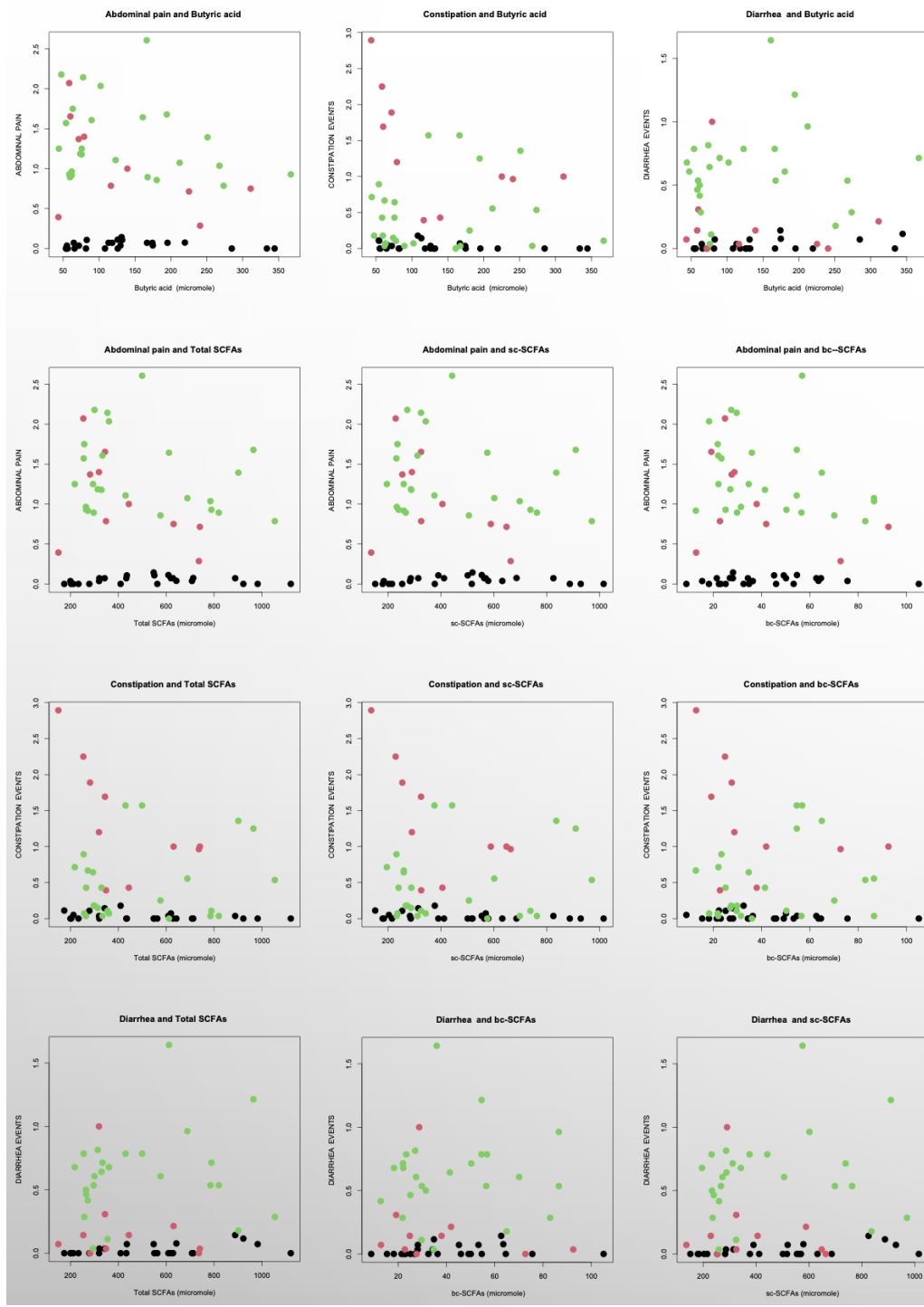


Figure 3a. The relationship of total sc-SCFA, total bc-SCFA, and total SCFA with self-reported GI symptoms.

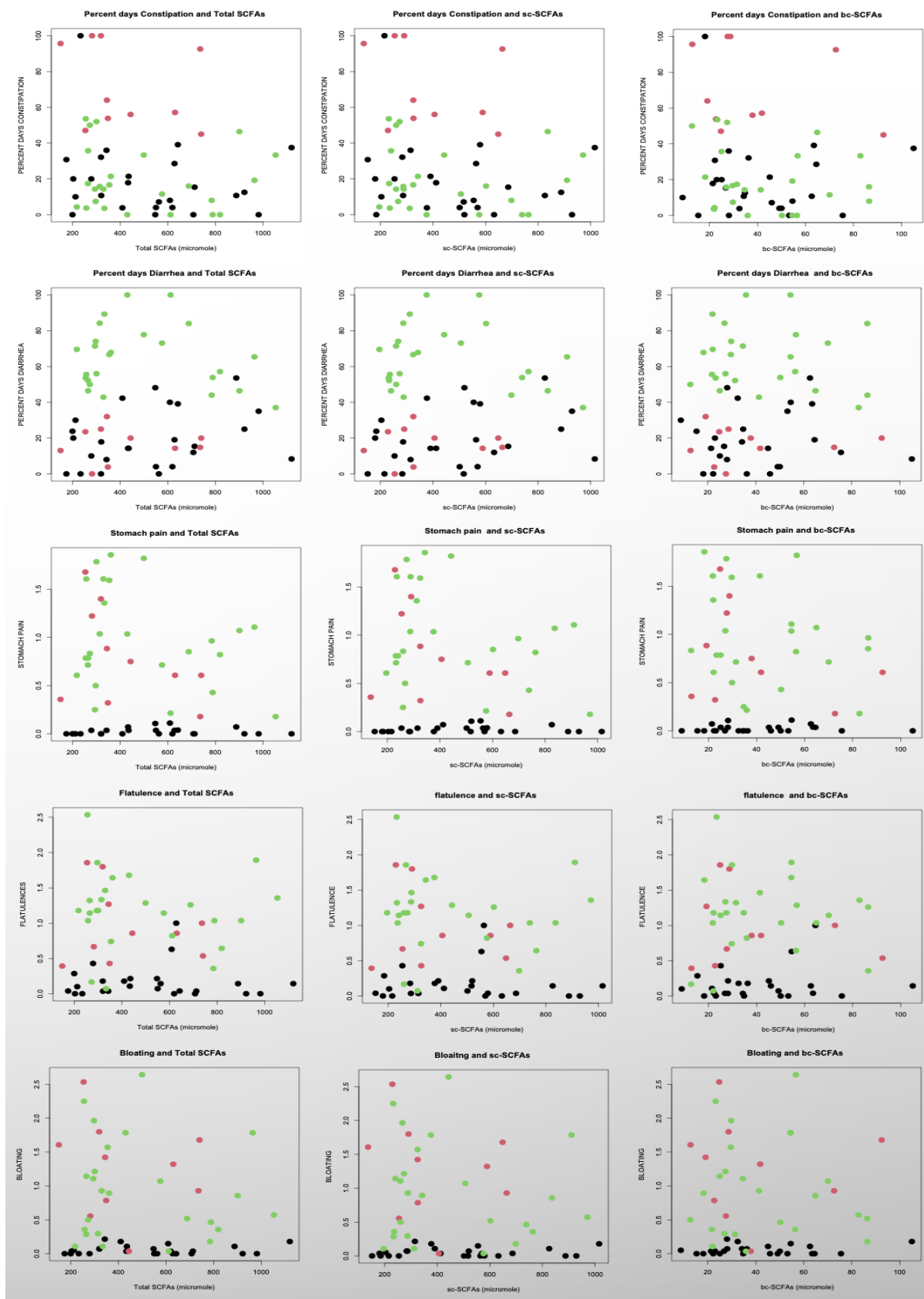


Figure 3b. The relationship of total sc-SCFA, total bc-SCFA, and total SCFA with self-reported GI symptoms.

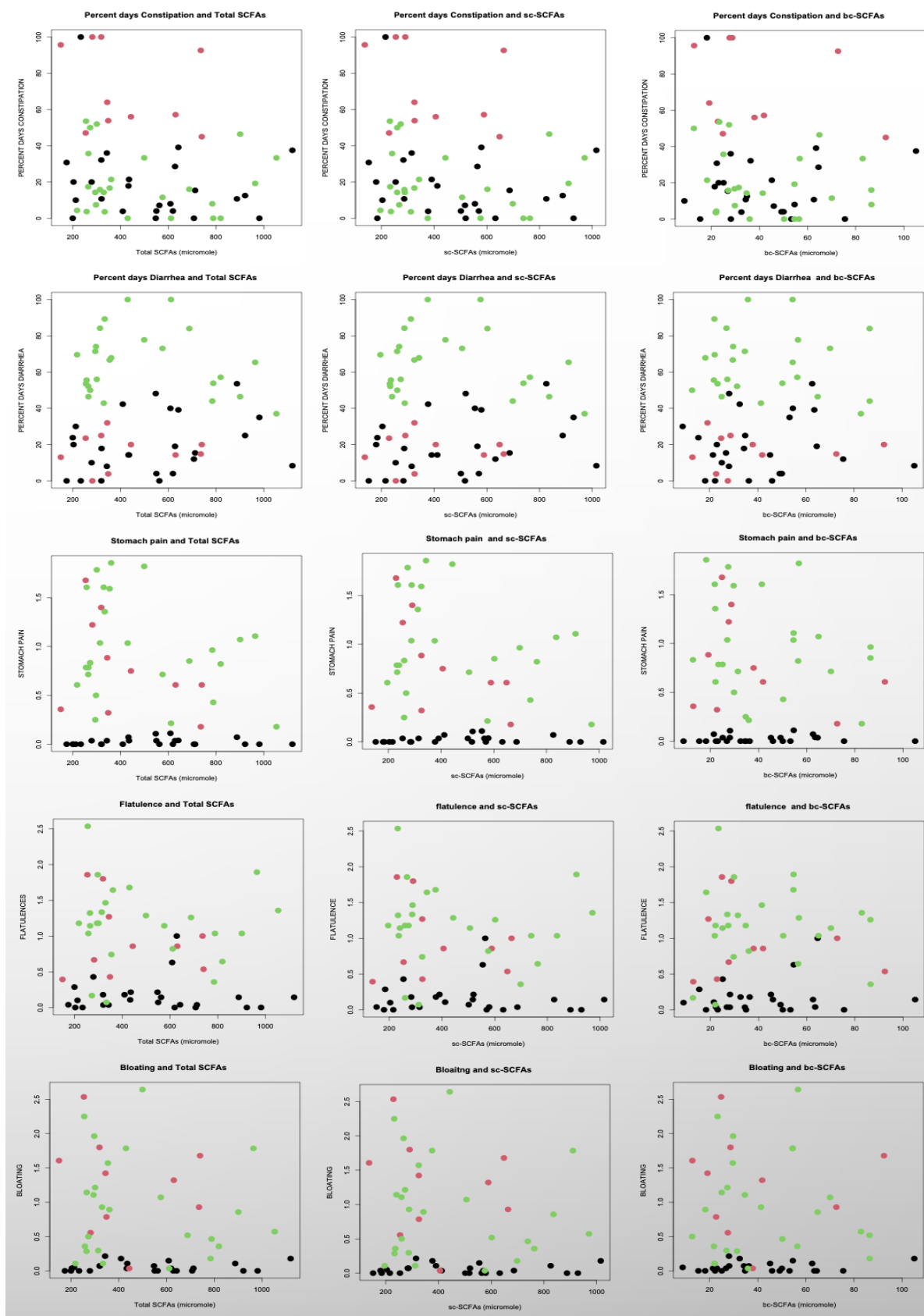


Figure 3c. The relationship of total sc-SCFA, total bc-SCFA, and total SCFA with self-reported GI symptoms.

AIM2a DISCUSSION

There is no specific laboratory test or technology that can be used to diagnose IBS. IBS patients frequently go through unnecessary and costly medical procedures and diagnostic testing (endoscopy, colonoscopy, gynecological laparoscopy) due to concern about potential misdiagnosis. To evaluate the role of the microbiome on the symptom generation of IBS, the association of SCFA and GI symptoms were investigated. It is hoped that the links observed in this study may help IBS health experts target specific biochemical components involved in SCFA metabolism to alleviate symptoms of IBS, especially in women.

The inconsistency of associations of fecal SCFA levels and symptoms reported in literature may reflect the heterogeneous nature of IBS; IBS itself is a cluster of symptoms and the clinical presentation of IBS varies over time (Matto et al. 2005). This variability in symptoms may be due to several factors including variability in microbial and its metabolome composition.

In this study, the GI symptom scores were obtained from the average of 28-day symptom diary filled out by each participant in the MAP study (Microbiome & Abdominal Pain, NR014479). The advantage for using this 28-day symptom measurement is to decrease retrospective bias associated with recalled symptoms. The 28-day symptom diary included 26 items that were grouped into 5 domains: GI pain, stool characteristics, psychological items, somatic symptoms and stool consistency. Under the GI pain, abdominal pain is the most prevalent symptom in IBS (Longstreth et al., (2006) and understanding its attributes is key to finding a cure for IBS. Not surprising, IBS status (via Pearson correlation multivariate analysis) was found to be strongly correlated with abdominal pain ($r = 0.83$, CI: 0.74, 0.90) and percent days with diarrhea ($r = 0.57$, CI: 0.36, 0.9) but not as strongly related to percent days with constipation ($r = 0.27$ CI: 0.02, 0.49). Additional GI symptoms that correlated with abdominal pain included bloating, flatulence ($r = 0.65$ and $r = 0.73$, respectively) with stomach pain ($r = 0.91$) having the strongest relationship. Moreover, the extra-intestinal symptoms (listed from highest to lowest: hard to concentrate, urgency, depression, life interference, stress, stress with muscle pain) correlated strongly with abdominal pain.

Stool consistency (bowel pattern) assessed by the Bristol Stool Scale is considered a surrogate marker for colonic transit time (Russo et al., 2013; Menees & Chey, 2018). Since stool consistency is closely related

to transit time, it was expected that higher total SCFA would be associated with diarrhea, while lower total SCFA would be associated with constipation. Indeed, the SCFA results were able to differentiate the bowel patterns (stool characteristics) in IBS. IBS-C was associated with lower total SCFA, while IBS-D was associated with higher total SCFA.

There is accumulating evidence about the positive physiological relevance of SCFA in both humans and animal models. Both animal studies and in vitro experiments indicate that butyrate is involved in GI homeostasis. Fecal transplantation studies in IBS mice models and humans have provided evidence of strong relationship and causality that the gut microbiome caused IBS symptoms (de Palma et al., 2017; Mazzawai et al., 2019). Understanding butyrate's role in improving gut integrity is key in alleviating symptoms of IBS, in particular abdominal pain.

The present study hypothesized that lower total SCFA would be associated with higher abdominal pain severity. While a positive relationship between propionic and butyric acid with stomach pain was found, these relationships were non-significant after adjusting for multiple comparison in the IBS group. Of the GI symptoms, a significant correlation (FDR adj. $p < 0.001$) was found in the proportion of propionic acid and flatulence when data from all women ($n=60$) were combined. To examine this further, fecal SCFA were used to categorize participants into two groups, i.e., those with high and those with low-concentration, and an inverse relationship between high fecal individual short chain (sc)-SCFA with abdominal pain severity score (demonstrated via bi-plot and boxplots) was found. In addition, there was a significant difference in abdominal pain severity scores ($p = 0.046$), stomach pain ($p = 0.002$), and bloating ($p = 0.03$) when all women with low total SCFA were compared with all women with high total SCFA. In these analyses, it is noteworthy that the negative relationship of abdominal pain with butyric acid was stronger in magnitude than with propionic acid. These relationships support the current literature related to the positive influence of individual SCFA on health (in terms of magnitude: butyrate > propionate > acetate) (Cummings et al., 2004; Korpela, Gibson, den Besten, van der Beek et al., 2017).

It can be speculated that the inverse relationship between butyrate and abdominal pain reflects an important role for butyrate in the context of inflammation. Inflammation has been identified as one potential

pathophysiologic factor leading to IBS (El-Salhy, 2015; Ng et al., 2018; Siddiqui & Cresci (2021). Butyrate is known to upregulate the transcription of tight junction genes (Wang et al.; 2012). Depending on the micro-environment, butyrate can promote normal cell division of normal colonocytes or reduce the abnormal growth of colonocytes (Schlörmann et al., 2023; Zeng et al., 2020; Wu et al., 2018; Feng et al., 2018; Fung et al., 2012; Scharlau et al., 2009; Hague et al., 1995; Heerdt et al., 1997).

The results from healthy controls parallel what is known in the literature about the physiological effects of the individual sc-SCFA. In this study, IBS had more lactate than healthy controls. The accumulation of lactic acid is known to reduce pH in the gut. Ringel-Kulka (2016) showed that CTT is positively associated with colonic pH (measured using wireless motility capsule) and therefore, a shorter CTT is associated with watery, acidic stool. Furthermore, these investigators found that CTT is negatively correlated with SCFA; thus, a shorter CTT translates to higher measured SCFA. In this study, a higher amount of fecal SCFA was associated with reduction in abdominal pain and percent days with constipation compared to lower amount of fecal SCFA in IBS-C.

Looking at the relationship of lactic acid and diarrhea within the “healthy gut” of the healthy controls is key to understanding the evolution of a disordered bowel patterns and its related symptoms. Within the healthy control group, there was a positive association between diarrhea and butyric acid or (diarrhea and acetic acid). Acetic acid is positively correlated with butyric acid. This is logical since acetic acid is converted to butyric acid during anaerobic fermentation in the colon by the gut bacteria. Steady state Infusion study was done using rats infused with carbon labeled butyrate (40 mM) was investigated by Fitch & Fleming to study the in-vivo SCFA metabolism under the context of high fiber diet (40 mol/L). They found that beta oxidation of butyrate can regenerate acetate back in the form of acetoacetate, lactate, CO₂ and mostly 3-hydroxybutyrate (>50%) (Fitch & Fleming, 1999). It is worth noting that excess butyric acid can be converted to ketoacids such as acetoacetate and 3-hydroxybutyrate. These two ketoacids along with acetone were first discovered in diabetic patients due to impaired insulin signaling, but also present in circulation during starvation in normal human beings (excluding those who have genetic deficiency in hepatic glycogen synthase who are unable to synthesize glycogen). These ketoacids become significant source of fuel for the brain (supply 70% of ATP

demand) when stored energy is scarce (e.g., depleted glycogen stores due to low rate of gluconeogenesis) and as a signaling molecule, prevents further muscle degradation during starvation (Huang et al, 2013) and downregulate hepatic β -oxidation of fat to regulate serum load; a healthy ketone serum concentration is between 2-8 mM. Under high protein diet the branched-chain α -keto acid dehydrogenase (BCKAD) is highly expressed resulting in the formation of increased ketoacids (i.e. α -ketoisocaproate (ketoleucine), α -keto- β -methylvalerate (ketoisoleucine) and α -ketoisovalerate (ketovaline)), however, in order to reduce protein turnover, BCKAD activity is reduced during prolonged starvation but only to the point when lipid stores are not exhausted (Holecek et al., 2000). Other environmental contexts where BCAA metabolism is increased is during intense exercise, administration of catabolic hormone-glucocorticoid, and during inflammation (i.e., sepsis, trauma, endotoxemia, cancer, high TNF- α treatment). Animal studies done by Nakao et al. (2019) have shown that animals fed with keto diet (high fat/low carb/low protein) increases production of cortisol by increasing glucocorticoid receptor target genes and increased ROS production due to increase oxidative stress (oxidized redox environment), In summary, fatty acid oxidation increases BKAD activities and reduced liver gluconeogenesis. Individuals who are under anti-diabetic diet or weight lost management diet with keto diet regimen are likely to have high BKACD activities, enhancing their changes o increased inflammatory state and increased diarrhea events as was reported in literature (Cavaleri and Bashar, 2018). Despite the negative effects of living on a ketogenic lifestyle, Cavaleri and Bashar purported the preferred use of beta hydroxy butyrate-butyric acid (BHB-BA) supplementation for folks who wants to induce self-ketosis that is more beneficial than the KD fad diet. This BHB-BA may be of benefit for older adults because of their higher risk of developing dementia and sarcopenia.

The metabolism of acetic acid to butyric acid is accomplished by acetate-consuming bacteria in the gut. In clinical practice, butyrate capsules are currently prescribed to treat constipation because of their efficacy in inducing bowel movement. In this current study, univariate analyses showed a positive association between lactic acid and formic acid with diarrhea in healthy controls; however; however, this relationship was better explained by the graphical visualization (i.e. scatter plot) produced from linear regression where it clearly demonstrated that only few healthy women were responsible for this positive relationship. Looking at scatter

plots data, this study found a positive relationship between diarrhea events with formic acid and with acetic acid in IBS-D women. Interestingly, there were two IBS-D women and one healthy individual that had very high lactate levels (more than 2 to 4 times higher than what is considered normal physiological level). It may be possible that these three individuals have abnormal level of lactate acid-producing bacteria and/or perhaps have other health-associated problem such as small intestinal bowel overgrowth (SIBO) that preferentially select the overgrowth of lactate acid-producing bacteria. It has been reported that IBS-D patients with SIBO produce higher SCFA than healthy controls (Hoverstad et al., 1985). Animal (rat) studies show that lactic acid stimulates the motility of both the small and large bowel intestine, while the 3 main SCFA stimulate only colonic motility (Yokokura et al., 1977). Interestingly, higher amount of lactate was associated with mean abdominal pain in IBS-D women, while this trend was reversed in healthy women; this result could mean that the healthy women and IBS-D women utilize different metabolic pathways for lactic acid metabolism.

Only a few studies have examined the association between fecal SCFA and IBS symptoms, however, the majority reported negative findings or lack of associations [Ahluwalia et al., 2021; Zhang et al., 2019; Mujagic et al., 2017; Ringel-Kulka et al., 2015; Farup et al., (2017) Vernia et al. 1996]. The present study found an inverse correlation between total SCFA and abdominal pain in IBS. It may be that symptom measures used by the investigators (i.e., IBS-SSS, IBSSI, etc.) may be capturing a different aspect of IBS symptom experience compared to the 28-day symptom diary that the MAP study employed. For example, Valeur et al. 2010 found no correlations between SCFA (micromole/g or proportions of each individual SCFA) to any GI symptom scores; however, this group found a positive trend in butyric acid proportion correlation with IBS symptoms when the IBS Symptom Severity Score (IBS-SSS) was categorized as high versus low. In their sample of 35 IBS patients (30 female, 5 male), Valeur et al. (2010) also reported that a higher proportion of butyrate was associated with higher IBS-SSS score compared to those with a lower IBS-SSS score in the IBS group. However, this trend was not found in their healthy control group (11 female, 4 male). The present study found a positive relationship between butyric acid and abdominal pain in healthy women, while an inverse, significant relationship was found in the IBS total group and for each subtype of IBS; IBS-C was driving the significant inverse relationship. Similarly, Tana et al., (2010) reported positive relationships between butyric and propionic

acids with IBS-SI GI symptoms (i.e., abdominal severity, bloating) and reduced quality of life (SF-36) in IBS. These relationships were also found to be significant when compared to a healthy control group. Just like Valeur (2016), Tana found the same positive associations between butyric acid and abdominal pain when using the proportions of butyric and propionic acid. Interestingly, in this study, when butyric acid is dichotomized to high (n=33) and low (n=27) butyric acid producers, percent days with constipation and stomach pain were significantly reduced ($p=0.04$) in women labeled as “high butyric acid producers” and although not significant, abdominal pain was also reduced ($p=0.058$) compared to “low butyric acid producers.” Furthermore, these women with high fecal butyric acids reported reduction in other symptoms (i.e., urgency, anxiety, depression, muscle pain, fatigue, hard to concentrate), as well as significant reduction in reported scores of activity interference and significantly higher reported scores for quality of sleep as compared to the low fecal butyric acid producers. An older RCT done by Banasiewicz and colleagues (2010) that enrolled 66 IBS-C patients (12-weeks duration) found a statistically significant reduction in abdominal pain and improvement in stool consistency in IBS-C patients who received capsules of butyrate compared to IBS-C patients with placebo treatment (Pituch, Walkowiak, & Banaszkiewicz, A. 2013).

A more recent study done in Beijing (Zhu et al. 2019) reported that metabolites (i.e., amino butyric acid) clustered into two modules (or related groups) were found to be significantly correlated with abdominal pain and its characteristics (duration, intensity) and with stool consistency/stool form. Although this study used state-of-the-art metabolic profiling used (GC-TOFMS), their sample size did not make the 80% power cut-off and their findings may be the result of randomness—spurious correlations and as such, need to be interpreted cautiously. In addition, their IBS-D cohort was predominantly male with high symptom severity; the lack of representation from less symptomatic IBS-D patients may have made their findings not generalizable.

Fiber RCTs studies (Kajander et al., (2007); Halmost et.al, (2015), Hustoft et al., (2016, Born et al., 1999) have also investigated the relationship of symptoms to SCFA but have not found significant relationships. An early RCT (double-blind, crossover design) done by Mortensen et. al., (1987) studied the effect of wheat bran (compared to placebo) on IBS symptoms (18 IBS vs. 9 healthy controls) and found no significant on effect of bran on symptoms or on two dichotomized groups of IBS SCFA producers (high and

low). Bran diet increased fecal mass and shortened the gut transit time (measured via fecal radiopaque plastic marker), but bran was not correlated with fecal SCFA concentration. They found that the IBS group had lower fecal SCFA overall than the healthy controls independent of the randomized assignment and that patients with lower fecal SCFA tended to have worse abdominal GI symptoms compared to the placebo treated IBS group; the investigators reported lower level of fecal SCFA to be correlated with higher abdominal GI symptoms, while high fecal SCFA producers had lower abdominal pain scores at baseline, but more 'rumbling' GI sounds reports post bran intervention. Consistent with the present study findings, the Mortenson study also found reduced abdominal pain scores in women with high fecal SCFA or butyric acid concentrations. Zhang et al (2019) found isovalerate to be positively correlated with severity and frequency of abdominal pain. In contrast, this study, higher valeric acid was significantly and negatively correlated with markedly reduced abdominal pain in both IBS subtypes and as a composite, however, the opposite trend was observed in healthy women, but the association was weak.

The Mortensen study sample was not described with respect to IBS bowel pattern subtype. However, it was reported that IBS individuals with lower fecal mean mass and longer transit time had significantly lower fecal SCFA concentrations while those patients with higher fecal mean mass and shorter transit time displayed significantly higher level of fecal SCFA compared to the reference group (healthy individuals). Consistent with Mortensen's finding on wheat bran, the current study also found no correlation of resistant grain and fecal SCFA levels. It can be assumed that resistant grain-like wheat bran only contributed to fecal bulking and not much to the colonic fermentation process. Also, in this present study, simple regression showed that the major SCFA are positively correlated with fecal dry weight (variable that was accounted for during the analyses for the relative concentration of the metabolites. Higher fecal mass means higher number of bacterial cells, and thus it made sense to have higher SCFA with higher amount of fecal sample. Although this may not be true for people who have induced diarrhea either by taking antibiotics or laxatives.

Another RCT study (double-blind, parallel group-crossover design with 3-week washout period) by Kajander et al. (2007) in Finland showed a reduction in IBS symptoms post probiotic intervention (5 weeks, Bifidobacteria and Lactobacillus) in IBS (n=86, 39 were women, Rome II), but this effect was not associated

with a change in fecal SCFA. Samples were processed at three timepoints (1 baseline and 2 during intervention) and measured via GLC platform. Although the subjects in this study kept a diary for 8-weeks, it was not clear whether subjects continued taking their medications (i.e., antidiarrhea, laxatives, antispasmodics) including fiber supplements that may have influenced the findings. Another probiotic intervention study by Cremon et.al (2018) reported significant increase in fecal butyrate and improved GI symptoms post intervention. This finding is in line with the results of Staudacher et.al, (2012) who reported improvement of global IBS symptoms (70% reduction in symptom severity and normalized bowel patterns) after a low FODMAP intervention. However, unlike Cremon, they found no change in fecal SCFA as measured via GLC from baseline to follow-up.

Cremon and Gargari et al (2016) did an RCT with *Lactobacillus* probiotics intervention in IBS (Rome III, n=40) and observed a statistically significant increase in fecal butyrate levels from baseline to post intervention. However, patients in this trial who received probiotics improved GI symptoms when compared to the placebo group. The change in butyrate levels suggests that one type of bacterial genera may be able to have an impact on the overall microbial composition, altering proportions of individual SCFA. A systematic review done by Dale et al. (2019) reported that 7 out of 11 probiotic supplementation studies in IBS (all diagnosed under Rome III criteria) significantly improved IBS symptoms (i.e., abdominal pain, bloating) compared to placebo treated IBS subjects and observed that multi-strain probiotics (that included *Lactobacillus* and *Bifidobacteria*) was more effective than the single strain probiotic supplementation.

The findings of several studies support the notion that butyrate and its associated metabolites regulate GI motility. Chassard et.al (2012) reported that the presence of butyrate-producing bacteria resulted in faster colonic transit. Convergence of research evidence has shown that bacteria taxa belonging to *Clostridia* and *Ruminococcus*, are known to have the enzyme tryptophan decarboxylase and are able to convert tryptophan to its indole form--tryptamine, a potent molecule that stimulates the production of serotonin (Hou et al., 2023; Roeger & Licht 2018; Marcoba et al., 2012; Yano et al., 2015) and has been found to stimulate ion secretions via Ussing chamber experiment with murine colon mucosa (Williams et al., 2014) and decrease transit time in

gnotobiotic mice (Bhattara et al., 2018). Fukumoto et al., (2003) showed that SCFA accelerated gut motility by regulating the release of 5-hydroxytryptamine (5HT).

While several known species of *Clostridia* can metabolize tryptophan into its acid derivatives (i.e., indole, skatole, indoleacetic, indolepropionic, indolelactic, indoleacrylic, tryptamine), *Lactobacillus* is known to produce indole-3-aldehyde, indoleacetic, and skatole. *Bifidobacteria* on the other hand, is known to convert tryptophan to indolelactic and indoleacetic acids (Roeger & Licht 2018). An older study with rats found that *Bifidobacteria infantis* modulates tryptophan metabolism by attenuating the abnormal behavior experienced from early maternal separation (early life stressors-related behaviors). (Desbonnet et al., 2010). They found that the rats that were separated early from their mothers had reduced DOPA found in the periamygdaloid cortex and reduced 5-HIAA, the main metabolite of serotonin metabolism in the frontal cortex (Desbonnet et al., (2010); their previous study demonstrated an increased peripheral tryptophan level in rats treated with *Bifidobacteria* (Desbonnet et al., 2008). Desbonnet et al. study implicated that *Bifidobacteria* have anti-depressant properties. Zhang et al., (2021) reported a high amount of *Alistipes*, *Bacteroides*, *Parabacteroides* and lower abundance in *Prevotella* to be associated with depression, while *Coprococcus*, *Acidobacterium*, *Blautia* and *Dorea* were found to be associated with sleep disturbances. Interestingly in this study, the relative abundances of these bacteria paralleled to the microbial signatures of BS-D women.

A more recent experiment on germ free mice inoculated with spore-forming bacteria (largely composed of *Clostridia*) showed causality—microbiota upregulates tryptophan metabolism as evidenced provided by both in vitro and in vivo experiments; butyrate, propionate, tyramine and other metabolites (i.e., alpha-tocopherol, cholate, deoxycholate, p-aminobenzoate) up regulated 5HT synthesis by the enterochromaffin cells and influenced GI motility (Yano et al., 2015). Still others implicated other metabolites (i.e., nitric oxide, substance P, endocannabinoids) that can influence gut motility (Quigley 2011). Other researchers have studied the inter-relatedness of butyrate and tryptophan, where butyrate is implicated to control tryptophan expression during inflammation (Dang et al., 2021). Although not significant, this current study found that higher soluble fiber intake (11-15 g) was also associated with higher mean tryptophan concentration compared to lower soluble fiber intake (2-9 g) intake in all women. Simple linear regression of butyrate and tryptophan showed strong,

positive co-linearity. It may be that butyrate modulates tryptophan's activity or vice versa. There are few studies in IBS where investigators also found tryptophan's derivatives in addition to SCFA in their metabolic profiling and others found that tryptophan is associated with high fiber diet. (Huang et al., 2023; Peron et al., 2022). In one study people with IBS (n=26) had significantly higher fecal levels of butyric acid, propionic acid, indole, p-cresol, but significantly lower acetone in their feces (Walton et al., (2013) compared to healthy volunteers (n=19). However, Walton's study measured metabolites by GC-MS giving nanomolar concentrations. Soret et.al. (2010) and Reigstad et al. (2015) proposed that butyrate accelerates colonic motility by stimulating the release of serotonin or by promoting cholinergic pathways. Serotonin's role in GI motility is supported by an in vitro study with colonic epithelial cells (Ussing chamber) obtained from IBS-C subjects ;5HT stimulation on IBS-C derived mucosa showed significantly lower secretion (reduced ionic flux) compared to HC (Mars's et al (2021). These investigators found that samples collected during an IBS flare had higher Bray-Curtis Dissimilarity index (meaning higher beta diversity between subjects over time), lower Shannon alpha-diversity index (lower microbial richness and evenness/distribution of each microbe), higher alcohol dehydrogenase (means high lactate production and altered energy metabolism), higher xanthine dehydrogenase/oxidase (means high stress response pathways involve), high serum tryptamine (means increased GI motility and hypersensitivity), and higher fecal bile acids (means high BSH activities , higher intestinal fluid secretion, also increased GI hypersensitivity (Camilleri 2014; Mars et.al., 2021). It is well known that the interactions of immune cells (adaptive and innate) with the enteric neurons modulate GI motility; this is mediated by the gut microbiome due to its dynamic interactions with the mucosal lumen (Muller et al., 2014). It has been reported that the mucosal lining is regenerated via the interactions of mucosa-glycan consuming bacteria and the colonocytes and that starvation compromises the thickness of these linings (Shroeder, 2019). On a side note, the IBS-D samples in this study are characterized with significantly high amount of secondary bile acids compared to healthy women. In this present study, there were other metabolites identified (i.e., tryptophan, 3-phenylacetic acid) that were also found to be higher in mean concentration in IBS than in healthy women, although these did not reach statistical significance. In literature, deconjugated bile acids are known to alter gut sensorimotor activity (Appleby & Walters, 2014). These molecules have important clinical implications.

For example, indole acetic acid (IAA) and phenyl acetic acid (PAA) belong to the same family of biomolecules (auxins), and both co-regulate auxin responsive genes (i.e., β -glucuronidase genes or commonly called “the GUS systems”). The GUS systems are what the bacteria use when they need to remove sugar rings on metabolites (endogenous and exogenous molecules such as hormones, xenobiotic drugs, etc.) including the deconjugation of bile acids, making the metabolites more potent and they are and could be very harmful to health upon reabsorption. In summary, it is helpful to test other metabolites, not just the SCFA to better understand IBS symptoms generation or the pathobiology behind IBS.

Mars and colleagues (2021) reported that at times of symptom flares, high tryptamine, cholic and chenodeoxycholic acid were elevated in both IBS subtypes. Mars’ group also studied gene expressions from the colonic biopsy obtained from participants; they found that there was an enrichment in human leukocyte antigen class II genes in IBS. This gives a good rationale for IBS patients with this marker to avoid gluten-enriched diet. Furthermore, Mars’ group reported that the KEGG pathway enrichment analysis highlighted the presence of inflammation-related pathways in IBS (prostaglandin D2 synthesis). These groups also carried out epigenetics study where they found differentially methylated genes that are involved in epithelial electrolyte transports, intestinal secretions, aquaporins, and voltage-gated potassium channel involved in neuronal excitability in IBS-D patients. In summary, Mars group elucidated possible mechanisms of dysregulated SCFA metabolism in women with IBS-D.

Conclusion

Due to the nature of this study, it is still unclear whether bacterial activity, as reflect in SCFA production is associated with symptom generation in IBS, However, the comparison of high butyric acid producer (HBAP) vs low butyric acid producer (LBAP) using all women provided >80% power and thus, it can be concluded that having high level of butyric acid is associated with lower reported GI and non-GI symptoms in all women in this study.. It is evident in this study that high butyric level using the relative concentration normalized with fecal dry weight gave the best association with several health indicators reported by all women. High fecal butyric acid level appeared to have the most significant impact in influencing GI and non-GI symptoms in IBS-C women.

Most of the observational studies that evaluated these relationships included a limited number of subjects with varying IBS phenotypes. Furthermore, RCT studies that employed nutritional interventions (i.e., probiotics and/or fiber supplementation) that measured fecal SCFA failed to show significant associations between SCFA and abdominal pain. However, most were not powered to do so.

Aim2b) Relationship of Diet and SCFA Concentration

The proposed central hypothesis of this project was that high intake of fiber would correlate with higher concentrations of short chain SCFA (sc-SCFA) and lower levels of branched chain (bc-SCFA) in both IBS and healthy control women. When dietary fiber intake was divided into high (25g/day) vs. low (<25g/day), a positive relationship between high fiber intake and measured SCFA was found in IBS as a composite, but not in HC; this trend was driven by the IBS-C women. Within the IBS-C women, the Fiber High group (Aim2b Table 1) had higher amount of individual fecal SCFA (absolute concentrations) and total SCFA (absolute concentration) when compared to Fiber Low group; butyric acid may be significantly reduced in the Fiber Low group ($p=0.05$). IBS-D women with high fiber intake (High Fiber group) had significantly higher caproic acid ($p=0.03$) compared to HC women in the same dietary fiber category. Also, in the IBS-D group, there was a trend for those with higher fiber intake (Fiber High group) to have higher mean (absolute) levels of fecal propionic acid ($p=0.14$), acetic acid ($p=0.29$), butyric acid ($p=0.39$), and valeric acid ($p=0.06$) when compared to HC women with a similar DF intake. The Fiber Low group in the IBS cohort as a composite had markedly high ($p=0.06$) concentration of lactic acid compared to the Fiber High group.

Within HC, the absolute concentrations of propionic ($p=0.03$) and valeric acids ($p=0.02$) were significantly higher in the Fiber Low group compared to the Fiber High group. Also, the HC Fiber Low group had significantly higher individual relative concentrations of fecal SCFA (acetic ($p=0.02$), propionic ($p=0.01$), valeric ($p=0.009$), and butyric acid ($p=0.05$) when compared with HC Fiber High group.

Both the IBS-C and IBS-D women in the Fiber Low group showed lower propionic, butyric, valeric, caproic, and total SCFA than the Fiber High group.

The High Fiber IBS-C women had significantly higher mean fecal dry weight ($p=0.02$) than the Low Fiber IBS-C women. The Fiber High IBS-C women had higher mean relative concentrations of butyric acid ($p=0.14$) compared to Fiber Low IBS-C women. The Fiber Low IBS-D women had higher mean fecal dry weight ($p=0.10$) compared to the Fiber High IBS-D women. The Fiber High IBS-D women also had higher mean relative concentration of butyric acid compared to Fiber Low IBS-D women.

These findings of higher SCFA in lower fiber dietary intake in healthy subjects were in contrast with several observational international studies that studied the effects of plant-based foods vs. animal-based diet (de Filippo et. al. 2010). The rationale for these findings is unclear but may represent a microbiome difference, other dietary and/or supplemental intake (i.e fat, caffeine, alcohol, probiotics and prebiotics intake), and other behavioral-related practices (e.g., smoking, antibiotics use). For example, the Fiber Low group in HC had higher microbial diversity index (i.e., Inverse Simpson, Shannon) than the HC Fiber Low group. However, the Fiber Low group in HC had significantly lower intake in these other food components: carbohydrate (($p=0.009$), pectin ($p=0.002$), caffeine ($p=0.03$), starch ($p=0.04$), glucose ($p=0.01$), fructose ($p=0.04$), sucrose ($p=0.03$), and tagatose ($p=0.02$); but had higher mean lactose and lower mean fat intake compared to HC Fiber High group. It is worth noting that the High Fiber HC women had approximately 2 mg lower fecal dry weight and is composed of older women and had higher mean weight than the Low Fiber HC women. In addition to the contribution of higher amount of lactose and fat intake and higher microbial diversity index, it may be that variables such as age, weight, and fecal dry weight exert greater influence on the relationship of high fiber intake and the measured fecal SCFA in HC women.

The IBS in the Fiber High group had markedly reduced intake of total carbohydrates ($p = 0.09$) when compared to the HC Fiber high group. Interestingly, the observed trend in HC women is consistent with the study done by Wu et.al. (2016) that reported higher consumptions of fermentable diet consumed by vegans was not associated with higher levels of fecal SCFA. In summary, the positive relationship of high fiber intake (>25g/day) and high fecal SCFA observed in IBS-C women (also happened to have significantly higher *Bacteroides* relative abundance compared to HC) is consistent with de Filippo's report on higher microbial signatures that included *Bacteroides* in rural dwellers of Burkina Faso children (Africa) with higher fiber intake compared to urban dwellers of Italian children with lower fiber intake. A newer study done by Calderon and colleagues (2022) reported no association between fiber intake and SCFA.

Table 1. The Associations of Metabolites (micromole) and Dietary Fiber (High vs. Low) in HC, IBS, and Subtypes of IBS

	HEALTHY CONTROL (n=25)					IBS COMBINED (n=35)				
Variables	FIBER HIGH (n=10)	FIBER LOW (n=15)	Mean Difference (High-Low)	High vs Low p	p after Age, BMI, Bifidobacteria adj.	IBS FIBER High (n=9)	IBS FIBER LOW (n=26)	Mean (High-Low)	High vs Low p	p after Age, BMI, Bifidobacteria
Acetic Acid	194.69 ± 106.26	285.11 ± 140.55	-90.42	0.05	0.37	199.21 ± 119.41	195.20 ± 101.94	4.00	0.47	0.51
Propionic Acid	73.09 ± 27.83	107.60 ± 51.33	-34.51	0.03	0.23	100.42 ± 47.83	100.51 ± 63.51	-0.09	0.09	0.66
Butyric Acid	113.96 ± 63.09	155.09 ± 92.88	-41.13	0.11	0.84	157.82 ± 103.22	124.41 ± 82.46	33.41	0.15	0.39
Caproic Acid	16.85 ± 12.07	20.45 ± 13.59	-3.60	0.25	0.63	24.86 ± 14.27	16.79 ± 11.62	8.06	0.11	0.26
Valeric Acid	16.19 ± 6.49	25.78 ± 12.17	-9.59	0.02	0.21	23.27 ± 12.41	22.15 ± 12.14	1.13	0.08	0.72
Lactic Acid	32.99 ± 20.68	57.13 ± 61.75	-24.14	0.10	0.25	34.93 ± 17.62	51.35 ± 49.03	-16.42	0.42	0.06
Formic Acid	1.10 ± 0.76	1.64 ± 2.49	-0.54	0.23	0.26	0.74 ± 0.24	0.93 ± 0.61	-0.19	0.09	0.56
Tryptophan	9.82 ± 4.01	9.06 ± 4.08	0.76	0.33	0.17	10.83 ± 4.66	10.94 ± 4.74	-0.10	0.31	0.80
2-Aminobutyric Acid	5.46 ± 2.39	8.31 ± 5.11	-2.85	0.05	0.28	6.66 ± 2.72	9.44 ± 11.70	-2.78	0.17	0.93
sc-SCFA	381.74 ± 191.19	547.80 ± 265.77	-166.06	0.05	0.43	457.44 ± 237.85	420.12 ± 226.29	37.32	0.23	0.44
bc-SCFA	33.04 ± 17.33	46.23 ± 23.69	-13.19	0.07	0.71	48.13 ± 25.40	38.94 ± 22.06	9.19	0.09	0.43
sc- + bc-SCFA	414.79 ± 202.42	594.03 ± 283.74	0.00	0.05	0.44	505.57 ± 259.39	459.06 ± 243.97	46.51	0.21	0.43
	IBS-CONSTIPATION (n=10)					IBS-DIARRHEA (n=25)				
Variables	FIBER HIGH (n=4)	FIBER LOW (n=6)	Mean Difference (High-Low)	High vs Low p-v	P after Age, BMI, Bifidobacteria	IBS FIBER High (n=5)	IBS FIBER LOW (n=20)	Mean (High-Low)	p-value	p after Age, BMI, Bifidobacteria
Acetic Acid	179.89 ± 93.68	137.70 ± 34.20	42.19	0.23	0.38	214.66 ± 145.83	212.45 ± 109.58	2.21	0.49	0.78

Propionic Acid	124.53 ± 63.38	79.83 ± 15.96	44.7	0.13	0.07	81.12 ± 22.74	106.71 ± 71.17	-25.59	0.12	0.45
Butyric Acid	205.04 ± 114.05	87.55 ± 33.03	117.49	0.07	0.05	120.05 ± 86.84	135.46 ± 90.02	-15.42	0.37	0.60
Caproic Acid	26.13 ± 18.22	10.35 ± 4.66	15.77	0.09	0.21	23.84 ± 12.47	18.73 ± 12.45	5.11	0.23	0.98
Valeric Acid	28.83 ± 17.26	16.43 ± 2.68	12.4	0.12	0.13	18.83 ± 5.38	23.86 ± 13.37	-5.03	0.13	0.53
Lactic Acid	39.94 ± 21.90	39.75 ± 29.65	0.19	0.50	0.80	30.93 ± 14.70	54.83 ± 53.63	-23.9	0.08	0.15
Formic Acid	0.62 ± 0.22	0.70 ± 0.38	-0.08	0.35	0.54	0.83 ± 0.23	1.00 ± 0.66	-0.17	0.20	0.95
Tryptophan	11.94 ± 5.30	9.52 ± 3.21	2.42	0.24	0.11	9.95 ± 4.50	11.36 ± 5.10	-1.41	0.29	0.61
2-Aminobutyric Acid	5.04 ± 2.12	4.37 ± 1.29	0.67	0.31	0.65	7.96 ± 2.59	10.96 ± 13.01	-3.00	0.20	0.70
sc-SCFA	509.46 ± 250.66	305.08 ± 62.59	204.38	0.10	0.009	415.83 ± 247.28	454.63 ± 246.81	-38.8	0.38	0.78
bc-SCFA	54.95 ± 34.98	26.78 ± 6.45	28.17	0.10	0.18	42.67 ± 17.01	42.59 ± 23.85	0.08	0.50	0.74
sc- + bc-SCFA	564.41 ± 281.30	331.86 ± 65.92	232.55	0.10	0.02	458.50 ± 262.65	497.22 ± 265.58	-38.72	0.39	0.78

Table 2 The Associations of SCFA (micromole) and Dietary Fiber in All Women

Variables	FIBER HIGH (>25g/day) (n=19)	FIBER LOW (<25g/day) (n=41)	Mean Difference (High-Low)	High vs Low p-value
Acetic Acid	196.83 ± 109.49	228.10 ± 123.82	-31.27	0.17
Propionic Acid	86.03 ± 40.01	103.10 ± 58.78	-17.07	0.10
Butyric Acid	134.74 ± 85.04	135.63 ± 86.56	-0.89	0.49
Caproic Acid	20.65 ± 13.43	18.13 ± 12.34	2.51	0.25
Valeric Acid	19.55 ± 10.14	23.48 ± 12.13	-3.93	0.10
Lactic Acid	33.91 ± 18.78	53.47 ± 53.34	-19.55	0.03
Formic Acid	0.93 ± 0.59	1.19 ± 1.59	-0.26008	0.19
Tryptophan	10.29 ± 4.25	10.25 ± 4.56	0.04352	0.49
2-Aminobutyric Acid	6.03 ± 2.55	9.02 ± 9.75	-3.00	0.04
sc-SCFA	417.60 ± 211.96	466.83 ± 246.18	-49.23	0.22
bc-SCFA	40.19 ± 22.29	41.61 ± 22.65	-1.42	0.41
sc-SCFA+ bc-SCFA	457.79 ± 229.26	508.44 ± 264.03	-50.65	0.23

Table 3. The Associations of Metabolites (micromole) and Dietary Fat (High vs. Low) in HC, IBS, and Subtypes of IBS

	HEALTHY CONTROL (n=25)				IBS (n=35)			
Metabolites	FAT High (n=11)	FAT Low (n=14)	Mean Difference	p-value	IBS FAT High (n=17)	IBS FAT Low (n=18)	Mean Difference	p-value

Acetic Acid	269.46 ± 154.05	232.83 ± 118.21	36.62	0.26	230.17 ± 133.16	164.18 ±55.41	65.99	0.04
Propionic Acid	100.80 ± 59.50	88.30 ± 33.57	12.50	0.27	115.87 ± 72.84	85.96 ± 39.44	29.91	0.08
Butyric Acid	150.44 ± 97.76	129.36 ± 72.56	21.09	0.28	141.26 ± 83.85	125.20 ± 93.29	16.07	0.30
Caproic Acid	19.83 ± 14.85	18.37 ± 11.62	1.47	0.40	21.15 ± 14.62	16.71 ± 10.43	4.44	0.16
Valeric Acid	24.04 ± 13.71	20.30 ± 8.94	3.74	0.23	25.38 ± 13.63	19.66 ± 9.92	5.71	0.09
Lactic Acid	57.55 ± 70.94	39.56 ± 25.44	17.99	0.22	62.61 ± 57.85	32.51 ± 13.08	30.09	0.03
Formic Acid	2.14 ± 2.80	0.85 ± 0.61	1.29	0.08	0.90 ± 0.61	0.87 ± 0.49	0.03	0.44
2-Aminobutyric Acid	7.57 ± 4.94	6.85 ± 4.10	0.71	0.35	12.02 ± 13.91	5.61 ± 2.15	6.41	0.04
Tryptophan	10.64 ± 4.21	8.36 ± 3.64	2.29	0.09	11.41 ± 5.44	10.44 ± 3.87	0.97	0.28
sc-SCFA	520.70 ± 300.68	450.49 ± 205.48	70.21	0.26	487.30 ± 270.87	375.33 ± 164.24	111.9	0.08
bc-SCFA	43.87 ± 26.38	38.66 ± 18.55	5.21	0.30	46.53 ± 25.50	36.37 ± 19.70	10.1	0.10
sc-SCFA+ bc-SCFA	564.57 ± 322.37	489.15 ± 217.39	75.42	0.26	533.83 ± 292.10	411.70 ± 179.08	122.13	0.08
IBS-CONSTIPATION (n=10)				IBS-DIARRHEA (n=25)				
Metabolites	FAT High (n=5)	FAT Low (n=5)	Mean Differen ce (High-Low)	High vs Low p-value	IBS FAT High (n=14)	IBS FIAT Low (n=11)	Mean Differen ce (High-Low)	High vs Low p-value
Acetic Acid	142.27 ±78.35	166.88 ± 50.95	-24.61	0.29	266.80 ± 136.32	163.14 ± 58.99	103.66	0.01
Propionic Acid	84.69 ± 51.39	110.72 ± 38.28	-26.03	0.21	128.86 ± 78.30	76.43 ± 36.89	52.43	0.03
Butyric Acid	94.84 ± 82.11	174.25 ± 93.37	-79.41	0.11	160.60 ± 79.93	106.33 ± 89.64	54.27	0.07
Caproic Acid	13.41 ± 12.47	19.91 ± 15.61	-6.5	0.25	24.37 ± 14.68	15.48 ± 8.19	8.89	0.05
Valeric Acid	17.97 ± 11.76	24.81 ± 12.54	-6.84	0.21	28.46 ± 13.58	17.68 ± 8.46	10.78	0.02
Lactic Acid	44.67 ± 33.99	34.98 ± 15.63	9.69	0.30	70.08 ± 65.12	31.56 ± 12.55	38.52	0.03
Formic Acid	0.79 ± 0.36	0.54 ± 0.22	0.25	0.13	0.94 ± 0.70	0.99 ± 0.51	-0.05	0.42

2-Aminobutyric Acid	4.42 ± 2.06	4.85 ± 1.15	-0.43	0.35	15.18 ± 15.58	5.90 ± 2.41	9.28	0.03
Tryptophan	9.21 ± 4.11	11.76 ± 4.06	-2.55	0.19	12.33 ± 5.81	9.93 ± 3.83	2.4	0.13
sc-SCFA	321.80 ± 202.84	451.86 ± 159.27	-130.06	0.16	556.26 ± 272.25	345.89 ± 162.41	210.37	0.02
bc-SCFA	31.38 ± 23.76	44.72 ± 27.75	-13.34	0.23	52.84 ± 24.34	33.16 ± 15.90	19.68	0.02
sc-SCFA+ bc-SCFA	353.18 ± 225.62	496.58 ± 182.95	-143.4	0.17	609.10 ± 290.83	379.06 ± 173.55	230.04	0.02

Table 4. The Associations of Metabolites (micromole) and Dietary Fat Intake (High vs. Low) in All Women

Variables	FAT HIGH (n=28)	FAT LOW (n=32)	Mean Difference (High-Low)	High vs Low p-value
Acetic Acid	245.60 ± 140.28	194.21 ± 93.49	51.39	0.06
Propionic Acid	109.95 ± 67.17	86.98 ± 36.42	22.97	0.06
Butyric Acid	144.87 ± 87.90	127.02 ± 83.57	17.85	0.21
Caproic Acid	20.63 ± 14.44	17.44 ± 10.82	3.20	0.17
Valeric Acid	24.85 ± 13.42	19.94 ± 9.36	4.91	0.06
Lactic Acid	60.62 ± 62.07	35.60 ± 19.44	25.02	0.03
Formic Acid	1.39 ± 1.88	0.86 ± 0.53	0.53	0.08
Tryptophan	11.11 ± 4.92	9.53 ± 3.85	1.58	0.09
2-Aminobutyric Acid	10.27 ± 11.34	6.15 ± 3.16	4.11	0.04
sc-SCFA	500.42 ± 277.92	408.21 ± 184.21	92.21	0.07
bc-SCFA	45.49 ± 25.39	37.38 ± 18.93	8.11	0.09
sc-SCFA+ bc-SCFA	545.91 ± 298.81	445.59 ± 197.30	100.32	0.07

Table 5. Adjusted p-Values for Metabolites Comparison under Dietary Fiber Intake (High vs. Low) in All Women

Variables	HC (n=25)	IBS (n=35)	IBS-C (n=10)	IBS-D (n=25)	ALL (N=60)
Acetic Acid	0.25	0.49	0.38	0.49	0.38
Propionic Acid	0.13	0.13	0.13	0.13	0.13
Butyric Acid	0.25	0.25	0.25	0.46	0.49
Caproic Acid	0.25	0.25	0.25	0.25	0.25
Valeric Acid	0.1	0.13	0.13	0.13	0.13
Lactic Acid	0.17	0.5	0.5	0.17	0.15
Formic Acid	0.29	0.29	0.35	0.29	0.29
Tryptophan	0.41	0.41	0.41	0.41	0.49
2-Aminobutyric Acid	0.13	0.25	0.31	0.25	0.13
sc-SCFA	0.25	0.29	0.25	0.38	0.29
bc-SCFA	0.17	0.17	0.17	0.5	0.5
sc-SCFA+ bc-SCFA	0.25	0.2875	0.25	0.39	0.29

Table 6. Adjusted p-values for Metabolites under Dietary Fat intake (High vs. Low) in All Women

Variables	HC (n=25)	IBS (n=35)	IBS-C (n=10)	IBS-D (n=25)	ALL (N=60)
Acetic Acid	0.15	0.49	0.38	0.49	0.15
Propionic Acid	0.13	0.13	0.13	0.13	0.13
Butyric Acid	0.25	0.25	0.25	0.37	0.26
Caproic Acid	0.25	0.25	0.25	0.25	0.25
Valeric Acid	0.1	0.13	0.13	0.13	0.13
Lactic Acid	0.17	0.5	0.5	0.17	0.15
Formic Acid	0.29	0.23	0.35	0.29	0.23
Tryptophan	0.33	0.33	0.33	0.33	0.33
2-Aminobutyric Acid	0.13	0.25	0.31	0.25	0.13
sc-SCFA	0.17	0.29	0.17	0.38	0.17
bc-SCFA	0.13	0.13	0.13	0.5	0.13
sc-SCFA+ bc-SCFA	0.17	0.26	0.17	0.39	0.17

The analysis for all women demonstrated that more SCFA are associated with high fat intake (<74 g/day) intake more than the contribution of high fiber diet intake. IBS-C group showed reduced concentration of total SCFA and individual SCFA under high fat diet while an increased concentration is observed under high fiber diet. The finding in IBS-C is supported by the 3-day food record, where the IBS-C women appeared to have healthier eating habits (avoiding fatty, fried foods and gluten) than IBS-D and/or the HC group who reported higher intake of meat and dairies. On the average, the IBS-D and HC showed higher fecal SCFA under high fat diet (>74 g/day) intake. The metabolites Tryptophan and Lactic acid are higher in all cohorts who consumed high fat diet.

High vs. Low SCFA Producers in All women. When the women are dichotomized as High SCFA Producers and Low SCFA Producers, high fecal SCFA was significantly associated with lower abdominal pain in all women (See Table below).

Fairly similar amount of fiber intake was found between high SCFA producer all women (n= 21) and low producer SCFA women (n=39). The fiber intake of High SCFA Producers vs Low SCFA Producers is as follows: Fiber: 23.1g vs. 21.5 g; Insoluble Fiber: 16.7 g vs. 15.3 g; Soluble Fiber: 6.4 g vs 6.1 g). Interestingly, the fat

intake of High SCFA Producer (84 gram) was significantly higher ($p=0.02$) than the Low SCFA Producer HC women 70 gram). The only diet that was significantly different was lactose (11.1 grams for High Producer vs. 6.4 grams for Low Producer; $p = 0.04$). High SCFA Producer had higher caloric intake ($p=0.07$) and total sugar intake ($p=0.18$) compared to Low SCFA Producer. The consumption of water, added sugar, resistant grain and gluten are almost exactly similar in both groups.

Table 7. Baseline Characteristics of Women (High SCFA Producers vs. Low SCFA Producers)

Variables	ALL women (N=60) Mean $\pm$ SD		HC (n=25) Mean $\pm$ SD		IBS-C + D (n=35) Mean $\pm$ SD		IBS-C (n=10) Mean $\pm$ SD		IBS-D (n=25) Mean $\pm$ SD	
	High SCFA (n=21)	Low SCFA (n=39)	High SCFA (n=10)	Low SCFA (n=15)	High SCFA(n=11)	Low SCFA (n=24)	High SCFA (n=3)	Low SCFA (n=7)	High SCFA (n=8)	Low SCFA (n=17)
Age	30 $\pm$ 6.28	28 $\pm$ 6.86	28.50 $\pm$ 4.90	30.87 $\pm$ 8.17	31.36 $\pm$ 7.27	26.54 $\pm$ 5.45	27 $\pm$ 8.72	25 $\pm$ 2.83	33.00 $\pm$ 6.52	27.18 $\pm$ 6.19
Weight	144.33 $\pm$ 33.54	137.90 $\pm$ 20.87	135.40 $\pm$ 28.30	136.18 $\pm$ 11.07	152.45 $\pm$ 37.10	138.98 $\pm$ 25.33	135.33 $\pm$ 14.50	130.79 $\pm$ 12.27	158.88 $\pm$ 41.64	142.35 $\pm$ 28.71
BMI	22.80 $\pm$ 3.23	23.09 $\pm$ 3.71	23.06 $\pm$ 3.21	22.34 $\pm$ 2.29	25.27 $\pm$ 5.87	23.09 $\pm$ 3.71	21.24 $\pm$ 1.14	23.10 $\pm$ 2.30	26.78 $\pm$ 6.26	23.08 $\pm$ 4.22
BMR	1429.72 $\pm$ 93.78	1441.46 $\pm$ 105.66	1411.30 $\pm$ 128.16	1410.93 $\pm$ 70.14	1477.09 $\pm$ 169.57	1441.46 $\pm$ 105.66	1432.00 $\pm$ 119.15	1405.57 $\pm$ 58.90	1494.00 $\pm$ 189.27	1456.24 $\pm$ 118.12
Fat intake	83.83 $\pm$ 26.63	70.14 $\pm$ 19.03	81.25 $\pm$ 31.60	67.67 $\pm$ 20.80	86.17 $\pm$ 22.52	71.68 $\pm$ 18.12	74.67 $\pm$ 10.63	73.77 $\pm$ 17.80	90.49 $\pm$ 24.78	70.82 $\pm$ 18.72
Fiber intake	23.13 $\pm$ 9.09	21.48 $\pm$ 7.95	24.31 $\pm$ 11.36	23.27 $\pm$ 9.26	22.07 $\pm$ 6.82	20.36 $\pm$ 6.98	28.53 $\pm$ 2.76	21.38 $\pm$ 4.87	19.64 $\pm$ 6.30	19.95 $\pm$ 7.77
Carbohydrates	224.14 $\pm$ 84.73	207.85 $\pm$ 69.02	237.05 $\pm$ 105.23	208.93 $\pm$ 73.54	212.42 $\pm$ 63.83	207.17 $\pm$ 67.65	201.95 $\pm$ 35.61	208.31 $\pm$ 35.13	216.34 $\pm$ 73.44	206.71 $\pm$ 78.20
Alcohol intake	11.15 $\pm$ 13.92	10.48 $\pm$ 14.92	16.13 $\pm$ 15.06	10.45 $\pm$ 14.12	6.61 $\pm$ 1.66	10.50 $\pm$ 15.69	0.02 $\pm$ 0.04	6.77 $\pm$ 2.67	9.09 $\pm$ 2.99	12.03 $\pm$ 16.89
Lactose intake	11.06 $\pm$ 10.83	6.37 $\pm$ 5.76	13.15 $\pm$ 8.99	6.15 $\pm$ 4.62	9.17 $\pm$ 2.39	6.51 $\pm$ 6.46	6.03 $\pm$ 9.51	9.15 $\pm$ 6.99	10.34 $\pm$ 13.70	5.43 $\pm$ 6.12

Table 8. P-values for Dietary Lactose & Fat Intake (High SCFA Producers vs. Low SCFA Producers)

Variab les	ALL Women High vs. Low SCFA Producers	Healthy Women High vs. Low SCFA Producers	IBS-C & IBS-D High vs. Low SCFA Producers	IBS-C High vs. Low SCFA Producers	IBS-D High vs. Low SCFA Producers
Lactose	0.04	0.02	0.006	0.15	0.18
Fat	0.02	0.13	0,05	0.47	0.04

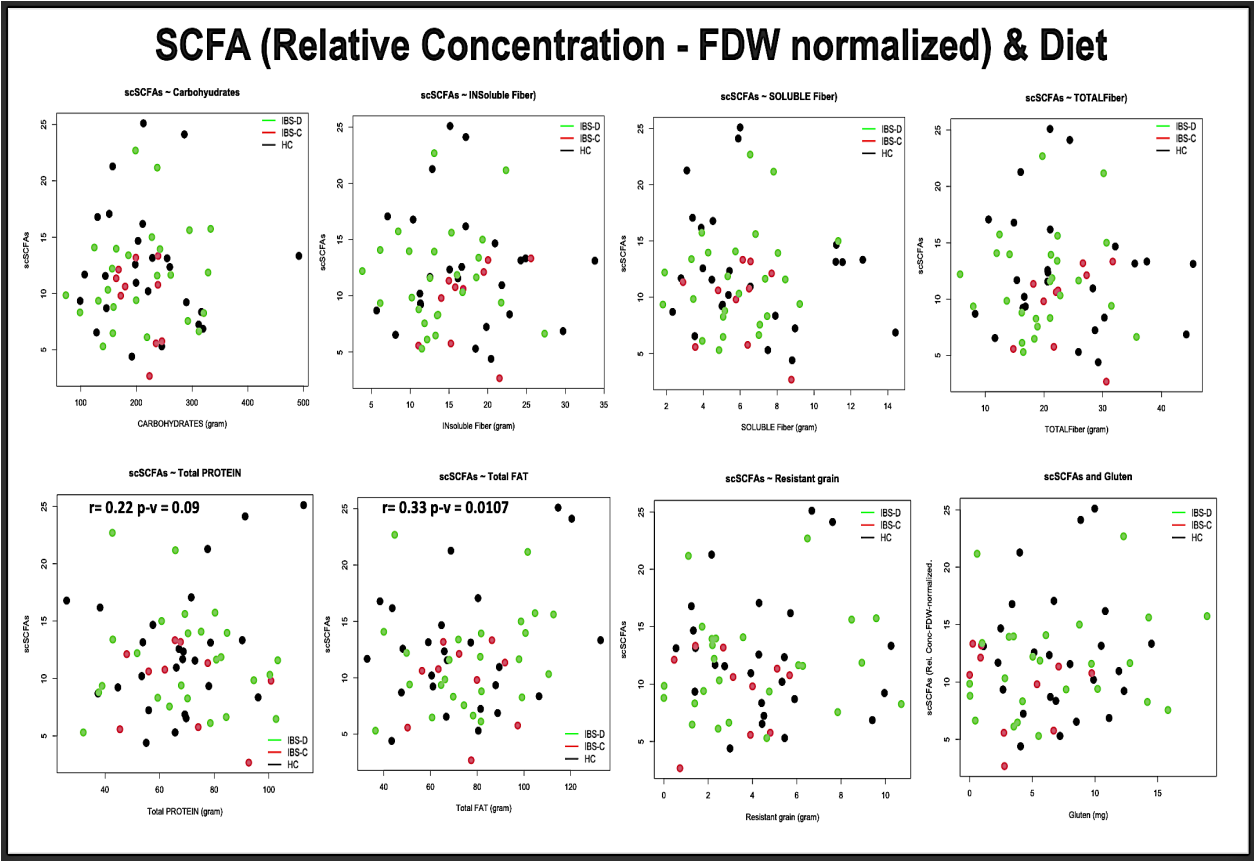


Figure 1. The relationships of dietary intake and SCFA.

AIM2b DISCUSSION

The relationship of dietary fiber intake with fecal SCFA is well established. The production of SCFA can be studied *in silico* (Diener et al., 2020). However, the strongest evidence of this relationship was derived from fermentation activities of well-characterized SCFA-producing bacteria (single or co-culture of two bacterial species) that were incubated with different amount and types of fibers where the proportion of SCFA were measured. (Cummings, Gibson, Macfarlane & Cummings 1987, 1991). Currently, the production of SCFA can be carried out in large, state of the art anaerobic fermenters where fluxes of nutrients enter the chamber and SCFA products can be produced continuously and measured efficiently. Although there are a few infusion studies done in humans to capture the stoichiometry of anaerobic fermentation (Wolever et al., 1997; Boets et al., 2015), the proportions of these SCFA in humans still needs to be well-characterized most especially in the context of GI disorders such as IBS.

Besides the presence of SCFA-producing microbes, the physical and chemical characteristics of fibers (i.e., solubility, digestibility) influence the rate of SCFA production. The intake of fermentable fibers (oats, coarse wheats,) and fiber supplements (inulin, xylan, pectin, high-amylose maize, arabinoxylan-oligosaccharides, psyllium) are known to increase colonic SCFA (Cummings & Macfarlane 1991). It was reported in literature that for every gram of mixed carbohydrate that enters the colon corresponds to about 1.5 kilo calories (or 6.2 kJ/g mixed carbohydrates); 20 grams of unavailable mixed carbohydrates would yield about 122 millimole or 150 KJ in terms of metabolizable energy (Livesey, 1990; Livesey 1989; NEED TO CHECK). In humans, the anaerobic fermentation in the gut adds complexity to the measured proportion of these metabolites. One must account for the contributions of transit time, the presence of other metabolic byproducts both from the host and bacterial activities.

The 3-day food record obtained by the MAP study was used to test the relationship of dietary intake with SCFA. The proposed central hypothesis of the study was that high intake of carbohydrates (high content of polysaccharides and oligosaccharides) would correlate with higher concentrations of short-chain SCFA (sc-SCFA) and lower levels of branched chain (bc-SCFA) in both IBS and healthy control women. It was hypothesized that higher intake of DF above 25g/day would significantly increase SCFA fecal concentration

(~50mM/10g DF). The expectation that higher DF intake would reflect a higher production of SCFA is conditional in that it would also require the necessary repertoire of SCFA-producing bacteria with the necessary genes and metabolic diversities for the metabolism of SCFA.

The dietary intake pattern of the subjects that participated in the MAP study revealed that on the average, healthy women consumed more total carbohydrates and lactose than the IBS women. The average dietary fiber (DF) intake of healthy women was comparable to IBS-C women (~ 24 g/day) while on the average IBS-D women consumed approximately 19 g fiber/day. Taken together, there were no significant differences found in the DF intake between women with IBS and healthy controls (HC or healthy women).

IBS-D women had lowest daily DF intake among the three groups and had significantly higher consumption of added sugar compared to healthy controls. Running multivariate analysis (Pearson's correlation) on R-software showed that only dietary fat was correlated with SCFA: propionic acid ($r=0.4$) and acetic acid ($r=0.33^*$); this relationship was driven by the IBS-D phenotype because of their higher mean of daily fat intake compared to healthy controls and IBS-C and only in this cohort where high fat diet resulted in higher means of SCFA; fat and added sugar became the major contributors for SCFA production in IBS-D women. IBS-D women had higher absolute amount of the bc-SCFA (caproic and valeric acids) than the healthy controls; this indicates that the oxidation of fat and protein in the gut were the main source of SCFA for the IBS-D women. A study carried out by Born et.al (1999) recruited 25 patients (9 men and 16 women) complaining of abdominal pain, flatulence, and diarrhea found that the proportion of butyrate and proportion of propionate (derived from the absolute concentration quantified from the fecal matter with GLC metabolic profiling) was positively associated with higher fructose degradation activity (less amount of fructose left in the bacteria culture media). However, the study failed to significantly differentiate the symptomatic IBS from the non-symptomatic IBS patients, meaning that all the IBS-D subjects were not that different from each other. However, Born et. al. mentioned that their earlier study on healthy controls showed significant difference between reported symptoms of carbohydrate-absorbers vs carbohydrate malabsorbers; higher fructose degradation activity significantly correlated with higher symptoms reported by the healthy individuals with carbohydrate malabsorption. This could mean that only the healthy individuals who were able to ferment most

of the sugar were able to produce SCFA that generated their GI symptoms compared to fructose malabsorbers who were unable to produce SCFA (non-symptomatic). Findings from the Born group infer that the production of SCFA generates GI disturbances, perhaps as the effect of gas (i.e., H₂, CH₄) responsible for bloating and flatulence and other GI discomfort. Perhaps the symptom experience detailed in that study were not precisely measured by the IBS symptom measurement scale used. Another likely explanation is that the participants were not able to absorb these SCFA, hence the higher amount of butyric acid in their feces compared to healthy controls. A strength of Born et al. study was their bacterial culture experiments where they measured the amount of fructose degraded by the gut bacteria, confirming that the degradation of these sugars was done only by the non-negative bacteria.

Human controlled infusion studies have shown that high fat intake results in higher acetic and propionic acids both in fecal and serum in people with obesity (Wolever et al., 1997; Boets et al., 2015). Interestingly, the IBS-D women in the present study as a group had higher body weights when compared to the IBS-C women and healthy control women. Although non-significant, the IBS-D women also tended to have higher fat intake and had more fecal propionic acid among the three groups. Using multi-regression on R software, lactose was found to be significantly associated with propionic acid. This could mean that the acrylate pathway is mostly utilized by the gut bacteria for the conversion of lactate to propionate (Lous & Flint 2017). The dichotomization of SCFA producers (High vs. Low) revealed that the high SCFA producers (n=21) consumed a significant amount of lactose when compared to low SCFA producers (n=39). Within the IBS, the high SCFA producers (n=11) also had significantly higher amount of lactose intake compared to the low SCFA producers (n=14). Investigating the high SCFA producers within the IBS cohort revealed that the high SCFA producers-IBS women consumed approximately similar amount of lactose when compared to the high SCFA producers-healthy control women. These results are suggestive of high bacterial fermentation activity by the lactate-utilizing bacteria responsible for the higher amount of the measured fecal SCFA. These results may also have important clinical implications because a specific amount of lactose intake corresponded to a higher amount of SCFA production both in IBS and healthy controls. Looking at the effect of caloric intake, strong positive correlations were found for all SCFA in all women, however several of women in the IBS-D and

healthy control cohorts consumed high calories, (i.e., above 2500 kcal/day up to 3500 kcal/day) while the IBS-C women did not.

Using linear regression (R-package version 3.61), DF was not correlated with the three individual SCFA. In contrast, fat (independent variable) was significantly and positively correlated with acetic acid and propionic acid. However, the significant relationship of butyrate and fat was lost due to the presence of DF in the multiple regression; only the propionic and acetic acid remained significantly associated with fat in the presence of DF as a covariate. It was reported in literature that the digestibility of fat is reduced by the presence of DF (Baer et al, 1999); hence, the pool of SCFA is reduced under the context of high fat intake. In the presence of both fat and DF intake, acetic acid is significantly higher in healthy controls compared to IBS-C while butyric acid was significantly higher in healthy controls compared to IBS-D. Moreover, linear regression showed beta coefficients that demonstrated lower beta estimate for acetic acid in IBS-C, lower beta estimate for butyric acid in IBS-D, and lower beta estimate for propionic acid in healthy controls in the context of either fat or DF or both, with fat providing a lower contribution to SCFA pool compared to DF.

Interestingly, when women were categorized as either DF High and DF Low, a positive association of DF intake (most especially insoluble fiber) and fecal SCFA was found in the IBS cohort (Note: IBS-D showed positive relationships with caproic acid with the overall high DF intake). Within the IBS-C cohort, higher intake of insoluble DF was positively associated with butyric acid ($p=0.07$). It can be speculated that the gut microbiome of IBS-C cohort was efficient at extracting energy from fiber. To do this, would require the right consortia of high SCFA-producing bacteria. Interestingly, the IBS-C women with high DF had the highest relative abundance of *Bacteroides* and enriched *Clostridia* which are characterized by a higher number of energy-metabolizing genes relative to other bacteria. This pair of high fiber-degrading bacteria (primary and secondary fiber degraders) are associated with high SCFA in IBS-C under high DF environment. It may be that IBS-C possessed higher microbial richness in the *Clostridia* family.

Compared to those with IBS-D, the IBS-C women had a greater microbial diversity index. Prior studies have shown that constipated individuals have higher microbial diversity than normal, healthy individuals; however, this study showed that healthy controls had higher microbial diversity compared to IBS-C

women. Perhaps, this can be attributed to the primer used (V4) to identify the OTUs and did not capture the other bacteria that are identified by other primers currently used to amplify the 16S ribosomal gene. Overall, IBS-C bacterial community signatures included higher *Bacteroides*, *Clostridium XIVa*, *Butyricoccus*, *Clostridium IV*, *Clostridium XVIII*, and *Eubacteria* compared to IBS-D and healthy controls. In addition, IBS-C also had higher *Ruminococcus2* than healthy controls. Studies done by Parthasarathy et.al. (2016) also reported higher level of *Bacteroidetes* in the colonic mucosa in women with IBS-C. Similarly, Li et.al. (2021) also reported that constipated women of reproductive age had higher abundance of *Bacteroides*. Clinically it can be conjectured that higher production of butyrate and propionate increase water and electrolyte absorption more than acetate and this may contribute to the constipation phenotype (Biner & Mehta 1989). It's been reported from literature that higher concentrations of butyric acid prevent the oxidation of acetic acid (Cavaleri & Bashar, 2018). This notion may be explained by the pattern observed in the present study; IBS-C women (the Fiber High IBS-C group) had higher butyric acid (205 micromole) but lower acetic acid (180 micromole), while in HC Fiber High group, there is more acetic acid (195 micromole) than butyric acid (114 micromole) in the Fiber High HC group. IBS-D followed similar trends as in HC High Fiber group. The data showed that high fecal butyric acid (>100 -400 micromole, as a consequence of high DF intake) is significantly associated with reduced abdominal pain in IBS-C compared to the effect of low butyric acid (40 - <100 micromole), while a high acetic acid (as a consequence of high DF intake) is associated with reduced mean abdominal pain in HC, and reduced mean diarrhea events in IBS-D women, although not significant.

When other dietary intakes were dichotomized (High vs. Low), more meaningful associations were observed. Within the healthy control group, a higher intake of protein (75-113g/day) was associated with significantly higher amount of butyric acid and although not significant, higher amount of other SCFA. This suggests that protein fermentation adds to the fecal SCFA pool, particularly propionic acid via the acrylate pathway and succinate pathway including the propanediol pathway (Louis & Flint, 2016). Due to the cross-feeding activities of the gut bacteria metabolites from amino acids leucine, valine, and alanine that produce bc-SCFA can be converted into the sc-SCFA. Excessive amino acid levels may be derived from bacterial turnovers, the host's pancreatic and gastric secretions, debris from intestinal mucosa turnovers, as well as

bacterial amino acid biosynthesis activities. Although non-significant, it is puzzling that the higher intake of insoluble DF and added sugar were associated with lower sc-SCFA in HC, but their higher intake of soluble DF and gluten were associated with higher amount of SCFA. This may be attributed to their higher intake of fat in concomitant with their higher intake of DF and individualized microbiota composition profile.

In the IBS-D group, higher intake of added sugar (40-110 g/day) was associated with higher acetic acid; however, their higher intake of insoluble or soluble DF was also associated with higher mean butyric acid. In summary, the high sugar intake and the resulting higher fecal acetic in IBS-D could have important negative implications for health. Higher intake of added sugar could increase the SCFA pool in the gut to the point that higher amount of acetic acid is absorbed and contributes to lipogenesis and may increase the risk of developing metabolic diseases. Hence, this result could inform the women with IBS-D phenotype the benefits of increasing their dietary fiber intake because it correlates well with increased amount of butyric acid production.

Despite the increasing body of literature that supports DF alters the gut microbiome in the positive direction, majority of women in this observational study did not meet the ADA recommendation of daily DF intake (25g/day). Although the presence of bacteria specialists that produce and consume SCFA ultimately decide the ultimate concentrations or proportions of these acids in the gut, these bacteria rely on a high amount of unavailable carbohydrates to benefit the host and vice versa.

The results from this study are in contrast with the observational study carried out by Calderon et al. (2022) with a sample size of 48 of which 39 were women; 18 were healthy volunteers and 30 subjects with IBS (13 IBS and 17 IBS-D; Rome IV); age 18-65 years old. Calderon's team did not find associations of habitual DF intake or other dietary intake such as starch with total or individual SCFA. One reason for the difference is that this team used a food frequency questionnaire and did not gather detailed components of the dietary intake and mean DF intake was only 12 g fiber/day (ranges: 7.8 grams to 16.7 grams). Such a short range of fiber intake may not have enough variability to capture significant differences. In addition, the DF intake of participants was markedly below the recommended fiber intake where SCFA are known to impact gut health. Furthermore, their study subjects were older (older adults at age 65) and it is well-established in literature that

older age has lower microbial diversity index. As people age, the microbiome becomes unstable due to accumulated exposures of environmental toxins and a life-time accumulation of stress. Without the use of probiotics and prebiotics, people beyond age 40 are likely to have reduced microbial diversity (Lozupone et al., 2012; Faith et al., 2013).

AIM2c RESULT: Relationship between Dietary Fiber and GI Symptoms

For Aim2c, it was hypothesized that higher intake of insoluble and slowly fermentable DF intake will be associated with lower abdominal pain. Also, a high and rapidly fermentable and soluble DF intake will be positively correlated with fecal SCFA concentrations and IBS abdominal pain severity.

Baseline Characteristics & Demographics. The demographic characteristics of the sample are shown in Table 1. Overall, the women (fecal donors) were mostly of white descent (n =51, 89.5%), 10.5% (n=6) are from non-White descent (3 did not specify their ethnic background). With the exceptions of body weight and calculated basal metabolic rate (BMR), few differences between IBS and HCs were found. As a group, women with IBS had a higher body weight and higher BMR as compared to HCs. Boxplots center represents median and box upper border and lower border as interquartile ranges (IQRs).

When subgroups of women with IBS (IBS-D, IBS-C) were compared only education level differed between them (Table 1). The IBS-C cohort tended to be younger when compared to the IBS-D (p=0.06) group. The IBS-D cohort had a higher mean body weight when compared to HC (p=0.0007) and IBS-C (p=0.001) and higher BMR compared to IBS-C group. These group differences remained significant after multiple comparisons testing using the Benjamin-Hochberg procedure (False Discovery Rate [FDR]) (see Table 1A). The FDR method is used to control expected proportion of false discoveries when doing a multiple hypothesis test. This method reports the FDR-adjusted p-values and the original p-values. The FDR values controlled the expected proportion of false positives among the rejected hypothesis to reduce Type 1 Error.

Table 1. Education and Income for the 4 Groups.

HC	IBS	IBS-C	IBS-D	Income
48% (MS & above)	28.6% (MS & above)	30 % (MS degree)	24% have (MS degree)	No differences in income in all groups (MS and above)
52% (BA & below)	71.4 % (BA & below)	70% (BA & below)	76% (BA & below)	70% of all groups earned < \$30,000 (BA and below)
28% (below BA)	60% (below BA)	20% below Bachelor's	32% are below Bachelor's	

As shown in Table 3, there were no significant IBS versus HC group differences in dietary intake of protein, fat, and carbohydrates. However, the IBS group reported a higher sugar intake ($p=.04$; FDR adj. $p = 0.05$). When subgroup comparisons were made some differences emerged. When compared to IBS-C and HC groups, the IBS-D cohort had higher mean daily total added sugar intake ($p<0.001$; $p=0.01$, respectively). These differences remained significant after adjusting for multiple comparisons (see Table 2). The IBS-D group reported higher intake of insoluble fiber ($p=0.04$) when compared to HC ($p=0.04$) and IBS-C groups ($p=0.03$). The IBS-C group had lower daily mean gluten intake (a type of soluble fiber) compared to HC ($p=0.01$) and IBS-D cohorts ($p=0.04$). Based on the World Health Organization dietary recommendation, sugars (<https://www.calculator.net/carbohydrate-calculator.html>), women in the IBS-D group consumed on average takes less than 20 g of fiber per day while women with IBS-C group and HCs had an average take of approximately 24 g fiber daily. Note: p-values in purple indicates clinical significance due to small size of the study.

Table 2. Baseline Characteristics of Fecal Donors: IBS (n=35) and HC (n=25)

Fecal Donors Variables	MEAN $\pm$ SD		HC (n=25) and IBS (n=35) p	FDR p
	Normal/ HC, n =25)	IBS-C + BS-D (n= 35)		
BMI	22.6 $\pm$ 2.7	23.8 $\pm$ 4.5	0.41	0.46
Age	29.9 $\pm$ 7.0	28.1 $\pm$ 6.4	0.15	0.20
Height (in.)	65.0 $\pm$ 2.5	65.1 $\pm$ 2.8	0.30	0.30
Weight (kg)	135.9 $\pm$ 19.3	143.2 $\pm$ 29.7	0.01	0.01
BMR*	1411.1 $\pm$ 95.0	1452.7 $\pm$ 127.6	0.003	0.01
Race	21 white, 3 Non-white, 2 NA (87.5% white)	30 white, 3 non-white, 2 NA (90.1% white)	NS	NS
Education	14 below, 11 above (56% BA & below)	24 below, 11 above (68.6% BA & below)	NS	MS
Income	14 below, 7 above, 4 NA (66.7% \$30,000 & below)	17 below, 13 above, 5 NA (56.7% \$30,000 & below)	NS	NS

Table 3. Comparison of 3-day Food Record Dietary intake in IBS and HC

Variables	MEAN $\pm$ SD		HC and IBS p-value Differences	FDR p
	Normal/HC,	IBS-C + BS-D		

Carbohydrates (g)	220.2± 86.6	208.8± 65.6	0.29	0.43
Fiber (g)	23.7± 9.9	20.9± 6.9	0.12	0.16
Insoluble Fiber (g)	17.0 ± 6.9	14.9 ± 5.3	0.11	0.15
Resistant grain	4.6 ± 2.7	3.8 ± 2.8	0.14	0.21
Soluble Fiber (g)	6.6 ± 3.4	5.9 ± 2.1	0.17	0.29
Gluten	7. 2± 3.6	5.9 ± 5.1	0.24	0.32
Added Sugar (g)	34.3 ± 25.7	46.6 ± 27.1	0.04	0.08
Total Sugar (g)	76.3 ± 40	80.1 ± 34.2	0.35	0.39
Lactose (g)	9.0 ± 7.4	7.4 ± 8.7	0.22	0.44
Fat (g)	73.1± 25.9	76.2± 20.4	0.31	0.44
Protein (g)	73.1± 19.5	69.4± 19.6	0.30	0.47
Alcohol (oz.)	12.73± 14.5	9.3± 14.5	0.19	0.25
Caloric intake	1849 ± 594	1820 ± 425	0.47	0.5
Water (ml)	1533 ± 776	1435 ± 606	0.30	0.39

Table 4. Baseline Characteristics of Fecal Donors: IBS-C (n=10), IBS-D (n=25), and HC (n=25)

Variables	MEAN ± SD			HC vs IBS-C	HC vs IBS-D	IBS-C vs. IBS-D	HC vs IBS-C	HC vs IBS-D	IBS-C vs. IBS-D
	HC (n=25)	IBS-C (n=25)	IBS-D (n=25)	p	p	p	FDR p	FDR p	FDR p
BMI	22.6± 2.7	22.5± 2.2	24.3 ± 5.1	0.46	0.08	0.09	0.46	0.18	0.18
Age	29.9 ± 7.0	25.6 ± 4.8	29.0 ± 6.8	0.03	0.33	0.06	0.12	0.33	0.12
Height (in.)	65.0 ± 2.5	64.4 ± 3.4	65.8 ± 1.7	0.30	0.11	0.12	0.30	0.24	0.24
Weight (kg)	135.9 ± 19.3	132.2 ± 12.3	160.0 ± 27.1	0.26	0.0007	0.001	0.26	0.002	0.0002

BMR*	1411.1 ± 95.0	1413.5 ± 75.0	1523.8 ± 107.6	0.47	0.08	0.004	0.47	0.11	0.008
Race	21 White 3 none-White 1 NA (87.5% white)	8 White 1 none-White 1 NA (89% white)	22 White 2 none-White 1 NA (921% white)						
Education (<BA)	14 below 11 above (56% BA & below) 44% Masters' and above	7 below 3 above (70% BA & below) 30 % Master's	18 below 7 above (72% BA & below) 28% Master's						
Income (<30L)	14 below 7 above 4 NA (66.7% \$30,000 & below)	4 below 3 above 3 NA (40% \$30,000 & below)	13 below 10 above 2 NA (56.5% \$30,000 & below)						

Table 5. Comparison of 3-day Food Record Dietary intake in IBS-C, IBS-D, and HC Women

Variables	Mean ± SD			HC vs IBS-C	HC vs IBS-D	IBS-C vs. IBS-D	HC vs IBS-C	HC vs IBS-D	IBS-C vs. IBS-D
	HC (n=25)	IBS-C (n=25)	IBS-D (n=25)	p	p	p	FDR p	FDR p	FDR p
Carbohydrates (g)	220.2 ± 86.6	206.4 ± 33.4	209.8 ± 75.3	0.26	0.33	0.43	0.43	0.43	0.43
Fiber (g)	23.7 ± 9.9	23.5 ± 5.4	19.9 ± 7.2	0.48	0.07	0.07	0.48	0.14	0.14
Insoluble Fiber (g)	17.0 ± 6.9	17.5 ± 4.2	13.9 ± 5.4	0.41	0.04	0.03	0.41	0.08	0.08
Resistant grain (g)	4.6 ± 2.7	3.2 ± 1.8	4.1 ± 3.1	0.05	0.27	0.16	0.2	0.27	0.213333
Soluble Fiber (g)	6.6 ± 3.4	5.9 ± 1.8	5.9 ± 2.3	0.22	0.19	0.5	0.29333333	0.2933333	0.5
Gluten	7.2 ± 3.6	3.7 ± 3.4	6.8 ± 5.4	0.01	0.38	0.04	0.04	0.38	0.08
Added Sugar (g)	34.3 ± 25.7	38.1 ± 24.2	49.9 ± 27.86	0.34	0.02	0.12	0.34	0.08	0.16
Lactose	9.0 ± 7.4	8.2 ± 7.4	7.0 ± 9.2	0.39	0.20	0.35	0.39	0.39	0.39
Total Sugar (g)	76.3 ± 40	79.2 ± 40.2	76.9 ± 32.5	0.43	0.07	0.44	0.44	0.28	0.44
Fat (g)	73.1 ± 25.9	74.0 ± 15.4	77.1 ± 22.4	0.45	0.28	0.33	0.45	0.44	0.44
Protein (g)	73.1 ± 19.5	69.0 ± 18.0	69.6 ± 20.5	0.37	0.30	0.47	0.47	0.47	0.47
Alcohol (oz.)	12.73 ± 14.5	4.7 ± 10.8	11.1 ± 15.5	0.05	0.35	0.10	0.2	0.35	0.2

Caloric intake	1849 $\pm$ 594	1746 $\pm$ 212	1850 $\pm$ 486	0.39	0.50	0.20	0.5	0.5	0.5
Water (ml)	1533 $\pm$ 776	1456 $\pm$ 700	1534 $\pm$ 776	0.39	0.34	0.39	0.39	0.39	0.39

Characteristics and Diet Intake

Table 3 and Table 5 summarize the comparisons of dietary patterns of women who self-identified as White vs non-White descent. As a group, women who reported non-White descent reported less income than White women. When relationships among sociodemographic characteristics and diet were examined, several trends emerged. When compared to women with lower education level (Bachelors' and below), women with higher education status (Masters' and above) reported higher mean of daily carbohydrate intake ($p=0.047$), fiber intake ($p=0.08$), insoluble fiber intake ($p=0.07$) and soluble fiber intake ($p=0.12$). As compared to those with lower education status, those with higher education report higher intake of gluten, fiber, resistant fiber, and added sugar. Women with lower education consumed a higher mean fat intake above RDA recommendation (see boxplot); while women with higher education had intake that was consistent with RDA recommendation.

Like education, differences in income level were also associated with diet. Those with higher income ($>\$30,000$ and above) had significantly higher intake of soluble fiber intake (6.6g vs. 5.3g; $p=0.05$). Women with higher income also tended to have higher mean of daily carbohydrate intake (228g vs. 202g; $p=0.12$), total added sugar intake (49g vs. 37g; $p=0.07$) and fat intake (81g vs. 71g; $p=0.08$). With respect to race, self-identified White women tended to report higher mean of daily protein intake (see Figure x). With removal of the one outlier the non-White group reported lower mean daily total protein intake ($p=0.005$). It is important to note that the non-White group is composed of 80% women with low income and 67% with low education status.

Table 6: Comparison of Diet Intake by Race, Education and Income on Dietary Intake in All Women (n=60)

Variable s	White (n=51)	Non-White (n=6)	MS & Above (n=21)	BA & Below (n=39)	30K and above (n=20)	30K and below (n=31)	Race	Edu	Inc om e	Race	Edu	Inco me
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	p	p	p	FDR p	FDR p	FDR p
Carbohydrates (g)	215.99 $\pm$ 79.31	193.52 $\pm$ 35.26	236.20 $\pm$ 73.69	201.36 $\pm$ 73.10	228.53 $\pm$ 80.60	202.68 $\pm$ 71.33	0.134	0.047	0.13	0.14	0.14	0.14

Fiber (g)	22.30 + 8.84	20.16 + 5.07	24.20 + 8.76	20.91 + 7.96	22.44 + 8.96	20.27 + 7.40	0.21	0.08	0.19	0.21	0.21	0.21
Insoluble Fiber(g)	15.78 + 6.42	15.05 + 4.51	17.36 + 6.35	14.91 + 5.81	15.79 + 6.92	14.70 + 5.44	0.37	0.08	0.28	0.37	0.24	0.37
Resistant Grain(g)	4.04 + 2.92	4.07 + 2.20	4.33 + 2.52	4.01 + 2.94	4.12 + 2.78	4.32 + 2.78	0.49	0.33	0.40	0.49	0.49	0.49
Soluble Fiber (g)	6.41 + 2.81	5.09 + 1.46	6.76 + 2.80	5.89 + 2.64	6.62 + 2.52	5.40 + 2.35	0.06	0.13	0.049	0.018	0.13	0.0735
Gluten (g)	6.16 + 4.53	6.88 + 5.06	7.23 + 4.58	6.00 + 4.53	6.32 + 4.94	6.61 + 4.46	0.38	0.17	0.42	0.42	0.42	0.42
Total Added Sugar (g)	42.43 + 27.83	37.94 + 24.27	43.00 + 23.84	40.59 + 28.80	49.04 + 23.30	37.14 + 30.10	0.35	0.37	0.06	0.37	0.37	0.18
Lactose (g)	7.89 8.29	9.25 6.93	7.84 8.44	8.11 8.08	9.25 8.32	7.85 8.90	0.34	0.45	0.29	0.45	0.45	0.45
Fat (g)	75.55 + 21.92	68.20 + 29.63	72.82 + 26.78	76.07 + 20.53	80.70 + 24.12	70.85 + 23.32	0.29	0.32	0.08	0.32	0.32	0.24
Protein (g)	69.87 + 17.54	55.96 + 30.62	66.73 + 16.17	69.09 + 21.12	72.15 + 20.38	65.70 + 19.92	0.16	0.32	0.14	0.24	0.32	0.24

The majority of women in this study were of White descent (n=51, 89.5%). All cohorts (N=60) were analyzed to maximize the effect size (or the variability) among group comparison (White vs. non-White). Although the data is not powered to statistically compare the dietary pattern intake of non-White to White, it is worthwhile to look at the differences in their dietary intake which may help explain the heterogeneity of GI symptoms reported by different subtypes of IBS. Despite the small number of non-White women in this study, looking at the reported baseline characteristics of the participants may help establish a baseline GI symptoms and dietary intake pattern among non-White women with IBS. The boxplots below shows that mean carbohydrate intake by women of white descent is 25.5 g higher than non-White women (p=0.14), 1.3g higher in mean soluble fiber intake than non-White (p=0.07), 7.4g higher in mean total fat (p=0.29), and 14.0g higher mean protein intake (p=0.11) than non-White women. See boxplot on the left.

Table 7. The Influence of Race, Education, and Income on GI Symptoms in All Women

Variables	White (n=51)	Non- White (n=6)	MS & Above (n=21)	BA & Below (n=39)	30K and above (n=20)	30K and below (n=31)	Race	Edu	Inco me	Race	Edu	Inco me
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	p	p	p	FDR p	FDR p	FDR p
Abdo minal Pain	0.75 $\pm$ 0.72	0.67 $\pm$ 0.75	0.55 $\pm$ 0.57	0.87 $\pm$ 0.78	0.78 $\pm$ 0.68	0.78 $\pm$ 0.75	0.41	0.04	0.5	0.5	0.12	0.5
Consti pation	0.42 $\pm$ 0.63	0.34 $\pm$ 0.49	0.31 $\pm$ 0.43	0.51 $\pm$ 0.74	0.43 $\pm$ 0.53	0.36 $\pm$ 0.61	0.36	0.10	0.33	0.36	0.3	0.36
Perce nt Consti pation	27.82 $\pm$ 26.99	28.62 $\pm$ 39.80	25.07 $\pm$ 24.18	29.04 $\pm$ 29.65	31.02 $\pm$ 26.51	20.79 $\pm$ 24.06	0.48	0.29	0.09	0.48	0.435	0.27
Diarrh ea	0.29 $\pm$ 0.33	0.52 $\pm$ 0.67	0.20 $\pm$ 0.31	0.35 $\pm$ 0.40	0.33 $\pm$ 0.39	0.32 $\pm$ 0.39	0.22	0.06	0.46	0.33	0.18	0.46
Perce nt Diarrh ea	37.40 $\pm$ 27.58	44.43 $\pm$ 30.00	33.50 $\pm$ 28.98	40.14 $\pm$ 27.22	38.61 $\pm$ 27.34	41.20 $\pm$ 28.29	0.30	0.20	0.37	0.37	0.37	0.37
Stoma ch Pain	0.56 $\pm$ 0.59	0.42 $\pm$ 0.57	0.38 $\pm$ 0.48	0.65 $\pm$ 0.63	0.61 $\pm$ 0.58	0.55 $\pm$ 0.61	0.30	0.04	0.36	0.36	0.12	0.36
Flatule nce	0.72 $\pm$ 0.63	0.51 $\pm$ 0.69	0.60 $\pm$ 0.61	0.78 $\pm$ 0.66	0.87 $\pm$ 0.68	0.69 $\pm$ 0.65	0.25	0.15	0.18	0.25	0.25	0.25
Bloatin g	0.60 $\pm$ 0.71	0.42 $\pm$ 0.70	0.48 $\pm$ 0.65	0.69 $\pm$ 0.78	0.60 $\pm$ 0.65	0.56 $\pm$ 0.74	0.29	0.14	0.42	0.42	0.42	0.42

The demographic characteristics are shown in Table 1. With the exceptions of body weight and calculated basal metabolic rate (BMR), few differences between IBS and HCs were found. As a group, women with IBS had a higher body weight and higher calculated BMR.

When subgroups of women with IBS were divided based on bowel subtype those in the IBS-D group (28% master's degree [MS], 72% bachelor's degree [BA], 32% below BA are less likely to have BA degree as compared to those in the IBS-C group (30 % MS, 70% have BA & below: 50% BA, 20% below BA). In HC

44% have MS and above, 56% have BA and below (28% are BA). However, there were no differences in income. Combined IBS cohort has 31.4% MS and above, 68.6 % BA and below (43% have BA). The IBS-C cohort is younger compared to HC ($p = 0.03$) and IBS-D ($p = 0.06$). IBS-D and HC groups are similar in age and older than the IBS-C cohort. The IBS-D cohort has a higher mean weight when compared to HC ($p = 0.0007$) and IBS-C ($p = 0.001$) and higher mean calculated BMR compared to IBS-C groups. These group differences remain significant after multiple comparisons testing (see Table 2).

There were no IBS versus HC group differences in dietary protein, fat, and carbohydrates. However, the IBS group reports a higher sugar intake ($p = 0.04$; FDR adj. $p = 0.05$). When subgroup comparisons are made some differences emerge. When compared to IBS-C and HC groups, the IBS-D cohort has higher mean daily total added sugar intake ($p < 0.001$; $p = 0.01$, respectively). These differences remain significant after adjusting for multiple comparisons (see Table 1 & 2). The IBS-D group reported higher intake of insoluble fiber ($p = 0.04$) when compared to HC ($p = 0.04$) and IBS-C groups ($p = 0.03$). The IBS-C group has lower daily mean gluten intake (a type of soluble fiber) compared to HC ($p = 0.01$) and IBS-D cohorts ($p = 0.04$).

Table 3 summarizes the comparisons of dietary patterns of women who are of White vs non-White descent. Women who report non-White descent are economically poorer with the white group reporting higher income. When relationships among sociodemographic characteristics and diet were examined, several trends emerged. When compared to women with lower education level (BA and below), women with higher education status (MS and above) report higher mean of daily carbohydrate intake ($p = 0.047$), fiber intake ($p = 0.08$), insoluble fiber intake ($p = 0.07$) and soluble fiber intake ($p = 0.12$). There is a trend for fat intake to be associated with education. As compared to those who hold a lower education status, those with higher education report higher intake of gluten, fiber, resistant fiber, and added sugar.

Table 8. The Influence of Race, Education, and Income on Dietary Intake in All Women

Variables	White (n=51)	Non-White (n=6)	MS & Above (n=21)	BA & Below (n=39)	30K and above (n=20)	30K and below (n=31)	Race	Edu	Income	Race	Edu	Income
	Mean + SD	Mean + SD	Mean + SD	Mean + SD	Mean + SD	Mean + SD	p	p	p	FDR p	FDR p	FDR p

Carb ohyd rates	215.99 ± 79.31	193.52 ± 35.26	236.20 ± 73.69	201.36 ± 73.10	228.53 ± 80.60	202.68 ± 71.33	0.14	0.047	0.13	0.14	0.14	0.14
Fiber	22.30 ± 8.84	20.16 ± 5.07	24.20 ± 8.76	20.91 ± 7.96	22.44 ± 8.96	20.27 ± 7.40	0.21	0.08	0.19	0.21	0.21	0.21
Insol uble Fiber	15.78 ± 6.42	15.05 ± 4.51	17.36 ± 6.35	14.91 ± 5.81	15.79 ± 6.92	14.70 ± 5.44	0.37	0.08	0.28	0.37	0.24	0.37
Resis tant Grain	4.04 ± 2.92	4.07 ± 2.20	4.33 ± 2.52	4.01 ± 2.94	4.12 ± 2.78	4.32 ± 2.78	0.49	0.33	0.40	0.49	0.49	0.49
Solu ble Fiber	6.41 ± 2.81	5.09 ± 1.46	6.76 ± 2.80	5.89 ± 2.64	6.62 ± 2.52	5.40 ± 2.35	0.00 6	0.13	0.049	0.01 8	0.13	0.073 5
Glute n	6.16 ± 4.53	6.88 ± 5.06	7.23 ± 4.58	6.00 ± 4.53	6.32 ± 4.94	6.61 ± 4.46	0.38	0.17	0.42	0.42	0.42	0.42
Total Adde d Suga r	42.43 ± 27.83	37.94 ± 24.27	43.00 ± 23.84	40.59 ± 28.80	49.04 ± 23.30	37.14 ± 30.10	0.35	0.37	0.06	0.37	0.37	0.18
Lacto se	7.89 8.29	9.25 6.93	7.84 8.44	8.11 8.08	9.25 8.32	7.85 8.90	0.34	0.45	0.29	0.45	0.45	0.45
Fat	75.55 ± 21.92	68.20 ± 29.63	72.82 ± 26.78	76.07 ± 20.53	80.70 ± 24.12	70.85 ± 23.32	0.29	0.32	0.08	0.32	0.32	0.24
Prote in	69.87 ± 17.54	55.96 ± 30.62	66.73 ± 16.17	69.09 ± 21.12	72.15 ± 20.38	65.70 ± 19.92	0.16	0.32	0.14	0.24	0.32	0.24

Difference in income level also influences the dietary pattern of donors in this study. Those with higher income (>\$30,000 and above) seems to have higher mean of daily carbohydrate intake (228 g vs. 202 g; p= 0.12), soluble fiber intake ((6.6 g vs. 5.3g; p = 0.047), total added sugar intake (49 g vs. 37g; p = 0.07) and fat intake (81g vs. 71g; p = 0.08). Differences in dietary pattern are also associated with race. White women tended to report higher mean of daily protein intake (see Figure x). With removal of the one outlier the non-White group reports lower mean daily total protein intake (p = 0.005) and daily protein intake is 44.60 g ± 14.31 for non-White descents. This group is composed of 80% women with low income and 67% lower education status.

Table 9. The Influence of Race, Education, and Income on GI Symptoms in All Women

Variables	White (n=51)	Non- White (n=6)	MS & Above (n=21)	BA & Below (n=39)	30K and above (n=20)	30K and below (n=31)	Race	Edu	Income	Race	Edu	Inco me
	Mean + SD	Mean + SD	Mean + SD	Mean + SD	Mean + SD	Mean + SD	p	p	p	FDR p	FDR p	FDR p
abdomi nal pain	0.75 ± 0.72	0.67 ± 0.75	0.55 ± 0.57	0.87 ± 0.78	0.78 ± 0.68	0.78 ± 0.75	0.41	0.04	0.5	0.5	0.12	0.5
constipa tion	0.42 ± 0.63	0.34 ± 0.49	0.31 ± 0.43	0.51 ± 0.74	0.43 ± 0.53	0.36 ± 0.61	0.36	0.10	0.33	0.36	0.3	0.36
percent constipa tion	27.82 ± 26.99	28.62 ± 39.80	25.07 ± 24.18	29.04 ± 29.65	31.02 ± 26.51	20.79 ± 24.06	0.48	0.29	0.09	0.48	0.435	0.27
diarrhea	0.29 ± 0.33	0.52 ± 0.67	0.20 ± 0.31	0.35 ± 0.40	0.33 ± 0.39	0.32 ± 0.39	0.22	0.06	0.46	0.33	0.18	0.46
percent diarrhea	37.40 ± 27.58	44.43 ± 30.00	33.50 ± 28.98	40.14 ± 27.22	38.61 ± 27.34	41.20 ± 28.29	0.30	0.20	0.37	0.37	0.37	0.37
stomach pain	0.56 ± 0.59	0.42 ± 0.57	0.38 ± 0.48	0.65 ± 0.63	0.61 ± 0.58	0.55 ± 0.61	0.30	0.04	0.36	0.36	0.12	0.36
flatulen ce	0.72 ± 0.63	0.51 ± 0.69	0.60 ± 0.61	0.78 ± 0.66	0.87 ± 0.68	0.69 ± 0.65	0.25	0.15	0.18	0.25	0.25	0.25
bloating	0.60 ± 0.71	0.42 ± 0.70	0.48 ± 0.65	0.69 ± 0.78	0.60 ± 0.65	0.56 ± 0.74	0.29	0.14	0.42	0.42	0.42	0.42

When looking at the 28-day GI symptom diary using the MetaboAnalyst 5.0 (R-based), women with IBS-D have higher self-reported severity in abdominal pain, stomach pain, and higher frequency in percent days diarrhea, urgency, anxiety, and flatulence than women with IBS-C and HCs. On the other hand, women with IBS-C have higher frequency in percent days of constipation, bloating, hard to concentrate, and stress than women with IBS-D and HCs. The Random Forest algorithm showed consistency with the produced clustering dendrogram.

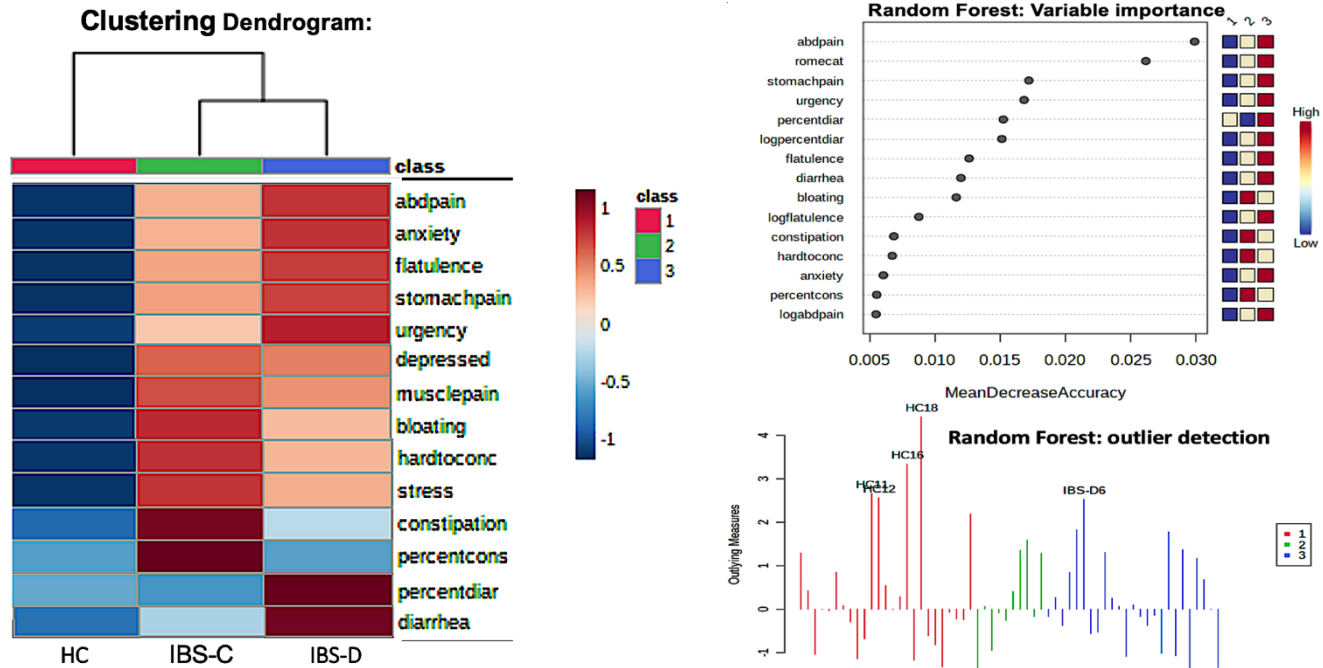


Figure 1. Heatmap of Symptoms in IBS (red, 1 = HC; green, 2=IBS-C; blue, 3=HC)

Individual correlations are performed to test the relationship for a GI symptom and a particular diet. Total fiber consists of insoluble and soluble fiber and a specific example of insoluble fiber such as resistant grain and an example of a soluble fiber such as gluten are included in this analysis. Since Fiber is considered as carbohydrate, other macronutrients (i.e. carbohydrates, fats, protein) are also included in this analysis to capture a better understanding of the outcome variables (GI symptoms).

In the HC group, positive trends are noted between fat intake and percent of days with diarrhea ($r = 0.35$, $p = 0.08$), added sugar intake and percent days with constipation ($r = 0.36$, $p = 0.08$), and carbohydrate intake and flatulence ($r = 0.54$, $p = 0.005$). Flatulence is also correlated with gluten intake ($r = 0.41$, $p = 0.04$), total added sugar ($r = 0.49$, $p = 0.01$), and fat ($r = 0.46$, $p = 0.02$). After adjusting for multiple comparisons, the carbohydrate intake remains significantly associated with flatulence (FDR adj. $p = 0.04$). Note: Added sugar intake maybe significantly associated to flatulence (FDR adj. $p = 0.11$).

Table 10: Univariate Analysis between Diet and GI Symptoms in Healthy Control (HC) Women

Variables	HC (n =25)															
	Abdominal pain		Constip.		Percent Constip.		Diarrhea		Percent diarrhea		Stomach pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Carbohydrates	0.07	0.74	0.01	0.96	0.22	0.28	-0.02	0.93	0.07	0.75	0.10	0.63	0.54	0.05	0.14	0.50
Fiber	0.19	0.36	-0.05	0.82	0.09	0.66	0.05	0.80	0.12	0.58	0.01	0.95	0.19	0.36	0.25	0.23
Insoluble Fiber	0.14	0.50	-0.01	0.97	0.05	0.80	0.01	0.94	0.06	0.78	-0.06	0.79	0.15	0.49	0.28	0.17
Resistant Grain	-0.07	0.75	0.13	0.52	0.00	0.98	0.01	0.98	0.05	0.83	0.06	0.79	0.33	0.11	0.19	0.37
Soluble Fiber	0.25	0.22	-0.12	0.57	0.18	0.39	0.07	0.74	0.18	0.38	0.14	0.52	0.28	0.18	0.18	0.38
Gluten (soluble fiber)	-0.19	0.37	0.22	0.28	-0.02	0.92	0.00	1.00	0.23	0.27	0.01	0.97	0.41	0.04	0.25	0.23
Total Added Sugar	-0.02	0.93	0.33	0.11	0.36	0.08	-0.16	0.44	-0.15	0.47	0.04	0.84	0.49	0.01	0.09	0.68
Fat	-0.06	0.78	-0.05	0.80	0.07	0.75	0.33	0.10	0.35	0.08	0.03	0.90	0.46	0.02	0.06	0.78
Protein	-0.08	0.72	-0.09	0.68	-0.15	0.48	0.34	0.10	0.18	0.40	0.16	0.45	0.19	0.35	0.14	0.51

Table 11: FDR Adjusted p-value for Diet and GI Symptoms in HC Women

DIET	HC FDR Adjusted P-values							
	Abdominal pain	Constip.	Percent days with Constip.	Diarrhea	Percent days with diarrhea	Stomach pain	Flatulence	Bloating
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p
Carbohydrates	0.96	0.96	0.96	0.96	0.96	0.96	0.04	0.96
Fiber	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93
Insoluble Fiber	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Resistant Grain	0.98	0.98	0.98	0.98	0.98	0.98	0.86	0.98
Soluble Fiber	0.62	0.65	0.62	0.74	0.62	0.65	0.62	0.62
Gluten	0.59	0.56	1.00	1.00	0.56	1.00	0.35	0.56

Total Added Sugar	0.93	0.29	0.29	0.75	0.75	0.93	0.11	0.91
Fat	0.90	0.90	0.90	0.27	0.27	0.90	0.17	0.90
Protein	0.72	0.72	0.67	0.67	0.67	0.67	0.67	0.67

Table 12: GI Symptoms under DF Intake (High vs Low) in HC Women

	HC (n=25)										
	Abdominal pain	Const.	Percent days with Const.	Diarrhea	Percent days w/ Diarr.	Stomach pain	Flatulence	Bloating	RACE (white)	Education ($\leq$ BA)	Income (<30 k)
Fiber intake	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD			
High Fiber intake (> 25/day)	0.04 0.05	0.04 0.07	24.84 29.61	0.02 0.03	21.57 16.91	0.02 0.03	0.23 0.31	0.04 0.06	9 W 1 NW 90%	5 below 5 above 50 %	3 below 4 above 3 NA 75 %
Low Fiber intake (< 25/day)	0.05 0.04	0.02 0.04	14.75 12.44	0.03 0.05	17.90 15.88	0.02 0.04	0.12 0.16	0.07 0.07	12 W 2 NW 1 NA 85.6 %	9 below 6 above 60 %	11 below 3 above 1 NA 79 %
p-value	0.30	0.22	0.17	0.27	0.30	0.5	0.16	0.14			
FDR Adj. p-value	0.31	0.31	0.21	0.27	0.47	0.50	0.28	0.23			
Difference	0.00	0.01	10.09	-0.01	3.67	0.00	0.11	-0.03			
Adj. age, BMI, BIFIDO, untransform	0.91	0.66	0.35	0.79	0.43	0.98	0.26	0.33			
Adj. age, BMI, BIFIDO log transformed	0.0084	0.93	0.16	0.76	0.80	0.99	0.27	0.04			

In HC, there is no association found among high fiber intake (> 25 g/day) and any of the GI symptoms.

Table 13: Univariate Analysis between Diet and GI Symptoms in IBS-C Women

DIET	IBS-C (n=10)															
	Abdominal pain		Constip.		Percent Constip.		Diarrhea		Percent diarrhea		Stomach pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Carbohydrates	-0.42	0.23	-0.04	0.91	0.71	0.02	0.02	0.95	0.62	0.06	0.27	0.46	-0.33	0.35	0.31	0.39

Fiber	-0.86	0.00	0.05	0.88	0.09	0.80	-0.64	0.04	-0.35	0.33	-0.82	0.00	-0.67	0.03	-0.21	0.57
Insoluble Fiber	-0.81	0.00	0.00	1.00	0.06	0.87	-0.63	0.05	-0.25	0.48	-0.75	0.01	-0.52	0.13	-0.15	0.67
Resistant Grain	0.69	0.03	-0.12	0.75	-0.19	0.59	0.11	0.77	0.06	0.86	0.55	0.10	0.30	0.40	0.01	0.98
Soluble Fiber	-0.74	0.01	0.14	0.70	0.16	0.65	-0.49	0.15	-0.48	0.16	-0.75	0.01	-0.85	0.00	-0.27	0.45
Gluten	0.50	0.14	0.20	0.58	-0.10	0.79	0.10	0.78	0.31	0.38	0.32	0.38	0.06	0.88	0.14	0.69
Total Added Sugar	-0.19	0.60	0.21	0.57	0.67	0.04	0.04	0.90	-0.59	0.07	0.00	1.00	-0.12	0.73	0.05	0.89
Fat	0.21	0.56	0.58	0.08	0.18	0.63	-0.55	0.10	-0.27	0.46	0.15	0.67	0.01	0.98	0.15	0.69
Protein	0.19	0.59	0.69	0.03	0.05	0.89	-0.36	0.31	0.18	0.62	0.02	0.95	-0.11	0.76	0.23	0.52

Table 14. FDR Adjusted p-value for Diet and GI Symptoms in IBS-C Women

DIET	IBS-C FDR Adjusted p-values							
	Abdominal pain	Constip.	Percent Constip.	Diarrhea	Percent diarrhea	Stomach pain	Flatulence	Bloating
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p
Carbohydrates	0.61	0.95	0.16	0.95	0.22	0.61	0.61	0.61
Fiber	0.01	0.88	0.88	0.09	0.52	0.01	0.09	0.75
Insoluble Fiber	0.04	1.00	1.00	0.14	0.77	0.05	0.25	0.90
Resistant Grain	0.22	0.98	0.98	0.98	0.98	0.40	0.98	0.98
Soluble Fiber	0.04	0.70	0.70	0.25	0.25	0.04	0.02	0.60
Gluten	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Total Added Sugar	1.00	1.00	0.28	1.00	0.28	1.00	1.00	1.00
Fat	0.78	0.39	0.78	0.39	0.78	0.78	0.98	0.78
Protein	0.95	0.22	0.95	0.95	0.95	0.95	0.95	0.95

In women with IBS-C phenotype, abdominal pain is negatively correlated with fiber intake ($r = -0.85$, $p < 0.001$), and also negatively correlated with insoluble fiber $r = -0.85$, $p < 0.001$),, soluble fiber $r = -0.74$, $p =$

0.01),, and resistant grains $r = -0.85$, $p = 0.03$), The associations of total fiber intake and insoluble fiber intake with abdominal pain remain significant after adjusting for multiple comparisons (FDR = 0.1, 0.04 respectively). Percent days constipation is positively associated with carbohydrates ($r = -0.71$, $p = 0.02$) and total added sugar intake $r = -0.67$, $p = 0.04$). Stomach pain is negatively associated with total fiber intake, insoluble fiber intake, and soluble fiber intake and these associations remain significant after multiple comparison adjustments. There was a negative correlation between flatulence and fiber intake and with soluble fiber intake and remained significant after adjusting for multiple comparisons.

In IBS-C, there is a significant association between abdominal pain and high fiber intake ($p < 0.0001$) and with stomach pain ($p = 0.009$) and flatulence ($p = 0.02$) after adjusting for covariates (age, BMI, Bifidobacteria relative abundance).

Table 15. GI Symptoms under DF Intake (High vs Low) in IBS-C Women

IBS-C (n=10)											
Variables	Abdominal Pain	Constipation	Percent days with Const.	Diarrhea	Percent days with Diarr.	Stomach Pain	Flatulence	Bloating	RACE (white)	Education ($\leq$ BA)	Income (<30 k)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD			
High Fiber intake (> 25 /day)	0.54 0.23	1.46 0.95	72.60 25.38	0.08 0.09	15.54 3.07	0.44 0.21	0.70 0.28	1.38 0.34	4 White 100%	3 below 1 above 75 %	2 below 2 NA 100 %
Low Fiber intake (< 25 /day)	1.38 0.46	1.31 0.77	70.15 23.74	0.27 0.37	17.40 12.66	1.04 0.49	1.15 0.60	1.19 0.91	4 White 1 NW 1 NA 80%	4 below 2 above 66.7 %	4 below 1 above 1 NA 80 %
p-value	0.02	0.40	0.44	0.16	0.38	0.04	0.10	0.34			
FDR Adj. p-value	0.10	0.40	0.44	0.20	0.47	0.20	0.28	0.34			
Difference	-0.84	0.16	2.45	-0.19	-1.86	-0.61	-0.45	0.19			
Adj. age, BMI, BIFIDO, untransformed	6.32e-06	0.55	0.69	0.45	0.20	0.007	0.07	0.36			
Adj. age, BMI, BIFIDO log	0.001	0.09	0.72	0.09	0.06	0.06	0.11	0.05			

transformed											
-------------	--	--	--	--	--	--	--	--	--	--	--

Table 16. Univariate Analysis between Diet and GI Symptoms in IBS-D Women

Variables	IBS-D (n=25)															
	Abdominal pain		Constip.		Percent days with Constip.		Diarrhea		Percent days with diarrhea		Stomach pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Carbohydrates	0.29	0.16	-0.07	0.74	0.07	0.74	-0.09	0.68	0.02	0.92	0.35	0.09	0.04	0.84	0.12	0.57
Fiber	0.35	0.09	0.32	0.12	0.40	0.05	-0.04	0.86	0.06	0.79	0.20	0.35	0.23	0.26	0.54	0.01
Insoluble Fiber	0.38	0.06	0.24	0.26	0.40	0.04	-0.03	0.87	0.01	0.95	0.19	0.37	0.19	0.37	0.45	0.02
Resistant Grain	-0.25	0.22	0.04	0.85	0.24	0.24	-0.02	0.93	0.04	0.85	0.11	0.61	0.01	0.96	0.05	0.81
Soluble Fiber	0.15	0.48	0.47	0.02	0.25	0.23	-0.04	0.85	0.16	0.45	0.15	0.48	0.29	0.16	0.62	0.00
Gluten	-0.14	0.51	0.15	0.48	0.21	0.31	-0.08	0.70	0.11	0.60	0.04	0.86	0.16	0.46	0.11	0.59
Total Added Sugar	0.35	0.09	0.07	0.74	0.04	0.84	0.09	0.67	0.02	0.91	0.38	0.06	0.27	0.21	0.17	0.42
Fat	0.24	0.25	0.29	0.16	0.05	0.83	-0.07	0.72	0.19	0.37	0.23	0.26	0.05	0.83	0.14	0.49
Protein	-0.14	0.52	0.04	0.85	0.18	0.39	-0.29	0.16	0.36	0.07	0.07	0.75	0.13	0.53	0.29	0.16

Table 17. FDR Adjusted p-value for Diet and GI Symptoms in IBS-D Women

Variables	IBS-D FDR Adjusted p-values							
	Abdominal pain	Constipation	Percent days with Constip.	Diarrhea	Percent days with diarrhea	Stomach pain	Flatulence	Bloating
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p
Carbohydrates	0.62	0.92	0.92	0.92	0.92	0.62	0.92	0.92
Fiber	0.24	0.25	0.20	0.86	0.86	0.46	0.41	0.04
Insoluble Fiber	0.15	0.50	0.15	0.95	0.95	0.50	0.50	0.15
Resistant Grain	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96

Soluble Fiber	0.55	0.07	0.46	0.85	0.55	0.55	0.43	0.01
Gluten	0.80	0.80	0.80	0.80	0.80	0.86	0.80	0.80
Total Added Sugar	0.36	0.91	0.91	0.91	0.91	0.36	0.55	0.84
Fat	0.69	0.69	0.83	0.83	0.75	0.69	0.83	0.78
Protein	0.70	0.85	0.70	0.43	0.43	0.85	0.70	0.43

Table 18. GI Symptoms under DF Intake (High vs Low) in IBS-D Women

IBS- D (n=25)											
Variables	Ab do mi nal Pai n	Cons tipati on	Perce nt days with Const.	Diarr hea	Perce nt days with Diarr.	Stomach Pain	Flatul ence	Bloati ng	RACE (white)	Educati on ($\leq$ BA)	Income (<30 k)
Mean $\pm$ SD	Me an $\pm$ S D	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ S D	Mean $\pm$ SD	Mean $\pm$ SD			
High Fiber intake (> 25/day)	1.66 0.71	0.79 0.65	30.69 19.49	0.43 0.31	65.14 13.35	1.09 0.72	1.31 0.32	1.56 0.73	4 W 1 NA 100%	2 <BA 3 >BA 40 %	4 less 1 NA 80 %
Low Fiber intake (< 25/day)	1.28 0.41	0.40 0.44	16.23 15.98	0.67 0.34	64.63 18.80	0.96 0.46	1.14 0.59	0.76 0.65	18 W 2 NW 90%	16 <BA 4 >BA 76.2 %	9 less 10 moe 47.4 %
p-value	0.16	0.14	0.10	0.10	0.47	0.36	0.22	0.04			
FDR Adj. p-value	0.27	0.31	0.17	0.17	0.47	0.45	0.28	0.10			
Difference	0.39	0.39	14.46	-0.24	0.51	0.13	0.17	0.80			
Adj. age, BMI, BIFIDO, untransform	0.45	0.13	0.15	0.19	0.79	0.96	0.95	0.04			
Adj. age, BMI, BIFIDO, log transformed	0.39	1.0	0.40	0.54	0.91	0.79	0.35	0.008			

In women with IBS-D phenotype, significant association is found between total fiber intake and percent days constipation ($r = 0.4$, $p = 0.05$) and bloating ($r = 0.54$, $p = 0.01$); insoluble fiber and percent days constipation ($r = 0.4$, $p = 0.04$) and with bloating ($r = 0.45$, $p = 0.02$); soluble fiber and constipation ($r = 0.47$, p

= 0.02) and with bloating ($r = 0.62$, $p = 0.001$). After adjusting for multiple comparison, the associations of bloating and soluble fiber intake and with soluble fiber remain significant. Looking at the comparisons among these group of women and high fiber intake, there is a significant association between high fiber intake (>25 g/day) and bloating ($p = 0.04$; FDR adj. $p_v = 0.07$). There is a weak association between high fiber intake and percent constipation ($p = 0.09$) and with bloating ($p = 0.07$) after adjusting for covariates (age, BMI, Bifidobacteria relative abundance).

Table 19. Univariate Analysis between Diet and GI Symptoms in IBS Women

Variable s	IBS (n=35)															
	Abdominal pain		Constipation		Percent days with Constipation		Diarrhea		Percent days with diarrhea		Stomach pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Carbohydrates	0.17	0.34	-0.06	0.74	0.09	0.62	-0.05	0.78	-0.02	0.90	0.25	0.14	-0.07	0.67	0.05	0.77
Fiber	-0.02	0.90	0.32	0.06	0.37	0.03	0.26	0.13	0.20	0.25	0.06	0.72	-0.01	0.97	0.41	0.01
Insoluble Fiber	-0.01	0.96	0.30	0.08	0.42	0.01	0.29	0.10	0.27	0.12	0.07	0.68	0.36	0.03	-0.08	0.66
Resistant Grain	0.07	0.70	-0.18	0.29	0.23	0.18	0.03	0.86	0.06	0.75	0.15	0.40	-0.05	0.79	0.12	0.50
Soluble Fiber	-0.08	0.66	0.29	0.10	0.14	0.42	0.12	0.50	0.03	0.85	0.05	0.76	0.03	0.85	0.41	0.02
Gluten	-0.01	0.97	-0.10	0.56	0.34	0.05	0.12	0.49	0.23	0.18	0.02	0.91	0.05	0.77	0.00	0.99
Total Added Sugar	0.24	0.16	-0.10	0.58	0.06	0.75	0.17	0.33	0.10	0.56	0.31	0.07	-0.18	0.29	0.15	0.38
Fat	0.24	0.17	0.25	0.15	0.01	0.97	0.10	0.56	0.06	0.75	0.22	0.20	0.05	0.77	0.12	0.48
Protein	-0.04	0.82	0.16	0.37	0.07	0.68	0.25	0.14	0.15	0.40	0.05	0.76	0.08	0.67	0.27	0.12

Table 20. FDR Adjusted p-value for Diet and GI Symptoms in IBS Women

Variables	IBS FDR Adjusted P-values							
	Abdominal pain	Constip.	Percent days with Constip.	Diarrhea	Percent days with diarrhea	Stomach pain	Flatulence	Bloating
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p

Carbohydrates	0.89	0.89	0.89	0.89	0.90	0.89	0.89	0.89
Fiber	0.97	0.17	0.11	0.27	0.40	0.95	0.97	0.11
Insoluble Fiber	0.96	0.19	0.10	0.19	0.20	0.78	0.13	0.78
Resistant Grain	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Soluble Fiber	0.85	0.38	0.85	0.85	0.85	0.85	0.85	0.12
Gluten	0.99	0.99	0.36	0.99	0.72	0.99	0.99	0.99
Total Added Sugar	0.60	0.66	0.75	0.60	0.66	0.57	0.60	0.60
Fat	0.52	0.52	0.97	0.88	0.88	0.52	0.88	0.88
Protein	0.82	0.79	0.82	0.57	0.79	0.82	0.82	0.57

Table 21. GI Symptoms under DF Intake (High vs Low) in IBS Women

IBS (n=35)											
Variables	Abdominal Pain	Constipation	Percent days w/ Cons.	Diarrhea	Percent days w/ Diarr.	Stomach Pain	Flatulence	Bloating	RACE (white)	Education ($\leq$ BA)	Income (<30 k)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD			
High Fiber intake (> 25/day)	1.16 0.79	1.09 0.83	49.32 30.32	0.27 0.29	43.09 27.86	0.80 0.63	1.04 0.43	1.48 0.57	8 W 1 NA 100%	5 <BA 4 >BA 55.6 %	4 below 2 above 3 NA 66.7 %
Low Fiber intake (< 25/day)	1.30 0.42	0.61 0.65	28.67 29.04	0.58 0.38	53.73 26.70	0.98 0.46	1.14 0.58	0.86 0.72	22 W 3 NW 1 NA 88 %	19 <BA 7 >BA 73.1 %	13 below 11 above 2 NA 54.2 %
p-value	0.31	0.08	0.06	0.02	0.17	0.23	0.30	0.02			
FDR Adj. p-value	0.31	0.31	0.17	0.05	0.43	0.38	0.30	0.10			
Difference	- 0.14	0.48	20.64	-0.30	-10.64	-0.18	-0.11	0.62			
Adj. IBS, age, BMI, BIFIDO, all untransform	0.45	0.13	0.41	0.13	0.67	0.24	0.51	0.04			
Adj. IBS, age, BMI, BIFIDO, all log transformed	0.29	0.95	0.28	0.34	0.62	0.28	0.92	0.13			

In the IBS group there is no significant relationship between diet and abdominal pain. In the IBS group, total fiber intake ($r = 0.37$, $p = 0.03$) and insoluble fiber ($r = 0.42$, $p = 0.01$) are positively correlated with percent days with constipation. After adjusting for multiple testing, fiber is positively associated with percent days with constipation (FDR adj. $p = 0.11$). Insoluble fiber is positively correlated with percent days with constipation (FDR adj. $p = 0.10$), flatulence (FDR adj. $p = 0.13$). Soluble fiber is positively associated with bloating (FDR adj. $p = 0.12$).

Table 22. Univariate Analysis between Diet and GI Symptoms in All Women

Variables	All Women (N=60)															
	Abdominal pain		Constip.		Percent days with Constip.		Diarrhea		Percent days with diarrhea		Stomach pain		Flatulence		Bloating	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Carbohydrates	-0.00	1.00	-0.07	0.58	0.11	0.41	-0.07	0.57	-0.04	0.79	0.05	0.68	0.00	0.97	0.09	0.82
Fiber	-0.14	0.28	0.08	0.57	0.18	0.16	-0.23	0.08	0.15	0.24	0.15	0.25	-0.09	0.48	0.04	0.58
Insoluble Fiber	-0.14	0.28	0.07	0.58	0.20	0.12	-0.26	0.05	0.21	0.10	0.16	0.21	-0.11	0.39	0.03	0.67
Resistant Grain	-0.08	0.53	-0.19	0.15	-0.18	0.16	-0.07	0.62	0.03	0.80	0.03	0.83	-0.07	0.58	0.05	0.26
Soluble Fiber	-0.13	0.32	0.06	0.62	0.10	0.44	-0.13	0.30	0.01	0.95	0.12	0.36	-0.04	0.78	0.07	0.52
Gluten	-0.12	0.35	-0.14	0.28	-0.28	0.03	-0.01	0.94	0.11	0.41	0.10	0.47	-0.03	0.83	0.03	0.53
Total Added Sugar	0.29	0.02	0.07	0.60	0.13	0.32	-0.24	0.07	0.15	0.25	-0.33	0.01	0.15	0.27	0.02	0.64
Fat	0.14	0.27	0.18	0.18	0.04	0.77	0.01	0.96	0.04	0.22	0.15	0.25	0.15	0.26	0.06	0.40
Protein	0.04	0.75	0.14	0.30	0.02	0.88	-0.09	0.50	0.00	0.99	0.02	0.85	0.12	0.37	0.03	0.14

Table 23. FDR Adjusted p-value for Diet and GI Symptoms in All Women

Variables	All Women FDR Adjusted P-values							
	Abdominal pain	Constip.	Percent days with Constip.	Diarrhea	Percent days with diarrhea	Stomach pain	Flatulence	Bloating
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p
Carbohydrates	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fiber	0.45	0.58	0.45	0.45	0.45	0.45	0.58	0.58
Insoluble Fiber	0.44	0.66	0.32	0.32	0.32	0.42	0.52	0.67
Resistant Grain	0.82	0.63	0.63	0.82	0.83	0.83	0.82	0.69
Soluble Fiber	0.83	0.83	0.83	0.83	0.95	0.83	0.89	0.83
Gluten	0.71	0.71	0.23	0.94	0.71	0.71	0.94	0.71
Total Added Sugar	0.10	0.64	0.42	0.18	0.42	0.09	0.42	0.64
Fat	0.54	0.54	0.88	0.96	0.56	0.54	0.54	0.56
Protein	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Table 24. GI Symptoms under DF Intake (High vs Low) in All Women

All Women (N=60)											
Variables	Abdominal Pain	Constipation	Percent days w/ Cons.	Diarrhea	Percent days w/ Diar.	Stomach Pain	Flatulence	Bloating	RACE (white)	Education (≤ BA)	Income (<30 k)
Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD			
High Fiber intake (> 25/day)	0.57 0.78	0.54 0.77	36.43 31.69	0.14 0.24	31.77 24.69	0.39 0.58	0.61 0.55	0.72 0.83	17 W 1 NW 1 NA 94.4%	10<BA 9>BA 52.6 %	7 less 6 more 6 NA 53.8 %
Low Fiber intake (< 25/day)	0.84 0.69	0.40 0.59	23.58 25.05	0.38 0.40	40.62 28.97	0.63 0.59	0.77 0.68	0.57 0.69	34 W 5 NW 2 NA 87.2 %	29<BA 12>BA 70.1 %	24 less 14 more 3 NA 63.2 %
p-value	0.11	0.25	0.07	0.005	0.12	0.08	0.17	0.25			
FDR Adj. p-value	0.27	0.31	0.17	0.03	0.43	0.20	0.28	0.31			
Difference	- 0.27	0.14	12.85	-0.24	-8.86	-0.24	-0.16	0.15			

P after Adjusting for IBS, age, BMI, BIFIDO, untransform	0.39	0.56	0.06	0.09	0.63	0.23	0.82	0.10			
P after Adjusting for IBS, age, BMI, BIFIDO, log transform	0.38	0.39	0.04	0.27	0.58	0.37	0.44	0.40			

Looking at data in ALL cohorts, total fiber intake and insoluble fiber are negatively correlated ($r = -0.23$, $p = 0.08$; $r = -0.26$, $p = 0.05$, respectively) with days with diarrhea. Total gluten intake is negatively correlated with percent days with constipation ($r = -0.28$, $p = 0.03$). Total added sugar intake is positively correlated with abdominal pain ($r = 0.29$, $p = 0.02$) and stomach pain ($r = 0.33$, $p = 0.01$) and trending with days with diarrhea ($r = 0.24$, $p = 0.07$). After adjustment for multiple testing, total added sugar only trends are found (stomach pain, FDR adj. $p = 0.09$; diarrhea FDR adj. $p = 0.18$).

When comparing women with high fiber intake ($>25g$) with low fiber ($<25g$) intake, there is a significant difference in reported mean diarrhea frequency between these two groups ($p = 0.005$, FDR adj. $p = 0.03$). Also, there is also a weak difference in reported mean stomach pain among these two groups ($p = 0.08$) and mean percent constipation ($p = 0.07$).

Looking at the association between individual GI symptoms and high fiber intake (25g/day) using the data from all groups should be able to capture the best relationships between the outcome (GI Symptom) and the predictor (high fiber intake). Using linear regression on R software, percent constipation is positively correlated with high fiber intake ($p = 0.07$) after adjusting for other covariates (BMI, age, IBS status, Bifidobacteria). Having the IBS status ($p = 0.007$) was driving the significant association of high fiber intake and GI symptoms. Without adjusting for IBS status, the strength of association between high fiber diet and percent constipation ($p = 0.14$) after adjusting for similar covariates.

AIM2c DISCUSSION

The global prevalence of IBS has been increasing and currently is now at 15% with a higher prevalence of 20% in the US (Zhang et al., 2022). For many individuals with IBS, high fiber intake may serve as a trigger for symptoms, however, a low fiber diet has been linked to symptoms such as constipation and abdominal pain. Currently, strong evidence exists for the effectiveness of dietary fiber (DF) in reducing IBS symptoms such as constipation (Ford et al., 2008). However, the relationship between SCFA and constipation remains unclear; whether high amount of SCFAs mediates the effect of high DF intake in relieving IBS symptoms remains to be investigated. The link between diet and IBS symptoms has focused on the impact of dietary constituents and gut microbiome (El-Salhy et al., 2017).

There are numerous clinical trial studies and systematic reviews that provide strong evidence for the efficacy of high fiber intake (functional fibers or natural fibers) in improving bowel patterns, reducing abdominal pain, and influencing transit times (McRae 2020; Nagarajan et al., 2015; Bijkerk et al., 2004, Suares & Ford 2011, Manning et al., 1997; Romano et al., 2013; Polymeros et al., 2014; Kamiya et al., 2014; Prior, A., & Whorwell, 1987; Cann et al., 1984; Arffmann et al., 1985; Rees et al., 2005) in people with constipation including IBS-C patients. However, there are a limited number of studies that are in support of fiber (mainly soluble such as psyllium, isphagula husk, partially hydrolyzed guar gum or PHGG, soy fiber) supplementation in relieving diarrhea events and other IBS symptoms but with no significant difference when compared to placebo (Bijkerk et al., 2004; Giannini et al., 2006; McRorie et al., 2020). It is important to note that the most recent review by McRae (2020) that included 18 meta-analyses of fiber supplementation in patients with IBS-C also found heterogeneity and publication bias in published studies. Thus, the positive role of fiber supplementation in IBS may still be questionable and need further exploration.

People with IBS sometimes avoid certain foods (i.e., spicy foods, high gluten-containing bread, foods high in lactose) that they believe exacerbate their GI system including the “extra organ”, the gut microbiome. People with IBS often seek out every venue to relieve their symptoms and dietary changes is a common one. It could be that they are very familiar with FODMAP diets and will try to avoid them. High FODMAP diet includes: artichokes, asparagus, peas, broccoli, cabbage, onions; cow, sheep, goat milk; ricotta cheese, cottage cheese,

apples, pears, mango, peaches, honey, high fructose corn syrup, onions, cherry, lychee, persimmons, tomatoes, cucumbers, red grapes, black grapes, wheat & rye, chickpeas, cherries, plums, prunes, avocado, mushrooms, cauliflower, watermelon, peaches, couscous, pasta, legumes, beetroots, garlic, and chickpeas.

Although there is an extensive body of evidence regarding the efficacy of low FODMAP diet in reducing the global symptoms of mild to moderate IBS (Eswaran et al. ,2017 RCT; systematic review by So. et al., 2022; Altomare et al., 2021), majority of investigators have also recommended that foods high in FODMAP be re-introduced back into the individual's daily intake incrementally. This is because of the concern that long term low FODMAP diet could affect the gut microbiome (i.e., reduction of SCFA-producing bacteria, Bifidogenic bacteria). Examples of low FODMAP diet includes the following: carrot, celery, eggplant, green beans, lettuce, tomato, gluten free bread, cereals, spelt bread, muesli; lactose-free drinks, rice milk, brie cheese, lactose free yogurt, sorbet, gelato; banana, blueberry, grapefruit, honeydew, orange, strawberry, maple syrup and polyol-containing: lemon, lime, orange, raspberry, sugar, glucose, sucralose, saccharin, aspartame. By virtue of their physio-chemical properties, the high FODMAP diet can result in abdominal pain, bloating, and excessive flatulence. In addition, excessive intake of high FODMAP diet can increase the amount of “unavailable carbohydrates” which translates to increases in colonic bacterial fermentation.

A percentage of IBS patients are also diagnosed with small intestinal bacterial overgrowth (SIBO). Increased microbial activities in the upper GI tract have also been linked to altered gas production (post-lactulose ingestion) and the decreased rate of hydrogen disposal may contribute to abdominal pain (Pimentel et al., 2003). Despite the negative impact that antibiotics has on the gut microbiome, the current patient care in treating SIBO is the administration of an oral antibiotic, (i.e., amoxicillin, rifaximin); however, its efficacy is questionable because patients are advised to repeat the regimen when there is worsening of IBS symptoms (Acosta et al., 2016). An RCT done by Acosta et al. (2016) found that rifaximin reduced butyrate level in IBS (Rome III, n=12 IBS Rifaximin arm vs. 12 IBS Placebo arm) and marginally increased bile acid secretion per gram of stool post rifaximin treatment in when compared to baseline. Acosta reported that the microbiome of the IBS irrespective of treatment was unstable over time during the study.

Experts in the field of IBS do not recommend high intake of insoluble fiber for IBS-D phenotype because it may worsen their symptoms; however, such assessments may have been inferred based on the results of the clinical trials done only with IBS-C phenotype. The rationale would be that insoluble fiber contains a high amount of lignin and only a portion is digested by the gut microbiome, so its incomplete fermentation stimulates GI motility and speeds up transit time. However, giving nutritional advice without accounting for the microbiota profile of a person with IBS may prove to be detrimental, i.e., increase symptoms.

Whether the types and amount of fiber play a role in IBS symptom generation requires further exploration. This dissertation focused on evaluating the relationships of GI symptoms and DF and leveraging the 28-day Symptom Diary collected by the MAP study. Investigating the relationships of DF and GI symptoms may provide insights regarding decisions about food avoidance and antibiotic therapies. The study hypotheses were 1) patients with higher intake of insoluble and slowly fermentable DF intake would have lower abdominal pain and 2) a high and rapidly fermentable and soluble DF intake would be positively correlated with IBS abdominal pain severity.

The results revealed that different types of DF were associated with stool pattern. The result supported the hypothesis that higher intake of insoluble fiber would significantly lower abdominal pain, but it was only seen in IBS-C and not in IBS-D nor in healthy controls. It was unexpected that insoluble fiber was correlated with higher abdominal pain in IBS-D although non-significant ($p=0.1$) but significantly correlated with higher bloating (see boxplot under supplemental figures). It is worth noting that insoluble fiber was also significantly correlated with lower mean scores of other symptoms (i.e., stomach pain, stress, depression and depression correlated symptoms (i.e., anxiety, muscle pain) in IBS-C; this result reinforces the current recommendation for increase insoluble intake for IBS-C to help alleviate their symptoms. However, the second hypothesis was not expected. Although non-significant, a negative relationship between soluble fiber and abdominal pain in IBS-D was unexpected (hypothesis 2), but soluble pain was significantly and positively associated with percent days with diarrhea, bloating, flatulence, and diarrhea (see boxplot under supplemental figures). This negative association of soluble fiber and abdominal pain in IBS-D was not significant and could not confirmed the

literature that soluble fiber helps to improve stool form in IBS-D phenotype (Bijkerk et al., 2004; Altomare et al., 2021).

Although non-significant, the findings of positive correlation of insoluble fiber and percent days with constipation in this present study contradicts the current recommendation that insoluble fiber should be avoided by persons with IBS-D. The negative relationship of insoluble fiber and diarrhea from the analysis of 60 women should alert experts in the field to reassess their reviews on IBS-D dietary treatment. Although insoluble DF attracts water in the colon, they are largely not fermented by the gut bacteria when compared to more soluble forms of carbohydrates (i.e., oligosaccharides, inulin, beta-glucans, pectin, psyllium), and thus there is less production of volatile SCFA which translate to less bloating and less flatulence.

In looking at the contributions of bacteria among the IBS-D women, there was a reduced relative abundance of methanogens and so it is understandable that they complained of bloating due to excessive gas production. A person with IBS-D needs to be informed that there are some opportunity costs involved during the production of higher concentrations of SCFA and that includes gas formation. The role of the nurse scientist is to help allay their fears of having flatulence and help them understand that it is a normal process.

Although Low-FODMAP diet has been shown to alleviate GI symptoms of IBS (i.e., flatulence, bloating, gas production), it has not been as effective in reducing abdominal pain (Staudacher et.al., 2017; Halmos et.al., 2014) and it negatively impacted the gut microbiota consortia—reduced growth of beneficial bacteria such as *Bifidobacteria*. Although readily accessible, over-the-counter medication such as loperamide can be taken to control diarrhea, it is not as effective in reducing abdominal pain (Cangemi et al. 2019). High DF diet promotes the growth of SCFA-producing bacteria including the serotonin-producing bacteria which may help regulate GI motility.

A more recent RCT done by So et.al., 2022 in Australia (7 IBS-C; 8 IBS-D, 8 IBS-M; 3 unknown), Rome IV, 3-arms, cross-over, double-blind design, 2-weeks intervention, 3-weeks washout period, 14-day symptom using VAS, and Fatigue Impact Scale; daily food diary) provided evidence of the positive effects of concomitant intake of higher fiber diet (above the current ADA recommendation of 25g/day) on alleviating the physical symptoms of IBS under the context of low FODMAP. So et al (2022) showed that intake of fibers

(22g/day, 33g/day, 45g/day) under low-FODMAP diet resulted in normalized stool patterns, normalized slow colonic transit time, and increased fecal bulking in those who complete the study (n=20) when compared to baseline; however, group comparison did not reach significance difference. However, their DF intervention failed to improve psychological aspects of IBS symptoms (i.e., anxiety, depression stress) using the IBS Quality of Life, Depression Anxiety Stress Scales (short form). Their result was opposite to what was found in this current observation study when analysis was done regarding the effect of high fiber intake on the psychological aspects of IBS.

How does fiber alleviate symptoms in IBS can be answered by looking at the mediators that interact with fibers that escape digestion in the upper GI tract, namely the bacteria that ferments these substrates upon entry into the colon. Interestingly, a post-hoc analysis of the data found a moderately positive correlation of abdominal pain and the bacteria genera, *Ruminococcus2* ($p < 0.001$, $r = 0.46$); this strong positive relationship can be visualized by a biplot creation using R software. Also, this study found a significantly lower abundance of beneficial, saccharolytic bacteria (i.e., *Bifidobacteria*, *Lactobacillus*, and *Prevotella*), but significantly higher abundance of mucosa-associated bacteria (i.e., *Bacteroides* and *Parabacteroides*) which are also known to alter bile acid metabolisms. Furthermore, IBS women had higher abundance of several *Clostridia* of which some are well-known to increase gut permeability and low-grade inflammation by producing toxins (Lee et al., 2015). The lower abundance of *Faecalibacteria*, known to maintain gut barrier integrity could also add to symptom exacerbation in IBS (Zhong et al., 2019; Riviere et al., 2016). In summary, all these bacteria play a role in SCFA, bile acid, and tryptophan metabolism and the alteration of these metabolites may prove to be the culprit of IBS symptoms development and exacerbations.

Strength of the current study. Results from this study are different than others (So et al., 2022) in that fiber intake were measured using dietary recalls and/or checklist marks, while this current study used a 3-day food diary record and components of the food were analyzed by MAP collaborators at FHCRC. Other studies did not use daily diaries to examine symptoms like the current study. In addition, earlier investigators did not specifically examine fiber intake with symptoms. Having IBS drives the relationship positive relationship b/w percent constipation and high fiber intake

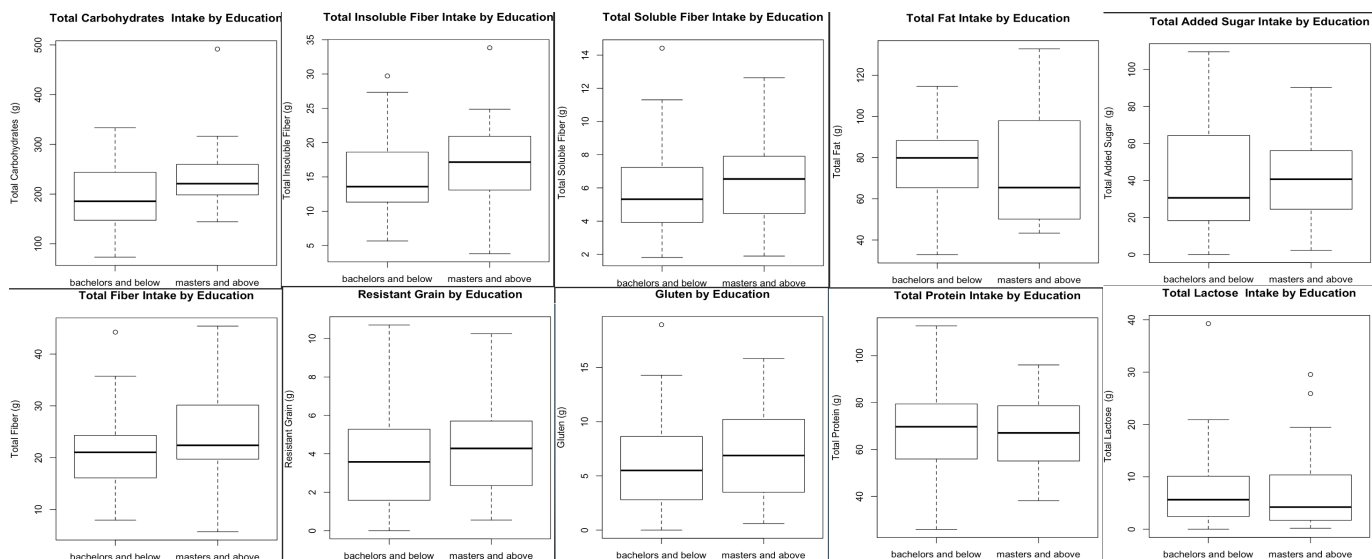


Figure 1: All Cohorts Boxplots: The Influence of Education on Dietary Intake

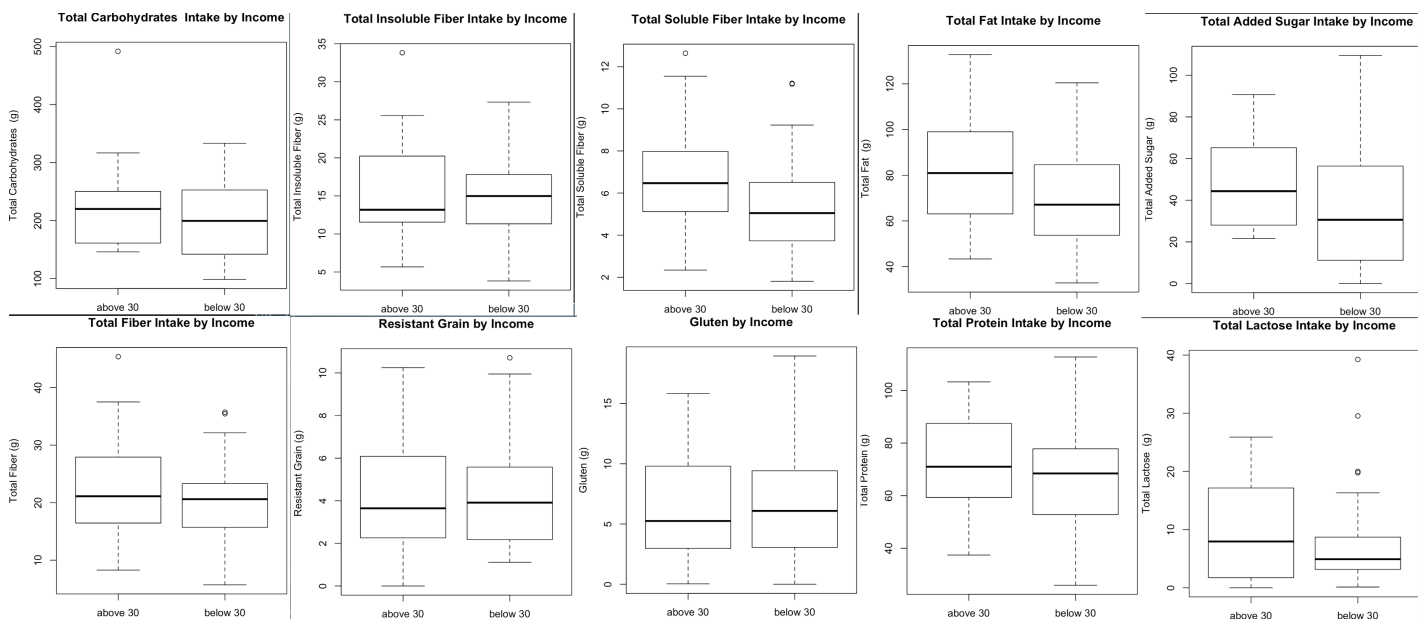


Figure 2: All Cohorts Boxplots: The Influence of Income on Dietary Intake

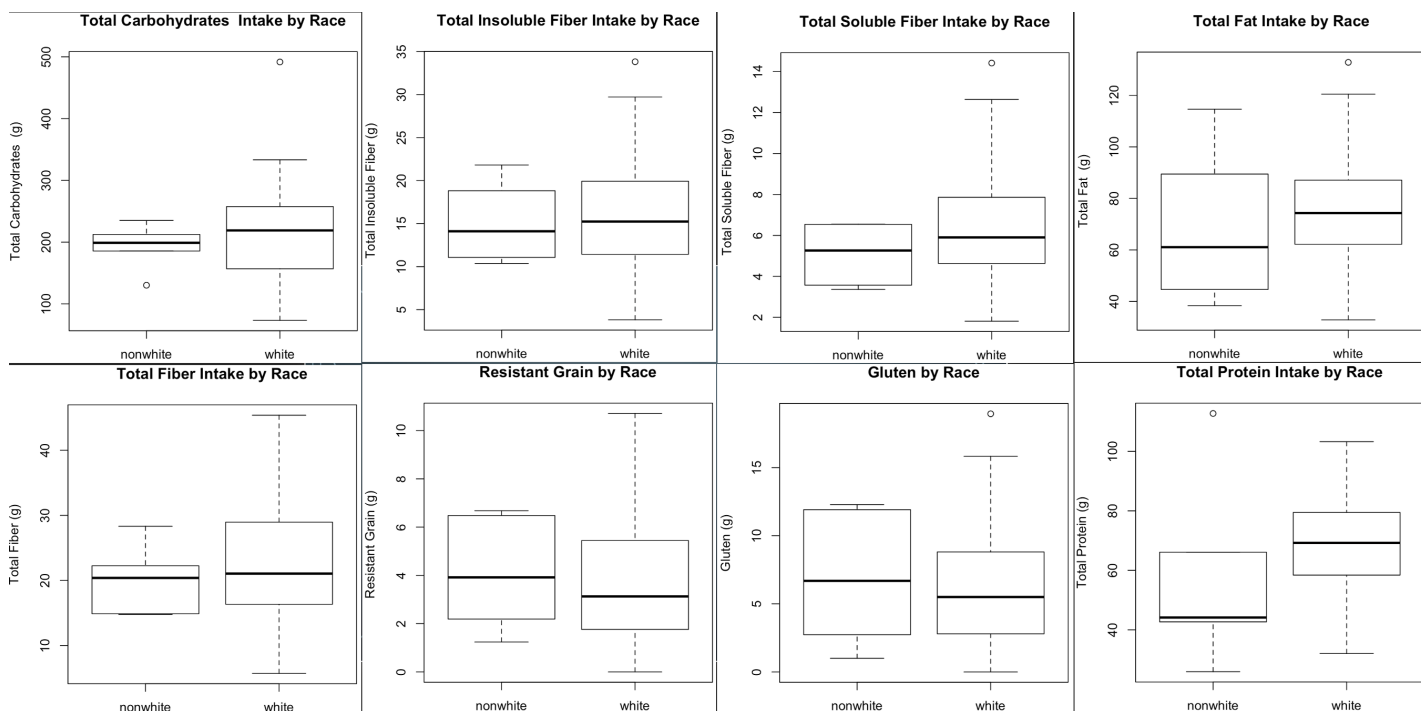


Figure 3. The dietary intake preferences by race in all women.

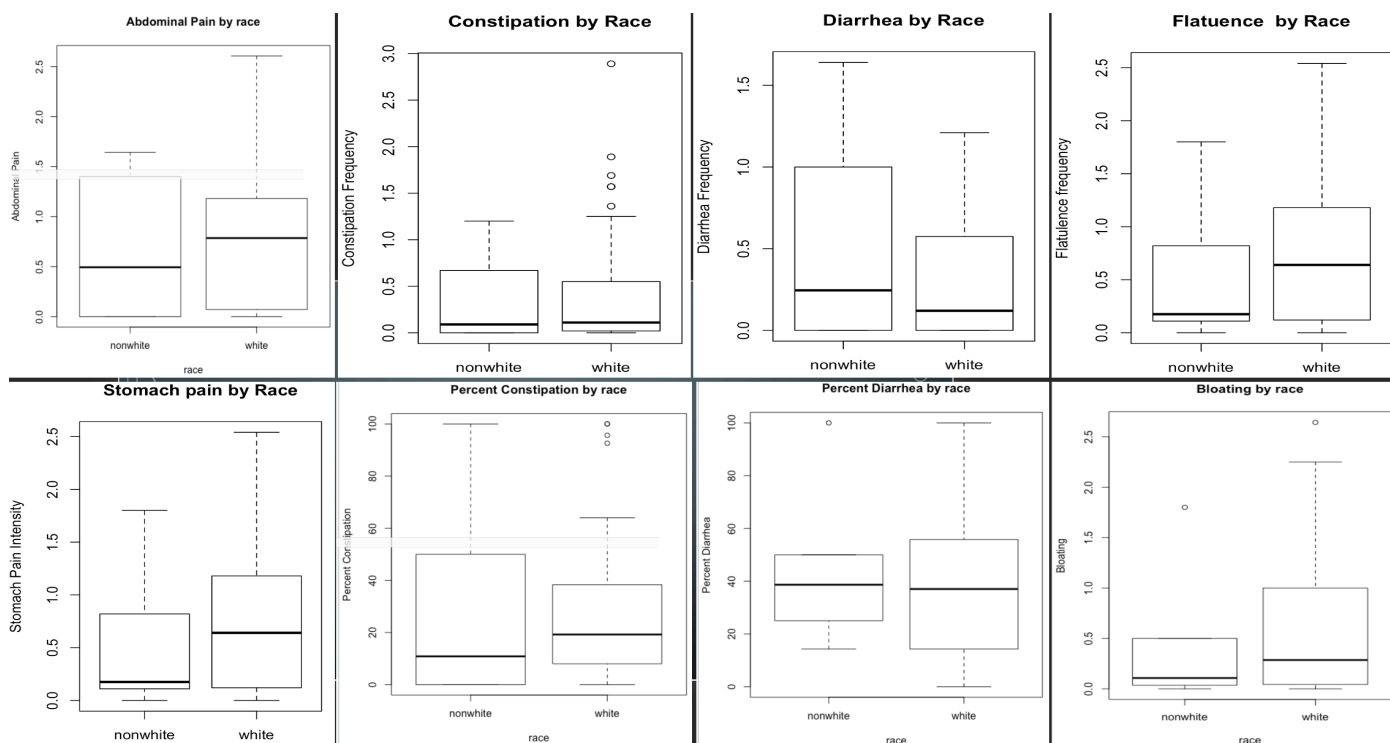


Figure 4. All Cohorts: The Influence of Race on GI Symptoms from All Cohorts.

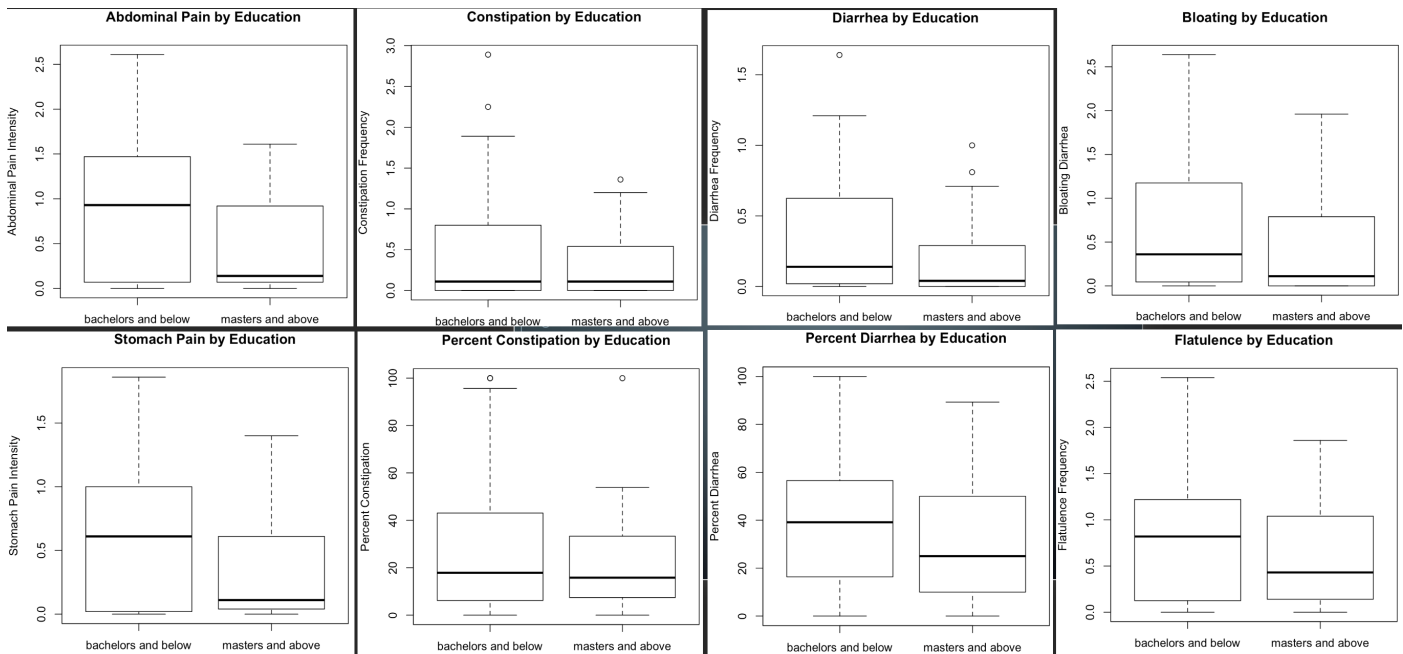


Figure 5. The Influence of Education level on GI Symptoms from All Cohorts

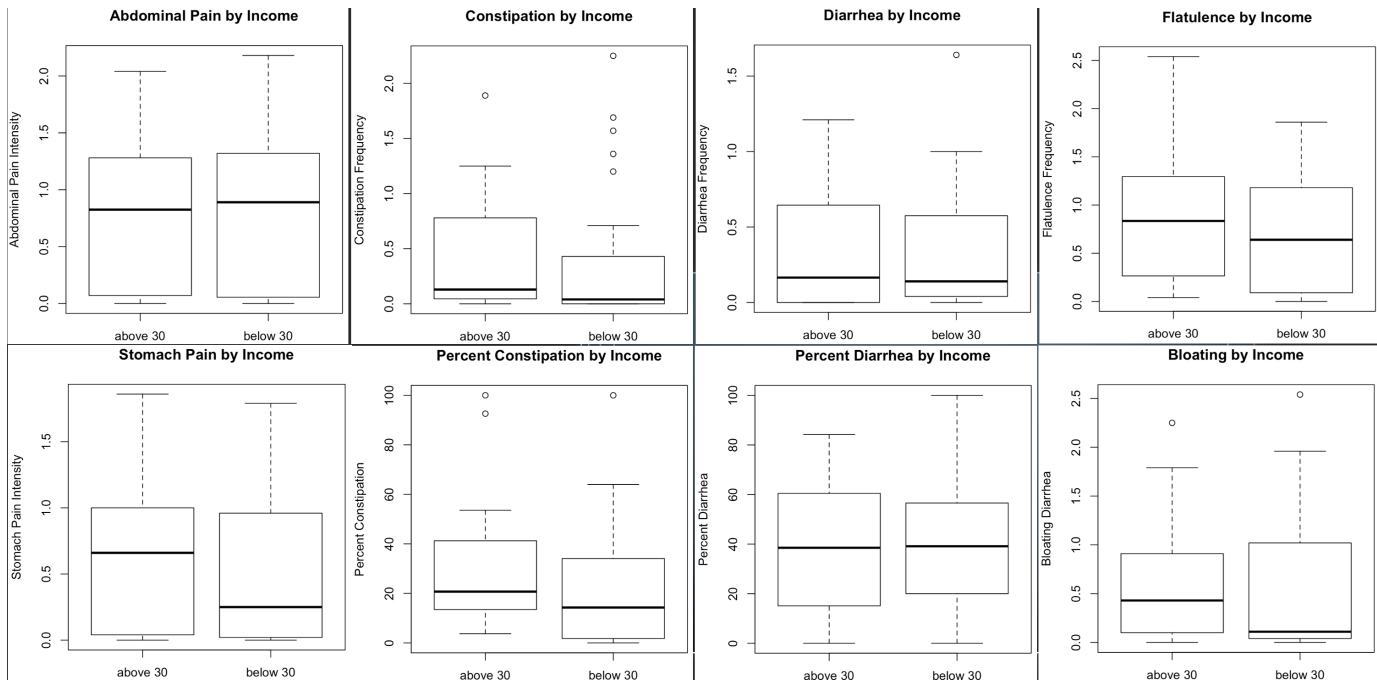


Figure 6. The Influence of Income level on GI Symptoms from All Cohorts

IBS-C

IBS-D

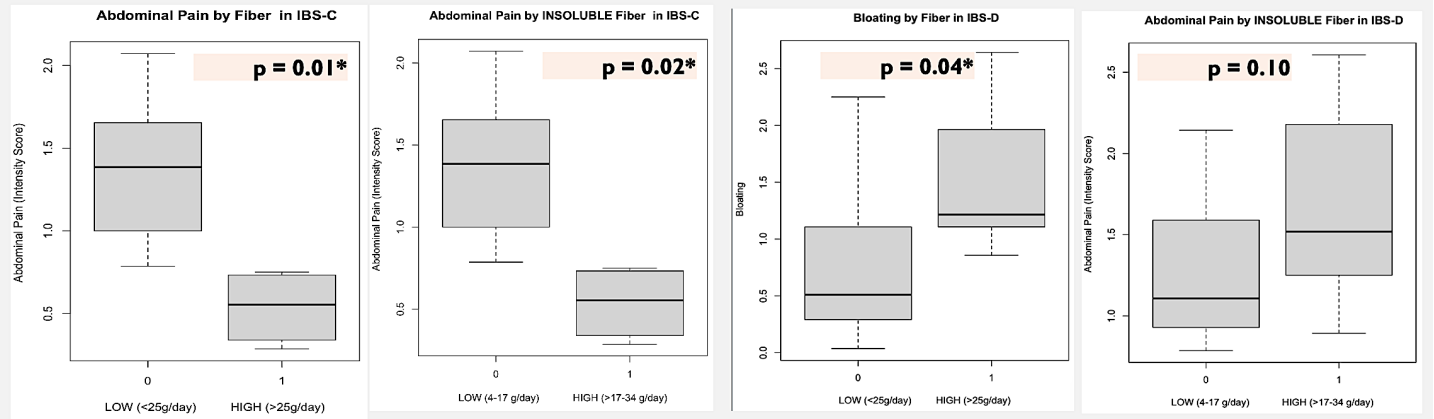


Figure 7. The relationship of DF and GI symptoms.

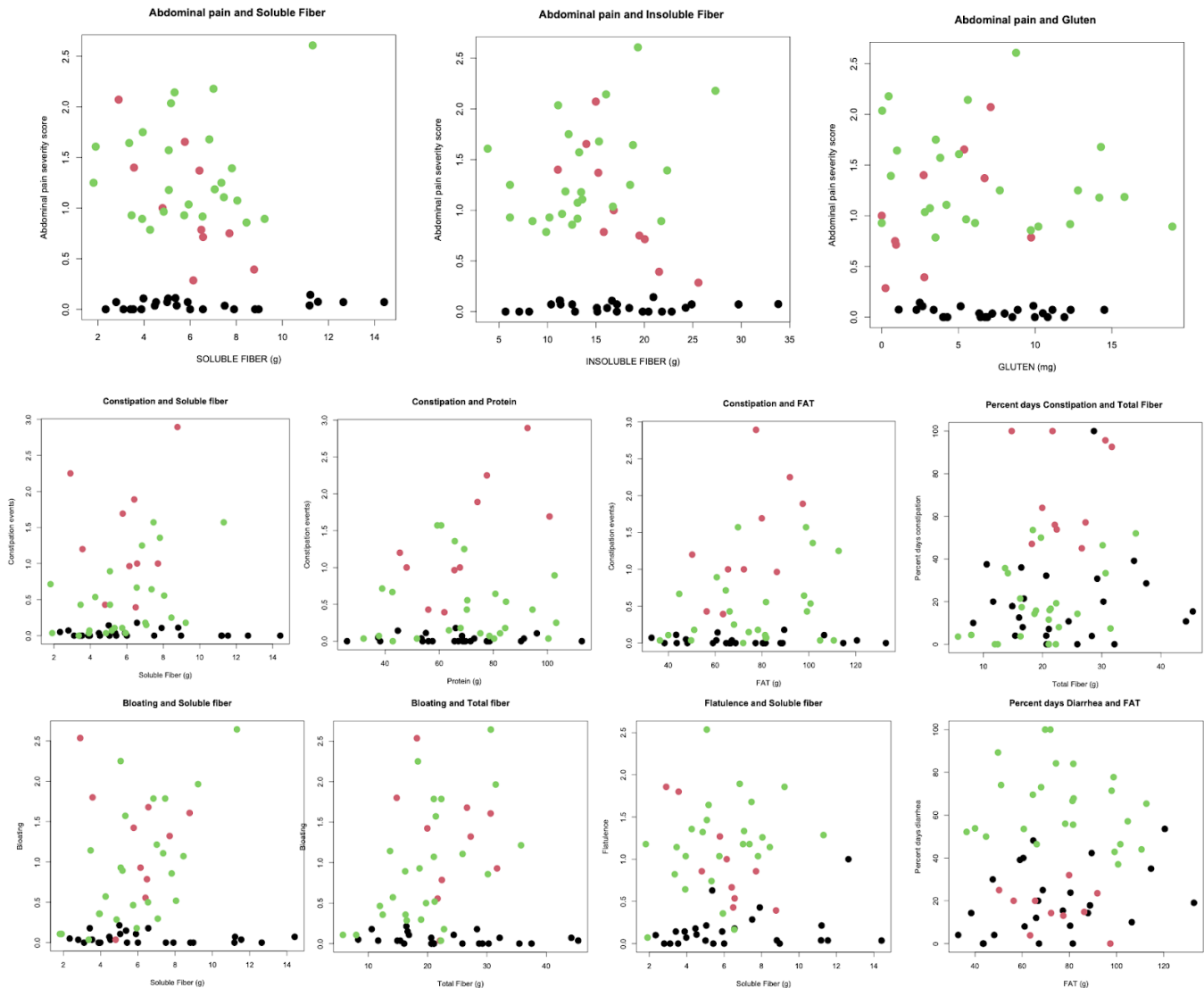
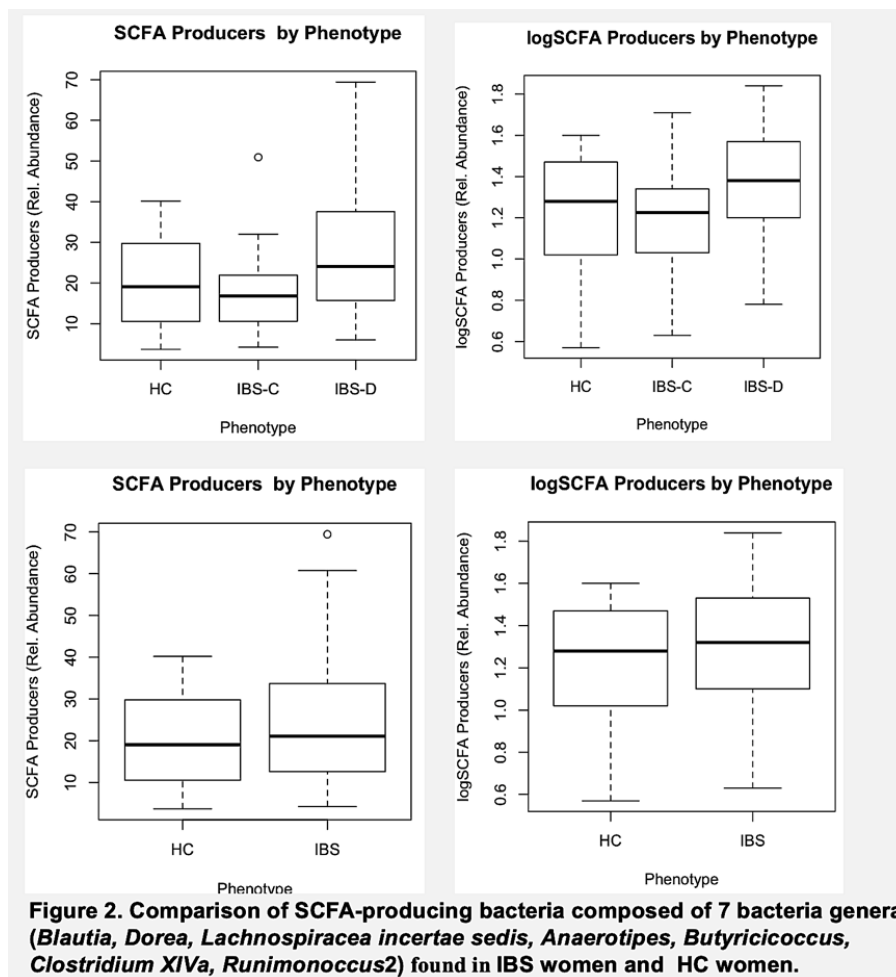
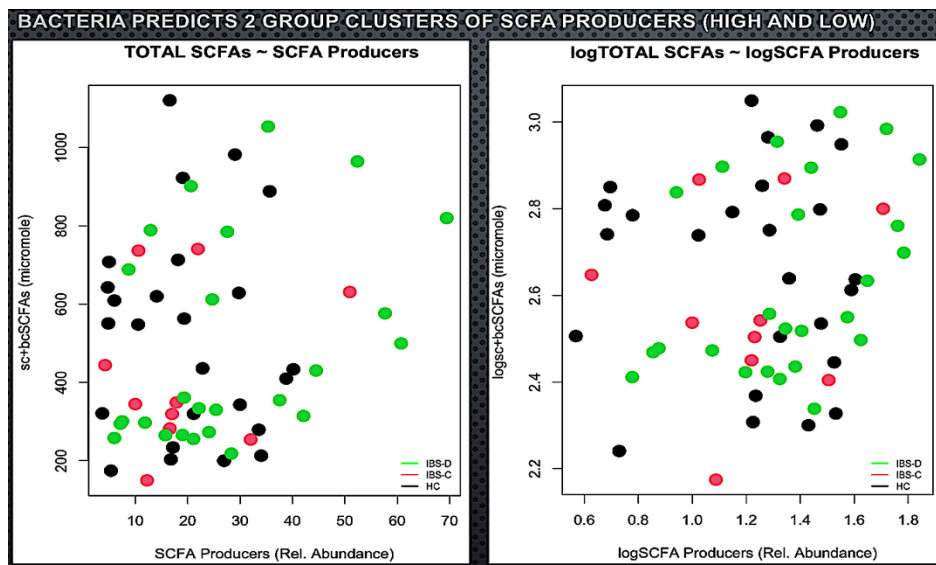


Figure 8. The relationship of diet and GI symptoms.

AIM3 RESULT: Explore the Relationships among Diet, SCFA, Bacteria, & Symptoms

Aim 3 is exploratory. Q-q plot (see Figure 3) was examined to see if the interaction of untransformed relative abundances of two SCFA-producing bacteria clusters is normal. A cluster of 7 SCFA-producing bacteria were grouped (*Blautia*, *Dorea*, *Lachnospiraceae insertia sedis*, *Anaerotipes*, *Butyrivibrio*, *Clostridium XIVa*, *Clostridium XIVb*, *Runimonococcus*) because they are known to produce butyrate. This group of SCFA-producing bacteria was plotted against the total fecal total SCFA for all women (see Figure 1). The scatter plot in Figure 1 shows that there are two groups of SCFA producers (High and Low) under the environment given by the 8 groups of bacteria genera. The two groups are probably a consequence of the differences in dietary intake of each person along with differences in lifestyle (e.g., medications, alcohol, dysregulated sleeping behavior, etc.). Looking at the Figure 2 below, the IBS phenotype has a higher mean relative abundance of SCFA-producing bacteria compared to healthy women (also called HC group); this trend is primarily driven by the IBS-D cohort because the IBS-C women had lower mean relative abundance of these SCFA-producing bacteria. Significance threshold was set at 0.05. However, results with significant differences must be interpreted with caution because of the potential for latent variables not included in the analysis. After performing group comparison using the Tukey method for three group comparisons, there were no significant differences in microbial diversity (see Table 2). The boxplots in Figure 4 show that the IBS-D phenotype had lower mean Shannon index and lower inverse Simpson index scores compared to IBS-C or to HC groups. Higher Shannon index and lower Simpson index means higher microbial diversity. Inverse Simpson index was analyzed for easier interpretation of results.



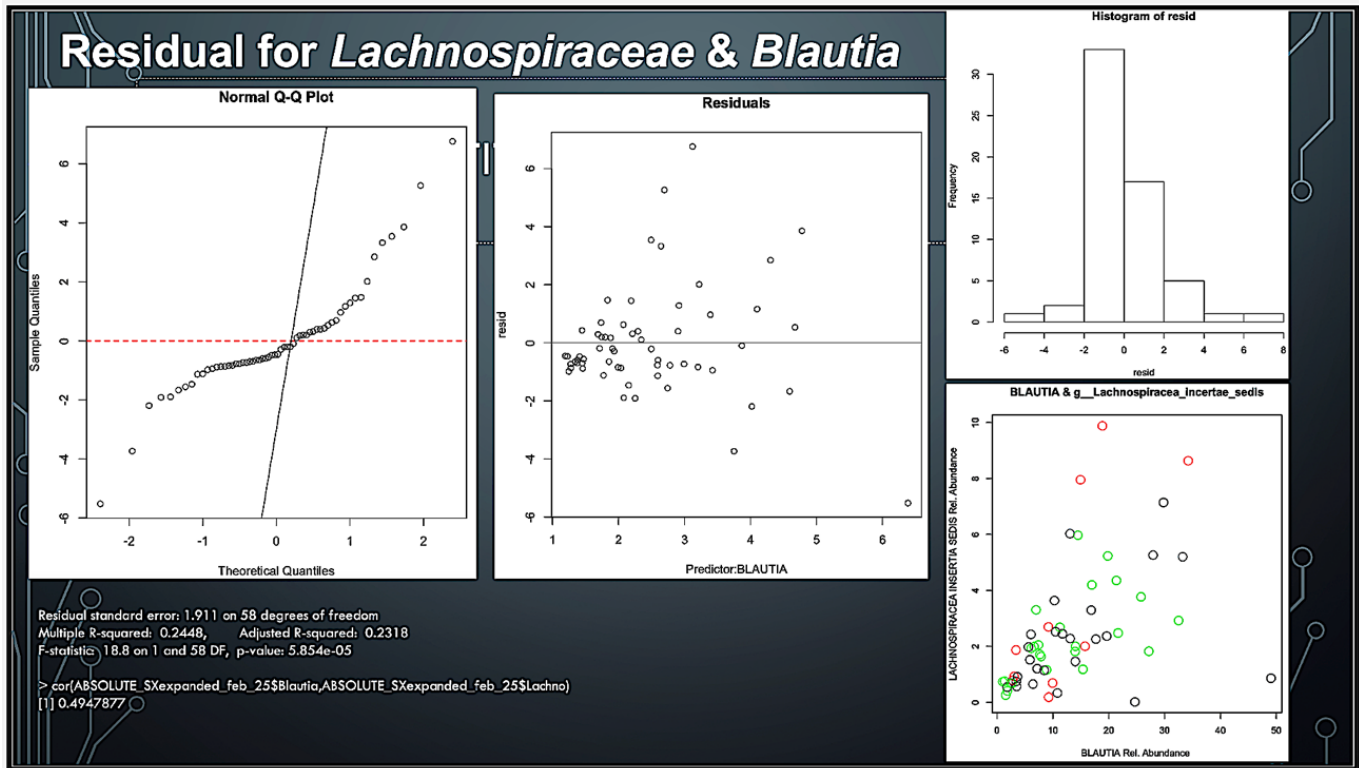


Figure 3. Q-q plot of wo SCFA-producing bacteria (*Lachnospiraceae insertia sedis* and *Blautia*).

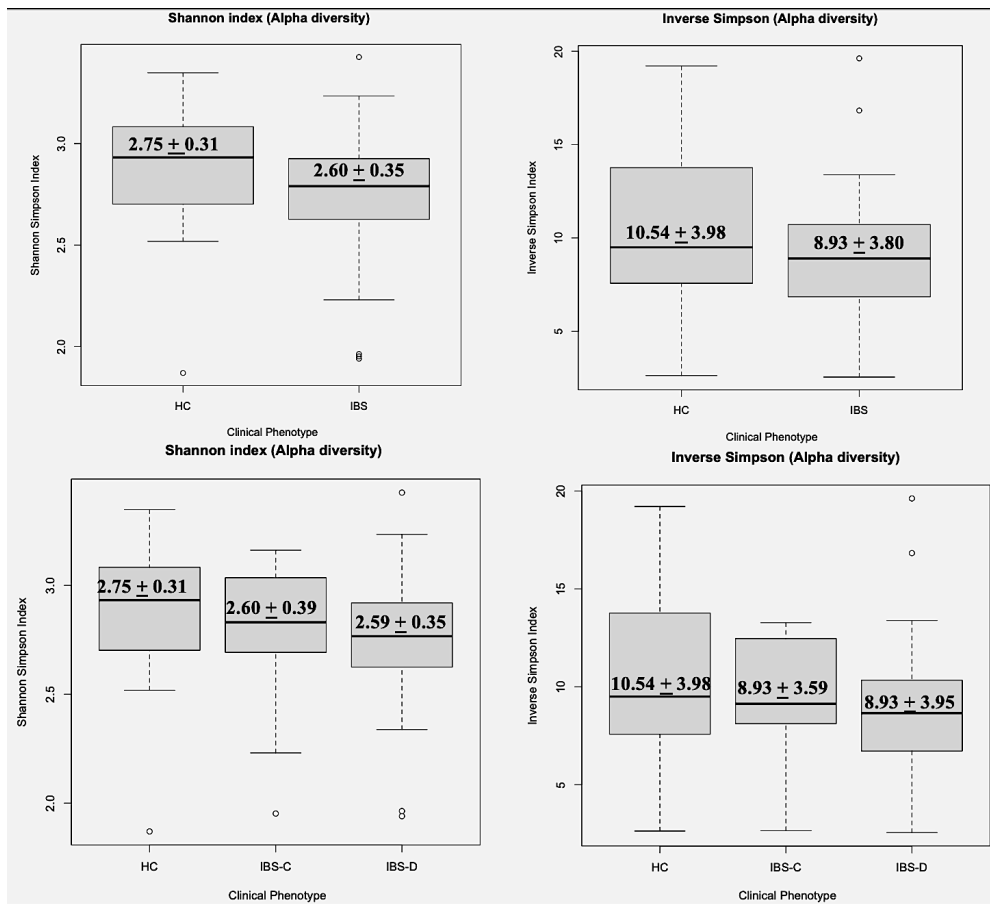


Figure 4a. Boxplots of microbial alpha diversity indices for IBS and HC women.

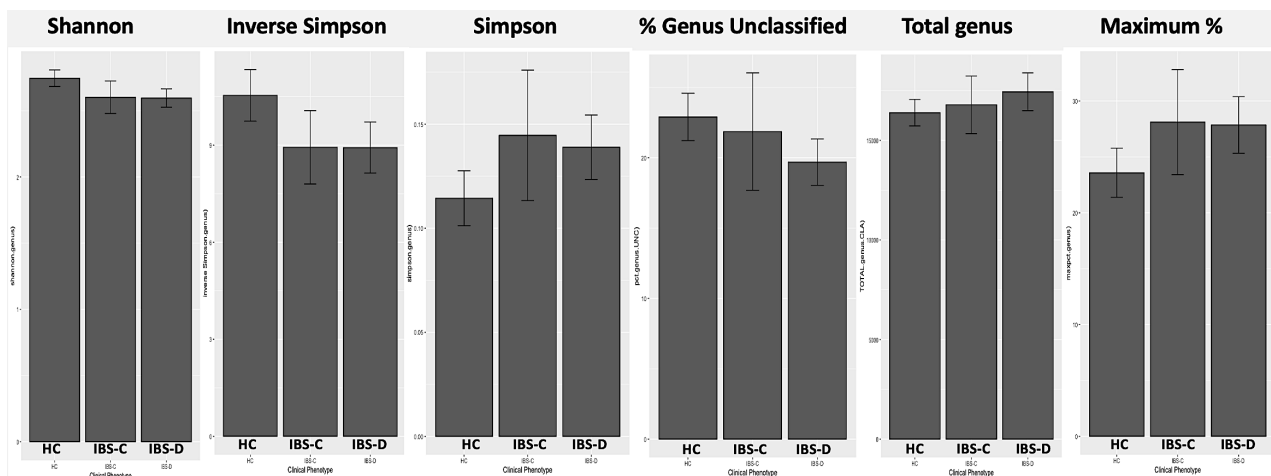


Figure 4b. Histograms of microbial alpha diversity indices for IBS and HC women.

Table 1. Ranges for Microbial Diversity indices and Other Microbial Measures

Metabolites	HC (n=25)	IBS-C (n=10)	IBS-D (n= 25)
shannon.genus	1.72 - 3.21	1.80 - 3.03	1.77 - 3.28
shannon0genus	1.87 - 3.35	1.95 - 3.16	1.94 - 3.43
inversesimpson.genus	2.63 - 19.21	2.65 - 13.27	2.55 - 19.62
simpson.genus	0.05 - 0.38	0.08 - 0.38	0.05 - 0.39
TOTAL.genus.CLA	10561 - 23652	10877 - 22458	11585 - 30682
TOTAL.genus	14572 - 29274	14291 - 28164	15690 - 35776
Maximum %	8.18 - 59.69	13.76 - 60.35	9.79 - 61.65

Table 2. Tukey Multiple Comparisons of Means for Microbial Indices Using ANOVA: 95% Family-Wise Confidence Level

Variables	HC -IBS-C			HC-IBSD D			IBS-C-IBS-D			
	Mean Diff.	CI	p-adj.	Diff	CI	p-adj.	Mean Diff.	CI	p-adj.	F-stat*
Total genus	155	-4152, 446	1.00	418	-2837,3673	0.95	263	-4043, 4570	0.99	0.05
Ttotal genus (CLA)	396	-3359, 4151	0.97	1051	-1787, 3890	0.65	656	-3099, 4410	0.91	0.40
Maximum %	4.53	-6.6, 15.7	0.59	4.27	-4.15, 12.7	0.44	-0.25	-11.4, 10.9	1.00	0.90
Percent.g Unc.bacteria	-1.04	-9.4, 7.3	0.95	-3.21	-9.52, 3.09	0.44	-2.17	-10.5, 6.2	0.81	0.77
Simpson	0.03	-0.04, 0.10	0.55	0.024	-0.028, 0.08	0.50	-0.006	-0.08, 0.06	0.98	0.86
Inverse Simpson	-1.61	-5.12, 1.91	0.52	-1.612	-4.27, 1.05	0.32	-0.006	-3.53, 3.51	1.00	1.24
Shannon	-0.14	-0.45, 0.16	0.50	-0.150	-0.38, 0.08	0.27	-0.006	-0.31, 0.30	1.00	1.39
Shannon.0	-0.141	-0.44, 0.16	0.50	-0.145	-0.37, 0.08	0.28	-0.004	-0.30, 0.30	1.00	0.36

* The critical value for F-statistics is 3.34, for N=60 total observation, comparing 3 groups at two-sided significance level.

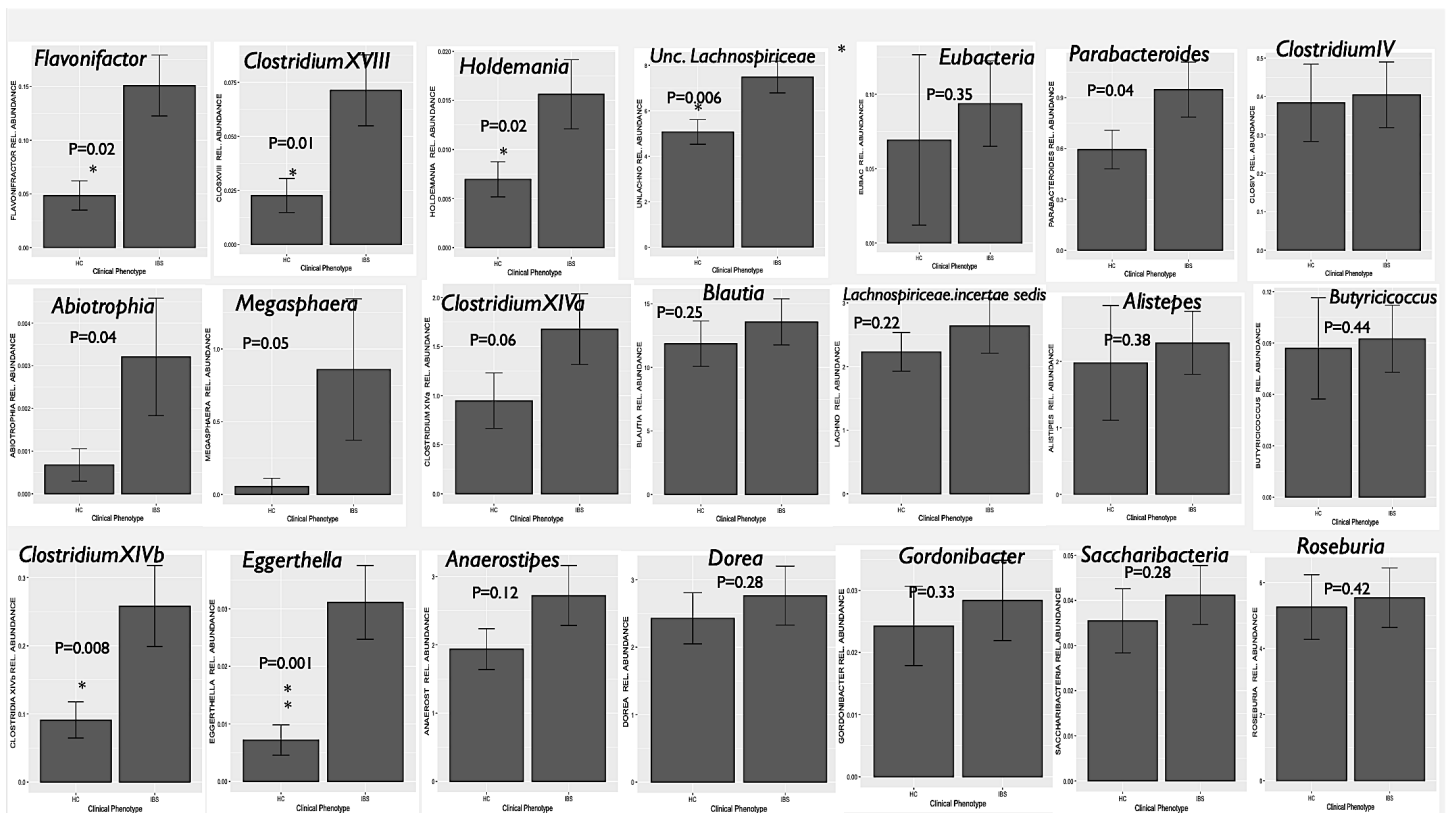


Figure 5a. The relative abundances of bacteria in HC vs. IBS.

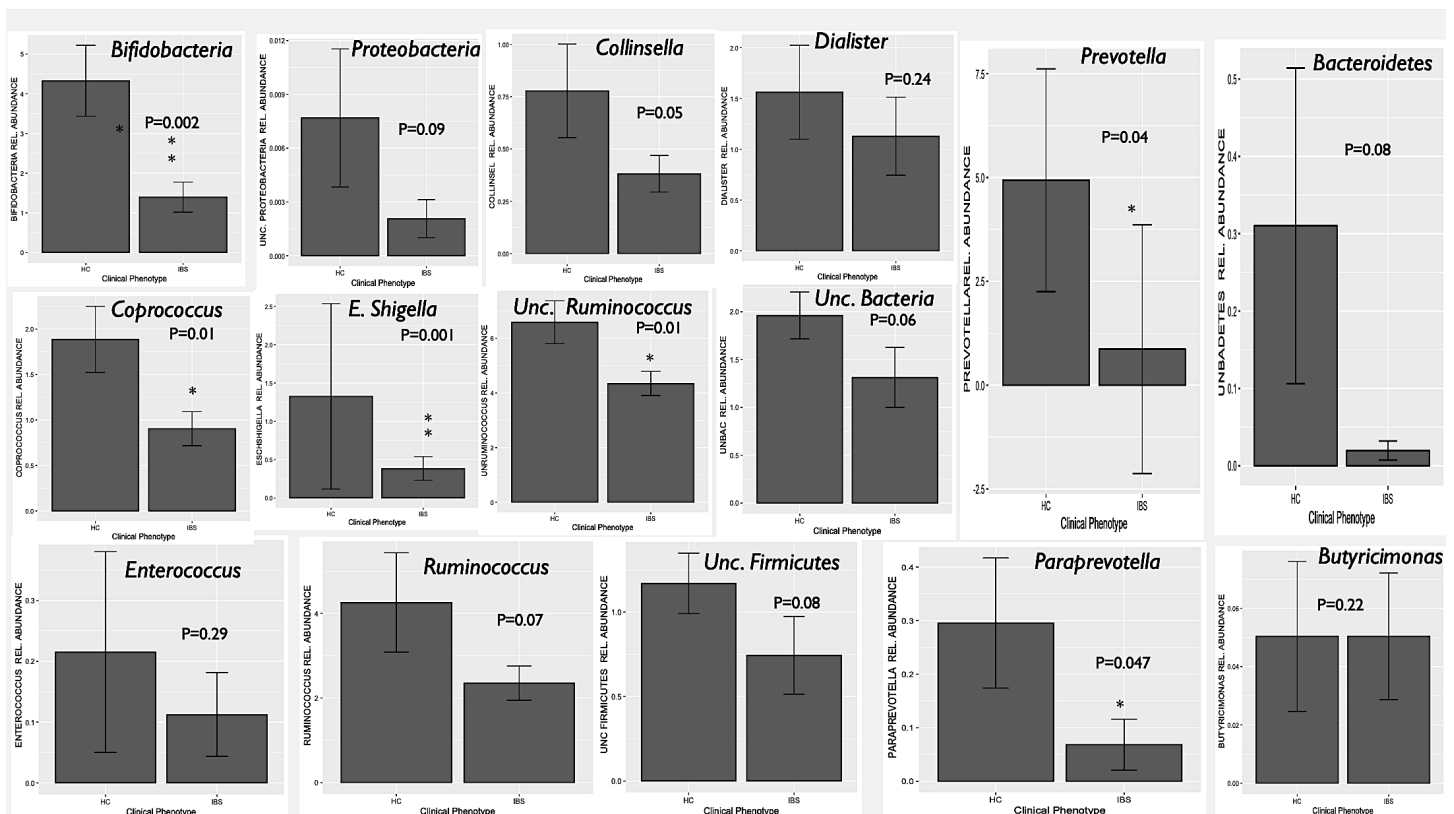


Figure 5b. The relative abundances of bacteria in HC vs. IBS.

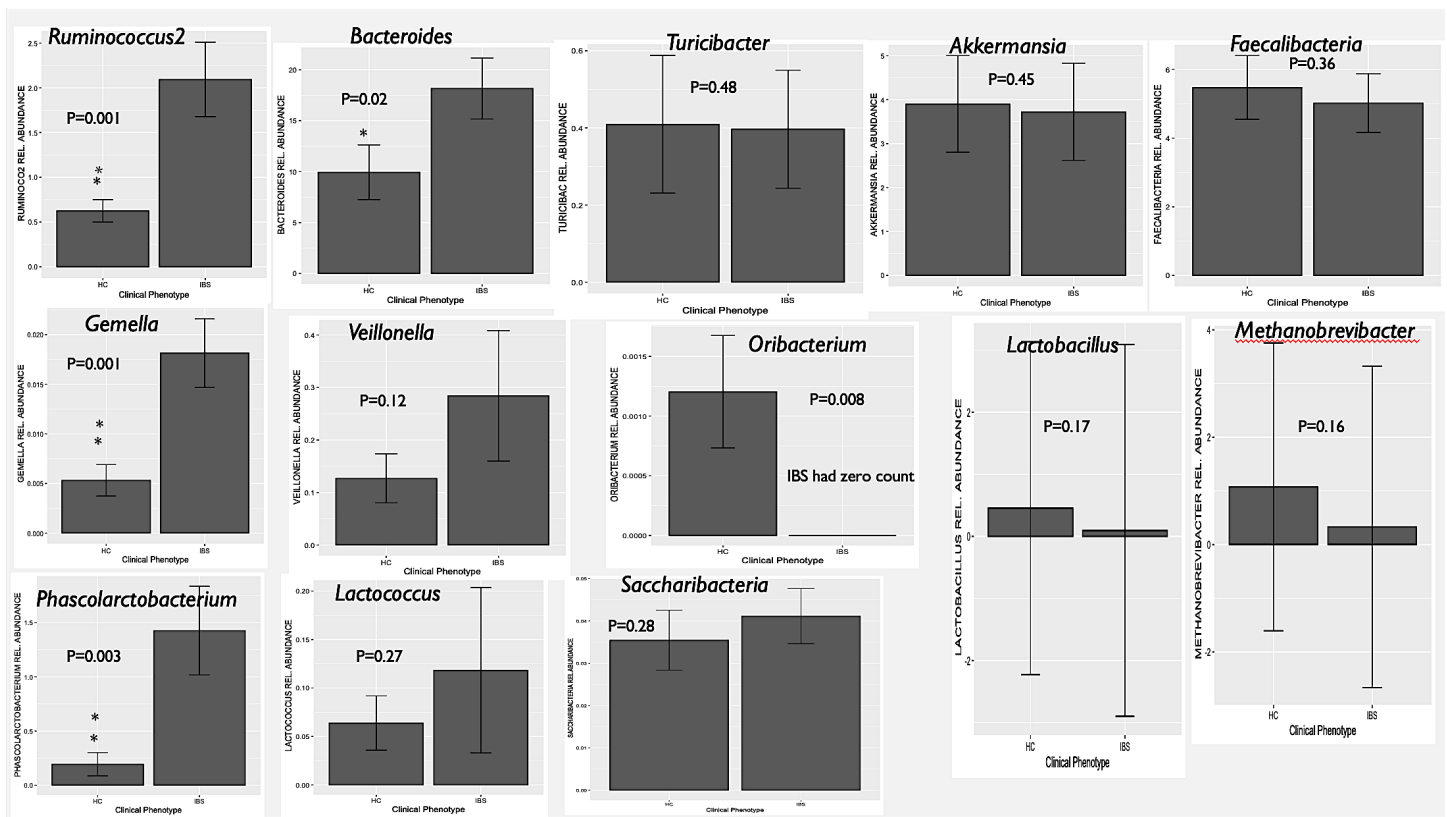


Figure 5c. The relative abundances of bacteria in HC vs. IBS.

Bacteria Profile. Comparison of bacteria genera for the IBS as a composite (and each subtype) with HC was visualized via histograms with confidence intervals or error bars (See figure 5 and Figure 6). As shown in Table 3, IBS-D women had significantly lower ($p = 0.002$) relative abundance of *Bifidobacteria* when compared to the HC group.

Differences in the gut microbiota existed at the genus level between IBS and HC groups. (see Figure 5). Several bacteria are significantly increased in IBS (i.e., *Bacteroides* ($p = 0.02$), *Parabacteroides* ($p = 0.04$), *Ruminococcus2* ($p = 0.001$), *Flavonifactor* ($p = 0.02$), *Clostridium XVIII* ($p = 0.01$), *Clostridium XIVb* ($p = 0.008$), *Abotrophia* ($p = 0.04$), *Holdemania* ($p = 0.02$), *Gemella* ($p = 0.001$), *Eggerthella* ($p = 0.001$), *Phascolarctobacterium* ($p = 0.003$), while IBS was found to be significantly reduced in relative abundance of *Bifidobacteria* ($p = 0.002$), *Coprococcus* ($p = 0.01$), *Prevotella* ($p = 0.04$), *Unc. Ruminococcus* ($p = 0.01$) and *E. Shigella* ($p = 0.001$). In the IBS-D group there was a lower level of *Bifidobacteria* ($p = 0.002$), *Prevotella*, *Paraprevotella*, *Coprococcus*, *Ruminococcus2*, and *unclassified Ruminococcus* in IBS-D while a higher level of

Clostridia genera (*Clostridium XIVb*, *Clostridium XVIII*, *Unclassified Lachnospiraceae*), *Flavonifractor*, *Unclassified Parabacteroides* when compared with the HC group. (see Table 3 below and Figure 6 for visualization with error bars). However, after accounting for multiple comparisons, only *Bifidobacteria*, *Coprococcus*, *Ruminococcus2*, *Flavonifractor*, *Gemella*, *Phascolarctobacterium*, *Oribacterium*, *Unc. Ruminococcus*, *Ruminococcus*, *Unc. Lachnospiraceae*, *Unc. Bacteria*, and *Clostridia XIVb* remained statistically significant (see FDR adjusted p-value on Table 4).

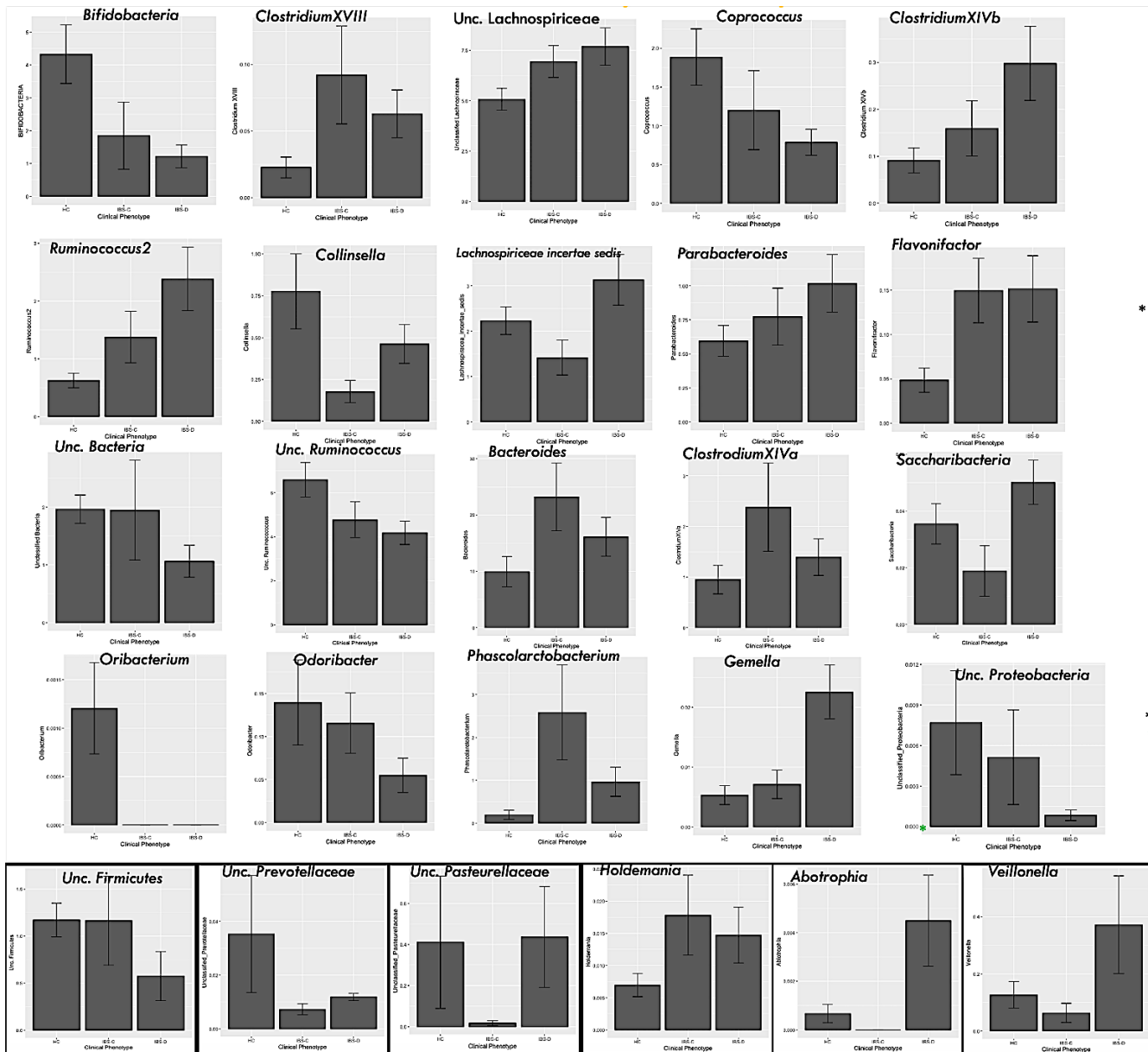


Figure 6. The relative abundances of bacteria in HC, IBS-C, IBS-D

IBS-C and IBS-D appeared to share these bacteria profiles (*Abiotrophia*, *Megasphaera*, *Clostridia XIVa*, *Clostridia XIVb*, *Ruminococcus2*, *Flavonifactor*, *Holdemania*). IBS-D and HC appeared to share similar abundances in *Sacharribacteria*, *Turicibacter*, *Clostridia XI*, *Clostridium stricto sensui*, and *Finegoldia*, which were found to be lower in the IBS-C group. Although not significant, IBS-D had higher *Alistipes*; overgrowth of this bacteria can cause harm to the host. Interestingly the IBS-C group had higher abundance *Gemmiger* as compared to the others groups.

Rajilic-Sstojanovic et al., (2011) reported differences of microbiota profile in subgroups of IBS compared to HC. They reported higher *Ruminococcus gnavus* and *Ruminococcus torques* in IBS-D (this is consistent with the result obtained from the most current phylogenetic analysis done from a bigger sample of women (n=118) originated from the MAP study. A smaller sample study (n=30) done by Zhu et al. 2019 reported higher *Lachnospiracea* and *Clostridia* in IBS (Rome III) than HC, but found higher alpha diversity in IBS than HC, however, they did not find differences in SCFA profile. Similar microbiota compositions were reported by Han et al. (2022); *Lachnospiracea*, *Bacteroides*, *Ruminococcus gnavus* were associated with IBS.

Table 3. Bacteria Taxa Relative Abundances Derived from 16S Data from the MAP Study

BACTERIA GENERA	Mean $\pm$ SD			HC (n=25) vs. IBS-C (n=10)	HC (n=25) vs. IBS- D (n=25)	IBS-C (n=10) vs. IBS- D (n=25)
	Healthy (n =25)	IBS-C (n= 10)	IBS-D (n= 25))	p	p	p
<i>Bifidobacteria</i>	4.33 $\pm$ 4.47	1.85 $\pm$ 3.23	1.27 $\pm$ 1.76	0.05	0.002	0.30
<i>Bilophila</i>	0.06 $\pm$ 0.09	0.14 $\pm$ 0.22	0.11 $\pm$ 0.17	0.15	0.10	0.35
<i>Blautia</i>	11.87 $\pm$ 8.92	9.72 $\pm$ 7.36	15.13 $\pm$ 11.58	0.24	0.14	0.14
<i>Lactobacillus</i>	0.45 $\pm$ 1.83	0.28 $\pm$ 0.81	0.02 $\pm$ 0.05	0.36	0.13	0.17
<i>Akkermansia</i>	3.90 $\pm$ 5.49	3.52 $\pm$ 5.85	3.80 $\pm$ 6.92	0.43	0.31	0.45
<i>Alistipes</i>	1.98 $\pm$ 4.31	1.69 $\pm$ 1.54	2.51 $\pm$ 3.17	0.39	0.31	0.17
<i>Anaerostipes</i>	1.94 $\pm$ 1.50	2.13 $\pm$ 1.47	2.96 $\pm$ 2.92	0.37	0.07	0.15
<i>Dorea</i>	2.43 $\pm$ 1.90	2.17 $\pm$ 2.02	3.00 $\pm$ 2.78	0.37	0.20	0.18
<i>Dialister</i>	1.56 $\pm$ 2.31	0.78 $\pm$ 1.39	1.27 $\pm$ 2.55	0.13	0.34	0.24
<i>Abiotrophia</i>	0.001 $\pm$ 0.002	0.00 $\pm$ 0.00	0.004 $\pm$ 0.009	0.02	0.06	0.03
<i>Acidaminoco</i>	0.02 $\pm$ 0.08	0.06 $\pm$ 0.12	0.01 $\pm$ 0.04	0.18	0.29	0.11
<i>Actinomyces</i>	0.07 $\pm$ 0.11	0.05 $\pm$ 0.04	0.08 $\pm$ 0.07	0.23	0.35	0.07
<i>Faecalibacteria</i>	5.48 $\pm$ 4.64	4.60 $\pm$ 4.33	5.19 $\pm$ 5.40	0.30	0.42	0.37

<i>Megasphaera</i>	0.06 + 0.28	0.72 + 2.26	0.92 + 3.12	0.19	0.09	0.42
<i>Mogibacteria</i>	0.005± 0.009	0.004 ± 0.008	0.007±0.012	0.38	0.26	0.21
<i>Lachnospiraceae incertae sedis</i>	2.23 ± 1.52	1.42 ± 1.21	3.13 ± 2.80	0.07	0.09	0.02
<i>Turcibacter</i>	0.41 ± 0.89	0.17 ± 0.31	0.49 ± 1.04	0.31	0.39	0.10
<i>Veillonella</i>	0.13 ± 0.23	0.06 ± 0.11	0.37± 0.86	0.13	0.10	0.06
<i>Roseburia</i>	5.26 ± 4.91	5.27 ± 5.67	5.64 ± 5.32	0.50	0.40	0.43
<i>Bacteroides</i>	9.95 ± 13.41	23.20 ± 18.92	16.15 ± 17.19	0.04	0.08	0.17
<i>Parabacteroides</i>	0.59 ± 0.57	0.77 ± 0.66	1.02± 1.06	0.23	0.04	0.21
<i>Eubacteria</i>	0.07 ± 0.29	0.19 ± 0.26	0.06 ± 0.10	0.13	0.44	0.08
<i>Clostridium XIVa</i>	0.97 ± 1.44	2.38 ± 2.75	1.40 ± 1.81	0.08	0.17	0.16
<i>Clostridium XIVb</i>	0.09 ± 0.13	0.16 ± 0.19	0.30 ± 0.40	0.16	0.01	0.10
<i>Clostridium XVIII</i>	0.02 ± 0.04	0.09 ± 0.12	0.06 ± 0.09	0.05	0.03	0.25
<i>Clostridium IV</i>	0.38± 0.50	0.50 ± 0.62	0.37 ± 0.46	0.30	0.47	0.28
<i>Eggerthella</i>	0.031± 0.01	0.03 ± 0.03	0.03 ± 0.04	0.46	0.45	0.50
<i>Butyricicoccus</i>	0.09 ± 0.15	0.14 ± 0.11	0.07± 0.11	0.15	0.30	0.06
<i>Enterococcus</i>	0.22 ± 0.83	0.02 ± 0.03	0.15 ± 0.48	0.13	0.36	0.11
<i>Streptococcus</i>	1.09 ± 1.23	1.45 ± 1.58	1.35 ± 1.99	0.27	0.29	0.44
<i>UNBadetes</i>	0.31 ± 1.02	0.05 ± 0.13	0.01 ± 0.02	0.12	0.08	0.18
<i>UNBac</i>	1.96 ± 1.22	1.94 ± 2.73	1.06 ± 1.38	0.49	0.01	0.18
<i>Unc. Lachnospiraceae</i>	5.07 ± 2.71	6.95 ± 2.50	7.70 ± 4.65	0.04	0.01	0.28
<i>Unc. Pasteurellaceae</i>	0.41 ± 1.61	0.02 ± 0.04	0.44 ± 1.23	0.13	0.47	0.06
<i>UNProteo</i>	0.008 ± 0.019	0.005 ± 0.011	0.001 ± 0.002	0.29	0.04	0.14
<i>Unclassified Porphyromonadaceae</i>	0.69 ± 2.15	0.12 ± 0.25	0.04 ± 0.13	0.11	0.07	0.18
<i>Unc. Prevotellaceae</i>	0.035 ± 0.108	0.007± 0.007	0.012± 0.007	0.11	0.15	0.04
<i>UncFirmicutes</i>	1.17 ± 0.89	1.16 ± 1.50	0.58 ± 1.30	0.49	0.04	0.16
<i>Unc. Erysipelotrichaceae</i>	0.73 ± 1.26	0.39 ± 0.61	0.70 ± 0.81	0.16	0.46	0.12
<i>UNCRuminococo</i>	6.59 ± 3.93	4.78 ± 2.60	4.18 ± 2.66	0.07	0.009	0.29
<i>Ruminoco</i>	4.26 ± 5.88	2.00 ± 1.98	2.49 ± 2.60	0.06	0.09	0.28
<i>Ruminoco2</i>	0.62 ± 0.63	1.38 ± 1.41	2.38 ± 2.75	0.07	0.002	0.10
<i>Coprococcus</i>	1.89 ± 1.82	1.20 ± 1.61	0.79 ± 0.84	0.15	0.005	0.23
<i>EschShigella</i>	1.33 ± 6.04	0.12 ± 0.30	0.49 ± 1.05	0.17	0.25	0.07
<i>Prevotella</i>	4.93 ± 10.51	1.39 ± 4.34	0.66 ± 2.20	0.10	0.03	0.31
<i>Collinsella</i>	0.78 ± 1.12	0.18 ± 0.21	0.46 ± 0.58	0.02	0.11	0.03
<i>Flavonifractor</i>	0.05 ± 0.07	0.15 ± 0.12	0.15 ± 0.19	0.02	0.01	0.5
<i>Oscillibacter</i>	0.28 ± 0.34	0.73 ± 1.07	0.27 ± 0.67	0.11	0.47	0.12
<i>Gemmiger</i>	1.51 ± 1.51	1.93 ± 2.00	1.55 ± 1.67	0.28	0.46	0.30
<i>Odoribacter</i>	0.14 ± 0.25	0.12 ± 0.11	0.05 ± 0.10	0.38	0.05	0.06
<i>Saccharibacteria</i>	0.04 ± 0.04	0.02 ± 0.03	0.05 ± 0.04	0.07	0.19	0.02
<i>Finegoldia</i>	0.0001±0.0007	0.00 ± 0.00	0.001±0.004	0.25	0.14	0.12
<i>Gemella</i>	0.0053±0.008	0.00711±0.008	0.0225±0.022	0.2703	0.0007	0.007
<i>Butyricimonas</i>	0.0646 ± 0.1225	0.0646 ± 0.1225	0.0646 ± 0.1225	0.0396	0.3461	0.10
<i>Solobacterium</i>	0.0067 ± 0.0236	0.0024 ± 0.0050	0.0021 ± 0.0044	0.206	0.175	0.43
<i>Helicobacteria</i>	0.0019 ± 0.0028	0.0016 ± 0.0021	0.0008 ± 0.0019	0.356	0.0485	0.15

<i>Holdemania</i>	0.0070 $\pm$ 0.0089	0.0178 $\pm$ 0.0196	0.0147 $\pm$ 0.0216	0.0632	0.0555	0.35
<i>Rothia</i>	0.0063 $\pm$ 0.0195	0.0045 $\pm$ 0.0034	0.0090 $\pm$ 0.0136	0.331	0.290	0.08
<i>Pseudoflavonifactor</i>	0.0030 $\pm$ 0.0067	0.0028 $\pm$ 0.0044	0.0006 $\pm$ 0.0032	0.462	0.066	0.10
<i>Phascolarctobacterium</i>	0.1946 $\pm$ 0.5344	2.583 $\pm$ 3.500	0.9645 $\pm$ 1.706	0.0301	0.0208	0.098
<i>Phorphyromonas</i>	0.0022 $\pm$ 0.0064	0.0009 $\pm$ 0.0018	0.0057 $\pm$ 0.0107	0.186	0.088	0.0293
<i>Olsenella</i>	0.0062 $\pm$ 0.0199	0.0005 $\pm$ 0.0016	0.0000 $\pm$ 0.0000	0.095	0.066	0.172
<i>Oribacterium</i>	0.0012 $\pm$ 0.0024	0.0000 $\pm$ 0.0000	0.0000 $\pm$ 0.0000	0.0154	0.0086	NA
<i>Fusobacterium</i>	0.0000 $\pm$ 0.0000	0.0026 $\pm$ 0.0082	0.0014 $\pm$ 0.0048	0.172	0.086	0.333
<i>Hydrogenoanaerobac</i>	0.0000 $\pm$ 0.0000	0.0004 $\pm$ 0.0011	0.0000 $\pm$ 0.0000	0.172	NA	0.172
<i>Methanobacteria</i>	1.0749 $\pm$ 3.524	0.5458 $\pm$ 1.7247	0.2423 $\pm$ 1.0197	0.284	0.134	0.307
<i>Gardnerella</i>	0.0002 $\pm$ 0.0011	0.0000 $\pm$ 0.0000	0.00016 $\pm$ 0.0008	0.172	0.429	0.172
<i>Parasuteralla</i>	0.0894 $\pm$ 0.1872	0.2798 $\pm$ 0.7482	0.2122 $\pm$ 0.5588	0.224	0.154	0.401

Table 4. FDR Adjusted p-value for the 3 Groups Comparisons

Bacteria Genera	HC (n=25) Vs. IBS-C (n=10)	HC (n=25) Vs. IBS-D (n=25))	IBS-C (n=10) Vs. IBS-D (n=25)
	FDR adj. p	FDR adj. p	FDR adj. p
<i>Bifidobacteria_adjusted</i>	0.075	0.006	0.3
<i>Bilophila_adjusted</i>	0.225	0.225	0.35
<i>Blautia_adjusted</i>	0.24	0.21	0.21
<i>Lactobacillus_adjusted</i>	0.36	0.255	0.255
<i>Akkermansia_adjusted</i>	0.45	0.45	0.45
<i>Alistipes_adjusted</i>	0.39	0.39	0.39
<i>Anaerostepes_adjusted</i>	0.37	0.21	0.225
<i>Dorea_adjusted</i>	0.37	0.3	0.3
<i>Dialister_adjusted</i>	0.34	0.34	0.34
<i>Abiotrophia_adjusted</i>	0.045	0.06	0.045
<i>Acidaminoco_adjusted</i>	0.27	0.29	0.27
<i>Actinomyces_adjusted</i>	0.345	0.35	0.21
<i>Faecalibacteria_adjusted</i>	0.42	0.42	0.42
<i>Megasphaera_adjusted</i>	0.285	0.27	0.42

<i>Mogibacteria_adjusted</i>	0.38	0.38	0.38
<i>Lachnospiraceaesedis_adjusted</i>	0.09	0.09	0.06
<i>Turicibacter_adjusted</i>	0.39	0.39	0.3
<i>Veillonella_adjusted</i>	0.13	0.13	0.13
<i>Roseburia_adjusted</i>	0.5	0.5	0.5
<i>Bacteroides_adjusted</i>	0.12	0.12	0.17
<i>Parabacteroides_adjusted</i>	0.23	0.12	0.23
<i>Eubacteria_adjusted</i>	0.195	0.44	0.195
<i>ClostridiumXIVa_adjusted</i>	0.17	0.17	0.17
<i>ClostridiumXIVb_adjusted</i>	0.16	0.03	0.15
<i>ClostridiumXVIII_adjusted</i>	0.075	0.075	0.25
<i>ClostridiumIV_adjusted</i>	0.45	0.47	0.45
<i>Eggerthella_adjusted</i>	0.5	0.5	0.5
<i>Butyricicoccus_adjusted</i>	0.225	0.3	0.18
<i>Enterococcus_adjusted</i>	0.195	0.36	0.195
<i>Streptococcus_adjusted</i>	0.435	0.435	0.44
<i>UNBacterioidetes_adjusted</i>	0.18	0.18	0.18
<i>UNBacteria_adjusted</i>	0.49	0.03	0.27
<i>UNLachno_adjusted</i>	0.06	0.03	0.28
<i>UNPasteur_adjusted</i>	0.195	0.47	0.18
<i>UNProteo_adjusted</i>	0.29	0.12	0.21
<i>UncPorphyromonadaceae_adjusted</i>	0.165	0.165	0.18
<i>UncPrevotellaceae_adjusted</i>	0.15	0.15	0.12
<i>UncFirmicutes_adjusted</i>	0.49	0.12	0.24
<i>UncErysipelotrichaceae_adjusted</i>	0.24	0.46	0.24
<i>UNCRuminococo_adjusted</i>	0.105	0.027	0.289
<i>Ruminoco1_adjusted</i>	0.135	0.135	0.28
<i>Ruminoco2_adjusted</i>	0.1	0.006	0.1
<i>Coproccoccus_adjusted</i>	0.225	0.015	0.23
<i>EschShigella_adjusted</i>	0.25	0.25	0.21
<i>Prevotella_adjusted</i>	0.15	0.09	0.31
<i>Collinsella_adjusted</i>	0.045	0.11	0.045
<i>Flavonifractor_adjusted</i>	0.03	0.03	0.5
<i>Oscillibacter_adjusted</i>	0.18	0.47	0.18

<i>Gemmiger_adjusted</i>	0.45	0.46	0.45
<i>Odoribacter_adjusted</i>	0.38	0.09	0.09
<i>Saccharibacteria_adjusted</i>	0.105	0.19	0.06
<i>Finegoldia_adjusted</i>	0.25	0.21	0.21
<i>Gemella_adjusted</i>	0.2703	0.00207	0.01065
<i>Butyricimonas_adjusted</i>	0.1188	0.3461	0.15495
<i>Solobacterium_adjusted</i>	0.309	0.309	0.43
<i>Helicobacteria_adjusted</i>	0.356	0.1455	0.2265
<i>Holdemania_adjusted</i>	0.0948	0.0948	0.346
<i>Rothia_adjusted</i>	0.331	0.331	0.237
<i>Pseudoflavonifactor_adjusted</i>	0.462	0.147	0.147
<i>Phascolarctobacterium_adjusted</i>	0.04515	0.04515	0.098
<i>Phorphyromonas_adjusted</i>	0.186	0.132	0.0879
<i>Olsenella_adjusted</i>	0.1425	0.1425	0.172
<i>Oribacterium_adjusted</i>	0.0154	0.0154	NA
<i>Fusobacterium_adjusted</i>	0.258	0.258	0.333
<i>Hydrogenoanaerobac_adjusted</i>	0.172	NA	0.172
<i>Methanobacteria_adjusted</i>	0.307	0.307	0.307
<i>Gardnerella_adjusted</i>	0.258	0.429	0.258
<i>Parasuteralla_adjusted</i>	0.336	0.336	0.401

Table 5. Tukey Multiple Comparisons of Means Using ANOVA: 95% Family-Wise Confidence Level

Bacteria Genera	HC -IBS-C			HC-IBSD			IBS-C-IBS-D			
	Mean Diff.	CI	p- adj.	Diff	CI	p- adj.	Mean Diff.	CI	p- adj.	F-stat
<i>Gemella</i>	0.002	-0.012, 0.016	0.95	0.017	0.007, 0.028	0.001	0.015	0.001, 0.030	0.03	8.27
<i>Phascolarctobacterium</i>	2.389	0.758, 4.020	0.00 2	0.770	-0.463, 2.003	0.30	-1.62	-3.250, 0.012	0.05	6.23
<i>Bifidobacteria</i>	-2.480	-5.509, 0.549	0.13	-3.110	-5.400, -0.820	0.005	-0.63	-3.660, 2.399	0.87	5.66
<i>Ruminococcus2</i>	0.751	-0.971, 2.473	0.55	1.758	0.456, 3.059	0.005	1.007	-0.716, 2.729	0.34	5.30
<i>Oribacterium</i>	-0.0012	-0.003, 0.0002	0.10	-0.001	-0.002, -0.0002	0.02	-0.000	-0.001, 0.0014	1.00	4.53
<i>Coprococcus</i>	-0.686	-1.993, 0.621	0.42	-1.100	-2.088, -0.112	0.03	-0.41	-1.721, 0.893	0.73	3.63
<i>Flavonifractor</i>	0.101	-0.022, 0.225	0.13	0.103	0.010, 0.196	0.03	0.002	-0.122, 0.125	1.00	4.07
<i>Unc. Ruminocococcus</i>	-1.816	-4.737, 1.106	0.30	-2.411	-4.620, -0.203	0.029	-0.60	-3.517, 2.326	0.88	3.60
<i>Unc. Lachno</i>	1.883	-1.387, 5.152	0.36	2.625	0.154, 5.097	0.03	0.74	-2.527, 4.013	0.85	3.37

<i>ClostridiumXIVb</i>	0.0682	-0.184, 0.321	0.79	0.207	0.0160, 0.398	0.03	0.139	-0.114, 0.391	0.39	3.47
<i>ClostridiumXVIII</i>	0.0695	- 0.0012, 0.140	0.05	0.040	- 0.0132, 0.094	0.17	-0.03	-0.010, 0.0414	0.58	3.30
<i>Abiotrophia</i>	-0.0007	-0.006, 0.005	0.95	0.004	- 0.0004, 0.008	0.08	0.005	-0.001, 0.0101	0.14	3.08
<i>Saccharibacteria</i>	-0.017	-0.049, 0.016	0.44	0.015	-0.001, 0.0392	0.33	0.03	-0.001, 0.064	0.06	2.88
<i>Bacteroides</i>	13.252	-1.172, 27.677	0.08	6.202	-4.702, 17.106	0.36	-7.05	- 21.475, 7.374	0.47	2.61
<i>Lachnospiraceae incertae sedis</i>	-0.813	-2.726, 1.099	0.57	0.901	-0.545, 2.346	0.30	1.714	-0.198, 3.626	0.09	2.60
<i>Prevotella</i>	-3.541	-10.00, 2.920	0.39	-4.274	-9.158, 0.610	0.10	-0.73	-7.194, 5.728	0.96	2.38
<i>Unc. Bacteria</i>	-0.017	-1.471, 1.436	1.00	-0.901	-1.100, 0.198	0.13	-0.88	-2.337, 0.570	0.32	2.25
<i>ClostridiumXIVa</i>	1.432	-0.232, 3.095	0.11	0.447	-0.811, 1.704	0.67	-0.95	-2.649, 0.679	0.34	2.15
<i>Collinsella</i>	-0.599	-1.340, 0.141	0.13	-0.315	-0.874, 0.245	0.37	0.286	-0.456, 1.025	0.63	2.12
<i>Unc. Firmicutes</i>	-0.0058	-1.071, 1.060	1.00	-0.593	-1.399, 0.212	0.19	-0.59	-1.653, 0.478	0.39	1.82
<i>Unc. Proteobacteria</i>	-0.0026	- 0.0145, 0.009	0.86	-0.007	-0.016, 0.002	0.17	-0.004	-0.016, 0.008	0.67	1.67
<i>Parabacteroides</i>	0.179	-0.563, 0.921	0.83	0.424	-0.137, 0.985	0.17	0.25	-0.497, 0.987	0.71	1.66

Age and SCFA. Age influences an individual's gut microbiota profile. It has been suggested that the gut microbiota can change throughout life and that older adults have remarkably different microbial profile compared to younger adults and thus, it would make sense that personalized probiotics regimen used to treat gut dysbiosis should be considering the impact of age to maintain a relatively stable gut microbiome (Dominguez-Bello et al., 2011). Figure 7 shows that the relative concentration of individual SCFA appeared to increase with age; propionic, valeric acids, and having IBS status are key drivers for this relationship. Butyric acid seemed to show a parabolic trend and look linear with age for IBS. Note: the HC data points were removed in the figures for easier visualization of the contribution of the IBS status. Figure 8 shows the relationship of butyric acid and age for IBS and HC women; the relationship of age and butyric acid becomes less positive because of the downward trend driven by the HC group.

Age is also positively related to microbial diversity. Zuluaga and colleagues (2019) analyzed a large data that was collected from four geographical locations (US, UK, Colombia, Chinese cohorts) has reported

that women have higher microbial diversity than men, and found higher microbial diversity in young adults, but not in middle-aged adults. Consistent with their reports, this study also found a positive relationship exist between age and microbial diversity indices from younger women at age 18 to 35. After age 35, this positive relationship becomes negative (see Figure 7b) in the HC group. Since the 3-day food diary included the intake of Greek yogurt, it may be that this person was supplementing herself with probiotics.

In Figure 9, the plot shows a negative relationship between microbial diversity indices and indicators for obesity such as weight and BMI. Having higher weight appeared to significantly correlate reduced microbial diversity indices; this is important because lower microbial diversity translates to lower metabolic capacity for the host harboring these bacteria. This data on weight and microbial indices is in line with current literature—higher weight is associated with lower microbial diversity as observed in several human and animal studies (Ley et al., 2005; Ley et al., 2006; Chen et al., 2020; Palmas et al., 2021; Obanda et al., 2021). Obanda and colleagues (2021) found that rats that are obesity-prone had higher total bacteria (reported higher glycan-degrading systems and LPS biosynthesis pathways) and SCFA, but lower alpha diversity index score as compared to obesity-resistant rats with similar calorie intake. Figure 10 show that there exists a positive relationship between butyric acid and microbial diversity index scores; HC women is the key driver for this positive relationship ($r=0.24$). Microbial diversity indices were strongly and positively associated with propionate, butyrate and valeric acids. Furthermore, higher concentration of SCFA was positively correlated with higher microbial diversity index scores and vice versa. Yamamura et. al. (2022) found positive association for fecal butyrate (identified via NMR) with inverse Simpson index and Shannon index from healthy subjects composed of 10 males and 2 females. The result in Figure 10 also seemed to be in line with Yamamura's study; this trend was observed in majority of the healthy individuals and IBS-C women. Butyrate's role in maintaining microbial diversity would make sense because of its role in maintaining the gut barrier (via stimulation of mucus secretion by the goblet cells) and several research have suggested that lower microbial diversity precedes gut dysbiosis and compromised gut integrity

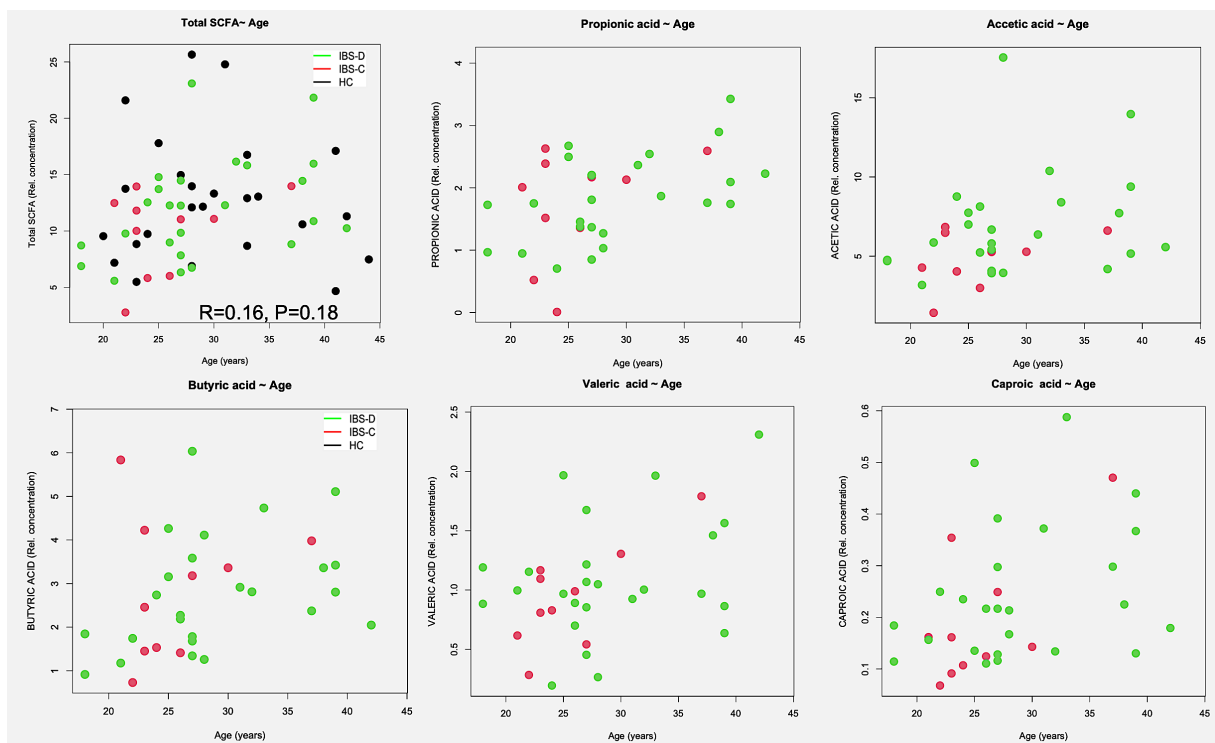


Figure 7. The relationship of SCFA (relative concentration) and age. IBS-D (green), IBS-C (red), HC (black).

Figure 7a. The relationship of SCFA and age.

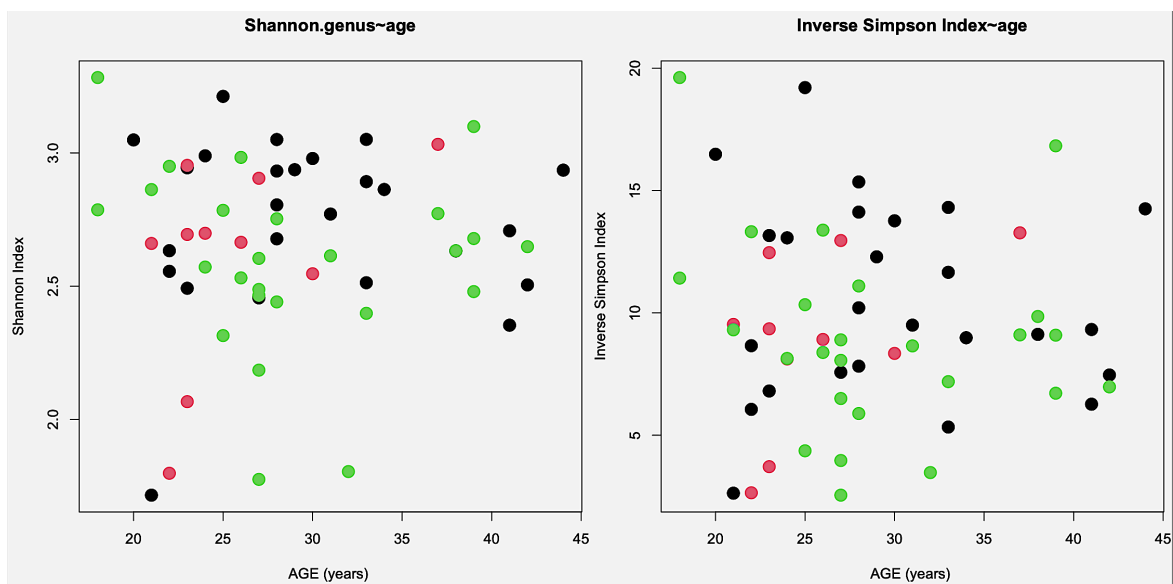


Figure 7b. The relationship of microbial diversity indices and age.

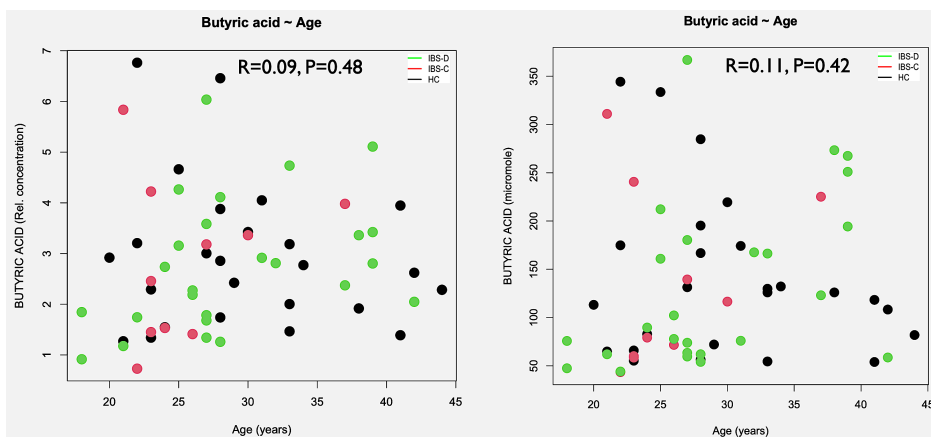


Figure 8. The relationship of butyric acid (absolute and relative concentrations) and age.

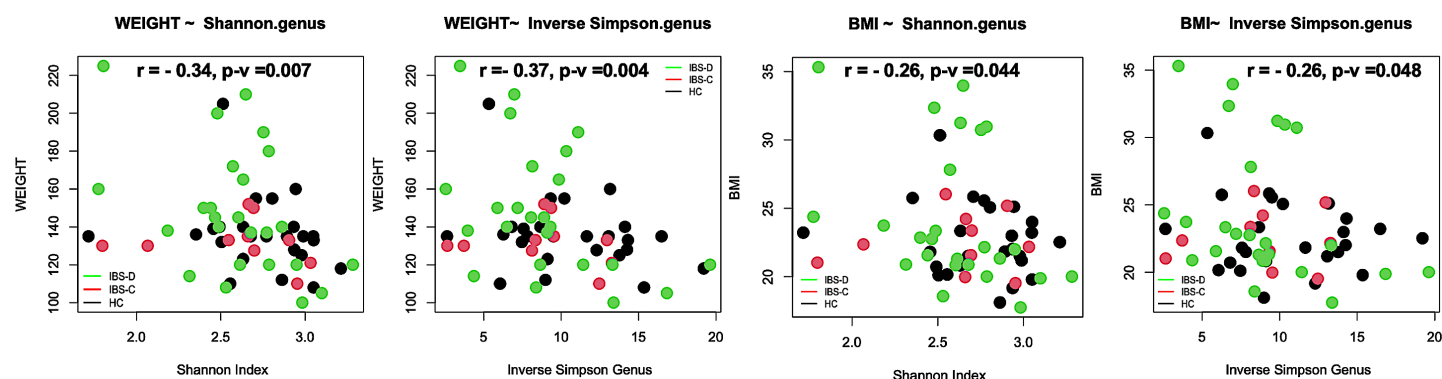


Figure 9. The relationship of the absolute concentration of SCFA and weight.

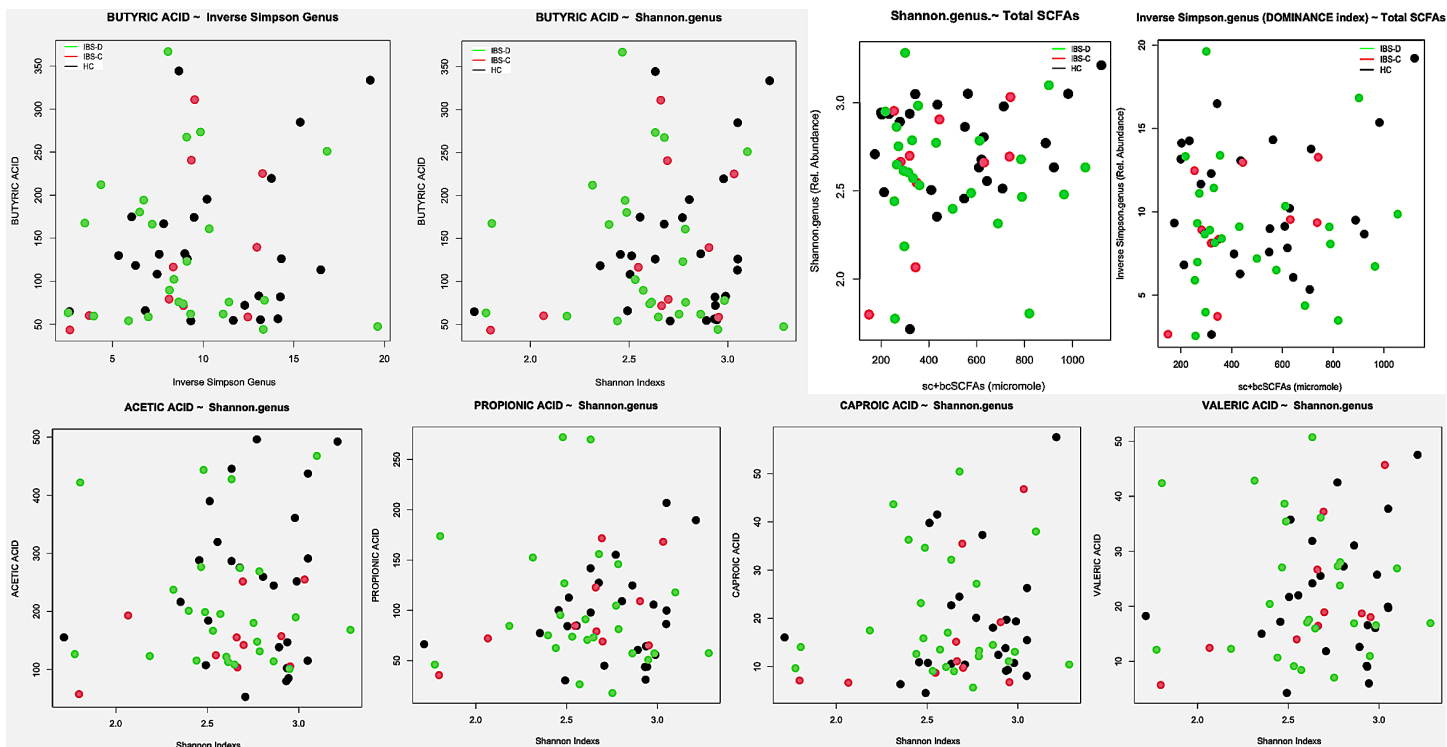


Figure 10. Relationship of individual SCFA and microbial diversity indices.

Yamamura et. al. (2022) found a positive association for fecal butyrate (identified via NMR) with inverse Simpson index and Shannon index from healthy subjects composed of 10 males and 2 females. The results (Figure 10) also seem to be in line with Yamamura's study; this trend was observed in majority of the HC individuals and IBS-C women. Butyrate's role in maintaining microbial diversity would make sense because of its role in maintaining the gut barrier (via stimulation of mucus secretion by the goblet cells) and several researchers have suggested that lower microbial diversity precedes gut dysbiosis and compromised gut integrity.

Positive linear relationship is also demonstrated when plotting butyric acid against specific SCFA-producing bacteria such as *Coprococcus*, and *Dorea* (see Figure 11).

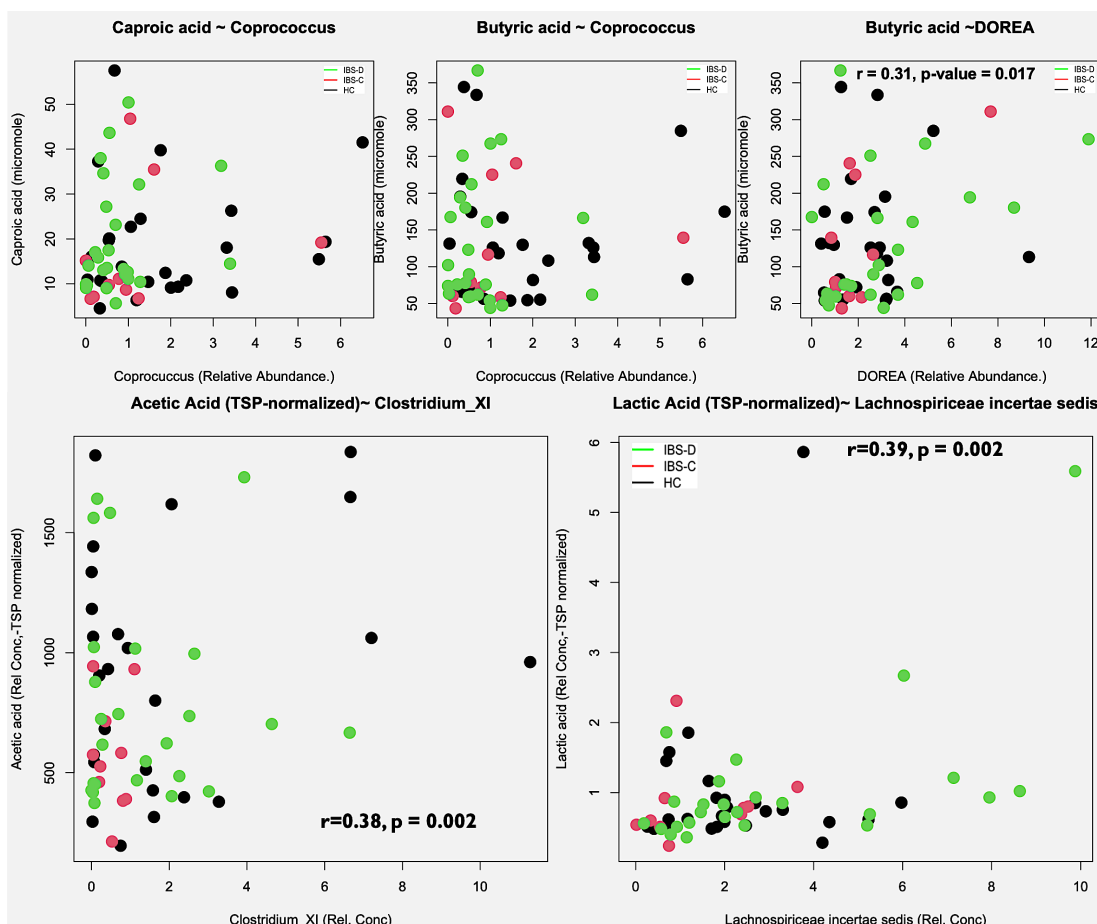


Figure 11. Relationship of individual SCFA and specific SCFA-producing bacteria.

This study found some evidence that fecal SCFA and its related metabolites are correlated with known SCFA-producing bacteria (*i.e. Bifidobacteria, Coprococcus, Blautia, Clostridia, Ruminococcus, Prevotella*,

Bacteroides, *Lachnospiracea*, *Dorea*, *Phascolarcobacteria*, *Anaerostipes*, *Roseburia*, *Coprococcus*, *Akkermansia*, *Faecalibacteria*, *Veillonella*). Positive and linear relationship was found when plotting butyric acid against specific SCFA-producing bacteria such as *Coprococcus*, and *Dorea* (see Figure 11).

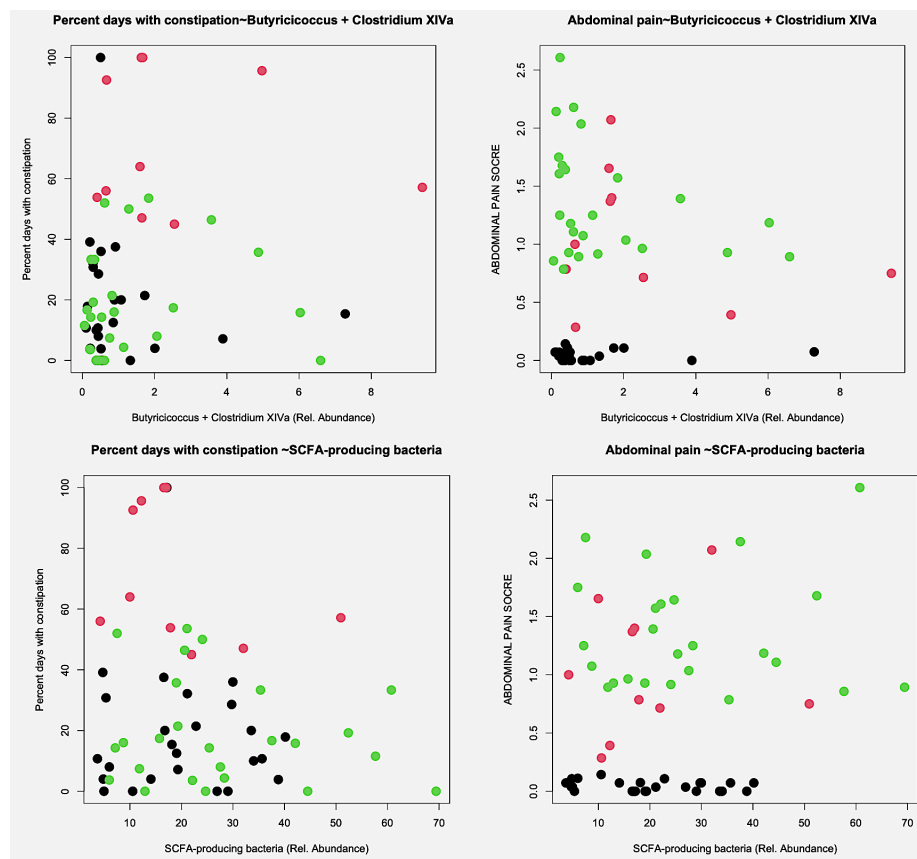


Figure 12a. Relationship of GI Symptom (percent days constipation and abdominal pain) with bacteria

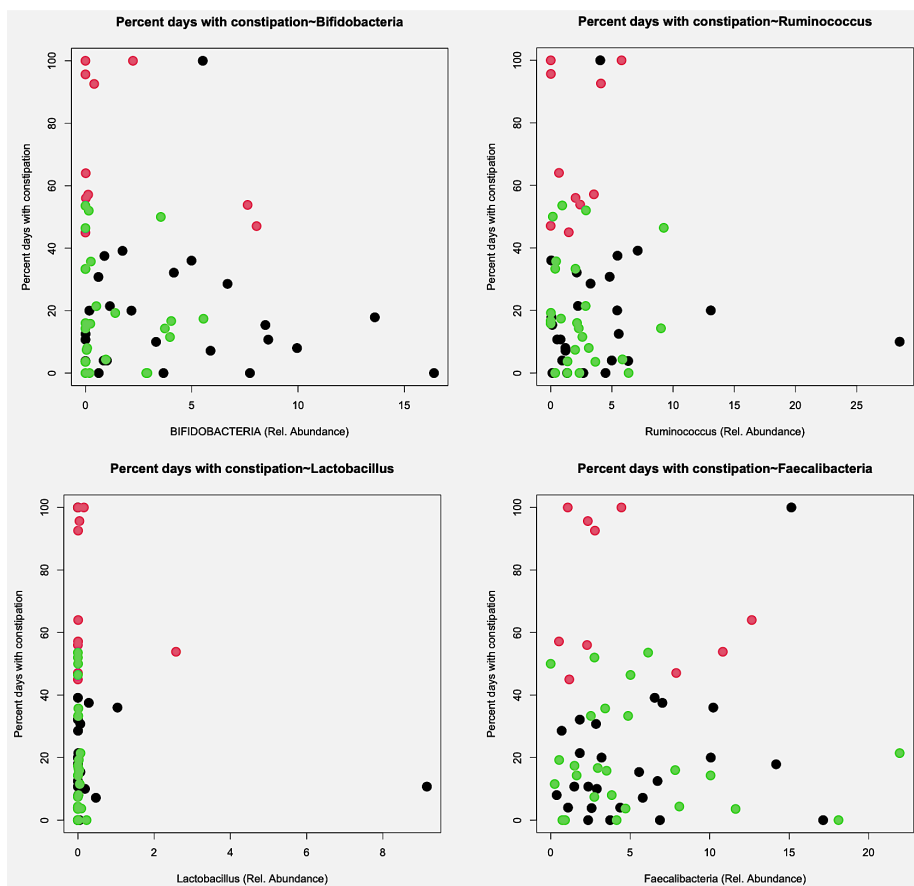


Figure 12b. Relationship of GI Symptom (percent days with constipation) and microbe.

Bacteria & GI Symptoms. As shown in Figure 12a and Figure 12b, this exploratory study is also in line with the results reported by Zhuang and colleagues (2019). Zhuang's group (2019) compared fecal SCFAs from constipated, healthy people from different age groups (infants, middle aged, older adults) and found that constipation was associated with reduced *Bifidobacteria*, *Lactobacillus*, *Faecalibacterium*, and *Ruminococaceae*, but increased *Desulfovibrionaceae*. Zhuang's group also reported that butyrate producing bacteria reduced constipation symptoms via SCFA. The figures above showed that the SCFA-producing bacteria clusters is associated with reduced abdominal pain and percent days with constipation. The two pairs of bacteria that included

In Figure 12c, *Parabacteroides*, *Bacteroides*, *Blautia*, *Lachnospiriceae incertae sedis*, and *Ruminococcus2* were both directly correlated with abdominal pain. *Lachnospiriceae incertae sedis* was found to be directly correlated with percent days diarrhea. On the other hand, higher relative abundance of

Bifidobacteria, *Roseburia*, *Prevotella*, and *Akkermansia* appeared to correlate with lower abdominal pain scores.

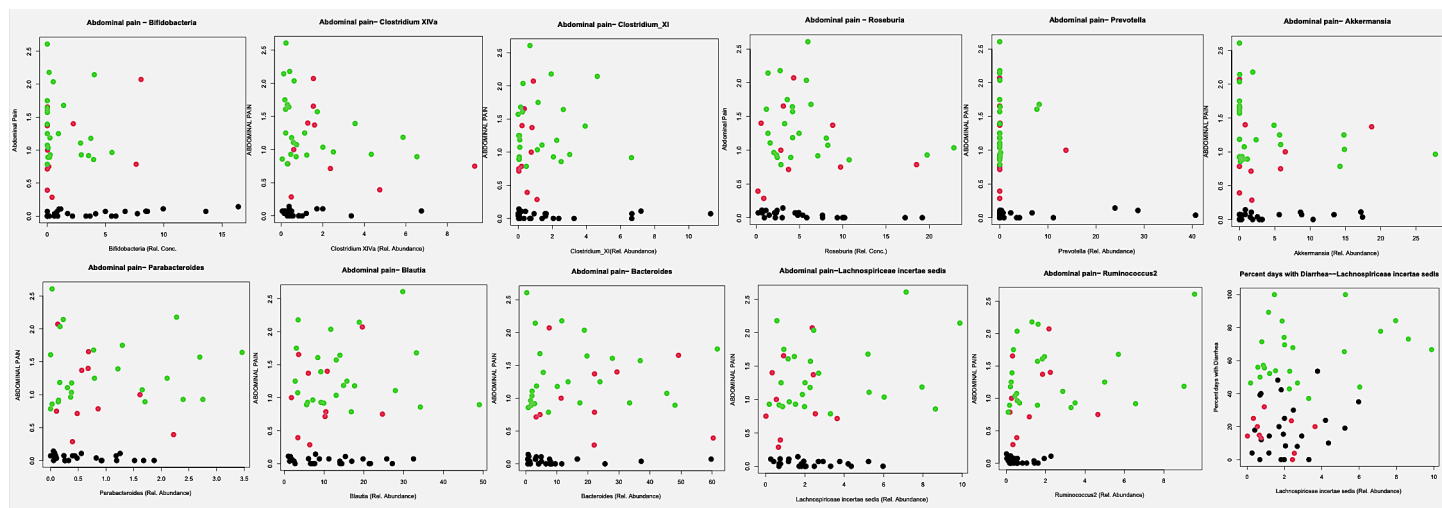


Figure 12c. Relationship of GI symptom (abdominal pain, percent days diarrhea) and microbe.

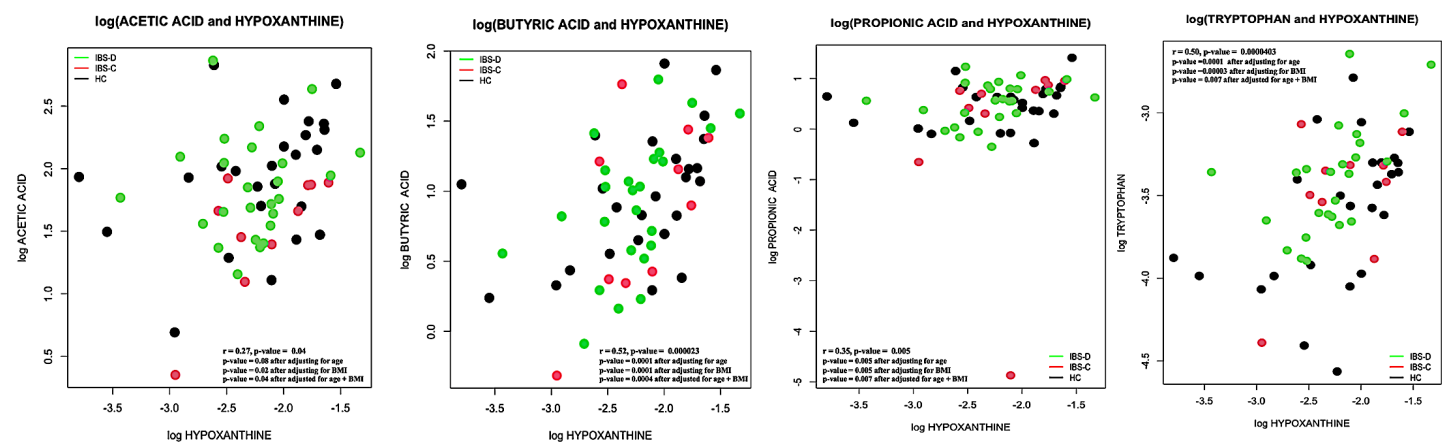


Figure 13a. Linear relationship of metabolites (absolute concentration)

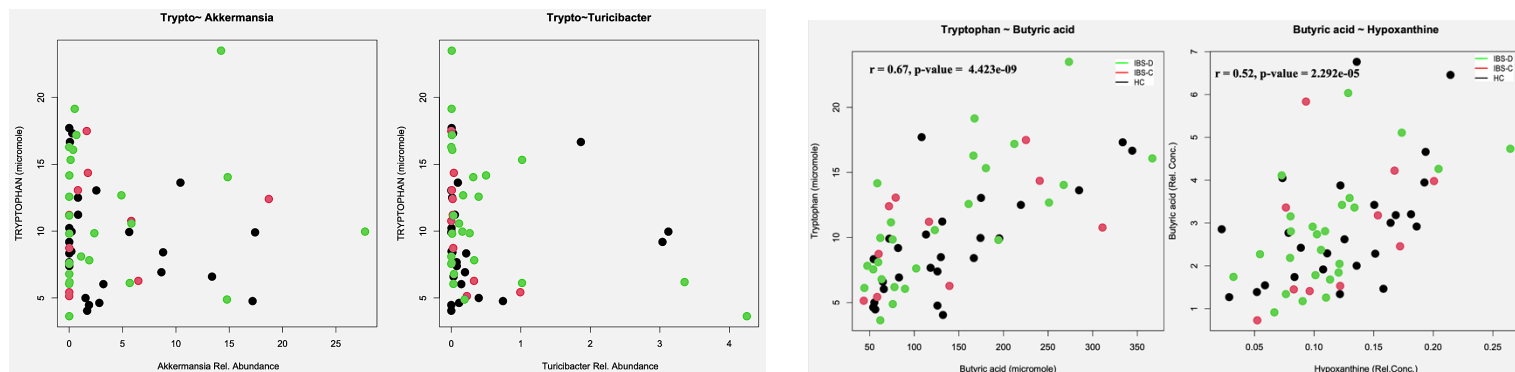


Figure 13b. Linear relationship of tryptophan and microbial tryptophan-metabolizers

There are reports in the literature about the relationship between butyric acid tryptophan metabolism. Tryptophan, an essential amino acid, is a precursor of serotonin (5HT). Aside from dietary sources, tryptophan can be derived from bacterial metabolic activity. Figure 13b shows a strong and positive relationship between tryptophan and butyric acid. The interactions of metabolic pathways involving tryptophan and butyrate metabolisms have been reported in literature (Liu et al., 2016; Morris et al., 2017; Mohseni et al., 2020). During inflammation, butyrate is known to suppress the degradation of tryptophan via suppressing IDO (indoleamine, 2-3 dioxygenase 1), multicomponent enzyme that catabolized the breakdown of tryptophan. Under inflammatory conditions, IDO1 is highly expressed. Butyrate as an anti-inflammatory, regulatory metabolite is also known to upregulate the levels of alpha-ketoglutarate, precursor of succinate known to regulate IDOs, Tryptophan is required for T cell survival and effector function. At high level, alpha-ketoglutarate is known to induce cell autophagy, a survival mechanism in times of scarce energy or limited nutrients availability. During an inflammatory process, increased glycolysis and lactate production (Warburg effect) provide the main source of energy for inflammatory immune cells, while during the resolution stage of inflammation, the cells rely on fatty acid oxidation as a main source for energy production.

This study found a serotonin-producing bacteria, *Turicibacter* to be positively associated with acetic, butyric acids, and tryptophan while a serotonin-consuming bacteria, *Alistipes* was positively associated with *Turicibacter* and *Akkermansia*. *Akkermansia* is well-known to positively correlated with tryptophan metabolism (Yin et al., 2021). Although non-significant, *Akkermansia* that is known to regulate the host's serotonin and has been shown to be lower in IBS (Wang et.al 2021). IBS-D women had higher counts of *Turicibacter* than IBS-C and slightly higher than healthy controls. Pearson correlation matrix showed that IBS is correlated with *Turicibacter* and propionic acid.

Using Pearson correlation, Figure 13c shows that butyric and valeric acids are negatively associated with abdominal pain in IBS ($r=-0,32$, $p=0,06$), while this negative association is also observed with tryptophan but weaker ($r =-0.28$, $p=0.098$). Although the p-values are not significant, the p-values of <0.1 may have clinical significance when considering the heterogeneity that comes with human samples.

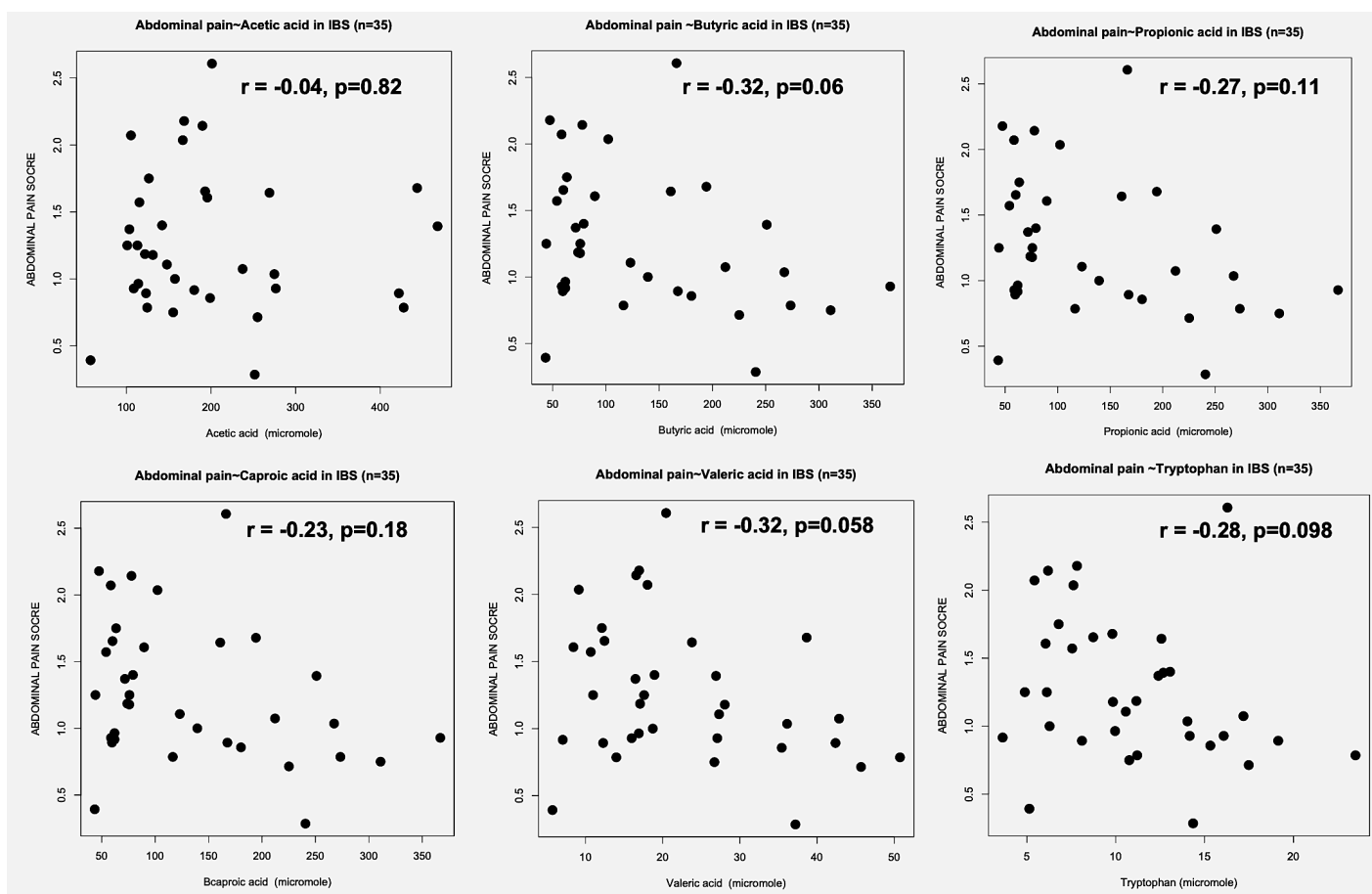


Figure 13c. Linear relationship of tryptophan and abdominal pain in IBS (n=35).

Figure 14 shows that several bacteria were found to be positively related with SCFA and other identified metabolites. In Figure 14a, bacteria genera such as *Alistipes* ($p=0.02$) and *Bacteroides* ($p=0.03$) showed significant negative relationships with acetic acid, while *Bifidobacteria* was positively and significantly correlated with acetic acid ($p=0.03$). In Figure 14c, total sc-SCFAs was found to be significantly correlated with *Lachnospiraceae incertae sedis* ($r=0.4$, $p=0.002$), while *Bacteroides* was significantly and negatively correlated with sc-SCFA. Consistent with existing literature, lactic acid-utilizing bacteria such as *Eubacteria*, *Veillonella*, *Coprococcus*, and *Anaerostipes*, appeared to be negatively correlated with lactic acid, while lactic acid-producing bacteria such as *Enterococcus*, *Lactobacillus*, and *Bifidobacteria* appeared to be positively correlated with lactic acid (see Figure 14d).

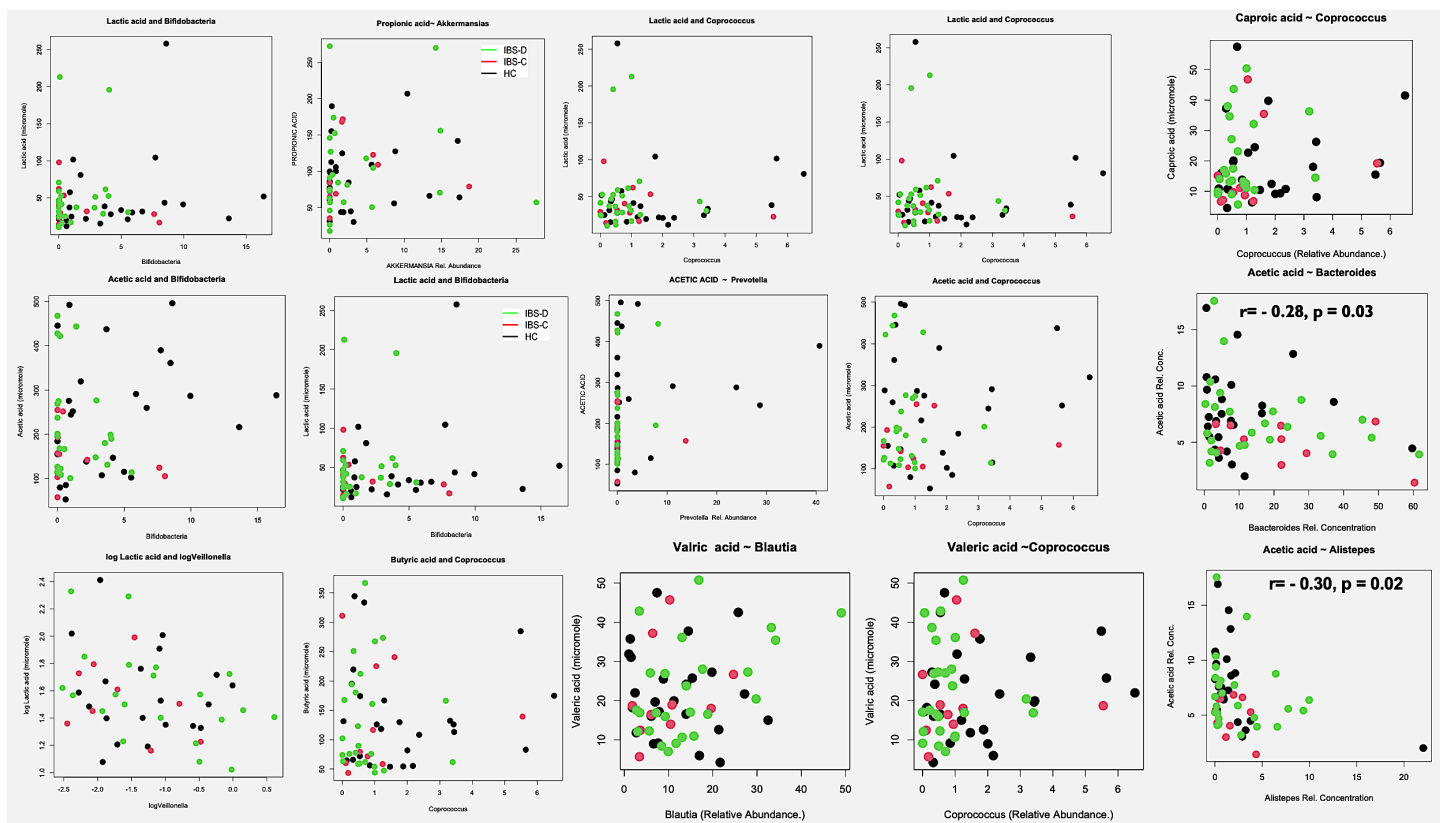
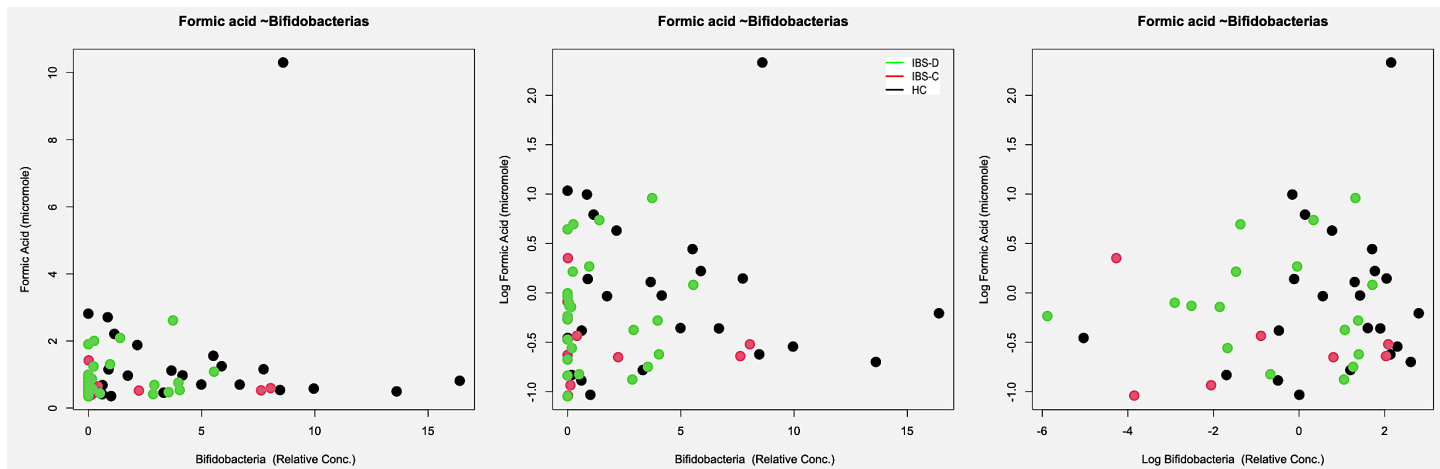


Figure 14a. Relationship of microbiota genera and its metabolic function



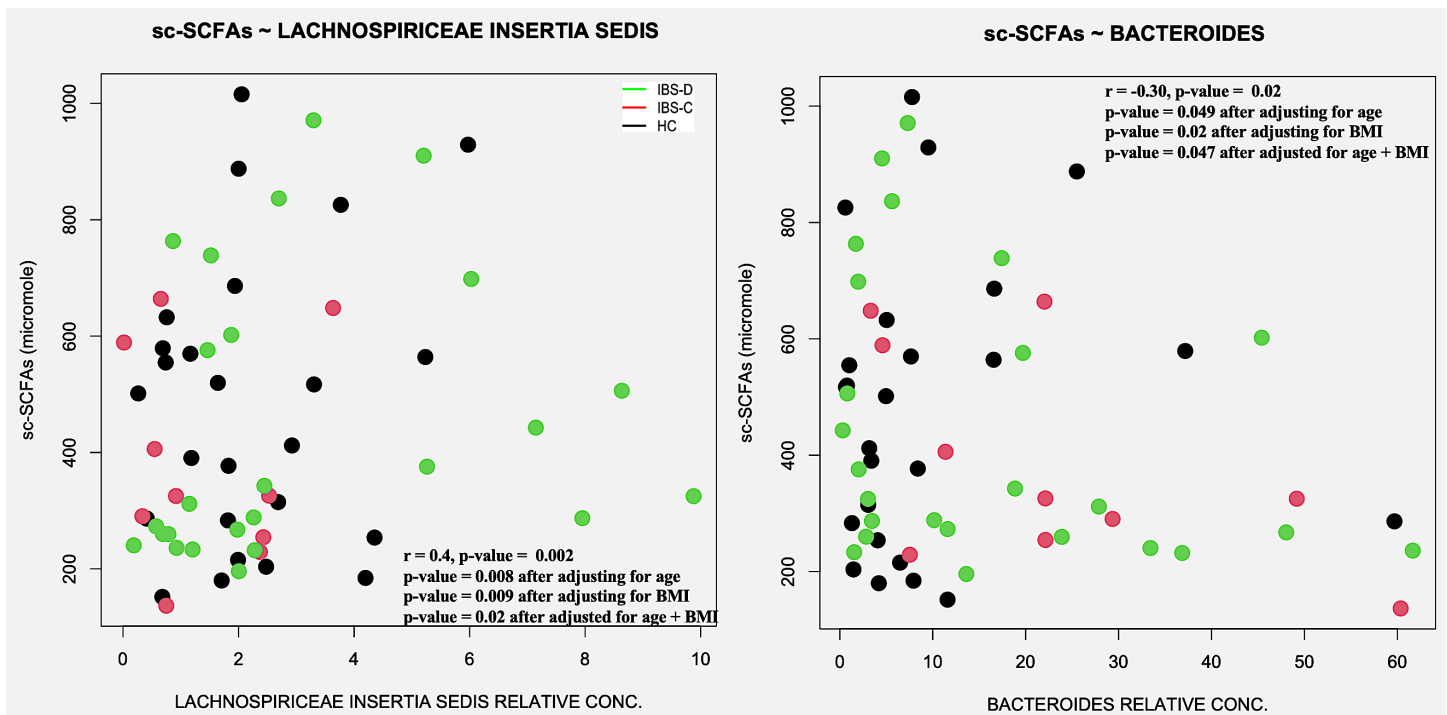


Figure 14b. Relationship of microbiota genera and its metabolic function

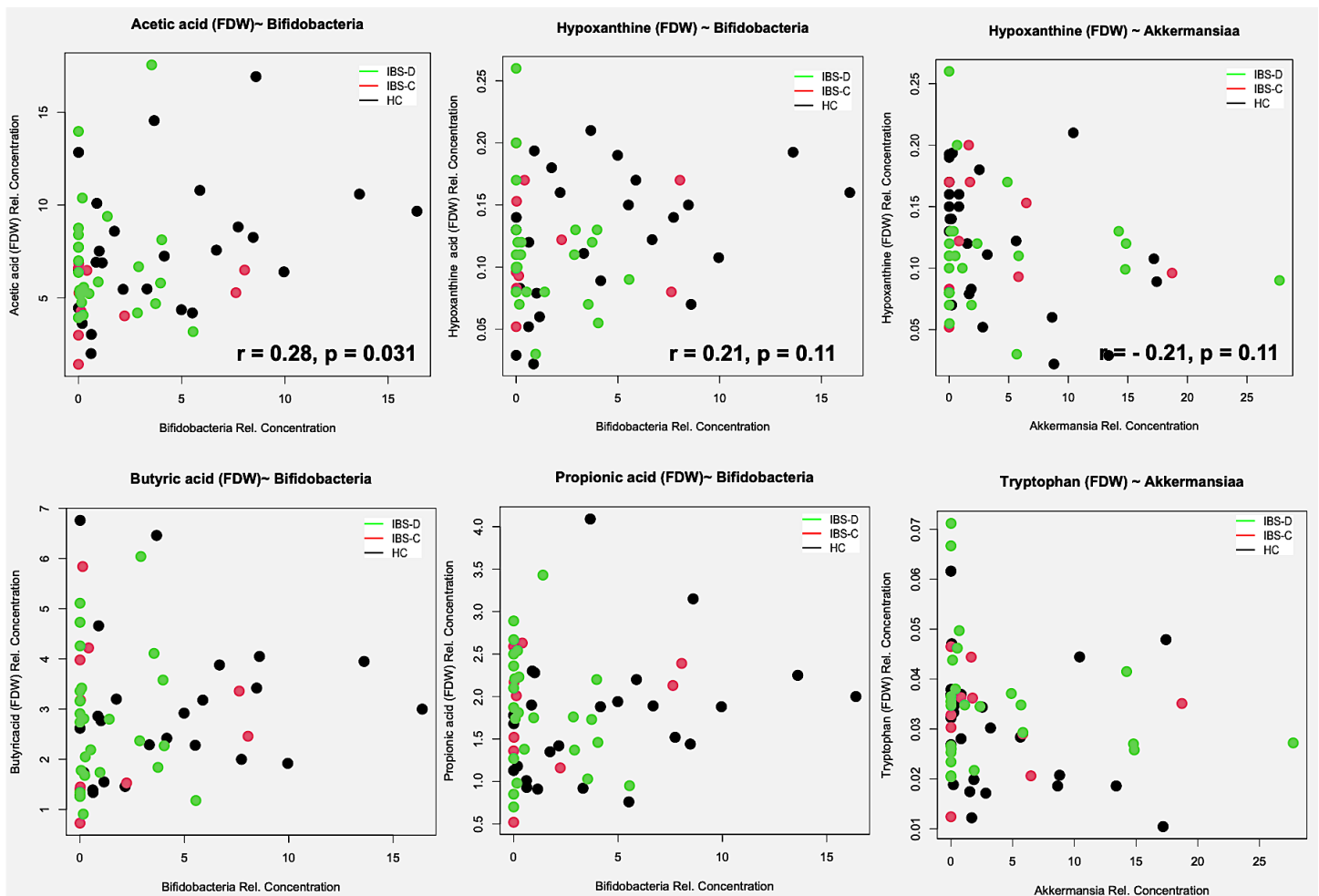


Figure 14c. Relationship of microbiota genera and its metabolic function

Bacteria-Metabolite Relationship. Pearson's matrix correlations using R found strong positive relationships between several of the *Clostridia* genera and the metabolite, putrescine ($r=0.45$ to 0.56). *Clostridium XVIII* is positively associated with succinic acid ($r=0.48$), *Blautia* ($r=0.37$), which is also positively associated with succinic acid ($r = 0.46$).

Using Pearson's matrix correlation, *Bifidobacteria* was found to be positively associated with 2-aminobutyric acid ($r = 0.39$), valeric acid ($r = 0.37$), propionic ($r = 0.35$) and acetic acid. *Blautia* was positively associated with lactic acid ($r = 0.35$). *Atopobium* is positively correlated with propionic acid ($r = 0.38$). *Unclassified Proteobacteria* was positively associated with caproic acid ($r=0.43$) and acetic acid ($r = 0.32^*$) and butyric acid ($r = 0.27$). Univariate analyses showed that *Clostridium XI* is positively associated with acetic acid normalized with TSP ($r = 0.38$, $p = 0.002$). *Lachnospiraceae incertae sedis* was also positively associated with lactic acid ($r = 0.39$, $p = 0.002$). *Unclassified Acidominocaceae* was positively correlated with tryptophan ($r=0.42$) and positively related to urocanic acid ($r = 0.46$). Note: Higher Tryptophan ($p = 0.02$) was observed in the High SCFA Producers HC women compared to its Low SCFA Producer women.

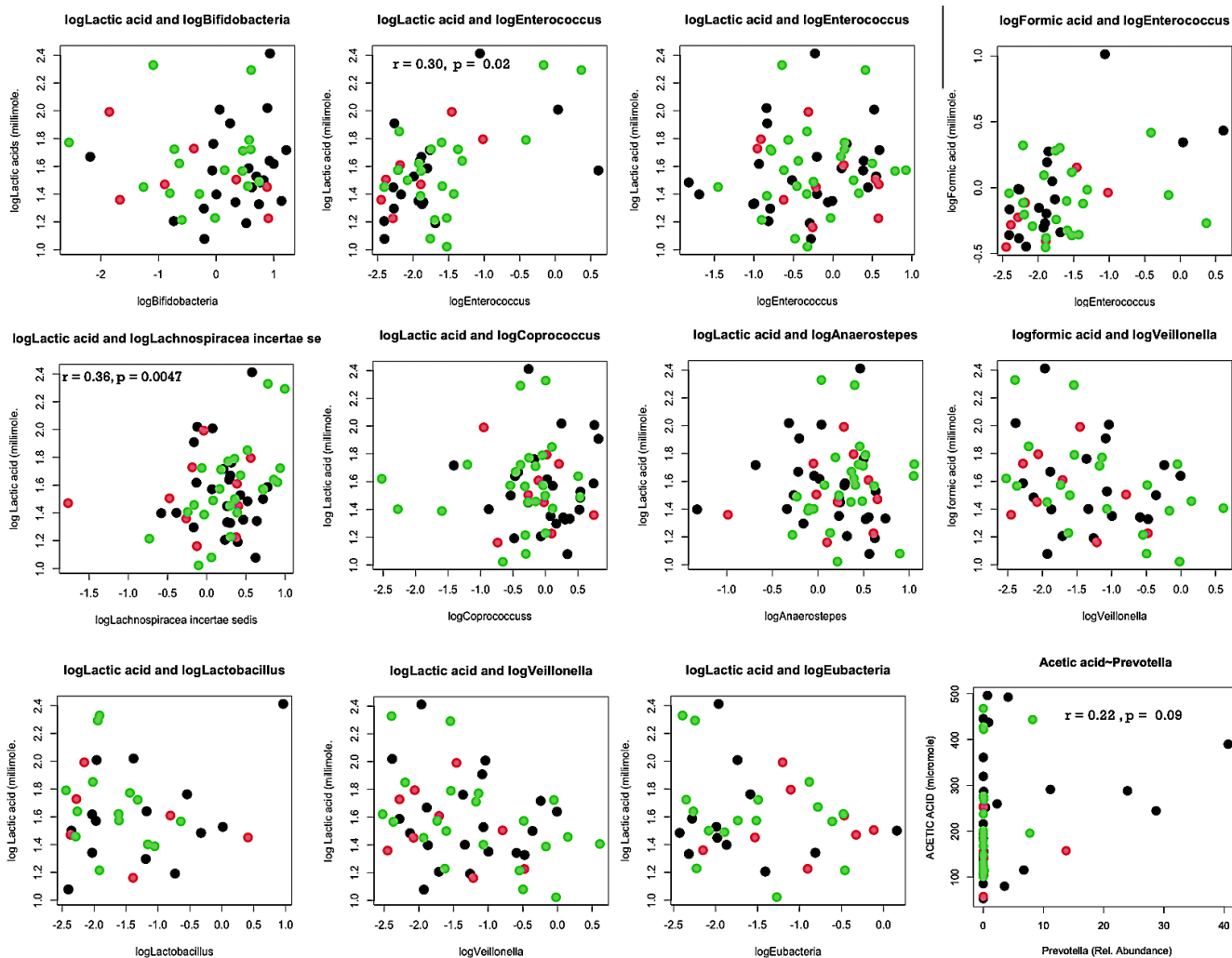


Figure 14d. Relationship of microbiota genera and its metabolic function

Bacteria-Bacteria Relationship. In Figure 15, bacterial genera, *Prevotella* and *Ruminococcus* are positively correlated, mostly driven by HC and IBS-C groups. The pairs of bacteria that were positively and significantly correlated are the following: *Blautia* and *Dorea*, *Lachnospiraceae incertae sedis* and *Dorea*, *Lachnospiraceae incertae sedis* and *Blautia*.

Pearson's matrix correlations (via R software 3.6.1). *Blautia* was positively correlated with *Anaerostipes* ($r = 0.39$), *Lachnospiraceae* ($r = 0.49$), *Dorea* ($r = 0.39$), and *Actinomyces* ($r = 0.37$). *Blautia* was negatively associated with *Bacteroides* ($r = -0.45$). *Bifidobacteria* is positively associated with *Unc. Pasteurellaceae* ($r = 0.39$) and with 2-minobutyric acid ($r = 0.39$), valeric acid ($r = 0.37$), propionic ($r = 0.35$) and acetic ($r = 0.27$). Literature reported that *Bifidobacteria* is known to produce GABA (Strandwitz, 2018). Interestingly,

Bifidobacteria is positively related to BMI ($r=0.31$). *Bilophila* ($r=0.4$), but negatively related to *Megasphaera* ($r = -0.37$).

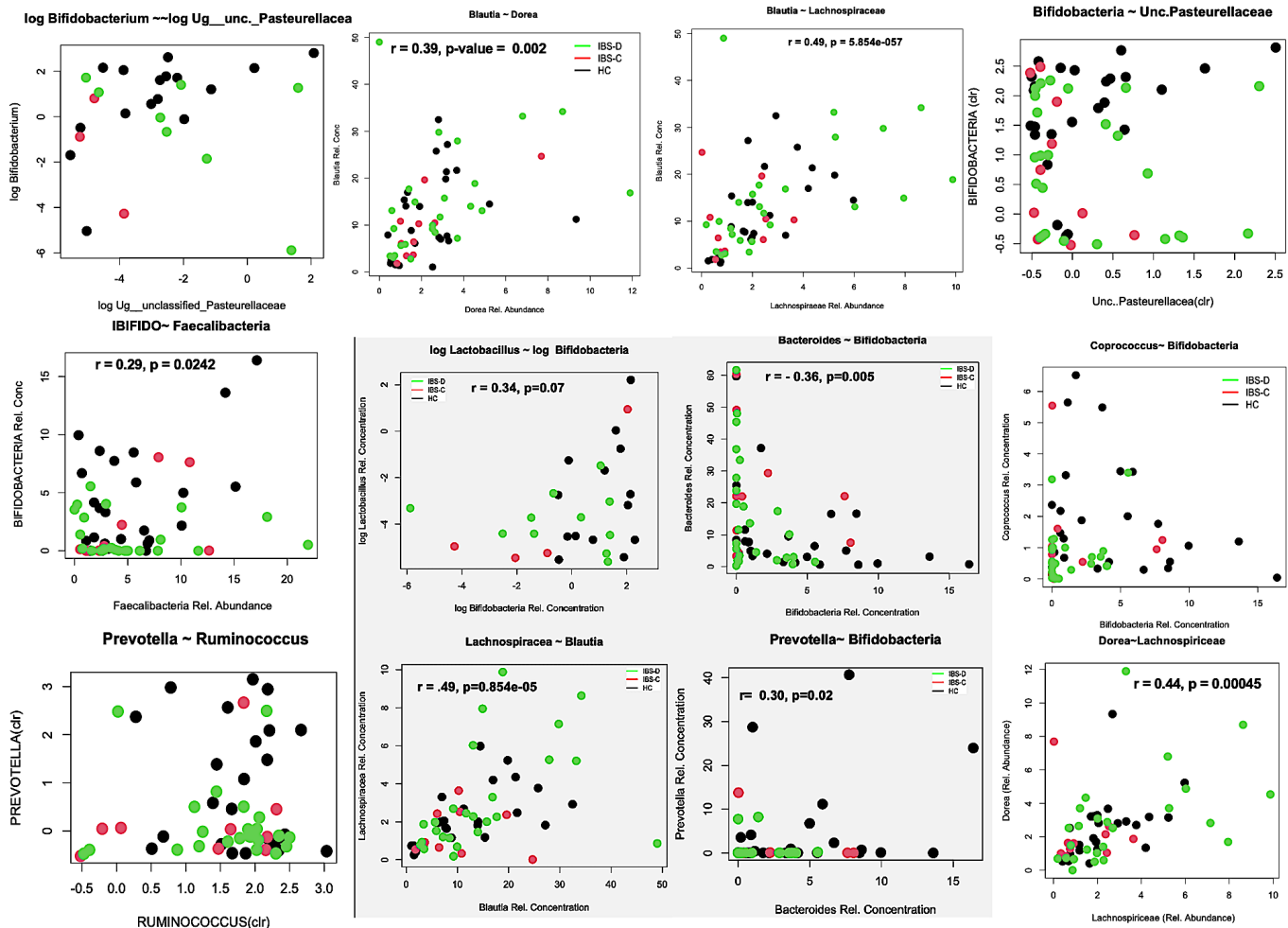


Figure 15. Relationship of microbe to microbe.

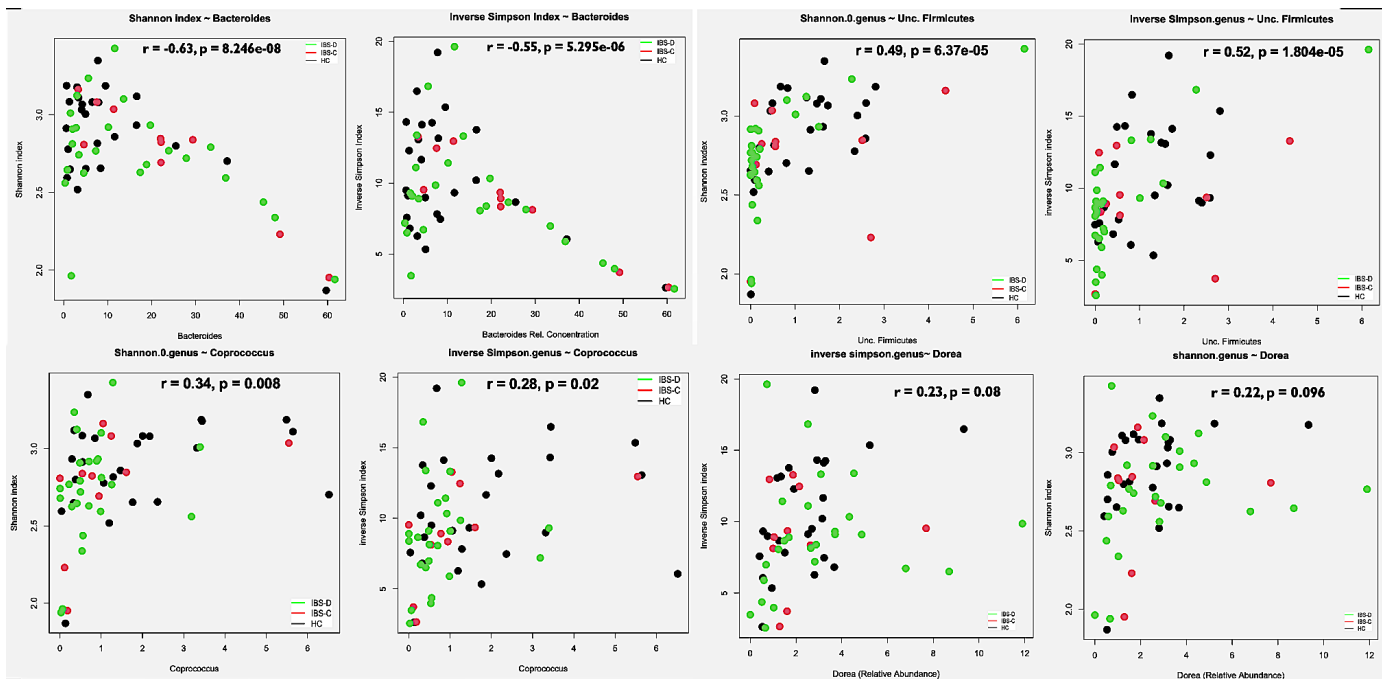


Figure 16a. Relationship of specific microbe and microbial diversity indices.

Gut microbial diversity is influenced by diet, colonic transit time, and the microbial-hosts cometabolites such as tryptophan, tyrosine, threonine, other amino acids (lysine, arginine, histidine, isoleucine, alanine, phenylalanine, proline, valine, glutamic acid), hypoxanthine, serotonin, glutamate, gamma-aminobutyric acid (GABA), dopamine, norepinephrine, and acetylcholine, serine proteases (Xiao et al., 2021; Morris et al., 2016; Strandwitz, 2018; Zhang et al., 2022; Corre et al., 2022; Ruiz-Perez & Nataro, 2014; Maiti et al., 1995). Having long colonic transit time (CTT) has been associated with increased microbial richness (Procházková et al., 2022).

Interestingly, high growth of *Blautia* and *Bacteroides* were associated with decreasing trend of alpha diversity indices, while *Coprococcus* follows the opposite trend. *Coprococcus*, a well-known microbe that is associated with wellness and health increases microbial diversity (see Figure 16a and Figure 16b).

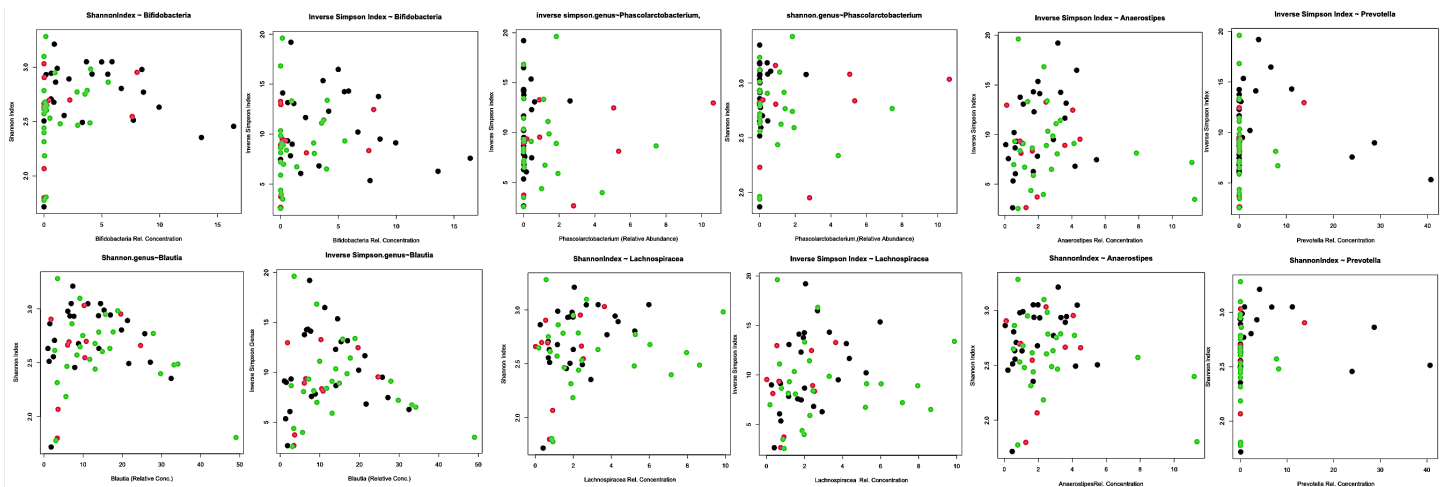


Figure 16b. Relationship of microbial diversity index and individual microbe.

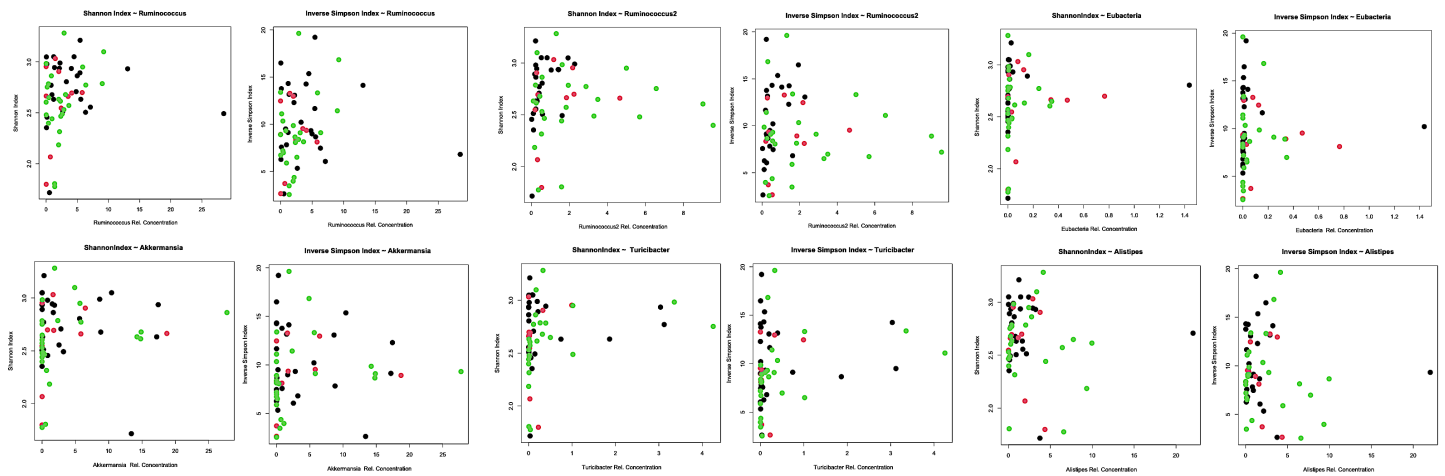


Figure 16c. Relationship of microbial diversity index and individual microbe.

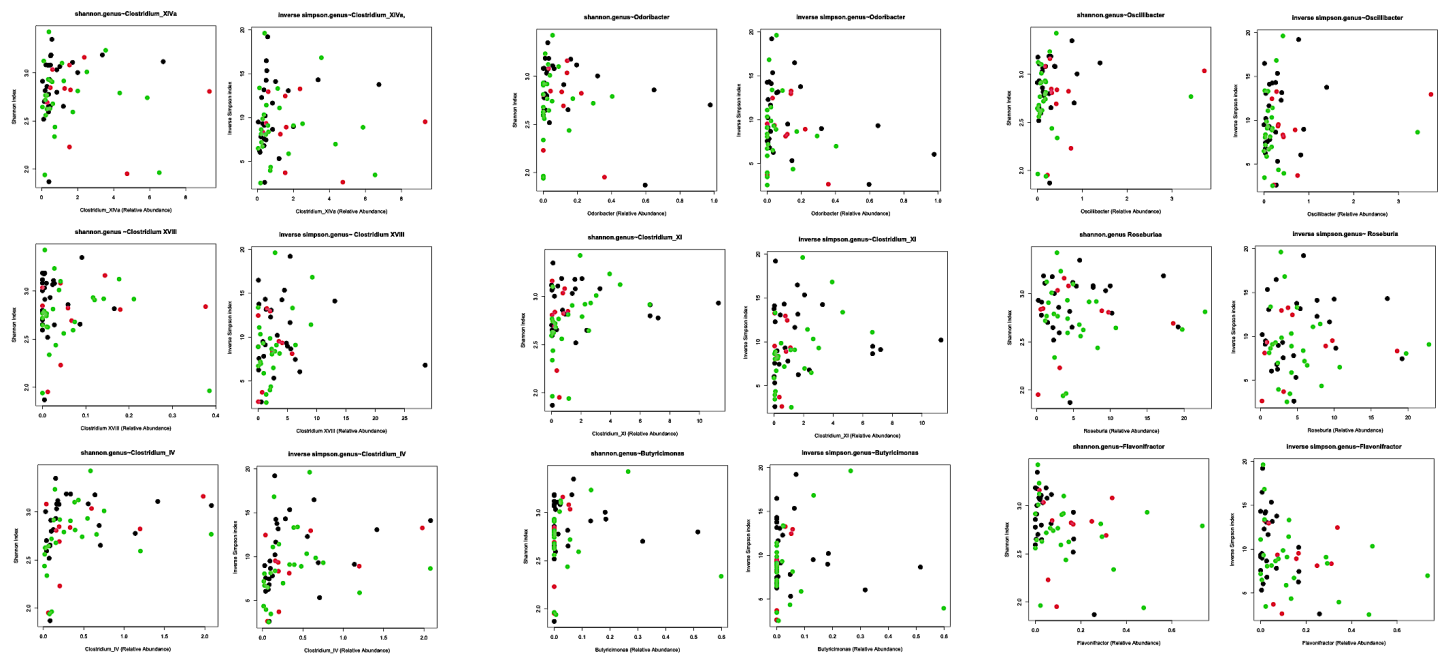


Figure 16d. Relationship of microbial diversity index and individual microbe.

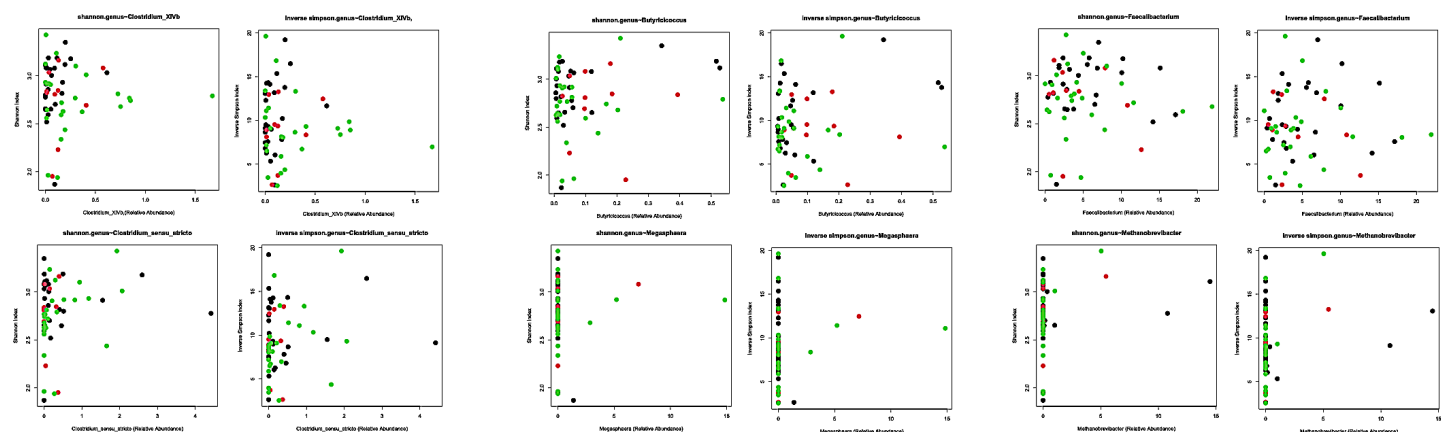


Figure 16e. Relationship of microbial diversity index and individual microbe.

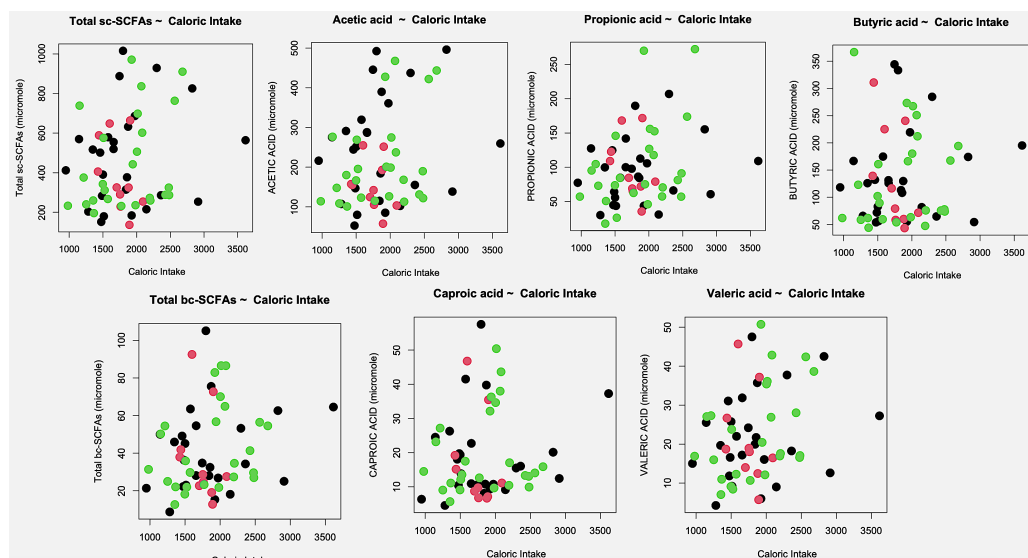


Figure 17. Relationship of caloric intake and SCFA.

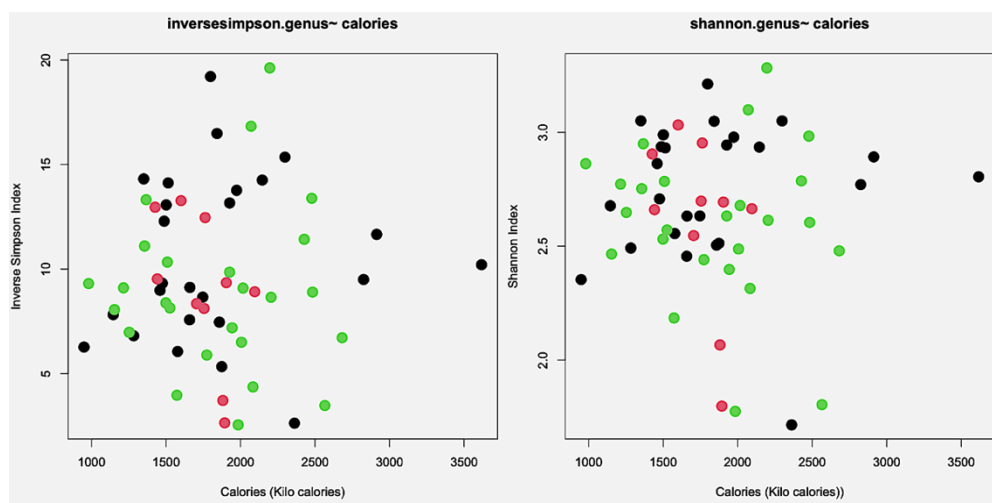


Figure 18. Relationship of caloric intake and microbial diversity indices.

In Figure 18, higher total caloric intake was negatively associated with microbial diversity.

As shown in Figure 20b, the relationship of dietary total fiber and total fat intake was plotted against total SCFA. As shown in the scatter plot, it appears that total fat may have higher contribution in the production of SCFA when compared to the dietary fiber's influence on SCFA production.

All Women. Bacteria that were enriched under the context of high fiber diet (>25g/day) include the following: *Saccharibacteria* (p = 0.07), *Unc. Porphyromonadaceae* (p=0.06), *Unc. Firmicutes* (p=0.09), *Odoribacter* (p=0.06), *Alistipes* (p = 0.07), *Clostridium XIVb* (p = 0.06), *Bacteroides* (p = 0.15), *Clostridium XIVa* (p = 0.22), *Eubacteria* (p =0.24), and *Veillonella* (p=0.11). On the other hand, the bacteria that were enriched under low DF (<25g/day) include: *Mogibacteria* (p =0.02), *Enterococcus* (p =0.04), *Eggerthella* (p = 0.05), *Megasphaera* p = 0.07), *Dorea* (p=0.07); *Lactobacillus* (p = 0.08), *Clostridia XVIII* (p=0.09), *Unc. Bacteria* (p =0.08), *Bilophila* (p = 0.12), *Streptococcus* (p =0.13), *Flavonifactor* (p = 0.14), *E. Shigella* (p = 0.16), *Prevotella* (p = 0.17), *Turicibacter* (p = 0.17), *Bifidobacteria* (p=0.24), *Akkermansia* (p=0.25), and *Unc. Prevotellaceae* (p = 0.24).

HC. On the average, HC women fiber intake a day is about 34g/day. Bacteria that were enriched under the context of high fiber diet include the following: *Bacteroides* (p = 0.04*), *Odoribacter* (p = 0.07), *Flavonifactor* (p = 0.07), *Eubacteria* (p = 0.10), *Veillonella* (p = 0.10), *Faecalibacteria* (p = 0.14), *Alistipes* (p = 0.18), *Megasphaera* (p = 0.2), *Roseburia* (p = 0.02), *Clostridium XIVb* (p = 0.14), *Megasphaera* (p = 0.17), *Oscillibacter* (p = 0.27).. On the other hand, the bacteria that were enriched under low DF include: *Ruminococcus2* (p = 0.06), *Unc. Proteobacteria* (p = 0.10), *Dorea*, (p = 0.08), *Enterococcus* (p = 0.12), and *Streptococcus* (p = 0.29).

IBS-C. On the average, HC women fiber intake a day is about 29 g/day. Bacteria that were enriched under the context of high DF were *Clostridium XIVa* (p = 0.10), *Unc. Bacteria* (p=0.13), *Unc. Pasteurellaceae* (p = 0.17), *Unc. Bacteroidetes* (p = 0.19), *Collinsella* (p = 0.20), *Dorea* (p=0.20), *Butyricicoccus* (p = 0.23), while *Faecalibacteria* (p = 0.04*), *Bifidobacteria* p = 0.08), *Dialister* (p = 0.07), *Mogibacteria* (p = 0.08), *Acidominococcus* (p = 0.11), *Atopobium* (p = 0.15), *E. Shigella* (p = 0.16), *Prevotella* (p = 0.19), *Turicibacter* (p

= 0.20), *Megasphaera* (p = 0.20), *Coprococcus* (p = 0.22), *Roseburia* (p = 0.24), *Clostridium XIVb* (p = 0.21), were enriched under low DF setting.

IBS-D. On the average, HC women fiber intake a day is about 20 g/day. Bacteria that were enriched under the context of high DF were: *Unc. Porphyromonadaceae* (p = 0.0007***), *Unc. Firmicutes* (p = 0.0003***), *Unc. Bacteria* (p = 0.13), *Unc. Bacteroidetes* (p = 0.08), *Bilophila* (p = 0.13), *Alistipes* (p = 0.07), *Veilonella* (p = 0.19), *Unc. Ruminococcus* (p = 0.12), *Unc. Ruminococcaceae* (p = 0.12), *Finegoldia* (p = 0.22), *Coprococcus* (p = 0.26), and *Clostridium IV* (p = 0.28).

On the other hand, the bacteria that were enriched under low DF include: *Bifidobacteria* (p = 0.01), *Eggerthella* (p = 0.02*), *Clostridium XVIII* (p = 0.04*), *Blautia* (p = 0.17), *Dorea* (p = 0.06), *Dialister* (p = 0.05), *Clostridium XIVb* (p = 0.05), *Roseburia* (p = 0.05), *Abiotrophia* (p = 0.04), *Faecalibacteria* (p = 0.06), *Acidominococcus* (p = 0.16), *Actinomyces* (p = 0.08), *Turcibacter* (p = 0.09), *Megasphaera* (p = 0.11), *Mogibacteria* (p = 0.21), *Enterococcus* (p = 0.12), *Unc. Pasteurellaceae* (p=0.15), *Prevotella* (p = 0.11), *Collinsella* (p = 0.25), *Flavonifactor* (p = 0.18), *Oscillibacter* (p = 0.13), and *Streptococcus* (p = 0.19).

SCFA (micromole) & Lactose intake

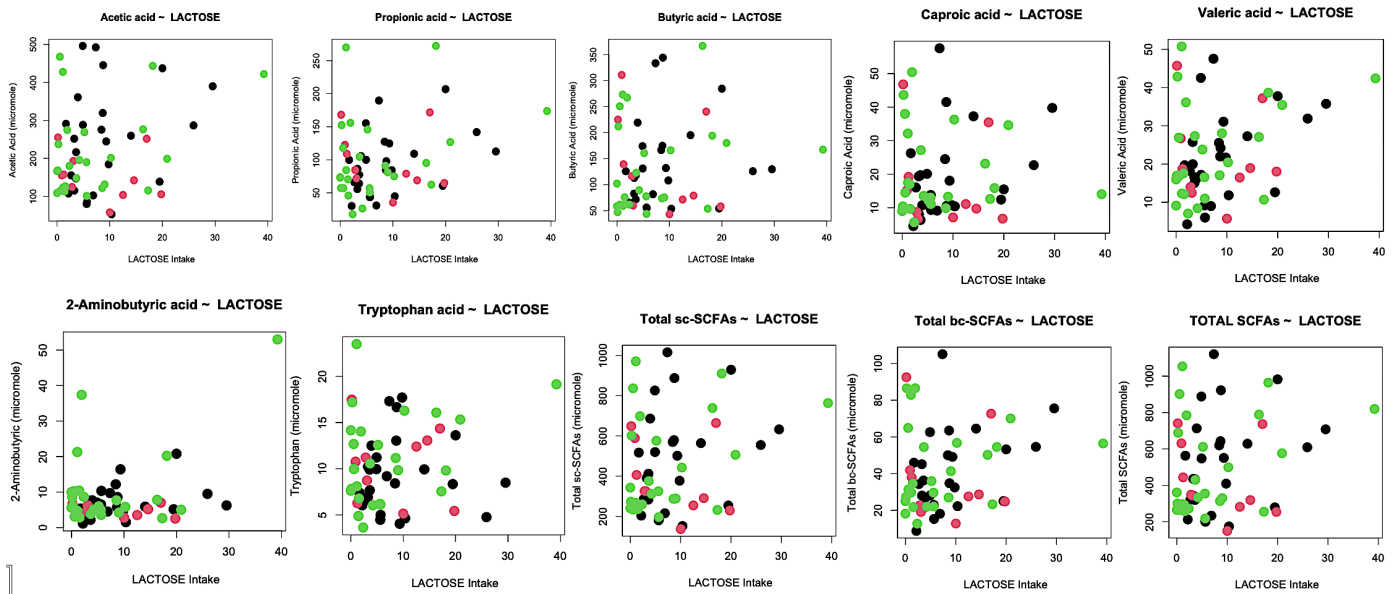


Figure 19. Relationship of lactose intake and SCFA.

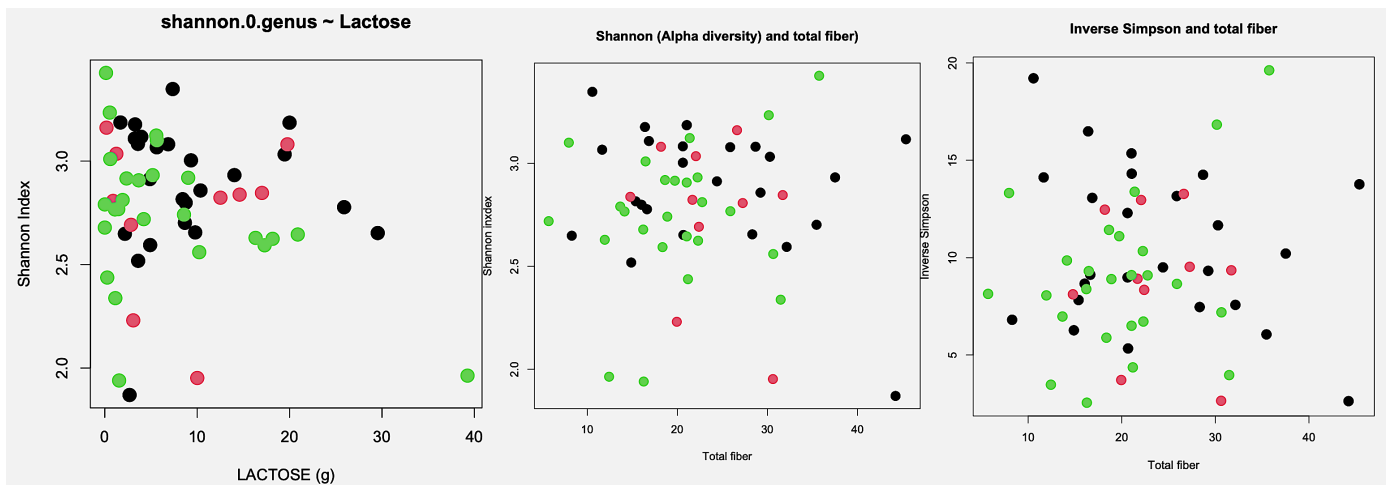
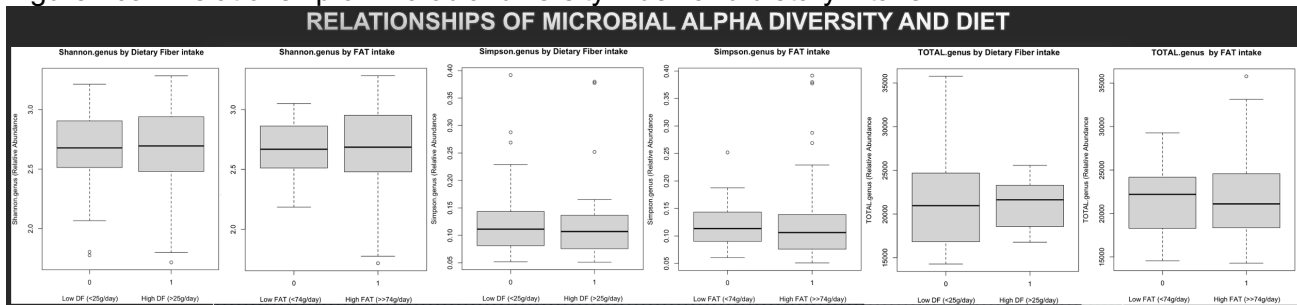


Figure 20a. Relationship of microbial diversity index and dietary intake.



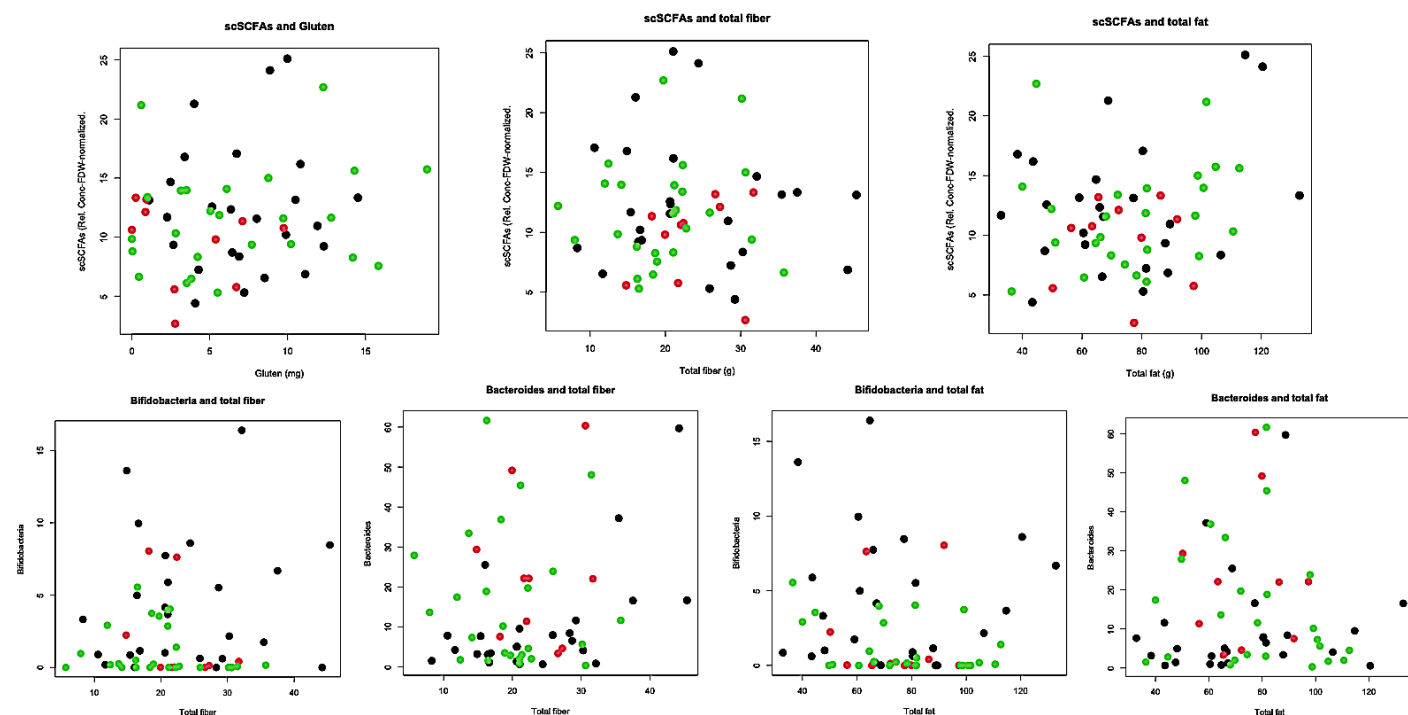


Figure 20b. Relationships of dietary fat and dietary fiber with total SCFA and microbes.

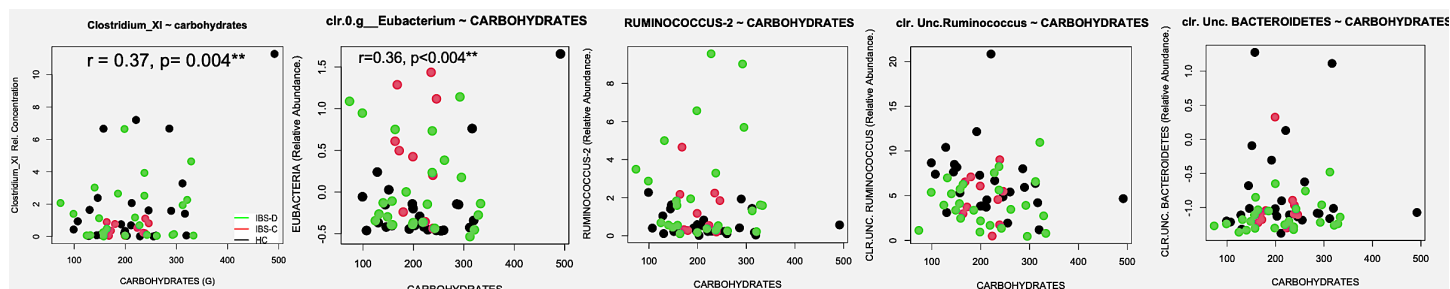


Figure 21. Relationship of carbohydrate-fermenting microbe and carbohydrate.

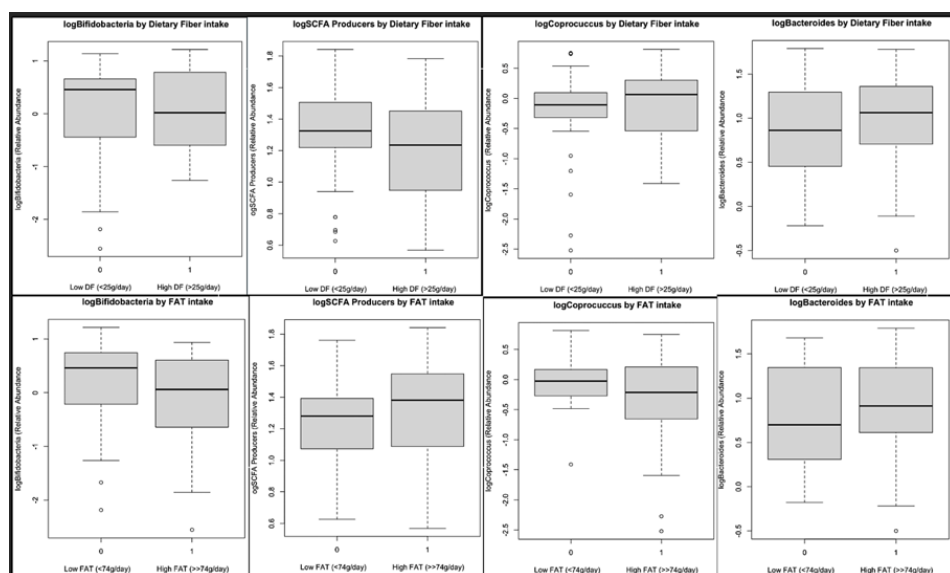


Figure 22a. Boxplots illustrating the Relative Abundance of Bacteria under high fiber diet (>25g/day).

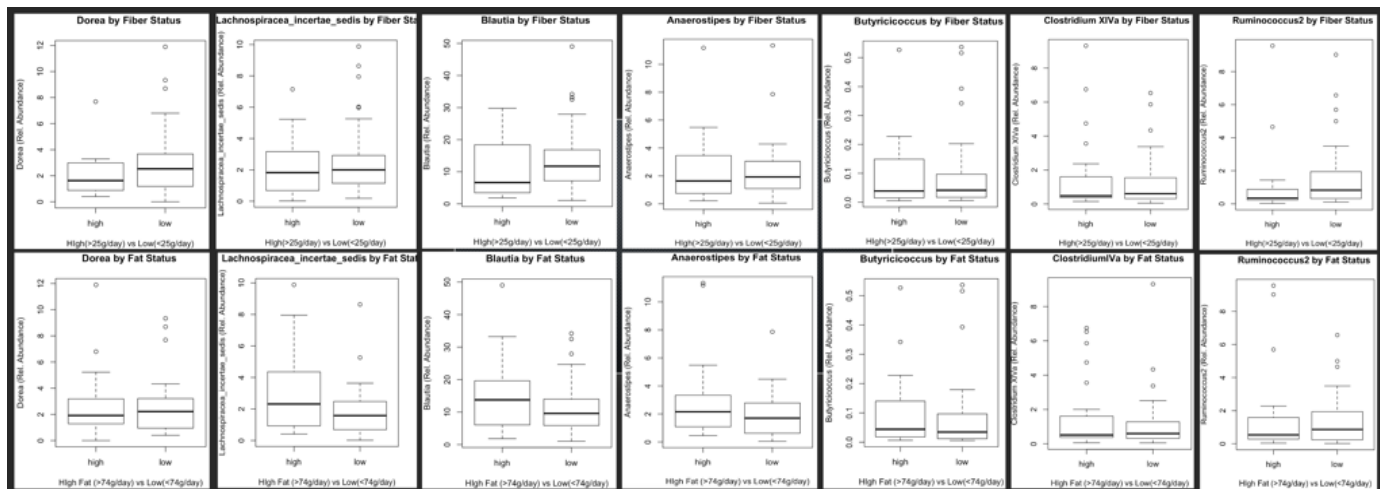


Figure 22b. Boxplots illustrating the Relative Abundance of Bacteria under high fiber diet (>25g/day)

The primary complex carbohydrate degraders from the unclassified phylum *Bacteroidetes*, and the secondary carbohydrate degraders, *Eubacteria*, *Clostridium XI*, *IRuminococcus2* were found to be positively correlated with carbohydrates (see Figure 21). In Figure 20, higher total fiber intake was found to be associated with higher microbial diversity. Unlike Wu et. al 2011 study, the current study found *Prevotella* to be associated with the higher fat intake diet (>74g/day) and low fiber intake diet (<25g/day) but not significant ($p=0.05$).

In Figure 23, microbial diversity is correlated with higher microbial SCFA Producers at midpoint, however, microbial diversity tends to go downward after the measured relative abundance at 25. Interestingly, the measured total fecal SCFA was associated with increasing trend of microbial diversity (See Figure 23).

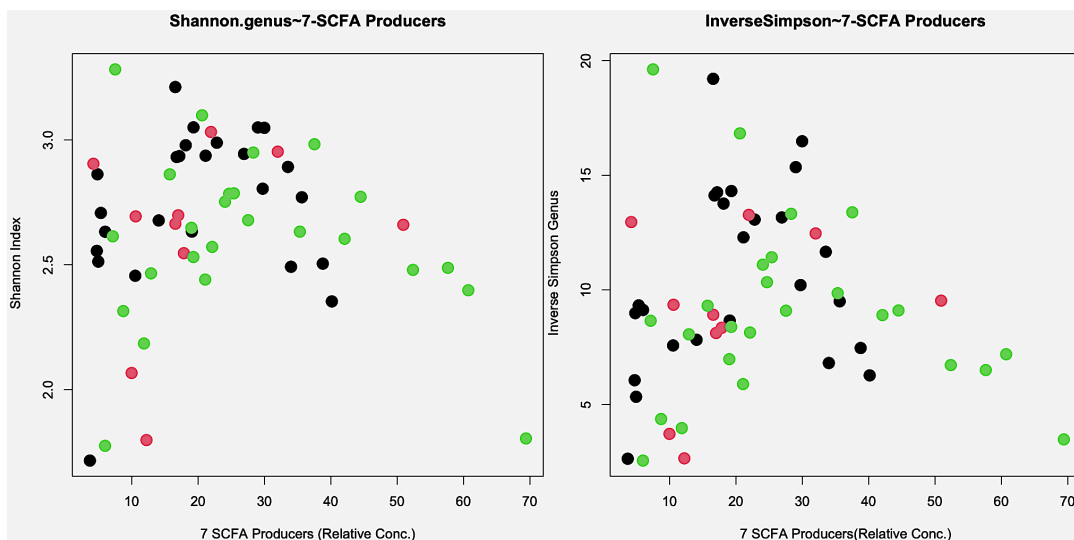


Figure 23. Relationship of the 7-SCFA Producers and Microbial Diversity Indices.

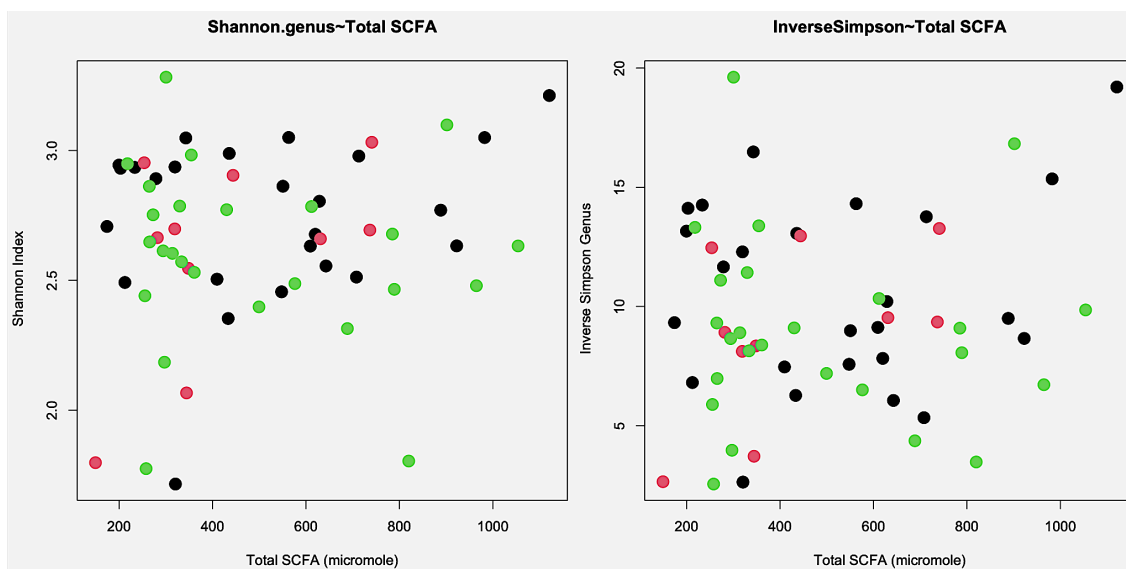


Figure 24. Relationship of the Total SCFA and Microbial Diversity Indices.

MetaboAnalyst 5.0 Result. MetaboAnalyst 5.0 software (<https://www.metaboanalyst.ca>) was used to look at the metabolic pathways that were enriched in IBS relative to HC. Pathways associated with SCFA metabolism include the following: aspartate metabolism, amino sugar metabolism, ethanol degradation, pterine biosynthesis, androgen and estrogen metabolism, androstenedione metabolism, tryptophan metabolism, fatty acid biosynthesis and pyruvate metabolism were found.

The PLS-DA analysis demonstrated that the metabolome of the IBS-D women appeared to deviate the most and that IBS-C women the least relative to healthy control women. This trend is reflected in the gut microbiota profile that was reported by Lyra et al., (2009).

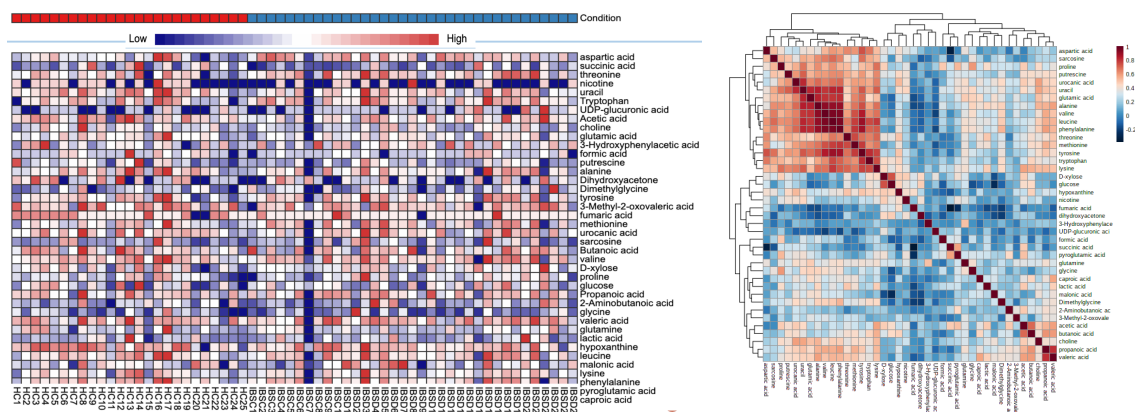


Figure 25. Heatmap of the metabolites (relative abundance) identified via NMR

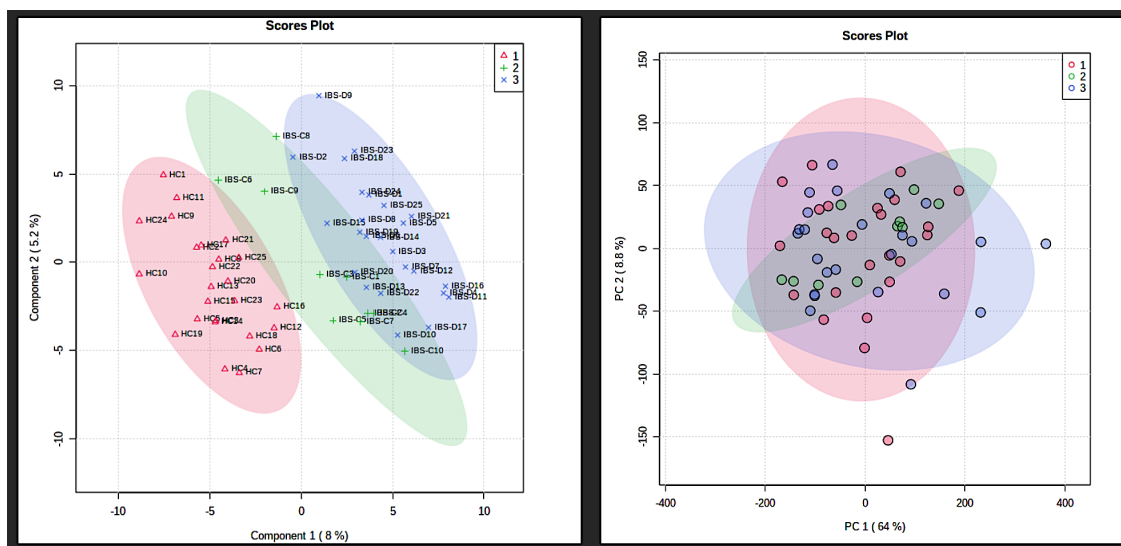


Figure 26. Principal component analysis from MetaboAnalyst 5.0 revealed separations of the metabolites (relative abundance) in the three groups of women.

According to the authors of the MetaboAnalyst5.0, the enrichment analysis is based on Global Test; p-values were approximated based on the asymptotic distribution without using permutations (Goeman & Bühlmann,2007). The rank of the pathway is primary focus instead of its absolute p-values. The selected node importance measure for topological analysis is relative betweenness centrality which measures the number of shortest paths going through the node of global metabolic networks. The interconnectedness of metabolites tells the molecular status of health and disease.

The metabolic profiles of healthy women differed with that of women with IBS. Because a person with IBS-C phenotype is distinctly different from that of a woman with IBS-D, it is best to look at the metabolic profile

of each IBS subtype with respect to healthy women or healthy controls (HC). The distinct separation of metabolites may indicate degrees of dysbiosis. Below is the pathway analysis result for HC vs IBS combined.

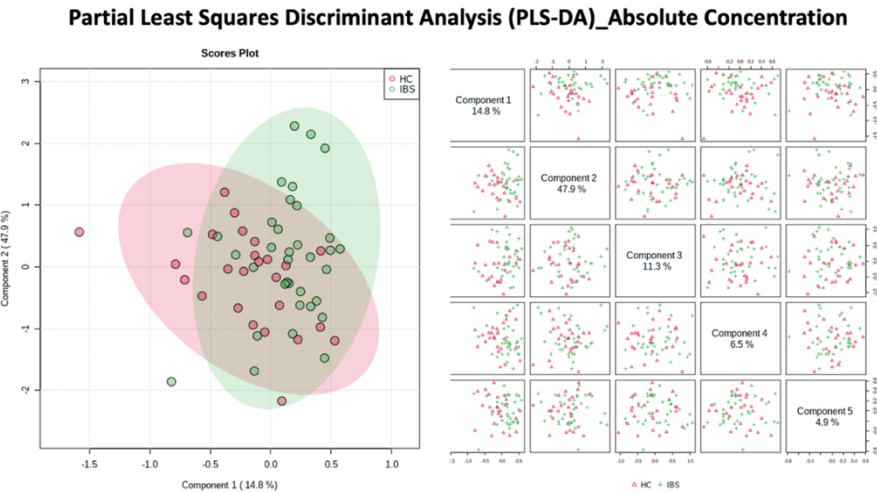


Figure 27. Principal component analysis of HC vs. IBS women.

Analysis: HC/IBS

fold_change-3

	Fold Change	log2(FC)
formic acid	1.6103	0.68733
acetic acid	1.2686	0.34328
2-Aminobutanoic acid	0.82159	-0.2835

Sig. threshold

At least 2 fold difference

Parameter

FC = 2 or FC = 0.5

Formula

$M1/M2 > 2$ or $M1/M2 < 1/2$

At least 5 fold difference

FC = 5 or FC = 0.2

$M1/M2 > 5$ or $M1/M2 < 1/5$

Any difference between two groups

FC = 1.0

$M1/M2 > 1$ or $M1/M2 < 1$

PLSDA Absolute Concentration

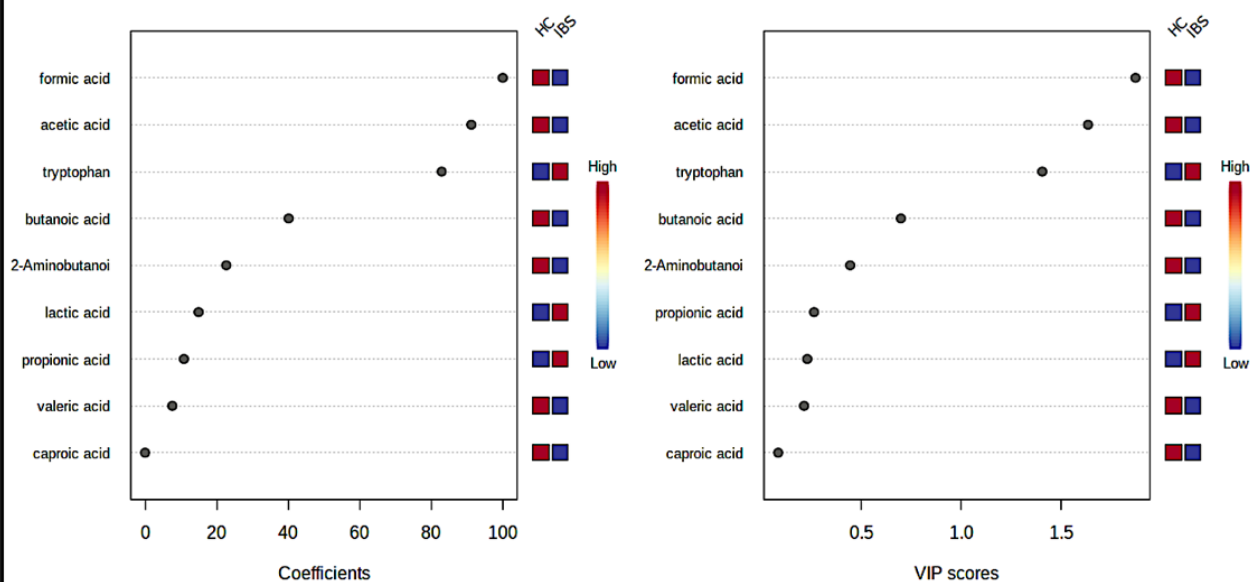


Figure 28. Metabolic biomarkers (absolute concentration) in IBS vs. HC women.

Partial Least Squares Discriminant Analysis (PLS-DA) for the 9 Metabolites of Interest (Absolute Concentration)

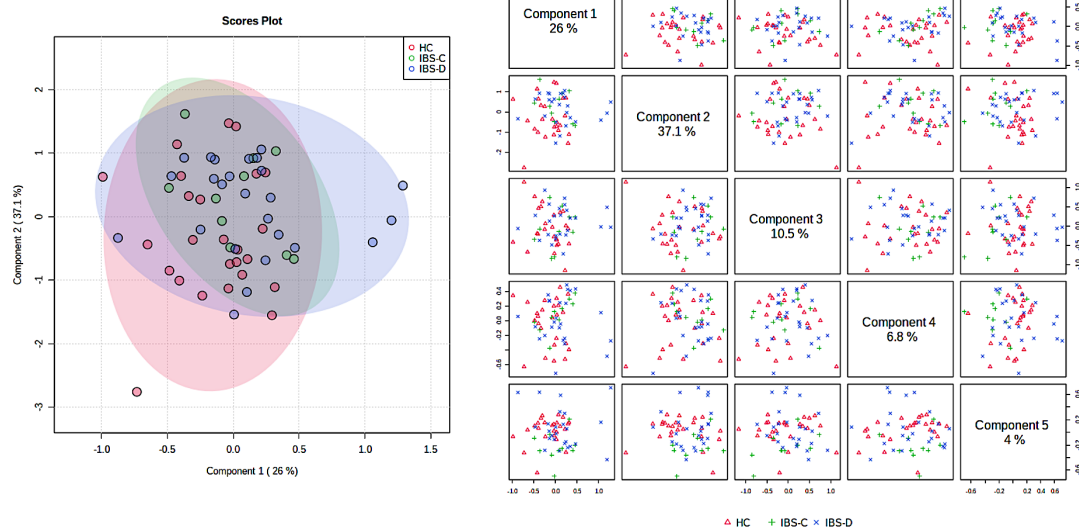


Figure 29. Principal component analysis of absolute concentration of metabolites.

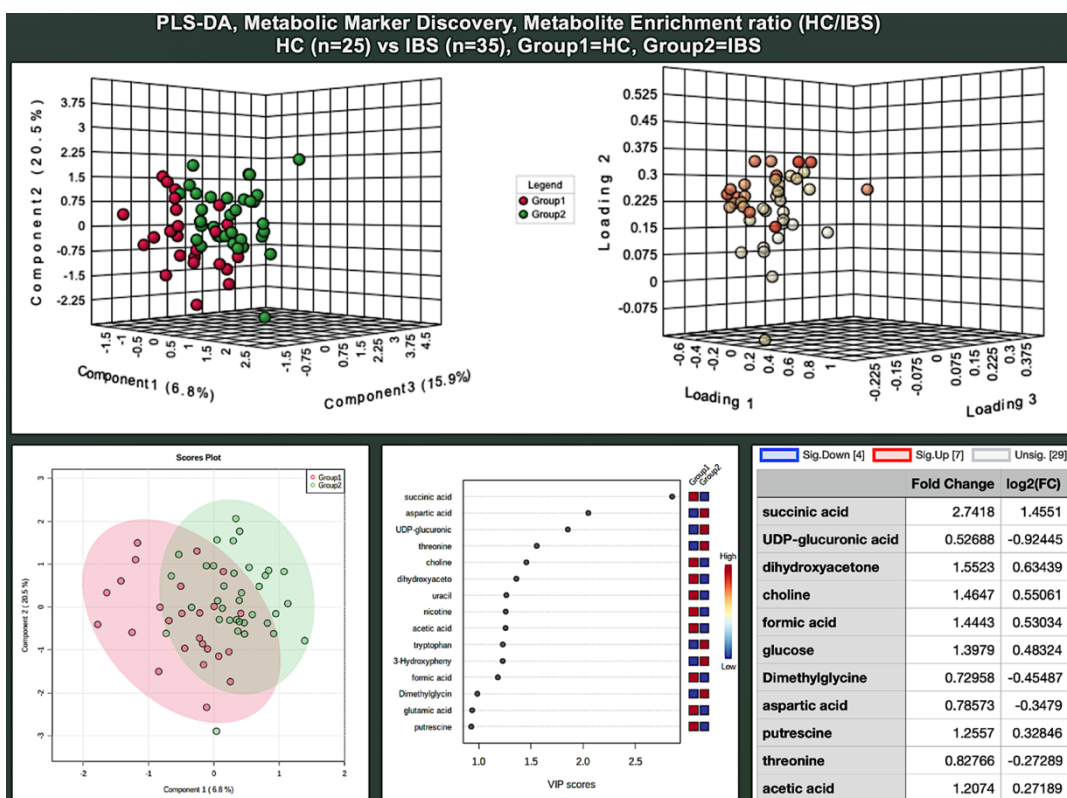


Figure 30. Principal component analysis of metabolites (relative concentration) in IBS and HC women. Succinic acid was found to be almost 3-fold higher in HC women compared to IBS.

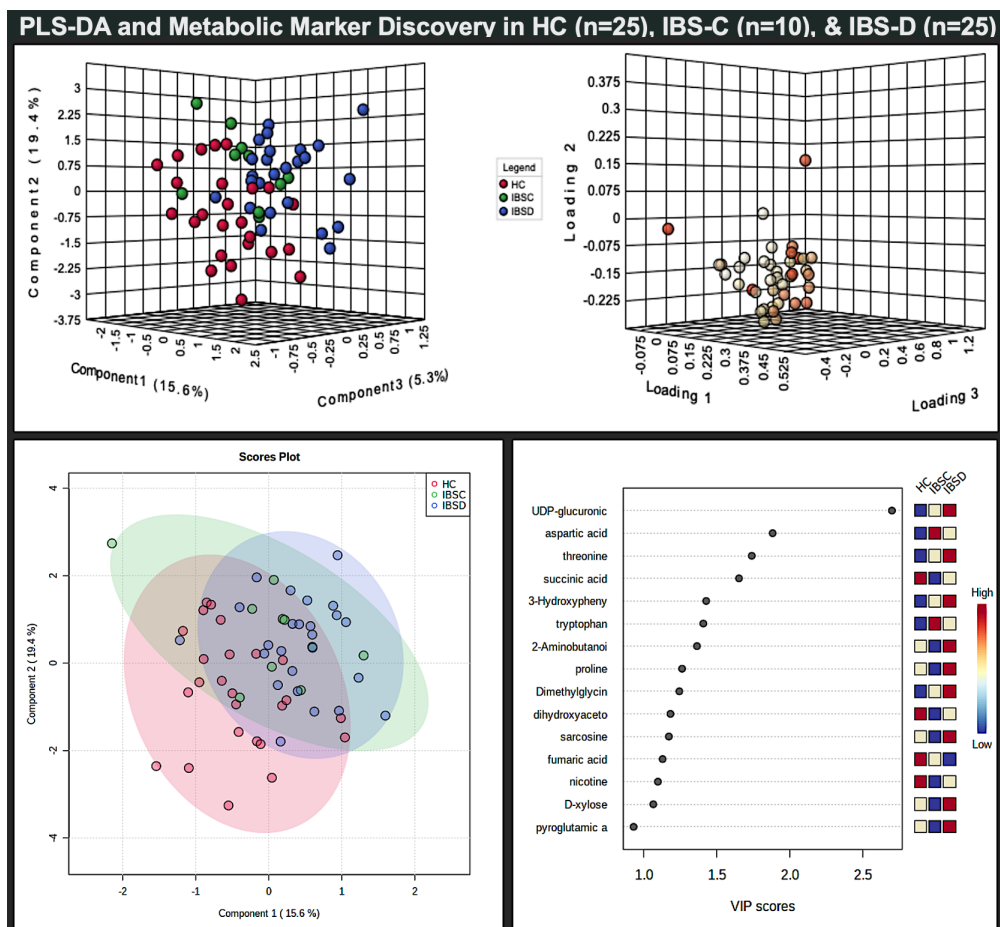


Figure31. Principal component analysis from MetaboAnalyst 5.0 revealed separations of the metabolites (relative abundance) in the three groups of women.

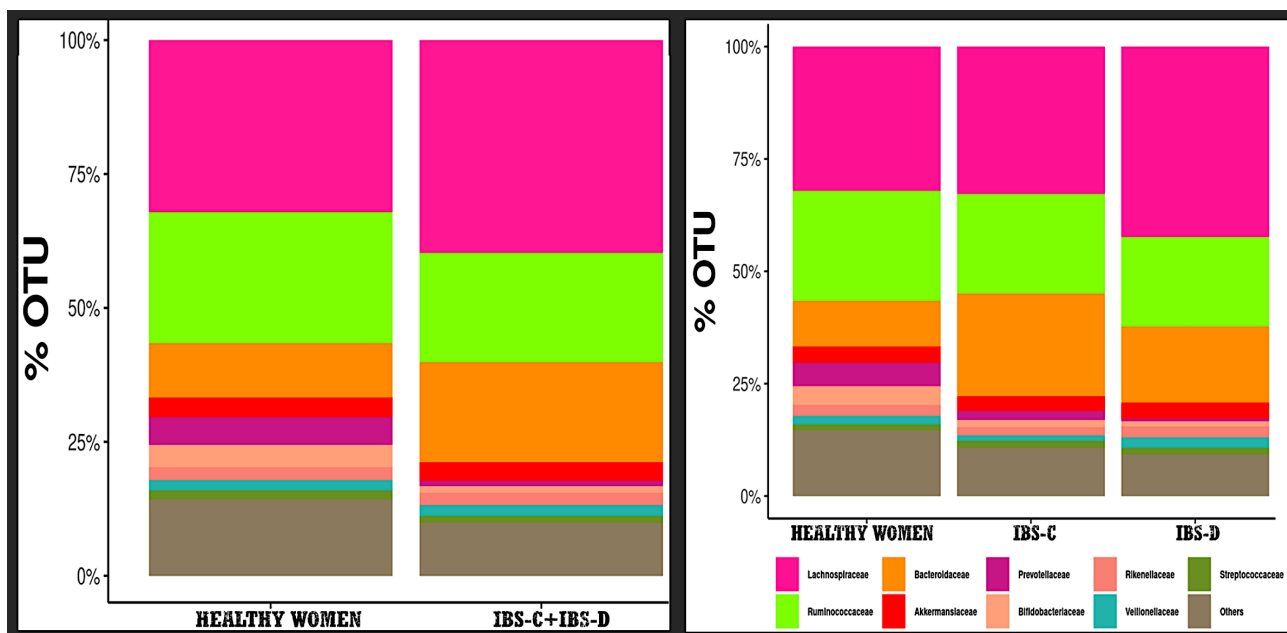


Figure 32. The 10 most abundant taxa of the gut bacteria composition in all 3 groups based on un-normalized 16S ribosomal RNA gene sequencing data.

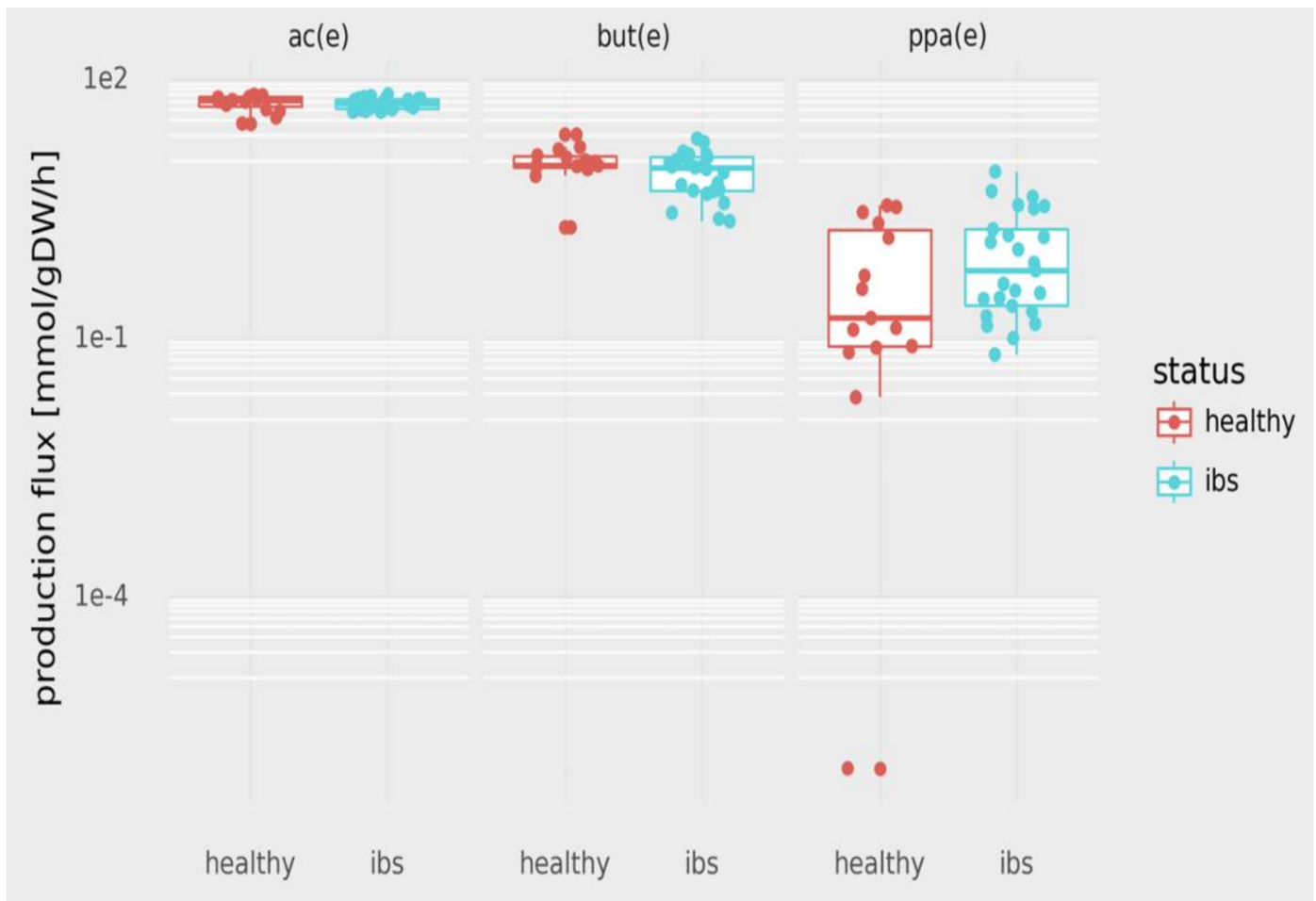


Figure 33. Box plots of predicted SCFA metabolites in the context of a Western diet intake and providing that every person consumed the same nutrients. The p-value for a Mann-Whitney test between HC and IBS for the major SCFAs: acetate = 0.23, butyrate = 0.1, and propionate = 0.052

AIM3 DISCUSSION

The symbiotic relationship of humans and microbes is the very essence of healthy living. Humans rely on the presence of the microbial consortia equipped with a plethora of metabolic genes necessary for nutrient acquisition, toxic compound conversions and elimination, and the provision of important metabolites and vitamins that the human genome cannot encode. Given the right dietary environment, gut microbial activities are indispensable for humans to age well. Existing knowledge supports the idea that infants acquire and shape his/her “adult microbiome” signature at age 3, originally acquired from the mother’s microbial profile after going through vaginal birth and breastfeeding (Yatsunenکو et al. 2012; Mariat et al., 2009; Wang et al. 2020). The keystone or core bacteria in the gut are known to be very resilient to change at early adulthood as compared to middle and late adulthood (Lozupone et al., 2012; Faith et al., 2013; Derrien et al., 2019). Although host genetics can shape the microbiome profile of a person with IBS, the gut environment plays a major role in determining the relative abundance (or absolute abundance) of bacteria taxa. Most of the time, the absolute microbial abundances are not known due to detection limitations of current technology, hence, relative abundance (setting the sum of each sample) is commonly used to estimate microbial composition, but literature reports that this method can lead to spurious correlations between variables when using classical statistical techniques (Bruinjing et.al., 2022). Relative abundances depend on the microbe of interest but also on the absolute abundances of the entire bacterial community (all taxa), which is unknown. Furthermore, there is the possibility of selection bias in the detection of different taxa. In this retrospective study, Relative Abundance was defined as $100 * (\text{count for bacteria taxa}) / (\text{sum of counts for all taxa for one fecal donor})$.

Aging well means maintaining a healthy microbiome, the very key players in the maintenance of gut homeostasis. Having a healthy microbiome requires one to practice a healthy lifestyle (e.g., committing to daily exercise, having adequate sleep, adequate high fiber dietary intake with low saturated fat intake, and avoiding excessive barbecued animal products). Because the trajectory of health and disease is dictated by the host-microbiome interactions, one should be aware of the several mechanisms where this interaction is perturbed. In this present study, butyric acid tended to decrease beginning at the age of 35 and onward (see

scatter plot of butyrate vs. age). *In vivo* research (infusion of carbon-14 butyrate) in older animals showed that the transport of butyrate is markedly reduced when compared to young animals (Fitz & Fleming, 1999). Other studies have reported that the colonocytes of older animals have abnormally high rates of fatty acid oxidation and that butyrate suppresses the oxidation of other fatty acids (Fleming & Gill, 1997; Clausen & Mortensen, 1994). These studies further support the importance of butyrate on colonic health and the overall health of older adults.

Host-microbe interaction. The luminal surface of the colonic mucosa is where the host and bacteria interact and influence each other; the host generally benefits from such a relationship. The host interacts with the gut microbiome by pattern recognition receptors such as toll-like receptors (TLRs); through these receptors, the immune system (both innate and adaptive) is developed and trained, however, the overactivation of these receptors along with the host nuclear receptors may serve as the route for setting the stage of inflammatory disorders under a high fat diet (Mukherji et al., 2013; Yiu et al., 2017; Dvořák et al., 2020; Wickstead et al. 2022). This is because the host depends on the microbial metabolites for various physiological processes. It is worth noting that for every human gene, there are about 100 bacterial genes, ten bacterial cells for every human cell. Humans harbor roughly more than 100 trillion of bacteria (of which 40 trillion are estimated to reside in the gut) and to date, the human genome only have 23,000 genes and bacteria genes is estimated to be around 3 million genes (reported to go up to 9 million). This is the very reason why humans are called “meta-organism” or “superorganism” (Goodacre 2007; Qin et al., 2010; Yang et al. 2009). Since most of the bacteria are unculturable, current findings regarding microbial counts based on the Human Microbiome Project have just barely touched the tip of the iceberg and because bacteria have the agility to continuously swap/acquire genes, the number of bacterial genes could reach to infinity (Tettelin et al. 2005). Besides the TLRs, humans have developed receptors, the SCFA-sensing G protein-coupled receptors or GPCRs, located on the surface of immune cells, adipocytes, intestinal epithelial cells, endothelial cells, neuronal cells, and other organs (i.e., cardiovascular, kidney, liver, etc.) for bacterial metabolite recognition, a sign that humans co-evolved with the gut microbiota to help regulate mammalian homeostasis (Ganapathy et

al. 2013; O'Riordan et al., 2022). It is through these receptors that the host-microbiota communicate and modulate each other.

Host immune system-bacteria relationship. How the host keeps the commensal bacteria in the gut without mounting a full immune response is an area of intense research. Since the commensals co-evolved with the host, the bacteria developed several mechanisms to avoid eliciting an inflammatory response. One of several mechanisms by which the gut microbiota modulates the host immune system towards a tolerogenic phenotype is through the actions of microbial metabolites such as butyrate. Butyrate is known to inhibit the activation of the central mediator of immune and inflammatory response, NF- κ B by preventing the degradation of I κ B via the ubiquitin-proteasome pathway (Place et al., 2005; Schwab et al., 2007). Microbial metabolites also modulate the immune system via the activation of GPCRs (i.e., GCPR41/FFAR43, GCPR43/FFAR2, GRR109A). The binding of butyrate to GPR109A (also called hydroxy carboxylic receptor, encoded by the niacin receptor gene) found in macrophages and dendritic cells induces regulatory T cells (Tregs) via the FOXP3 pathway and IL-10 production by T cells to promote intestinal immune tolerance to commensal bacteria (Singh et al., 2014). Furthermore, microbes such as *B. fragilis* can exert intestinal immune tolerance by modulating the production of Type 1 interferon gamma by the host immune system (i.e., macrophages and dendritic cells) which also activates the production of IL-10 and Tregs (Ayala et al., 2023). Other microbes with protective, anti-inflammatory function include the *Clostridium IV* (now Ruminococcaceae; species such as *Faecalibacterium prausnitzii*, and genera *Butyricicoccus*), and *Clostridium XIVa* (now *Lachnospiraceae*, genera such as *Coprococcus*, *Dorea*, *Eubacteria*) (Singh et al, Guo et al., 2020). Moreover, the activation of GPR109A mediated by butyrate (the only SCFA that binds to this receptor) results in the induction of IL-18 which facilitates colonic repair and maintains gut homeostasis (Sing et al, 2014; Marcia et al., 2015). The importance of GRP109A receptor in mediating intestinal inflammation via the action of butyrate and other bacterial metabolites such as niacin/vitamin B3 supports the positive interaction between host-gut-microbiome. By acting as a histone deacetylase (HDAC) inhibitor, butyrate can influence the transcription of genes (i.e., upregulate IL-10 and/or inhibit the NF κ B signaling cascade) necessary to maintain immune tolerance. Furthermore, SCFA can regulate gene expressions via modulation of nuclear receptor (e.g., peroxisome

proliferator activated receptor (PPAR), pregnane X receptor (PXR), aryl hydrocarbon receptor (AhR), farnesoid X receptor (FXR), vitamin D receptor (VDR) (Marinelli et al. 2019). PXR and VDR are known to inhibit NF- κ B activities (Nepalska et al., 2017; Schwab et al., 2007). Antimicrobial peptides can have anti-inflammatory properties (Schwab et al., 2007). Butyrate has also been shown to downregulate the expression of TLR2 and TLR4 (Ye et al., 2018); these microbial pattern recognition receptors are documented to elicit immune responses. As cited by Dey et al. (2023), commensals can be coated with immunoglobulin A to resist immune response from the host.

Butyrate dual role. The functionality of butyrate as an anti-inflammatory factor depends on the context of the colonic epithelium. In a normal intestinal milieu, butyrate promotes the normal growth and differentiation of normal colonic cells, upregulates tumor suppressor genes, and downregulates the expressions of metastatic related genes while it induces cell cycle arrest in butyrate-treated cancer cells (Joseph et al., 2004; Li & Li, 2006; Tabuchi et al., 2002). When butyrate is diffused inside abnormal colonocytes, it acts as an HDAC inhibitor and upregulates the inflammatory cascade and potentiates the function of cytotoxic T cells, resulting in abnormal cell apoptosis (Scharlau et al., 2009; Bailón et al., 2010; Donohoe et al., 2012; Park et al., 2015; Luu et al., 2018; Wu et al., 2018; Zeng et al., 2020; Schlörmann et al., 2023).

Attributes of IBS. The presence of an intact gut microbiota (eubiosis) including normal bio-physiological functioning predates gut dysbiosis. The normal functioning of the neuro-endocrino-immuno-gut-liver-microbiome axis is necessary prior to the development of IBS. Disruptions of these communication pathways account for the complex IBS symptoms. The circadian system is made up of transcription factors derived from oscillating neurons (central clocks) that generate molecular-cellular rhythms (regulated via positive and negative feedback loops) upon sensing metabolites/nutrients/environmental cues such as nutrients, light, temperature, pH and moisture changes. The oscillators of the circadian system live in the suprachiasmatic nucleus (SCN) located above the optic chiasm at the base of the hypothalamus which receives information about the day-night cycle through the retino-hypothalamic tract where the retinal ganglion cells get activated to release photopigments---melanopsin (Saini et al., 2019; Pachos et al. 2010). Evidence exists where there is a marked reduction or loss of neuromelanin in the pons (locus coeruleus) and in the midbrain (substantia nigra)

in Alzheimer's disease (AD) mice model and in post-mortem brain studies in patients with AD, Parkinson's Disease, and Lewy body dementia patients (Berg & Berg, 2023; Lannitelli & Weinshenker, 2023; Calafate et al., 2023; Li et al., 2022). The circadian clock genes govern the timely regulation of gene expressions and translations and have the power to influence the microbial fingerprints of a person that can be either result in health or disease. Studies have reported that disruptions of circadian rhythms have been linked to pathologies in humans such as metabolic disorders including mental illness (Pachos et al., 2010; Levi & Schibler 2007, Klerman 2005; Curtis & Fitzgerald, 2006). In theory, the circadian clocks should be established first in order for this entity to impose a rhythm on cell metabolisms and as well as the rhythmic activity of the GI tract (i.e., gastric emptying, motility, gastric secretion, gastric absorption, gastric detoxification systems such as the cytochrome P450s) and its bacterial residents. Most importantly, melatonin, a synchronizer of the SCN clockwork has diurnal variations with its maximum secretion from the pineal gland at night (Pachos et al., 2010). Besides the influence of internal factors, external environmental factors such as antibiotics, proton pump inhibitors, xenobiotics, pollution, and stress, as well as adaptation to these factors can disturb the gut microbiota and lead to an altered gut microbial composition and function (dysbiosis). With time, in an individual, the disruption of internal clocks and gut dysbiosis can result in chronic gut inflammation. Thais and colleagues (2014) showed that misalignments of biological clocks in mice and humans cause dysbiosis and such factors (i.e., jet-lagged/frequent flying, aberrant feeding behavior) promote obesity and glucose intolerance. Thais (2014) found significant diurnal variations of bacterial operational taxonomic units (OTUs) in mice (15%) and humans (10%) that were influenced/controlled by diurnal feeding behavior with the use of Jonckheere-Terpstra-Kendall (JTK) cycle software. The JTK statistical analysis software (R-programming) is a non-parametric algorithm used to detect oscillations of physiology, behavior, and metabolism that have periods of lengths of 24 hours (Hughes et al. 2010). Such significant diurnal microbiota oscillations result in diurnal fluctuations of gene expressions, metabolites and their associated biological pathways (i.e., vitamins and nucleotide metabolism, secretion systems, detoxification systems, DNA repair, cell wall synthesis, etc.). Moreover, Thais and colleagues demonstrated a reversal of phenotype of a double-knockout mice (*Per1* & *2*^{-/-}) that lost rhythmic expressions of OTUs with restricted feeding. In addition, other studies in mice have shown

that disturbances of circadian rhythms cause significant dysfunction in gut barrier and reduce alpha diversity index with an increased abundance of *Ruminococcus torques*, a major mucin degrader (Deaver et al., 2018); increased abundance of *Odoribacter* and *Prevotella* in diabetic mice with sleep disturbances (Geurts et al., 2011). Recently, Zhou and colleagues (2022) identified and reported sleep-related metabolites that were significantly associated with bacteria signatures from healthy individuals and individuals with insomnia (n=24). This group found that bacteria genera such as *Gemmiger* and *Fusicatenibacter* predominate in individuals with insomnia, while healthy individuals without sleep disturbances (n=22) had higher abundance of *Coprococcus*, *Clostridium XI*, and *Oscillibacter*. Deaver and colleagues observed downregulation of expressions of genes involved in SCFA (i.e., 3-hydroxybutyryl-CoA dehydrogenase) and tryptophan (i.e., acetaldehyde dehydrogenase) metabolism including the up-regulation of genes involved in LPS production. Further studies have shown that the intake of cellobiose-containing diet is able to adjust the misalignment of peripheral circadian clocks during the light/active period (Tahara et al., 2018). Thus, a disturbance of biological clocks as seen in individual exposed to constant light including continuous access to food, is in danger of having an altered microbiome, increasing their susceptibility to inflammatory and metabolic diseases. These studies further support that gut dysbiosis and its consequences (i.e., inflammation, altered tryptophan and SCFA metabolism), circadian rhythm disturbances, impacts the attributes of IBS symptoms, and thus, the therapeutic importance of dietary fibers along with having proper sleep may be key to preventing and alleviating IBS symptoms.

There are several potential mechanisms that could explain the pathobiology of IBS. As a heterogeneous condition, IBS is a disorder with multi-component etiologies that include chronic gut inflammation as supported by altered immune biomarkers (Nakov et al., 2022; Martin-Viñas et al., 2016). This low-grade inflammation related to mucosa irritation which can be caused by food types is oftentimes exacerbated due to host genetics and a heightened physiological response. Heightened sensitivity to certain foods may be attributed to inadequate enzyme capacity for carbohydrate degradation and having inefficient transport mechanisms (i.e., mutation of sucrase gene that transports sugars in the small intestine). As a result, faster transit time occurs. On the other hand, since humans rely on the metabolism of the gut microbiome, the lack of certain bacterial

species or the predominance of specific groups of bacteria may contribute to this heightened sensitivity. Moreover, the excessive intake of proton pump inhibitors may also contribute to increased small intestine bacterial fermentation because a higher pH can result in the overgrowth of small intestine bacteria (i.e., *Prevotella*, *Streptococcus*, *Veillonella*). Another contributing factor for IBS is heightened susceptibility to intestinal infection based on host genetic predisposition (i.e., ABO secretor vs non-secretor); the ABO secretors are known to have higher susceptibility to infection compared to non-secretors. A long history of antibiotic treatments may permanently change the gut microbiome states (in terms of energy level or pathology level) where the microbes themselves can exacerbate the IBS condition. Without rectifying extreme gut dysbiosis (via dietary modification or microbiome supplementation), symptoms of IBS become worse because of the crucial role that the gut microbiome plays in health and disease. However, not all factors are fully understood, in particular--the interaction of diet, the mediating bacteria as well as the whole repertoire of other colonic microorganisms capable of transferring genetic materials laterally, vertically and horizontally.

SCFA production. To some degree SCFA can be produced by human metabolism (minor contributor to plasma levels), but most (95%) are produced by anaerobic fermentation by colonic bacteria. The production of SCFA by the host usually happens during starvation or in the presence of a metabolic disorder (i.e., diabetes (type 1 and type 2), glucose intolerance, insulin resistance) where the branched-chain (bc) amino acids (i.e., valine, leucine, isoleucine) are converted to bc-keto acids and then to bc acyl-CoA esters (Holeček, (2020). Depending on the presence of disease/disorder, the host's SCFA contribution can be significant or not significant. The focus for this dissertation was to evaluate the microbial SCFA production (fecal SCFA as a proxy measure) and its benefits to the host harboring such microbes under the availability of the right substrates, i.e., high complex carbohydrates or high fiber diet. Microbial SCFA production relies heavily on the bacterial GUS system. Note: humans also express a GUS enzyme in the lysosomes of the first-pass tissues (i.e., liver and intestines) and is responsible for the degradation of endogenous glycosaminoglycans (Jain et al., 1996). Inborn metabolic disease such as Sly Syndrome is caused by germline mutation of human GUS enzymes (Sly et al., 1973).

Probiotics supplementation. The use of probiotics in treating IBS abdominal pain and other related symptoms is mounting (SR review by Wang, 2022; Liang et al., 2020; Dale et al. 2019), and thus it is important to look at associations between diet and the microbiome. Due to co-dependence of the host circadian rhythm with the gut microbiome (Mukherji et al. 2013), taking probiotics (effective dose: >10 billions/capsule) should be taken at the right time to maximize colonization. Furthermore, eating high fiber diet during the day prior to taking probiotics may be more beneficial for the person because the SCFA metabolic machinery are more efficient at certain hours and reserve the night period as a “caloric restriction” phase; the night period is the time for proper functioning of the cell’s detoxification machineries (Pachos et al., 2010). When the available, fermentable substrates from complex carbohydrates or from high fat/protein diet, these bacteria can be “friendly” or “harmful” to the host. It is believed that the intake of probiotics such as *Bifidobacterium infantis*, and *Lactobacillus plantarum* in the context of a high fiber diet can help stabilize the microbiome, help correct inflammation and the dysregulated immune response, promote the growth of “disappearing/compromised state” commensals, and prevent the colonization of opportunistic pathogenic bacteria and/or antagonize the pathogenic ones by modulating the immune-neuroendocrine metabolic pathways which may help restore the delicate balance of the gut microbial ecosystem (Dey et al., 2023; Lozupone et al., 2012; Faith et al., 2013; Ford et al., 2014; Thais et al., 2014; Vollaard & Clasener, 1994). Depending on the cause of perturbation (genetic vs environmental insults), clinicians may recommend the use of probiotics and higher fiber diet to maintain host-bacteria homeostasis. An IBS condition induced by small intestinal bacterial overgrowth (SIBO) or infection may need to take antibiotics first prior to taking probiotics or its related products called postbiotics. Despite the positive and beneficial effects of the current over-the-counter probiotics (i.e., *Bifidobacteria longum*, *Bifidobacteria infantis*, *Lactobacillus plantarum*) or the next generation of probiotics (e.g., *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*), one must be cautious because such strains could easily become a vector of unwanted genetic materials (i.e., antibiotic resistant genes, transposable elements, viruses) and cause disease. (Jacobsen et al., 2007; Egerværn et al. 2010; Suez, et al., 2019; Kothari et al., 2019). Such commensal bacteria could evolve from being friendly bacteria to becoming an opportunistic pathogen, a phenomenon that changes the host-bacteria symbiotic relationships that can either activate or suppress the

host immune response. Overactivation of the host immune response leads to inflammatory diseases while suppression may be a risk factor for cancer development.

Diet-bacteria relationship. It is important to consider the role of dietary fiber and its impact on the metabolism of bacteria. The intake of Western diets high in fat and processed foods could tip the balance of residential bacteria, promote the bioconversion of “good metabolites” into “toxic metabolites.” For example, the biotransformation of primary bile acids into toxic secondary bile acids or the conversion of tryptophan metabolites via the tryptophan-kynurenine metabolic pathway occurs under chronic low-grade inflammation (Tanaka et al., 2021; Ducastel et al. 2020). Due to their high genetic pool, commensals easily adapt to the new, high-fat diet environment rapidly and certain species can expand their nutritional repertoire and physiological niches within the gut colonic lumen. Therefore, the versatile commensal become the key player for maintaining chronic low-grade inflammation. It has been reported that commensals can become pathogenic when they escape from their original niche (Little et al. 2022), which may happen when glycans in the colon are scarce.

Aim3 purpose. This study sought to explore these relationships (e.g., microbe-microbe, microbe-diet, metabolite-microbe, metabolite-metabolite relationships) and their impact on the host’s GI physiological responses using simple linear regression and Pearson’s bivariate correlations. An examination of the associations of SCFA with the microbes that produce them and how they are related to diet and host characteristics (i.e., weight, dietary patterns) may help unravel the mystery of IBS. Studying the relationship of microbial community structure with 16S ribosomal DNA gene sequencing data along with fecal SCFA metabolome from the Metabolomics-NMR platform provides new insights on microbial structures and their metabolic functions. Both “omics” technologies complement each other and help to reveal important mechanisms that may underlie the pathogenesis of IBS symptoms.

Discussion of current results: Microbe-metabolite. There are several microbes that can generate SCFA from carbohydrate and protein fermentation. In the present study, many of these bacteria demonstrated linear, positive relationships with fecal SCFA. Several bacteria can both produce and use these metabolites, while other bacteria are only consumers. In this study, linear regression (biplot) showed a positive relationship

between microbial diversity index (Shannon index) and each of the individual three major SCFA (acetic acid, propionic acid, and butyric acid). These positive associations of bacteria with SCFA confirmed existing literature. For example, *Bifidobacteria* is known to consume lactose available from the colonic environment. Furthermore, *Bifidobacteria* produces acetic acid and lactic acid. Lactic acid produced by lactic acid producers such as *Lactobacillus* and *Enterococcus* is metabolized by lactic acid utilizers (i.e., *Coprococcus*, *Veillonella*, *Eubacteria*, *Lachnospiricea*). Microbe-microbe. Interestingly, the current study found significant positive associations of SCFA-producing bacteria; *Bifidobacteria* with *Lactobacillus* and with *Prevotella*, but an inverse association of *Bifidobacteria* with *Bacteroides*. *Bacteroides* are the most versatile gut microbes because they can use a plethora of substrates (animal-derived glycans, host mucins, complex carbohydrates), and can survive with low fiber diet as compared to other SCFA producers; however, they are not the primary SCFA producers. There is mounting evidence that *Bacteroides* are associated with meat-based Western diets. Considering all women (n=60) *Bacteroides* was negatively associated with dietary fiber intake and positively associated with protein intake; this negative association may have been driven by the high dietary fat consumption of healthy control and IBS-D women.

Consistent to what is known in current literature, this study found a significant inverse relationship of *Faecalibacteria* and *Akkermansia*, mucin-degrading bacteria which lives in proximity to the colonic mucosa. Previous study done by Yin et al., (2021) showed that *Akkermansia* grows well in the presence of tryptophan. *Akkermansia* is known to metabolize tryptophan to its indole derivatives in the gut. Interestingly, this current study found a negative relationship between *Akkermansia* and *Turicibacter*. *Turicibacter* is defined more as an opportunistic than a commensal (Chu et al., 2023). It is known to promote serotonin biosynthesis by the host's enterochromaffin cells (Reigstad et al. (2015). This relationship is supported by the current findings in which an inverse relationship of *Turicibacteria* with tryptophan was found. *Turicibacter* is known to consume tryptophan (Vasquez-Medina et al., 2024) and it has been shown that neuro-psychiatric disorders such as depression are positively linked to high relative abundance of *Turicibacter* (Getachew et al., 2018). Ye and colleagues (2018) reported that methionine-choline deficient diet (MCD) disrupts barrier homeostasis and causes microbiome dysbiosis; these investigators noted that MCD is known to favor the growth of *Turicibacter*,

Bilophila and other pro-inflammatory bacteria. Ye et al (2018) found that butyrate treatment in a mouse model of non-alcoholic steatohepatitis (NASH) induced by MCD diet reduced *Bilophila* and promoted the growth of keystone bacteria known to maintain gut stability (i.e., *Akkermansia*, *Coprococcus*, *Roseburia*). Tryptophan is a precursor for serotonin, and *Turicibacter* expresses transporters for the uptake of serotonin (Fung et al., (2019), and thus *Turicibacter* depletes the serotonin pool.

Microbe-metabolite. Consistent with existing literature, this study also found significant positive associations between acetic acid (FDW normalized) and *Bifidobacteria* along with other SCFA-producing bacteria (i.e., *Lachnospiraceae*, *Clostridia XI*, *Megasphaera*). Moreover, this study also found a negative association between acetic acid and the bacteria belonging to *Bacteroidales* (i.e., *Bacteroides*, *Alistipes*). These microbes have been implicated in gut dysbiosis and inflammatory conditions such as appendicitis, abdominal and rectal abscess, including chronic intestinal inflammation (Didari et al., 2014; Kothari et al., 2019). In line with current literature, this study also found *Dorea* to be positively associated with butyric acid and propionic acid, while the lactic acid-utilizers such as *Veillonella* and *Enterococcus* were positively associated with lactic acid. Moreover, consistent with existing research, this study found a significant positive association of formic acid and *Enterococcus*. Certain species of *Enterococcus* are known to ferment a variety of sugar substrates in wide pH and temperature ranges and are generally bile salts resistant, facultative anaerobes able to use different pathways to produce formic acid and other intermediates for SCFA production (Moshokoa et al., 2023). This commensal can be very friendly to the host but can cause GI symptoms such as bloating and abdominal distention. The formation of excess gas can be uncomfortable when the host lacks the microbes that convert these gases into more tolerable metabolites. In terms of associations, the current study also looked at the contribution of certain bacteria genera in maintaining the microbial diversity of the gut ecosystem. These results confirmed the increasing body of literature that health is associated with richness and microbial diversity.

Microbiome-GI symptoms. In support of the findings by Malinen and colleagues (2010), this study also found a significant negative correlation between *Clostridium Cluster XIVa* with abdominal pain in IBS patients as a composite (n=35). Similarly, this current study was also consistent with Saulnier et al. (2011) findings

where they reported reduced abdominal pain in IBS individuals that harbored *Faecalibacteria* and increased abdominal pain with the increased presence of *Ruminococcus*

Diet-metabolome. Looking at the dietary contribution to the mechanistic link that promotes the positive interaction of the host-gut microbiome, several significant relationships between dietary constituents and SCFA were discovered. *Bifidobacteria*, a known commensal, thrives on lactose (Hidalgo-Cantabrana et al., 2018). Consistent with literature, this study found dietary lactose to be positively associated with propionic acid and acetic acid, valeric acid, and aminobutyric acid. Furthermore, the calculated basal metabolic rate or BMR was negatively associated with insoluble fiber dietary intake while it was positively associated with total added sugar intake. This would make sense because a reduction in BMR under high fiber diet is advantageous to the host because less intake of calories is required to maintain basic physiological functions at rest and to maintain a stable body weight. Less intake of food translates to less metabolic activities and thus, less production of metabolic byproducts and less damage to mitochondria (Bolisetty & Jaimes 2013). This finding can have clinical implication in that a reduction in BMR with adequate intake of insoluble fiber may potentially reduce the production of reactive species (normal by-products of metabolism), and therefore, a nurse scientist must advocate for reduced intake of added sugars and increased fiber intake to reduce the negative effects of oxidative phosphorylation (Bolisetty & Jaimes 2013).

There were strong and positive correlations among butyric acid, tryptophan and hypoxanthine. Butyrate is known to upregulate tryptophan metabolism, preventing its oxidation during inflammation (Martin-Gallausiaux et al., 2018; Martin-Gallausiaux et al. 2023; Hou et al., 2023). Existing body of knowledge implicated that butyrate regulates tryptophan metabolism (Reigstad et al., 2015; Fukumoto et al. 2003; Yano et al., 2015). In this exploratory study, high concentration of butyrate was found to be correlated with several symptoms (i.e., stress, muscle pain, depression, anxiety, hard to concentrate) and was also generally reduced in IBS-C.

Conclusion

The gut microbiome is the primary link between diet and IBS. The disruption of the gut-microbiota axis may lead to IBS disorder. While the findings from the present study are quite interesting, such associations need to be validated by conducting functional studies to generalize results. QT-RT-PCR and bacteria culture experimentation should be used to validate findings.

CHAPTER 6. IMPLICATIONS

A well-controlled feeding study is still needed to validate the impact of dietary fiber intake on IBS symptoms. In addition to having adequate sleep, the results in this present study supported the importance of taking adequate vegetables and fruits to maintain health and help prevent early development of inflammatory diseases related to aging. Indeed, a personalized dietary recommendation designed to increase butyrate production and minimize gut dysbiosis may help a person with IBS, especially those who have altered circadian rhythms and sleep disturbances. The intake of probiotics that are well characterized may be necessary to help reestablish a more diverse microbial consortia for IBS who experienced more severe symptoms because a diverse microbiome helps digest foods that are important for gut health.

REFERENCES

1. Abell, G. C., Cooke, C. M., Bennett, C. N., Conlon, M. A., & McOrist, A. L. Phylotypes related to *Ruminococcus bromii* are abundant in the large bowel of humans and increase in response to a diet high in resistant starch. *FEMS microbiology ecology*. 2008;66(3), 505-515.
2. Abellan-Schneyder, I., Machado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., ... & Neuhaus, K. (2021). Primer, pipelines, parameters: issues in 16S rRNA gene sequencing. *Mosphere*, 6(1), e01202-20.
3. Abeysekara, S., Naylor, J. M., Wassef, A. W., Isak, U., & Zello, G. A. (2007). D-Lactic acid-induced neurotoxicity in a calf model. *American Journal of Physiology-Endocrinology and Metabolism*, 293(2), E558-E565.
4. Abi, I., Ashiekaa, M., Abi, E., Adeniyi, O. S., & Saalu, L. C. (2022). High Fat Diet Alteration of Gut Microbiota Impacts Learning, Memory and Anxiety Response in Mice: Cannabidiol and Omega 3 Possible Remedies. *Advances in Alzheimer's Disease*, 11(1), 1-9.
5. Acosta, A., Camilleri, M., Shin, A., Nord, S. L., O'Neill, J., Gray, A. V., ... & Zinsmeister, A. R. Tu1793 Effects of Rifaximin on Transit, Permeability, Fecal Microbiome, and Organic Acid Excretion in Irritable Bowel Syndrome: A Randomized, Double-Blinded Trial. *Gastroenterology*. 2016;150(4), S948-S949.
6. Aagaard, K., Petrosino, J., Keitel, W., Watson, M., Katancik, J., Garcia, N., ... & Harris, E. The Human Microbiome Project strategy for comprehensive sampling of the human microbiome and why it matters. *The FASEB Journal*. 2013;27(3), 1012-1022.
7. Adeva-Andany, M., López-Ojén, M., Funcasta-Calderón, R., Ameneiros-Rodríguez, E., Donapetry-García, C., Vila-Altesor, M., & Rodríguez-Seijas, J. (2014). Comprehensive review on lactate metabolism in human health. *Mitochondrion*, 17, 76-100.
8. Adlanmerini, M., & Lazar, M. A. (2023). The REV-ERB nuclear receptors: timekeepers for the core clock period and metabolism. *Endocrinology*, 164(6), bqad069.
9. Aerssens, J., Camilleri, M., Talloen, W., Thielemans, L., Göhlmann, H. W., Van den Wyngaert, I., ... & Carlson, P. J. (2008). Alterations in mucosal immunity identified in the colon of patients with irritable bowel syndrome. *Clinical Gastroenterology and Hepatology*, 6(2), 194-205.
10. Agazzi, A., De Ponti, F., De Giorgio, R., Candura, S. M., Anselmi, L., Cervio, E., ... & Tonini, M. (2003). Review of the implications of dietary tryptophan intake in patients with irritable bowel syndrome and psychiatric disorders. *Digestive and liver disease*, 35(8), 590-595.
11. Aguirre, M., Eck, A., Koenen, M. E., Savelkoul, P. H., Budding, A. E., & Venema, K. (2016). Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. *Research in microbiology*, 167(2), 114-125.
12. Ahmadi, S., Taghizadieh, M., Mehdizadehfard, E., Hasani, A., Fard, J. K., Feizi, H., ... & Memar, M. Y. (2024). Gut microbiota in neurological diseases: Melatonin plays an important regulatory role. *Biomedicine & Pharmacotherapy*, 174, 116487.
13. Ahmed, I., Greenwood, R., de Lacy Costello, B., Ratcliffe, N. M., & Probert, C. S. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. *PloS one*. 2013;8(3), e58204
14. Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., ... & Theodorou, V. (2012). Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology*, 37(11), 1885-1895
15. Akhtar, M., Chen, Y., Ma, Z., Zhang, X., Shi, D., Khan, J. A., & Liu, H. (2021). Gut microbiota-derived short chain fatty acids are potential mediators in gut inflammation. *Animal Nutrition*.
16. Alam, R., Abdolmaleky, H. M., & Zhou, J. R. Microbiome, inflammation, epigenetic alterations, and mental diseases. *American Journal of Medical Genetics Part B Neuropsychiatric Genetics*. 2017;174(6), 651-660. doi:10.1002/ajmg.b.32567
17. Allali I, Arnold JW, Roach J, et al. A comparison of sequencing platforms and bioinformatics pipelines for compositional analysis of the gut microbiome. *BMC Microbiology*. 2017;17:194. doi:10.1186/s12866-017-1101-8.

18. Allen, J. M., Mailing, L. J., Niemi, G. M., Moore, R., Cook, M. D., White, B. A., ... & Woods, J. A. (2018). Exercise alters gut microbiota composition and function in lean and obese humans. *Med Sci Sports Exerc*, 50(4), 747-57.
19. Aller, R., de Luis, D. A., Izaola, O., la Calle, F., del Olmo, L., Fernandez, L., ... & Hernandez, J. G. Effects of a high-fiber diet on symptoms of irritable bowel syndrome: a randomized clinical trial. *Nutrition*. 2004;20(9), 735-737.
20. Altomare, A., Di Rosa, C., Imperia, E., Emerenziani, S., Cicala, M., & Guarino, M. P. L. (2021). Diarrhea predominant-irritable bowel syndrome (IBS-D): effects of different nutritional patterns on intestinal dysbiosis and symptoms. *Nutrients*, 13(5), 1506.
21. American College of Gastroenterology Chronic Constipation Task Force. (2005). An evidence-based approach to the management of chronic constipation in North America. *American Journal of Gastroenterology*, 100, S1-S4.
22. Amiri, P., Arefhosseini, S., Bakhshimoghaddam, F., Jamshidi Gurvan, H., & Hosseini, S. A. (2022). Mechanistic insights into the pleiotropic effects of butyrate as a potential therapeutic agent on NAFLD management: A systematic review. *Frontiers in Nutrition*, 9, 1037696.
23. Andoh, A., Tsujikawa, T., & Fujiyama, Y. (2003). Role of dietary fiber and short-chain fatty acids in the colon. *Current pharmaceutical design*, 9(4), 347-358.
24. Andrews, C. N., & Storr, M. (2011). The pathophysiology of chronic constipation. *Canadian Journal of Gastroenterology and Hepatology*, 25(Suppl B), 16B-21B.
25. Aoyama, M., Kotani, J., & Usami, M. (2010). Butyrate and propionate induced activated or non-activated neutrophil apoptosis via HDAC inhibitor activity but without activating GPR-41/GPR-43 pathways. *Nutrition*, 26(6), 653-661.
26. Arffmann, S., Andersen, J. R., Hegnhøj, J., Schaffalitzky de Muckadell, O. B., Mogensen, N. B., & Krag, E. The Effect of Coarse Wheat Bran in the Irritable Bowel Syndrome I: A Double-Blind Cross-Over Study. *Scandinavian journal of gastroenterology*. 1985;20(3), 295-298.
27. Alam, R., Abdolmaleky, H. M., & Zhou, J. R. (2017). Microbiome, inflammation, epigenetic alterations, and mental diseases. *Am J Med Genet B Neuropsychiatr Genet*, 174(6):651-660. doi:10.1002/ajmg.b.32567
28. Alam, N. H., Meier, R., Schneider, H., Sarker, S. A., Bardhan, P. K., Mahalanabis, D., ... & Gyr, N. (2000). Partially hydrolyzed guar gum—supplemented oral rehydration solution in the treatment of acute diarrhea in children. *Journal of pediatric gastroenterology and nutrition*, 31(5), 503-507.
29. Alexea, O., Bacarea, V., & Piqué, N. (2016). The combination of oligo- and polysaccharides and reticulated protein for the control of symptoms in patients with irritable bowel syndrome: Results of a randomised, placebo-controlled, double-blind, parallel group, multicentre clinical trial. *United European gastroenterology journal*, 4(3), 455-465.
30. Allison, M. J., Bryant, M. P., & Doetsch, R. N. Studies on the metabolic function of branched-chain volatile fatty acids, growth factors for ruminococci I: Incorporation of Isovalerate into leucine. *Journal of Bacteriology*. 1962;83(3), 523-532.
31. Altayar, O., Sharma, V., Prokop, L. J., Sood, A., & Murad, M. H. (2015). Psychological therapies in patients with irritable bowel syndrome: a systematic review and meta-analysis of randomized controlled trials. *Gastroenterology Research and Practice*
32. Altomare, A., Di Rosa, C., Imperia, E., Emerenziani, S., Cicala, M., & Guarino, M. P. L. (2021). Diarrhea predominant-irritable bowel syndrome (IBS-D): effects of different nutritional patterns on intestinal dysbiosis and symptoms. *Nutrients*, 13(5), 1506
33. Amiri, P., Arefhosseini, S., Bakhshimoghaddam, F., Jamshidi Gurvan, H., & Hosseini, S. A. (2022). Mechanistic insights into the pleiotropic effects of butyrate as a potential therapeutic agent on NAFLD management: A systematic review. *Frontiers in Nutrition*, 9, 1037696.
34. Andresen, V., Keller, J., Pehl, C., Schemann, M., Preiss, J., & Leyer, P. Irritable bowel syndrome--the main recommendations. *Deutsches Arzteblatt International*. 2011;108(44):751-760. doi:10.3238/arztebl.2011.0751
35. Ang, Q. Y., Alexander, M., Newman, J. C., Tian, Y., Cai, J., Upadhyay, V., ... & Turnbaugh, P. J. (2020). Ketogenic diets alter the gut microbiome resulting in decreased intestinal Th17 cells. *Cell*, 181(6), 1263-1275.

36. Aoyama, M., Kotani, J., & Usami, M. (2010). Butyrate and propionate induced activated or non-activated neutrophil apoptosis via HDAC inhibitor activity but without activating GPR-41/GPR-43 pathways. *Nutrition*, 26(6), 653-661.
37. Appleby, R. N., & Walters, J. R. (2014). The role of bile acids in functional GI disorders. *Neurogastroenterology & Motility*, 26(8), 1057-1069.
38. Arffmann, S., Andersen, J. R., Hegnhøj, J., Schaffalitzky de Muckadell, O. B., Mogensen, N. B., & Krag, E. The Effect of Coarse Wheat Bran in the Irritable Bowel Syndrome IA Double-Blind Cross-Over Study. *Scandinavian journal of gastroenterology*. 1985;20(3), 295-298.
39. Arjmandi, B. H., Ahn, J., Nathani, S., & Reeves, R. D. (1992). Dietary soluble fiber and cholesterol affect serum cholesterol concentration, hepatic portal venous short-chain fatty acid concentrations and fecal sterol excretion in rats. *The Journal of nutrition*, 122(2), 246-253.
40. Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., ... & Bertalan, M. Enterotypes of the human gut microbiome. *Nature*. 2011;473(7346), 174-180.
41. Attaluri, A., Donahoe, R., Valestin, J., Brown, K., & Rao, S. S. C. (2011). Randomised clinical trial: dried plums (prunes) vs. psyllium for constipation. *Alimentary pharmacology & therapeutics*, 33(7), 822-828.
42. Aunan, J. R., Cho, W. C., & Søreide, K. (2017). The biology of aging and cancer: a brief overview of shared and divergent molecular hallmarks. *Aging and disease*, 8(5), 628.
43. Ayeni, F. A., Biagi, E., Rampelli, S., Fiori, J., Soverini, M., Audu, H. J., ... & Turrone, S. (2018). Infant and adult gut microbiome and metabolome in rural Bassa and urban settlers from Nigeria. *Cell Rep* 23: 3056–3067.
44. Azad, M., Kalam, A., Sarker, M., Li, T., & Yin, J. Probiotic species in the modulation of gut microbiota: an overview. *BioMed research international*. 2018.
45. Azpiroz, F., Dubray, C., Bernalier-Donadille, A., Cardot, J. M., Accarino, A., Serra, J., ... & Dapoigny, M. (2017). Effects of sc FOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. *Neurogastroenterology & Motility*, 29(2), e12911.
46. Badiali, D., Corazziari, E., Habib, F. I., Tomei, E., Bausano, G., Magrini, P., ... & Torsoli, A. (1995). Effect of wheat bran in treatment of chronic nonorganic constipation. *Digestive diseases and sciences*, 40(2), 349-356.
47. Baer, D. J., Rumpler, W. V., Miles, C. W., & Fahey Jr, G. C. (1997). Dietary fiber decreases the metabolizable energy content and nutrient digestibility of mixed diets fed to humans. *The Journal of nutrition*, 127(4), 579-586.
48. Bailón, E., Cueto-Sola, M., Utrilla, P., Rodríguez-Cabezas, M. E., Garrido-Mesa, N., Zarzuelo, A., ... & Comalada, M. (2010). Butyrate in vitro immune-modulatory effects might be mediated through a proliferation-related induction of apoptosis. *Immunobiology*, 215(11), 863-873.
49. Bajor, A., Törnblom, H., Rudling, M., Ung, K. A., & Simrén, M. (2015). Increased colonic bile acid exposure: a relevant factor for symptoms and treatment in IBS. *Gut*, 64(1), 84-92.
50. Ballou, S., Alhassan, E., Hon, E., Lembo, C., Rangan, V., Singh, P., ... & Lembo, A. (2018). Sleep disturbances are commonly reported among patients presenting to a gastroenterology clinic. *Digestive diseases and sciences*, 63, 2983-2991.
51. Banasiewicz, T., Borycka-Kiciak, K., Dobrowolska-Zachwieja, A., Friediger, J., Kiciak, A., Krokowicz, P., ... & Zabielski, R. (2010). Clinical aspects of sodium butyrate application in dietary treatment of bowel diseases. *Gastroenterology Review/Przegląd Gastroenterologiczny*, 5(6), 329-334.
- Bandura, A. (1999). Social cognitive theory: An agentic perspective. *Asian journal of social psychology*, 2(1), 21-41.
52. Basnayake, C. (2018). Treatment of irritable bowel syndrome. *Australian Prescriber*, 41(5), 145.
53. Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *MBio*. 2019;10(1), e02566-18. Baxter, N.
54. Bajka, B. H., Clarke, J. M., Topping, D. L., Cobiac, L., Abeywardena, M. Y., & Patten, G. S. Butyrylated starch increases large bowel butyrate levels and lowers colonic smooth muscle contractility in rats. *Nutrition research*. 2010;30(6), 427-434.

55. Ballou, S., & Keefer, L. (2017). Psychological Interventions for Irritable Bowel Syndrome and Inflammatory Bowel Diseases. *Clin Transl Gastroenterol*, 8(1), e214. doi:10.1038/ctg.2016.69
56. Balsari, A., Ceccarelli, A., Dubini, F., Fesce, E., & Poli, G. (1982). The fecal microbial population in the irritable bowel syndrome. *Microbiologica*, 5(3), 185-194.
57. Banasiewicz, T., Krokowicz, Ł., Stojcev, Z., Kaczmarek, B. F., Kaczmarek, E., Maik, J., ... & Drews, M. (2013). Microencapsulated sodium butyrate reduces the frequency of abdominal pain in patients with irritable bowel syndrome. *Colorectal Disease*, 15(2), 204-209.
58. Barandouzi, Z. A., Lee, J., Maas, K., Starkweather, A. R., & Cong, X. S. (2021). Altered gut microbiota in irritable bowel syndrome and its association with food components. *Journal of Personalized Medicine*, 11(1), 35.
59. Baranska, A., Mujagic, Z., Smolinska, A., Dallinga, J. W., Jonkers, D. M., Tigchelaar, E. F., . . . van Schooten, F. J. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. *Alimentary Pharmacology & Therapeutics*. 2016;44(1):45-56. doi:10.1111/apt.13654.
60. Barbara, G., Cremon, C., & Azpiroz, F. Probiotics in irritable bowel syndrome: Where are we? *Neurogastroenterology & Motility*. 2018;30(12), e13513.
61. Barbara, G., Feinle-Bisset, C., Ghoshal, U. C., Santos, J., Vanner, S. J., Vergnolle, N., ... & Quigley, E. M. (2016). The intestinal microenvironment and functional gastrointestinal disorders. *Gastroenterology*. 2016;150(6), 1305-1318.
62. Barik, S. (2020). The uniqueness of tryptophan in biology: properties, metabolism, interactions and localization in proteins. *International journal of molecular sciences*, 21(22), 8776.
63. Barrett, J. S., Gearry, R. B., Muir, J. G., Irving, P. M., Rose, R., Rosella, O., . . . Gibson, P. R. Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon. *Alimentary Pharmacology & Therapeutics*. 2010;31(8): 874-882. doi:10.1111/j.1365-2036.2010.04237.x
64. Barroso, E., Cueva, C., Peláez, C., Martínez-Cuesta, M. C., & Requena, T. Development of human colonic microbiota in the computer-controlled dynamic SIMulator of the GastroIntestinal tract SIMGI. *LWT-Food Science and Technology*. 2015; 61(2), 283-289.
65. Bashashati, M., Rezaei, N., Andrews, C. N., Chen, C. Q., Daryani, N. E., Sharkey, K. A., & Storr, M. A. (2012). Cytokines and irritable bowel syndrome: where do we stand? *Cytokine*. 2012;57(2):201-209.
66. Bassotti, G., Fruganti, A., Maconi, G., Marconi, P., & Fettucciari, K. (2021). Clostridioides difficile infection in patients with inflammatory bowel disease may be favoured by the effects of proinflammatory cytokines on the enteroglial network. *Journal of Inflammation Research*, 7443-7453.
67. Baumann, A., Jin, C. J., Brandt, A., Sellmann, C., Nier, A., Burkard, M., ... & Bergheim, I. (2020). Oral supplementation of sodium butyrate attenuates the progression of non-alcoholic steatohepatitis. *Nutrients*, 12(4), 951.
68. Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *MBio*. 2019;10(1), e02566-18. Baxter, N.
69. Bayer, S. B., Gearry, R. B., Drummond, L. N., Birgit, S., Gearry, R. B., Drummond, L. N., ... & Bayer, S. B. Putative mechanisms of kiwifruit on maintenance of normal gastrointestinal function Putative mechanisms of kiwifruit on maintenance of normal gastrointestinal function.
70. Bayrer, J. R., Castro, J., Venkataraman, A., Touhara, K. K., Rossen, N. D., Morrie, R. D., ... & Julius, D. (2023). Gut enterochromaffin cells drive visceral pain and anxiety. *Nature*, 1-6.
71. Beger RD, Dunn W, Schmidt MA, et al. Metabolomics enables precision medicine: "A White Paper, Community Perspective." *Metabolomics*. 2016;12(9):149. doi:10.1007/s11306-016-1094-6.
72. Belenguer, A., Duncan, S. H., Calder, A. G., Holtrop, G., Louis, P., Lobley, G. E., & Flint, H. J. Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. *Applied and Environmental Microbiology*. 2006;72(5), 3593-3599.
73. Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157(1), 121-141.

74. Bellono, N. W., Bayrer, J. R., Leitch, D. B., Castro, J., Zhang, C., O'Donnell, T. A., ... & Julius, D. (2017). Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell*, 170(1), 185-198.
75. Berg, S. Z., & Berg, J. (2023). Melanin: a unifying theory of disease as exemplified by Parkinson's, Alzheimer's, and Lewy body dementia. *Frontiers in Immunology*, 14, 1228530.
76. Bergman, E. N. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological reviews*. 1990;70(2), 567-590.
77. Bernstein, C. N., Fried, M., Krabshuis, J. H., Cohen, H., Eliakim, R., Fedail, S., ... & LeMair, A. W. (2010). World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. *Inflammatory bowel diseases*, 16(1), 112-124.
78. Beukema, M., Faas, M. M., & de Vos, P. (2020). The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells. *Experimental & molecular medicine*, 52(9), 1364-1376.
79. Bhadra, U., Thakkar, N., Das, P., & Bhadra, M. P. (2017). Evolution of circadian rhythms: from bacteria to human. *Sleep medicine*, 35, 49-61.
80. Bhattarai, Y., Williams, B. B., Battaglioli, E. J., Whitaker, W. R., Till, L., Grover, M., ... & Kashyap, P. C. (2018). Gut microbiota-produced tryptamine activates an epithelial G-protein-coupled receptor to increase colonic secretion. *Cell host & microbe*, 23(6), 775-785.
81. Biernat, K. A., Pellock, S. J., Bhatt, A. P., Bivins, M. M., Walton, W. G., Tran, B. N. T., ... & Redinbo, M. R. (2019). Structure, function, and inhibition of drug reactivating human gut microbial β -glucuronidases. *Scientific reports*, 9(1), 825.
82. Biesiekierski, J. R., Rosella, O., Rose, R., Liels, K., Barrett, J. S., Shepherd, S. J., . . . Muir, J. G. Quantification of fructans, galacto-oligosaccharides and other short-chain carbohydrates in processed grains and cereals. *Journal of Human Nutrition and Dietetics*. 2011;24(2):154-176. doi:10.1111/j.1365-277X.2010.01139.x
83. Bien, J., Palagani, V., & Bozko, P. (2013). The intestinal microbiota dysbiosis and *Clostridium difficile* infection: is there a relationship with inflammatory bowel disease?. *Therapeutic advances in gastroenterology*, 6(1), 53-68
84. Binder, H. J., & Mehta, P. (1989). Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. *Gastroenterology*, 96(4), 989-996.
85. Bishehsari, F., Engen, P. A., Preite, N. Z., Tuncil, Y. E., Naqib, A., Shaikh, M., ... & Keshavarzian, A. (2018). Dietary fiber treatment corrects the composition of gut microbiota, promotes SCFA production, and suppresses colon carcinogenesis. *Genes*, 9(2), 102.
86. Black, C. J., Thakur, E. R., Houghton, L. A., Quigley, E. M., Moayyedi, P., & Ford, A. C. (2020). Efficacy of psychological therapies for irritable bowel syndrome: systematic review and network meta-analysis. *Gut*, 69(8), 1441-1451.
87. Blackwood, A. D., Salter, J., Dettmar, P. W., & Chaplin, M. F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *The journal of the Royal Society for the Promotion of Health*, 120(4), 242-247.
88. Blanchard, E. B., Lackner, J. M., Jaccard, J., Rowell, D., Carosella, A. M., Powell, C., . . . Kuhn, E. The role of stress in symptom exacerbation among IBS patients. *Journal of Psychosomatic Research*. 2008; 64(2):119-128. doi:10.1016/j.jpsychores.2007.10.010.
89. Blekhman, R., Goodrich, J. K., Huang, K., Sun, Q., Bukowski, R., Bell, J. T., ... & Clark, A. G. (2015). Host genetic variation impacts microbiome composition across human body sites. *Genome biology*, 16(1), 191.
90. Blackshaw, L. A. (2009). The ion channel TRPA1 is required for normal mechanosensation and is modulated by algogenic stimuli. *Gastroenterology*, 137(6), 2084-2095.
91. Bleser, S. D. (2006). Chronic constipation: let symptom type and severity direct treatment: how various therapies fit into the scheme of things; what is unlikely to help. *Journal of family practice*, 55(7), 587-594.
92. Bliss, D. Z., Jung, H. J., Savik, K., Lowry, A., LeMoine, M., Jensen, L., ... & Schaffer, K. (2001). Supplementation with dietary fiber improves fecal incontinence. *Nursing research*, 50(4), 203-213.

93. Boesmans, L., Valles-Colomer, M., Wang, J., Eeckhaut, V., Falony, G., Ducatelle, R., ... & Verbeke, K. (2018). Butyrate producers as potential next-generation probiotics: safety assessment of the administration of *Butyricicoccus pullicaecorum* to healthy volunteers. *Msystems*, 3(6), e00094-18.
94. Bolisetty, S., & Jaimes, E. A. (2013). Mitochondria and reactive oxygen species: physiology and pathophysiology. *International journal of molecular sciences*, 14(3), 6306-6344.
95. Bonfrate, L., Tack, J., Grattagliano, I., Cuomo, R., & Portincasa, P. (2013). Microbiota in health and irritable bowel syndrome: current knowledge, perspectives and therapeutic options. *Scandinavian journal of gastroenterology*, 48(9), 995-1009.
96. Born, P., Bauch, C., Ulm, K., Kamereck, K., Classen, M., & Scheppach, W. (2000). Fecal bacterial activity in symptomatic carbohydrate malabsorption: Effect on the fecal short-chain fatty acid ratio. *Zeitschrift für Gastroenterologie*, 38(08), 623-626.
97. Bosch, G., Wrigglesworth, D. J., Cone, J. W., Pellikaan, W. F., & Hendriks, W. H. (2013). Effects of preservation conditions of canine feces on in vitro gas production kinetics and fermentation end products. *Journal of animal science*, 91(1), 259-267.
98. Bottacini, F., Ventura, M., Van Sinderen, D., & Motherway, M. O. C. Diversity, ecology and intestinal function of bifidobacteria. In *Microbial cell factories*. 2014;(Vol. 13, No. 1, p. S4). BioMed Central.
99. Bouhnik Y, Raskine L, Simoneau G, et al. The capacity of nondigestible carbohydrates to stimulate fecal Bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *American Journal of Clinical Nutrition*. 2004;80:1658–1664.
100. Bourassa, M. W., Alim, I., Bultman, S. J., & Ratan, R. R. Butyrate, neuroepigenetics and the gut microbiome: can a high fiber diet improve brain health? *Neuroscience letters*. 2016;625, 56-63.
101. Bijkerk, C. J., De Wit, N. J., Muris, J. W. M., Whorwell, P. J., Knottnerus, J. A., & Hoes, A. W. BMJ: Soluble or insoluble fibre in irritable bowel syndrome in primary care? Randomised placebo controlled trial." *Alternative Medicine Review*. 2009;14(4), 409-410.
102. Boeckxstaens, G. E., Drug, V., Dumitrascu, D., Farmer, A. D., Hammer, J., Hausken, T., ... & COST Action BM1106 GENIEUR members. (2016). Phenotyping of subjects for large scale studies on patients with IBS. *Neurogastroenterology & Motility*, 28(8), 1134-1147.
103. Böhn, L., Störsrud, S., Törnblom, H., Bengtsson, U., & Simrén, M. (2013). Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. *Official journal of the American College of Gastroenterology| ACG*, 108(5), 634-641.
104. Bohn, L., Storsrud, S., Liljebo, T., Collin, L., Lindfors, P., Tornblom, H., & Simren, M. Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial. *Gastroenterology*. 2015;149(6):1399-1407.e1392. doi:10.1053/j.gastro.2015.07.054
105. Böhn, L., Störsrud, S., & Simrén, M. Nutrient intake in patients with irritable bowel syndrome compared with the general population. *Neurogastroenterology & motility*. 2013;25(1), 23-e1.
106. Bonaz, B., Pellissier, S., Sinniger, V., Clarençon, D., Peinnequin, A., & Canini, F. (2012). The irritable bowel syndrome: how stress can affect the amygdala activity and the brain-gut axis. *The amygdala-A discrete multitasking manager*.
107. Borg, J., Stenger, A., & Toazara, J. (1986). Uptake and metabolism of L-[3H] pyroglutamic acid in neuronal and glial cells in culture. *Neurochemistry international*, 8(3), 397-402.
108. Born, P., Bauch, C., Ulm, K., Kamereck, K., Classen, M., & Scheppach, W. (2000). Fecal bacterial activity in symptomatic carbohydrate malabsorption: Effect on the fecal short-chain fatty acid ratio. *Zeitschrift für Gastroenterologie*, 38(08), 623-626.
109. Borrell-Carrio, F., Suchman, A. L., & Epstein, R. M. The biopsychosocial model 25 years later: principles, practice, and scientific inquiry. *The Annals of Family Medicine*. 2004;2(6), 576-582. doi:10.1370/afm.245.
110. Bosaeus, I. (2004). Fibre effects on intestinal functions (diarrhoea, constipation and irritable bowel syndrome). *Clinical Nutrition Supplements*, 1(2), 33-38.
111. Bosi, A., Banfi, D., Bistoletti, M., Giaroni, C., & Baj, A. (2020). Tryptophan metabolites along the microbiota-gut-brain axis: an interkingdom communication system influencing the gut in health and disease. *International Journal of Tryptophan Research*, 13, 1178646920928984.

112. Brodribb, A. J. M. Treatment of symptomatic diverticular disease with a high-fibre diet. *The Lancet*. 1977;309(8013), 664-666.
113. Born, P., Bauch, C., Ulm, K., Kamereck, K., Classen, M., & Scheppach, W. Fecal bacterial activity in symptomatic carbohydrate malabsorption: effect on the fecal short-chain fatty acid ratio. *Zeitschrift für Gastroenterologie*. 2000;38(08), 623-626.
114. Bottacini, F., Ventura, M., Van Sinderen, D., & Motherway, M. O. C. Diversity, ecology and intestinal function of bifidobacteria. In *Microbial cell factories*. 2014;(Vol. 13, No. 1, p. S4). BioMed Central.
115. Bouhnik Y, Raskine L, Simoneau G, et al. The capacity of nondigestible carbohydrates to stimulate fecal Bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am J Clin Nutr*. 2004;80:1658–1664.
116. Bourassa MW, Alim I, Bultman SJ, Ratan RR. Butyrate, Neuroepigenetics and the Gut Microbiome: Can a High Fiber Diet Improve Brain Health? *Neuroscience letters*. 2016;625:56-63.
doi:10.1016/j.neulet.2016.02.009
- Brooks, A. N., Turkarslan, S., Beer, K. D., Yin Lo, F., & Baliga, N. S. (2011). Adaptation of cells to new environments. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 3(5), 544-561.
117. Bradley, H. K., Wyatt, G. M., Bayliss, C. E., & Hunter, J. O. (1987). Instability in the faecal flora of a patient suffering from food-related irritable bowel syndrome. *Journal of Medical Microbiology*, 23(1), 29-32.
118. Brierley, S. M., Hughes, P. A., Page, A. J., O'Donnell, T. A., Martin, C. M., Kwan, K. Y., ... & Blackshaw, L. A. (2008). 50 Novel and Specific Roles for the Ion Channel TRPA1 in Visceral Sensory Transduction. *Gastroenterology*, 4(134), A-8.
119. Brigidi, P., Vitali, B., Swennen, E., Bazzocchi, G., & Matteuzzi, D. (2001). Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. *Research in Microbiology*, 152(8), 735-741.
120. Brownawell, A. M., Caers, W., Gibson, G. R., Kendall, C. W., Lewis, K. D., Ringel, Y., & Slavin, J. L. (2012). Prebiotics and the health benefits of fiber: current regulatory status, future research, and goals. *The Journal of nutrition*, 142(5), 962-974.
121. Brownlee, I. A. (2011). The physiological roles of dietary fibre. *Food hydrocolloids*, 25(2), 238-250.
122. Bruijning, M., Ayroles, J. F., Henry, L. P., Koskella, B., Meyer, K. M., & Metcalf, C. J. E. (2022). Relative abundance data can misrepresent heritability of the microbiome. *bioRxiv*.
123. Brunk, E., Sahoo, S., Zielinski, D. C., Altunkaya, A., Dräger, A., Mih, N., ... & Prlić, A. (2018). Recon3D enables a three-dimensional view of gene variation in human metabolism. *Nature biotechnology*, 36(3), 272.
124. Brunk, U. T., & Terman, A. (2002). Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radical Biology and Medicine*, 33(5), 611-619.
125. Bubier, J. A., Philip, V. M., Quince, C., Campbell, J., Zhou, Y., Vishnivetskaya, T., ... & Chesler, E. J. (2020). A microbe associated with sleep revealed by a novel systems genetic analysis of the microbiome in collaborative cross mice. *Genetics*, 214(3), 719-733.
126. Buono, J. L., Mathur, K., Averitt, A. J., & Andrae, D. A. (2017). Economic Burden of Irritable Bowel Syndrome with Diarrhea: Retrospective Analysis of a U.S. Commercially Insured Population. *Journal of Managed Care & Specialty Pharmacy*, 23(4), 453-460. doi:10.18553/jmcp.2016.16138
127. Burden, S. (2001). Dietary treatment of irritable bowel syndrome: current evidence and guidelines for future practice. *Journal of Human Nutrition and Dietetics*, 14(3), 231-241.
128. Burkitt, D. P., Walker, A. R. P., & Painter, N. S. (1972). Effect of dietary fibre on stools and transit-times, and its role in the causation of disease. *The Lancet*, 300(7792), 1408-1411.
129. Burks, A. W., Vanderhoof, J. A., Mehra, S., Ostrom, K. M., & Baggs, G. (2001). Randomized clinical trial of soy formula with and without added fiber in antibiotic-induced diarrhea. *The Journal of pediatrics*, 139(4), 578-582.
130. Burr, R. L., Gu, H., Cain, K., Djukovic, D., Zhang, X., Han, C., ... & Heitkemper, M. (2019). Tryptophan metabolites in irritable bowel syndrome: an overnight time-course study. *Journal of Neurogastroenterology and Motility*, 25(4), 551.

131. Bustos, A. Y., Saavedra, L., de Valdez, G. F., Raya, R. R., & Taranto, M. P. Relationship between bile salt hydrolase activity, changes in the internal pH and tolerance to bile acids in lactic acid bacteria. *Biotechnology letters*. 2012;34(8), 1511-1518.
132. Cai, J., Zhang, J., Tian, Y., Zhang, L., Hatzakis, E., Krausz, K. W., ... & Patterson, A. D. Orthogonal comparison of GC-MS and ¹H NMR spectroscopy for short chain fatty acid quantitation. *Analytical chemistry*. 2017;89(15), 7900-7906.
133. Cai, Y., Folkerts, J., Folkerts, G., Maurer, M., & Braber, S. (2020). Microbiota-dependent and-independent effects of dietary fibre on human health. *British journal of pharmacology*, 177(6), 1363-1381.
134. Calafate, S., Özturan, G., Thrupp, N., Vanderlinden, J., Santa-Marinha, L., Morais-Ribeiro, R., ... & de Wit, J. (2023). Early alterations in the MCH system link aberrant neuronal activity and sleep disturbances in a mouse model of Alzheimer's disease. *Nature neuroscience*, 26(6), 1021-1031.
135. Calderon, G., Patel, C., Camilleri, M., James-Stevenson, T., Bohm, M., Siwiec, R., ... & Shin, A. (2022). Associations of habitual dietary intake with fecal short-chain fatty acids and bowel functions in irritable bowel syndrome. *Journal of clinical gastroenterology*, 56(3), 234-242.
136. Calzadilla, N., Comiskey, S. M., Dudeja, P. K., Saksena, S., Gill, R. K., & Alrefai, W. A. (2022). Bile acids as inflammatory mediators and modulators of intestinal permeability. *Frontiers in Immunology*, 13, 1021924.
137. Camilleri, M. (2002). Serotonergic modulation of visceral sensation: lower gut. *Gut*, 51(suppl 1), i81-i86.
138. Camilleri, M., & Di Lorenzo, C. (2012). Brain-gut axis: from basic understanding to treatment of IBS and related disorders. *Journal of pediatric gastroenterology and nutrition*, 54(4), 446-453.
139. Camilleri, M. (2014). Advances in understanding of bile acid diarrhea. *Expert review of gastroenterology & hepatology*, 8(1), 49-61.
140. Camilleri, M., Ford, A. C., Mawe, G. M., Dinning, P. G., Rao, S. S., Chey, W. D., ... & Chang, L. (2017). Chronic constipation. *Nature Reviews Disease Primers*, 3(1), 1-19.
141. Canakis, A., Haroon, M., & Weber, H. C. (2020). Irritable bowel syndrome and gut microbiota. *Current Opinion in Endocrinology, Diabetes and Obesity*, 27(1), 28-35.
142. Canavan, C., West, J. & Card, T. The epidemiology of irritable bowel syndrome. *Clin. Epidemiol.* 6, 71 (2014).
143. Cani PD, de Vos WM. Next-Generation Beneficial Microbes: The Case of *Akkermansia muciniphila*. *Frontiers in Microbiology*. 2017;8:1765. doi:10.3389/fmicb.2017.01765.
144. Cann, P. A., Read, N. W., & Holdsworth, C. D. What is the benefit of coarse wheat bran in patients with irritable bowel syndrome? *Gut*. 1984;25(2), 168-173.
145. Cao Y, Gao X, Zhang W, et al. Dietary fiber enhances TGF- β signaling and growth inhibition in the gut. *American Journal of Physiology - Gastrointestinal and Liver Physiology*. 2011;301(1):G156-G164. doi:10.1152/ajpgi.00362.2010
146. Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of gastroenterology: quarterly publication of the Hellenic Society of Gastroenterology*, 28(2), 203.
147. Carbonero, F., Benefiel, A. C., Alizadeh-Ghamsari, A. H., & Gaskins, H. R. (2012). Microbial pathways in colonic sulfur metabolism and links with health and disease. *Frontiers in physiology*, 3, 448.
148. Carlson, A. B., & Kraus, G. P. (2018). Physiology, cholinergic receptors.
149. Carmody, R. N., Bisanz, J. E., Bowen, B. P., Maurice, C. F., Lyalina, S., Louie, K. B., ... & Turnbaugh, P. J. (2019). Cooking shapes the structure and function of the gut microbiome. *Nature microbiology*, 4(12), 2052-2063.
150. Carmody, R. N., Gerber, G. K., Luevano Jr, J. M., Gatti, D. M., Somes, L., Svenson, K. L., & Turnbaugh, P. J. Diet dominates host genotype in shaping the murine gut microbiota. *Cell host & microbe*. 2015;17(1), 72-84.
151. Canani, R. B., Di Costanzo, M., Leone, L., Pedata, M., Meli, R., & Calignano, A. (2011). Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World journal of gastroenterology: WJG*, 17(12), 1519.

152. Carroll, Ian M., Tamar Ringel-Kulka, Jennica P. Siddle, and Yehuda Ringel. "Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome." *Neurogastroenterology & Motility* 24, no. 6 (2012): 521-e248.
153. Carroll, I. M., Ringel-Kulka, T., Keku, T. O., Chang, Y. H., Packey, C. D., Sartor, R. B., & Ringel, Y. (2011). Molecular analysis of the luminal-and mucosal-associated intestinal microbiota in diarrhea-predominant irritable bowel syndrome. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 301(5), G799-G807.
154. Cashion, A. K., Gill, J., Hawes, R., Henderson, W. A., & Saligan, L. National Institutes of Health Symptom Science Model sheds light on patient symptoms. *Nursing outlook*. 2016;64(5), 499-506.
155. Catassi, G., Lionetti, E., Gatti, S., & Catassi, C. The low FODMAP diet: many question marks for a catchy acronym. *Nutrients*. 2017;9(3), 292.
156. Cao, H., Liu, X., An, Y., Zhou, G., Liu, Y., Xu, M., ... & Wang, B. (2017). Dysbiosis contributes to chronic constipation development via regulation of serotonin transporter in the intestine. *Scientific reports*, 7(1), 10322.
157. Centanni, M., Hutchison, J. C., Carnachan, S. M., Daines, A. M., Kelly, W. J., Tannock, G. W., & Sims, I. M. Differential growth of bowel commensal *Bacteroides* species on plant xylans of differing structural complexity. *Carbohydrate polymers*. 2017;157, 1374-1382.
158. Cesbron, N., Royer, A. L., Guitton, Y., Sydor, A., Le Bizec, B., & Dervilly-Pinel, G. (2017). Optimization of fecal sample preparation for untargeted LC-HRMS based metabolomics. *Metabolomics*, 13(8), 99.
159. Chadwick, D. J., & Goode, J. A. (Eds.). (2003). *Mechanisms and Biological Significance of Pulsatile Hormone Secretion*. John Wiley & Sons.
160. Champe, P. C., Harvey, R. A., & Ferrier, D. R. (2005). *Biochemistry*. Lippincott Williams & Wilkins.
161. Champ, Martine, Anna-Maria Langkilde, Fred Brouns, Bernd Kettlitz, and Yves Le Bail Collet. "Advances in dietary fibre characterisation. 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects." *Nutrition Research Reviews* 16, no. 1 (2003): 71-82.
162. Chang, L., Sundaresh, S., Elliott, J., Anton, P. A., Baldi, P., Licudine, A., ... & Mayer, E. A. (2009). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in irritable bowel syndrome. *Neurogastroenterology & Motility*, 21(2), 149-159.
163. Chang, C. C., Lin, Y. T., Lu, Y. T., Liu, Y. S., & Liu, J. F. (2010). Kiwifruit improves bowel function in patients with irritable bowel syndrome with constipation. *Asia Pacific journal of clinical nutrition*, 19(4), 451.
164. Charrier, C., Duncan, G. J., Reid, M. D., Rucklidge, G. J., Henderson, D., Young, P., ... & Louis, P. (2006). A novel class of CoA-transferase involved in short-chain fatty acid metabolism in butyrate-producing human colonic bacteria. *Microbiology*, 152(1), 179-185.
165. Chassaing, B., Kumar, M., Baker, M. T., Singh, V., & Vijay-Kumar, M. (2014). Mammalian gut immunity. *Biomedical journal*, 37(5), 246.
166. Chassard, C., Dapoigny, M., Scott, K. P., Crouzet, L., Del'homme, C., Marquet, P., ... & Dubray, C. Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome. *Alimentary pharmacology & therapeutics*. 2012;35(7), 828-838.
167. Chaussade, S., Khyari, A., Roche, H., Garret, M., Gaudric, M., Couturier, D., & Guerre, J. (1989). Determination of total and segmental colonic transit time in constipated patients. *Digestive diseases and sciences*, 34(8), 1168-1172.
168. Chen, H. W., Zhou, R., Cao, B. F., Liu, K., Zhong, Q., Huang, Y. N., ... & Wu, X. B. (2023). The predictive, preventive, and personalized medicine of insomnia: gut microbiota and inflammation. *EPMA Journal*, 14(4), 571-583.
169. Chen, X., Sun, H., Jiang, F., Shen, Y., Li, X., Hu, X., ... & Wei, P. (2020). Alteration of the gut microbiota associated with childhood obesity by 16S rRNA gene sequencing. *PeerJ*, 8, e8317.
170. Chen, T., Kim, C. Y., Kaur, A., Lamothe, L., Shaikh, M., Keshavarzian, A., & Hamaker, B. R. (2017). Dietary fibre-based SCFA mixtures promote both protection and repair of intestinal epithelial barrier function in a Caco-2 cell model. *Food & function*, 8(3), 1166-1173.
171. Chen, T. F., Chen, R. M., Tsai, J. J., & Hu, R. M. (2016, October). Fine Classification of Human Gut Microbiota by Using Hierarchical Clustering Approach. In 2016 IEEE 16th International Conference on Bioinformatics and Bioengineering (BIBE) (pp. 109-112). IEEE.

172. Chen, J., & Vitetta, L. (2018). Inflammation-modulating effect of butyrate in the prevention of colon cancer by dietary fiber. *Clinical colorectal cancer*, 17(3), e541-e544.
173. Chen, X., Su, W., Wan, T., Yu, J., Zhu, W., Tang, F., ... & Zheng, S. G. (2017). Sodium butyrate regulates Th17/Treg cell balance to ameliorate uveitis via the Nrf2/HO-1 pathway. *Biochemical pharmacology*, 142, 111-119.
174. Chen, M. X., Wang, S. Y., Kuo, C. H., & Tsai, I. L. Metabolome analysis for investigating host-gut microbiota interactions. *Journal of the Formosan Medical Association*. 2019;118, S10-S22.
175. Cherbut, C. Motor effects of short-chain fatty acids and lactate in the gastrointestinal tract. *Proceedings of the Nutrition Society*. 2003;62(1), 95-99.
176. Cheskin, L. J., Mitola, A. H., Ridoré, M., Kolge, S., Hwang, K., & Clark, B. (2009). A naturalistic, controlled, crossover trial of plum juice versus psyllium versus control for improving bowel function. *Internet J Nutr Wellness*, 7(2), 5447.
177. Chey, W. D., Keefer, L., Whelan, K., & Gibson, P. R. (2021). Behavioral and diet therapies in integrated care for patients with irritable bowel syndrome. *Gastroenterology*, 160(1), 47-62.
178. Chey, W. Y., Jin, H. O., Lee, M. H., Sun, S. W., & Lee, K. Y. (2001). Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. *Official journal of the American College of Gastroenterology* | ACG, 96(5), 1499-1506.
179. Chey, W. D. Food: the main course to wellness and illness in patients with irritable bowel syndrome. *The American journal of gastroenterology*. 2016;111(3):366.
180. Chey, W. D., Kurlander, J., & Eswaran, S. (2015). Irritable bowel syndrome: a clinical review. *Jama*, 313(9), 949-958.
181. Chey, W. D., Shah, E. D., & DuPont, H. L. (2020). Mechanism of action and therapeutic benefit of rifaximin in patients with irritable bowel syndrome: a narrative review. *Therapeutic Advances in Gastroenterology*, 13, 1756284819897531.
182. Chiang, J. Y. (2013). Bile acid metabolism and signaling. *Comprehensive Physiology*, 3(3), 1191-1212.
183. Chiarioni, G., Popa, S. L., Ismaiel, A., Pop, C., Dumitrascu, D. I., Brata, V. D., ... & Surdea-Blaga, T. (2023). The Effect of Polyphenols, Minerals, Fibers, and Fruits on Irritable Bowel Syndrome: A Systematic Review. *Nutrients*, 15(18), 4070.
184. CHIRA A, FILIP M, DUMITRAȘCU DL. Patterns of alternation in irritable bowel syndrome. *Clujul Medical*. 2016;89(2):220-223. doi:10.15386/cjmed-589.
185. Cho, S., & Almeida, N. (2012). *Dietary fiber and health*. CRC Press.
186. Chong, P. P., Chin, V. K., Looi, C. Y., Wong, W. F., Madhavan, P., & Yong, V. C. (2019). The microbiome and irritable bowel syndrome—a review on the pathophysiology, current research and future therapy. *Frontiers in microbiology*, 10, 1136.
187. Chong, J., Wishart, D. S., & Xia, J. Using metaboanalyst 4.0 for comprehensive and integrative metabolomics data analysis. *Current protocols in bioinformatics*. 2019;68(1), e86.
188. Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., ... & Xia, J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic acids research*. 2018
189. Chong, E. Y., Huang, Y., Wu, H., Ghasemzadeh, N., Uppal, K., Quyyumi, A. A., Jones, D. P., & Yu, T. (2015). Local false discovery rate estimation using feature reliability in LC/MS metabolomics data. *Scientific reports*, 5, 17221. <https://doi.org/10.1038/srep17221>
190. Choung, Rok Seon et al. "Irritable bowel syndrome and chronic pelvic pain: a population-based study." *Journal of clinical gastroenterology* vol. 44,10 (2010): 696-701. doi:10.1097/MCG.0b013e3181d7a368
191. Christodoulides, S., Dimidi, E., Fragkos, K. C., Farmer, A. D., Whelan, K., & Scott, S. M. (2016). Systematic review with meta-analysis: effect of fibre supplementation on chronic idiopathic constipation in adults. *Alimentary pharmacology & therapeutics*, 44(2), 103-116.
192. Christopher, M. M., Eckfeldt, J. H., & Eaton, J. W. (1990). Propylene glycol ingestion causes D-lactic acidosis. *Laboratory investigation; a journal of technical methods and pathology*, 62(1), 114-118.
193. Chu, C., Li, T., Yu, L., Li, Y., Li, M., Guo, M., Zhao, J., Zhai, Q., Tian, F. and Chen, W., 2023. A low-protein, high-carbohydrate diet exerts a neuroprotective effect on mice with 1-methyl-4-phenyl-1, 2, 3, 6-

- tetrahydropyridine-Induced Parkinson's disease by regulating the microbiota-metabolite-brain axis and fibroblast growth factor 21. *Journal of Agricultural and Food Chemistry*, 71(23), pp.8877-8893.
194. Chumpitazi, B. P., Cope, J. L., Hollister, E. B., Tsai, C. M., McMeans, A. R., Luna, R. A., . . . Shulman, R. J. Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*. 2015; 42(4):418-427. doi:10.1111/apt.13286
 195. Ciorba, M. A. (2018). *Gastrointestinal Disease. Pathophysiology of Disease: An Introduction to Clinical Medicine* 8E, 369, chapter 13.
 196. Clausen, M. R., & Mortensen, P. B. (1994). Kinetic studies on the metabolism of short-chain fatty acids and glucose by isolated rat colonocytes. *Gastroenterology*, 106(2), 423-432.
 197. Cleave, T. L. (1941). Natural Bran for Constipation. *British medical journal*, 1(4185), 461.
 198. Cockburn, D. W., & Koropatkin, N. M. (2016). Polysaccharide degradation by the intestinal microbiota and its influence on human health and disease. *Journal of molecular biology*, 428(16), 3230-3252.
 199. Codling, C., O'Mahony, L., Shanahan, F., Quigley, E. M., & Marchesi, J. R. (2010). A molecular analysis of fecal and mucosal bacterial communities in irritable bowel syndrome. *Digestive diseases and sciences*, 55, 392-397.
 200. Collins, S. M. A role for the gut microbiota in IBS. *Nat Rev Gastroenterology & Hepatology*. 2014;11(8):497-505. doi:10.1038/nrgastro.2014.40.
 201. Collins, S. M. (2002). A case for an immunological basis for irritable bowel syndrome. *Gastroenterology*, 122(7), 2078-2080.
 202. Conley, B. A., Egorin, M. J., Tait, N., Rosen, D. M., Sausville, E. A., Dover, G., ... & Van Echo, D. A. (1998). Phase I study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. *Clinical cancer research: an official journal of the American Association for Cancer Research*, 4(3), 629-634.
 203. Conrad, J., & Nöthlings, U. Innovative approaches to estimate individual usual dietary intake in large-scale epidemiological studies. *Proceedings of the Nutrition Society*. 2017;76(3), 213-219.
 204. Cook, S. I., & Sellin, J. H. short chain fatty acids in health and disease. *Alimentary pharmacology & therapeutics*. 1998;12(6), 499-507.
 205. Cook, I. J., Talley, N. J., Benninga, M. A., Rao, S. S., & Scott, S. M. (2009). Chronic constipation: overview and challenges. *Neurogastroenterology & Motility*, 21, 1-8.
 206. Coppola, S., Avagliano, C., Calignano, A., & Berni Canani, R. (2021). The protective role of butyrate against obesity and obesity-related diseases. *Molecules*, 26(3), 6
 207. Corbin, K. D., Carnero, E. A., Dirks, B., Igudesman, D., Yi, F., Marcus, A., ... & Smith, S. R. (2023). Host-diet-gut microbiome interactions influence human energy balance: a randomized clinical trial. *Nature Communications*, 14(1), 3161.
 208. Corre, M. H., Bachmann, V., & Kohn, T. (2022). Bacterial matrix metalloproteases and serine proteases contribute to the extra-host inactivation of enteroviruses in lake water. *The ISME Journal*, 16(8), 1970-1979.
 209. Corsetti, M., & Whorwell, P. (2017). The global impact of IBS: time to think about IBS-specific models of care?. *Therapeutic advances in gastroenterology*, 10(9), 727-736.
 210. Couch, R. D., Navarro, K., Sikaroodi, M., Gillevet, P., Forsyth, C. B., Mutlu, E., ... & Keshavarzian, A. (2013). The approach to sample acquisition and its impact on the derived human fecal microbiome and VOC metabolome. *PloS one*, 8(11).
 211. Correa, R. O., Vieira, A., Sernaglia, E. M., Lancellotti, M., Vieira, A. T., Avila-Campos, M. J., ... & Vinolo, M. A. R. (2017). Bacterial short-chain fatty acid metabolites modulate the inflammatory response against infectious bacteria. *Cellular microbiology*, 19(7), e12720.
 212. Correia, A. S., & Vale, N. (2022). Tryptophan metabolism in depression: a narrative review with a focus on serotonin and kynurenine pathways. *International journal of molecular sciences*, 23(15), 8493.
 213. Costa, R. L., Moreira, J., Lorenzo, A., & Lamas, C. C. (2018). Infectious complications following probiotic ingestion: a potentially underestimated problem? A systematic review of reports and case series. *BMC complementary and alternative medicine*, 18(1), 1-8.

214. Costantini, L., Molinari, R., Farinon, B., & Merendino, N. (2017). Impact of omega-3 fatty acids on the gut microbiota. *International journal of molecular sciences*, 18(12), 2645.
215. Cox, L. M., & Weiner, H. L. (2018). Microbiota signaling pathways that influence neurologic disease. *Neurotherapeutics*, 15(1), 135-145.
216. Cowgill, G. R., & Anderson, W. E. (1932). LAXATIVE EFFECTS OF WHEAT BRAN AND WASHED BRAN IN HEALTHY MEN: A COMPARATIVE STUDY. *Journal of the American Medical Association*, 98(22), 1866-1875.
217. Crawford, I. P., & Stauffer, G. V. (1980). Regulation of tryptophan biosynthesis. *Annual Review of Biochemistry*, 49(1), 163-195.
218. Cremon, C., Guglielmetti, S., Gargari, G., Taverniti, V., Castellazzi, A. M., Valsecchi, C., ... & Gambaccini, D. (2018). Effect of *Lactobacillus paracasei* CNCM I-1572 on symptoms, gut microbiota, short chain fatty acids, and immune activation in patients with irritable bowel syndrome: A pilot randomized clinical trial. *United European gastroenterology journal*, 6(4), 604-613.
219. Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews neuroscience*, 13(10), 701-712.
220. Cummings, J. H. Constipation, dietary fibre and the control of large bowel function. (1984). *Postgraduate Medical Journal*, 60(709), 811.
221. Cummings, J. H. (2001). The effect of dietary fiber on fecal weight and composition. *CRC handbook of dietary fiber in human nutrition*, 3rd ed., 183-252.
222. Cummings, J. H., & Macfarlane, G. T. (1991). The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology*, 70(6), 443-459.
223. Cummings, J. H., Roberfroid, M. B., Andersson, H., Barth, C., Ferro-Luzzi, A., Ghos, Y., ... & Lairon, D. A new look at dietary carbohydrate: chemistry, physiology and health. *European Journal of Clinical Nutrition*. 1997;51(7), 417.
224. Cummings, J. H., & Englyst, H. N. (1987). Fermentation in the human large intestine and the available substrates. *The American Journal of Clinical Nutrition*, 45(5), 1243-1255.
225. Cummings, J. H., Rombeau, J. L., & Sakata, T. (Eds.). (2004). *Physiological and clinical aspects of short-chain fatty acids*. Cambridge University Press.
226. Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., ... & Gibson, G. R. (2021). Shaping the future of probiotics and prebiotics. *Trends in microbiology*, 29(8), 667-685.
227. Dai, C., Zheng, C. Q., Jiang, M., Ma, X. Y., & Jiang, L. J. (2013). Probiotics and irritable bowel syndrome. *World Journal of Gastroenterology: WJG*, 19(36), 5973.
228. Dale, H. F., Rasmussen, S. H., Asiller, Ö. Ö., & Lied, G. A. (2019). Probiotics in irritable bowel syndrome: an up-to-date systematic review. *Nutrients*, 11(9), 2048.
229. Dang, G., Wu, W., Zhang, H., & Everaert, N. (2021). A new paradigm for a new simple chemical: butyrate & immune regulation. *Food & Function*, 12(24), 12181-12193.
230. Danneskiold-Samsøe, N. B., Barros, H. D. D. F. Q., Santos, R., Bicas, J. L., Cazarin, C. B. B., Madsen, L., ... & Júnior, M. R. M. (2019). Interplay between food and gut microbiota in health and disease. *Food Research International*, 115, 23-31.
231. D'Argenio, G., & Mazzacca, G. Short-chain fatty acid in the human colon. In *Advances in Nutrition and Cancer*. 1999; 2(pp. 149-158). Springer, Boston, MA.
232. Darley-Usmar, V. M., Mason, R. P., Chamulitrat, W., Hogg, N., & Kalyanaraman, B. (1995). Lipid peroxidation and cardiovascular disease. In *Immunopharmacology of Free Radical Species* (pp. 23-37). Academic Press.
233. Daskalaki, E., Pilon, N. J., Krook, A., Wheelock, C. E., & Checa, A. (2018). The influence of culture media upon observed cell secretome metabolite profiles: The balance between cell viability and data interpretability. *Analytica chimica acta*, 1037, 338-350.
234. Daubner, S. C., Le, T., & Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of biochemistry and biophysics*, 508(1), 1-12.
235. Daujat-Chavanieu, M., & Gerbal-Chaloin, S. (2020). Regulation of CAR and PXR Expression in Health and Disease. *Cells*, 9(11), 2395.

236. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014; 505, 559-63.
237. Davies, G. J., Crowder, M., Reid, B., & Dickerson, J. W. Bowel function measurements of individuals with different eating patterns. *Gut*. 1986;27(2), 164-169.
238. Deaver, J. A., Eum, S. Y., & Toborek, M. (2018). Circadian disruption changes gut microbiome taxa and functional gene composition. *Frontiers in microbiology*, 9, 316714.
239. Deechakawan, W., Cain, K. C., Jarrett, M. E., Burr, R. L., & Heitkemper, M. M. (2013). Effect of self-management intervention on cortisol and daily stress levels in irritable bowel syndrome. *Biological Research for Nursing*, 15(1), 26-36. doi:10.1177/1099800411414047
240. De la Cuesta-Zuluaga, J., Mueller, N. T., Álvarez-Quintero, R., Velásquez-Mejía, E. P., Sierra, J. A., Corrales-Agudelo, V., ... & Escobar, J. S. (2018). Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. *Nutrients*, 11(1), 51.
241. Del Chierico, F., Nobili, V., Vernocchi, P., Russo, A., De Stefanis, C., Gnani, D., ... & Putignani, L. (2017). Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology*, 65(2), 451-464.
242. Del Rio, B., Redruello, B., Linares, D. M., Ladero, V., Ruas-Madiedo, P., Fernandez, M., ... & Alvarez, M. A. (2019). The biogenic amines putrescine and cadaverine show in vitro cytotoxicity at concentrations that can be found in foods. *Scientific reports*, 9(1), 120.
243. den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of lipid research*, 54(9), 2325-2340.
244. den Besten, G., Lange, K., Havinga, R., van Dijk, T. H., Gerding, A., van Eunen, K., ... & Reijngoud, D. J. (2013). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 305(12), G900-G910.
245. De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I. B., La Storia, A., Laghi, L., ... & Turrone, S. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut*. 2016;65(11):1812-1821.
246. De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poulet, J. B., Massart, S., ... & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences*, 107(33), 14691-14696.
247. De Palma, G., Lynch, M. D., Lu, J., Dang, V. T., Deng, Y., Jury, J., ... Bercik, P. Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. *Science Translational Medicine*. 2017; 9(379). doi:10.1126/scitranslmed.aaf6397
248. Depoortère, R., Meleire, M., Bardin, L., Aliaga, M., Muller, E., Ardid, D., & Newman-Tancredi, A. (2011). Milnacipran is active in models of irritable bowel syndrome and abdominal visceral pain in rodents. *European journal of pharmacology*, 672(1-3), 83-87.
249. De Preter, V. Metabolomics in the clinical diagnosis of inflammatory bowel disease. *Digestive Diseases*. 2015; 33(Suppl. 1): 2-10.
250. Derrien, M., Alvarez, A. S., & de Vos, W. M. (2019). The gut microbiota in the first decade of life. *Trends in microbiology*, 27(12), 997-1010.
251. Desai, M. S., Seekatz, A. M., Koropatkin, N. M., Kamada, N., Hickey, C. A., Wolter, M., ... & Young, V. B. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell*. 2016;167(5), 1339-1353.
252. Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. (2010). Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience*, 170(4), 1179-1188.
253. Dey, P., & Ray Chaudhuri, S. (2023). The opportunistic nature of gut commensal microbiota. *Critical reviews in microbiology*, 49(6), 739-763.
254. DeVries, J. W., Prosky, L., Li, B., & Cho, S. (1999). A historical perspective on defining dietary fiber. *Cereal foods world*, 44, 367-369.
255. Didari, T., Solki, S., Mozaffari, S., Nikfar, S., & Abdollahi, M. (2014). A systematic review of the safety of probiotics. *Expert opinion on drug safety*, 13(2), 227-239.

256. Diener, C., Gibbons, S. M., & Resendis-Antonio, O. MICOM: metagenome-scale modeling to infer metabolic interactions in the gut microbiota. *Msystems*. 2020;5(1).
257. Dimidi, E., Rossi, M., & Whelan, K. (2017). Irritable bowel syndrome and diet: where are we in 2018?. *Current opinion in clinical nutrition and metabolic care*, 20(6), 456-463.
258. Dimidi, Eirini, Stephanos Christodoulides, S. Mark Scott, and Kevin Whelan. "Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation." *Advances in Nutrition* 8, no. 3 (2017): 484-494.
259. Dinan TG, Quigley EMM, Ahmed SMM, et al. Hypothalamic- pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*. 2006;130: 304–311.
260. Dionne, J., Ford, A. C., Yuan, Y., Chey, W. D., Lacy, B. E., Saito, Y. A., ... & Moayyedi, P. (2018). A systematic review and meta-analysis evaluating the efficacy of a gluten-free diet and a low FODMAPs diet in treating symptoms of irritable bowel syndrome. *Official journal of the American College of Gastroenterology| ACG*, 113(9), 1290-1300.
261. Dior, M., Delagrèverie, H., Duboc, H., Jouet, P., Coffin, B., Brot, L., ... & Sabate, J. M. (2016). Interplay between bile acid metabolism and microbiota in irritable bowel syndrome. *Neurogastroenterology & Motility*, 28(9), 1330-1340.
262. Distrutti, E., Monaldi, L., Ricci, P., & Fiorucci, S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. *World Journal of Gastroenterology*. 2016;22(7):2219–2241.
263. Dixon, E., Clubb, C., Pittman, S., Ammann, L., Rasheed, Z., Kazmi, N., ... & Couch, R. D. (2011). Solid-phase microextraction and the human fecal VOC metabolome. *PloS one*, 6(4).
264. Ducastel, S., Touche, V., Trabelsi, M. S., Boulinguez, A., Butruille, L., Nawrot, M., ... & Lestavel, S. (2020). The nuclear receptor FXR inhibits Glucagon-Like Peptide-1 secretion in response to microbiota-derived Short-Chain Fatty Acids. *Scientific reports*, 10(1), 174.
265. Dobbin, A., Dobbin, J., Ross, S. C., Graham, C., & Ford, M. J. (2013). Randomised controlled trial of brief intervention with biofeedback and hypnotherapy in patients with refractory irritable bowel syndrome. *Journal of the Royal College of Physicians Edinburgh*, 43(1), 15-23. doi:10.4997/jrcpe.2013.104
266. Dominguez-Bello, Maria Gloria, Martin J. Blaser, Ruth E. Ley, and Rob Knight. "Development of the human gastrointestinal microbiota and insights from high-throughput sequencing." *Gastroenterology* 140, no. 6 (2011): 1713-1719.
267. Donaldson, G. P., Lee, S. M., & Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology*, 14(1), 20-32.
268. Dong, W., Jia, Y., Liu, X., Zhang, H., Li, T., Huang, W., ... & Wu, H. (2017). Sodium butyrate activates NRF2 to ameliorate diabetic nephropathy possibly via inhibition of HDAC. *J Endocrinol*, 232(1), 71-83.
269. Donohoe, D. R., Collins, L. B., Wali, A., Bigler, R., Sun, W., & Bultman, S. J. (2012). The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Molecular cell*, 48(4), 612-626.
270. Donnachie, E., Schneider, A., Mehring, M., & Enck, P. Incidence of irritable bowel syndrome and chronic fatigue following GI infection: a population-level study using routinely collected claims data. *Gut*. 2018;67(6), 1078-1086.
271. Donovan, S. M. (2017). Introduction to the special focus issue on the impact of diet on gut microbiota composition and function and future opportunities for nutritional modulation of the gut microbiome to improve human health. *Gut Microbes*, 8(2), 75-81.
272. Drossman, D. A., Tack, J., Ford, A. C., Szigethy, E., Tornblom, H., & Van Oudenhove, L. Neuromodulators for Functional Gastrointestinal Disorders (Disorders of Gut-Brain Interaction): A Rome Foundation Working Team Report. *Gastroenterology*. 2018;154(4):1140-1171.e1141. doi:10.1053/j.gastro.2017.11.279
273. Drossman, D. A., & Hasler, W. L. Rome IV—functional GI disorders: disorders of gut-brain interaction. *Gastroenterology*. 2016;150(6), 1257-1261.
274. Drossman, A.D., Chang, L, Chey, W.D., Kellow, J., Tack, J., Whitehead, W.E. (2016). Rome IV. Functional Gastrointestinal Disorders: Disorders of Gut-Brain Interactions. (4th ed.)
275. Drossman, A.D., Chang, L, Chey, W.D., Kellow, J., Tack, J., Whitehead, W.E. Rome IV. Functional Gastrointestinal Disorders: Disorders of Gut-Brain Interactions. 4th ed. 2016.

276. Drossman, D. A. (Gastrointestinal illness and the biopsychosocial model. *Journal of clinical gastroenterology*. 1996;22(4), 252-254.
277. Drossman, D. A., & Thompson, W. G. (1992). The irritable bowel syndrome: review and a graduated multicomponent treatment approach. *Annals of Internal Medicine*, 116(12_Part_1), 1009-1016.
278. Duboc, H., Rainteau, D., Rajca, S., Humbert, L., Farabos, D., Maubert, M., . . . Sabate, J. M. Increase in fecal primary bile acids and dysbiosis in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterology & Motility*. 2012;24(6):513-520.
279. Duckworth, J., & Godden, W. J. (1941). The influence of dietary fibre on secretory activities of the alimentary tract: Observations on faecal phosphatase excretion and calcium and nitrogen balances of rats. *Biochemical Journal*, 35(1-2), 16.
280. Duncan, S. H., Louis, P., & Flint, H. J. (2004). Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and environmental microbiology*, 70(10), 5810-5817.
281. Duncan, S. H., Belenguer, A., Holtrop, G., Johnstone, A. M., Flint, H. J., & Lobley, G. E. (2007). Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Applied and environmental microbiology*, 73(4), 1073-1078.
282. Duncan, S. H., Louis, P., Thomson, J. M., & Flint, H. J. The role of pH in determining the species composition of the human colonic microbiota. *Environmental microbiology*. 2009;11(8), 2112-2122.
283. Duncan, P. I., Enters-Weijnen, C. F., Emami, N., McLean, P., Nunes, T., Beaumont, M., ... & Weits, T. (2018). Short-term daily intake of polydextrose fiber does not shorten intestinal transit time in constipated adults: a randomized controlled trial. *Nutrients*, 10(7), 920.
284. Dunwell, Jim M., Sawsan Khuri, and Paul J. Gane. "Microbial relatives of the seed storage proteins of higher plants: conservation of structure and diversification of function during evolution of the cupin superfamily." *Microbiology and Molecular Biology Reviews* 64, no. 1 (2000): 153-179.
285. Dupont, H. L., Jiang, Z. D., Dupont, A. W., & Utay, N. S. (2020). The intestinal microbiome in human health and disease. *Transactions of the American Clinical and Climatological Association*, 131, 178.
286. Durbán, A., Abellán, J. J., Jiménez-Hernández, N., Artacho, A., Garrigues, V., Ortiz, V., ... & Moya, A. (2013). Instability of the faecal microbiota in diarrhoea-predominant irritable bowel syndrome. *FEMS microbiology ecology*, 86(3), 581-589.
287. Duszka, K., & Wahli, W. Enteric microbiota–gut–brain axis from the perspective of nuclear receptors. *International journal of molecular sciences*. 2018;19(8), 2210.
288. Dvořák, Z., Sokol, H., & Mani, S. (2020). Drug mimicry: promiscuous receptors PXR and AhR, and microbial metabolite interactions in the intestine. *Trends in pharmacological sciences*, 41(12), 900-908.
289. Eastwood, M. A., Walton, B. A., Brydon, W. G., & Anderson, J. R. Faecal weight, constituents, colonic motility, and lactose tolerance in the irritable bowel syndrome. *Digestion*. 1984;30(1), 7-12.
290. Eastwood, M. A., Kirkpatrick, J. R., Mitchell, W. D., Bone, A., & Hamilton, T. (1973). Effects of dietary supplements of wheat bran and cellulose on faeces and bowel function. *Br Med J*, 4(5889), 392-394.
291. Edelman, M. J., Bauer, K., Khanwani, S., Tait, N., Trepel, J., Karp, J., ... & Van Echo, D. (2003). Clinical and pharmacologic study of tributyrin: an oral butyrate prodrug. *Cancer chemotherapy and pharmacology*, 51, 439-444.
292. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). (2010). Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA journal*, 8(3), 1462
293. Eghbalnia HR, Romero PR, Westler WM, Baskaran K, Ulrich EL, Markley JL. Increasing rigor in NMR-based metabolomics through validated and open source tools. *Current opinion in biotechnology*. 2017;43:56-61. doi:10.1016/j.copbio.2016.08.005.
294. Eilam, O., Zarecki, R., Oberhardt, M., Ursell, L. K., Kupiec, M., Knight, R., ... & Ruppín, E. (2014). Glycan degradation (GlyDeR) analysis predicts mammalian gut microbiota abundance and host diet-specific adaptations. *MBio*, 5(4), e01526-14
295. Elamin, E. E., Masclee, A. A., Dekker, J., Pieters, H. J., & Jonkers, D. M. (2013). Short-chain fatty acids activate AMP-activated protein kinase and ameliorate ethanol-induced intestinal barrier dysfunction in Caco-2 cell monolayers. *The Journal of nutrition*, 143(12), 1872-1881.

296. Elsenbruch, S., & Enck, P. (2017). The stress concept in gastroenterology: from Selye to today. *F1000Res*, 6, 2149. doi:10.12688/f1000research.12435.1
297. El-Salhy, M., Ystad, S. O., Mazzawi, T., & Gundersen, D. Dietary fiber in irritable bowel syndrome. *International journal of molecular medicine*. (2017);40(3), 607-613.
298. Eslick, S., Williams, E. J., Berthon, B. S., Wright, T., Karihaloo, C., Gately, M., & Wood, L. G. (2022). Weight Loss and Short-Chain Fatty Acids Reduce Systemic Inflammation in Monocytes and Adipose Tissue Macrophages from Obese Subjects. *Nutrients*, 14(4), 765.
299. El Oufir, L., Flourie, B., Des Varannes, S. B., Barry, J. L., Cloarec, D., Bornet, F., & Galmiche, J. P. Relations between transit time, fermentation products, and hydrogen consuming flora in healthy humans. *Gut*. 1996;38(6), 870-877.
300. El-Salhy, M., Valeur, J., Hausken, T., & Gunnar Hatlebakk, J. (2021). Changes in fecal short-chain fatty acids following fecal microbiota transplantation in patients with irritable bowel syndrome. *Neurogastroenterology & Motility*, 33(2), e13983.
301. Elsenbruch, S., & Enck, P. (2017). The stress concept in gastroenterology: from Selye to today. *F1000Research*. 2017; 6:2149. doi:10.12688/f1000research.12435.1
302. Emwas A-H, Luchinat C, Turano P, et al. Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review. *Metabolomics*. 2015;11(4):872-894. doi:10.1007/s11306-014-0746-7.
303. Encarnação, J. C., Pires, A. S., Amaral, R. A., Gonçalves, T. J., Laranjo, M., Casalta-Lopes, J. E., ... & Botelho, M. F. (2018). Butyrate, a dietary fiber derivative that improves irinotecan effect in colon cancer cells. *The Journal of Nutritional Biochemistry*, 56, 183-192.
304. Enck P., Aziz Q., Barbara G., Farmer, A.D., Fukudo, S., Mayer, E.A., Niesler, B., Quigley, M.M., Stojanovic, M.R., Schemann, M., . . . Spiller, R.C. Irritable bowel syndrome. *Nature Reviews Disease Primers*. 2016;2:16014. doi:10.1038/nrdp.2016.14.
305. Eswaran, S. L., Chey, W. D., Han-Markey, T., Ball, S., & Jackson, K. A Randomized Controlled Trial Comparing the Low FODMAP Diet vs. Modified NICE Guidelines in US Adults with IBS-D. *American Journal of Gastroenterology*. 2016;111(12):1824-1832. doi:10.1038/ajg.2016.434
306. Eswaran, S., Chey, W. D., Jackson, K., Pillai, S., Chey, S. W., & Han-Markey, T. (2017). A diet low in fermentable oligo-, di-, and monosaccharides and polyols improves quality of life and reduces activity impairment in patients with irritable bowel syndrome and diarrhea. *Clinical Gastroenterology and Hepatology*, 15(12), 1890-1899.
307. Eswaran, S., Muir, J., & Chey, W. D. (2013). Fiber and functional gastrointestinal disorders. *American journal of gastroenterology*, 108(5), 718-727.
308. Everhart, J. E., Go, V. L. W., Johannes, R. S., Fitzsimmons, S. C., Roth, H. P., & White, L. R. (1989). A longitudinal survey of self-reported bowel habits in the United States. *Digestive diseases and sciences*, 34, 1153-1162.
309. Faintuch, J., & Faintuch, S. (Eds.). (2019). *Microbiome and Metabolome in Diagnosis, Therapy, and Other Strategic Applications*. Academic Press.
310. Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological conservation*, 61(1), 1-10.
311. Faith, D (n.d.). Phylogenetic diversity. Retrieved April 29, 2023, from <https://danielpfaith.wordpress.com/phylogenetic-diversity/>
312. Faith, D. P. (2018). Phylogenetic diversity and conservation evaluation: perspectives on multiple values, indices, and scales of application. *Phylogenetic diversity: applications and challenges in biodiversity science*, 1-26.
313. Faith, J. J., Guruge, J. L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A. L., ... & Gordon, J. I. (2013). The long-term stability of the human gut microbiota. *Science*, 341(6141), 1237439.
314. Faith, D. P., & Richards, Z. T. (2012). Climate change impacts on the tree of life: changes in phylogenetic diversity illustrated for *Acropora* corals. *Biology*, 1(3), 906-932.
315. Faith, D. P. (2018). Phylogenetic diversity and conservation evaluation: perspectives on multiple values, indices, and scales of application. *Phylogenetic diversity: applications and challenges in biodiversity science*, 1-26.

316. Falony, G., Vlachou, A., Verbrugghe, K., & De Vuyst, L. Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. *Applied and Environmental Microbiology*. 2006;72(12), 7835-7841.
317. Fan, W. T., Ding, C., Xu, N. N., Zong, S., Ma, P., & Gu, B. Close association between intestinal microbiota and irritable bowel syndrome. *European Journal of Clinical Microbiology & Infectious Diseases*. 2017;36(12), 2303-2317.
318. Fang, X., Han, L., Zhao, L., Zhou, Y., Yang, C., Xiong, T., ... & Bian, Z. (2020). Integrated Multi-Omics Data Analysis Reveals Altered Metabolome Activity and Microbiome Composition in Irritable Bowel Syndrome.
319. Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre?. *Nutrition research reviews*, 23(1), 65-134.
320. Farré, R., & Vicario, M. Abnormal barrier function in gastrointestinal disorders. In *Gastrointestinal Pharmacology*. 2016. 193-217.
321. Farup, P. G., Rudi, K., & Hestad, K. Faecal short-chain fatty acids-a diagnostic biomarker for irritable bowel syndrome?. *BMC gastroenterology*. 2016;16(1), 51.
322. Farup, P. G., Ueland, T., Rudi, K., Lydersen, S., & Hestad, K. Functional bowel disorders are associated with a central immune activation. *Gastroenterology research and practice*, 2017.
323. Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39, 175-191.
324. Faure, C., Patey, N., Gauthier, C., Brooks, E. M., & Mawe, G. M. (2010). Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. *Gastroenterology*, 139(1), 249-258.
325. FDA (n.d). Alosetron Labeling Information. Retrieved May 7, 2024, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2002/21107s5lbl.pdf,
326. FDA (n.d). Tenapanor Labeling Information. Retrieved May 7, 2024, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/211801s004lbl.pdf
327. FDA (n.d). Plecanatide Labeling Information. Retrieved May 7, 2024, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/208745lbl.pdf
328. FDA (n.d). Rifaximin Labeling Information. Retrieved May 7, 2024, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021361s023lbl.pdf
329. Feng, Y., Wang, Y., Wang, P., Huang, Y., & Wang, F. (2018). Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy. *Cellular Physiology and Biochemistry*, 49(1), 190-205.
330. Figueira, I., Garcia, G., Pimpão, R. C., Terrasso, A. P., Costa, I., Almeida, A. F., ... & Santos, C. N. (2021). Correction to: Polyphenols journey through blood-brain barrier towards neuronal protection. *Scientific reports*, 11(1), 17112.
331. Filosa, S., Di Meo, F., & Crispi, S. (2018). Polyphenols-gut microbiota interplay and brain neuromodulation. *Neural regeneration research*, 13(12), 2055.
332. Findlay, J. M., Mitchell, W. D., Smith, A. N., Anderson, A. J. B., & Eastwood, M. A. (1974). Effects of unprocessed bran on colon function in normal subjects and in diverticular disease. *The Lancet*, 303(7849), 146-149.
333. Fiorucci, S., & Distrutti, E. (2015). Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. *Trends in molecular medicine*. 2015;21(11), 702-714.
334. Fitch, M. D., & Fleming, S. E. (1999). Metabolism of short-chain fatty acids by rat colonic mucosa in vivo. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 277(1), G31-G40.
335. FitzGerald, L. Z., Kehoe, P., & Sinha, K. (2009). Hypothalamic—pituitary—adrenal axis dysregulation in women with irritable bowel syndrome in response to acute physical stress. *Western Journal of Nursing Research*, 31(7), 818-836.
336. Fleming, S. E., & Gill, R. (1997). Aging Stimulates Fatty Acid Oxidation in Rat Colonocytes But Does Not Influence the Response to... *Journals of Gerontology Series A: Biological Sciences & Medical Sciences*, (6).

337. Flint, H. J., Duncan, S. H., Scott, K. P., & Louis, P. Links between diet, gut microbiota composition and gut metabolism. *Proceedings of the Nutrition Society*. 2015;74(1), 13-22.
338. Flint, H. J., Duncan, S. H., Scott, K. P., & Louis, P. Interactions and competition within the microbial community of the human colon: links between diet and health. *Environmental microbiology*. 2007;9(5), 1101-1111.
339. Flint, H. J., & Duncan, S. H. (2011). The importance of cultured representatives of the human intestinal microbiota in the metagenomic era. *The Bulletin of BISMIS*, 2(part 2), 117-123.
340. Flint, H. J. The impact of nutrition on the human microbiome. *Nutrition reviews*. 2012; 70(suppl_1), S10-S13.
341. Flint, H. J., Duncan, S. H., & Louis, P. (2009). Gut microbial ecology. In *Designing Functional Foods* (pp. 38-67). Woodhead Publishing.
342. Ford, A. C., Talley, N. J., Spiegel, B. M., Foxx-Orenstein, A. E., Schiller, L., Quigley, E. M., & Moayyedi, P. (2008). Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. *Bmj*, 337, a2313.
343. Ford, A. C., Forman, D., Bailey, A. G., Axon, A. T. R., & Moayyedi, P. (2008). Fluctuation of gastrointestinal symptoms in the community: a 10-year longitudinal follow-up study. *Alimentary pharmacology & therapeutics*, 28(8), 1013-1020.
344. Ford, A. C., Quigley, E. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., ... & Moayyedi, P. (2014). Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Official journal of the American College of Gastroenterology| ACG*, 109(10), 1547-1561.
345. Ford, A. C., Moayyedi, P., Chey, W. D., Harris, L. A., Lacy, B. E., Saito, Y. A., & Quigley, E. M. American College of Gastroenterology monograph on management of irritable bowel syndrome. *American Journal of Gastroenterology*. 2018;113, 1-18.
346. Ford, A. C., Moayyedi, P., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., ... & Quigley, E. M. (2014). American College of Gastroenterology monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. *The American journal of gastroenterology*. 2014;109(S1), S2.
347. Ford, CF, Lacy, BE, Talley NJ. Irritable Bowel Syndrome. *The New England Journal of Medicine*. 2017;376:2566-78. DOI: 10.1056/NEJMr1607547
348. Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of stress*.
349. Fowler, S., Hoedt, E. C., Talley, N. J., Keely, S., & Burns, G. L. (2022). Circadian rhythms and melatonin metabolism in patients with disorders of gut-brain interactions. *Frontiers in neuroscience*, 16, 825246.
350. Fowlie, S., Eastwood, M. A., & Prescott, R. (1992). Irritable bowel syndrome: assessment of psychological disturbance and its influence on the response to fibre supplementation. *Journal of psychosomatic research*, 36(2), 175-180.
351. Frame, L. A., Costa, E., & Jackson, S. A. (2020). Current explorations of nutrition and the gut microbiome: a comprehensive evaluation of the review literature. *Nutrition reviews*, 78(10), 798-812.
352. Francis, C. Y., & Whorwell, P. J. (1994). Bran and irritable bowel syndrome: time for reappraisal. *The Lancet*, 344(8914), 39-40.
353. Frank, L. E., Blaser, M. J., Hirschhorn, K., Moros, D. A., Rhodes, M. E., Philpott, S., ... & Benkov, K. The human microbiome: science, history and research. In *The Human Microbiome Ethical, Legal and Social Concerns* (pp. 16-55). Oxford University Press. 2013
354. Fredericks, E., Theunissen, R., & Roux, S. (2020). Short chain fatty acids and monocarboxylate transporters in irritable bowel syndrome. *The Turkish Journal of Gastroenterology*, 31(12), 840.
355. Fukudo, S., & Suzuki, J. (1987). Colonic motility, autonomic function, and gastrointestinal hormones under psychological stress on irritable bowel syndrome. *The Tohoku journal of experimental medicine*, 151(4), 373-385.
356. Fukui H, Xu X, Miwa H. Role of Gut Microbiota-Gut Hormone Axis in the Pathophysiology of Functional Gastrointestinal Disorders. *Journal of Neurogastroenterology and Motility*. 2018;24(3):367-386. doi:10.5056/jnm18071

357. Fukumoto, S., Tatewaki, M., Yamada, T., Fujimiya, M., Mantyh, C., Voss, M., ... & Takahashi, T. (2003). Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 284(5), R1269-R1276.
358. Fuller, R. (1989). Probiotics in man and animals. *The Journal of applied bacteriology*, 66(5), 365-378.
359. Fukushima, A., Aizaki, Y., & Sakuma, K. Short-chain fatty acids induce intestinal transient receptor potential vanilloid type 6 expression in rats and Caco-2 cells. *The Journal of nutrition*. 2008;139(1), 20-25.
360. Fung, K. Y., Cosgrove, L., Lockett, T., Head, R., & Topping, D. L. (2012). A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *British Journal of Nutrition*, 108(5), 820-831.
361. Fung, T. C., Vuong, H. E., Luna, C. D., Pronovost, G. N., Aleksandrova, A. A., Riley, N. G., ... & Hsiao, E. Y. (2019). Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nature microbiology*, 4(12), 2064-2073.
362. Ganapathy, V., Thangaraju, M., Prasad, P. D., Martin, P. M., & Singh, N. (2013). Transporters and receptors for short-chain fatty acids as the molecular link between colonic bacteria and the host. *Current opinion in pharmacology*, 13(6), 869-874.
363. Gao, X., Pujos-Guillot, E., Martin, J. F., Galan, P., Juste, C., Jia, W., & Sebedio, J. L. (2009). Metabolite analysis of human fecal water by gas chromatography/mass spectrometry with ethyl chloroformate derivatization. *Analytical biochemistry*, 393(2), 163-175.
364. Gargari, G., Taverniti, V., Balzaretto, S., Ferrario, C., Gardana, C., Simonetti, P., & Guglielmetti, S. Consumption of a *Bifidobacterium bifidum* strain for 4 weeks modulates dominant intestinal bacterial taxa and fecal butyrate in healthy adults. *Applied Environmental Microbiology* 2016;82(19), 5850-5859.
365. Gargari, G., Taverniti, V., Gardana, C., Cremon, C., Canducci, F., Pagano, I., ... & Tagliacarne, S. C. Fecal Clostridiales distribution and short-chain fatty acids reflect bowel habits in irritable bowel syndrome. *Environmental microbiology*. 2018;20(9), 3201-3213.
366. Garner, C. E., Smith, S., de Lacy Costello, B., White, P., Spencer, R., Probert, C. S., & Ratcliffe, N. M. (2007). Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. *The FASEB Journal*, 21(8), 1675-1688.
367. Ganapathy, V., Thangaraju, M., Prasad, P. D., Martin, P. M., & Singh, N. (2013). Transporters and receptors for short-chain fatty acids as the molecular link between colonic bacteria and the host. *Current opinion in pharmacology*. 2013;13(6), 869-874.
368. Gao, J. H., Guo, L. J., Huang, Z. Y., Rao, J. N., & Tang, C. W. Roles of cellular polyamines in mucosal healing in the gastrointestinal tract. *Journal of Physiology & Pharmacology*. 2013;64(6):681-693.
369. Gao, X., Pujos-Guillot, E., Martin, J. F., Galan, P., Juste, C., Jia, W., & Sebedio, J. L. Metabolite analysis of human fecal water by gas chromatography/mass spectrometry with ethyl chloroformate derivatization. *Analytical biochemistry*. 2009;393(2), 163-175.
370. Gao, X., Lin, H., Revanna, K., & Dong, Q. (2017). A Bayesian taxonomic classification method for 16S rRNA gene sequences with improved species-level accuracy. *BMC bioinformatics*, 18(1), 1-10.
371. Garner, C. E., Smith, S., de Lacy Costello, B., White, P., Spencer, R., Probert, C. S., & Ratcliffe, N. M. Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. *The FASEB Journal*. 2007;21(8), 1675-1688.
372. Geirnaert A, Calatayud M, Grootaert C, et al. Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. *Scientific Reports*. 2017;7:11450. doi:10.1038/s41598-017-11734-8.
373. Gershon, M. D. (2004). serotonin receptors and transporters—roles in normal and abnormal gastrointestinal motility. *Alimentary pharmacology & therapeutics*, 20, 3-14.
374. Gershon, M. D., & Tack, J. (2007). The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology*, 132(1), 397-414.
375. Getachew, B., Aubee, J. I., Schottenfeld, R. S., Csoka, A. B., Thompson, K. M., & Tizabi, Y. (2018). Ketamine interactions with gut-microbiota in rats: relevance to its antidepressant and anti-inflammatory properties. *BMC microbiology*, 18, 1-10.

376. Geurts, L., Lazarevic, V., Derrien, M., Everard, A., Van Roye, M., Knauf, C., ... & Cani, P. D. (2011). Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. *Frontiers in microbiology*, 2, 10960.
377. Ghoshal, U. C., Shukla, R., Ghoshal, U., Gwee, K. A., Ng, S. C., & Quigley, E. M. (2012). The gut microbiota and irritable bowel syndrome: friend or foe?. *International journal of inflammation*, 2012.
378. Giaccari, S., Grasso, G., Tronci, S., Allegretta, L., Sponziello, G., Montefusco, A., ... & Chiri, S. (2001). Partially hydrolyzed guar gum: a fiber as coadjuvant in the irritable colon syndrome. *La Clinica terapeutica*, 152(1), 21-25.
379. Giannini, E. G., Mansi, C., Dulbecco, P., & Savarino, V. (2006). Role of partially hydrolyzed guar gum in the treatment of irritable bowel syndrome. *Nutrition*, 22(3), 334-342.
380. Gibson, P. R., & Shepherd, S. J. Personal view: food for thought—western lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. *Alimentary pharmacology & therapeutics*. 2005;21(12):1399-1409.
381. Gibson, K. M., Nguyen, B. N., Neumann, L. M., Miller, M., Buss, P., Daniels, S., ... & Pukazhenthi, B. (2019). Gut microbiome differences between wild and captive black rhinoceros—implications for rhino health. *Scientific Reports*, 9(1), 7570.
382. Gibson, G. R., & Roberfroid, M. B. (Eds.). (1999). *Colonic microbiota, nutrition and health* (pp. 101-124). Dordrecht:: Kluwer Academic.
383. Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition*, 125(6), 1401-1412.
384. Gibson, P., & Rosella, O. (1995). Interleukin 8 secretion by colonic crypt cells in vitro: response to injury suppressed by butyrate and enhanced in inflammatory bowel disease. *Gut*, 37(4), 536-543.
385. Glancy, B., Kane, D. A., Kavazis, A. N., Goodwin, M. L., Willis, W. T., & Gladden, L. B. (2021). Mitochondrial lactate metabolism: history and implications for exercise and disease. *The Journal of Physiology*, 599(3), 863-888.
386. Goel, S. K., Lalwani, N. D., & Reddy, J. K. (1986). Peroxisome proliferation and lipid peroxidation in rat liver. *Cancer research*, 46(3), 1324-1330.
387. Goeman, J. J., & Bühlmann, P. (2007). Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics*, 23(8), 980-987.
388. Gonçalves, P., Araújo, J. R., & Di Santo, J. P. (2018). A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. *Inflammatory bowel diseases*, 24(3), 558-572.
389. Gonsalkorale, W. M., Miller, V., Afzal, A., & Whorwell, P. J. (2003). Long term benefits of hypnotherapy for irritable bowel syndrome. *Gut*, 52(11), 1623-1629.
390. Gonsalkorale, W.M., & Whorwell, P.J. (2005). Hypnotherapy in the treatment of irritable bowel syndrome. *European Journal of Gastroenterology Hepatology*, 17, 15-20.
391. Gonsalkorale, W. M., Toner, B. B., & Whorwell, P. J. (2004). Cognitive change in patients undergoing hypnotherapy for irritable bowel syndrome. *Journal of Psychosomatic Research*, 56, 271–278. [https://doi.org/10.1016/S0022-3999\(03\)00076-X](https://doi.org/10.1016/S0022-3999(03)00076-X)
392. Gonsalkorale, W. M., Perrey, C., Pravica, V., Whorwell, P. J., & Hutchinson, I. V. (2003). Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component?. *Gut*, 52(1), 91-93.
393. Goo, Y. A., Cain, K., Jarrett, M., Smith, L., Voss, J., Tolentino, E., ... & Shulman, R. J. Urinary proteome analysis of irritable bowel syndrome (IBS) symptom subgroups. *Journal of proteome research*. 2012;11(12), 5650-5662.
394. Goodacre, R. (2007). Metabolomics of a superorganism. *The Journal of nutrition*, 137(1), 259S-266S.
395. Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., ... & Spector, T. D. (2014). Human genetics shape the gut microbiome. *Cell*, 159(4), 789-799.
396. Gordon, D. (2007). Dietary fiber definitions at risk. *Cereal foods world*, 52(3), 112.
397. Gowda GAN, Shanaiah N, Raftery D. Isotope Enhanced Approaches in Metabolomics. *Advances in experimental medicine and biology*. 2012;992:147-164. doi:10.1007/978-94-007-4954-2_8.
398. Gowda GAN. Profiling Redox and Energy Coenzymes in Whole Blood, Tissue and Cells Using NMR Spectroscopy. *Metabolites*. 2018;8(2):32. doi:10.3390/metabo8020032.

399. Gowda GAN, Raftery D. Can NMR solve some significant challenges in metabolomics? *Journal of magnetic resonance* (San Diego, Calif : 1997). 2015;260:144-160. doi:10.1016/j.jmr.2015.07.014.
400. Grace, R. F., & Glader, B. (2018). Red blood cell enzyme disorders. *Pediatric Clinics*, 65(3), 579-595.
401. Gratton, J., Phetcharaburanin, J., Mullish, B. H., Williams, H. R., Thursz, M., Nicholson, J. K., ... & Li, J. V. Optimized sample handling strategy for metabolic profiling of human feces. *Analytical chemistry*. 2016;88(9), 4661-4668.
402. Graham, D. Y., Moser, S. E., & Estes, M. K. (1982). The effect of bran on bowel function in constipation. *American Journal of Gastroenterology*, 77(9).
403. Grider, J. R., & Piland, B. E. (2007). The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 292(1), G429-G437.
404. Groh, H., Schade, K., & Hörhold-Schubert, C. (1993). Steroid metabolism with intestinal microorganisms. *Journal of basic microbiology*, 33(1), 59-72.
405. Gropper, S. S., & Smith, J. L. (2012). *Advanced nutrition and human metabolism*. Cengage Learning.
406. Grotz, R. L., Pemberton, J. H., Talley, N. J., Rath, D. M., & Zinsmeister, A. R. (1994). Discriminant value of psychological distress, symptom profiles, and segmental colonic dysfunction in outpatients with severe idiopathic constipation. *Gut*, 35(6), 798-802.
407. Gu H, Nagana Gowda GA, Neto FC, Opp MR, Raftery D. RAMSY: Ratio Analysis of Mass Spectrometry to Improve Compound Identification. *Analytical chemistry*. 2013;85(22):10.1021/ac4019268. doi:10.1021/ac4019268.
408. Guo, Y., Huang, Z. P., Liu, C. Q., Qi, L., Sheng, Y., & Zou, D. J. (2018). Modulation of the gut microbiome: a systematic review of the effect of bariatric surgery. *European journal of endocrinology*, 178(1), 43-56.
409. Guo, P., Zhang, K., Ma, X., & He, P. (2020). Clostridium species as probiotics: potentials and challenges. *Journal of animal science and biotechnology*, 11(1), 1-10.
410. Gupta, V. K., Paul, S., & Dutta, C. Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Frontiers in microbiology*. 2017;8, 1162.
411. Gurry, T., Yu, X., & Alm, E. J. Functional heterogeneity in the fermentation capabilities of the healthy human gut microbiota. *bioRxiv*. 2020
412. Guthman, J., & Mansfield, B. (2013). The implications of environmental epigenetics: A new direction for geographic inquiry on health, space, and nature-society relations. *Progress in Human Geography*, 37(4), 486-504
413. Ha, C. W., Lam, Y. Y., & Holmes, A. J. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. *World Journal of Gastroenterology: WJG*. 2014;20(44), 16498.
414. Hagiwara T, Saito S, Ujiie Y, et al. HPLC Retention time prediction for metabolome analysis. *Bioinformatics*. 2010;5(6):255-258.
415. Hague, A., Elder, D. J., Hicks, D. J., & Paraskeva, C. (1995). Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *International journal of cancer*, 60(3), 400-406.
416. Halland, M., & Saito, Y. A. Irritable bowel syndrome: new and emerging treatments. *The British Medical Journal*. 2015;350, h1622.
417. Halili, M. A., Andrews, M. R., Labzin, L. I., Schroder, K., Matthias, G., Cao, C., ... & Sweet, M. J. (2010). Differential effects of selective HDAC inhibitors on macrophage inflammatory responses to the Toll-like receptor 4 agonist LPS. *Journal of leukocyte biology*, 87(6), 1103-1114.
418. Halmos, E. P., Christophersen, C. T., Bird, A. R., Shepherd, S. J., Gibson, P. R., & Muir, J. G. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut*. 2015;64(1), 93-100.
419. Halmos, E. P., Power, V. A., Shepherd, S. J., Gibson, P. R., & Muir, J. G. (2014). A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology*. 2014;146(1):67-75.e65. doi:10.1053/Journal of Gastroenterology. 2014;2013.09.046
420. Halpert, A., Dalton, C. B., Palsson, O., Morris, C., Hu, Y., Bangdiwala, S., ... & Drossman, D. (2007). What patients know about irritable bowel syndrome (IBS) and what they would like to know. *National Survey on Patient Educational Needs in IBS and development and validation of the Patient Educational*

Needs Questionnaire (PEQ). Official journal of the American College of Gastroenterology| ACG, 102(9), 1972-1982.

421. Hamer HM, De Preter V, Windey K, Verbeke K. Functional analysis of colonic bacterial metabolism: relevant to health? *American Journal of Physiology - Gastrointestinal and Liver Physiology*. 2012;302(1):G1-G9. doi:10.1152/ajpgi.00048.2011.
422. Hamer, Henrike M., et al. "The role of butyrate on colonic function." *Alimentary pharmacology & therapeutics*. 2008; 27.2:104-119
423. Han, L.; Zhao, L.; Zhou, Y.; Yang, C.; Xiong, T.; Lu, L.; Deng, Y.; Luo, W.; Chen, Y.; Qiu, Q.; et al. Altered metabolome and microbiome features provide clues in understanding irritable bowel syndrome and depression comorbidity. *ISME J*. 2022, 16, 983–996
424. Harper, A., Naghibi, M. M., & Garcha, D. (2018). The Role of Bacteria, Probiotics and Diet in Irritable Bowel Syndrome. *Foods*, 7(2). doi:10.3390/foods7020013.
425. Harris, L. A., & Baffy, N. (2017). Modulation of the gut microbiota: a focus on treatments for irritable bowel syndrome. *Postgraduate medicine*, 129(8), 872-888.
426. Harty, R. F. (2013). Energy, oxidative stress, and inflammation in the colon. *Digestive Diseases and Sciences*, 58(12), 3386-3388.
427. Harvey, R. F., Pomare, E. W., & Heaton, K. W. Effects of increased dietary fibre on intestinal transit. *The Lancet*, 301(7815). 1973;1278-1280.
428. Harvie, R. A reduction in FODMAP intake correlates strongly with a reduction in IBS symptoms—The FIBS study (Doctoral dissertation Abstract, University of Otago). 2014.
429. Hauser, G., Pletikoscic, S., & Tkalcic, M. (2014). Cognitive behavioral approach to understanding irritable bowel syndrome. *World Journal of Gastroenterology: WJG*, 20(22), 6744.
430. Hauser, A., Eisenmann, P., Muhle-Goll, C., Luy, B., Dötsch, A., Graf, D., & Tzvetkova, P. Efficient Extraction from Mice Feces for NMR Metabolomics Measurements with Special Emphasis on SCFAs. *Metabolites*. 2019;9(3), 55.
431. Hayes, P., Corish, C., O'mahony, E., & Quigley, E. M. M. (2014). A dietary survey of patients with irritable bowel syndrome. *Journal of human nutrition and dietetics*, 27, 36-47
432. Haworth, J. J., Pitcher, C. K., Ferrandino, G., Hobson, A. R., Pappan, K. L., & Lawson, J. L. (2022). Breathing new life into clinical testing and diagnostics: perspectives on volatile biomarkers from breath. *Critical Reviews in Clinical Laboratory Sciences*, 59(5), 353-372.
433. He, Y., Tan, Y., Zhu, J., Wu, X., Huang, Z., Chen, H., ... & Chen, D. (2021). Effect of sodium butyrate regulating IRAK1 (interleukin-1 receptor-associated kinase 1) on visceral hypersensitivity in irritable bowel syndrome and its mechanism. *Bioengineered*, 12(1), 1436-1444.
434. Heerdt, B. G., Houston, M. A., & Augenlicht, L. H. (1997). Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. *Cell Growth and Differentiation-Publication American Association for Cancer Research*, 8(5), 523-532.
435. Heath, G., Roach, G. D., Dorrian, J., Ferguson, S. A., Darwent, D., & Sargent, C. (2012). The effect of sleep restriction on snacking behaviour during a week of simulated shiftwork. *Accident Analysis & Prevention*, 45, 62-67.
436. Heaton, K. W., Radvan, J., Cripps, H., Mountford, R. A., Braddon, F. E., & Hughes, A. O. (1992). Defecation frequency and timing, and stool form in the general population: a prospective study. *Gut*, 33(6), 818-824.
437. Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., ... & Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences*, 108(7), 3047-3052.
438. Heitkemper, M. M., Cain, K. C., Shulman, R. J., Burr, R. L., Ko, C., Hollister, E. B., ... & Jarrett, M. E. (2018). Stool and urine trefoil factor 3 levels: associations with symptoms, intestinal permeability, and microbial diversity in irritable bowel syndrome. *Beneficial microbes*, 9(3), 345-355.
439. Heitkemper, M. M., Han, C. J., Jarrett, M. E., Gu, H., Djukovic, D., Shulman, R. J., ... Cain, K. C. Serum Tryptophan Metabolite Levels During Sleep in Patients With and Without Irritable Bowel Syndrome (IBS). *Biological Research for Nursing*. 2016;18(2):193-198. doi:10.1177/1099800415594251.

440. Heitkemper, M., Cain, K. C., Shulman, R., Burr, R., Poppe, A., & Jarrett, M. (2011). Subtypes of irritable bowel syndrome based on abdominal pain/discomfort severity and bowel pattern. *Digestive diseases and sciences*, 56, 2050-2058.
441. Heitkemper, M. M., & Chang, L. (2009). Do fluctuations in ovarian hormones affect gastrointestinal symptoms in women with irritable bowel syndrome?. *Gender medicine*, 6, 152-167.
442. Heitkemper, M., Carter, E., Ameen, V., Olden, K., & Cheng, L. (2002). Women with irritable bowel syndrome: differences in patients' and physicians' perceptions. *Gastroenterology Nursing*, 25(5), 192-200.
443. Heitkemper, M., Jarrett, M., Bond, E. F., & Chang, L. (2003). Impact of sex and gender on irritable bowel syndrome. *Biological Research for Nursing*, 5(1), 56-65. doi:10.1177/1099800403005001006
444. Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., ... & Myers, B. (2016). Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Comprehensive physiology*, 6(2), 603.
445. Henao-Mejia, J., Elinav, E., Thaiss, C. A., & Flavell, R. A. (2014). Inflammasomes and metabolic disease. *Annual review of physiology*, 76, 57-78.
446. Henning, S. J., & Hird, F. J. R. (1972). Ketogenesis from butyrate and acetate by the caecum and the colon of rabbits. *Biochemical Journal*, 130(3), 785-790.
447. Herrera-Cazarez, L. A. (2019). Untargeted metabolomic evaluation of mango bagasse and mango bagasse based confection under simulated colonic fermentation. Instituto de Ciencias Biomédicas.
448. Herter, C. A., & Kendall, A. I. (1910). The Influence of Dietary Alternations on the Types of Intestinal Flora: Plates I-III (Fellow of the Rockefeller Institute for Medical Research). *Journal of Biological Chemistry*, 7(3), 203-239.
449. Hidalgo-Cantabrana, C., Delgado, S., Ruiz, L., Ruas-Madiedo, P., Sánchez, B., & Margolles, A. (2018). Bifidobacteria and their health-promoting effects. *Bugs as Drugs: Therapeutic Microbes for the Prevention and Treatment of Disease*, 73-98.
450. Hijova, E., & Chmelarova, A. (2007). Short chain fatty acids and colonic health. *Bratislavské lekárske listy*, 108(8), 354.
451. Hill P, Muir JG, Gibson PR. Controversies and Recent Developments of the Low-FODMAP Diet. *Gastroenterology & Hepatology*. 2017;13(1):36-45.
452. Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., ... & Sterne, J. A. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj*, 343, d5928.
453. Hillman, L. C., Stace, N. H., Fisher, A., & Pomare, E. W. (1982). Dietary intakes and stool characteristics of patients with the irritable bowel syndrome. *The American journal of clinical nutrition*, 36(4), 626-629.
454. Hillman, L. C., Stace, N. H., & Pomare, E. W. (1984). Irritable bowel patients and their long-term response to a high fiber diet. *American Journal of Gastroenterology*, 79(1).
455. Hinton, J. M., Lennard-Jones, J. E., & Young, A. C. (1969). A new method for studying gut transit times using radioopaque markers. *Gut*, 10(10), 842-847.
456. Hodgkinson, K., El Abbar, F., Dobranowski, P., Manoogian, J., Butcher, J., Figeys, D., ... & Stintzi, A. (2023). Butyrate's role in human health and the current progress towards its clinical application to treat gastrointestinal disease. *Clinical Nutrition*, 42(2), 61-75.
457. Holeček, M. (2020). Why are branched-chain amino acids increased in starvation and diabetes? *Nutrients* 12: 3087.
458. Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut microbes*, 8(2), 172-184.
459. Hoffman, R., & Gerber, M. (2013). *The Mediterranean diet: health and science*. John Wiley & Sons.
460. Hollister, E. B., Cain, K. C., Shulman, R. J., Jarrett, M. E., Burr, R. L., Ko, C., . . . Heitkemper, M. M. Relationships of Microbiome Markers With Extraintestinal, Psychological Distress and Gastrointestinal
461. Holmes, E., Li, J. V., Marchesi, J. R., & Nicholson, J. K. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell metabolism*. 2012;16(5), 559-564.
462. Holster, S., Repsilber, D., Geng, D., Hyötyläinen, T., Salonen, A., Lindqvist, C. M., ... & König, J. (2021). Correlations between microbiota and metabolites after faecal microbiota transfer in irritable bowel syndrome. *Beneficial Microbes*, 12(1), 17-30.

463. Holtmann, G. J., Ford, A. C., & Talley, N. J. Pathophysiology of irritable bowel syndrome. *The Lancet Gastroenterology & Hepatology*. 2016;1(2):133-146.
464. Hong, Y. S., Hong, K. S., Park, M. H., Ahn, Y. T., Lee, J. H., Huh, C. S., . . . Kim, J. S. Metabonomic understanding of probiotic effects in humans with irritable bowel syndrome. *J Clinical of Gastroenterology*. 2011;45(5):415-425. doi:10.1097/MCG.0b013e318207f76c.
465. Hongisto, S. M., Paajanen, L., Saxelin, M., & Korpela, R. (2006). A combination of fibre-rich rye bread and yoghurt containing *Lactobacillus* GG improves bowel function in women with self-reported constipation. *European journal of clinical nutrition*, 60(3), 319-324.
466. Hornung, B., dos Santos, V. A. M., Smidt, H., & Schaap, P. J. Studying microbial functionality within the gut ecosystem by systems biology. *Genes & nutrition*. 2018; 13(1), 5
467. Houghton, L. A., Heitkemper, M., Crowell, M. D., Emmanuel, A., Halpert, A., McRoberts, J. A., & Toner, B. (2016). Age, gender, and women's health and the patient. *Gastroenterology*, 150(6), 1332-1343.
468. Hou, Y., Li, J., & Ying, S. (2023). Tryptophan Metabolism and Gut Microbiota: A Novel Regulatory Axis Integrating the Microbiome, Immunity, and Cancer. *Metabolites*, 13(11), 116
469. Høverstad, T., Fausa, O., Bjørneklett, A., & Bøhmer, T. Short-chain fatty acids in the normal human feces. *Scandinavian journal of gastroenterology*. 1984;19(3), 375-381
470. Hsu, C. H., Jeng, Y. M., & Ni, Y. H. (2012). *Clostridium difficile* infection in a patient with Crohn disease. *Journal of the Formosan Medical Association*, 111(6), 347-349.
471. Huang, Z., Boekhorst, J., Fogliano, V., Capuano, E., & Wells, J. M. (2023). Impact of high-fiber or high-protein diet on the capacity of human gut microbiota to produce tryptophan catabolites. *Journal of Agricultural and Food Chemistry*, 71(18), 6956-6966.
472. Huang, Z., Boekhorst, J., Fogliano, V., Capuano, E., & Wells, J. M. (2023). Distinct effects of fiber and colon segment on microbiota-derived indoles and short-chain fatty acids. *Food Chemistry*, 398, 133801.
473. Huda-Faujan, N., Abdulmir, A. S., Fatimah, A. B., Anas, O. M., Shuhaimi, M., Yazid, A. M., & Loong, Y. Y. (2010). The impact of the level of the intestinal short chain fatty acids in inflammatory bowel disease patients versus healthy subjects. *The open biochemistry journal*, 4, 53.
474. Hughes, M. E., Hogenesch, J. B., & Kornacker, K. (2010). JTK_CYCLE: an efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets. *Journal of biological rhythms*, 25(5), 372-380.
475. Hukla, R., Ghoshal, U., Ranjan, P., & Ghoshal, U. C. (2018). Expression of toll-like receptors, pro-, and anti-inflammatory cytokines in relation to gut microbiota in irritable bowel syndrome: the evidence for its micro-organic basis. *Journal of Neurogastroenterology and Motility*. 2018;24(4), 628.
476. Hume, M. P., Nicolucci, A. C., & Reimer, R. A. Prebiotic supplementation improves appetite control in children with overweight and obesity: a randomized controlled trial-. *The American journal of clinical nutrition*. 2017;105(4), 790-799.
477. Hupcey, J. E., & Penrod, J. (2005). Concept analysis: examining the state of the science. *Research and Theory for Nursing Practice*, 19(2), 197-208.
478. Hussain, M. M., & Pan, X. (2009). Clock genes, intestinal transport and plasma lipid homeostasis. *Trends in Endocrinology & Metabolism*, 20(4), 177-185.
479. Husted, A. S., Trauelsen, M., Rudenko, O., Hjorth, S. A., & Schwartz, T. W. (2017). GPCR-mediated signaling of metabolites. *Cell metabolism*, 25(4), 777-796.
480. Hustoft, T. N., Hausken, T., Ystad, S. O., Valeur, J., Brokstad, K., Hatlebakk, J. G., & Lied, G. A. Effects of varying dietary content of fermentable short-chain carbohydrates on symptoms, fecal microenvironment, and cytokine profiles in patients with irritable bowel syndrome. *Neurogastroenterology & motility*. 2017;29(4), e12969.
481. Huynh, T., & Alderson, M. (2009). Concept analysis of human ecology. *Nursing Forum*, 44(2), 115-128. doi:10.1111/j.1744-6198.2009.00134.x
482. Iannitelli, A. F., & Weinshenker, D. (2023). Riddles in the dark: Decoding the relationship between neuromelanin and neurodegeneration in locus coeruleus neurons. *Neuroscience & Biobehavioral Reviews*, 105287.

483. Ibrahim, M., & Anishetty, S. (2012). A meta-metabolome network of carbohydrate metabolism: interactions between gut microbiota and host. *Biochemical and biophysical research communications*, 428(2), 278-284.
484. Ideker, T., Galitski, T., & Hood, L. (2001). A new approach to decoding life: systems biology. *Annual review of genomics and human genetics*, 2(1), 343-372.
485. Ioniță-Mîndrican, C. B., Ziani, K., Mititelu, M., Oprea, E., Neacșu, S. M., Moroșan, E., ... & Negrei, C. (2022). Therapeutic benefits and dietary restrictions of fiber intake: A state of the art review. *Nutrients*, 14(13), 2641.
486. Ippolito, J. E., & Piwnica-Worms, D. (2014). A fluorescence-coupled assay for gamma aminobutyric acid (GABA) reveals metabolic stress-induced modulation of GABA content in neuroendocrine cancer. *PloS one*, 9(2), e88667.
487. Jabs, S., Biton, A., Bécavin, C., Nahori, M. A., Ghazlane, A., Pagliuso, A., ... & Chaze, T. Impact of the gut microbiota on the m 6 A epitranscriptome of mouse cecum and liver. *Nature Communications*. 2020;11(1), 1-16.
488. Jacobs, D. M., Deltimple, N., van Velzen, E., van Dorsten, F. A., Bingham, M., Vaughan, E. E., & van Duynhoven, J. (2008). 1H NMR metabolite profiling of feces as a tool to assess the impact of nutrition on the human microbiome. *NMR in Biomedicine: An International Journal Devoted to the Development and Application of Magnetic Resonance In vivo*. 2008;21(6), 615-626.
489. Jacobsen, L., Wilcks, A., Hammer, K., Huys, G., Gevers, D., & Andersen, S. R. (2007). Horizontal transfer of tet (M) and erm (B) resistance plasmids from food strains of *Lactobacillus plantarum* to *Enterococcus faecalis* JH2-2 in the gastrointestinal tract of gnotobiotic rats. *FEMS microbiology ecology*, 59(1), 158-166.
490. Jailwala, J., Imperiale, T. F., & Kroenke, K. (2000). Pharmacologic treatment of the irritable bowel syndrome: a systematic review of randomized, controlled trials. *Annals of Internal medicine*, 133(2), 136-147.
491. Jain, S., Drendel, W. B., Chen, Z. W., Mathews, F. S., Sly, W. S., & Grubb, J. H. (1996). Structure of human β -glucuronidase reveals candidate lysosomal targeting and active-site motifs. *Nature structural biology*, 3(4), 375-381.
492. Jalanka-Tuovinen, J., Salonen, A., Nikkilä, J., Immonen, O., Kekkonen, R., Lahti, L., ... & De Vos, W. M. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PloS one*. 2011; 6(7).
493. James, S. C., Fraser, K., Young, W., McNabb, W. C., & Roy, N. C. (2019). Gut Microbial Metabolites and Biochemical Pathways Involved in Irritable Bowel Syndrome: Effects of Diet and Nutrition on the Microbiome. *The Journal of Nutrition*.
494. Jan, G. B. A. S., Belzacq, A. S., Haouzi, D., Rouault, A., Metivier, D., Kroemer, G., & Brenner, C. (2002). Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. *Cell Death & Differentiation*, 9(2), 179-188.
495. Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol*, 45(9), 2761-2764. doi:10.1128/jcm.01228-07
496. Jarrett, M. O. N. I. C. A., Heitkemper, M. M., Bond, E. F., & Georges, J. A. N. E. (1994). Comparison of diet composition in women with and without functional bowel disorder. *Gastroenterology nursing: the official journal of the Society of Gastroenterology Nurses and Associates*, 16(6), 253-258.
497. Jarrett, M. E., Burr, R. L., Cain, K. C., Hertig, V., Weisman, P., & Heitkemper, M. M. (2003). Anxiety and depression are related to autonomic nervous system function in women with irritable bowel syndrome. *Digestive Diseases and Sciences*, 48, 386-394.
498. Jeffery, I. B., O'Toole, P. W., Ohman, L., Claesson, M. J., Deane, J., Quigley, E. M., & Simren, M. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut*. 2012;61(7):997-1006. doi:10.1136/gutjnl-2011-301501
499. Jenkins, T. A., Nguyen, J. C., Polglaze, K. E., & Bertrand, P. P. (2016). Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients*, 8(1), 56.

500. Jeon, Jeong Ryae, and Joon Hyuk Choi. "Lactic acid fermentation of germinated barley fiber and proliferative function of colonic epithelial cells in loperamide-induced rats." *Journal of Medicinal Food* 13, no. 4 (2010): 950-960.
501. Jiang, H. Y., Zhang, X., Yu, Z. H., Zhang, Z., Deng, M., Zhao, J. H., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of psychiatric research*, 104, 130-136.
502. Jiang, W., Wu, J., Zhu, S., Xin, L., Yu, C., & Shen, Z. (2022). The role of short chain fatty acids in irritable bowel syndrome. *Journal of Neurogastroenterology and Motility*, 28(4), 540.
503. Jin, S. O. N. G., Tianyuan, W. A. N. G., Zhang, X., Bo, L. I., Chunyang, Z. H. U., & Zhang, S. (2020). Upregulation of gastric norepinephrine with β -adrenoceptors and gastric dysmotility in a rat model of functional dyspepsia. *Physiological Research*, 69(1), 135.
504. Jin, C. J., Engstler, A. J., Sellmann, C., Ziegenhardt, D., Landmann, M., Kanuri, G., ... & Bergheim, I. (2016). Sodium butyrate protects mice from the development of the early signs of non-alcoholic fatty liver disease: role of melatonin and lipid peroxidation. *British journal of nutrition*, 116(10), 1682-1693.
505. Johnsen, P. H., Hilpüsch, F., Cavanagh, J. P., Leikanger, I. S., Kolstad, C., Valle, P. C., & Goll, R. (2018). Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. *The lancet Gastroenterology & hepatology*, 3(1), 17-24.
506. Jonathan, M. C., van den Borne, J. J., van Wiechen, P., da Silva, C. S., Schols, H. A., & Gruppen, H. In vitro fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. *Food Chemistry*. 2012;133(3), 889-897.
507. Jones, B. V., Begley, M., Hill, C., Gahan, C. G., & Marchesi, J. R. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proceedings of the national academy of sciences*, 105(36), 13580-13585.
508. Jones, J. M. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutrition journal*. 2014;13(1), 34.
509. Joseph, J., Mudduluru, G., Antony, S., Vashistha, S., Ajitkumar, P., & Somasundaram, K. (2004). Expression profiling of sodium butyrate (NaB)-treated cells: identification of regulation of genes related to cytokine signaling and cancer metastasis by NaB. *Oncogene*, 23(37), 6304-6315.
510. Jost, T., Lacroix, C., Braegger, C., & Chassard, C. (2015). Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health. *Nutrition reviews*, 73(7), 426-437.
511. Jumpertz, R., Le, D. S., Turnbaugh, P. J., Trinidad, C., Bogardus, C., Gordon, J. I., & Krakoff, J. (2011). Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *The American journal of clinical nutrition*, 94(1), 58-65.
512. Kaczmarek, J. L., Musaad, S. M., & Holscher, H. D. (2017). Time of day and eating behaviors are associated with the composition and function of the human gastrointestinal microbiota. *The American journal of clinical nutrition*, 106(5), 1220-1231.
513. Kado, D. M. (2024). Night-to-night sleep duration variability and gut microbial diversity: more evidence for a brain-gut microbiome-sleep connection. *Sleep*, zsae005.
514. Kajander, K., KROGIUS-KURIKKA, L., Rinttilä, T., Karjalainen, H., Palva, A., & Korpela, R. Effects of multispecies probiotic supplementation on intestinal microbiota in irritable bowel syndrome. *Alimentary pharmacology & therapeutics*. 2007;26(3), 463-473.
515. Kajander, K., Hatakka, K., Poussa, T., Färkkilä, M., & Korpela, R. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Alimentary pharmacology & therapeutics*. 2005;22(5), 387-394.
516. Kajikawa, A., Suzuki, S., & Igimi, S. (2018). The impact of motility on the localization of *Lactobacillus agilis* in the murine gastrointestinal tract. *BMC microbiology*, 18, 1-7.
517. Kamiya, T., Shikano, M., Tanaka, M., Ozeki, K., Ebi, M., Katano, T., ... & Mori, Y. Therapeutic effects of biobran, modified arabinoxylan rice bran, in improving symptoms of diarrhea predominant or mixed type irritable bowel syndrome: a pilot, randomized controlled study. *Evidence-Based Complementary and Alternative Medicine*. 2014.

518. Kamp, K. J., Cain, K. C., Utleg, A., Burr, R. L., Raftery, D., Luna, R. A., ... & Heitkemper, M. M. (2021). Bile acids and microbiome among individuals with irritable bowel syndrome and healthy volunteers. *Biological Research for Nursing*, 23(1), 65-74.
519. Kannampalli, P., Shaker, R., & Sengupta, J. N. (2011). Colonic butyrate-algesic or analgesic?. *Neurogastroenterology & Motility*, 23(11), 975-979.
520. Kafsack BFC, Llinás M. Eating at the Table of Another: Metabolomics of Host/Parasite Interactions. *Cell host & microbe*. 2010;7(2):90-99. doi:10.1016/j.chom.2010.01.008.
521. Kamath, P. S., Phillips, S. F., & Zinsmeister, A. R. (1988). Short-chain fatty acids stimulate ileal motility in humans. *Gastroenterology*, 95(6), 1496-1502.
522. Kanemitsu, T., Tsurudome, Y., Kusunose, N., Oda, M., Matsunaga, N., Koyanagi, S., & Ohdo, S. (2017). Periodic variation in bile acids controls circadian changes in uric acid via regulation of xanthine oxidase by the orphan nuclear receptor PPAR α . *Journal of Biological Chemistry*, 292(52), 21397-21406.
523. Karu, Naama, et al. "A Review on Human Fecal Metabolomics: Methods, Applications and the Human Fecal Metabolome Database." *Analytica Chimica Acta*. 2018.
524. Karuppiyah, S., & Pomianowski, K. (2017). Rifaximin (Xifaxan) for Irritable Bowel Syndrome. *American Family Physician*, 95(4), 258-259.
525. Kassinen A, Kroquius-Kurikka L, Mökivuokko H et al. The faecal microbiota of irritable bowel syndrome patients differs significantly from that of health subjects. *Gastroenterology* 2007; 133: 24–33.
526. Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in Cellular Neuroscience*. 2015;9:392. doi:10.3389/fncel.2015.00392.
527. Kerckhoffs AP, Samsom M, van der Rest ME, et al. Lower Bifidobacteria counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients. *World J Gastroenterol*. 2009;15(23):2887-92.
528. Kellogg, M. D., Ellervik, C., Morrow, D., Hsing, A., Stein, E., & Sethi, A. A. (2015). Preanalytical considerations in the design of clinical trials and epidemiological studies. *Clinical chemistry*, 61(6), 797-803.
529. Kellow, J. E., & Phillips, S. F. (1987). Altered small bowel motility in irritable bowel syndrome is correlated with symptoms. *Gastroenterology*, 92(6), 1885-1893.
530. Kempner, W. (1939). The nature of leukemic blood cells as determined by their metabolism. *The Journal of clinical investigation*, 18(3), 291-300.
531. Kennedy, P. J., Cryan, J. F., Quigley, E. M. M., Dinan, T. G., & Clarke, G. (2014). A sustained hypothalamic–pituitary–adrenal axis response to acute psychosocial stress in irritable bowel syndrome. *Psychological medicine*, 44(14), 3123-3134.
532. Kesika, P., Suganthi, N., Sivamaruthi, B. S., & Chaiyasut, C. (2021). Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. *Life sciences*, 264, 118627.
533. Kettle, H., Louis, P., Holtrop, G., Duncan, S. H., & Flint, H. J. (2015). Modelling the emergent dynamics and major metabolites of the human colonic microbiota. *Environmental microbiology*, 17(5), 1615-1630.
534. Keys, A., Grande, F., & Anderson, J. T. (1961). Fiber and pectin in the diet and serum cholesterol concentration in man. *Proceedings of the Society for Experimental Biology and Medicine*, 106(3), 555-558.
535. Khoo, X. H., Chong, C. W., Talha, A. M., Philip, K., Teh, C. S. J., Isa, A. M., ... & Mahadeva, S. (2023). The impact of diet and ethnicity on Gut Microbiota Variation in Irritable Bowel Syndrome: A Multi-centre Study. *Journal of Gastroenterology and Hepatology*.
536. Kilkens, TOC, Honig, A., Van Nieuwenhoven, M. A., Riedel, W. J., & Brummer, R. M. (2004). Acute tryptophan depletion affects brain-gut responses in irritable bowel syndrome patients and controls. *Gut*, 53(12), 1794-1800.
537. Kim, J., & Jeong, J. (2019). MetPC: Metabolite Pipeline Consisting of Metabolite Identification and Biomarker Discovery Under the Control of Two-Dimensional FDR. *Metabolites*, 9(5), 103.
538. Kim, C. H. Immune regulation by microbiome metabolites. *Immunology*. 2018;154(2), 220-229.

539. Kinsinger, S. W. (2017). Cognitive-behavioral therapy for patients with irritable bowel syndrome: current insights. *Psychology Research and Behavior Management*, 10, 231–237.
<http://doi.org/10.2147/PRBM.S120817>
540. Klaassen, C. D., & Cui, J. Y. (2015). Mechanisms of how the intestinal microbiota alters the effects of drugs and bile acids. *Drug Metabolism and Disposition*, 43(10), 1505-1521.
541. Kleessen, B., Stoof, G., Proll, J., Schmiedl, D., Noack, J., & Blaut, M. Feeding resistant starch affects fecal and cecal microflora and short-chain fatty acids in rats. *Journal of animal science*. 1997; 75(9), 2453-2462
542. Kobayashi, K. S., Chamaillard, M., Ogura, Y., Henegariu, O., Inohara, N., Nuñez, G., & Flavell, R. A. (2005). Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science*, 307(5710), 731-734.
543. Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., ... & Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences*, 108(supplement_1), 4578-4585.
544. Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*. 2016;165(6), 1332-1345.
545. Koide A, Yamaguchi T, Odaka T et al. Quantitative analysis of bowel gas using plain abdominal radiograph in patients with irritable bowel syndrome. *Am J Gastroenterol* 2000; 95: 1735–41.
546. König, J., Wells, J., Cani, P. D., García-Ródenas, C. L., MacDonald, T., Mercenier, A., ... & Brummer, R. J. (2016). Human intestinal barrier function in health and disease. *Clinical and translational gastroenterology*, 7(10), e196.
547. Kong, C., Elderman, M., Cheng, L., de Haan, B. J., Nauta, A., & de Vos, P. (2019). Modulation of intestinal epithelial glycocalyx development by human milk oligosaccharides and non-digestible carbohydrates. *Molecular nutrition & food research*, 63(17), 1900303.
548. Konturek, P. C., Brzozowski, T., & Konturek, S. J. (2011). Gut clock: implication of circadian rhythms in the gastrointestinal tract. *J Physiol Pharmacol*, 62(2), 139-150.
549. Kopečný, J., & Šimůnek, J. Cellulolytic bacteria in human gut and irritable bowel syndrome. *Acta Veterinaria Brno*. 2002;71(4), 421-427.
550. Korecka, A. (2014). The Microbiome, PPAR γ and AhR in the inflammation-metabolism interface within the gastrointestinal tract. Karolinska Institutet (Sweden).
551. Korpela, K. (2018). Diet, microbiota, and metabolic health: Trade-off between saccharolytic and proteolytic fermentation. *Annual review of food science and technology*. 2018;9, 65-84.
552. Kumar, S., Shukla, R., Ranjan, P., & Kumar, A. (2017). Interleukin-10: a compelling therapeutic target in patients with irritable bowel syndrome. *Clinical therapeutics*, 39(3), 632-643.
553. Kurdi, P., Tanaka, H., W van Veen, H., Asano, K., Tomita, F., & Yokota, A. Cholic acid accumulation and its diminution by short-chain fatty acids in bifidobacteria. *Microbiology*. 2003;149, 2031-2037.
554. Kwon, Y. J., Lee, H. S., Park, J. Y., & Lee, J. W. (2020). Associating intake proportion of carbohydrate, fat, and protein with all-cause mortality in Korean adults. *Nutrients*, 12(10), 3208.
555. Lackner, J., Jaccard, J., Baum, C., Smith, A., Krasner, S., Katz, L., . . . Powell, C. Patient-reported outcomes for irritable bowel syndrome are associated with patients' severity ratings of gastrointestinal symptoms and psychological factors. *Clinical Gastroenterology and Hepatology*. 2011;9(11):957-964.e951. doi:10.1016/j.cgh.2011.06.014
556. Lacy, B. E., Chey, W. D., & Lembo, A. J. New and Emerging Treatment Options for Irritable Bowel Syndrome. *Gastroenterology & Hepatology*. 2015;11(4 Suppl 2):1–19.
557. Lacy, B. E. The Science, Evidence, and Practice of Dietary Interventions in Irritable Bowel Syndrome. *Clinical Gastroenterology & Hepatology*. 2015;13(11):1899-1906. doi:10.1016/j.cgh.2015.02.043
558. Lamichhane, S., Sundekilde, U. K., Blædel, T., Dalsgaard, T. K., Larsen, L. H., Dragsted, L. O., ... & Bertram, H. C. Optimizing sampling strategies for NMR-based metabolomics of human feces: pooled vs. unpooled analyses. *Analytical methods*. 2017;9(30), 4476-4480.
559. Lampe, J. W., Fredstrom, S. B., Slavin, J. L., & Potter, J. D. Sex differences in colonic function: a randomised trial. *Gut*. 1993;34(4), 531-536.

560. Lampe, J. W., Effertz, M. E., Larson, J. L., & Slavin, J. L. (1992). Gastrointestinal effects of modified guar gum and soy polysaccharide as part of an enteral formula diet. *Journal of Parenteral and Enteral Nutrition*, 16(6), 538-544.
561. Lan, G., Chen, H., Chen, S., & Tian, J. (2012). Chemical composition and physicochemical properties of dietary fiber from *Polygonatum odoratum* as affected by different processing methods. *Food Research International*, 49(1), 406-410.
562. Langhendries, J. P., Detry, J., Van Hees, J., Lamboray, J. M., Darimont, J., Mozin, M. J., ... & Senterre, J. (1995). Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. *Journal of pediatric gastroenterology and nutrition*, 21(2), 177-181
563. Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients*, 2(12), 1266-1289.
564. Le Leu, R. K., Brown, I. L., Hu, Y., Bird, A. R., Jackson, M., Esterman, A., & Young, G. P. (2005). A synbiotic combination of resistant starch and *Bifidobacterium lactis* facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. *The Journal of nutrition*, 135(5), 996-1001.
565. Leahy, S. C., Higgins, D. G., Fitzgerald, G. F., and van Sinderen, D. Getting better with bifidobacteria. *Journal of Applied Microbiology*.2005;98:1303–1315. doi: 10.1111/j.1365-2672.2005.02600.x
566. Le Blay, G., Blottière, H. M., Ferrier, L., Le Foll, E., Bonnet, C., Galmiche, J. P., & Cherbut, C. (2000). Short-chain fatty acids induce cytoskeletal and extracellular protein modifications associated with modulation of proliferation on primary culture of rat intestinal smooth muscle cells. *Digestive diseases and sciences*, 45, 1623-1630.
567. Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., . . . Pedersen, O. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013;500(7464):541-546. doi:10.1038/nature12506.
568. Lee, W. J., Lattimer, L. D., Stephen, S., Borum, M. L., & Doman, D. B. (2015). Fecal microbiota transplantation: a review of emerging indications beyond relapsing *clostridium difficile* toxin colitis. *Gastroenterology & hepatology*, 11(1), 24
569. Lee, J. S., Kim, S. Y., Chun, Y. S., Chun, Y. J., Shin, S. Y., Choi, C. H., & Choi, H. K. (2020). Characteristics of fecal metabolic profiles in patients with irritable bowel syndrome with predominant diarrhea investigated using 1H-NMR coupled with multivariate statistical analysis. *Neurogastroenterology & Motility*, 32(6), e13830.
570. Lee, H. H., Choi, Y. Y., & Choi, M. G. (2014). The efficacy of hypnotherapy in the treatment of irritable bowel syndrome: a systematic review and meta-analysis. *Journal of neurogastroenterology and motility*, 20(2), 152.
571. Lee, L. C., Liong, C. Y., & Jemain, A. A. (2018). Partial least squares-discriminant analysis (PLS-DA) for classification of high-dimensional (HD) data: a review of contemporary practice strategies and knowledge gaps. *Analyst*, 143(15), 3526-3539.
572. Lee, Jae Soung, Seok-Young Kim, Yoon Shik Chun, Young-Jin Chun, Seung Yong Shin, Chang Hwan Choi, and Hyung-Kyoon Choi. "Characteristics of fecal metabolic profiles in patients with irritable bowel syndrome with predominant diarrhea investigated using 1H-NMR coupled with multivariate statistical analysis." *Neurogastroenterology & Motility* 32, no. 6 (2020): e13830.
573. Lembo, A., Pimentel, M., Rao, S. S., Schoenfeld, P., Cash, B., Weinstock, L. B., ... & Forbes, W. P. (2016). Repeat treatment with rifaximin is safe and effective in patients with diarrhea-predominant irritable bowel syndrome. *Gastroenterology*, 151(6), 1113-1121.
574. Lenhart, Adrienne, and William D. Chey. "A systematic review of the effects of polyols on gastrointestinal health and irritable bowel syndrome." *Advances in Nutrition*. 2017;8.4: 587-596.
575. Leonel, A. J., & Alvarez-Leite, J. I. Butyrate: implications for intestinal function. *Current Opinion in Clinical Nutrition & Metabolic Care*. 2012;15(5), 474-479.
576. LessLikely (n.d.). False Positives, FWER, and FDR Explained. Retrieved March 2, 2020, from <https://lesslikely.com/statistics/multiplicity-explained/>
577. Le Leu, R. K., Brown, I. L., Hu, Y., Bird, A. R., Jackson, M., Esterman, A., & Young, G. P. (2005). A synbiotic combination of resistant starch and *Bifidobacterium lactis* facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. *The Journal of nutrition*, 135(5), 996-1001.

578. Lewandowski, K., Kaniewska, M., Karłowicz, K., Rosołowski, M., & Rydzewska, G. (2022). The effectiveness of microencapsulated sodium butyrate at reducing symptoms in patients with irritable bowel syndrome. *Gastroenterology Review/Przegląd Gastroenterologiczny*, 16(1).
579. Levast, B., Li, Z., & Madrenas, J. (2015). The role of IL-10 in microbiome-associated immune modulation and disease tolerance. *Cytokine*, 75(2), 291-301.
580. Levene, P. A., and Meyer, G. M., On the action of leukocytes on glucose. *J. Biol. Chem.*, 1912, 12, 265
581. Levy, M., Thaïss, C. A., Katz, M. N., Suez, J., & Elinav, E. (2015, January). Inflammasomes and the microbiota—partners in the preservation of mucosal homeostasis. In *Seminars in immunopathology* (Vol. 37, No. 1, pp. 39-46). Springer Berlin Heidelberg.
582. Levy, R. L., Olden, K. W., Naliboff, B. D., Bradley, L. A., Francisconi, C., Drossman, D. A., & Creed, F. (2006). Psychosocial aspects of the functional gastrointestinal disorders. *Gastroenterology*, 130(5), 1447-1458.
583. Lewis, J. D., Albenberg, L., Lee, D., Kratz, M., Gottlieb, K., & Reinisch, W. The importance and challenges of dietary intervention trials for inflammatory bowel disease. *Inflammatory bowel diseases*. 2017; 23(2), 181-191.
584. Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. *nature*, 444(7122), 1022-1023.
585. Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, 124(4), 837-848.
586. Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the national academy of sciences*, 102(31), 11070-11075.
587. Li, C. J., Zhang, Z., Zhan, P. C., Lv, A. P., Li, P. P., Liu, L., ... & Zhi, X. Y. (2023). Comparative genomic analysis and proposal of *Clostridium yunnanense* sp. nov., *Clostridium rhizosphaerae* sp. nov., and *Clostridium paridis* sp. nov., three novel *Clostridium* sensu stricto endophytes with diverse capabilities of acetic acid and ethanol production. *Anaerobe*, 79, 102686.
588. Li, Z., Yi, C. X., Katiraei, S., Kooijman, S., Zhou, E., Chung, C. K., ... & Wang, Y. (2017). Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut*, gutjnl-2017.
589. Li, B., Liang, L., Deng, H., Guo, J., Shu, H., & Zhang, L. (2020). Efficacy and safety of probiotics in irritable bowel syndrome: a systematic review and meta-analysis. *Frontiers in pharmacology*, 11, 332.
590. Li, H., Myeroff, L., Smiraglia, D., Romero, M. F., Pretlow, T. P., Kasturi, L., ... & Markowitz, S. D. (2003). SLC5A8, a sodium transporter, is a tumor suppressor gene silenced by methylation in human colon aberrant crypt foci and cancers. *Proceedings of the National Academy of Sciences*, 100(14), 8412-8417.
591. Li, J., Zhu, W., Liu, W., Wu, Y., & Wu, B. (2016). Rifaximin for irritable bowel syndrome: a meta-analysis of randomized placebo-controlled trials. *Medicine*. 2016;95(4).
592. Li, R. W., & Li, C. (2006). Butyrate induces profound changes in gene expression related to multiple signal pathways in bovine kidney epithelial cells. *BMC genomics*, 7, 1-14.
593. Li, M., van Esch, B., Wagenaar, G. T. M., Garssen, J., Folkerts, G., & Henricks, P. A. J. Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells. *European Journal of Pharmacology*. 2018;831:52-59. doi:10.1016/j.ejphar.2018.05.003
594. Li, Q., Khambadkone, S. G., Zhu, Y., Ceuleers, H., Liu, L., Goodman, E. J., ... & Pasricha, P. J. (2018). 917-Butyrate-Producing Synbiotics Reduce Visceral Hyperalgesia and Anxiety-Like Behaviors in a Mouse Model of Irritable Bowel Syndrome, Associated with Inhibition of TRPV1 Currents in Sensory Neurons. *Gastroenterology*, 154(6), S-182.
595. Li, X. Q., Zhu, Y. H., Zhang, H. F., Yue, Y., Cai, Z. X., Lu, Q. P., ... & Wang, J. F. (2012). Risks associated with high-dose *Lactobacillus rhamnosus* in an *Escherichia coli* model of piglet diarrhoea: intestinal microbiota and immune imbalances.
596. Li, H., Chen, J., Ren, X., Yang, C., Liu, S., Bai, X., ... & Dong, X. (2021). Gut microbiota composition changes in constipated women of reproductive age. *Frontiers in Cellular and Infection Microbiology*, 10, 557515.
597. Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., ... & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS medicine*, 6(7), e1000100.

598. Lied GA. Indication of immune activation in patients with perceived food hypersensitivity. (2014). *Digestive Diseases & Sciences*, 59:259–266.
599. Liegl, G., Plessen, C. Y., Leitner, A., Boeckle, M., & Pieh, C. Guided self-help interventions for irritable bowel syndrome: a systematic review and meta-analysis. *European Journal of Gastroenterology & Hepatology*. 2015;27(10):1209-1221. doi:10.1097/meg.0000000000000428
600. Little, M., Dutta, M., Li, H., Matson, A., Shi, X., Mascarinas, G., ... & Cui, J. Y. (2022). Understanding the physiological functions of the host xenobiotic-sensing nuclear receptors PXR and CAR on the gut microbiome using genetically modified mice. *Acta Pharmaceutica Sinica B*, 12(2), 801-820.
601. Liu, H., Huang, D., & Wen, J. (2016). Integrated intracellular metabolic profiling and pathway analysis approaches reveal complex metabolic regulation by *Clostridium acetobutylicum*. *Microbial Cell Factories*, 15, 1-14.
602. Liu, T., Gu, X., Li, L. X., Li, M., Li, B., Cui, X., & Zuo, X. L. (2020). Microbial and metabolomic profiles in correlation with depression and anxiety co-morbidities in diarrhoea-predominant IBS patients. *BMC microbiology*, 20, 1-14.
603. Liu, H. N., Wu, H., Chen, Y. Z., Chen, Y. J., Shen, X. Z., & Liu, T. T. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: a systematic review and meta-analysis. *Digestive and Liver Disease*. 2017;49(4), 331-337.
604. Liu, N., Sun, S., Wang, P., Sun, Y., Hu, Q., & Wang, X. (2021). The mechanism of secretion and metabolism of gut-derived 5-hydroxytryptamine. *International journal of molecular sciences*, 22(15), 7931.
605. Livesey, G. (1995). Short-chain fatty acids as an energy source in the colon: metabolism and clinical implication. *Physiological and clinical aspects of short-chain fatty acids*, 427-481.
606. Livesey, G., I. R. Davies, J. C. Brown, R. M. Faulks, and S. Southon. "Energy balance and energy values of α -amylase (EC 3. 2. 1. 1)-resistant maize and pea (*Pisum sativum*) starches in the rat." *British journal of Nutrition* 63, no. 3 (1990): 467-480.
607. Livesey, G. (1989). Procedure for calculating the digestible and metabolisable energy values of food components making a small contribution to dietary intake. *Journal of the Science of Food and Agriculture*, 48(4), 475-481.
608. Livesey, G. (1995). Short-chain fatty acids as an energy source in the colon: metabolism and clinical implication. *Physiological and clinical aspects of short-chain fatty acids*, 427-481.
609. Livesey, G. (1990). Energy values of unavailable carbohydrate and diets: an inquiry and analysis. *The American Journal of Clinical Nutrition*, 51(4), 617-637.
610. Lofffield, E., Vogtmann, E., Sampson, J. N., Moore, S. C., Nelson, H., Knight, R., ... & Sinha, R. Comparison of collection methods for fecal samples for discovery metabolomics in epidemiologic studies. *Cancer Epidemiology and Prevention Biomarkers*. 2016;25(11), 1483-1490.
611. Loman, N. J., Misra, R. V., Dallman, T. J., Constantinidou, C., Gharbia, S. E., Wain, J., & Pallen, M. J. (2012). Performance comparison of benchtop high-throughput sequencing platforms. *Nat Biotechnol*, 30(5), 434-439. doi:10.1038/nbt.2198
612. Long, X., Li, M., Li, L. X., Sun, Y. Y., Zhang, W. X., Zhao, D. Y., & Li, Y. Q. (2018). Butyrate promotes visceral hypersensitivity in an IBS-like model via enteric glial cell-derived nerve growth factor. *Neurogastroenterology & Motility*, 30(4), e13227.
613. Longstreth, G. F., Thompson, W. G., Chey, W. D., Houghton, L. A., Mearin, F., & Spiller, R. C. (2006). Functional bowel disorders. *Gastroenterology*, 130(5), 1480-1491.
614. LOPES, S. S., MISZPUTEN, S. J., SACHS, A., LIMA, M. M., & AMBROGINI JR, O. (2019). Evaluation of carbohydrate and fiber consumption in patients with irritable bowel syndrome in outpatient treatment. *Arquivos de gastroenterologia*, 56(1), 3-9.
615. Lopetuso, L. R., Scaldaferri, F., Petito, V., & Gasbarrini, A. (2013). Commensal *Clostridia*: leading players in the maintenance of gut homeostasis. *Gut pathogens*, 5(1), 1-8.
616. Lopez, D. E. G., Lashinger, L. M., Weinstock, G. M., & Bray, M. S. (2021). Circadian rhythms and the gut microbiome synchronize the host's metabolic response to diet. *Cell Metabolism*, 33(5), 873-887.
617. López, J. R., Martínez, A. G., Luque, A., Pons, J. M., Vargas, A. A., Iglesias, J. R., ... & Villegas, J. A. (2008). The effect of a fibre enriched dietary milk product in chronic primary idiopathic constipation. *Nutricion hospitalaria*, 23(1), 12-19.

618. Louis, P., Duncan, S. H., Sheridan, P. O., Walker, A. W., & Flint, H. J. (2022). Microbial lactate utilisation and the stability of the gut microbiome. *Gut Microbiome*, 3, e3.
619. Louis, P., Hold, G. L., & Flint, H. J. The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews Microbiology*. 2014;12(10): 661.
620. Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental microbiology*, 19(1), 29-41.
621. Louis, P., & Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS microbiology letters*. 2009;294(1), 1-8.
622. Louis, P., Young, P., Holtrop, G., & Flint, H. J. (2010). Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA: acetate CoA-transferase gene. *Environmental microbiology*. 2010;12(2):304-314.
623. Louis, P., McCrae, S. I., Charrier, C., & Flint, H. J. (2007). Organization of butyrate synthetic genes in human colonic bacteria: phylogenetic conservation and horizontal gene transfer. *FEMS microbiology letters*, 269(2), 240-247.
624. Louis, P., Scott, K. P., Duncan, S. H., & Flint, H. J. (2007). Understanding the effects of diet on bacterial metabolism in the large intestine. *Journal of applied microbiology*, 102(5), 1197-1208.
625. Louis, P., & Flint, H. J. (2007). Development of a semiquantitative degenerate real-time PCR-based assay for estimation of numbers of butyryl-coenzyme A (CoA) CoA transferase genes in complex bacterial samples. *Appl. Environ. Microbiol.*, 73(6), 2009-2012.
626. Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, 489(7415), 220-230.
627. Lu, W., Su, X., Klein, M. S., Lewis, I. A., Fiehn, O., & Rabinowitz, J. D. (2017). Metabolite Measurement: Pitfalls to Avoid and Practices to Follow. *Annual review of biochemistry*, 86, 277–304.
628. Lukić, I., Ivković, S., Mitić, M., & Adžić, M. (2022). Tryptophan metabolites in depression: Modulation by gut microbiota. *Frontiers in behavioral neuroscience*, 16, 987697.
629. Lunn, J., & Buttriss, J. L. Carbohydrates and dietary fibre. *Nutrition Bulletin*. 2007;32(1), 21-64.
630. Lupton, J. R. (2004). Microbial degradation products influence colon cancer risk: the butyrate controversy. *The Journal of nutrition*, 134(2), 479-482.
631. Luu, M., Weigand, K., Wedi, F., Breidenbend, C., Leister, H., Pautz, S., ... & Visekruna, A. (2018). Regulation of the effector function of CD8+ T cells by gut microbiota-derived metabolite butyrate. *Scientific reports*, 8(1), 14430.
- 632.
633. Lynch, J. B., Gonzalez, E. L., Choy, K., Faull, K. F., Jewell, T., Arellano, A., ... & Hsiao, E. Y. (2023). Gut microbiota *Turicibacter* strains differentially modify bile acids and host lipids. *Nature Communications*, 14(1), 3669.
634. Lyra, A., Rinttilä, T., Nikkilä, J., Krogius-Kurikka, L., Kajander, K., Malinen, E., ... & Palva, A. (2009). Diarrhoea-predominant irritable bowel syndrome distinguishable by 16S rRNA gene phylotype quantification. *World journal of gastroenterology: WJG*, 15(47), 5936.
635. Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, 33(8), 574-581.
636. Ma, N., Guo, P., Zhang, J., He, T., Kim, S. W., Zhang, G., & Ma, X. Nutrients Mediate Intestinal Bacteria–Mucosal Immune Crosstalk. *Frontiers in Immunology*. 2018; 9: 5
637. Macagnan, F. T., da Silva, L. P., & Hecktheuer, L. H. (2016). Dietary fibre: The scientific search for an ideal definition and methodology of analysis, and its physiological importance as a carrier of bioactive compounds. *Food Research International*, 85, 144-154.
638. Macedo, D., Chaves Filho, A. J. M., de Sousa, C. N. S., Quevedo, J., Barichello, T., Júnior, H. V. N., & de Lucena, D. F. (2017). Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. *Journal of affective disorders*, 208, 22-32.
639. Macfarlane, S., & Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society*. 2003;62(1): 67-72.

640. Macfarlane, G. T., Gibson, G. R., Beatty, E., & Cummings, J. H. (1992). Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. *FEMS microbiology ecology*, 10(2), 81-88.
641. Macfarlane, G. T., & Macfarlane, S. (2012). Bacteria, colonic fermentation, and gastrointestinal health. *Journal of AOAC International*, 95(1), 50-60.
642. Macia, L., Tan, J., Vieira, A. T., Leach, K., Stanley, D., Luong, S., ... & Binge, L. (2015). Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nature communications*, 6, 6734.
643. Marcobal, A., De Las Rivas, B., Landete, J. M., Tabera, L., & Muñoz, R. (2012). Tyramine and phenylethylamine biosynthesis by food bacteria. *Critical reviews in food science and nutrition*, 52(5), 448-467.
644. Madden, J. A. J., & Hunter, J. O. (2002). A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. *British Journal of Nutrition*, 88(S1), s67-s72.
645. Magnúsdóttir, S., Heinken, A., Kutt, L., Ravcheev, D. A., Bauer, E., Noronha, A., ... & Fleming, R. M. (2017). Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nature biotechnology*, 35(1), 81.
646. Magnúsdóttir, S., Heinken, A., Kutt, L., Ravcheev, D. A., Bauer, E., Noronha, A., ... & Fleming, R. M. (2017). Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nature biotechnology*, 35(1), 81.
647. Maguire, M., & Maguire, G. (2019). Gut dysbiosis, leaky gut, and intestinal epithelial proliferation in neurological disorders: towards the development of a new therapeutic using amino acids, prebiotics, probiotics, and postbiotics. *Reviews in the Neurosciences*, 30(2), 179-201.
648. Mahadevan, V., & Zieve, L. Determination of volatile free fatty acids of human blood. *Journal of lipid research*. 1969;10(3), 338-341.
649. Maier, S., Reich, E., Martin, R., Bachem, M., Altug, V., Hautmann, R. E., & Gschwend, J. E. (2000). Tributyrin induces differentiation, growth arrest and apoptosis in androgen-sensitive and androgen-resistant human prostate cancer cell lines. *International journal of cancer*, 88(2), 245-251.
650. Maiti, T. K., Roy, A., Mukherjee, S. K., & Chatterjee, S. P. (1995). Microbial production of L-tyrosine: a review. *Hindustan Antibiotics Bulletin*, 37(1-4), 51-65.
651. Mairinger, M., Maget, A., Wagner-Skacel, J., Mörk, S., Dalkner, N., Hellinger, T., ... & Bengesser, S. (2023). Gut microbiome composition and its association with sleep in major psychiatric disorders. *Neuropsychobiology*, 82(4), 220-233.
652. Majima, A., Handa, O., Naito, Y., Suyama, Y., Onozawa, Y., Higashimura, Y., ... & Itoh, Y. (2017). Real-time monitoring of trans-epithelial electrical resistance in cultured intestinal epithelial cells: the barrier protection of water-soluble dietary fiber. *Journal of Digestive Diseases*, 18(3), 151-159.
653. Makki, K., Deehan, E. C., Walter, J., & Bäckhed, F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell host & microbe*. 2018;23(6), 705-715
654. Malago, J. J., Koninkx, J. F. J. G., Douma, P. M., Dirkzwager, A., Veldman, A., Hendriks, H. G. C. J. M., & Van Dijk, J. E. (2003). Differential modulation of enterocyte-like Caco-2 cells after exposure to short-chain fatty acids. *Food Additives & Contaminants*, 20(5), 427-437.
655. Malinen, E., Rinttilä, T., Kajander, K., Mättö, J., Kassinen, A., Krogus, L., ... & Palva, A. (2005). Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Official journal of the American College of Gastroenterology| ACG*, 100(2), 373-382.
656. Malinen, E., Krogus-Kurikka, L., Lyra, A., Nikkilä, J., Jääskeläinen, A., Rinttilä, T., ... & Palva, A. Association of symptoms with gastrointestinal microbiota in irritable bowel syndrome. *World journal of gastroenterology: WJG*. 2010;16(36), 4532.
657. Mandl, P., & Kiss, J. P. (2007). Role of presynaptic nicotinic acetylcholine receptors in the regulation of gastrointestinal motility. *Brain research bulletin*, 72(4-6), 194-200.
658. Manly, K. F., Nettleton, D., & Hwang, J. G. (2004). Genomics, prior probability, and statistical tests of multiple hypotheses. *Genome research*, 14(6), 997-1001.
659. Manoharan, I., Prasad, P. D., Thangaraju, M., & Manicassamy, S. (2021). Lactate-dependent regulation of immune responses by dendritic cells and macrophages. *Front. Immunol.* 12, 691134.

660. Mariat, D., Firmesse, O., Levenez, F., Guimarães, V. D., Sokol, H., Doré, J., ... & Furet, J. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC microbiology*, 9, 1-6.
661. Marinelli, L. (2018). Activation mechanism of AhR pathway in intestinal epithelial cells by commensal bacteria (Doctoral dissertation, Sorbonne université).
662. Marinelli, L., Martin-Gallausiaux, C., Bourhis, J. M., Béguet-Crespel, F., Blottière, H. M., & Lapaque, N. (2019). Identification of the novel role of butyrate as AhR ligand in human intestinal epithelial cells. *Scientific reports*, 9(1), 643.
663. Marra, F., & Ng, K. (2015). Controversies around epidemiology, diagnosis and treatment of *Clostridium difficile* infection. *Drugs*, 75, 1095-1118.
664. Marsh, A., Eslick, E. M., & Eslick, G. D. Does a diet low in FODMAPs reduce symptoms associated with functional gastrointestinal disorders? A comprehensive systematic review and meta-analysis. *European journal of nutrition*. 2016; 55(3), 897-906.
665. Martin, B. (2020). The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells. *Experimental and Molecular Medicine*, 52, 1-13.
666. Martin-Subero, M., Anderson, G., Kanchanatawan, B., Berk, M., & Maes, M. (2016). Comorbidity between depression and inflammatory bowel disease explained by immune-inflammatory, oxidative, and nitrosative stress; tryptophan catabolite; and gut–brain pathways. *CNS spectrums*, 21(2), 184-198.
667. MathStackExchange (n.d.). Mathematics. Retrieved March 2, 2020, from <https://math.stackexchange.com/questions/1252849/what-does-ssb-and-ssw-of-anova-tell-you-about-your-data>
668. Martin F-PJ, Collino S, Rezzi S, Kochhar S. Metabolomic Applications to Decipher Gut MicrobialMetabolic Influence in Health and Disease. *Frontiers in Physiology*. 2012;3:113. doi:10.3389/fphys.2012.00113.
669. Mayeur, C., Gratadoux, J. J., Bridonneau, C., Chegdani, F., Larroque, B., Kapel, N., ... & Joly, F. (2013). Faecal D/L lactate ratio is a metabolic signature of microbiota imbalance in patients with short bowel syndrome. *PloS one*, 8(1), e54335.
670. Mazhar, M., Zhu, Y., & Qin, L. (2023). The interplay of dietary fibers and intestinal microbiota affects type 2 diabetes by generating short-chain fatty acids. *Foods*, 12(5), 1023.
671. Moloney, R. D., Johnson, A. C., O'mahony, S. M., Dinan, T. G., Greenwood-Van Meerveld, B., & Cryan, J. F. (2016). Stress and the microbiota–gut–brain axis in visceral pain: relevance to irritable bowel syndrome. *CNS neuroscience & therapeutics*, 22(2), 102-117.
672. Manning, A. P., Harvey, R. F., Heaton, K. W., & Uglow, P. Wheat fibre and irritable bowel syndrome: a controlled trial. *The Lancet*. 1977;310(8035), 417-418.
673. Mardinoglu, A., Wu, H., Bjornson, E., Zhang, C., Hakkarainen, A., Räsänen, S. M., ... & Boren, J. (2018). An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell metabolism*, 27(3), 559-571.
674. Maroni, L., Agostinelli, L., Saccomanno, S., Pinto, C., Giordano, D. M., Rychlicki, C., ... & Marzioni, M. (2017). Nlrp3 activation induces IL-18 synthesis and affects the epithelial barrier function in reactive cholangiocytes. *The American journal of pathology*, 187(2), 366-376.
675. \Martin AM, Lumsden AL, Young RL, Jessup CF, Spencer NJ, Keating DJ. Regional differences in nutrient-induced secretion of gut serotonin. *Physiological Reports*. 2017;5(6):e13199. doi:10.14814/phy2.13199.
676. Mansueto, P., Seidita, A., D'alcamo, A., & Carroccio, A. Role of FODMAPs in patients with irritable bowel syndrome. *Nutrition in Clinical Practice*. 2015;30(5):665-682.
677. Mantovani, A., & Marchesi, F. (2014). IL-10 and macrophages orchestrate gut homeostasis. *Immunity*, 40(5), 637-639.
678. Marriott, B. P., Olsho, L., Hadden, L., & Connor, P. (2010). Intake of added sugars and selected nutrients in the United States, National Health and Nutrition Examination Survey (NHANES) 2003—2006. *Critical reviews in food science and nutrition*, 50(3), 228-258.

679. Mars, R. A., Yang, Y., Ward, T., Houtti, M., Priya, S., Lekatz, H. R., ... & Kashyap, P. C. (2020). Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome. *Cell*, 182(6), 1460-1473.
680. Martens, E. (2018). The Diet-microbiota-mucus Layer Axis as a Mediator of Intestinal Health and Disease. *The FASEB Journal*, 32, 101-3.
681. Martínez, I., Kim, J., Duffy, P. R., Schlegel, V. L., & Walter, J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PloS one*. 2010;5(11), e15046.
682. Matenchuk, B. A., Mandhane, P. J., & Kozyrskyj, A. L. (2020). Sleep, circadian rhythm, and gut microbiota. *Sleep Medicine Reviews*, 53, 101340.
683. Mättö, J., Maunuksela, L., Kajander, K., Palva, A., Korpela, R., Kassinen, A., & Saarela, M. (2005). Composition and temporal stability of gastrointestinal microbiota in irritable bowel syndrome—a longitudinal study in IBS and control subjects. *FEMS Immunology & Medical Microbiology*, 43(2), 213-222.
684. Maukonen, J., Satokari, R., Mättö, J., Söderlund, H., Mattila-Sandholm, T., & Saarela, M. (2006). Prevalence and temporal stability of selected clostridial groups in irritable bowel syndrome in relation to predominant faecal bacteria. *Journal of Medical Microbiology*, 55(5), 625-633.
685. Mazzawi, T., Hausken, T., Hov, J. R., Valeur, J., Sangnes, D. A., El-Salhy, M., ... & Lied, G. A. (2019). Clinical response to fecal microbiota transplantation in patients with diarrhea-predominant irritable bowel syndrome is associated with normalization of fecal microbiota composition and short-chain fatty acid levels. *Scandinavian Journal of Gastroenterology*.
686. Mazzawi, T., & El-Salhy, M. Effect of diet and individual dietary guidance on gastrointestinal endocrine cells in patients with irritable bowel syndrome. *International journal of molecular medicine*. 2017;40(4), 943-952.
687. McOrist, A. L., Abell, G. C., Cooke, C., & Nyland, K. Bacterial population dynamics and faecal short-chain fatty acid (SCFA) concentrations in healthy humans. *British journal of nutrition*. 2008;100(1), 138-146.
688. McEwen, B. S. (2016). In pursuit of resilience: stress, epigenetics, and brain plasticity. *Annals of New York Academy of Sciences*, 1373(1), 56-64. doi:10.1111/nyas.13020
689. McFarland, L. V., Karakan, T., & Karatas, A. (2021). Strain-specific and outcome-specific efficacy of probiotics for the treatment of irritable bowel syndrome: A systematic review and meta-analysis. *EClinicalMedicine*, 41.
690. McGarr, S. E., Ridlon, J. M., & Hylemon, P. B. (2005). Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. *Journal of clinical gastroenterology*, 39(2), 98-109.
691. McIntyre, A., Vincent, R. M., Perkins, A. C., & Spiller, R. C. (1997). Effect of bran, ispaghula, and inert plastic particles on gastric emptying and small bowel transit in humans: the role of physical factors. *Gut*, 40(2), 223-227.
692. McIntosh, K., Reed, D. E., Schneider, T., Dang, F., Keshteli, A. H., De Palma, G., . . . Vanner, S. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut*. 2017;66(7):1241-1251. doi:10.1136/gutjnl-2015-311339
693. McKenzie, Y. A., Thompson, J., Gulia, P., Lomer, M. C. E., & (IBS Dietetic Guideline Review Group on behalf of Gastroenterology Specialist Group of the British Dietetic Association). British Dietetic Association systematic review of systematic reviews and evidence-based practice guidelines for the use of probiotics in the management of irritable bowel syndrome in adults (2016 update). *Journal of human nutrition and dietetics*. 2016;29(5):576-592.
694. McKee, D. P., & Quigley, E. M. (1993). Intestinal motility in irritable bowel syndrome: Is IBS a motility disorder? Part 2. Motility of the small bowel, esophagus, stomach, and gall-ladder. *Digestive diseases and sciences*, 38, 1773-1782.
695. McNeil, N. I. (1984). The contribution of the large intestine to energy supplies in man. *The American journal of clinical nutrition*, 39(2), 338-342.
696. McOrist, A. L., Miller, R. B., Bird, A. R., Keogh, J. B., Noakes, M., Topping, D. L., & Conlon, M. A. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *The Journal of nutrition*. 2011;141(5), 883-889.

697. McRae, M. P. (2020). Effectiveness of fiber supplementation for constipation, weight loss, and supporting gastrointestinal function: a narrative review of meta-analyses. *Journal of chiropractic medicine*, 19(1), 58-64.
698. McRorie Jr, J. W., & McKeown, N. M. Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. *Journal of the Academy of Nutrition and Dietetics*. 2017;117(2), 251-264.
699. McRorie Jr, J. W., Fahey Jr, G. C., Gibb, R. D., & Chey, W. D. Laxative effects of wheat bran and psyllium: Resolving enduring misconceptions about fiber in treatment guidelines for chronic idiopathic constipation. *Journal of the American Association of Nurse Practitioners*. 2020;32(1), 15-23.
700. McRorie Jr, J. W. (2015). Evidence-based approach to fiber supplements and clinically meaningful health benefits, part 1: What to look for and how to recommend an effective fiber therapy. *Nutrition today*, 50(2), 82.
701. Mearin, F., Baró, E., Roset, M., Badía, X., Zárate, N., & Pérez, I. Clinical patterns over time in irritable bowel syndrome: symptom instability and severity variability. *The American journal of gastroenterology*. 2004;99(1):113.
702. Medawar, E., Haange, S. B., Rolle-Kampczyk, U., Engelmann, B., Dietrich, A., Thieleking, R., ... & Veronica Witte, A. (2021). Gut microbiota link dietary fiber intake and short-chain fatty acid metabolism with eating behavior. *Translational psychiatry*, 11(1), 500.
703. Menees, S., & Chey, W. (2018). The gut microbiome and irritable bowel syndrome. *F1000Research*, 7.
704. Metchnikoff, I. I. (2004). *The prolongation of life: optimistic studies*. Springer Publishing Company. <https://books.google.com/books?hl=en&lr=&id=dZPSCgAAQBAJ&oi=fnd&pg=PR7&ots=IU7iamX48-&sig=M0wMD5rol-79JC6PbxXh9za1-jQ#v=onepage&q&f=false>
705. MICOM. Retrieved June 18, 2020, from <https://github.com/micom-dev/q2-micom/blob/master/docs/README.md>
706. Min, Yang Won, and Poong-Lyul Rhee. "The role of microbiota on the gut immunology." *Clinical therapeutics*. 2015; 37(5):968-975.
707. Mitra, S., Dash, R., Al Nishan, A., Habiba, S. U., & Moon, I. S. (2023). Brain modulation by the gut microbiota: From disease to therapy. *Journal of Advanced Research*, 53, 153-173.
708. Miyauchi, S., Gopal, E., Fei, Y. J., & Ganapathy, V. (2004). Functional identification of SLC5A8, a tumor suppressor down-regulated in colon cancer, as a Na⁺-coupled transporter for short-chain fatty acids. *Journal of Biological Chemistry*, 279(14), 13293-13296.
709. Miyoshi, M., Sakaki, H., Usami, M., Iizuka, N., Shuno, K., Aoyama, M., & Usami, Y. Oral administration of tributyrin increases concentration of butyrate in the portal vein and prevents lipopolysaccharide-induced liver injury in rats. *Clinical nutrition*. 2011;30(2), 252-258.
710. Moens, F., Weckx, S., and De Vuyst, L. Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *International Journal of Food Microbiology*. 2016;231:76–85 doi: 10.1016/j.ijfoodmicro.2016.05.015
711. Moffett, J. R., & Namboodiri, M. A. (2003). Tryptophan and the immune response. *Immunology and cell biology*, 81(4), 247-265.
712. Mohan, R., Koebrick, C., Schildt, J., Mueller, M., Radke, M., & Blaut, M. Effects of *Bifidobacterium lactis* Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants. *Pediatric research*. 2008; 64(4):418.
713. Moll, J. M., Myers, P. N., Zhang, C., Eriksen, C., Wolf, J., Appelberg, K. S., ... & Hammarström, L. (2021). Gut microbiota perturbation in IgA deficiency is influenced by IgA-autoantibody status. *Gastroenterology*, 160(7), 2423-2434
714. Morris, G., Berk, M., Carvalho, A., Caso, J. R., Sanz, Y., Walder, K., & Maes, M. (2017). The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease. *Molecular neurobiology*, 54, 4432-4451.
715. Morrison, D. J., & Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*. 2016;7(3):189-200. doi:10.1080/19490976.2015.1134082
716. Mosińska, P., Fichna, J., & Storr, M. Inhibition of ileal bile acid transporter: an emerging therapeutic strategy for chronic idiopathic constipation. *World journal of gastroenterology: WJG*. 2015;21(24), 7436.

717. Martínez, I., Kim, J., Duffy, P. R., Schlegel, V. L., & Walter, J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PloS one*. 2010;5(11), e15046.
718. Mayer, E. A., & Tillisch, K. (2011). The brain-gut axis in abdominal pain syndromes. *Annual review of medicine*, 62, 381-396.
719. Min, Y. W., Rezaie, A., & Pimentel, M. (2022). Bile acid and gut microbiota in irritable bowel syndrome. *Journal of neurogastroenterology and motility*, 28(4), 549.
720. Moayyedi, P., Quigley, E. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., ... & Ford, A. C. (2014). The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. *American Journal of Gastroenterology*, 109(9), 1367-1374.
721. Moens, F., Weckx, S., and De Vuyst, L. Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *International Journal of Food Microbiology*. 2016;231:76–85 doi: 10.1016/j.ijfoodmicro.2016.05.015
722. Mohseni, A. H., Taghinezhad-S, S., & Fu, X. (2020). Gut microbiota-derived metabolites and colorectal cancer: New insights and updates. *Microbial Pathogenesis*, 149, 104569.
723. Monsbakken, K. W., Vandvik, P. O., & Farup, P. G. (2006). Perceived food intolerance in subjects with irritable bowel syndrome—etiology, prevalence and consequences. *European journal of clinical nutrition*, 60(5), 667-672.
724. Moya-Pérez, A., Perez-Villalba, A., Benítez-Páez, A., Campillo, I., & Sanz, Y. (2017). *Bifidobacterium* CECT 7765 modulates early stress-induced immune, neuroendocrine and behavioral alterations in mice. *Brain, behavior, and immunity*, 65, 43-56.
725. Mujagic, Z., Jonkers, D. M. A. E., Ludidi, S., Keszthelyi, D., Hesselink, M. A., Weerts, Z. Z. R. M., ... & Masclee, A. A. M. (2017). Biomarkers for visceral hypersensitivity in patients with irritable bowel syndrome. *Neurogastroenterology & Motility*, 29(12), e13137
726. Mujagic, Z., Tigchelaar, E. F., Zhernakova, A., Ludwig, T., Ramiro-Garcia, J., Baranska, A., ... & Jonkers, D. M. (2016). A novel biomarker panel for irritable bowel syndrome and the application in the general population. *Scientific reports*, 6(1), 26420.
727. Mohseni, A. H., Taghinezhad-S, S., & Fu, X. (2020). Gut microbiota-derived metabolites and colorectal cancer: New insights and updates. *Microbial Pathogenesis*, 149, 104569.
728. Mortenson, P. B., Hove, H., Clausen, M. R., & Holtug, T. K. (1991). Fermentation to short-chain fatty acids and lactate in human fecal batch cultures. *Scand. J. Gastroenterol*, 26, 1285-1294.
729. Mortensen, P. B., Andersen, J. R., Arffmann, S., & Krag, E. Short-chain fatty acids and the irritable bowel syndrome: the effect of wheat bran. *Scandinavian journal of gastroenterology*. 1987;22(2), 185-192.
730. Mortensen, P. B., Holtug, K., & Rasmussen, H. S. (1988). Short-chain fatty acid production from mono- and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans. *The Journal of nutrition*, 118(3), 321-325.
731. Moschen AR, Wieser V, Tilg H. Dietary Factors: Major Regulators of the Gut's Microbiota. *Gut Liver*. 2012;6(4):411-6.
732. Moshokoa, M. F., Daramola, M. O., Adeleke, R., Ndaba, B., & Roopnarain, A. (2023). Influence of environmental factors on *Enterococcus faecium* for production of succinic acid in a batch reactor: A preliminary investigation. *Materials Today: Proceedings*.
733. Muir, J. G., Yeow, E. G., Keogh, J., Pizzey, C., Bird, A. R., Sharpe, K., ... & Macrae, F. A. (2004). Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. *The American journal of clinical nutrition*, 79(6), 1020-1028.
734. Muir, J. G., Shepherd, S. J., Rosella, O., Rose, R., Barrett, J. S., & Gibson, P. R. Fructan and free fructose content of common Australian vegetables and fruit. *Journal of agricultural and food chemistry*. 2007;55(16): 6619-6627.
735. Muir, J. G., Rose, R., Rosella, O., Liels, K., Barrett, J. S., Shepherd, S. J., & Gibson, P. R. Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *Journal of agricultural and food chemistry*. 2009;57(2):554-565

736. Mujagic Z, Tigchelaar EF, Zhernakova A, et al. A novel biomarker panel for irritable bowel syndrome and the application in the general population. *Scientific Reports*. 2016;6:26420. doi:10.1038/srep26420.
737. Mukherji, A., Kobiita, A., Ye, T., & Chambon, P. (2013). Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell*, 153(4), 812-827.
738. Muller, P. A., Koscsó, B., Rajani, G. M., Stevanovic, K., Berres, M. L., Hashimoto, D., ... & Bogunovic, M. (2014). Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell*, 158(2), 300-313.
739. Müller, M., Hermes, G. D., Canfora, E. E., Smidt, H., Masclee, A. A., Zoetendal, E. G., & Blaak, E. E. (2020). Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 318(2), G361-G369.
740. Müller-Lissner, S. A. (1988). Effect of wheat bran on weight of stool and gastrointestinal transit time: a meta analysis. *Br Med J (Clin Res Ed)*, 296(6622), 615-617.]
741. Murali, A., Bhargava, A., & Wright, E. S. (2018). IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome*, 6(1), 1-14.
742. Nagana Gowda GA, Raftery D. Biomarker Discovery and Translation in Metabolomics. *Current Metabolomics*. 2013;1(3):227-240. doi:10.2174/2213235X113019990005.
743. Nagana Gowda GA, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D. Metabolomics-Based Methods for Early Disease Diagnostics: A Review. *Expert review of molecular diagnostics*. 2008;8(5):617-633. doi:10.1586/14737159.8.5.617.
744. Nagarajan, N., Morden, A., Bischof, D., King, E. A., Kosztowski, M., Wick, E. C., & Stein, E. M. (2015). The role of fiber supplementation in the treatment of irritable bowel syndrome: a systematic review and meta-analysis. *European journal of gastroenterology & hepatology*, 27(9), 1002-1010.
745. Nakov, R., Snegarova, V., Dimitrova-Yurukova, D., & Velikova, T. (2022). Biomarkers in Irritable Bowel Syndrome: biological rationale and diagnostic value. *Digestive Diseases*, 40(1), 23-32.
746. Nanayakkara, W. S., Skidmore, P. M., O'Brien, L., Wilkinson, T. J., & Geary, R. B. Efficacy of the low FODMAP diet for treating irritable bowel syndrome: the evidence to date. *Clinical and Experimental Gastroenterology*. 2016;9:131–142.
747. Natarajan, N., & Pluznick, J. L. (2014). From microbe to man: the role of microbial short chain fatty acid metabolites in host cell biology. *American Journal of Physiology-Cell Physiology*, 307(11), C979-C985.
748. Nelson, M. C., Morrison, H. G., Benjamino, J., Grim, S. L., & Graf, J. (2014). Analysis, optimization and verification of Illumina-generated 16S rRNA gene amplicon surveys. *PLoS One*, 9(4), e94249. doi:10.1371/journal.pone.0094249
749. Nepelska, M., De Wouters, T., Jacouton, E., Béguet-Crespel, F., Lapaque, N., Doré, J., ... & Blottière, H. M. (2017). Commensal gut bacteria modulate phosphorylation-dependent PPAR γ transcriptional activity in human intestinal epithelial cells. *Scientific Reports*, 7(1), 43199.
750. Neumann, A., Björck, L., & Frick, I. M. (2020). *Finnegoldia magna*, an anaerobic gram-positive bacterium of the normal human microbiota, induces inflammation by activating neutrophils. *Frontiers in microbiology*, 11, 505031.
751. Neumeister, A. (2003). Tryptophan depletion, serotonin, and depression: where do we stand?. *Psychopharmacology bulletin*, 37(4), 99-115.
752. Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science*, 336(6086), 1262-1267.
753. Niv, E., Halak, A., Tiomny, E., Yanai, H., Strul, H., Naftali, T., & Vaisman, N. (2016). Randomized clinical study: partially hydrolyzed guar gum (PHGG) versus placebo in the treatment of patients with irritable bowel syndrome. *Nutrition & metabolism*, 13(1), 10.
754. Nøhr, M. K., Egerod, K. L., Christiansen, S. H., Gille, A., Offermanns, S., Schwartz, T. W., & Møller, M. (2015). Expression of the short chain fatty acid receptor GPR41/FFAR3 in autonomic and somatic sensory ganglia. *Neuroscience*, 290, 126-137.
755. Nøhr, M. K., Pedersen, M. H., Gille, A., Egerod, K. L., Engelstoft, M. S., Husted, A. S., ... & Schwartz, T. W. (2013). GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in

enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology*, 154(10), 3552-3564.

756. Noronha, A., Modamio, J., Jarosz, Y., Guerard, E., Sompairac, N., Preciat, G., ... & Heinken, A. (2019). The Virtual Metabolic Human database: integrating human and gut microbiome metabolism with nutrition and disease. *Nucleic acids research*, 47(D1), D614-D624.
757. Noronha, A., Daníelsdóttir, A. D., Gawron, P., Jóhannsson, F., Jónsdóttir, S., Jarlsson, S., ... & Fleming, R. M. (2017). ReconMap: an interactive visualization of human metabolism. *Bioinformatics*, 33(4), 605-607.
758. Nozu, T., Miyagishi, S., Nozu, R., Takakusaki, K., & Okumura, T. (2019). Butyrate inhibits visceral allodynia and colonic hyperpermeability in rat models of irritable bowel syndrome. *Scientific reports*, 9(1), 1-13.
759. NW-MRC (n.d.). Assays and Fees. Retrieved March 2, 2020, from <https://nwmetabolomics.org/services/assays-fees/>
760. Obanda, D. N., Keenan, M. J., Page, R., Raggio, A. M., Taylor, C. M., Marx, B. D., ... & Husseneder, C. (2021). Gut microbiota composition and predicted microbial metabolic pathways of obesity prone and obesity resistant outbred Sprague-Dawley CD rats may account for differences in their phenotype. *Frontiers in Nutrition*, 8, 746515.
761. Oddsson, E., Rask-Madsen, J., & Krag, E. (1978). A secretory epithelium of the small intestine with increased sensitivity to bile acids in irritable bowel syndrome associated with diarrhoea. *Scandinavian journal of gastroenterology*, 13(4), 409-416
762. Odes, H. S., & Madar, Z. (1991). A double-blind trial of a celandin, aloe vera and psyllium laxative preparation in adult patients with constipation. *Digestion*, 49(2), 65-71.
763. Office of Disease Prevention and Health Promotion (n.d). Fiber. Retrieved February 15, 2020, from <https://www.healthypeople.gov/2020/About-Healthy-People/Development-Healthy-People-2030>
764. Ohata, A., Usami, M., & Miyoshi, M. (2005). Short-chain fatty acids alter tight junction permeability in intestinal monolayer cells via lipoxxygenase activation. *Nutrition*, 21(7-8), 838-847.
765. Ohara, K., Kiyotani, Y., Uchida, A., Nagasaka, R., Maehara, H., Kanemoto, S., ... & Ushio, H. (2011). Oral administration of γ -aminobutyric acid and γ -oryzanol prevents stress-induced hypoadiponectinemia. *Phytomedicine*, 18(8-9), 655-660.
766. Ohira, H., Fujioka, Y., Katagiri, C., Mamoto, R., Aoyama-Ishikawa, M., Amako, K., ... & Ikeda, M. (2013). Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. *Journal of atherosclerosis and thrombosis*, 20(5), 425-442.
767. Öhman, L., & Simren, M. (2007). New insights into the pathogenesis and pathophysiology of irritable bowel syndrome. *Digestive and liver disease*, 39(3), 201-215.
768. Öhman, L., & Simrén, M. (2010). Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nature reviews Gastroenterology & hepatology*, 7(3), 163-173.
769. Ohno, Y., Lee, J., Fusunyan, R. D., MacDermott, R. P., & Sanderson, I. R. (1997). Macrophage inflammatory protein-2: chromosomal regulation in rat small intestinal epithelial cells. *Proceedings of the National Academy of Sciences*, 94(19), 10279-10284.
770. Oka, P., Parr, H., Barberio, B., Black, C. J., Savarino, E. V., & Ford, A. C. (2020). Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *The lancet Gastroenterology & hepatology*, 5(10), 908-917.
771. O'Keefe, M., Jansen, C., Martin, L., Williams, M., Seemark, L., Staudacher, H. M., . . . Lomer, M. C. Long-term impact of the low-FODMAP diet on gastrointestinal symptoms, dietary intake, patient acceptability, and healthcare utilization in irritable bowel syndrome. *Neurogastroenterology & Motility*. 2018;30(1). doi:10.1111/nmo.13154
772. O'Keefe, S. J., Ou, J., DeLany, J. P., Curry, S., Zoetendal, E., Gaskins, H. R., & Gunn, S. Effect of fiber supplementation on the microbiota in critically ill patients. *World journal of gastrointestinal pathophysiology*. 2011;2(6), 138.
773. Ong, D. K., Mitchell, S. B., Barrett, J. S., Shepherd, S. J., Irving, P. M., Biesiekierski, J. R., . . . Muir, J. G. Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of

- symptoms in irritable bowel syndrome. *Journal of Gastroenterology and Hepatology*. 2010;25(8):1366-1373.
774. Okubo, T., Ishihara, N., Takahashi, H., Fujisawa, T., Kim, M., Yamamoto, T., & Mitsuoka, T. (1994). Effects of partially hydrolyzed guar gum intake on human intestinal microflora and its metabolism. *Bioscience, biotechnology, and biochemistry*, 58(8), 1364-1369.
 775. Oregon State University (n.d.). Linus Pauling Institute Micronutrient Information Center: Fiber. Retrieved 25, 2020, from website. <http://lpi.oregonstate.edu/mic/other-nutrients/fiber>
 776. Oregon State University (n.d.). Fiber. Retrieved February 15, 2020, from <https://lpi.oregonstate.edu/mic/other-nutrients/fiber>
 777. O'Riordan, K. J., Collins, M. K., Moloney, G. M., Knox, E. G., Aburto, M. R., Fülling, C., ... & Cryan, J. F. (2022). Short chain fatty acids: microbial metabolites for gut-brain axis signalling. *Molecular and Cellular Endocrinology*, 546, 111572.
 778. Ornstein, M. H., Littlewood, E. R., Baird, I. M., Fowler, J., North, W. R., & Cox, A. G. (1981). Are fibre supplements really necessary in diverticular disease of the colon? A controlled clinical trial. *Br Med J (Clin Res Ed)*, 282(6273), 1353-1356.
 779. Osadchiy, V., Martin, C. R., & Mayer, E. A. (2019). The gut–brain axis and the microbiome: mechanisms and clinical implications. *Clinical Gastroenterology and Hepatology*, 17(2), 322-332.
 780. O'Sullivan, A., Gibney, M. J., & Brennan, L. Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. *The American journal of clinical nutrition*. 2011;93(2), 314-321.
 781. Ott, S. J., Musfeldt, M., Timmis, K. N., Hampe, J., Wenderoth, D. F., & Schreiber, S. In vitro alterations of intestinal bacterial microbiota in fecal samples during storage. *Diagnostic microbiology and infectious disease*. 2004;50(4), 237-245.
 782. Palmas, V., Pisanu, S., Madau, V., Casula, E., Deledda, A., Cusano, R., ... & Velluzzi, F. (2021). Gut microbiota markers associated with obesity and overweight in Italian adults. *Scientific reports*, 11(1), 5532
 783. Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS biology*, 5(7), e177.
 784. Paschos, G. K., Baggs, J. E., Hogenesch, J. B., & FitzGerald, G. A. (2010). The role of clock genes in pharmacology. *Annual review of pharmacology and toxicology*, 50, 187-214.
 785. Palsson, O. S., Whitehead, W., Törnblom, H., Sperber, A. D., & Simren, M. (2020). Prevalence of Rome IV functional bowel disorders among adults in the United States, Canada, and the United Kingdom. *Gastroenterology*, 158(5), 1262-1273.
 786. Palsson, O. S., Baggish, J. S., Turner, M. J., & Whitehead, W. E. (2012). IBS patients show frequent fluctuations between loose/watery and hard/lumpy stools: implications for treatment. *The American journal of gastroenterology*, 107(2).
 787. Pan, Z., & Raftery, D. Comparing and combining NMR spectroscopy and mass spectrometry in metabolomics. *Analytical and bioanalytical chemistry*. 2007;387(2), 525-527.
 788. Panda, S., Hogenesch, J. B., & Kay, S. A. (2002). Circadian rhythms from flies to human. *Nature*, 417(6886), 329-335.
 789. Paripati, N., Nesi, L., Sterrett, J. D., Dawud, L. A. M., Kessler, L. R., Lowry, C. A., ... & Phadtare, S. (2023). Gut Microbiome and Lipidome Signatures in Irritable Bowel Syndrome Patients from a Low-Income, Food-Desert Area: A Pilot Study. *Microorganisms*, 11(10), 2503.
 790. Park, J., Kim, M., Kang, S. G., Jannasch, A. H., Cooper, B., Patterson, J., & Kim, C. H. (2015). Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. *Mucosal immunology*, 8(1), 80-93.
 791. Parker, B. J., Wearsch, P. A., Veloo, A. C., & Rodriguez-Palacios, A. (2020). The genus *Alistipes*: gut bacteria with emerging implications to inflammation, cancer, and mental health. *Frontiers in immunology*, 11, 906.
 792. Parkar, S. G., Kalsbeek, A., & Cheeseman, J. F. (2019). Potential role for the gut microbiota in modulating host circadian rhythms and metabolic health. *Microorganisms*, 7(2), 41.

793. Parker, T. J., Naylor, S. J., Riordan, A. M., & Hunter, J. O. (1995). Management of patients with food intolerance in irritable bowel syndrome: the development and use of an exclusion diet. *Journal of Human Nutrition and Dietetics*, 8(3), 159-166.
794. Parthasarathy, G., Chen, J., Chen, X., Chia, N., O'Connor, H. M., Wolf, P. G., ... & Bharucha, A. E. (2016). Relationship between microbiota of the colonic mucosa vs feces and symptoms, colonic transit, and methane production in female patients with chronic constipation. *Gastroenterology*, 150(2), 367-379.
795. Parisi, G. C., Zilli, M., Miani, M. P., Carrara, M., Bottona, E., Verdianelli, G., ... & Tonon, A. High-fiber diet supplementation in patients with irritable bowel syndrome (IBS): a multicenter, randomized, open trial comparison between wheat bran diet and partially hydrolyzed guar gum (PHGG). *Digestive diseases and sciences*. 2002;47(8), 1697-1704.
796. Parisi, G., Bottona, E., Carrara, M., Cardin, F., Faedo, A., Goldin, D., ... & Zilli, M. Treatment effects of partially hydrolyzed guar gum on symptoms and quality of life of patients with irritable bowel syndrome. A multicenter randomized open trial. *Digestive diseases and sciences*. 2005;50(6), 1107-1112.
797. Parrish, C.R., Thomas, J.R., Nanda, R. and Shu, L.H. A FODMAP Diet Update: Craze or Credible. Retrieved February 22, 2018, from https://med.virginia.edu/ginutrition/wp-content/uploads/sites/199/2014/06/Parrish_Dec_12.pdf. 2012.
798. Patnode, M. L., Beller, Z. W., Han, N. D., Cheng, J., Peters, S. L., Terrapon, N., ... & Meynier, A. Interspecies competition impacts targeted manipulation of human gut bacteria by fiber-derived glycans. *Cell*. 2019;179(1), 59-73.
799. Patrick, P. G., Gohman, S. M., Marx, S. C., DeLEGGE, M. H., & Greenberg, N. A. (1998). Effect of supplements of partially hydrolyzed guar gum on the occurrence of constipation and use of laxative agents. *Journal of the Academy of Nutrition and Dietetics*, 98(8), 912
800. Pattanaik, A. K., Kumar, S., & Rashmi, H. M. (2022). Probiotics, prebiotics, eubiotics and synbiotics for human and animal health and mitigation of antimicrobial resistance. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 43(1), 90-97.
801. Payler, D. K., Pomare, E. W., Heaton, K. W., & Harvey, R. F. The effect of wheat bran on intestinal transit. *Gut*. 1975;16(3), 209-213.
802. Pedersen, H. K., Gudmundsdottir, V., Nielsen, H. B., Hyotylainen, T., Nielsen, T., Jensen, B. A., ... & Le Chatelier, E. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*. 2016;535(7612), 376-381.
803. Pereira, F. L., Soares, S. C., Dorella, F. A., Leal, C. A., & Figueiredo, H. C. (2016). Evaluating the efficacy of the new Ion PGM Hi-Q Sequencing Kit applied to bacterial genomes. *Genomics*, 107(5), 189-198. doi:10.1016/j.ygeno.2016.03.004
804. Peron, G., Meroño, T., Gargari, G., Hidalgo-Liberona, N., Miñarro, A., Lozano, E. V., ... & Andrés-Lacueva, C. (2022). A Polyphenol-Rich Diet Increases the Gut Microbiota Metabolite Indole 3-Propionic Acid in Older Adults with Preserved Kidney Function. *Molecular Nutrition & Food Research*, 66(21), 2100349.
805. Pellock, S. J., & Redinbo, M. R. (2017). Glucuronides in the gut: Sugar-driven symbioses between microbe and host. *Journal of Biological Chemistry*, 292(21), 8569-8576.
806. Peter, J., Fournier, C., Durdevic, M., Knoblich, L., Keip, B., Dejacó, C., ... & Moser, G. (2018). A microbial signature of psychological distress in irritable bowel syndrome. *Psychosomatic medicine*, 80(8), 698.
807. Phillips, G. O., & Cui, S. W. (2011). An introduction: Evolution and finalisation of the regulatory definition of dietary fibre.
808. Philpott, H., Nandurkar, S., Lubel, J., & Gibson, P. R. (2015). Food, fibre, bile acids and the pelvic floor: An integrated low risk low cost approach to managing irritable bowel syndrome. *World Journal of Gastroenterology: WJG*, 21(40), 11379.
809. Phua, L. C., Koh, P. K., Cheah, P. Y., Ho, H. K., & Chan, E. C. Y. Global gas chromatography/time-of-flight mass spectrometry (GC/TOFMS)-based metabonomic profiling of lyophilized human feces. *Journal of Chromatography B*. 2013;937, 103-113.
810. Pier Luca Guarino, M., Altomare, A., Emerenziani, S., Di Rosa, C., Ribolsi, M., Balestrieri, P., ... & Cicala, M. Mechanisms of Action of Prebiotics and Their Effects on Gastro-Intestinal Disorders in Adults. *Nutrients*. 2020;12(4), 1037.

811. Pierantonelli, I., & Svegliati-Baroni, G. (2019). Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH. *Transplantation*, 103(1), e1-e13.
812. Pietzke, M., Meiser, J., & Vazquez, A. (2020). Formate metabolism in health and disease. *Molecular metabolism*, 33, 23-37
813. Pilichiewicz, A. N., Horowitz, M., Holtmann, G. J., Talley, N. J., & Feinle-Bisset, C. Relationship between symptoms and dietary patterns in patients with functional dyspepsia. *Clinical Gastroenterology and Hepatology*. 2009;7(3), 317-322.
814. Pimentel, M., Lembo, A., Chey, W. D., Zakko, S., Ringel, Y., Yu, J., ... & Forbes, W. P. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *New England Journal of Medicine*. 2011;364(1), 22-32.
815. Pimentel, M., Chow, E. J., & Lin, H. C. (2003). Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. *The American Journal of Gastroenterology*, 98(2), 412-419.
816. Pinart, M., Dötsch, A., Schlicht, K., Laudes, M., Bouwman, J., Forslund, S. K., ... & Nimptsch, K. (2021). Gut microbiome composition in obese and non-obese persons: a systematic review and meta-analysis. *Nutrients*, 14(1), 12.
817. Pinn, D. M., Aroniadis, O. C., & Brandt, L. J. (2014). Is fecal microbiota transplantation the answer for irritable bowel syndrome? A single-center experience. *Official Journal of the American College of Gastroenterology | ACG*, 109(11), 1831-1832.
818. Pinu, F. R., Beale, D. J., Paten, A. M., Kouremenos, K., Swarup, S., Schirra, H. J., & Wishart, D. (2019). Systems biology and multi-omics integration: Viewpoints from the metabolomics research community. *Metabolites*, 9(4), 76.
819. Pirozzi, Claudio, Lorena Coretti, Nicola Opallo, Maria Bove, Chiara Annunziata, Federica Comella, Luigia Turco et al. "Palmitoylethanolamide counteracts high-fat diet-induced gut dysfunction by reprogramming microbiota composition and affecting tryptophan metabolism." *Frontiers in Nutrition* 10 (2023): 1143004.
820. Pittayanon, R., Lau, J. T., Yuan, Y., Leontiadis, G. I., Tse, F., Surette, M., & Moayyedi, P. (2019). Gut microbiota in patients with irritable bowel syndrome—a systematic review. *Gastroenterology*, 157(1), 97-108.
821. Pituch, A., Walkowiak, J., & Banaszekiewicz, A. Butyric acid in functional constipation. *Przegląd gastroenterologiczny*. 2013;8(5), 295.
822. Place, R. F., Noonan, E. J., & Giardina, C. (2005). HDAC inhibition prevents NF-κB activation by suppressing proteasome activity: down-regulation of proteasome subunit expression stabilizes IκBα. *Biochemical pharmacology*, 70(3), 394-406.
823. Polymeros, D., Beintaris, I., Gaglia, A., Karamanolis, G., Papanikolaou, I. S., Dimitriadis, G., & Triantafyllou, K. Partially hydrolyzed guar gum accelerates colonic transit time and improves symptoms in adults with chronic constipation. *Digestive diseases and sciences*. 2014;59(9), 2207-2214.
824. Pomare, E. W., Branch, W. J., & Cummings, J. H. Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. *The Journal of clinical investigation*. 1985;75(5), 1448-1454.
825. Ponnusamy, K., Choi, J. N., Kim, J., Lee, S. Y., & Lee, C. H. Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *Journal of medical microbiology*. 2011;60(Pt 6), 817.
826. Posserud, I., Stotzer, P. O., Björnsson, E. S., Abrahamsson, H., & Simrén, M. (2007). Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut*, 56(6), 802-808.
827. Postler, Thomas Siegmund, and Sankar Ghosh. "Understanding the holobiont: how microbial metabolites affect human health and shape the immune system." *Cell metabolism* 26, no. 1 (2017): 110-130.
828. Poussin, C., Sierro, N., Boué, S., Battey, J., Scotti, E., Belcastro, V., ... & Hoeng, J. Interrogating the microbiome: experimental and computational considerations in support of study reproducibility. *Drug discovery today*. 2018;23(9), 1644-1657.

829. Pouteau, E., Nguyen, P., Ballèvre, O., & Krempf, M. (2003). Production rates and metabolism of short-chain fatty acids in the colon and whole body using stable isotopes. *Proceedings of the Nutrition Society*, 62(1), 87-93.
830. Pouteau, E., Vahedi, K., Messing, B., Flourié, B., Nguyen, P., Darmaun, D., & Krempf, M. (1998). Production rate of acetate during colonic fermentation of lactulose: a stable-isotope study in humans,—. *The American journal of clinical nutrition*, 68(6), 1276-1283.
831. Powell, N., Walker, M. M., & Talley, N. J. The mucosal immune system: master regulator of bidirectional gut–brain communications. *Nature Reviews Gastroenterology & Hepatology*. 2017; 14(3), 143.
832. Pozuelo, M., Panda, S., Santiago, A., Mendez, S., Accarino, A., Santos, J., . . . Manichanh, C. Reduction of butyrate- and methane-producing microorganisms in patients with Irritable Bowel Syndrome. *Science Reports*. 2015;5:12693. doi:10.1038/srep12693.
833. Prasad, K. N. (1980). Butyric acid: a small fatty acid with diverse biological functions. *Life sciences*, 27(15), 1351-1358.
834. Prasad, C., & Sundar, I. K. (2023). Regulating the Clock: REV-ERB Agonists as Promising Therapeutic Agents.
835. Primec, M., Mičetić-Turk, D., & Langerholc, T. Analysis of short-chain fatty acids in human feces: A scoping review. *Analytical biochemistry*. 2017;526, 9-21.
836. Prior, A., & Whorwell, P. J. Double blind study of ispaghula in irritable bowel syndrome. *Gut*. 1987;28(11), 1510-1513.
837. Principi, N., Cozzali, R., Farinelli, E., Brusaferrro, A., & Esposito, S. Gut dysbiosis and irritable bowel syndrome: The potential role of probiotics. *Journal of Infection*. 2018;76(2):111-120. doi:10.1016/j.jinf.2017.12.013
838. Ponnusamy, K., Choi, J. N., Kim, J., Lee, S. Y., & Lee, C. H. (2011). Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *Journal of medical microbiology*, 60(Pt 6), 817.
839. Pouteau, E., Nguyen, P., Ballèvre, O., & Krempf, M. Production rates and metabolism of short-chain fatty acids in the colon and whole body using stable isotopes. *Proceedings of the Nutrition Society*. 2003;62(1), 87-93.
840. Powell, N., Walker, M. M., & Talley, N. J. The mucosal immune system: master regulator of bidirectional gut–brain communications. *Nature Reviews Gastroenterology & Hepatology*. 2017;14(3), 143.
841. Pozuelo, M., Panda, S., Santiago, A., Mendez, S., Accarino, A., Santos, J., ... & Manichanh, C. (2015). Reduction of butyrate-and methane-producing microorganisms in patients with Irritable Bowel Syndrome. *Scientific reports*, 5(1), 1-12.
842. Priyadarshini, M., Thomas, A., Reisetter, A. C., Scholtens, D. M., Wolever, T. M., Josefson, J. L., & Layden, B. T. (2014). Maternal short-chain fatty acids are associated with metabolic parameters in mothers and newborns. *Translational Research*, 164(2), 153-157.
843. Procházková, N., Falony, G., Dragsted, L. O., Licht, T. R., Raes, J., & Roager, H. M. (2023). Advancing human gut microbiota research by considering gut transit time. *Gut*, 72(1), 180-191.
844. Przekop, P., Haviland, M. G., Zhao, Y., Oda, K., Morton, K. R., & Fraser, G. E. Self-reported physical health, mental health, and comorbid diseases among women with irritable bowel syndrome, fibromyalgia, or both compared with healthy control respondents. *The Journal of the American Osteopathic Association*. 2012;112(11):726-735.
845. Pulliam, S. R., Pellom Jr, S. T., Shanker, A., & Adunyah, S. E. (2016). Butyrate regulates the expression of inflammatory and chemotactic cytokines in human acute leukemic cells during apoptosis. *Cytokine*, 84, 74-87.
846. Putignani, L., Del Chierico, F., Vernocchi, P., Cicala, M., Cucchiara, S., Dallapiccola, B., & Dysbiotrack Study Group. (2016). Gut microbiota dysbiosis as risk and premorbid factors of IBD and IBS along the childhood–adulthood transition. *Inflammatory bowel diseases*, 22(2), 487-504.
847. Pylro, V. S., Roesch, L. F. W., Morais, D. K., Clark, I. M., Hirsch, P. R., & Tótola, M. R. (2014). Data analysis for 16S microbial profiling from different benchtop sequencing platforms. *Journal of microbiological methods*, 107, 30-37.

848. Qi, Q., Li, J., Yu, B., Moon, J. Y., Chai, J. C., Merino, J., ... & Kaplan, R. C. (2022). Host and gut microbial tryptophan metabolism and type 2 diabetes: An integrative analysis of host genetics, diet, gut microbiome and circulating metabolites in cohort studies. *Gut*, 71(6), 1095-1105.
849. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41(D1), D590-D596.
850. Quigley, E. M. M. (2018). The Gut-Brain Axis and the Microbiome: Clues to Pathophysiology and Opportunities for Novel Management Strategies in Irritable Bowel Syndrome (IBS). *Journal of Clinical Medicine*, 7(1). doi:10.3390/jcm7010006
851. Quigley, B. M., Sova, C. C., Brenner, D. M., Keefer, L. A., Sitrin, M. D., Radziwon, C. D., . . . Lackner, J. M. (Can't Get No) Patient Satisfaction: The Predictive Power of Demographic, GI, and Psychological Factors in IBS Patients. *Journal of Clinical Gastroenterology*. 2017. doi:10.1097/mcg.0000000000000906
852. Quigley, E. M., Abdel-Hamid, H., Barbara, G., Bhatia, S. J., Boeckxstaens, G., De Giorgio, R., ... & Gwee, K. A. (2012). A global perspective on irritable bowel syndrome: a consensus statement of the World Gastroenterology Organisation Summit Task Force on irritable bowel syndrome. *Journal of clinical gastroenterology*, 46(5), 356-366.
853. Quigley, E. M. M. (2011). The enteric microbiota in the pathogenesis and management of constipation. *Best practice & research clinical gastroenterology*, 25(1), 119-126.
854. Quigley, E. M. (2011). Microflora modulation of motility. *Journal of neurogastroenterology and motility*, 17(2), 140.
855. Quigley, E. M. (2009). Prebiotics for irritable bowel syndrome. *Expert Review of Gastroenterology & Hepatology*, 3(5), 487-492.
856. Quigley, E. M. M., & Flourie, B. (2007). Probiotics and irritable bowel syndrome: a rationale for their use and an assessment of the evidence to date. *Neurogastroenterology & Motility*, 19(3), 166-172.
857. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., ... & Wang, J. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *nature*, 464(7285), 59-65.
858. Rachek, S., Nikpoor, N., Gómez del Pulgar, E. M., Gonzaga, A., Sanz, Y., & Tompkins, T. A. (2021). Complete genome sequence of *phascolarctobacterium faecium* G 104, isolated from the stools of a healthy lean donor. *Microbiology Resource Announcements*, 10(4), 10-1128.
859. Radwanski, E. R., & Last, R. L. (1995). Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. *The Plant Cell*, 7(7), 921.
860. Raftery, D. (2016). *Mass Spectrometry in Metabolomics*. Humana.
861. Rahat-Rozenbloom, S., Fernandes, J., Cheng, J., Gloor, G. B., & Wolever, T. M. (2017). The acute effects of inulin and resistant starch on postprandial serum short-chain fatty acids and second-meal glycemic response in lean and overweight humans. *European journal of clinical nutrition*, 71(2), 227-233.
862. Rajilic-Stojanovic, M., Jonkers, D. M., Salonen, A., Hanevik, K., Raes, J., Jalanka, J., . . . Penders, J. (2015). Intestinal microbiota and diet in IBS: causes, consequences, or epiphenomena? *Am J Gastroenterol*, 110(2), 278-287. doi:10.1038/ajg.2014.427
863. Rajoka, M. S. R., Shi, J., Mehwish, H. M., Zhu, J., Li, Q., Shao, D., ... & Yang, H. (2017). Interaction between diet composition and gut microbiota and its impact on gastrointestinal tract health. *Food Science and Human Wellness*, 6(3), 121-130.
864. Rana SV, Sharma S, Sinha SK, Parsad KK, Malik A, Singh K. Pro- inflammatory and anti-inflammatory cytokine response in diarrhoea- predominant irritable bowel syndrome patients. *Tropical Gastroenterology*. 2012;33:251–256.
865. Rangel, I., Sundin, J., Fuentes, S., Repsilber, D., De Vos, W. M., & Brummer, R. J. (2015). The relationship between faecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients and healthy subjects. *Alimentary pharmacology & therapeutics*, 42(10), 1211-1221.
866. Ranhotra, H. S. (2017). Gut Microbiota and Host Nuclear Receptors Signalling. *Nuclear Receptor Research*, 4.
867. Ranjan, R., Rani, A., Metwally, A., McGee, H. S., & Perkins, D. L. (2016). Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem Biophys Res Commun*, 469(4), 967-977. doi:10.1016/j.bbrc.2015.12.083

868. Rao, S. S., Rattanakovit, K., & Patcharatrakul, T. (2016). Diagnosis and management of chronic constipation in adults. *Nature Reviews gastroenterology & hepatology*, 13(5), 295.
869. Rao, S. S. C., Yu, S., & Fedewa, A. (2015). Systematic review: dietary fibre and FODMAP-restricted diet in the management of constipation and irritable bowel syndrome. *Alimentary pharmacology & therapeutics*, 41(12), 1256-1270.
870. Ray, S., & Reddy, A. B. (2016). Cross-talk between circadian clocks, sleep-wake cycles, and metabolic networks: Dispelling the darkness. *Bioessays*, 38(4), 394-405.
871. Rivière, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Frontiers in microbiology*. 2016;7, 979.
872. Rea, K., Dinan, T. G., & Cryan, J. F. The microbiome: a key regulator of stress and neuroinflammation. *Neurobiology of stress*. 2016;4, 23-33.
873. Reigstad, C. S., Salmonson, C. E., Rainey III, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., ... & Kashyap, P. C. (2015). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, 29(4), 1395.
874. Rajilić-Stojanović, M., Jonkers, D. M., Salonen, A., Hanevik, K., Raes, J., Jalanka, J., ... & Philippou, E. Intestinal microbiota and diet in IBS: causes, consequences, or epiphenomena? *The American journal of gastroenterology*. 2015;110(2):278.
875. Rajilić-Stojanović, M., Biagi, E., Heilig, H. G., Kajander, K., Kekkonen, R. A., Tims, S., & de Vos, W. M. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology*. 2011;141(5), 1792-1801.
876. Rea, K., Dinan, T. G., & Cryan, J. F. The microbiome: a key regulator of stress and neuroinflammation. *Neurobiology of Stress*. 2016; 4: 23–33.
877. Reed, T. T. (2011). Lipid peroxidation and neurodegenerative disease. *Free Radical Biology and Medicine*, 51(7), 1302-1319
878. Reese, S. E., Archer, K. J., Therneau, T. M., Atkinson, E. J., Vachon, C. M., de Andrade, M., Kocher, J. P., & Eckel-Passow, J. E. (2013). A new statistic for identifying batch effects in high-throughput genomic data that uses guided principal component analysis. *Bioinformatics (Oxford, England)*, 29(22), 2877–2883. <https://doi.org/10.1093/bioinformatics/btt480>
879. Rees, G., Davies, J., Thompson, R., Parker, M., & Liepins, P. Randomised-controlled trial of a fibre supplement on the symptoms of irritable bowel syndrome. *The journal of the Royal Society for the Promotion of Health*. 2005;125(1), 30-34.
880. Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., Leitch, C. M., Scott, K. P., ... & Louis, P. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *The ISME journal*, 8(6), 1323-1335.
881. Reichardt, N., Vollmer, M., Holtrop, G., Farquharson, F. M., Wefers, D., Bunzel, M., ... & Preston, T. (2018). Specific substrate-driven changes in human faecal microbiota composition contrast with functional redundancy in short-chain fatty acid production. *The ISME journal*, 12(2), 610-622.
882. Reigstad, C. S., Salmonson, C. E., Rainey III, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., ... & Kashyap, P. C. (2015). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, 29(4), 1395.
883. Relman, D. A. Microbiology: learning about who we are. *Nature*. 2012;486(7402), 194.
884. Reynolds, A., Mann, J., Cummings, J., Winter, N., Mete, E., & Te Morenga, L. (2019). Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *The Lancet*, 393(10170), 434-445.
885. Ribeiro, S. C., Domingos-Lopes, M. F., Stanton, C., Ross, R. P., & Silva, C. C. (2018). Production of-aminobutyric acid (GABA) by *Lactobacillus otakiensis* and other *Lactobacillus* sp. isolated from traditional Pico cheese. *International Journal of Dairy Technology*, 71(4), 1012-1017.
886. Ridlon, J. M. (2008). Enzymology and Molecular Biology of Bile Acid 7alpha-and 7beta-dehydroxylation by the Intestinal Bacteria *Clostridium Scindens* and *Clostridium Hylemonae*.

887. Ringel-Kulka, T., Choi, C. H., Temas, D., Kim, A., Maier, D. M., Scott, K., ... & Ringel, Y. Altered colonic bacterial fermentation as a potential pathophysiological factor in irritable bowel syndrome. *The American journal of gastroenterology*. 2015;110(9), 1339.
888. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilán CG, Salazar N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Frontiers in Microbiology*. 2016;7:185. doi:10.3389/fmicb.2016.00185
889. Rios-Covian, D., González, S., Nogacka, A. M., Arbolea, S., Salazar, N., Gueimonde, M., & de Los Reyes-Gavilán, C. G. (2020). An overview on fecal branched short-chain fatty acids along human life and as related with body mass index: associated dietary and anthropometric factors. *Frontiers in microbiology*, 11, 973.
890. Rizzello, F., Ricci, C., Scandella, M., Cavazza, E., Giovanardi, E., Valerii, M. C., ... & Spisni, E. (2018). Dietary geraniol ameliorates intestinal dysbiosis and relieves symptoms in irritable bowel syndrome patients: A pilot study. *BMC complementary and alternative medicine*, 18, 1-11.
891. Ritchie, J. A., & Truelove, S. C. (1979). Treatment of irritable bowel syndrome with lorazepam, hyoscine butylbromide, and ispaghula husk. *Br Med J*, 1(6160), 376-378.
892. Roager, H. M., & Licht, T. R. (2018). Microbial tryptophan catabolites in health and disease. *Nature communications*, 9(1), 3294.
893. Roberfroid, M., Gibson, G., Hoyles, L., McCartney, A., Rastall, R., Rowland, I., . . . Meheust, A. Prebiotic effects: Metabolic and health benefits. *British Journal of Nutrition*. 2010;104(S2): S1-S63. doi:10.1017/S0007114510003363
894. Roberts, L. D., Souza, A. L., Gerszten, R. E., & Clish, C. B. Targeted metabolomics. *Current protocols in molecular biology*. 2012;98(1), 30-2.
895. Robertson, M. D., Bickerton, A. S., Dennis, A. L., Vidal, H., & Frayn, K. N. (2005). Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism—. *The American journal of clinical nutrition*, 82(3), 559-567.
896. Rodiño-Janeiro, B. K., Vicario, M., Alonso-Cotoner, C., Pascua-García, R., & Santos, J. A review of microbiota and irritable bowel syndrome: future in therapies. *Advances in therapy*. 2018;35(3), 289-310.
897. Rogers, A. C., McDermott, F. D., Mohan, H. M., O'Connell, P. R., Winter, D. C., & Baird, A. W. The effects of polyamines on human colonic mucosal function. *European journal of pharmacology*. 2015;764:157-163.
898. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. *Hrbek T, ed. PeerJ*. 2016;4:e2584. doi:10.7717/peerj.2584.
899. Rohr, M. W., Narasimhulu, C. A., Rudeski-Rohr, T. A., & Parthasarathy, S. (2020). Negative effects of a high-fat diet on intestinal permeability: a review. *Advances in Nutrition*. 2020;11(1), 77-91.
900. Rojo D, Méndez-García C, Raczowska BA, et al. Exploring the human microbiome from multiple perspectives: factors altering its composition and function. *FEMS Microbiology Reviews*. 2017;41(4):453-478. doi:10.1093/femsre/fuw046.
901. ROI, R. (2011). Severity in Irritable Bowel Syndrome: A Rome Foundation Working Team Report.
902. Romano, C., Comito, D., Famiani, A., Calamarà, S., & Loddo, I. Partially hydrolyzed guar gum in pediatric functional abdominal pain. *World Journal of Gastroenterology*. 2013;19(2), 235.
903. Rombeau, J. L. (1995). Short-chain fatty acids and intestinal surgery: rationale and clinical implication. *Physiology and Clinical Aspects of Short-Chain Fatty Acids*, 401-425.
904. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nature reviews Immunology*. 2016;16(6):341-352. doi:10.1038/nri.2016.42.
905. Ropert, A., Cherbut, C., Roze, C., Le Quellec, A., Holst, J. J., Fu-Cheng, X., ... & Galmiche, J. P. Colonic fermentation and proximal gastric tone in humans. *Gastroenterology*. 1996;111(2), 289-296.
906. Ruiz-Perez, F., & Nataro, J. P. (2014). Bacterial serine proteases secreted by the autotransporter pathway: classification, specificity, and role in virulence. *Cellular and molecular life sciences*, 71, 745-770.
907. Ruppin, H., Bar-Meir, S., Soergel, K. H., Wood, C. M., & Schmitt, M. G. Absorption of short-chain fatty acids by the colon. *Gastroenterology*. 1980;78(6), 1500-1507

908. Russell, J. P., Mohammadi, E., Ligon, C., Latorre, R., Johnson, A. C., Hoang, B., ... & Greenwood-Van Meerveld, B. (2019). Enteric RET inhibition attenuates gastrointestinal secretion and motility via cholinergic signaling in rat colonic mucosal preparations. *Neurogastroenterology & Motility*, 31(4), e13479.
909. Russo, M., Martinelli, M., Sciorio, E., Botta, C., Miele, E., Vallone, G., & Staiano, A. (2013). Stool consistency, but not frequency, correlates with total gastrointestinal transit time in children. *The Journal of pediatrics*, 162(6), 1188-1192.
910. Rutten, J. M., Korterink, J. J., Venmans, L. M., Benninga, M. A., & Tabbers, M. M. (2015). Nonpharmacological treatment of functional abdominal pain disorders: a systematic review. *Pediatrics*, 135(3), 522-535.
911. Rutten, J. M., Vlieger, A. M., Frankenhuis, C., George, E. K., Groeneweg, M., Norbruis, O. F., ... Benninga, M. A. (2014). Gut-directed hypnotherapy in children with irritable bowel syndrome or functional abdominal pain (syndrome): a randomized controlled trial on self exercises at home using CD versus individual therapy by qualified therapists. *BMC Pediatrics*, 14, 140.
<http://doi.org.offcampus.lib.washington.edu/10.1186/1471-2431-14-140>
912. Saini, R., Jaskolski, M., & Davis, S. J. (2019). Circadian oscillator proteins across the kingdoms of life: structural aspects. *BMC biology*, 17, 1-39.
913. Saksena S, Theegala S, Bansal N, et al. Mechanisms underlying modulation of monocarboxylate transporter 1 (MCT1) by somatostatin in human intestinal epithelial cells. *American Journal of Physiology - Gastrointestinal and Liver Physiology*. 2009;297(5): G878-G885. doi:10.1152/ajpgi.00283.2009
914. Salonen, A., de Vos, W. M., & Palva, A. (2010). Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. *Microbiology*, 156(11), 3205-3215
915. Sato, T. K., Panda, S., Miraglia, L. J., Reyes, T. M., Rudic, R. D., McNamara, P., ... & Hogenesch, J. B. (2004). A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron*, 43(4), 527-537.
916. Savignac, H. M., Corona, G., Mills, H., Chen, L., Spencer, J. P., Tzortzis, G., & Burnet, P. W. (2013). Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. *Neurochemistry international*, 63(8), 756-764.
917. Salem, A. E., Singh, R., Ayoub, Y. K., Khairy, A. M., & Mullin, G. E. The gut microbiome and irritable bowel syndrome: State of art review. *Arab Journal of Gastroenterology*. 2018.Sawicki, C. M., Livingston, K. A., Obin, M., Roberts, S. B., Chung, M., & McKeown, N. M. (2017). Dietary fiber and the human gut microbiota: application of evidence mapping methodology. *Nutrients*, 9(2), 125.
918. Schumann, D., Klose, P., Lauche, R., Dobos, G., Langhorst, J., & Cramer, H. (2018). Low fermentable, oligo-, di-, mono-saccharides and polyol diet in the treatment of irritable bowel syndrome: A systematic review and meta-analysis. *Nutrition*, 45, 24-31.
919. Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological reviews*.
920. Sanger, G. J. (2008). 5-hydroxytryptamine and the gastrointestinal tract: where next?. *Trends in pharmacological sciences*, 29(9), 465-471.
921. Salipante, S. J., Kawashima, T., Rosenthal, C., Hoogestraat, D. R., Cummings, L. A., Sengupta, D. J., . . . Hoffman, N. G. (2014). Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. *Appl Environ Microbiol*, 80(24), 7583-7591. doi:10.1128/aem.02206-14
922. Saito, Y. A., Locke III, G. R., Weaver, A. L., Zinsmeister, A. R., & Talley, N. J. (2005). Diet and functional gastrointestinal disorders: a population-based case-control study. *American Journal of Gastroenterology*. 2005;100(12), 2743-2748.
923. Sanchez, S., Rodríguez-Sanoja, R., Ramos, A., & Demain, A. L. (2018). Our microbes not only produce antibiotics, they also overproduce amino acids. *The Journal of antibiotics*, 71(1), 26-36.
924. Santiago, A., Panda, S., Mengels, G., Martinez, X., Azpiroz, F., Dore, J., ... & Manichanh, C. Processing faecal samples: a step forward for standards in microbial community analysis. *BMC microbiology*. 2014;14(1), 112.
925. Sauerschnig, C., Doppler, M., Bueschl, C., & Schuhmacher, R. (2018). Methanol generates numerous artifacts during sample extraction and storage of extracts in metabolomics research. *Metabolites*, 8(1), 1.

926. Saulnier, D. M., Riehle, K., Mistretta, T. A., Diaz, M. A., Mandal, D., Raza, S., ... & Versalovic, J. (2011). Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology*, 141(5), 1782-1791.
927. Savović, J., Weeks, L., Sterne, J. A., Turner, L., Altman, D. G., Moher, D., & Higgins, J. P. (2014). Evaluation of the Cochrane Collaboration's tool for assessing the risk of bias in randomized trials: focus groups, online survey, proposed recommendations and their implementation. *Systematic reviews*, 3(1), 37.
928. Scharlau, D., Borowicki, A., Habermann, N., Hofmann, T., Klenow, S., Miene, C., ... & Glei, M. (2009). Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutation Research/Reviews in Mutation Research*, 682(1), 39-53.
929. Schaefer, R., Klose, P., Moser, G., & Häuser, W. (2014). Efficacy, tolerability, and safety of hypnosis in adult irritable bowel syndrome: systematic review and meta-analysis. *Psychosomatic medicine*, 76(5), 389-398.
930. Shaikh, S. D., Sun, N., Canakis, A., Park, W. Y., & Weber, H. C. (2023). Irritable bowel syndrome and the gut microbiome: a comprehensive review. *Journal of Clinical Medicine*, 12(7), 2558.
931. Schenck Eidam, H., Russell, J., Raha, K., DeMartino, M., Qin, D., Guan, H. A., ... & Cheung, M. (2018). Discovery of a first-in-class gut-restricted RET kinase inhibitor as a clinical candidate for the treatment of IBS. *ACS medicinal chemistry letters*, 9(7), 623-628.
932. Scheppach, W., Bartram, H. P., & Richter, F. (1995). Role of short-chain fatty acids in the prevention of colorectal cancer. *European Journal of Cancer*, 31(7-8), 1077-1080
933. Scheppach, W. Effects of short chain fatty acids on gut morphology and function. *Gut*. 1994;35(1 Suppl), S35-S38.
934. Scheppach, W., Pomare, E. W., Elia, M., & Cummings, J. H. (1991). The contribution of the large intestine to blood acetate in man. *Clinical Science*, 80(2), 177-182.
935. Schlörmann, W., Horlebein, C., Hübner, S.M., Wittwer, E. and Glei, M., 2023. Potential Role of ROS in Butyrate-and Dietary Fiber-Mediated Growth Inhibition and Modulation of Cell Cycle-, Apoptosis-and Antioxidant-Relevant Proteins in LT97 Colon Adenoma and HT29 Colon Carcinoma Cells. *Cancers*, 15(2), p.440.
936. Schmidt, F., List, M., Cukuroglu, E., Köhler, S., Göke, J., & Schulz, M. H. (2018). An ontology-based method for assessing batch effect adjustment approaches in heterogeneous datasets. *Bioinformatics*, 34(17), i908-i916.
937. Schmier, J. K., Perez, V., Cloran, S., Hulme-Lowe, C., & O'Sullivan, K. (2015). Cost savings of reduced constipation rates attributed to increased dietary fibre intakes in Europe: a decision-analytic model. *J Pharm Nutr Sci*, 5, 14-23.
938. Schmulson, M. J., & Drossman, D. A. (2017). What is new in Rome IV. *Journal of neurogastroenterology and motility*, 23(2), 151
939. Scholem, R. D., & Brown, G. K. (1983). Metabolism of malonic semialdehyde in man. *Biochemical Journal*, 216(1), 81.
940. Schroeder, B. O., Birchenough, G. M., Ståhlman, M., Arike, L., Johansson, M. E., Hansson, G. C., & Bäckhed, F. Bifidobacteria or fiber protects against diet-induced microbiota-mediated colonic mucus deterioration. *Cell host & microbe*. 2018;23(1), 27-40.
941. Schroeder, B. O., & Bäckhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nature Med*, 22, 1079. doi:10.1038/nm.4185
942. Schroeder, B. O. (2019). Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. *Gastroenterol Rep (Oxf)* 7: 3–12.
943. Schoenfeld, P. S. Advances in IBS 2016: a review of current and emerging data. *Gastroenterology & hepatology*. 2016;12(8 Suppl 3), 1.
944. Schubert, S. (2021). The effect of oral butyrate administration on the release of serotonin and dopamine in the gastrointestinal tract (Master's thesis, Itä-Suomen yliopisto)
945. Schumann, D., Klose, P., Lauche, R., Dobos, G., Langhorst, J., & Cramer, H. (2018). Low fermentable, oligo-, di-, mono-saccharides and polyol diet in the treatment of irritable bowel syndrome: A systematic review and meta-analysis. *Nutrition*. 2018;45, 24-31. doi:10.1016/j.nut.2017.07.004

946. Schwab, M., Reynders, V., Loitsch, S., Steinhilber, D., Stein, J., & Schröder, O. (2007). Involvement of different nuclear hormone receptors in butyrate-mediated inhibition of inducible NF κ B signalling. *Molecular immunology*, 44(15), 3625-3632.
947. Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., & Duncan, S. H. The influence of diet on the gut microbiota. *Pharmacological research*. 2013;69(1), 52-60.
948. Scully P, McKernan DP, Keohane J, et al. Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co- morbidity. *American Journal of Gastroenterology*. 2010;105:2235–2243.
949. Serdar, C. C., Cihan, M., Yücel, D., & Serdar, M. A. (2021). Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochemia medica*, 31(1), 27-53.
950. Shah, S. L., & Lacy, B. E. (2016). Dietary interventions and irritable bowel syndrome: a review of the evidence. *Current gastroenterology reports*, 18(8), 41.
951. Shah, B. R., Li, B., Al Sabbah, H., Xu, W., & Mráz, J. (2020). Effects of prebiotic dietary fibers and probiotics on human health: With special focus on recent advancement in their encapsulated formulations. *Trends in food science & technology*, 102, 178-192.
952. Shahdadi, H., Balouchi, A., & Shaykh, A. (2017). Comparison of Resilience and Psychological Wellbeing in Women with Irritable Bowel Syndrome and Normal Women. *Materia Socio Medica*, 29(2), 105-108. doi:10.5455/msm.2017.29.105-108
953. Shankar V, Reo NV, Paliy O. Simultaneous fecal microbial and metabolite profiling enables accurate classification of pediatric irritable bowel syndrome. *Microbiome*. 2015;3:73. doi:10.1186/s40168-015-0139-9.
954. Sheflin, A. M., Melby, C. L., Carbonero, F., & Weir, T. L. (2017). Linking dietary patterns with gut microbial composition and function. *Gut microbes*, 8(2), 113-129.
955. Shepherd, S. J., Parker, F. C., Muir, J. G., & Gibson, P. R. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. *Clinical Gastroenterology and Hepatology*. 2008;6(7):765-771. doi:10.1016/j.cgh.2008.02.058
956. Shepherd, S. J., & Gibson, P. R. Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management. *Journal of the American Dietetic Association*. 2006;106(10):1631-1639. doi:10.1016/j.jada.2006.07.010
957. Shapiro, H., Thaïss, C. A., Levy, M., & Elinav, E. The cross talk between microbiota and the immune system: metabolites take center stage. *Current opinion in immunology*. 2014;30:54-62
958. Shoaie, S., Ghaffari, P., Kovatcheva-Datchary, P., Mardinoglu, A., Sen, P., Pujos-Guillot, E., ... & Hoyles, L. Quantifying diet-induced metabolic changes of the human gut microbiome. *Cell metabolism*. 2015;22(2), 320-331.
959. Shimizu, J., Kubota, T., Takada, E., Takai, K., Fujiwara, N., Arimitsu, N., ... & Suzuki, N. Propionate-producing bacteria in the intestine may associate with skewed responses of IL10-producing regulatory T cells in patients with relapsing polychondritis. *PloS one*. 2018;13(9).
960. Shin, A., Camilleri, M., Vijayvargiya, P., Busciglio, I., Burton, D., Ryks, M., ... & Zinsmeister, A. R. Bowel functions, fecal unconjugated primary and secondary bile acids, and colonic transit in patients with irritable bowel syndrome. *Clinical Gastroenterology and Hepatology*. 2013;11(10), 1270-1275.
961. Shoaie, S., Ghaffari, P., Kovatcheva-Datchary, P., Mardinoglu, A., Sen, P., Pujos-Guillot, E., ... & Hoyles, L. Quantifying diet-induced metabolic changes of the human gut microbiome. *Cell metabolism*. 2015;22(2), 320-331.
962. Shukla, R., Ghoshal, U., Dhole, T. N., & Ghoshal, U. C. (2015). Fecal microbiota in patients with irritable bowel syndrome compared with healthy controls using real-time polymerase chain reaction: an evidence of dysbiosis. *Digestive diseases and sciences*, 60, 2953-2962.
963. Shulman, R. J., Hollister, E. B., Cain, K., Czyzewski, D. I., Self, M. M., Weidler, E. M., ... & Heitkemper, M. (2017). Psyllium fiber reduces abdominal pain in children with irritable bowel syndrome in a randomized, double-blind trial. *Clinical Gastroenterology and Hepatology*, 15(5), 712-719.
964. Si, J. M., Yu, Y. C., Fan, Y. J., & Chen, S. J. (2004). Intestinal microecology and quality of life in irritable bowel syndrome patients. *World journal of gastroenterology: WJG*, 10(12), 1802.

965. Siddiqui, M. T., & Cresci, G. A. (2021). The immunomodulatory functions of butyrate. *Journal of inflammation research*, 6025-6041.
966. Simpson, H. L., & Campbell, B. J. (2015). dietary fibre–microbiota interactions. *Alimentary pharmacology & therapeutics*, 42(2), 158-179.
967. Simren, M., Palsson, O. S., & Whitehead, W. E. (2017). Update on Rome IV Criteria for Colorectal Disorders: Implications for Clinical Practice. *Current Gastroenterology Rep*, 19(4), 15. doi:10.1007/s11894-017-0554-0
968. Silk, D. B. A., Davis, A., Vulevic, J., Tzortzis, G., & Gibson, G. R. (2009). Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Alimentary pharmacology & therapeutics*, 29(5), 508-518.
969. Simmler C, Kulakowski D, Lankin DC, McAlpine JB, Chen S-N, Pauli GF. Holistic Analysis Enhances the Description of Metabolic Complexity in Dietary Natural Products. *Advances in Nutrition*. 2016;7(1):179-189. doi:10.3945/an.115.009928.
970. Simren, M. (2006). Hypnosis for irritable bowel syndrome: the quest for the mechanism of action. *International Journal of Clinical and Experimental Hypnosis*, 54(1), 65-84. doi:10.1080/00207140500327239
971. Simren, M., & Tack, J. (2018). New treatments and therapeutic targets for IBS and other functional bowel disorders. *Nature Reviews Gastroenterology & Hepatology*, 1.
972. Sinagra, E., Morreale, G. C., Mohammadian, G., Fusco, G., Guarnotta, V., Tomasello, G., ... Raimondo, D. New therapeutic perspectives in irritable bowel syndrome: Targeting low-grade inflammation, immuno-neuroendocrine axis, motility, secretion and beyond. *World Journal of Gastroenterology*. 2017;23(36): 6593–6627.
973. Singh, J., Metrani, R., Shivanagoudra, S. R., Jayaprakasha, G. K., & Patil, B. S. Review on bile acids: effects of the gut microbiome, interactions with dietary fiber, and alterations in the bioaccessibility of bioactive compounds. *Journal of agricultural and food chemistry*. 2019;67(33), 9124-9138.
974. Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., ... & Ganapathy, V. (2014). Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity*, 40(1), 128-139.
975. Singh, P., & Lembo, A. (2021). Emerging role of the gut microbiome in irritable bowel syndrome. *Gastroenterology Clinics*, 50(3), 523-545.
976. Siu, L. L., Von Hoff, D. D., Rephaeli, A., Izbicka, E., Cerna, C., Gomez, L., ... & Eckhardt, S. G. (1998). Activity of pivaloyloxymethyl butyrate, a novel anticancer agent, on primary human tumor colony-forming units. *Investigational new drugs*, 16, 113-119.
977. Sivaprakasam, S., Prasad, P. D., & Singh, N. Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. *Pharmacology & therapeutics*. 2016; 164, 144-151.
978. Skoglund, J. Quantification of short chain fatty acids in serum and plasma. Online publication: <http://stud.epsilon.slu.se>. 2016.
979. Slattery, S. A., Niaz, O., Aziz, Q., Ford, A. C., & Farmer, A. D. Systematic review with meta-analysis: the prevalence of bile acid malabsorption in the irritable bowel syndrome with diarrhoea. *Alimentary pharmacology & therapeutics*. 2015;42(1), 3-11.
980. Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, 5(4), 1417-1435.
981. Slavin, J. L., & Greenberg, N. A. (2003). Partially hydrolyzed guar gum: clinical nutrition uses. *Nutrition*, 19(6), 549-552.
982. Sly, W. S., Quinton, B. A., McAlister, W. H., & Rimoim, D. L. (1973). Beta glucuronidase deficiency: report of clinical, radiologic, and biochemical features of a new mucopolysaccharidosis. *The Journal of pediatrics*, 82(2), 249-257.
983. Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-y, M., ... & Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*, 341(6145), 569-573.
984. Smith, E. A., & Macfarlane, G. T. (1998). Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. *FEMS microbiology ecology*, 25(4), 355-368.

985. Smith, N. W., Shorten, P. R., Altermann, E. H., Roy, N. C., & McNabb, W. C. (2019). Hydrogen cross-feeders of the human gastrointestinal tract. *Gut microbes*, 10(3), 270-288.
986. So, D., Yao, C., Gibson, P., & Muir, J. (2021, September). Combination of two fibers with different fermentabilities in patients with irritable bowel syndrome initiated on a low-FODMAP diet is well tolerated and promotes distal colonic fermentation, as shown telemetrically by colonic hydrogen concentrations but not pH. In *Journal of Gastroenterology and Hepatology* (Vol. 36, pp. 160-161). 111 RIVER ST, HOBOKEN 07030-5774, NJ USA: WILEY.
987. Soergel, K. H. Colonic fermentation: metabolic and clinical implications. *The clinical investigator*. 1994;72(10), 742-748.
988. Søltoft, J., Krag, B., Gudmand-Høyer, E., Kristensen, E., & Wulff, H. R. (1976). A double-blind trial of the effect of wheat bran on symptoms of irritable bowel syndrome. *The Lancet*, 307(7954), 270-272.
989. Sonnenburg, J. L., & Bäckhed, F. Diet–microbiota interactions as moderators of human metabolism. *Nature*. 2016;535(7610), 56-64.
990. Song, J., Ma, W., Gu, X., Zhao, L., Jiang, J., Xu, Y., ... & Yang, L. (2019). Metabolomic signatures and microbial community profiling of depressive rat model induced by adrenocorticotrophic hormone. *Journal of translational medicine*, 17, 1-12.
991. Song, Y., Könönen, E., Rautio, M., Liu, C., Bryk, A., Eerola, E., & Finegold, S. M. (2006). *Alistipes onderdonkii* sp. nov. and *Alistipes shahii* sp. nov., of human origin. *International journal of systematic and evolutionary microbiology*, 56(8), 1985-1990
992. Sonnenburg, E. D., & Sonnenburg, J. L. (2014). Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell metabolism*, 20(5), 779-786.
993. Soret, R., Chevalier, J., De Coppet, P., Poupeau, G., Derkinderen, P., Segain, J. P., & Neunlist, M. (2010). Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. *Gastroenterology*, 138(5), 1772-1782.
994. Soto-Herederó, G., Gomez de las Heras, M. M., Gabandé-Rodríguez, E., Oller, J., & Mittelbrunn, M. (2020). Glycolysis—a key player in the inflammatory response. *The FEBS journal*, 287(16), 3350-3369.
995. Southgate, D. A. T. (1975). (c) Dietary Fibre—Its Nature and Role in the Diet. *Royal Society of Health Journal*, 95(4), 191-194.
996. Sparberg M. Constipation. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd edition. Boston: Butterworths; 1990. Chapter 89. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK415/>
997. Spiegel, B. M., Gralnek, I. M., Bolus, R., Chang, L., Dulai, G. S., Mayer, E. A., & Naliboff, B. (2004). Clinical determinants of health-related quality of life in patients with irritable bowel syndrome. *Archives of Internal Medicine*, 164(16), 1773-1780. doi:10.1001/archinte.164.16.1773
998. Sperber, A. D., Bangdiwala, S. I., Drossman, D. A., Ghoshal, U. C., Simren, M., Tack, J., ... & Palsson, O. S. (2021). Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation Global Study. *Gastroenterology*, 160(1), 99-114.
999. Spiegel, B., Strickland, A., Naliboff, B. D., Mayer, E. A., & Chang, L. Predictors of patient-assessed illness severity in irritable bowel syndrome. *American Journal of Gastroenterology*. 2008;103(10): 2536-2543. doi:10.1111/j.1572-0241.2008.01997.
1000. Spiegel, B. M., Khanna, D., Bolus, R., Agarwal, N., Khanna, P., & Chang, L. Understanding gastrointestinal distress: a framework for clinical practice. *American Journal of Gastroenterology*. 2011; 106(3):380-385. doi:10.1038/ajg.2010.383
1001. Spiller, G. A. (Ed.). (2001). *CRC handbook of dietary fiber in human nutrition*. CRC press.
1002. Spiller, R. Irritable bowel syndrome: new insights into symptom mechanisms and advances in treatment. *F1000Research*. 2016;5. doi:10.12688/f1000research.7992.1
1003. Stanculete, M. F., Matu, S., Pojoga, C., & Dumitrascu, D. L. (2015). Coping strategies and irrational beliefs as mediators of the health-related quality of life impairments in irritable bowel syndrome. *Journal of Gastrointestinal of Liver Diseases*, 24(2), 159-164. doi:10.15403/jgld.2014.1121.242.strt
1004. Stasi C, Bellini M, Gambaccini D, et al. Neuroendocrine Dysregulation in Irritable Bowel Syndrome Patients: A Pilot Study. *Journal of Neurogastroenterology and Motility*. 2017;23(3):428-434. doi:10.5056/jnm16155.

1005. Stasi, C., Bellini, M., Costa, F., Mumolo, M. G., Ricchiuti, A., Grosso, M., ... & Marchi, S. (2013). Neuroendocrine markers and psychological features in patients with irritable bowel syndrome. *International journal of colorectal disease*, 28, 1203-1208.
1006. Stasi, C., Rosselli, M., Bellini, M., Laffi, G., & Milani, S. (2012). Altered neuro-endocrine-immune pathways in the irritable bowel syndrome: the top-down and the bottom-up model. *Journal of gastroenterology*, 47, 1177-1185.
1007. Stasi, C., Bellini, M., Bassotti, G., Blandizzi, C., & Milani, S. (2014). Serotonin receptors and their role in the pathophysiology and therapy of irritable bowel syndrome. *Techniques in coloproctology*, 18, 613-621.
1008. Staudacher, H. M., Irving, P. M., Lomer, M. C., & Whelan, K. (2017). The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. *Proceedings of the Nutrition Society*, 76(3), 203-212.
1009. Staudacher, H. M., Irving, P. M., Lomer, M. C., & Whelan, K. (2014). Mechanisms and efficacy of dietary FODMAP restriction in IBS. *Nature reviews Gastroenterology & hepatology*, 11(4), 256-266.
1010. Staudacher, H. M., Lomer, M. C., Anderson, J. L., Barrett, J. S., Muir, J. G., Irving, P. M., & Whelan, K. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *The Journal of Nutrition*. 2012;142(8):1510-1518.
1011. Staudacher, H. M., Lomer, M. C., Farquharson, F. M., Louis, P., Fava, F., Franciosi, E., ... & Whelan, K. (2017). A diet low in FODMAPs reduces symptoms in patients with irritable bowel syndrome and a probiotic restores bifidobacterium species: a randomized controlled trial. *Gastroenterology*, 153(4), 936-947.
1012. Staudacher, H. M., & Whelan, K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. *Gut*. 2017;66(8), 1517-1527. doi:10.1136/gutjnl-2017-313750
1013. Steele, R. D. (1982). Transaminative metabolism of α -amino-n-butyrate in rats. *Metabolism*, 31(4), 318-325.
1014. Stempelj, M., Keding, M., Augenlicht, L., & Klampfer, L. (2007). Essential role of the JAK/STAT1 signaling pathway in the expression of inducible nitric-oxide synthase in intestinal epithelial cells and its regulation by butyrate. *Journal of Biological Chemistry*, 282(13), 9797-9804.
1015. Stephen, A. M., Wiggins, H. S., & Cummings, J. H. Effect of changing transit time on colonic microbial metabolism in man. *Gut*. 1987; 28(5), 601-609.
1016. Stephen, A. M., & Cummings, J. H. (1980). Mechanism of action of dietary fibre in the human colon. *Nature*, 284(5753), 283-284.
1017. Stephen, A. M., & Cummings, J. H. (1979). Water-holding by dietary fibre in vitro and its relationship to faecal output in man. *Gut*, 20(8), 722-729.
1018. Stephen, A. M., Wiggins, H. S., Englyst, H. N., Cole, T. J., Wayman, B. J., & Cummings, J. H. (1986). The effect of age, sex and level of intake of dietary fibre from wheat on large-bowel function in thirty healthy subjects. *British Journal of Nutrition*, 56(2), 349-361.
1019. Stern, E. K., & Brenner, D. M. (2018). Gut microbiota-based therapies for irritable bowel syndrome. *Clinical and translational gastroenterology*, 9(2), e134.
1020. Stoessel, D., Stellmann, J. P., Willing, A., Behrens, B., Rosenkranz, S. C., Hodecker, S. C., ... & Pless, O. (2018). Metabolomic profiles for primary progressive multiple sclerosis stratification and disease course monitoring. *Frontiers in human neuroscience*, 12, 226
1021. Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain research*, 1693, 128-133.
1022. Strauss B. S. (2016). Biochemical Genetics and Molecular Biology: The Contributions of George Beadle and Edward Tatum. *Genetics*, 203(1), 13–20. <https://doi.org/10.1534/genetics.116.188995>
1023. Suares, N. C., & Ford, A. C. (2011). Systematic review: the effects of fibre in the management of chronic idiopathic constipation. *Alimentary pharmacology & therapeutics*, 33(8), 895-901.
1024. Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., ... & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *The Journal of physiology*, 558(1), 263-275.
1025. Suenart, P., Bulteel, V., Lemmens, L., Noman, M., Geypens, B., Van Assche, G., ... & Rutgeerts, P. (2002). Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. *The American journal of gastroenterology*, 97(8), 2000-2004.

1026. Suez, J., Zmora, N., Segal, E., & Elinav, E. (2019). The pros, cons, and many unknowns of probiotics. *Nature medicine*, 25(5), 716-729.
1027. Sugaya, N., Izawa, S., Saito, K., Shiotsuki, K., Nomura, S., & Shimada, H. (2015). Effect of prolonged stress on the adrenal hormones of individuals with irritable bowel syndrome. *BioPsychoSocial medicine*, 9, 1-9.
1028. Sun, M., Wu, W., Chen, L., Yang, W., Huang, X., Ma, C., ... & Yao, S. (2018). Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. *Nature communications*, 9(1), 1-15.
1029. Sun, Q., Jia, Q., Song, L., & Duan, L. Alterations in fecal short-chain fatty acids in patients with irritable bowel syndrome: A systematic review and meta-analysis. *Medicine*. 2019 98(7).
1030. Sun, Z., Wang, X., Feng, S., Xie, C., Xing, Y., Guo, L., ... & Ji, C. (2023). A review of neuroendocrine immune system abnormalities in IBS based on the brain–gut axis and research progress of acupuncture intervention. *Frontiers in Neuroscience*, 17, 934341.
1031. Sun, Q. H., Liu, Z. J., Zhang, L., Wei, H., Song, L. J., Zhu, S. W., ... & Duan, L. P. (2021). Sex-based differences in fecal short-chain fatty acid and gut microbiota in irritable bowel syndrome patients. *Journal of Digestive Diseases*, 22(5), 246-255.
1032. Sunderland, R. Irritable bowel syndrome in adults: symptoms, treatment and management. *Nursing Standard*. 2017;31(26).
1033. Swanson, H. I. (2015). Drug metabolism by the host and gut microbiota: a partnership or rivalry?. *Drug Metabolism and Disposition*, 43(10), 1499-1504.
1034. Szanton, S. L., & Gill, J. M. (2010). Facilitating resilience using a society-to-cells framework: a theory of nursing essentials applied to research and practice. *Advances in Nursing Science*, 33(4), 329-343.
1035. Sze, M. A., Topçuoğlu, B. D., Lesniak, N. A., Ruffin, M. T., & Schloss, P. D. Fecal short-chain fatty acids are not predictive of colonic tumor status and cannot be predicted based on bacterial community structure. *Mbio*. 2019;10(4), e01454-19.
1036. Tabuchi, Y., Arai, Y., Kondo, T., Takeguchi, N., & Asano, S. (2002). Identification of genes responsive to sodium butyrate in colonic epithelial cells. *Biochemical and biophysical research communications*, 293(4), 1287-1294.
1037. Tack, J., & Sarnelli, G. (2002). Serotonergic modulation of visceral sensation: upper gastrointestinal tract. *Gut*, 51(suppl 1), i77-i80.
1038. Tack, J., Müller-Lissner, S., Stanghellini, V., Boeckxstaens, G., Kamm, M. A., Simren, M., ... Fried, M. (2011). Diagnosis and treatment of chronic constipation--a European perspective. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society*, 23(8), 697–710. doi:10.1111/j.1365-2982.2011.01709.x
1039. Tahara, Y., Yamazaki, M., Sukigara, H., Motohashi, H., Sasaki, H., Miyakawa, H., ... & Shibata, S. (2018). Gut microbiota-derived short chain fatty acids induce circadian clock entrainment in mouse peripheral tissue. *Scientific reports*, 8(1), 1395.
1040. Takahashi, H., Wako, N., OKUBO, T., ISHIHARA, N., YAMANAKA, J., & YAMAMOTO, T. (1994). Influence of partially hydrolyzed guar gum on constipation in women. *Journal of nutritional science and vitaminology*, 40(3), 251-259.
1041. Takahashi, H., Ik Yang, S., Hayashi, C., Kim, M., Yamanaka, J., & Yamamoto, T. (1993). Effect of partially hydrolyzed guar gum on fecal output in human volunteers. *Nutrition Research*, 13(6), 649-657.
1042. Taleb, S. (2019). Tryptophan dietary impacts gut barrier and metabolic diseases. *Frontiers in immunology*, 10, 467946.
1043. Talley, N. J. (2020). What causes functional gastrointestinal disorders? A proposed disease model. *Official journal of the American College of Gastroenterology| ACG*, 115(1), 41-48.
1044. Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The role of short-chain fatty acids in health and disease. *Advances in immunology*, 121, 91-119.
1045. Tanahashi, Y., Komori, S., Matsuyama, H., Kitazawa, T., & Unno, T. (2021). Functions of muscarinic receptor subtypes in gastrointestinal smooth muscle: A review of studies with receptor-knockout mice. *International Journal of Molecular Sciences*, 22(2), 926.

1046. Tanaka, M., Tóth, F., Polyák, H., Szabó, Á., Mándi, Y., & Vécsei, L. (2021). Immune influencers in action: metabolites and enzymes of the tryptophan-kynurenine metabolic pathway. *Biomedicines*, 9(7), 734.
- 1047.
1048. Tarnowski, W., Borycka-Kiciak, K., Kiciak, A., Friediger, J., Cienciala, A., & Zabielski, R. Outcome of treatment with butyric acid in irritable bowel syndrome—preliminary report. *Gastroenterol Prakt.* 2011;1, 43-8.
1049. Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. The role of short-chain fatty acids in health and disease. *Advances in Immunology.* 2014;121:91-119. doi:10.1016/b978-0-12-800100-4.00003-9.
1050. Tana, C., Umesaki, Y., Imaoka, A., Handa, T., Kanazawa, M., & Fukudo, S. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterology & Motility.* 2010;22(5), 512-e115.
1051. Tanahashi, Y., Komori, S., Matsuyama, H., Kitazawa, T., & Unno, T. (2021). Functions of muscarinic receptor subtypes in gastrointestinal smooth muscle: A review of studies with receptor-knockout mice. *International Journal of Molecular Sciences*, 22(2), 926.
1052. Tanaka, Yukari & Kanazawa, Motoyori & Fukudo, Shin & Drossman, Douglas. (2011). Biopsychosocial Model of Irritable Bowel Syndrome. *Journal of Neurogastroenterology and Motility.* 17. 131-9. 10.5056/jnm.2011.17.2.131.
1053. Tap, J., Derrien, M., Tornblom, H., Brazeilles, R., Cools-Portier, S., Dore, J., . . . Simren, M. Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable Bowel Syndrome. *Gastroenterology.* 2017;152(1):111-123.e118. doi:10.1053/j.gastro.2016.09.049.
1054. Tappenden, K. A. Emerging therapies for intestinal failure. *Archives of surgery.* 2010;145(6), 528-532.
1055. Tavel, M. E. (2015). Somatic symptom disorders without known physical causes: one disease with many names?. *The American journal of medicine*, 128(10), 1054-1058.
1056. Tavella, T., Rampelli, S., Guidarelli, G., Bazzocchi, A., Gasperini, C., Pujos-Guillot, E., ... & Santoro, A. (2021). Elevated gut microbiome abundance of Christensenellaceae, Porphyromonadaceae and Rikenellaceae is associated with reduced visceral adipose tissue and healthier metabolic profile in Italian elderly. *Gut microbes*, 13(1), 1880221.
1057. Tebani A, Afonso C, Bekri S. Advances in metabolome information retrieval: turning chemistry into biology. Part I: analytical chemistry of the metabolome. *Journal of Inherited Metabolic Disease.* 2018;41(3):379-391. doi:10.1007/s10545-017-0074-y.
1058. Tennoune, N., Andriamihaja, M., & Blachier, F. (2022). Production of indole and indole-related compounds by the intestinal microbiota and consequences for the host: the good, the bad, and the ugly. *Microorganisms*, 10(5), 930.
1059. Tettelin, H., Massignani, V., Cieslewicz, M. J., Donati, C., Medini, D., Ward, N. L., ... & Fraser, C. M. (2005). Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial “pan-genome”. *Proceedings of the National Academy of Sciences*, 102(39), 13950-13955.
1060. Thau L, Gandhi J, Sharma S. Physiology, Cortisol. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538239/>
1061. TheHumanMetabolomeDatabase (n.d.). Retrieved March 2, 2020, from <http://www.hmdb.ca>
1062. The Nutrition Source. (n.d.). <https://www.hsph.harvard.edu/nutritionsource/pantothenic-acid-vitamin-b5/>
1063. Thiele, I., Sahoo, S., Heinken, A., Heirendt, L., Aurich, M. K., Noronha, A., & Fleming, R. M. (2018). When metabolism meets physiology: Harvey and Harvetta. *BioRxiv*, 255885.
1064. Thompson, W. G., Longstreth, G. F., Drossman, D. A., Heaton, K. W., Irvine, E. J., & Müller-Lissner, S. A. (1999). Functional bowel disorders and functional abdominal pain. *Gut*, 45(suppl 2), II43-II47.
1065. Thursby, E., & Juge, N. Introduction to the human gut microbiota. *Biochemical Journal.* 2017;474(11), 1823-1836.
1066. Tian, P., Chen, Y., Zhu, H., Wang, L., Qian, X., Zou, R., ... & Chen, W. (2022). *Bifidobacterium breve* CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: A randomized clinical trial. *Brain, behavior, and immunity*, 100, 233-241.

1067. Tian, Y., Gui, W., Koo, I., Smith, P. B., Allman, E. L., Nichols, R. G., ... & Patterson, A. D. (2020). The microbiome modulating activity of bile acids. *Gut microbes*, 1-18.\
1068. Timmons J, Chang ET, Wang J-Y, Rao JN. Polyamines and Gut Mucosal Homeostasis. *Journal of gastrointestinal & digestive system*. 2012;2(Suppl 7).
1069. Tiso, M., & Schechter, A. N. (2015). Nitrate reduction to nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions. *PloS one*, 10(3), e0119712.
1070. Tiwari, S. B., Hagen, G., & Guilfoyle, T. (2003). The roles of auxin response factor domains in auxin-responsive transcription. *The Plant Cell*, 15(2), 533-543.
1071. Tobin, G., Giglio, D., & Lundgren, O. (2009). Muscarinic receptor subtypes in the alimentary tract. *Acta physiologica Polonica*, 60(1), 3.
1072. Todays Dietician (n.d.). The Magazine for Nutrition Professionals. Retrieved April 15, 2020, from <https://www.todaysdietitian.com/newarchives/0816p34.shtml>
1073. Topping, D. L., & Clifton, P. M. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological reviews*. 2001;81(3), 1031-1064.
1074. Törnblom, H., & Drossman, D. A. (2021). Psychopharmacologic therapies for irritable bowel syndrome. *Gastroenterology Clinics*, 50(3), 655-669.
1075. Torres, M. J., Sabate, J. M., Bouchoucha, M., Buscail, C., Hercberg, S., & Julia, C. Food consumption and dietary intakes in 36,448 adults and their association with irritable bowel syndrome: Nutrinet-Sante study. *Therapeutic advances in gastroenterology*. 2018;11, 1756283X17746625.
1076. Tramonte, S. M., Brand, M. B., Mulrow, C. D., Amato, M. G., O'Keefe, M. E., & Ramirez, G. (1997). The treatment of chronic constipation in adults: a systematic review. *Journal of General Internal Medicine*, 12(1), 15-24.
1077. Treem, W. R., Ahsan, N., Kastoff, G., & Hyams, J. S. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. *Journal of pediatric gastroenterology and nutrition*. 1996;23(3), 280-286.
1078. Tremaroli, V., & Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012;489(7415), 242.
1079. Trivedi, D. K., Hollywood, K. A., & Goodacre, R. Metabolomics for the masses: the future of metabolomics in a personalized world. *New horizons in translational medicine*. 2017;3(6):294-305.
1080. Trowell, H. (1978). The development of the concept of dietary fiber in human nutrition. *The American journal of clinical nutrition*, 31(10), S3-S11.
1081. Trowell, H. (1976). Definition of dietary fiber and hypotheses that it is a protective factor in certain diseases. *The American journal of clinical nutrition*, 29(4), 417-427.
1082. Trumbo, P., Schlicker, S., Yates, A. A., & Poos, M. (2002). Food and Nutrition Board of the Institute of Medicine, The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc*, 102(11), 1621-30.
1083. Tsang, S. W., Auyeung, K. K. W., Bian, Z. X., & Ko, J. K. S. Pathogenesis, Experimental Models and Contemporary Pharmacotherapy of Irritable Bowel Syndrome: Story About the Brain-Gut Axis. *Current Neuropharmacology*. 2016;14(8):842–856.
1084. Tsavkelova, E. A., Klimova, S. Y., Cherdyntseva, T. A., & Netrusov, A. I. (2006). Hormones and hormone-like substances of microorganisms: a review. *Applied biochemistry and microbiology*, 42, 229-235.
1085. Tsugawa, H., Kabe, Y., Kanai, A., Sugiura, Y., Hida, S., Taniguchi, S. I., ... & Suematsu, M. (2020). Short-chain fatty acids bind to apoptosis-associated speck-like protein to activate inflammasome complex to prevent Salmonella infection. *PLoS biology*, 18(9), e3000813.
1086. Tu, Q., Heitkemper, M. M., Jarrett, M. E., & Buchanan, D. T. (2017). Sleep disturbances in irritable bowel syndrome: a systematic review. *Neurogastroenterology & Motility*, 29(3), e12946.
1087. Tuck, C.J., & Vanner, S. J. Dietary therapies for functional bowel symptoms: Recent advances, challenges, and future directions. *Neurogastroenterology & Motility*. 2018;30(1). doi:10.1111/nmo.13238
1088. Tuohy, K. M., Kolida, S., Lustenberger, A. M., & Gibson, G. R. (2001). The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides—a human volunteer study. *British Journal of Nutrition*, 86(3), 341-348.

1089. Tucker, D. M., Sandstead, H. H., Logan, G. M., Klevay, L. M., Mahalko, J., Johnson, L. K., ... & Inglett, G. E. (1981). Dietary fiber and personality factors as determinants of stool output. *Gastroenterology*, 81(5), 879-883.
1090. Umu, Ö. C., Rudi, K., & Diep, D. B. (2017). Modulation of the gut microbiota by prebiotic fibres and bacteriocins. *Microbial ecology in health and disease*, 28(1), 1348886.
1091. Undseth, R., Jakobsdottir, G., Nyman, M., Berstad, A., & Valeur, J. (2015). Low serum levels of short-chain fatty acids after lactulose ingestion may indicate impaired colonic fermentation in patients with irritable bowel syndrome. *Clinical and experimental gastroenterology*. 2015;8, 303.
1092. Uribarri, J., Oh, M. S., & Carroll, H. J. (1998). D-lactic acidosis: a review of clinical presentation, biochemical features, and pathophysiologic mechanisms. *Medicine-Baltimore*, 77(2), 73-82.
1093. Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Frontiers in Microbiology*, 11, 589.
1094. Uwada, J., Nakazawa, H., Muramatsu, I., Masuoka, T., & Yazawa, T. (2023). Role of muscarinic acetylcholine receptors in intestinal epithelial homeostasis: Insights for the treatment of inflammatory bowel disease. *International Journal of Molecular Sciences*, 24(7), 6508.
1095. Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M., & De Angelis, M. (2020). The controversial role of human gut lachnospiraceae. *Microorganisms*, 8(4), 573.
1096. Vahora, I. S., Tsouklidis, N., Kumar, R., Soni, R., & Khan, S. (2020). How serotonin level fluctuation affects the effectiveness of treatment in irritable bowel syndrome. *Cureus*, 12(8).
1097. Valeur, J., Smastuen, M. C., Knudsen, T., Lied, G. A., & Roseth, A. G. Exploring Gut Microbiota Composition as an Indicator of Clinical Response to Dietary FODMAP Restriction in Patients with Irritable Bowel Syndrome. *Digestive Diseases and Sciences*. 2018;63(2):429-436. doi:10.1007/s10620-017-4893-3
1098. Valeur, J., Røseth, A. G., Knudsen, T., Malmstrøm, G. H., Fiennes, J. T., Midtvedt, T., & Berstad, A. Fecal fermentation in irritable bowel syndrome: influence of dietary restriction of fermentable oligosaccharides, disaccharides, monosaccharides and polyols. *Digestion*. 2016;94(1), 50-56.
1099. Valeur, J., Smastuen, M. C., Knudsen, T., Lied, G. A., & Roseth, A. G. Exploring Gut Microbiota Composition as an Indicator of Clinical Response to Dietary FODMAP Restriction in Patients with Irritable Bowel Syndrome. *Digestive Diseases and Sciences*. 2018;63(2):429-436. doi:10.1007/s10620-017-4893-3
1100. Valeur, J., Morken, M. H., Norin, E., Midtvedt, T., & Berstad, A. Intestinal fermentation in patients with self-reported food hypersensitivity: painful, but protective? *Clinical and experimental gastroenterology*. 2010;3, 65.
1101. van den Berg, R. A., Hoefsloot, H. C., Westerhuis, J. A., Smilde, A. K., & van der Werf, M. J. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC Genomics*. 2006;7, 142.
1102. van den Berg, R. A., Hoefsloot, H. C., Westerhuis, J. A., Smilde, A. K., & van der Werf, M. J. Centering, Zierer, J., Jackson, M. A., Kastenmüller, G., Mangino, M., Long, T., Telenti, A., ... & Valdes, A. M. The fecal metabolome as a functional readout of the gut microbiome. *Nature genetics*. 2018;50(6), 790-795.
1103. van den Berg, R. A., Hoefsloot, H. C., Westerhuis, J. A., Smilde, A. K., & van der Werf, M. J. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC Genomics*. 2006;7:142.
1104. Vandeputte, D.; Joossens, M. Effects of Low and High FODMAP Diets on Human Gastrointestinal Microbiota Composition in Adults with Intestinal Diseases: A Systematic Review. *Microorganisms* 2020, 8, 1638
1105. van der Beek, C. M., Dejong, C. H., Troost, F. J., Masclee, A. A., & Lenaerts, K. (2017). Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. *Nutrition reviews*, 75(4), 286-305.
1106. van der Hee, B., & Wells, J. M. (2021). Microbial regulation of host physiology by short-chain fatty acids. *Trends in Microbiology*, 29(8), 700-712.
1107. van de Wouw, M., Boehme, M., Lyte, J. M., Wiley, N., Strain, C., O'Sullivan, O., ... & Cryan, J. F. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. *The Journal of physiology*. 2018.

1108. Vangay, P., Johnson, A. J., Ward, T. L., Al-Ghalith, G. A., Shields-Cutler, R. R., Hillmann, B. M., ... & Knights, D. (2018). US immigration westernizes the human gut microbiome. *Cell*, 175(4), 962-972.
1109. Vanhauwaert, Erika et al. "Low-residue and low-fiber diets in gastrointestinal disease management." *Advances in nutrition* (Bethesda, Md.) vol. 6,6 820-7. 13 Nov. 2015, doi:10.3945/an.115.009688
1110. Velázquez, O. C., Seto, R. W., & Rombeau, J. L. (1996). The scientific rationale and clinical application of short-chain fatty acids and medium-chain triacylglycerols. *Proceedings of the Nutrition Society*, 55(1B), 49-78.
1111. van Lanen, A. S., de Bree, A., & Greyling, A. (2021). Efficacy of a low-FODMAP diet in adult irritable bowel syndrome: a systematic review and meta-analysis. *European journal of nutrition*, 60, 3505-3522.
1112. Van Loo, J., Cummings, J., Delzenne, N., Englyst, H., Franck, A., Hopkins, M., ... & Roberfroid, M. Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). *British Journal of Nutrition*. 1999; 81(2), 121-132.
1113. Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., & Smits, G. (1995). On the presence of inulin and oligofructose as natural ingredients in the western diet. *Critical Reviews in Food Science & Nutrition*, 35(6), 525-552.
1114. Van Lanen, A. S., de Bree, A., & Greyling, A. (2021). Efficacy of a low-FODMAP diet in adult irritable bowel syndrome: a systematic review and meta-analysis. *European journal of nutrition*, 60, 3505-3522.
1115. Van Tilburg, A. J. P., De Rooij, F. W. M., Van Blankenstein, M., Van Den Berg, J. W. O., & Bosman-Jacobs, E. P. (1990). Na⁺-dependent bile acid transport in the ileum: the balance between diarrhea and constipation. *Gastroenterology*, 98(1), 25-32.
1116. Varel, V. H. (1987). Activity of fiber-degrading microorganisms in the pig large intestine. *Journal of animal science*, 65(2), 488-496.
1117. Vasant, D. H., Paine, P. A., Black, C. J., Houghton, L. A., Everitt, H. A., Corsetti, M., ... & Ford, A. C. (2021). British Society of Gastroenterology guidelines on the management of irritable bowel syndrome. *Gut*, 70(7), 1214-1240.
1118. Vasquez Ayala, A., Hsu, C. Y., Oles, R. E., Matsuo, K., Loomis, L. R., Buzun, E., ... & Chu, H. (2024). Commensal bacteria promote type I interferon signaling to maintain immune tolerance in mice. *Journal of Experimental Medicine*, 221(1).
1119. Vazquez-Medina, Alejandra, Nicole Rodriguez-Trujillo, Kiara Ayuso-Rodriguez, Fabiana Marini-Martinez, Roberto Angeli-Morales, Gerardo Caussade-Silvestrini, Filipa Godoy-Vitorino, and Nataliya Chorna. "Exploring the interplay between running exercises, microbial diversity, and tryptophan metabolism along the microbiota-gut-brain axis." *Frontiers in Microbiology* 15 (2024): 1326584.
1120. Veiga, P., Pons, N., Agrawal, A., Oozeer, R., Guyonnet, D., Brazeilles, R., ... & Ehrlich, S. D. (2014). Changes of the human gut microbiome induced by a fermented milk product. *Scientific reports*. 2014; 4, 6328.
1121. Velly, H., Britton, R. A., & Preidis, G. A. (2017). Mechanisms of cross-talk between the diet, the intestinal microbiome, and the undernourished host. *Gut microbes*, 8(2), 98-112.
1122. Venegas, D. P., Marjorie, K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., ... & Hermoso, M. A. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in immunology*. 2019;10.
1123. Venter, C. S., Vorster, H. H., & Cummings, J. H. (1990). Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *American Journal of Gastroenterology*, 85(5).
1124. Veraza, D. I., Calderon, G., Jansson-Knodell, C., Aljaras, R., Foster, E. D., Xu, H., ... & Shin, A. (2024). A systematic review and meta-analysis of diet and nutrient intake in adults with irritable bowel syndrome. *Neurogastroenterology & Motility*, 36(1), e14698.
1125. Vernia, P., Latella, G., Magliocca, F. M., Mancuso, G., & LI, R. C. Seeking clues for a positive diagnosis of the irritable bowel syndrome. *European journal of clinical investigation*. 1987;17(3), 189-193.
1126. Vernocchi, Pamela, Federica Del Chierico, and Lorenza Putignani. "Gut microbiota profiling: metabolomics based approach to unravel compounds affecting human health." *Frontiers in microbiology*. 2016;7:1144. doi:10.3389/fmicb.2016.01144.

1127. Vesth, T., Ozen, A., Andersen, S. C., Kaas, R. S., Lukjancenko, O., Bohlin, J., ... & Ussery, D. W. (2013). Veillonella, firmicutes: microbes disguised as gram negatives. *Standards in genomic sciences*, 9(2), 431-448.
1128. Vervier, K.; Moss, S.; Kumar, N.; Adoum, A.; Barne, M.; Browne, H.; Kaser, A.; Kiely, C.J.; Neville, B.A.; Powell, N.; et al. Two microbiota subtypes identified in irritable bowel syndrome with distinct responses to the low FODMAP diet. *Gut* 2022, 71, 1821–1830.
1129. Vieira, A. T., & Vinolo, M. A. R. (2019). Regulation of immune cell function by short chain fatty acids and their impact on arthritis. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (pp. 175-188). Academic Press.
1130. Vijayvargiya, P., Camilleri, M., Burton, D., Busciglio, I., Lueke, A., & Donato, L. J. Bile and fat excretion are biomarkers of clinically significant diarrhoea and constipation in irritable bowel syndrome. *Alimentary pharmacology & therapeutics*. 2019;49(6), 744-758.
1131. Vincent, A. D., Wang, X. Y., Parsons, S. P., Khan, W. I., & Huizinga, J. D. (2018). Abnormal absorptive colonic motor activity in germ-free mice is rectified by butyrate, an effect possibly mediated by mucosal serotonin. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 315(5), G896-G907.
1132. Vinelli, V., Biscotti, P., Martini, D., Del Bo', C., Marino, M., Meroño, T., ... & Riso, P. (2022). Effects of dietary fibers on short-chain fatty acids and gut microbiota composition in healthy adults: a systematic review. *Nutrients*, 14(13), 2559.
1133. Vinolo, M. A., Rodrigues, H. G., Nachbar, R. T., & Curi, R. (2011). Regulation of inflammation by short chain fatty acids. *Nutrients*, 3(10), 858-876.
1134. Voigt, R. M., Forsyth, C. B., Green, S. J., Engen, P. A., & Keshavarzian, A. Circadian rhythm and the gut microbiome. In *International review of neurobiology* (Vol. 131, pp. 193-205). 2016
1135. Vollaard, E. J., & Clasener, H. A. (1994). Colonization resistance. *Antimicrobial agents and chemotherapy*, 38(3), 409-414.
1136. Volta, U., Pinto-Sanchez, M. I., Boschetti, E., Caio, G., De Giorgio, R., & Verdu, E. F. Dietary triggers in irritable bowel syndrome: is there a role for gluten? *Journal of neurogastroenterology and motility*. 2016; 22(4):547.
1137. Von Engelhardt, W., Burmester, M., Hansen, K., Becker, G., & Rechkemmer, G. (1993). Effects of amiloride and ouabain on short-chain fatty acid transport in guinea-pig large intestine. *The Journal of Physiology*, 460(1), 455-466.
1138. Waitzberg, D. L., Pereira, C. C., Logullo, L., Jacintho, T. M., Almeida, D., SILVA, M., & Torrinhas, R. S. (2012). Microbiota benefits after inulin and partially hydrolyzed guar gum supplementation-a randomized clinical trial in constipated women. *Nutricion hospitalaria*.
1139. Wakade, C., Chong, R., Bradley, E., Thomas, B., & Morgan, J. (2014). Upregulation of GPR109A in Parkinson's disease. *PloS one*, 9(10), e109818.
1140. Wald, A. (2007). Chronic constipation: advances in management. *Neurogastroenterology & Motility*, 19(1), 4-10.
1141. Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X., ... & Louis, P. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *The ISME journal*. 2011;5(2), 220-230.
1142. WALKER, A. R. (1974). Dietary fibre and the pattern of diseases. *Annals of internal medicine*, 80(5), 663-664.
1143. Wall, G. C., Bryant, G. A., Bottenberg, M. M., Maki, E. D., & Miesner, A. R. (2014). Irritable bowel syndrome: a concise review of current treatment concepts. *World Journal of Gastroenterology: WJG*, 20(27), 8796.
1144. Walton, C., Fowler, D. P., Turner, C., Jia, W., Whitehead, R. N., Griffiths, L., ... & Cauchi, M. Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases. *Inflammatory bowel diseases*. 2013;19(10), 2069-2078.
1145. Wang, Ben, Shiwei Zhu, Zuoqing Liu, Hui Wei, Lu Zhang, Meibo He, Fei Pei, Jindong Zhang, Qinghua Sun, and Liping Duan. "Increased expression of colonic mucosal melatonin in patients with irritable bowel syndrome correlated with gut dysbiosis." *Genomics, Proteomics and Bioinformatics* 18, no. 6 (2020): 708-720.

1146. Wang, B., Yao, M., Lv, L., Ling, Z., & Li, L. The human microbiota in health and disease. *Engineering*. 2017;3(1), 71-82.
1147. Wang, B., Zhu, S., Liu, Z., Wei, H., Zhang, L., He, M., ... & Duan, L. (2020). Increased expression of colonic mucosal melatonin in patients with irritable bowel syndrome correlated with gut dysbiosis. *Genomics, proteomics & bioinformatics*, 18(6), 708-720.
1148. Wang, L., Cen, S., Wang, G., Lee, Y. K., Zhao, J., Zhang, H., & Chen, W. Acetic acid and butyric acid released in large intestine play different roles in the alleviation of constipation. *Journal of Functional Foods*. 2020;69, 103953.
1149. Wang, L., Alammari, N., Singh, R., Nanavati, J., Song, Y., Chaudhary, R., & Mullin, G. E. (2020). Gut microbial dysbiosis in the irritable bowel syndrome: a systematic review and meta-analysis of case-control studies. *Journal of the Academy of Nutrition and Dietetics*, 120(4), 565-586.
1150. Wang, M., Song, Z., Lai, S., Tang, F., Dou, L., & Yang, F. (2024). Depression-associated gut microbes, metabolites and clinical trials. *Frontiers in Microbiology*, 15, 1292004.
1151. Wang, H. B., Wang, P. Y., Wang, X., Wan, Y. L., & Liu, Y. C. (2012). Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Digestive diseases and sciences*, 57, 3126-3135.
1152. Wang, Z., Chen, W. D., & Wang, Y. D. (2021). Nuclear receptors: a bridge linking the gut microbiome and the host. *Molecular Medicine*, 27(1), 1
1153. Wangel, A. G., & Deller, D. J. (1965). Intestinal motility in man: III. Mechanisms of constipation and diarrhea with particular reference to the irritable colon syndrome. *Gastroenterology*, 48(1), 69-84.
1154. Waseem, R., James-Stevenson, T. N., Siwiec, R. M., Bohm, M., Rogers, N., Wo, J. M., ... & Shin, A. S. (2022). 407: MICROBIAL METABOLITES, ACETATE AND BUTYRATE, ARE LINKED TO ANXIETY IN PATIENTS WITH IRRITABLE BOWEL SYNDROME. *Gastroenterology*, 162(7), S-91.
1155. Watanabe, Y., Nagai, F., & Morotomi, M. Characterization of *Phascolarctobacterium succinatutens* sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. *Applied and Environmental Microbiology*. 2012;78(2), 511-518.
1156. Waters (n.d.). Untargeted Metabolomics & Lipidomics. Retrieved on June 29, 2018 from <http://www.waters.com/webassets/cms/library/docs/720005319en.pdf>
1157. Weaver, G. A., Krause, J. A., Miller, T. L., & Wolin, M. J. (1988). Short chain fatty acid distributions of enema samples from a sigmoidoscopy population: an association of high acetate and low butyrate ratios with adenomatous polyps and colon cancer. *Gut*, 29(11), 1539-1543.
1158. Wei, W., Wang, H.F., Zhang, Y., Zhang, Y.L., Niu, B.Y. and Yao, S.K., 2020. Altered metabolism of bile acids correlates with clinical parameters and the gut microbiota in patients with diarrhea-predominant irritable bowel syndrome. *World Journal of Gastroenterology*, 26(45), p.7153.
1159. Wei S, Zhang J, Liu L, et al. Ratio Analysis NMR Spectroscopy (RANSY) for Selective Metabolite Identification in Complex Samples. *Analytical chemistry*. 2011;83(20):7616-7623. doi:10.1021/ac201625f.
1160. Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., ... & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5(1), 1-18.
1161. Wenzl, H. H., Fine, K. D., Schiller, L. R., & Fordtran, J. S. (1995). Determinants of decreased fecal consistency in patients with diarrhea. *Gastroenterology*, 108(6), 1729-1738.
1162. Whelan, K., Martin, L. D., Staudacher, H. M., & Lomer, M. C. E. The low FODMAP diet in the management of irritable bowel syndrome: an evidence-based review of FODMAP restriction, reintroduction and personalisation in clinical practice. *Journal of Human Nutrition & Dietetics*. 2018;31(2):239-255. doi:10.1111/jhn.12530
1163. White, P. D. (2010). Chronic fatigue syndrome: Is it one discrete syndrome or many? Implications for the "one vs. many" functional somatic syndromes debate. *Journal of Psychosomatic Research*, 68(5), 455-459.
1164. Wickstead, E. S., Solito, E., & McArthur, S. (2022). Promiscuous receptors and neuroinflammation: the formyl peptide class. *Life*, 12(12), 2009
1165. Williams, E. A., Nai, X., & Corfe, B. M. (2011). Dietary intakes in people with irritable bowel syndrome. *BMC gastroenterology*. 2011;11(1), 9.

1166. Williams, E. A., Stimpson, J., Wang, D., Plummer, S., Garaiova, I., Barker, M. E., & Corfe, B. M. (2009). Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Alimentary pharmacology & therapeutics*, 29(1), 97-103.
1167. Williams, R. D., & Olmsted, W. H. (1936). The manner in which food controls the bulk of the feces. *Annals of Internal Medicine*, 10(6), 717-727.
1168. Williams, B. B., Van Benschoten, A. H., Cimermancic, P., Donia, M. S., Zimmermann, M., Taketani, M., ... & Fischbach, M. A. (2014). Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell host & microbe*, 16(4), 495-503.
1169. Whorwell, P. J., Prior, A., & Faragher, E. B. (1984). Controlled trial of hypnotherapy in the treatment of severe refractory irritable-bowel syndrome. *Lancet*, 2(8414), 1232-1234.
1170. Whorwell, P. J., Prior, A., & Colgan, S. M. (1987). Hypnotherapy in severe irritable bowel syndrome: further experience. *Gut*, 28(4), 423-425.
1171. Windgassen, S., Moss-Morris, R., Chilcot, J., Sibelli, A., Goldsmith, K., & Chalder, T. (2017). The journey between brain and gut: A systematic review of psychological mechanisms of treatment effect in irritable bowel syndrome. *British Journal of Health Psychology*, 22(4), 701-736. doi:10.1111/bjhp.12250
1172. Winge, K., Rasmussen, D., & Werdelin, L. (2003). Constipation in neurological diseases. *Journal of neurology, neurosurgery, and psychiatry*, 74(1), 13.
1173. Wishart DS, Feunang YD, Marcu A, et al. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Research*. 2018;46(Database issue):D608-D617. doi:10.1093/nar/gkx1089.
1174. Withrow, D., Bowers, S. J., Depner, C. M., González, A., Reynolds, A. C., & Wright Jr, K. P. (2021). Sleep and circadian disruption and the gut microbiome-possible links to dysregulated metabolism. *Current Opinion in endocrine and metabolic research*, 17, 26-37.
1175. Wolever, T. M., Josse, R. G., Leiter, L. A., & Chiasson, J. L. Time of day and glucose tolerance status affect serum short-chain fatty concentrations in humans. *Metabolism*. 1997;46(7), 805-811.
1176. Wong, B. S., Camilleri, M., Carlson, P., McKinzie, S., Busciglio, I., Bondar, O., ... & Zinsmeister, A. R. (2012). Increased bile acid biosynthesis is associated with irritable bowel syndrome with diarrhea. *Clinical gastroenterology and hepatology*. 2012;10(9), 1009-1015.
1177. Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. Colonic health: fermentation and short chain fatty acids. *Journal of clinical gastroenterology*. 2006;40(3), 235-243.
1178. Wong, R. K., Palsson, O. S., Turner, M. J., Levy, R., Feld, A. D., Von Korff, M., & Whitehead, W. E. (2010). Inability of the Rome III criteria to distinguish functional constipation from constipation subtype irritable bowel syndrome. *The American journal of gastroenterology*, 105(10), 2228.
1179. Woo, H. I., Kwak, S. H., Lee, Y., Choi, J. H., Cho, Y. M., & Om, A. S. (2015). A controlled, randomized, double-blind trial to evaluate the effect of vegetables and whole grain powder that is rich in dietary fibers on bowel functions and defecation in constipated young adults. *Journal of cancer prevention*, 20(1), 64.
1180. Worley, B., & Powers, R. Multivariate analysis in metabolomics. *Current Metabolomics*. 2013;1(1), 92-107.
1181. Wu, G. D., Compher, C., Chen, E. Z., Smith, S. A., Shah, R. D., Bittinger, K., ... & Lewis, J. D. (2016). Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut*, 65(1), 63-72.
1182. Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., ... & Sinha, R. Linking long-term dietary patterns with gut microbial enterotypes. *Science*. 2011;334(6052), 105-108.
1183. Wu, X., Wu, Y., He, L., Wu, L., Wang, X., & Liu, Z. (2018). Effects of the intestinal microbial metabolite butyrate on the development of colorectal cancer. *Journal of Cancer*, 9(14), 2510.
1184. Wu, L., Guo, C., & Wu, J. (2020). Therapeutic potential of PPAR γ natural agonists in liver diseases. *Journal of cellular and molecular medicine*, 24(5), 2736-2748.
1185. Xiao, L., Liu, Q., Luo, M., & Xiong, L. (2021). Gut microbiota-derived metabolites in irritable bowel syndrome. *Frontiers in cellular and infection microbiology*, 11, 729346.
1186. Xie, G., Keyhani, N. O., Bonner, C. A., & Jensen, R. A. (2003). Ancient origin of the tryptophan operon and the dynamics of evolutionary change. *Microbiology and Molecular Biology Reviews*, 67(3), 303-342.
1187. y Abreu, A. A., Milke-García, M. P., Argüello-Arévalo, G. A., Calderón-de la Barca, A. M., Carmona-Sánchez, R. I., Consuelo-Sánchez, A., ... & Vázquez-Frias, R. (2021). Dietary fiber and the microbiota: A

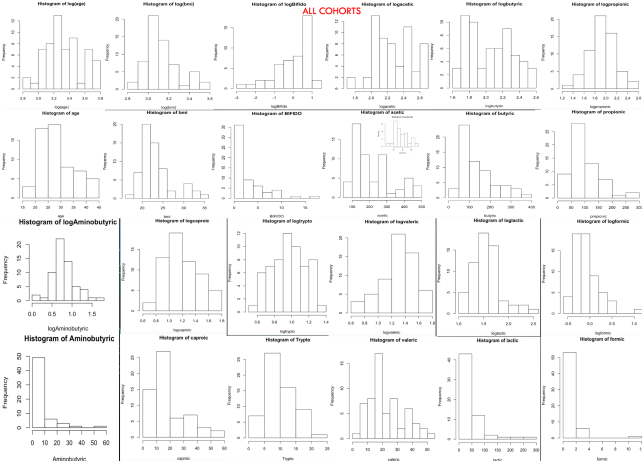
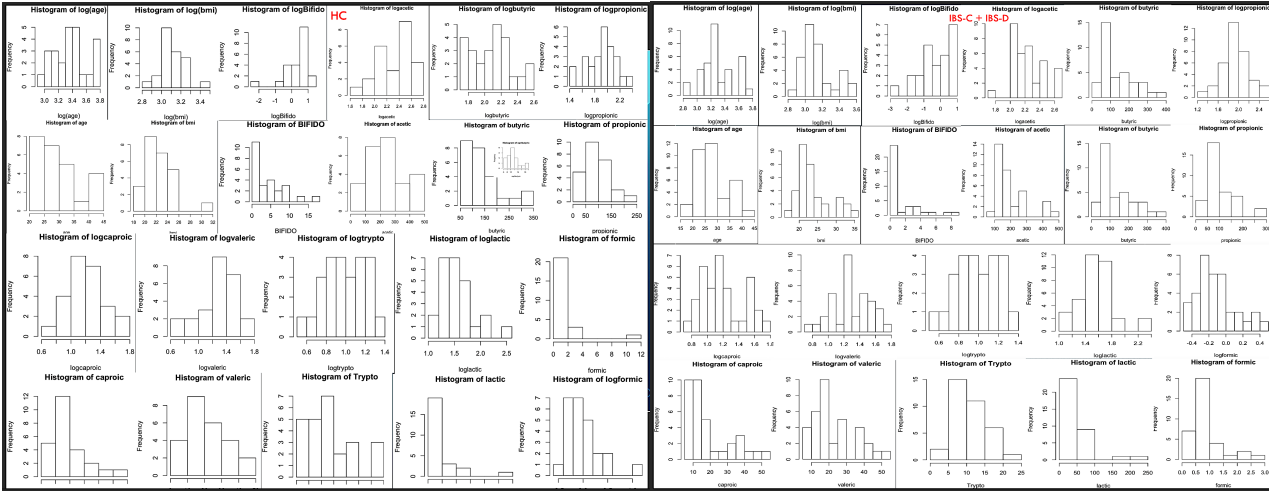
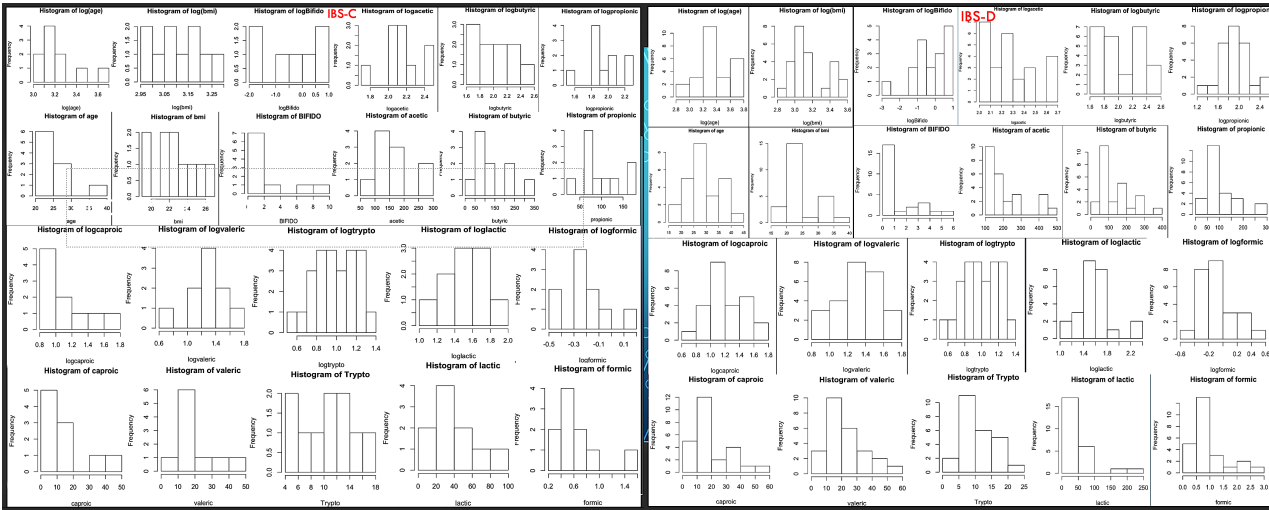
- narrative review by a group of experts from the Asociación Mexicana de Gastroenterología. *Revista de Gastroenterología de México (English Edition)*, 86(3), 287-304.
1188. Yamamura, R., Nakamura, K., Kitada, N., Aizawa, T., Shimizu, Y., Nakamura, K., ... & Tamakoshi, A. (2020). Associations of gut microbiota, dietary intake, and serum short-chain fatty acids with fecal short-chain fatty acids. *Bioscience of microbiota, food and health*, 39(1), 11-17.
 1189. Yan, R., Andrew, L., Marlow, E., Kunaratnam, K., Devine, A., Dunican, I. C., & Christophersen, C. T. (2021). Dietary fibre intervention for gut microbiota, sleep, and mental health in adults with irritable bowel syndrome: a scoping review. *Nutrients*, 13(7), 2159.
 1190. Yan, H., & Ajuwon, K. M. (2017). Butyrate modifies intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins and activation of the Akt signaling pathway. *PloS one*, 12(6), e0179586.
 1191. Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., ... & Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2), 264-276.
 1192. Yang, H., Shi, L., Yao, W., Wang, Y., Huang, L., Wan, D., & Liu, S. (2015). Differentiation of disaccharide isomers by temperature-dependent in-source decay (TDISD) and DART-Q-TOF MS/MS. *Journal of The American Society for Mass Spectrometry*, 26(9), 1599-1605.
 1193. Yang, X., Xie, L., Li, Y., & Wei, C. (2009). More than 9,000,000 unique genes in human gut bacterial community: estimating gene numbers inside a human body. *PloS one*, 4(6), e6074.
 1194. Yang, K., Xu, M., & Zhu, J. Evaluating the Impact of Four Major Nutrients on Gut Microbial Metabolism by a Targeted Metabolomics Approach. *Journal of Proteome Research*. 2020
 1195. Yang, J., Martínez, I., Walter, J., Keshavarzian, A., & Rose, D. J. In vitro characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe*. 2013;23, 74-81.
 1196. Yang, J., Wang, H. P., Zhou, L., & Xu, C. F. (2012). Effect of dietary fiber on constipation: a meta analysis. *World Journal of Gastroenterology: WJG*, 18(48), 7378.
 1197. Yang, J. C., Jacobs, J. P., Hwang, M., Sabui, S., Liang, F., Said, H. M., & Skupsky, J. (2023). Biotin deficiency induces intestinal dysbiosis associated with an inflammatory bowel disease-like phenotype. *Nutrients*, 15(2), 264.
 1198. Yangilar, F. (2013). The application of dietary fibre in food industry: structural features, effects on health and definition, obtaining and analysis of dietary fibre: a review. *Journal of Food and Nutrition Research*, 1(3), 13-23.
 1199. Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., ... & Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2), 264-276.
 1200. Yanofsky, C. (1960). The tryptophan synthetase system. *Bacteriological reviews*, 24(2), 221-245.
 1201. Yatsunenkov, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., ... & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *nature*, 486(7402), 222-227.
 1202. Ye, J., Lv, L., Wu, W., Li, Y., Shi, D., Fang, D., ... & Li, L. (2018). Butyrate protects mice against methionine–choline-deficient diet-induced non-alcoholic steatohepatitis by improving gut barrier function, attenuating inflammation and reducing endotoxin levels. *Frontiers in microbiology*, 9, 1967.
 1203. Yin, J., Song, Y., Hu, Y., Wang, Y., Zhang, B., Wang, J., ... & Wang, S. (2021). Dose-dependent beneficial effects of tryptophan and its derived metabolites on *Akkermansia* In Vitro: A preliminary prospective study. *Microorganisms*, 9(7), 1511.
 1204. Yin, X., Lee, B., Zaragoza, J., & Marco, M. L. (2017). Dietary perturbations alter the ecological significance of ingested *Lactobacillus plantarum* in the digestive tract. *Scientific reports*, 7(1), 7267.
 1205. Yokokura, T., Yajima, T., & Hashimoto, S. (1977). Effect of organic acid on gastrointestinal motility of rat invitro. *Life sciences*, 21(1), 59-61.
 1206. Yoshiuchi, K., Inada, S., & Kikuchi, H. Food diaries: use of new technology versus the written record. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. 2010;5(048), 1-5.
 1207. Yoshii, K., Hosomi, K., Sawane, K., & Kunisawa, J. (2019). Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. *Frontiers in nutrition*, 6, 48.

1208. You, A. (2015). Dietary guidelines for Americans. US Department of Health and Human Services and US Department of Agriculture, 7.
1209. Yousefi-Mashouf, R., Eley, A., & Duerden, B. I. (1992). Characterisation of non-pigmented species of the genus *Prevotella* by polyacrylamide gel electrophoresis. *Journal of medical microbiology*. 1992;37(2), 83-90.
1210. Yuan, X., Wang, L., Bhat, O. M., Lohner, H., & Li, P. L. (2018). Differential effects of short chain fatty acids on endothelial Nlrp3 inflammasome activation and neointima formation: antioxidant action of butyrate. *Redox Biology*, 16, 21-31
1211. Yurrita, L. C., Martín, I. S. M., Calle-Purón, M. E., & Cabria, M. H. (2014). Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled randomized clinical trials. *Nutricion hospitalaria*, 30(2), 244-252.
1212. Xu, D., Wu, X., Grabauskas, G., & Owyang, C. (2013). Butyrate-induced colonic hypersensitivity is mediated by mitogen-activated protein kinase activation in rat dorsal root ganglia. *Gut*, 62(10), 1466-1474.
1213. Załęski, A., Banaszkiewicz, A., & Walkowiak, J. Butyric acid in irritable bowel syndrome. *Przegląd gastroenterologiczny*. 2013;8(6), 350.
1214. Zaribaf, F., Keshteli, A. H., Esmailzadeh, A., Saneei, P., Feizi, A., Daghighzadeh, H., ... & Adibi, P. (2018). Empirically derived dietary habits are associated with irritable bowel syndrome. *European journal of clinical nutrition*, 72(11), 1537-1547.
1215. Zeber-Lubecka N, Kulecka M, Ambrozkiwicz F, et al. Limited prolonged effects of rifaximin treatment on irritable bowel syndrome-related differences in the fecal microbiome and metabolome. *Gut Microbes*. 2016;7(5):397-413. doi:10.1080/19490976.2016.1215805.
1216. Zeng, H., Umar, S., Rust, B., Lazarova, D., & Bordonaro, M. (2019). Secondary bile acids and short chain fatty acids in the colon: a focus on colonic microbiome, cell proliferation, inflammation, and cancer. *International journal of molecular sciences*, 20(5), 1214.
1217. Zeng, J., Y-Q. LI, X-L. ZUO, Y-B. ZHEN, J. Yang, and C-H. LIU. "Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome." *Alimentary pharmacology & therapeutics* 28, no. 8 (2008): 994-1002.
1218. Zeng, H., Hamlin, S. K., Safratowich, B. D., Cheng, W. H., & Johnson, L. K. (2020). Superior inhibitory efficacy of butyrate over propionate and acetate against human colon cancer cell proliferation via cell cycle arrest and apoptosis: Linking dietary fiber to cancer prevention. *Nutrition Research*, 83, 63-72.
1219. Zhan, Z., Liu, W., Pan, L., Bao, Y., Yan, Z., & Hong, L. (2022). Overabundance of *Veillonella parvula* promotes intestinal inflammation by activating macrophages via LPS-TLR4 pathway. *Cell Death Discovery*, 8(1), 251.
1220. Zhan, K., Jiang, M., Gong, X., & Zhao, G. Effect of short-chain fatty acids on the expression of genes involved in short-chain fatty acid transporters and inflammatory response in goat jejunum epithelial cells. *In Vitro Cellular & Developmental Biology-Animal*. 2018; 54(4):311-320. doi:10.1007/s11626-017-0226-2
1221. Zhang, Y., Li, Y., Barber, A. F., Noya, S. B., Williams, J. A., Li, F., ... & Sehgal, A. (2023). The microbiome stabilizes circadian rhythms in the gut. *Proceedings of the National Academy of Sciences*, 120(5), e2217532120.
1222. Zhang, Q., Yun, Y., An, H., Zhao, W., Ma, T., Wang, Z., & Yang, F. (2021). Gut microbiome composition associated with major depressive disorder and sleep quality. *Frontiers in Psychiatry*, 12, 645045.
1223. Zhang, W. X., Zhang, Y., Qin, G., Li, K. M., Wei, W., Li, S. Y., & Yao, S. K. (2019). Altered profiles of fecal metabolites correlate with visceral hypersensitivity and may contribute to symptom severity of diarrhea-predominant irritable bowel syndrome. *World journal of gastroenterology*, 25(43), 6416.
1224. Zhang, T., Ma, X., Tian, W., Zhang, J., Wei, Y., Zhang, B., ... & Tang, X. (2022). Global research trends in irritable bowel syndrome: a bibliometric and visualized study. *Frontiers in Medicine*, 9, 922063.
1225. Zhang, T., Zhang, C., Zhang, J., Sun, F., & Duan, L. (2022). Efficacy of probiotics for irritable bowel syndrome: a systematic review and network meta-analysis. *Frontiers in cellular and infection microbiology*, 12, 349.

1226. Zhang, Z., Hyun, J. E., Thiesen, A., Park, H., Hotte, N., Watanabe, H., ... & Madsen, K. L. (2020). Sex-specific differences in the gut microbiome in response to dietary fiber supplementation in il-10-deficient mice. *Nutrients*, 12(7), 2088.
1227. Zhang, X., Tian, H., Gu, L., Nie, Y., Ding, C., Ge, X., ... & Li, N. (2018). Long-term follow-up of the effects of fecal microbiota transplantation in combination with soluble dietary fiber as a therapeutic regimen in slow transit constipation. *Science China Life Sciences*, 61(7), 779-786.
1228. Zhang, J., Song, L., Wang, Y., Liu, C., Zhang, L., Zhu, S., ... & Duan, L. (2019). Beneficial effect of butyrate-producing Lachnospiraceae on stress-induced visceral hypersensitivity in rats. *Journal of gastroenterology and hepatology*, 34(8), 1368-1376.
1229. Zhang, Z., Tang, H., Chen, P., Xie, H., & Tao, Y. (2019). Demystifying the manipulation of host immunity, metabolism, and extraintestinal tumors by the gut microbiome. *Signal transduction and targeted therapy*, 4(1), 1-34.
1230. Zhang B, Powers R. Analysis of bacterial biofilms using NMR-based metabolomics. *Future medicinal chemistry*. 2012;4(10):1273-1306. doi:10.4155/fmc.12.59.
1231. Zhang, L. X., Cooney, R. V., & Bertram, J. S. (1991). Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis*, 12(11), 2109-2114
1232. Zhang, Yi-Wen, Feng Nie, Xiao-Yu Zheng, and Shu-Juan Zhao. "L-tyrosine metabolic pathway in microorganisms and its application in the biosynthesis of plant-derived natural products." *World Journal of Traditional Chinese Medicine* 8, no. 3 (2022): 386-394.
1233. Zhao, L., Yang, W., Chen, Y., Huang, F., Lu, L., Lin, C., ... & Bian, Z. (2020). A Clostridia-rich microbiota enhances bile acid excretion in diarrhea-predominant irritable bowel syndrome. *The Journal of clinical investigation*, 130(1), 438-450.
1234. Zhao, L., Yang, W., Chen, Y., Huang, F., Lu, L., Lin, C., ... & Bian, Z. (2020). A Clostridia-rich microbiota enhances bile acid excretion in diarrhea-predominant irritable bowel syndrome. *The Journal of clinical investigation*, 130(1), 438-450.
1235. Zhao, Y., Wu, J., Li, J. V., Zhou, N. Y., Tang, H., & Wang, Y. (2013). Gut microbiota composition modifies fecal metabolic profiles in mice. *Journal of proteome research*, 12(6), 2987-2999.
1236. Zheng, Z., Huang, C., Guo, Y., Niu, K., Momma, H., Kobayashi, Y., ... Nagatomi, R. (2015). Staple foods consumption and irritable bowel syndrome in Japanese adults: a cross-sectional study. *PloS one*, 10(3), e0119097. doi:10.1371/journal.pone.0119097
1237. Zheng, L., Kelly, C. J., Battista, K. D., Schaefer, R., Lanis, J. M., Alexeev, E. E., ... & Colgan, S. P. (2017). Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. *The Journal of Immunology*. 2017;199(8), 2976-2984.
1238. Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Research*, 1-15.
1239. Zheng X, Qiu Y, Zhong W, et al. A targeted metabolomic protocol for short-chain fatty acids and branched-chain amino acids. *Metabolomics : Official journal of the Metabolomic Society*. 2013;9(4):818-827. doi:10.1007/s11306-013-0500-6.
1240. Zhong, W., Lu, X., Shi, H., Zhao, G., Song, Y., Wang, Y., ... & Wang, S. (2019). Distinct microbial populations exist in the mucosa-associated microbiota of diarrhea predominant irritable bowel syndrome and ulcerative colitis. *Journal of clinical gastroenterology*, 53(9), 660-672.
1241. Zhou, Q., Zhang, B., & Verne, N. G. (2009). Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *Pain*, 146(1), 41-46.
1242. Zhou, D., Chen, Y. W., Zhao, Z. H., Yang, R. X., Xin, F. Z., Liu, X. L., ... & Fan, J. G. (2018). Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Experimental & molecular medicine*, 50(12), 1-12.
1243. Zhou, J., Wu, X., Li, Z., Zou, Z., Dou, S., Li, G., ... & Li, Y. (2022). Alterations in gut microbiota are correlated with serum metabolites in patients with insomnia disorder. *Frontiers in Cellular and Infection Microbiology*, 12, 722662.
1244. Zhu, L., Liu, W., Alkhouri, R., Baker, R. D., Bard, J. E., Quigley, E. M., & Baker, S. S. Structural changes in the gut microbiome of constipated patients. *Physiological genomics*. 2014;46(18), 679-686.

1245. Zhu, S., Liu, S., Li, H., Zhang, Z., Zhang, Q., Chen, L., ... & Zhang, S. (2019). Identification of gut microbiota and metabolites signature in patients with irritable bowel syndrome. *Frontiers in cellular and infection microbiology*, 9, 346.
1246. Zhuang, X., Xiong, L., Li, L., Li, M., & Chen, M. Alterations of gut microbiota in patients with irritable bowel syndrome: A systematic review and meta-analysis. *Journal of gastroenterology and hepatology*. 2017;32(1), 28-38.
1247. Zhuang, X., Xiong, L., Tian, Z., Li, L., Zeng, Z., & Chen, M. Fecal Microbiota Alterations Associated With Diarrhea-Predominant Irritable Bowel Syndrome. *Frontiers in microbiology*. 2018; 9, 1600.
1248. Zhuang, M., Shang, W., Ma, Q., Strappe, P., & Zhou, Z. (2019). Abundance of probiotics and butyrate-production microbiome manages constipation via short-chain fatty acids production and hormones secretion. *Molecular nutrition & food research*, 63(23), 1801187.
1249. Zia, J. K., Lenhart, A., Yang, P. L., Heitkemper, M. M., Baker, J., Keefer, L., ... & Chang, L. (2022). Risk Factors for Abdominal Pain Disorders of Gut Brain Interaction in Adults and Children: A Systematic Review. *Gastroenterology*.
1250. Zia, J., Chung, C. F., Xu, K., Dong, Y., Schenk, J. M., Cain, K., ... & Heitkemper, M. M. Inter-Rater Reliability of Provider Interpretations of Irritable Bowel Syndrome Food and Symptom Journals. *Journal of clinical medicine*. 2017;6(11):105.
1251. Zierer, J., Jackson, M. A., Kastenmuller, G., Mangino, M., Long, T., Telenti, A., . . Menni, C. The fecal metabolome as a functional readout of the gut microbiome. *Nature Genetics*. 2018; 50(6):790-795. doi:10.1038/s41588-018-0135-7
1252. Zimmer, J., Lange, B., Frick, J. S., Sauer, H., Zimmermann, K., Schwartz, A., ... & Enck, P. (2012). A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. *European journal of clinical nutrition*, 66(1), 53-60.
1253. Zimmermann, J., Kaleta, C., & Waschina, S. gapseq: Informed prediction of bacterial metabolic pathways and reconstruction of accurate metabolic models. *bioRxiv*. 2020.
1254. Zimmerman MA, Singh N, Martin PM et al. Butyrate suppresses colonic inflammation through HDAC1-dependent Fas upregulation and Fas- mediated apoptosis of T cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2012;302:61405–15.
1255. Zimmerman, M. A., Singh, N., Martin, P. M., Thangaraju, M., Ganapathy, V., Waller, J. L., ... & Liu, K. (2012). Butyrate suppresses colonic inflammation through HDAC1-dependent Fas upregulation and Fas-mediated apoptosis of T cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 302(12), G1405-G1415.
1256. Zuckerman, M. J. (2006). The role of fiber in the treatment of irritable bowel syndrome: therapeutic recommendations. *Journal of clinical gastroenterology*, 40(2), 104-108.

SUPPLEMENTAL



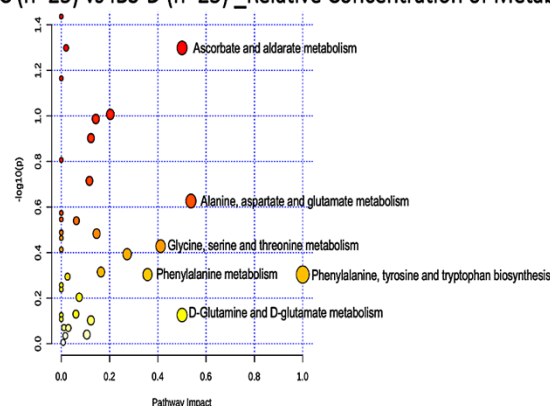
Results from HC vs IBS-D Pathway analysis using the human metabolic database (KEGG) using the Globaltest pathway enrichment analysis method from the Relative metabolite concentration that is normalized by fecal dry weight and TSP.

Biomarker analysis was also done for HC vs IBS-D to identify a metabolite or a set of metabolites capable of classifying conditions or disease with high sensitivity (true-positive rate) and specificity (true negative rate). Metabolite set enrichment ratios was done to identify significantly enriched metabolites from the NMR data. Ratios between two metabolite concentrations may provide more meaningful biological information. MetaboAnalystR generated ratios and the top ranked ratios (based on p-values) are listed first.

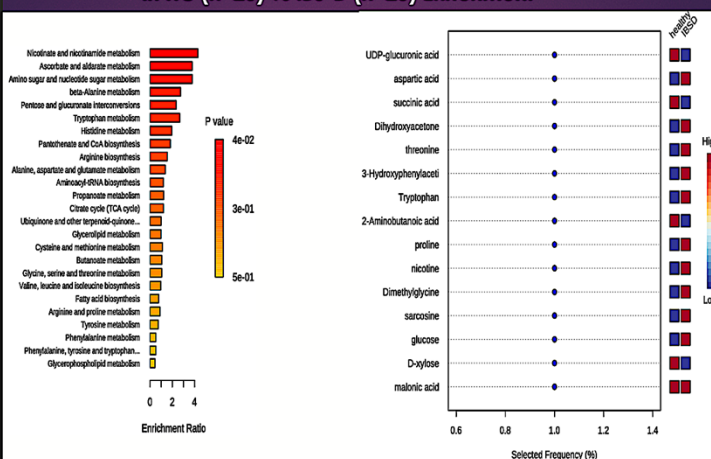
Table 2: Result from Quantitative Enrichment Analysis

	Total Compd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR
Nicotinate and nicotinamide metabolism	15	1	8.61	2.00	3.67E-02	1.00E+00	7.64E-01
Ascorbate and aldarate metabolism	8	1	7.59	2.00	5.03E-02	1.00E+00	7.64E-01
Amino sugar and nucleotide sugar metabolism	37	1	7.59	2.00	5.03E-02	1.00E+00	7.64E-01
beta-Alanine metabolism	21	2	5.48	2.00	6.85E-02	1.00E+00	7.19E-01
Pentose and glucuronate interconversions	18	2	4.69	2.00	9.84E-02	1.00E+00	7.22E-01
Tryptophan metabolism	41	1	5.33	2.00	1.03E-01	1.00E+00	7.22E-01
Histidine metabolism	16	3	3.93	2.00	1.25E-01	1.00E+00	7.51E-01
Pantothenate and CoA biosynthesis	19	3	3.62	2.00	1.56E-01	1.00E+00	8.09E-01
Arginine biosynthesis	14	4	3.09	2.00	1.93E-01	1.00E+00	8.09E-01
Alanine, aspartate and glutamate metabolism	28	6	2.72	2.00	2.36E-01	1.00E+00	8.09E-01
Aminoacyl-tRNA biosynthesis	48	14	2.39	2.00	2.67E-01	1.00E+00	8.09E-01
Propanoate metabolism	23	2	2.43	2.00	2.84E-01	1.00E+00	8.09E-01
Citrate cycle (TCA cycle)	20	2	2.40	2.00	2.88E-01	1.00E+00	8.09E-01
Ubiquinone and other terpenoid-quinone biosynthesis	9	1	1.99	2.00	3.24E-01	1.00E+00	8.09E-01
Glycerolipid metabolism	16	1	1.97	2.00	3.25E-01	1.00E+00	8.09E-01
Cysteine and methionine metabolism	33	2	2.23	2.00	3.29E-01	1.00E+00	8.09E-01
Butanoate metabolism	15	3	2.12	2.00	3.44E-01	1.00E+00	8.09E-01
Glycine, serine and threonine metabolism	33	5	2.10	2.00	3.73E-01	1.00E+00	8.09E-01
Valine, leucine and isoleucine biosynthesis	8	4	1.92	2.00	3.86E-01	1.00E+00	8.09E-01
Fatty acid biosynthesis	47	1	1.52	2.00	3.89E-01	1.00E+00	8.09E-01
Arginine and proline metabolism	38	3	1.80	2.00	4.04E-01	1.00E+00	8.09E-01
Tyrosine metabolism	42	2	1.46	2.00	4.84E-01	1.00E+00	8.53E-01
Phenylalanine metabolism	10	2	1.63	2.00	4.97E-01	1.00E+00	8.53E-01
Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	1.63	2.00	4.97E-01	1.00E+00	8.53E-01
Glycerophospholipid metabolism	36	1	0.90	2.00	5.08E-01	1.00E+00	8.53E-01
Lysine degradation	25	1	0.72	2.00	5.53E-01	1.00E+00	8.60E-01
Biotin metabolism	10	1	0.72	2.00	5.53E-01	1.00E+00	8.60E-01
Selenocompound metabolism	20	1	0.64	2.00	5.78E-01	1.00E+00	8.67E-01
Pyrimidine metabolism	39	2	0.92	2.00	6.25E-01	1.00E+00	8.73E-01
Glycolysis / Gluconeogenesis	26	2	0.65	2.00	7.06E-01	1.00E+00	8.73E-01
Galactose metabolism	27	1	0.25	2.00	7.28E-01	1.00E+00	8.73E-01
Starch and sucrose metabolism	18	1	0.25	2.00	7.28E-01	1.00E+00	8.73E-01
Neomycin, kanamycin and gentamicin biosynthesis	2	1	0.25	2.00	7.28E-01	1.00E+00	8.73E-01
Pyruvate metabolism	22	3	0.81	2.00	7.41E-01	1.00E+00	8.73E-01
D-Glutamine and D-glutamate metabolism	6	4	0.55	2.00	7.48E-01	1.00E+00	8.73E-01
Nitrogen metabolism	6	2	0.55	2.00	7.48E-01	1.00E+00	8.73E-01
Porphyry and chlorophyll metabolism	30	2	0.46	2.00	7.81E-01	1.00E+00	8.73E-01
Glutathione metabolism	28	4	0.78	2.00	7.90E-01	1.00E+00	8.73E-01
Valine, leucine and isoleucine degradation	40	3	0.35	2.00	8.50E-01	1.00E+00	9.16E-01
Glyoxylate and dicarboxylate metabolism	32	5	0.54	2.00	9.12E-01	1.00E+00	9.46E-01
Purine metabolism	65	2	0.16	2.00	9.23E-01	1.00E+00	9.46E-01
Primary bile acid biosynthesis	46	1	0.00	2.00	9.86E-01	1.00E+00	9.86E-01

HC (n=25) vs IBS-D (n=25) _Relative Concentration of Metabolites



Quantitative Enrichment Analysis and Metabolic Marker Discovery in HC (n=25) vs IBS-D (n=25) Enrichment



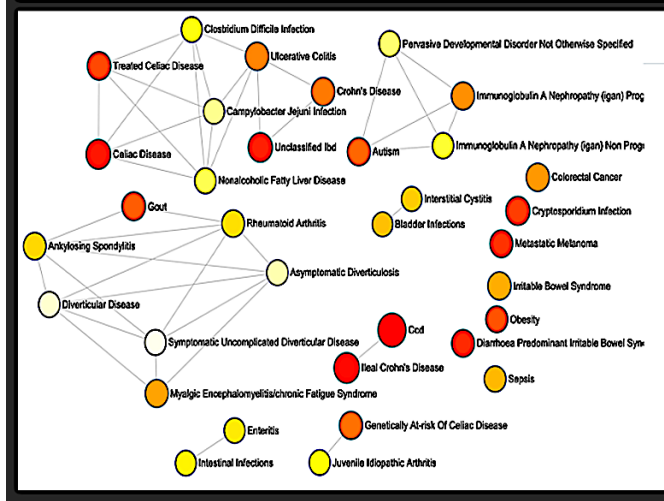
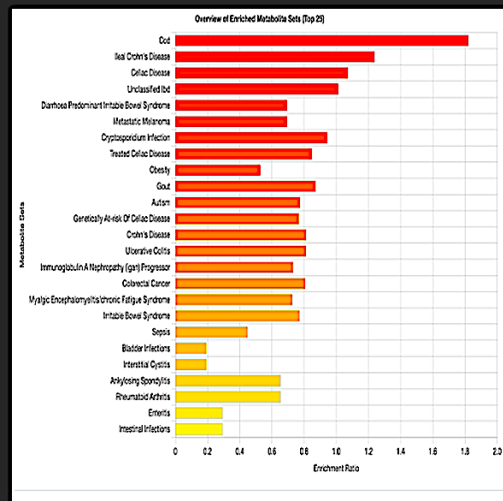
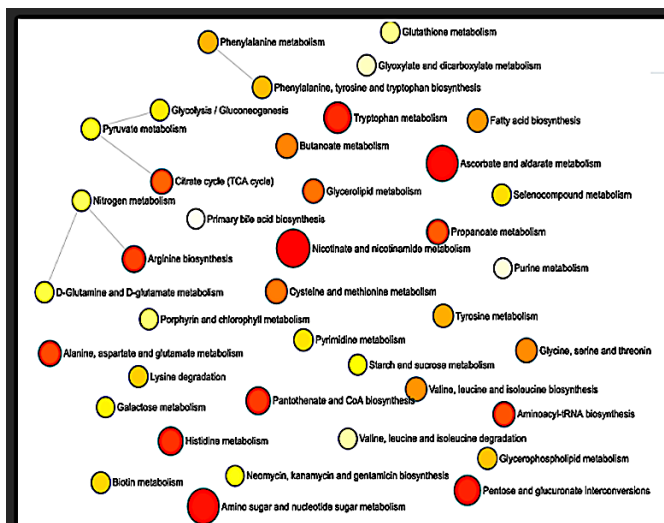


Table 2: Result from Pathway Analysis

Pathway	Total Compd	Hits	Raw p	-log10(p)	Benjamini adjusted	FDR	Impact
Nicotinate and nicotinamide metabolism	15	1	2.48E-02	1.61E+01	1.61E+01	3.51E-01	0.00
Beta-Alanine metabolism	21	2	2.83E-02	1.55E+01	1.55E+01	3.51E-01	0.00
Alanine, aspartate and glutamate metabolism	28	6	4.12E-02	1.39E+01	1.00E+01	3.51E-01	0.54
Butanoate metabolism	15	3	5.44E-02	1.26E+01	1.00E+01	3.51E-01	0.00
Propanoate metabolism	23	2	5.50E-02	1.26E+01	1.00E+01	3.51E-01	0.00
Citrate cycle (TCA cycle)	30	2	5.82E-02	1.24E+01	1.00E+01	3.51E-01	0.06
Histidine metabolism	15	3	6.46E-02	1.19E+01	1.00E+01	3.51E-01	0.12
Pantoic acid and CoA biosynthesis	19	3	8.11E-02	1.09E+01	1.00E+01	3.51E-01	0.00
Arginine biosynthesis	14	4	1.01E-01	9.94E+00	1.00E+01	4.28E-01	0.12
Tryptophan metabolism	41	1	1.39E-01	8.37E+00	1.00E+01	4.92E-01	0.14
Ascorbate and aldarate metabolism	8	1	1.56E-01	8.07E+00	1.00E+01	4.92E-01	0.20
Amino sugar and nucleotide sugar metabolism	37	1	1.56E-01	8.07E+00	1.00E+01	4.92E-01	0.02
Glycerophospholipid metabolism	30	1	1.68E-01	7.74E+00	1.00E+01	4.92E-01	0.03
Glycine, serine and threonine metabolism	33	5	2.61E-01	5.84E+00	1.00E+01	6.01E-01	0.41
Pyrimidine metabolism	30	2	2.61E-01	5.84E+00	1.00E+01	6.01E-01	0.07
Aminoacyl-tRNA biosynthesis	48	14	2.81E-01	5.52E+00	1.00E+01	6.01E-01	0.00
Pentose and glucuronate interconversions	18	2	2.83E-01	5.49E+00	1.00E+01	6.01E-01	0.20
Valine, leucine and isoleucine biosynthesis	8	4	3.36E-01	4.73E+00	1.00E+01	6.01E-01	0.00
Selenocompound metabolism	20	1	3.40E-01	4.60E+00	1.00E+01	6.01E-01	0.00
Glycerolipid metabolism	16	1	3.40E-01	4.60E+00	1.00E+01	6.01E-01	0.00
Glyoxylate and dicarboxylate metabolism	32	5	3.55E-01	4.50E+00	1.00E+01	6.01E-01	0.11
Ubiquinone and other terpenoid-quinone biosynthesis	9	1	3.94E-01	4.04E+00	1.00E+01	6.01E-01	0.00
Arginine and proline metabolism	38	3	4.01E-01	3.97E+00	1.00E+01	6.01E-01	0.27
D-Glutamine and D-glutamate metabolism	6	2	4.03E-01	3.95E+00	1.00E+01	6.01E-01	0.50
Nitrogen metabolism	6	2	4.03E-01	3.95E+00	1.00E+01	6.01E-01	0.00
Porphyria and chlorophyll metabolism	30	2	4.11E-01	3.86E+00	1.00E+01	6.01E-01	0.00
Pyruvate metabolism	22	3	4.50E-01	3.47E+00	1.00E+01	6.33E-01	0.06
Tyrosine metabolism	42	2	4.91E-01	3.09E+00	1.00E+01	6.66E-01	0.16
Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	5.69E-01	2.45E+00	1.00E+01	7.14E-01	1.00
Glutathione metabolism	10	2	5.69E-01	2.45E+00	1.00E+01	7.14E-01	0.36
Glycolysis / Gluconeogenesis	28	4	5.83E-01	2.35E+00	1.00E+01	7.14E-01	0.12
Oxidation-reduction	26	3	6.06E-01	2.17E+00	1.00E+01	7.20E-01	0.03
Valine, leucine and isoleucine degradation	40	3	6.77E-01	1.70E+00	1.00E+01	7.70E-01	0.01
Primary bile acid biosynthesis	46	1	7.01E-01	1.54E+00	1.00E+01	7.80E-01	0.01
Cysteine and methionine metabolism	33	2	7.27E-01	1.38E+00	1.00E+01	7.90E-01	0.15
Lysine degradation	25	1	8.72E-01	0.95E+00	1.00E+01	8.96E-01	0.00
Biotin metabolism	10	1	8.72E-01	0.95E+00	1.00E+01	8.96E-01	0.00
Purine metabolism	65	2	9.08E-01	0.90E+00	1.00E+01	9.08E-01	0.02

Enrichment Overview (top 25)

KEGG_HC vs. IBS-C

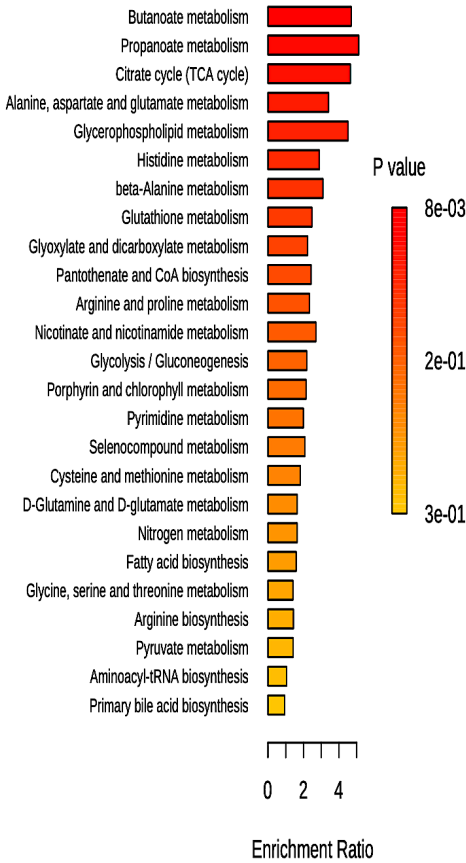


Figure 1: Summary plot for Quantitative Enrichment Analysis (QEA).

Table 2: Result from Quantitative Enrichment Analysis

	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR
Butanoate metabolism	15	3	13.78	2.94	8.00E-03	3.36E-01	1.49E-01
Propanoate metabolism	23	2	15.05	2.94	9.29E-03	3.81E-01	1.49E-01
Citrate cycle (TCA cycle)	20	2	13.66	2.94	1.42E-02	5.67E-01	1.49E-01
Alanine, aspartate and glutamate metabolism	28	6	10.05	2.94	1.42E-02	5.67E-01	1.49E-01
Glycerophospholipid metabolism	36	1	13.26	2.94	3.15E-02	1.00E+00	2.65E-01
Histidine metabolism	16	3	8.52	2.94	4.60E-02	1.00E+00	2.95E-01
beta-Alanine metabolism	21	2	9.10	2.94	4.92E-02	1.00E+00	2.95E-01
Glutathione metabolism	28	4	7.28	2.94	7.75E-02	1.00E+00	3.54E-01
Glyoxylate and dicarboxylate metabolism	32	5	6.57	2.94	7.87E-02	1.00E+00	3.54E-01
Pantothenate and CoA biosynthesis	19	3	7.15	2.94	9.11E-02	1.00E+00	3.54E-01
Arginine and proline metabolism	38	3	6.87	2.94	9.96E-02	1.00E+00	3.54E-01
Nicotinate and nicotinamide metabolism	15	1	7.94	2.94	1.01E-01	1.00E+00	3.54E-01
Glycolysis / Gluconeogenesis	26	2	6.43	2.94	1.22E-01	1.00E+00	3.81E-01
Porphyrin and chlorophyll metabolism	30	2	6.32	2.94	1.27E-01	1.00E+00	3.81E-01
Pyrimidine metabolism	39	2	5.86	2.94	1.40E-01	1.00E+00	3.92E-01
Selenocompound metabolism	20	1	6.12	2.94	1.52E-01	1.00E+00	3.99E-01
Cysteine and methionine metabolism	33	2	5.35	2.94	1.62E-01	1.00E+00	4.00E-01
D-Glutamine and D-glutamate metabolism	6	4	4.85	2.94	1.90E-01	1.00E+00	4.20E-01
Nitrogen metabolism	6	2	4.85	2.94	1.90E-01	1.00E+00	4.20E-01
Fatty acid biosynthesis	47	1	4.68	2.94	2.12E-01	1.00E+00	4.28E-01
Glycine, serine and threonine metabolism	33	5	4.14	2.94	2.14E-01	1.00E+00	4.28E-01
Arginine biosynthesis	14	4	4.22	2.94	2.24E-01	1.00E+00	4.28E-01
Pyruvate metabolism	22	3	4.17	2.94	2.35E-01	1.00E+00	4.29E-01
Aminoacyl-tRNA biosynthesis	48	14	3.09	2.94	3.15E-01	1.00E+00	5.51E-01
Primary bile acid biosynthesis	46	1	2.77	2.94	3.39E-01	1.00E+00	5.70E-01
Valine, leucine and isoleucine degradation	40	3	2.88	2.94	3.75E-01	1.00E+00	6.05E-01
Valine, leucine and isoleucine biosynthesis	8	4	2.71	2.94	4.00E-01	1.00E+00	6.23E-01
Glycerolipid metabolism	16	1	1.83	2.94	4.38E-01	1.00E+00	6.57E-01
Lysine degradation	25	1	1.14	2.94	5.42E-01	1.00E+00	7.58E-01
Biotin metabolism	10	1	1.14	2.94	5.42E-01	1.00E+00	7.58E-01
Tryptophan metabolism	41	1	0.86	2.94	5.96E-01	1.00E+00	7.68E-01
Galactose metabolism	27	1	0.75	2.94	6.22E-01	1.00E+00	7.68E-01
Starch and sucrose metabolism	18	1	0.75	2.94	6.22E-01	1.00E+00	7.68E-01
Neomycin, kanamycin and gentamicin biosynthesis	2	1	0.75	2.94	6.22E-01	1.00E+00	7.68E-01
Ascorbate and aldarate metabolism	8	1	0.21	2.94	7.96E-01	1.00E+00	9.27E-01
Amino sugar and nucleotide sugar metabolism	37	1	0.21	2.94	7.96E-01	1.00E+00	9.27E-01
Ubiquinone and other terpenoid-quinone biosynthesis	9	1	0.16	2.94	8.17E-01	1.00E+00	9.27E-01
Phenylalanine metabolism	10	2	0.24	2.94	8.61E-01	1.00E+00	9.27E-01
Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	0.24	2.94	8.61E-01	1.00E+00	9.27E-01
Pentose and glucuronate interconversions	18	2	0.35	2.94	8.91E-01	1.00E+00	9.36E-01
Purine metabolism	65	2	0.15	2.94	9.49E-01	1.00E+00	9.73E-01
Tyrosine metabolism	42	2	0.08	2.94	9.75E-01	1.00E+00	9.75E-01

Disease Signatures_HC vs. IBS-C

Table 2: Result from Quantitative Enrichment Analysis

	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR
Genetically At-risk Of Celiac Disease	5	5	10.06	2.94	2.17E-02	7.38E-01	2.93E-01
Sepsis	22	4	11.84	2.94	2.47E-02	8.14E-01	2.93E-01
Gout	28	16	8.26	2.94	2.79E-02	8.92E-01	2.93E-01
Cryptosporidium Infection	29	10	7.87	2.94	3.96E-02	1.00E+00	2.93E-01
Obesity	14	2	9.76	2.94	6.77E-02	1.00E+00	2.93E-01
Unclassified Ibd	84	18	6.32	2.94	8.23E-02	1.00E+00	2.93E-01
Crohn's Disease	522	71	5.62	2.94	1.02E-01	1.00E+00	2.93E-01
Enteritis	8	2	7.13	2.94	1.05E-01	1.00E+00	2.93E-01
Intestinal Infections	8	2	7.13	2.94	1.05E-01	1.00E+00	2.93E-01
Ulcerative Colitis	609	56	5.16	2.94	1.14E-01	1.00E+00	2.93E-01
Irritable Bowel Syndrome	186	56	5.05	2.94	1.16E-01	1.00E+00	2.93E-01
Myalgic Encephalomyelitis/chronic Fatigue Syndrome	22	17	5.32	2.94	1.21E-01	1.00E+00	2.93E-01
Campylobacter Jejuni Infection	150	3	5.99	2.94	1.21E-01	1.00E+00	2.93E-01
Asymptomatic Diverticulosis	22	13	4.86	2.94	1.41E-01	1.00E+00	2.93E-01
Diverticular Disease	22	13	4.86	2.94	1.41E-01	1.00E+00	2.93E-01
Symptomatic Uncomplicated Diverticular Disease	22	13	4.86	2.94	1.41E-01	1.00E+00	2.93E-01
Clostridium Difficile Infection	139	4	5.43	2.94	1.49E-01	1.00E+00	2.93E-01
Colorectal Cancer	1653	72	4.36	2.94	1.55E-01	1.00E+00	2.93E-01
Juvenile Idiopathic Arthritis	3	3	5.11	2.94	1.73E-01	1.00E+00	3.10E-01
Immunoglobulin A Nephropathy (igan) Non Progressor	66	5	3.82	2.94	2.49E-01	1.00E+00	3.85E-01
Nonalcoholic Fatty Liver Disease	344	10	3.82	2.94	2.49E-01	1.00E+00	3.85E-01
Pervasive Developmental Disorder Not Otherwise Specified	157	10	3.82	2.94	2.49E-01	1.00E+00	3.85E-01
Immunoglobulin A Nephropathy (igan) Progressor	73	11	3.63	2.94	2.75E-01	1.00E+00	4.06E-01
Treated Celiac Disease	98	8	3.42	2.94	2.90E-01	1.00E+00	4.11E-01
Autism	204	34	3.12	2.94	3.19E-01	1.00E+00	4.19E-01
Celiac Disease	109	11	3.23	2.94	3.20E-01	1.00E+00	4.19E-01
Ankylosing Spondylitis	21	15	2.90	2.94	3.85E-01	1.00E+00	4.68E-01
Rheumatoid Arthritis	42	30	2.90	2.94	3.85E-01	1.00E+00	4.68E-01
Bladder Infections	6	1	0.88	2.94	5.91E-01	1.00E+00	6.70E-01
Interstitial Cystitis	6	1	0.88	2.94	5.91E-01	1.00E+00	6.70E-01
Ccd	17	2	0.52	2.94	7.44E-01	1.00E+00	8.16E-01
Ileal Crohn's Disease	19	3	0.47	2.94	8.08E-01	1.00E+00	8.36E-01
Diarrhoea Predominant Irritable Bowel Syndrome	62	2	0.13	2.94	8.36E-01	1.00E+00	8.36E-01
Metastatic Melanoma	74	2	0.13	2.94	8.36E-01	1.00E+00	8.36E-01

Enrichment Overview (top 25)

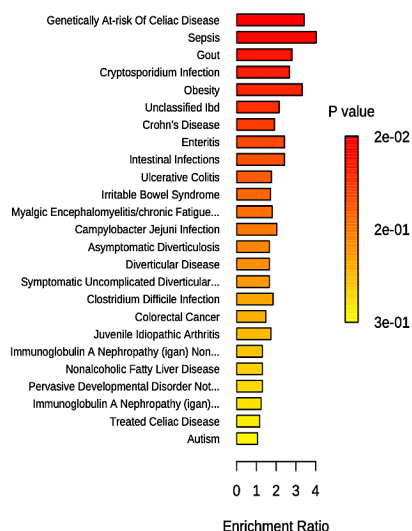
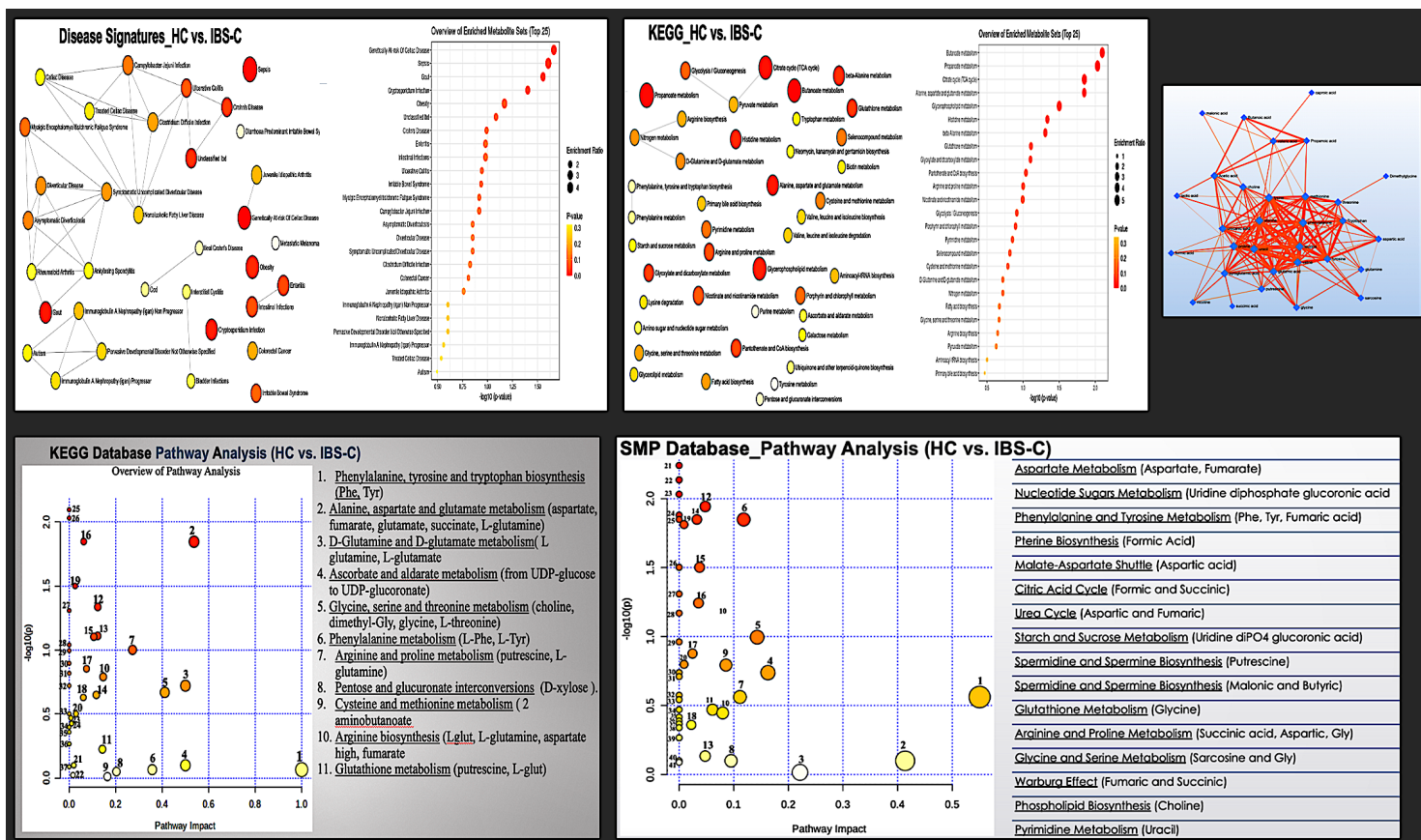


Figure 1: Summary plot for Quantitative Enrichment Analysis (QEA).



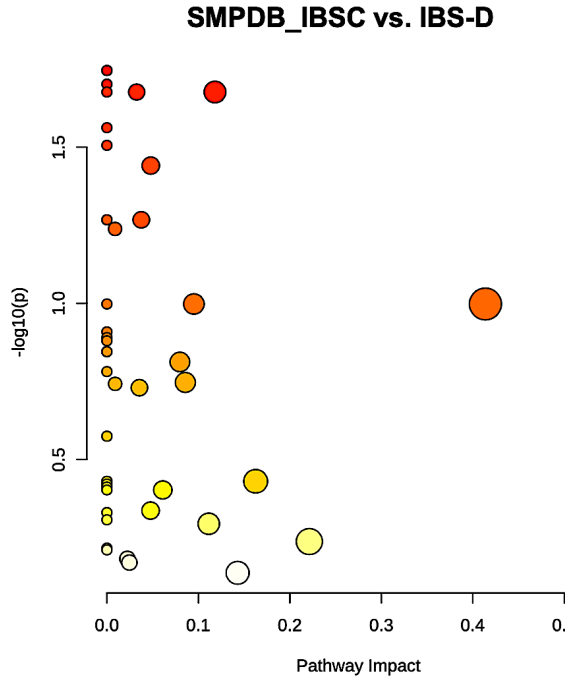
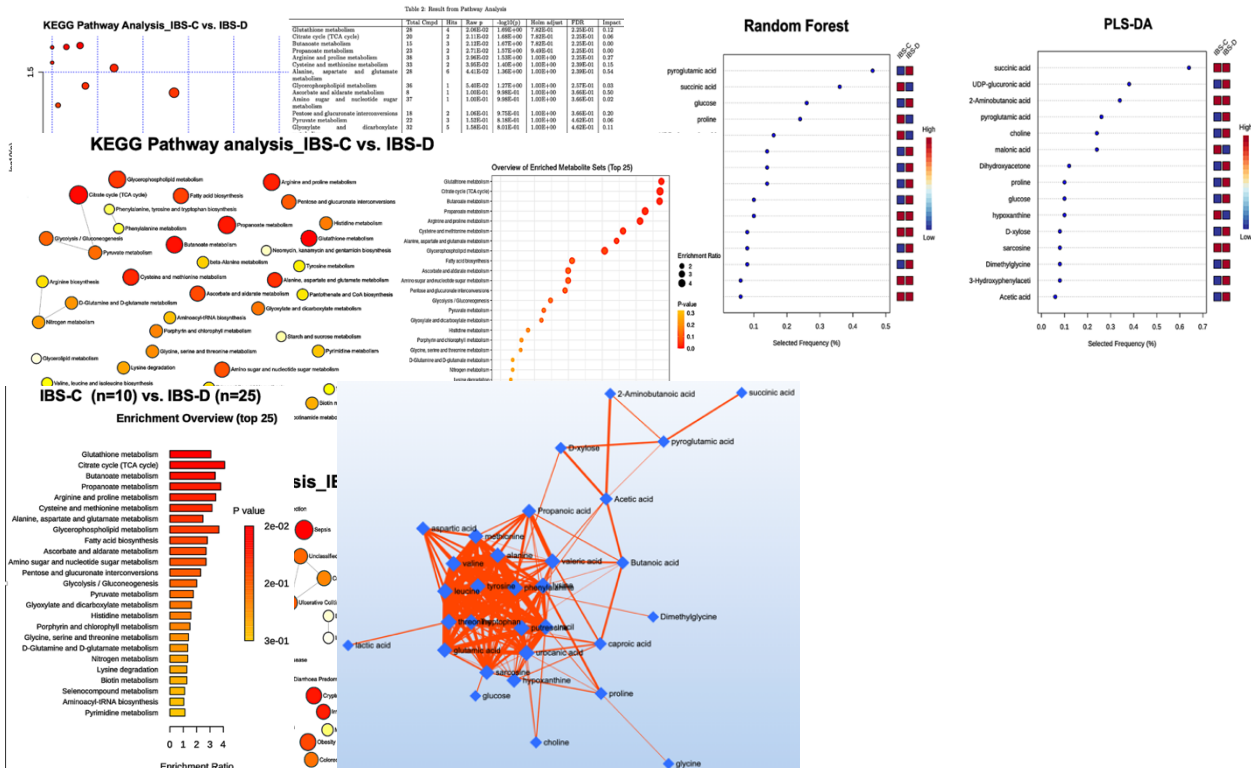


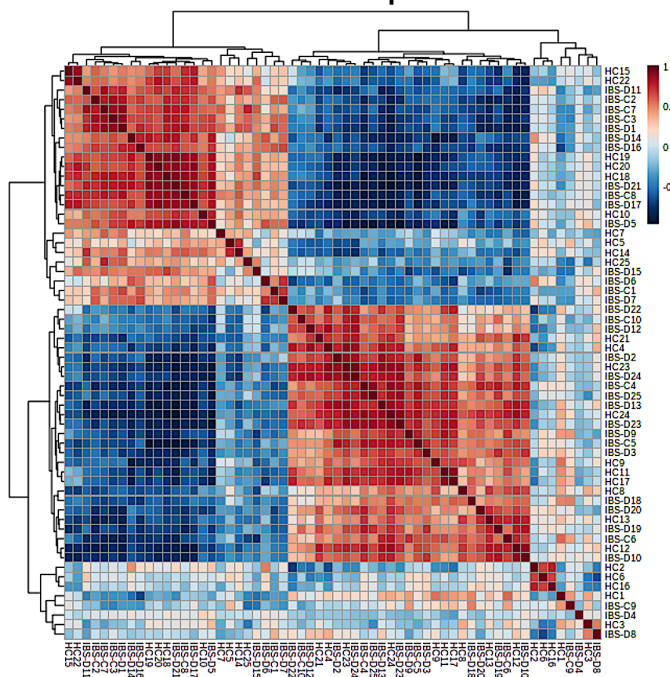
Figure 1: Summary of Pathway Analysis

Table 2: Result from Pathway Analysis

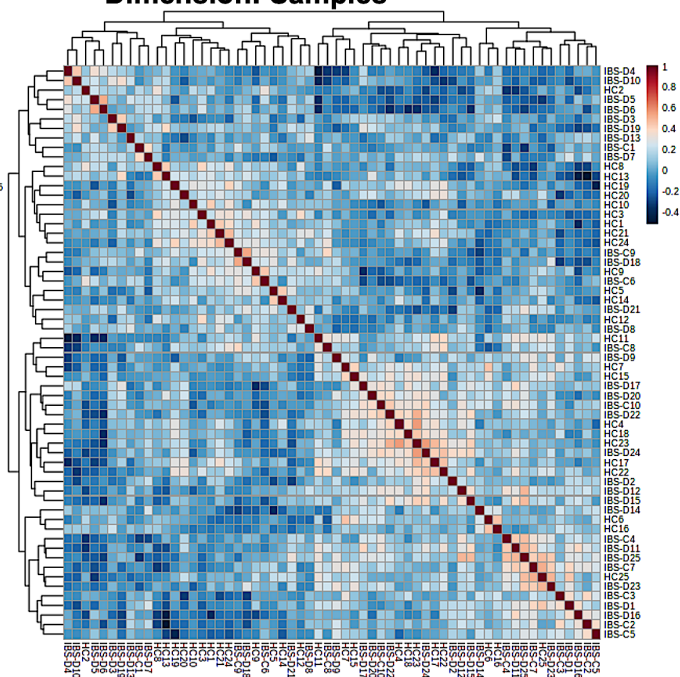
	Total Cmpd	Hits	Raw p	-log10(p)	Holm adjust	FDR	Impact
Oxidation of Branched Chain Fatty Acids	22	1	1.80E-02	1.75E+00	8.62E-01	1.44E-01	0.00
Ketone Body Metabolism	12	1	1.80E-02	1.75E+00	8.62E-01	1.44E-01	0.00
Phytanic Acid Peroxisomal Oxidation	19	1	1.80E-02	1.75E+00	8.62E-01	1.44E-01	0.00
Carnitine Synthesis	16	3	1.99E-02	1.70E+00	8.94E-01	1.44E-01	0.00
Citric Acid Cycle	26	2	2.11E-02	1.68E+00	9.27E-01	1.44E-01	0.12
Warburg Effect	49	2	2.11E-02	1.68E+00	9.27E-01	1.44E-01	0.03
Mitochondrial Electron Transport Chain	15	2	2.11E-02	1.68E+00	9.27E-01	1.44E-01	0.00
Butyrate Metabolism	16	2	2.74E-02	1.56E+00	1.00E+00	1.64E-01	0.00
Glutamate Metabolism	45	3	3.12E-02	1.51E+00	1.00E+00	1.66E-01	0.00
Arginine and Proline Metabolism	48	4	3.62E-02	1.44E+00	1.00E+00	1.74E-01	0.05
Phospholipid Biosynthesis	25	1	5.40E-02	1.27E+00	1.00E+00	1.98E-01	0.04
Phosphatidylcholine Biosynthesis	18	1	5.40E-02	1.27E+00	1.00E+00	1.98E-01	0.00
Phosphatidylethanolamine Biosynthesis	13	1	5.40E-02	1.27E+00	1.00E+00	1.98E-01	0.00
Valine, Leucine and Isoleucine Degradation	51	4	5.78E-02	1.24E+00	1.00E+00	1.98E-01	0.01
Nucleotide Sugars Metabolism	16	1	1.00E-01	9.98E-01	1.00E+00	2.84E-01	0.41
Starch and Sucrose Metabolism	26	1	1.00E-01	9.98E-01	1.00E+00	2.84E-01	0.10
Estrone Metabolism	20	1	1.00E-01	9.98E-01	1.00E+00	2.84E-01	0.00
Cysteine Metabolism	24	1	1.23E-01	9.09E-01	1.00E+00	3.11E-01	0.00
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	17	1	1.29E-01	8.90E-01	1.00E+00	3.11E-01	0.00
Porphyryn Metabolism	36	3	1.32E-01	8.80E-01	1.00E+00	3.11E-01	0.00
Androgen and Estrogen Metabolism	29	2	1.43E-01	8.45E-01	1.00E+00	3.11E-01	0.00
Androstenedione Metabolism	23	2	1.43E-01	8.45E-01	1.00E+00	3.11E-01	0.00
Fatty Acid Biosynthesis	33	3	1.54E-01	8.12E-01	1.00E+00	3.21E-01	0.08
Betaine Metabolism	18	2	1.65E-01	7.82E-01	1.00E+00	3.31E-01	0.00
Spermidine and Spermine Biosynthesis	14	1	1.79E-01	7.47E-01	1.00E+00	3.31E-01	0.09
Methionine Metabolism	39	5	1.81E-01	7.42E-01	1.00E+00	3.31E-01	0.01
Pyrimidine Metabolism	54	1	1.86E-01	7.30E-01	1.00E+00	3.31E-01	0.04
Lysine Degradation	20	1	2.66E-01	5.75E-01	1.00E+00	4.41E-01	0.00
Biotin Metabolism	7	1	2.66E-01	5.75E-01	1.00E+00	4.41E-01	0.00
Pterine Biosynthesis	18	1	3.71E-01	4.30E-01	1.00E+00	5.28E-01	0.16
Steroid Biosynthesis	43	1	3.71E-01	4.30E-01	1.00E+00	5.28E-01	0.00
Folate Metabolism	24	1	3.71E-01	4.30E-01	1.00E+00	5.28E-01	0.00
Bile Acid Biosynthesis	59	2	3.79E-01	4.21E-01	1.00E+00	5.28E-01	0.00
Beta-Alanine Metabolism	26	2	3.87E-01	4.12E-01	1.00E+00	5.28E-01	0.00
Glutathione Metabolism	19	1	3.90E-01	4.02E-01	1.00E+00	5.28E-01	0.06
Alanine Metabolism	14	1	3.96E-01	4.02E-01	1.00E+00	5.28E-01	0.00
Glycine and Serine Metabolism	50	3	4.61E-01	3.36E-01	1.00E+00	5.91E-01	0.05
Threonine and 2-Oxobutanoate Degradation	13	1	4.68E-01	3.30E-01	1.00E+00	5.91E-01	0.00
Tryptophan Metabolism	55	2	4.94E-01	3.06E-01	1.00E+00	5.95E-01	0.00
Aspartate Metabolism	34	2	5.09E-01	2.94E-01	1.00E+00	5.95E-01	0.55
Urea Cycle	23	2	5.09E-01	2.94E-01	1.00E+00	5.95E-01	0.11
Phenylalanine and Tyrosine Metabolism	25	3	5.80E-01	2.37E-01	1.00E+00	6.62E-01	0.00
Tyrosine Metabolism	55	3	6.09E-01	2.16E-01	1.00E+00	6.73E-01	0.00
Catecholamine Biosynthesis	14	1	6.17E-01	2.10E-01	1.00E+00	6.73E-01	0.00
Purine Metabolism	63	4	6.58E-01	1.82E-01	1.00E+00	7.02E-01	0.02
Ammonia Recycling	25	2	6.77E-01	1.70E-01	1.00E+00	7.06E-01	0.02
Malate-Aspartate Shuttle	7	1	7.30E-01	1.37E-01	1.00E+00	7.46E-01	0.14



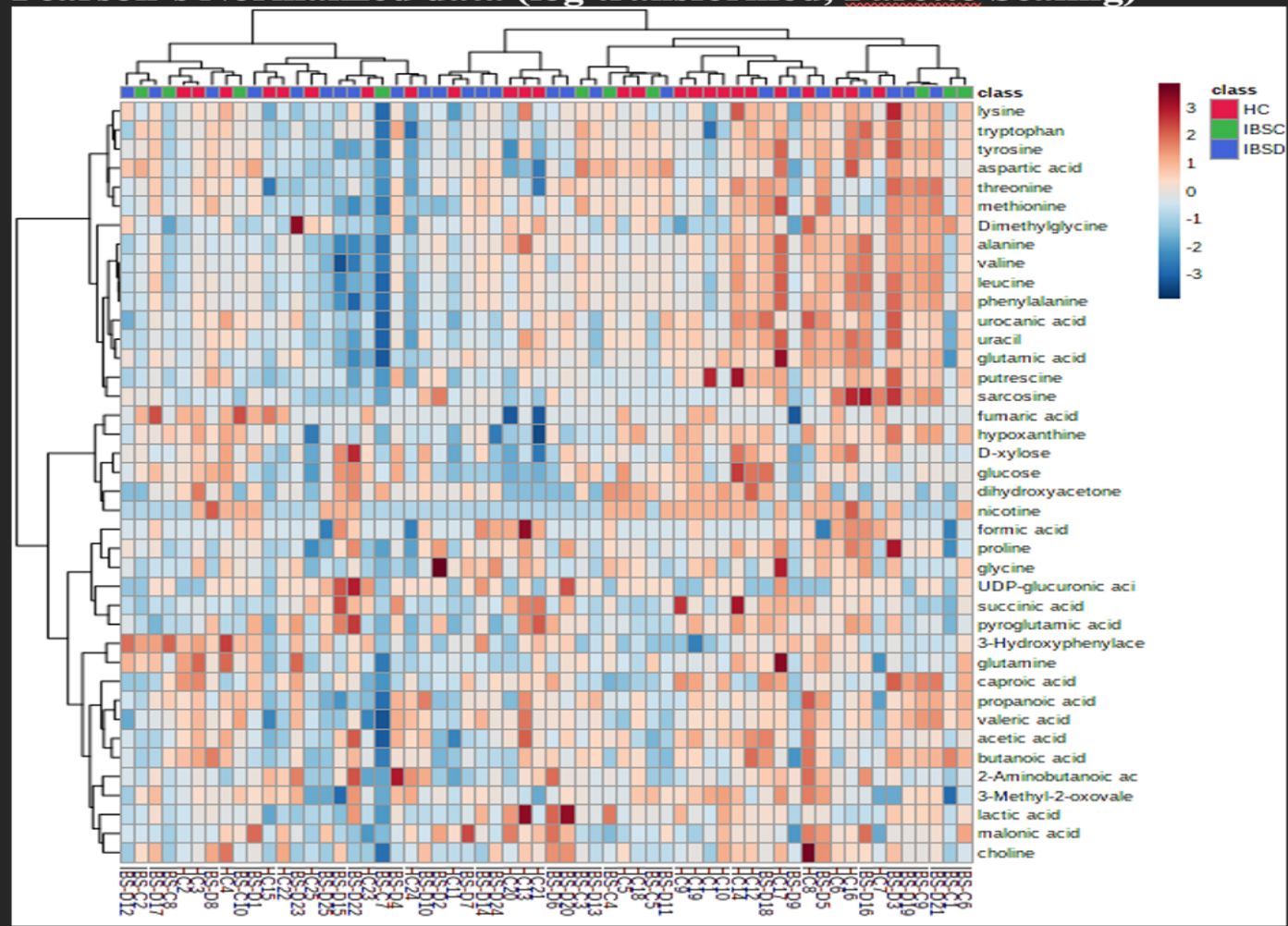
Correlation Heatmaps
Distance measure: Pearson
Dimension: Samples



Correlation Heatmaps
Distance measure: Spearman rank correl.
Dimension: Samples

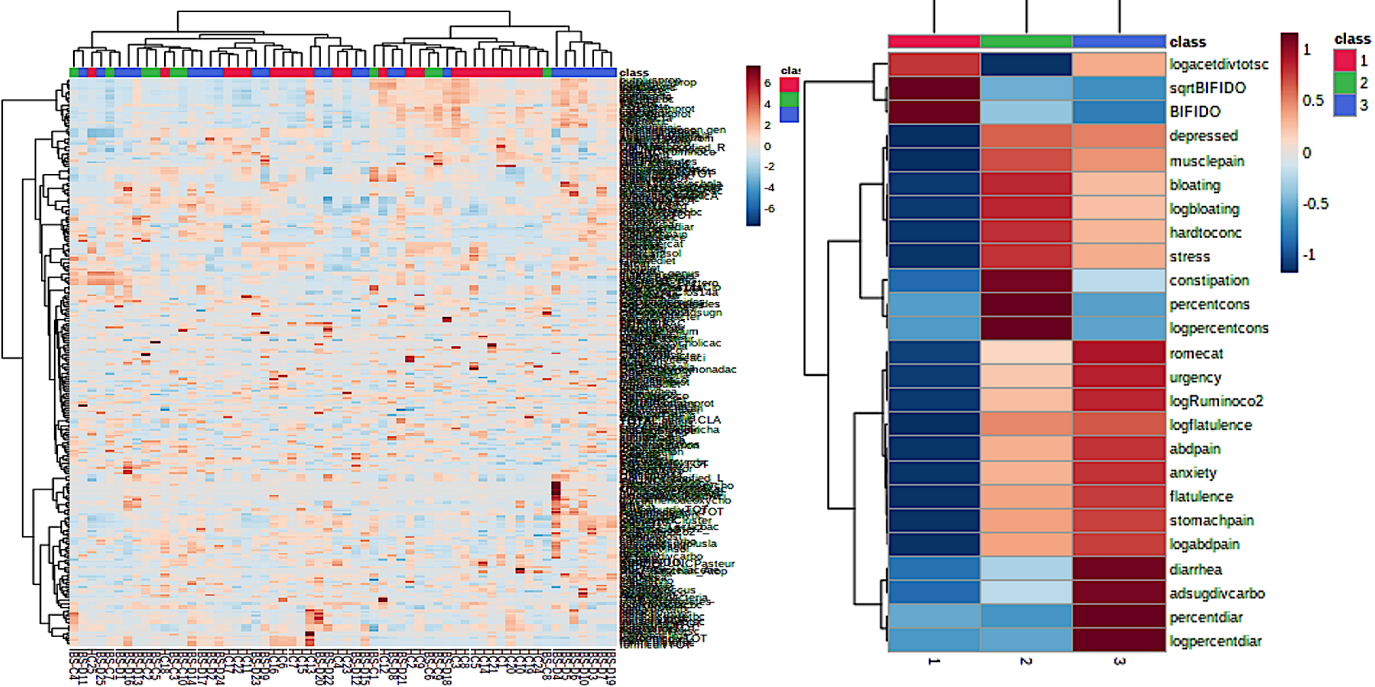


Pearson's Normalized data (log transformed, Pareto Scaling)

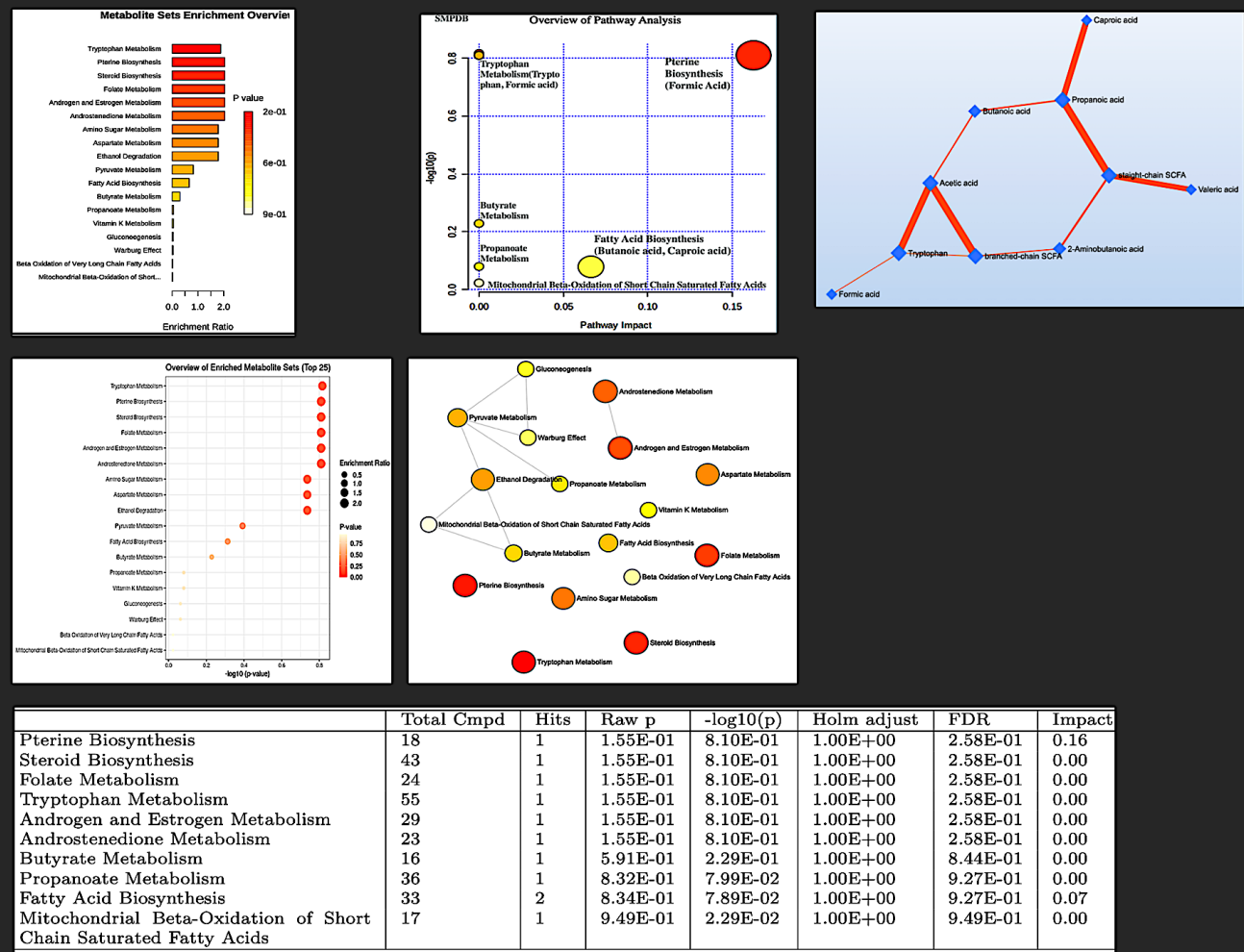


The figure displays two heatmaps of organic acid relative abundance. The top heatmap shows a clear separation between HC (green) and IBS (red) samples. The bottom heatmap shows a more mixed distribution of samples. The color scale ranges from -6 (blue) to 6 (red), with 0 being white. The acids listed on the right are: lactic acid, formic acid, 2-Aminobutanoic acid, caproic acid, tryptophan, propionic acid, valeric acid, acetic acid, and butanoic acid. The samples are numbered 1 to 30 on the x-axis.

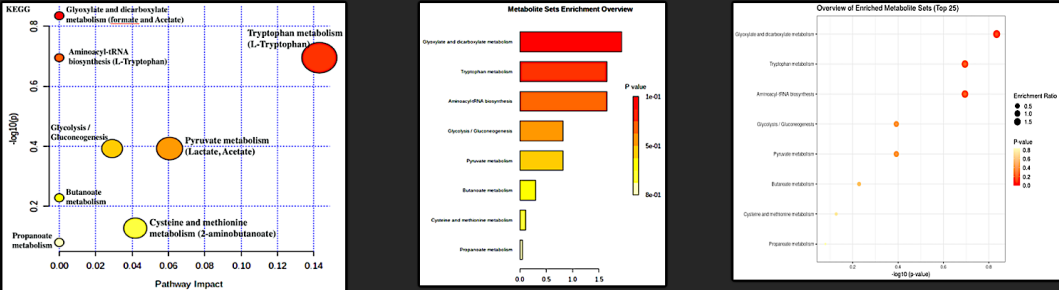
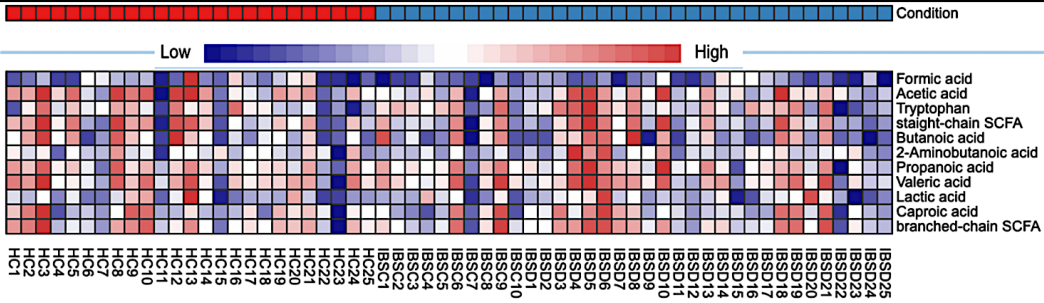
Clustering



SMP Database/Absolute Concentration: The gut microbiome functional capacity in terms of their metabolic signatures and their predicted metabolic pathways

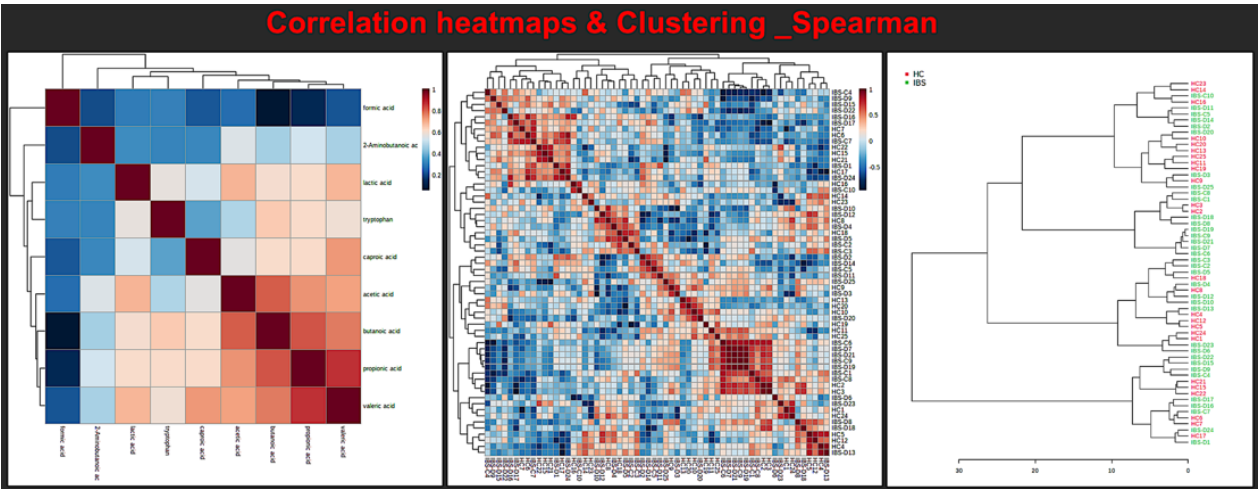


KEGG Database (Human database)/Absolute Concentration (HC vs. IBS). The gut microbiome functional capacity in terms of their metabolic signatures and their predicted metabolic pathways



msea_qea_result							
	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR
Glyoxylate and dicarboxylate metabolism	32	2	3.2591	1.6949	0.14625	1	0.53814
Tryptophan metabolism	41	1	2.7936	1.6949	0.2018	1	0.53814
Aminoacyl-tRNA biosynthesis	48	1	2.7936	1.6949	0.2018	1	0.53814
Glycolysis / Gluconeogenesis	26	2	1.3862	1.6949	0.40499	1	0.64799
Pyruvate metabolism	22	2	1.3862	1.6949	0.40499	1	0.64799
Butanoate metabolism	15	1	0.50145	1.6949	0.59081	1	0.78775
Cysteine and methionine metabolism	33	1	0.18259	1.6949	0.74581	1	0.83202
Propanoate metabolism	23	1	0.078214	1.6949	0.83202	1	0.83202

This is illustrating how the metabolites (Absolute Concentration) are correlated from each other. The women are clustered based on their metabolic profile. There are 6 networks nodes and 7 edges

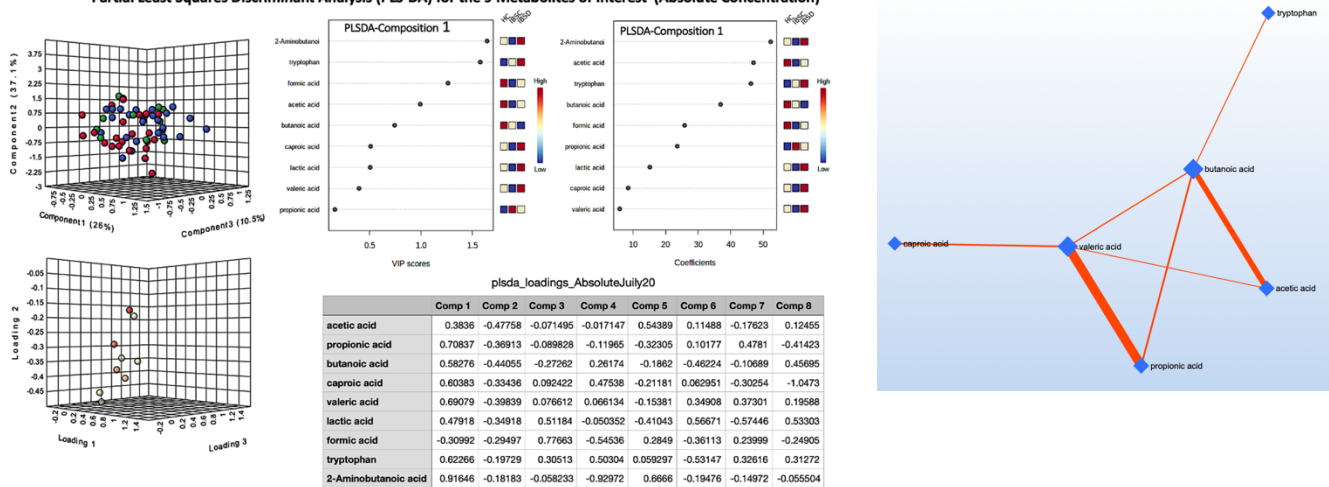


correlation_table-6

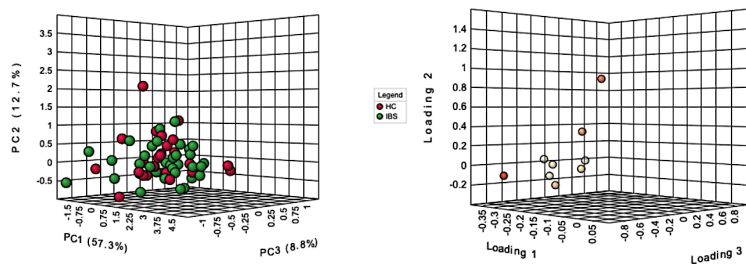
	formic acid	2-Aminobutanoic acid	lactic acid	tryptophan	caproic acid	acetic acid	butanoic acid	propionic acid	valeric acid
formic acid	1	0.21061	0.30181	0.31103	0.22862	0.26852	0.06783	0.13632	0.22445
2-Aminobutanoic acid	0.21061	1	0.33598	0.31698	0.32559	0.55365	0.46835	0.53476	0.4733
lactic acid	0.30181	0.33598	1	0.58016	0.54343	0.6913	0.60817	0.61689	0.69041
tryptophan	0.31103	0.31698	0.58016	1	0.37644	0.48563	0.65452	0.62056	0.59928
caproic acid	0.22862	0.32559	0.54343	0.37644	1	0.57144	0.61695	0.61945	0.73693
acetic acid	0.26852	0.55365	0.6913	0.48563	0.57144	1	0.81478	0.74182	0.72092
butanoic acid	0.06783	0.46835	0.60817	0.65452	0.61695	0.81478	1	0.82578	0.77727
propionic acid	0.13632	0.53476	0.61689	0.62056	0.61945	0.74182	0.82578	1	0.87502
valeric acid	0.22445	0.4733	0.69041	0.59928	0.73693	0.72092	0.77727	0.87502	1

PCA of two groups (HC and IBS) detected as samples with 9 (metabolites) as features or variables

Partial Least Squares Discriminant Analysis (PLS-DA) for the 9 Metabolites of Interest (Absolute Concentration)



Principal Component Analysis for the 9 Metabolites of Interest (Absolute Concentration)

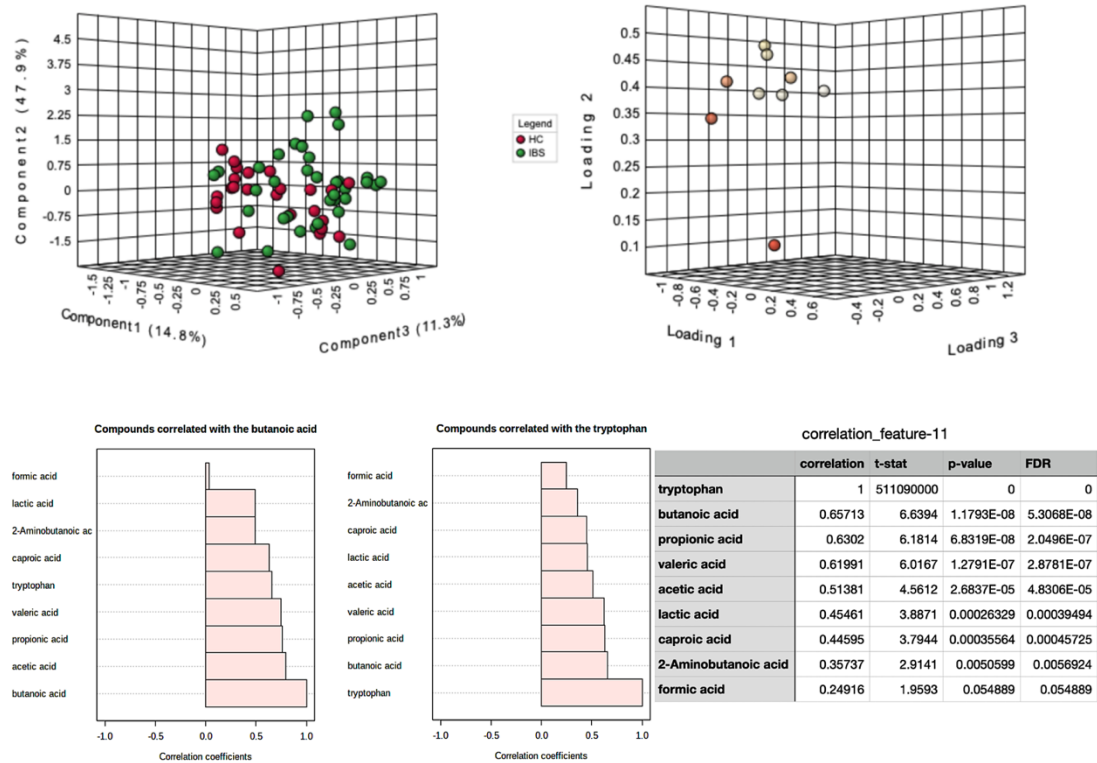


	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
acetic acid	-0.36829	0.028719	-0.12074	0.16236	-0.1933	0.62054	-0.099595	0.61362	0.11713
propionic acid	-0.37618	-0.16155	-0.013445	-0.20364	-0.043512	-0.26209	-0.56676	-0.12578	0.61897
butanoic acid	-0.38252	-0.27245	0.095504	-0.14564	-0.27144	0.45346	0.15137	-0.6467	-0.16932
caproic acid	-0.33896	-0.094417	0.32489	-0.1071	0.76116	0.073864	0.36757	0.083718	0.17954
valeric acid	-0.39642	-0.046501	0.1045	-0.10569	0.14176	-0.23221	-0.42708	0.16265	-0.73193
lactic acid	-0.33752	0.32098	0.33355	0.74182	-0.16523	-0.24408	0.087039	-0.15154	0.063882
formic acid	-0.13865	0.88007	-0.14798	-0.34303	0.092372	0.14186	-0.052699	-0.1875	0.0095589
tryptophan	-0.27032	0.020442	0.12466	-0.42467	-0.47677	-0.39493	0.50667	0.29975	0.028149
2-Aminobutanoic acid	-0.31348	-0.099	-0.84318	0.20001	0.15642	-0.21348	0.24774	-0.08942	-0.039593

PLS-DA of three samples (HC, IBS-C, IBS-D). The loadings plot shows the variables selected by the PLS-DA model for a given component. The variables are ranked by the absolute values of their loadings. There were

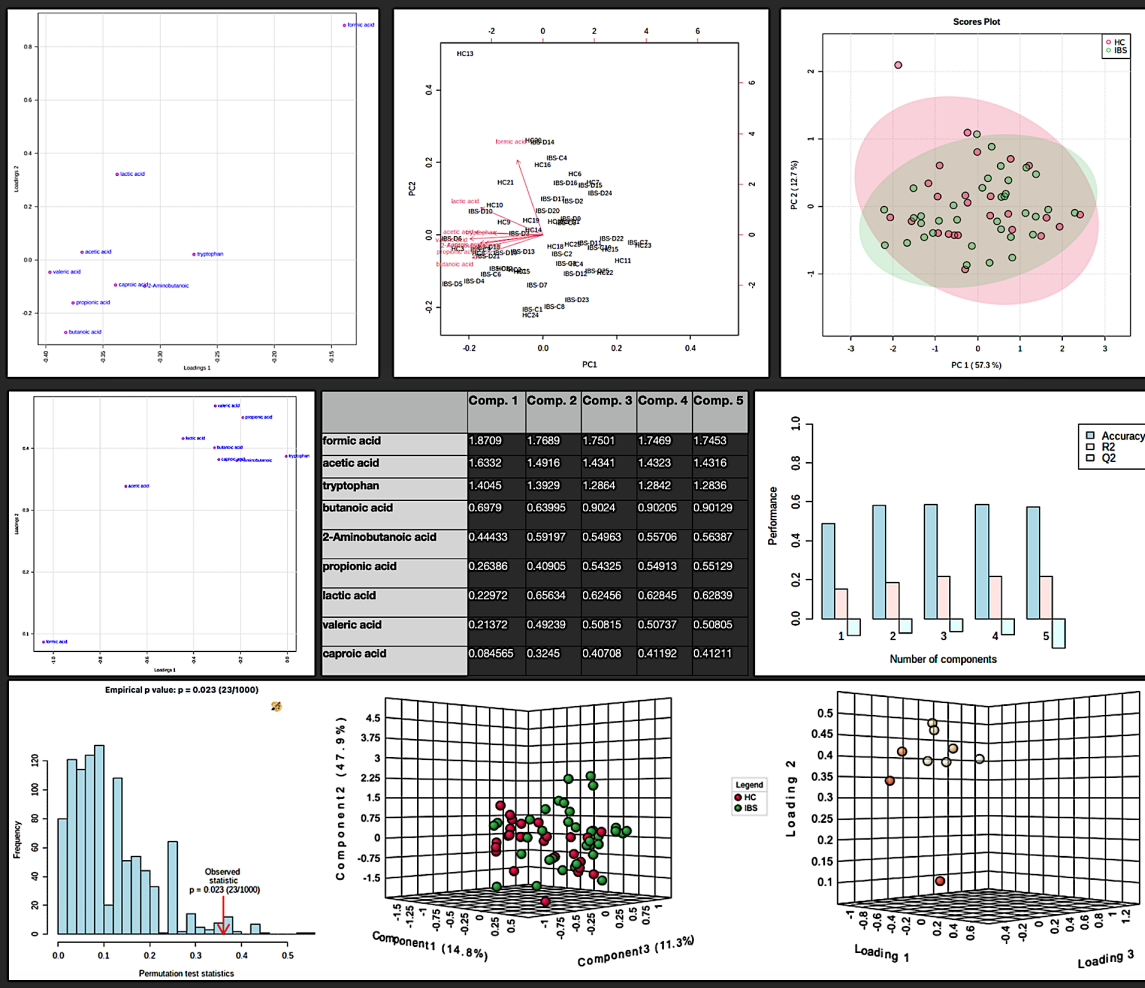
no missing values. By default, if there was a missing value, missing values are replaced by 1/5 of minimum positive values of their corresponding variables.

PLSDA

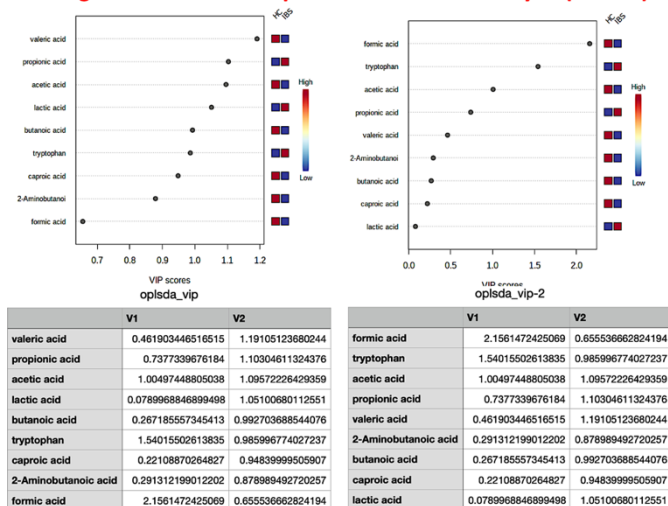


PCA & PLS-DA of two groups (HC and IBS) detected as samples with 9 (metabolites) as features or variables

Principal Component Analysis (PCA)

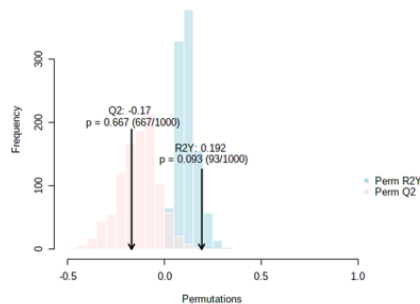


Orthogonal Partial Least Squares Discriminant Analysis (PLS-DA)

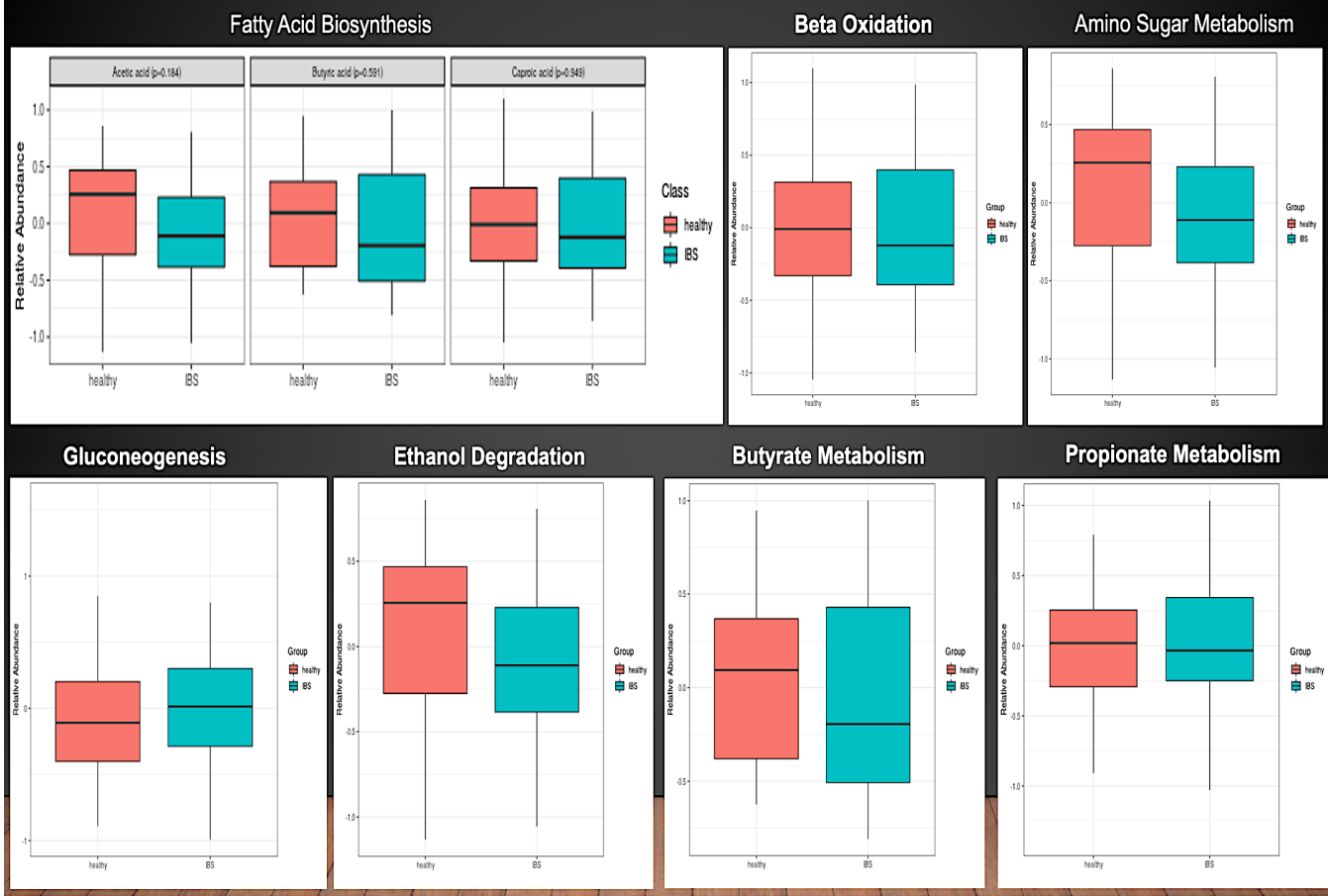


Set permutation numbers: 1000

Empirical p-values Q2: $p = 0.667$ (667/1000) and R2Y: $p = 0.093$ (93/1000)



HC (N=25) VS IBS (N=35) _9 METABOLITES (MICROMOLE)



From the Metabolites with Absolute concentration

Pterine Biosynthesis: Formic acid

Amino sugar metabolism: Acetic acid

Ethanol degradation: Acetic acid

Tryptophan metabolism: Formic and Tryptophan

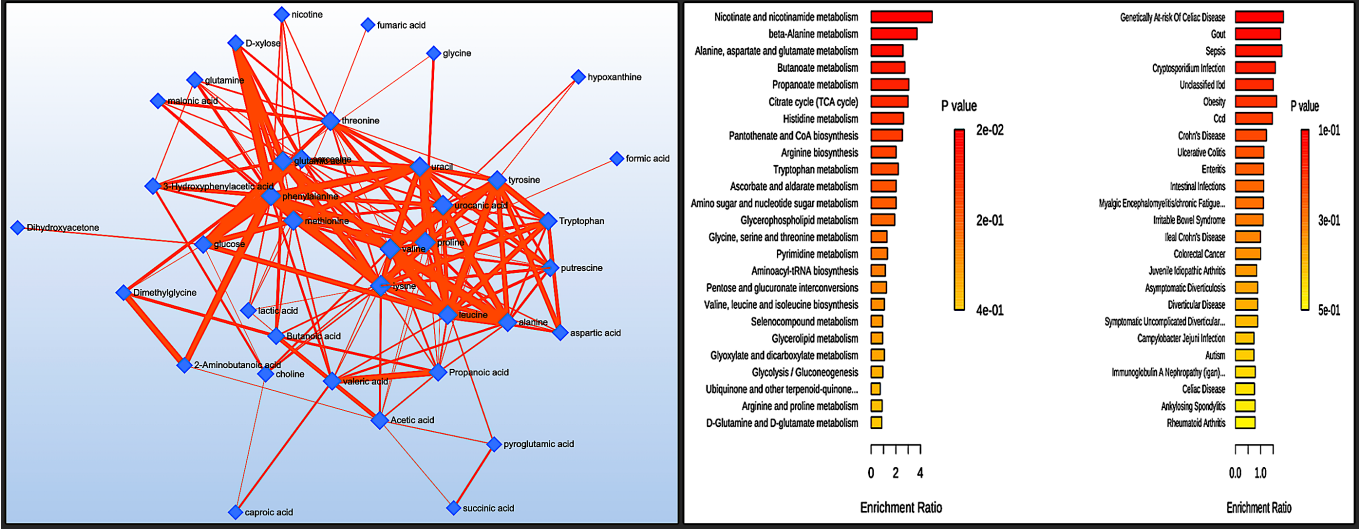
Fatty acid synthesis: butyric, acetic, caproic acids

Pyruvate metabolism: lactic and acetic acids

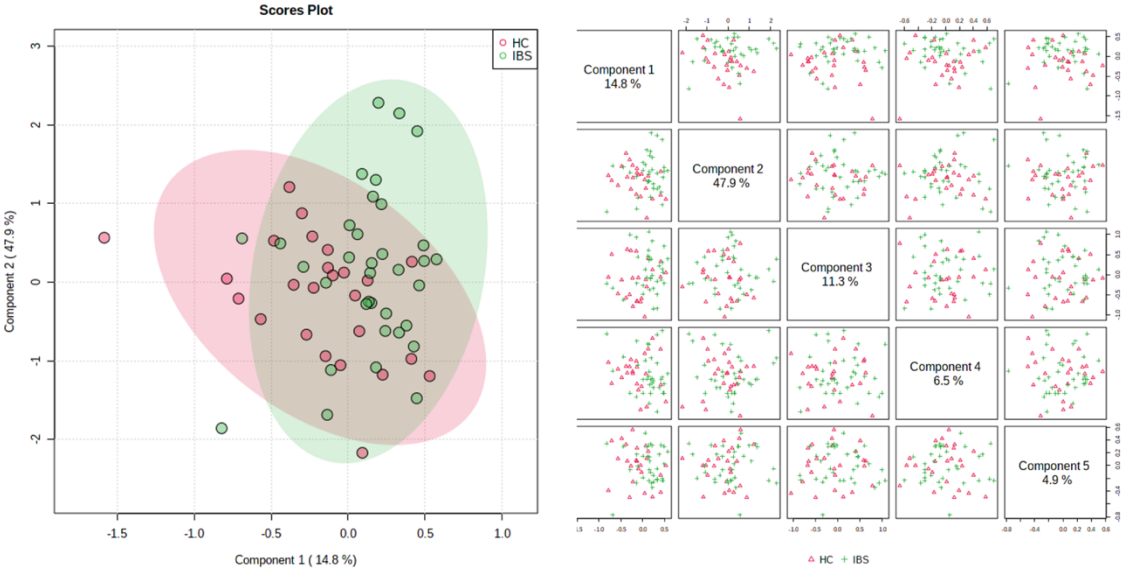
Butyrate metabolism: Ketone body metabolism, Succinic acid source, ATP, NADH regeneration

Propanoate metabolism: TCA, Pantothenate and CoA biosynthesis, methyl malonic acid, ATP, pyruvate metabolism, crotonyl CoA, NADH

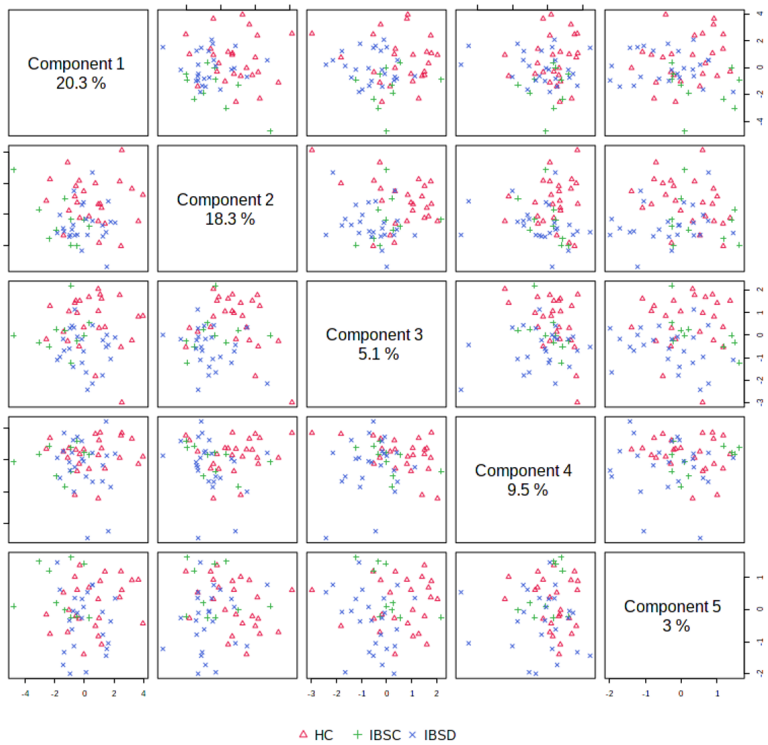
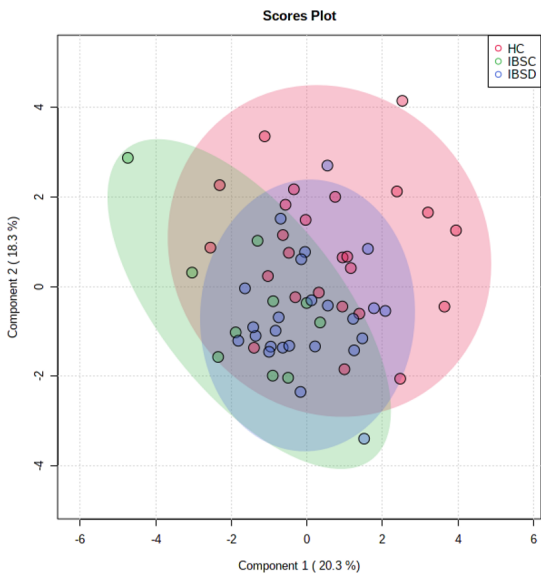
SMP Database/Relative Concentration (HC vs. IBS). Fecal Dry Weight Normalized) June 7, 2022



Partial Least Squares Discriminant Analysis (PLS-DA)_Absolute Concentration



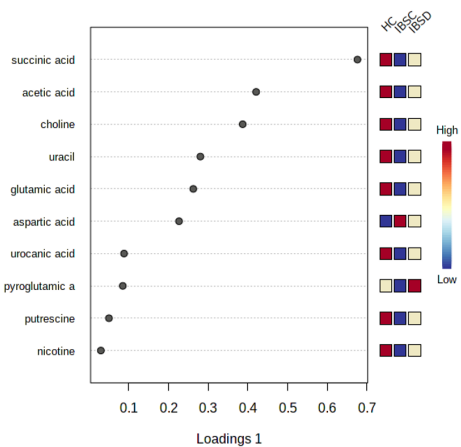
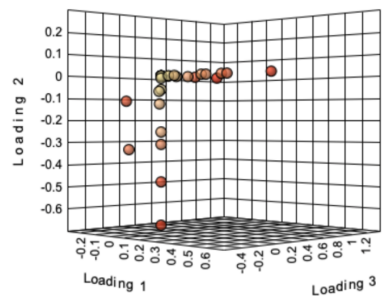
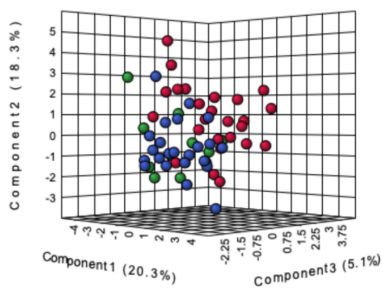
Sparse-PLSDA. Validation Method: 5-fold
CV, 5 components, 10 variables/component



FDW_Sparse_5foldCV_splsda_loadings

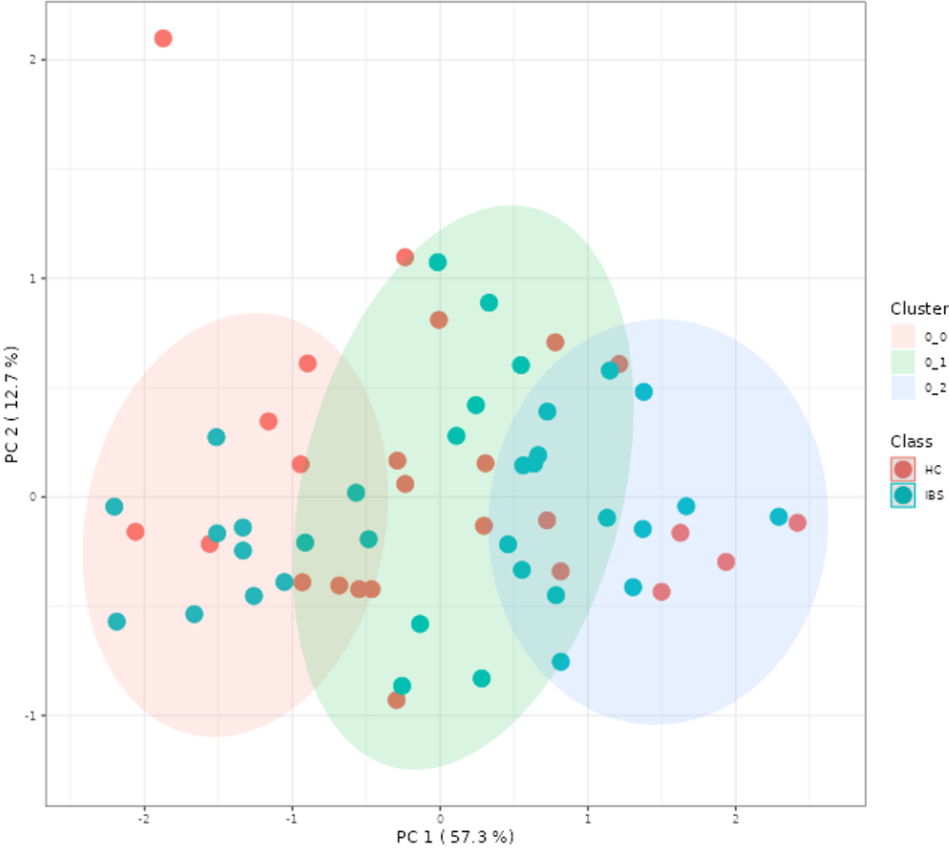
	comp 1	comp 2	comp 3	comp 4	comp 5
succinic acid	0.67583	0	0	0	0
acetic acid	0.42063	0	0	0	0
choline	0.38672	0	0	0	0
uracil	0.28014	0	0.022035	0	0
glutamic acid	0.26227	0	0	0.13901	0
aspartic acid	-0.2264	-0.33783	0	0	0
urocanic acid	0.087892	0	0	0	-0.14992
pyroglutamic acid	0.084692	-0.12844	-0.16961	0	0
putrescine	0.049867	0	0	0	0
nicotine	0.029478	0	0.27648	0	0
threonine	0	-0.6756	0	0	0
tryptophan	0	-0.47963	0	0	0.047731
proline	0	-0.30985	0	0	0
UDP-glucuronic acid	0	-0.25384	0	-0.094621	0
2-Aminobutanoic acid	0	-0.11693	-0.40878	0	-0.14869
3-Hydroxyphenylacetic acid	0	-0.070058	-0.024656	-0.51096	0
tyrosine	0	-0.061419	0	0	0
Dimethylglycine	0	-0.010034	0	0	0
propanoic acid	0	0	0	0	0
butanoic acid	0	0	0	0	0.4258
caproic acid	0	0	0	0	-0.33549
valeric acid	0	0	0	0	0
lactic acid	0	0	0	0	0.2215
formic acid	0	0	0	0.13143	0
fumaric acid	0	0	0.71063	0	0
malonic acid	0	0	0	0	-0.58666
dihydroxyacetone	0	0	0.085797	0	0
3-Methyl-2-oxovaleric acid	0	0	0	0	0
hypoxanthine	0	0	0.42382	0	0
D-xylose	0	0	0	0	0
valine	0	0	0.18591	0.55739	0
alanine	0	0	0	0.42855	0
leucine	0	0	0	0.22155	0
lysine	0	0	0	0	0
phenylalanine	0	0	0.0076411	0.30346	0
methionine	0	0	0	0	0
glutamine	0	0	0	0.0077437	0.39211
sarcosine	0	0	0	0	-0.27314
glycine	0	0	0	0.2404	0
glucose	0	0	0	0	0.19414

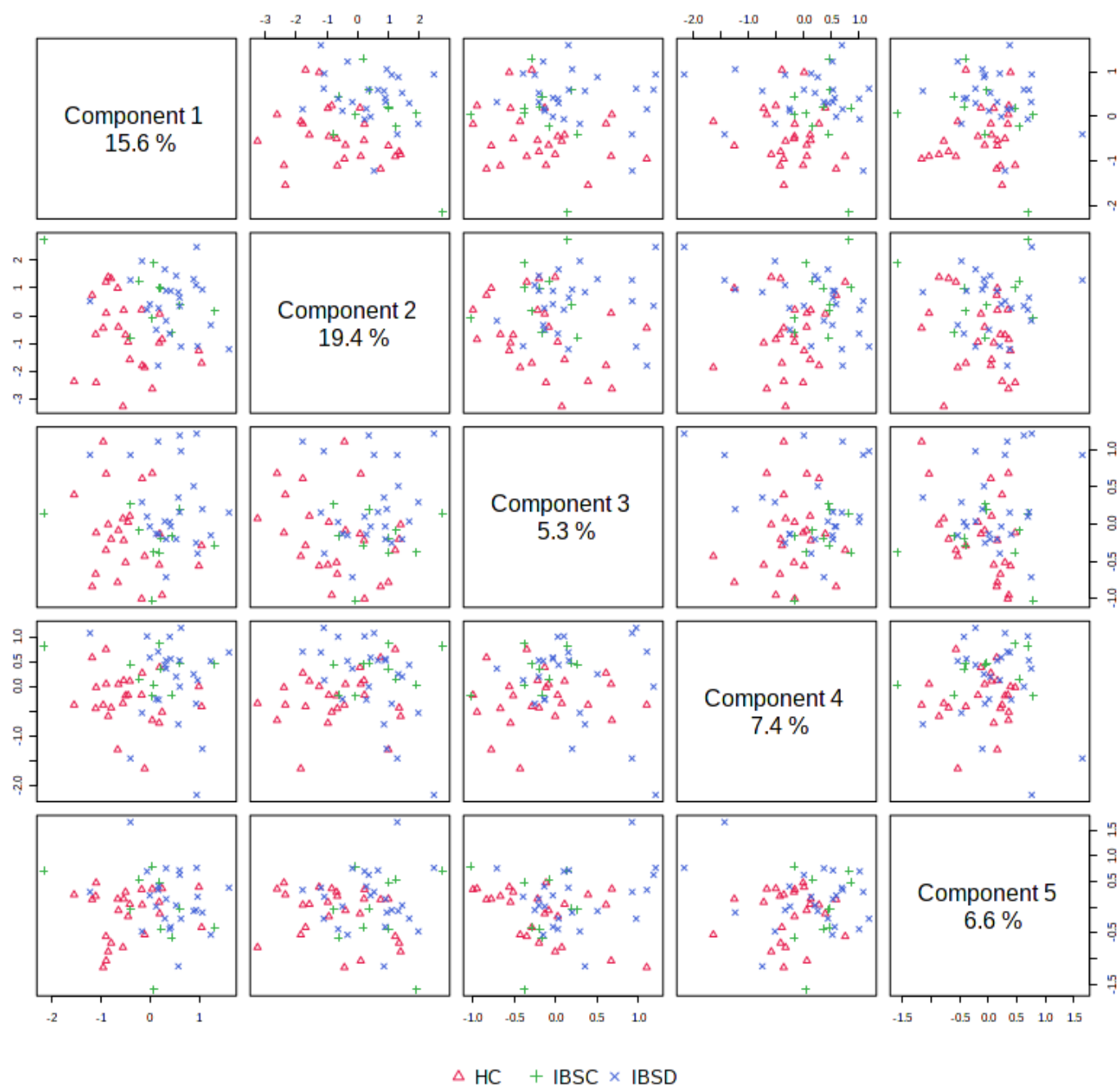
Sparse-PLSDA, 5-fold CV, 10 variables/component



Self Organizing Map (SOM)Clustering Details

Cluster	Members
Cluster (0, 0)	HC3 , HC8 , HC9 , HC10 , HC12 , HC13 , HC21 , IBS-C6 , IBS-C9 , IBS-D4 , IBS-D5 , IBS-D6 , IBS-D8 , IBS-D10 , IBS-D18 , IBS-D19 , IBS-D21
Cluster (0, 1)	HC1 , HC2 , HC5 , HC6 , HC14 , HC16 , HC17 , HC18 , HC19 , HC20 , HC24 , HC25 , IBS-C1 , IBS-C2 , IBS-C3 , IBS-C4 , IBS-C5 , IBS-C8 , IBS-D1 , IBS-D2 , IBS-D3 , IBS-D7 , IBS-D9 , IBS-D12 , IBS-D13 , IBS-D14 , IBS-D16 , IBS-D17 , IBS-D20
Cluster (0, 2)	HC4 , HC7 , HC11 , HC15 , HC22 , HC23 , IBS-C7 , IBS-C10 , IBS-D11 , IBS-D15 , IBS-D22 , IBS-D23 , IBS-D24 , IBS-D25





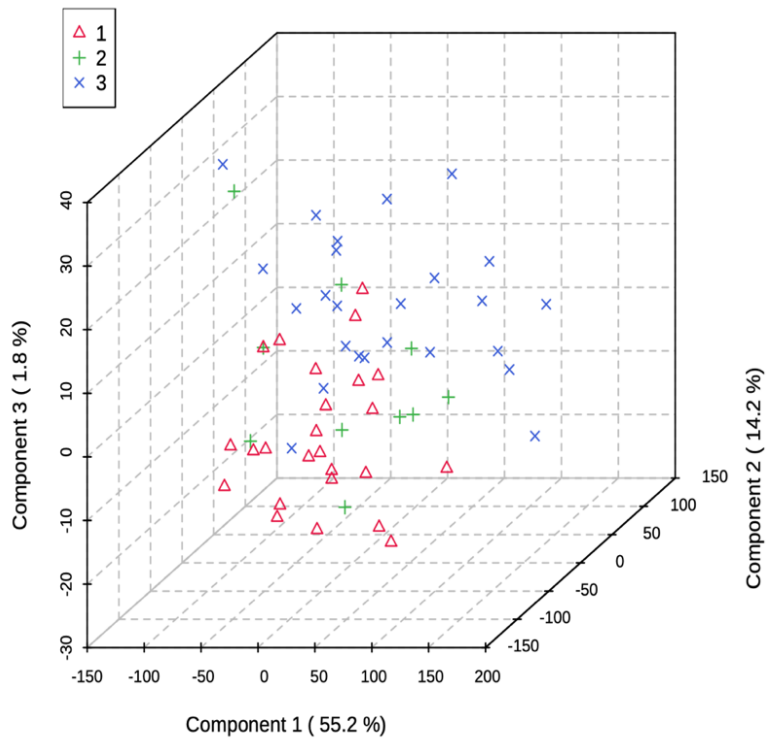
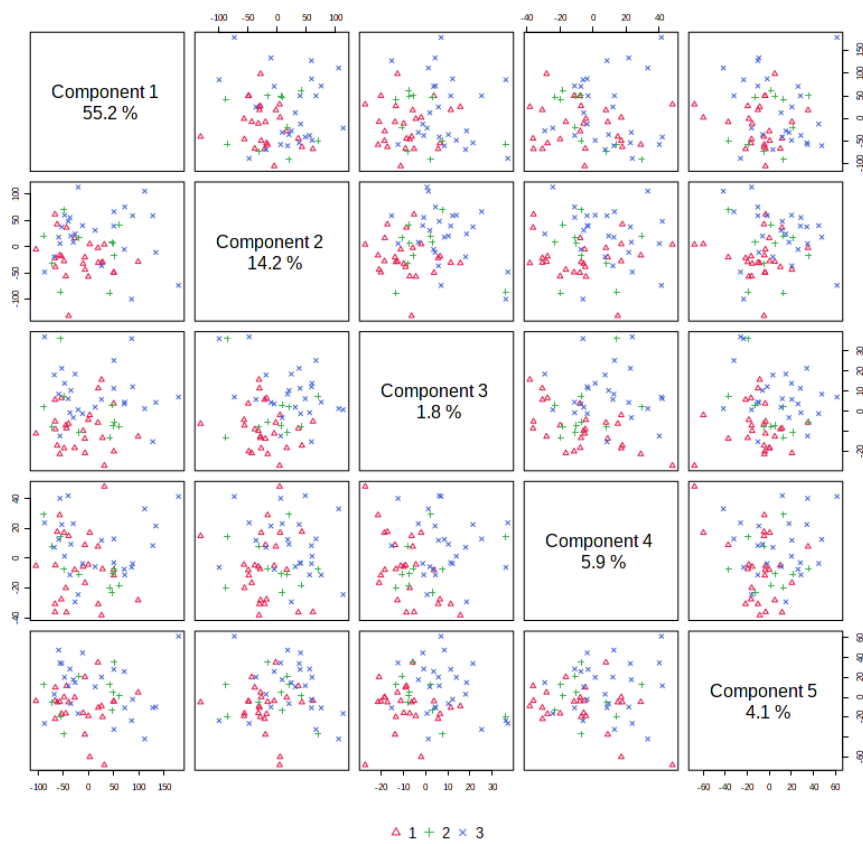
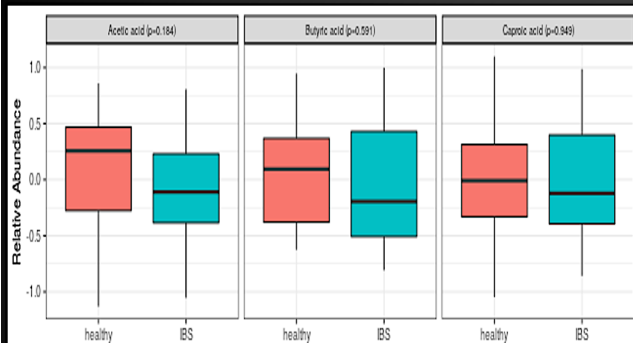


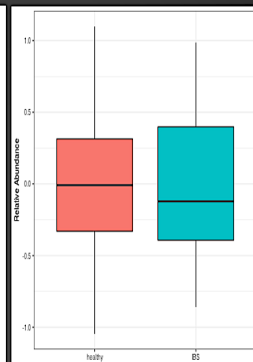
Figure 4: 3D scores plot between the selected PCs. The explained variances are shown in brackets.

HC (N=25) VS IBS (N=35)_9 METABOLITES (MICROMOLE)

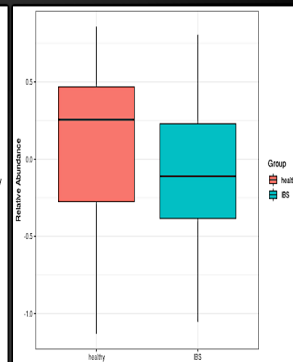
Fatty Acid Biosynthesis



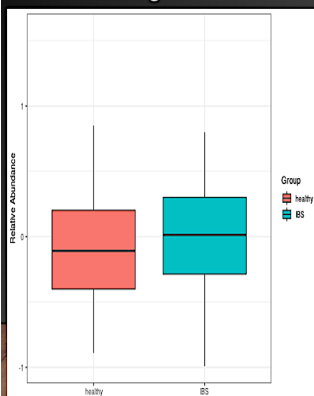
Beta Oxidation



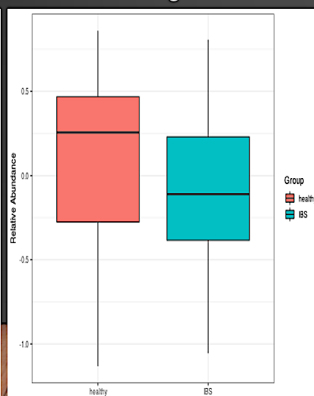
Amino Sugar Metabolism



Gluconeogenesis



Ethanol Degradation



Butyrate Metabolism

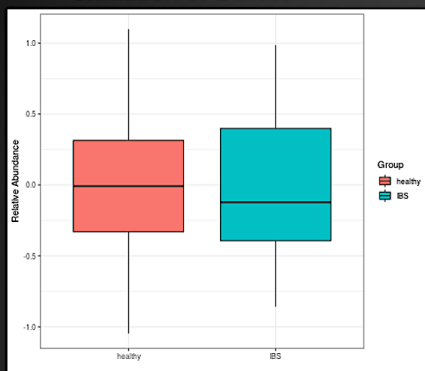


Propionate Metabolism

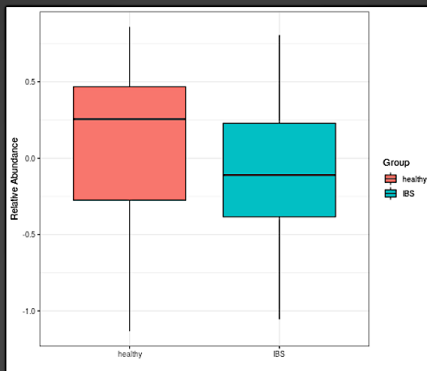


HC (N=25) VS IBS (N=35) _9 METABOLITES (MICROMOLE)

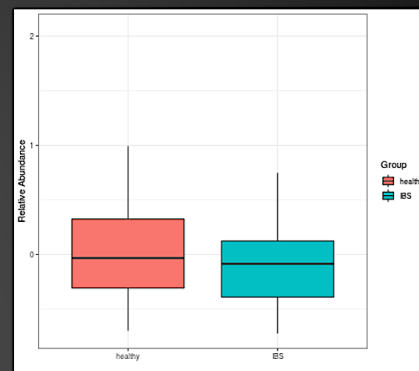
Mitochondrial Beta Oxidation of SCFAs



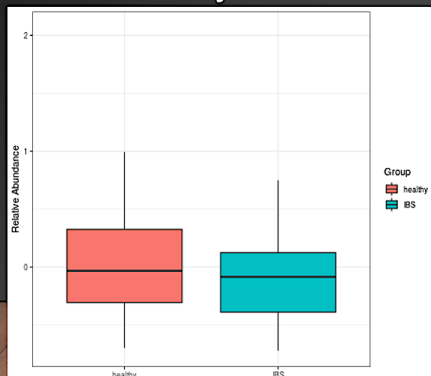
Aspartate Metabolism



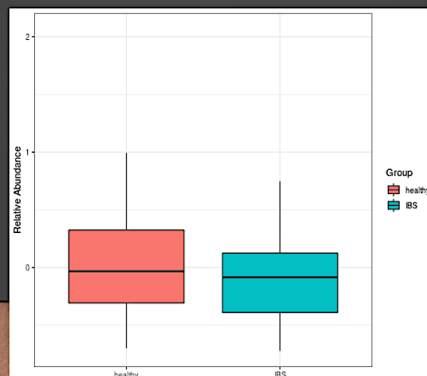
Folate Metabolism



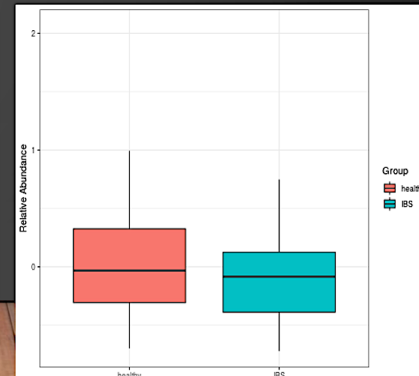
Steroid Biosynthesis



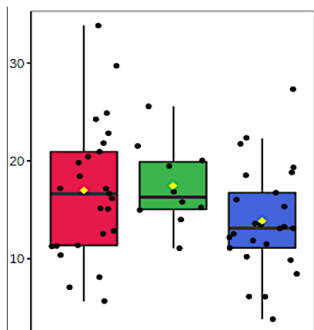
Androgen & Estrogen Metabolism



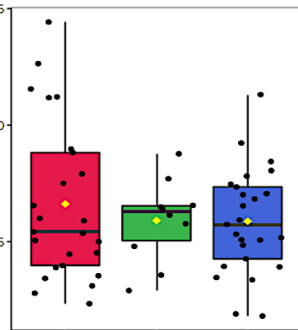
Androstenedione Metabolism



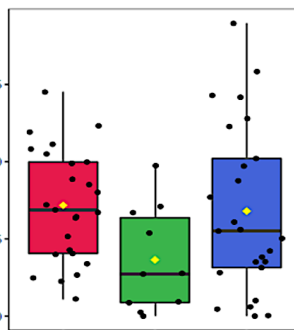
INSOLUBLE FIBER



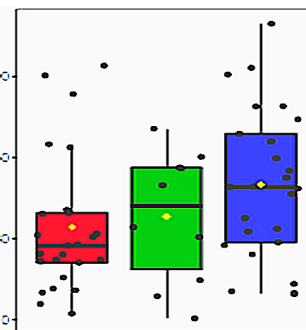
SOLUBLE FIBER



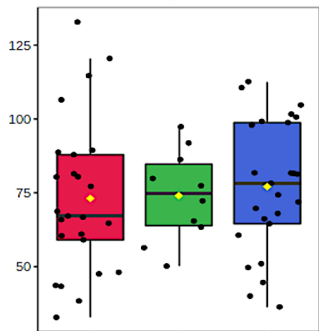
GLUTEN



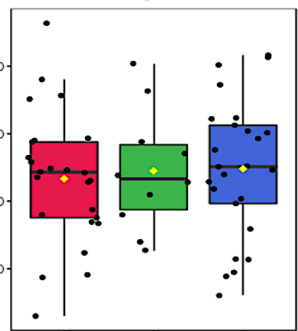
ADDED SUGAR



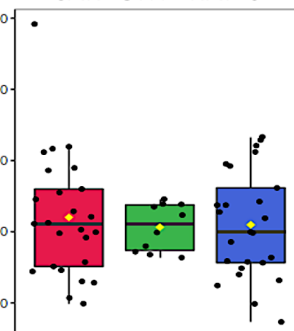
FAT



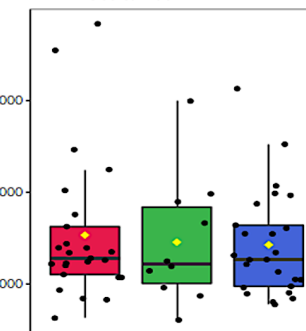
PROTEIN



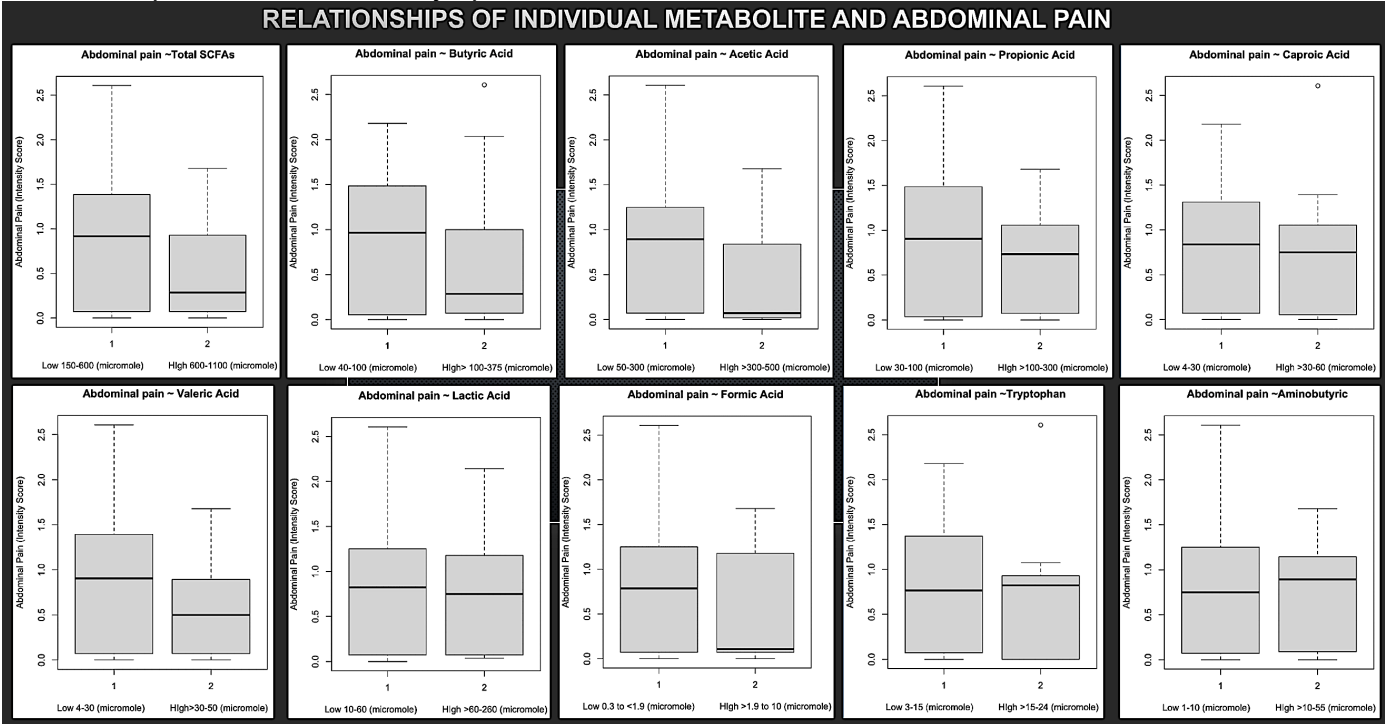
CARBOHYDRATES



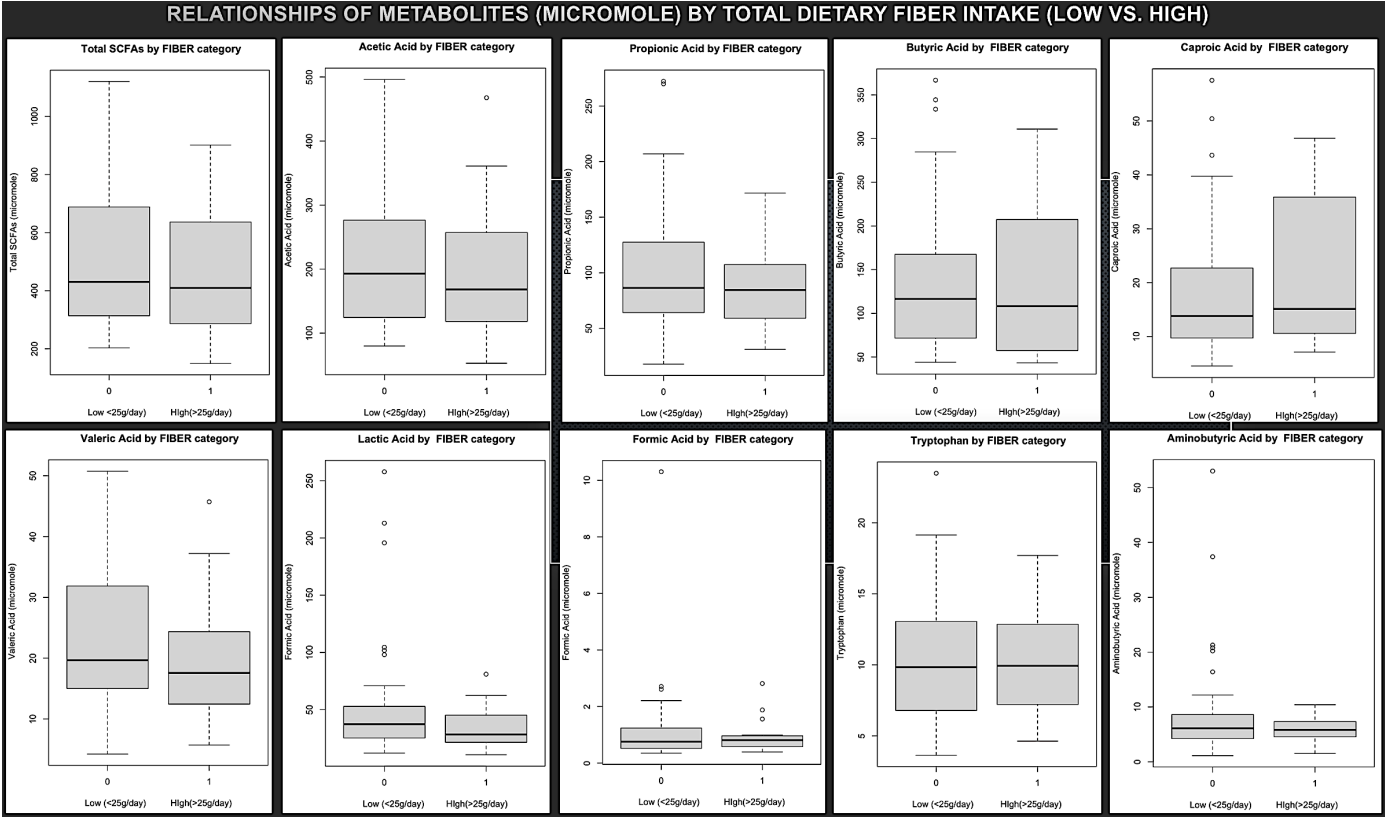
WATER



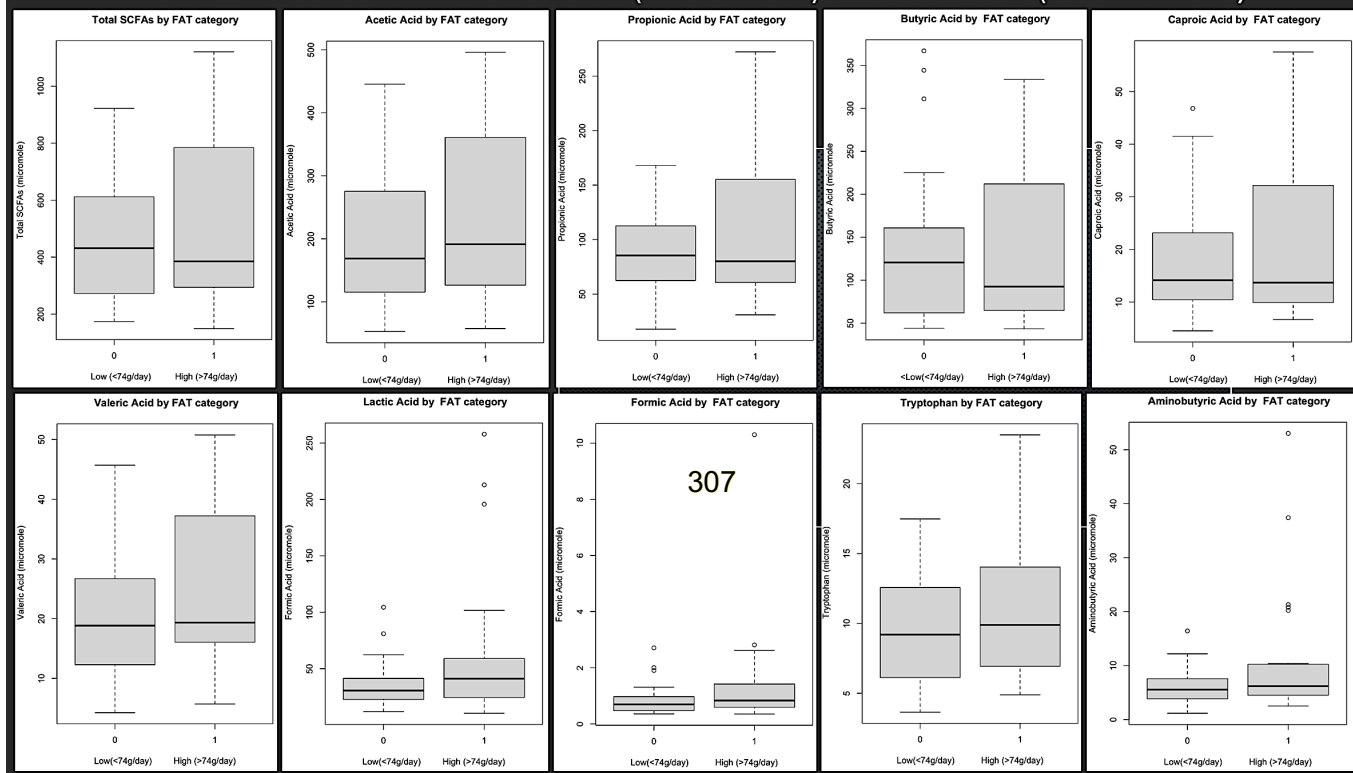
Aim2 a Boxplots: SCFA and GI Symptoms



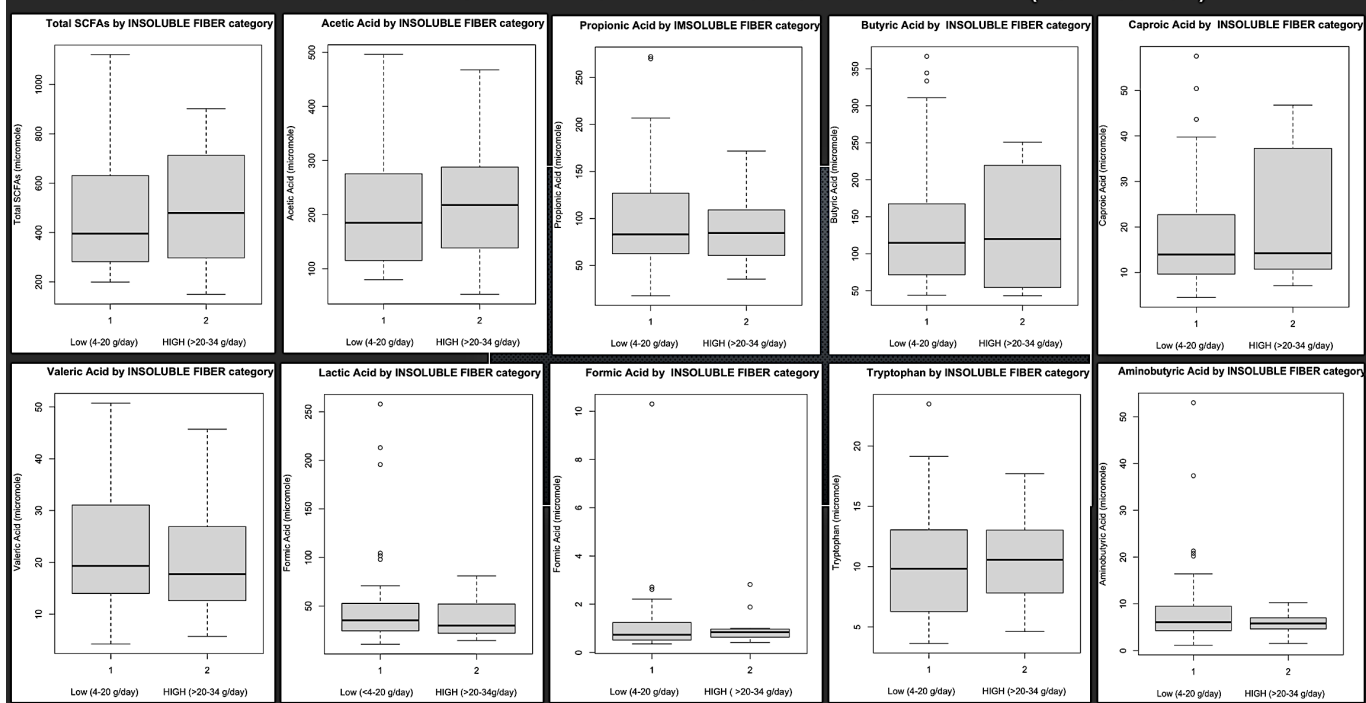
AIM2b: FIBER and SCFA



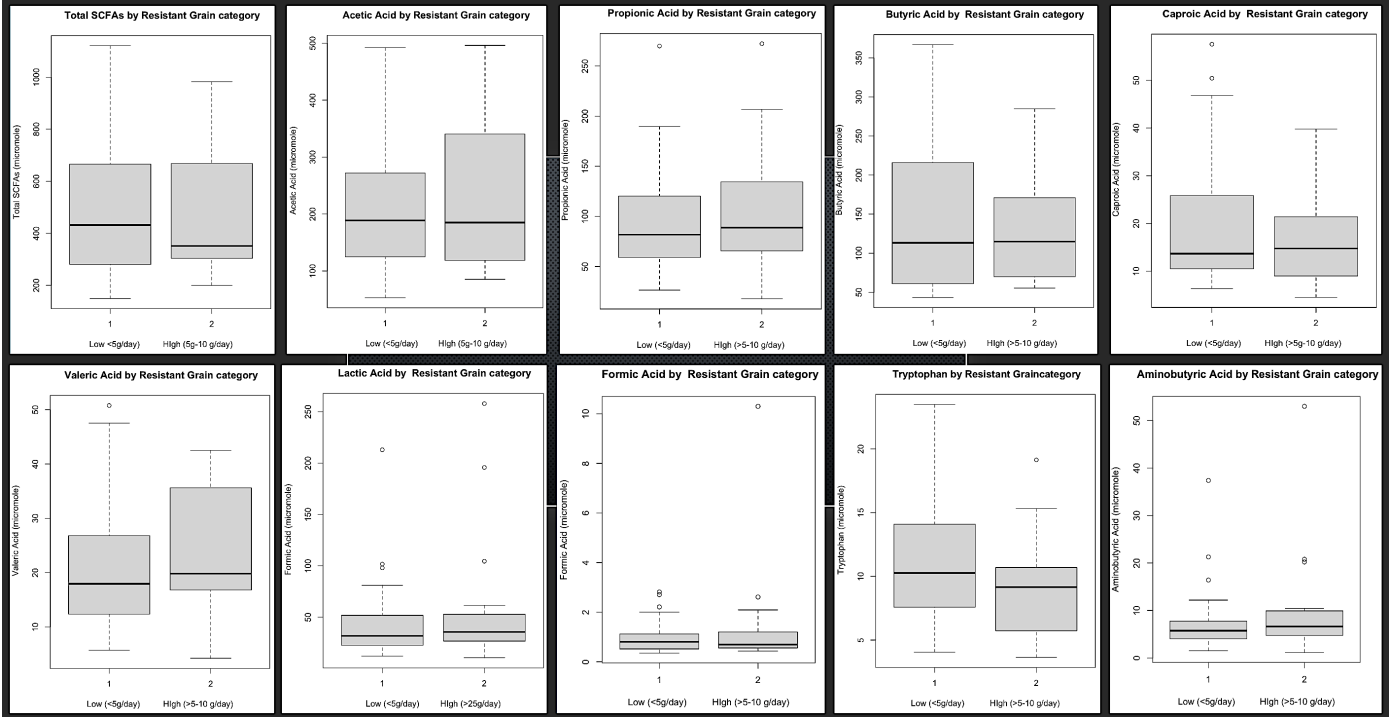
RELATIONSHIPS OF METABOLITES (MICROMOLE) BY FAT INTAKE (LOW VS. HIGH)



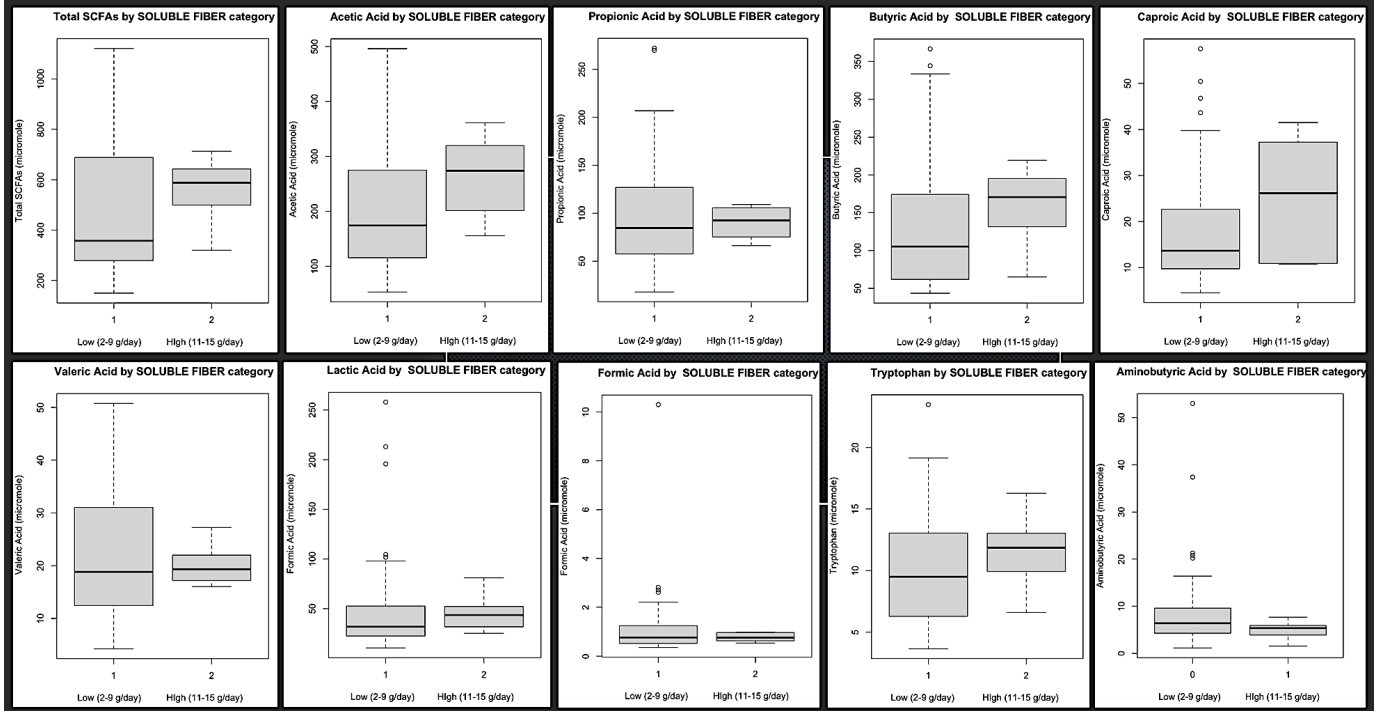
RELATIONSHIPS OF INDIVIDUAL METABOLITE AND INSOLUBLE FIBER INTAKE (LOW VS. HIGH)



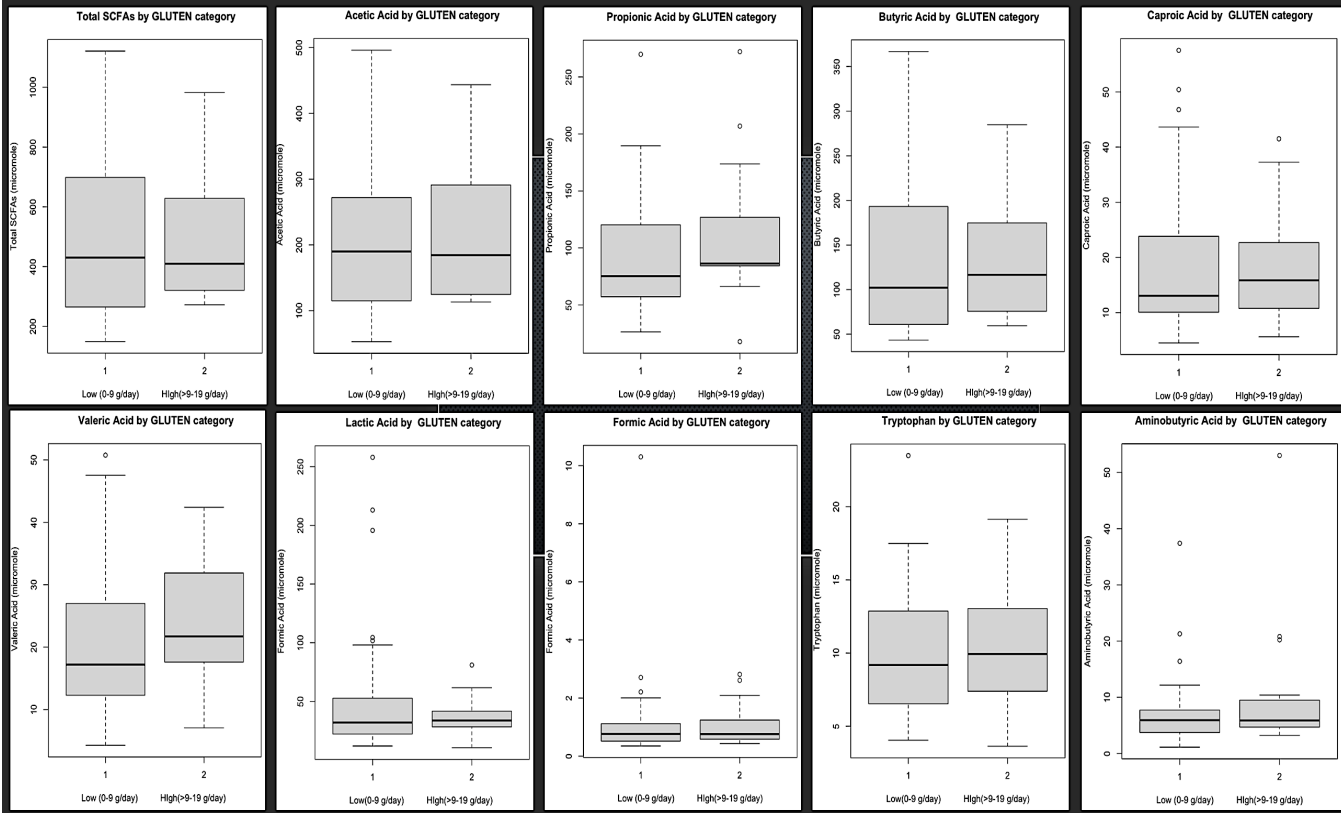
RELATIONSHIPS OF INDIVIDUAL METABOLITE AND RESISTANT GRAIN INTAKE (LOW VS. HIGH)



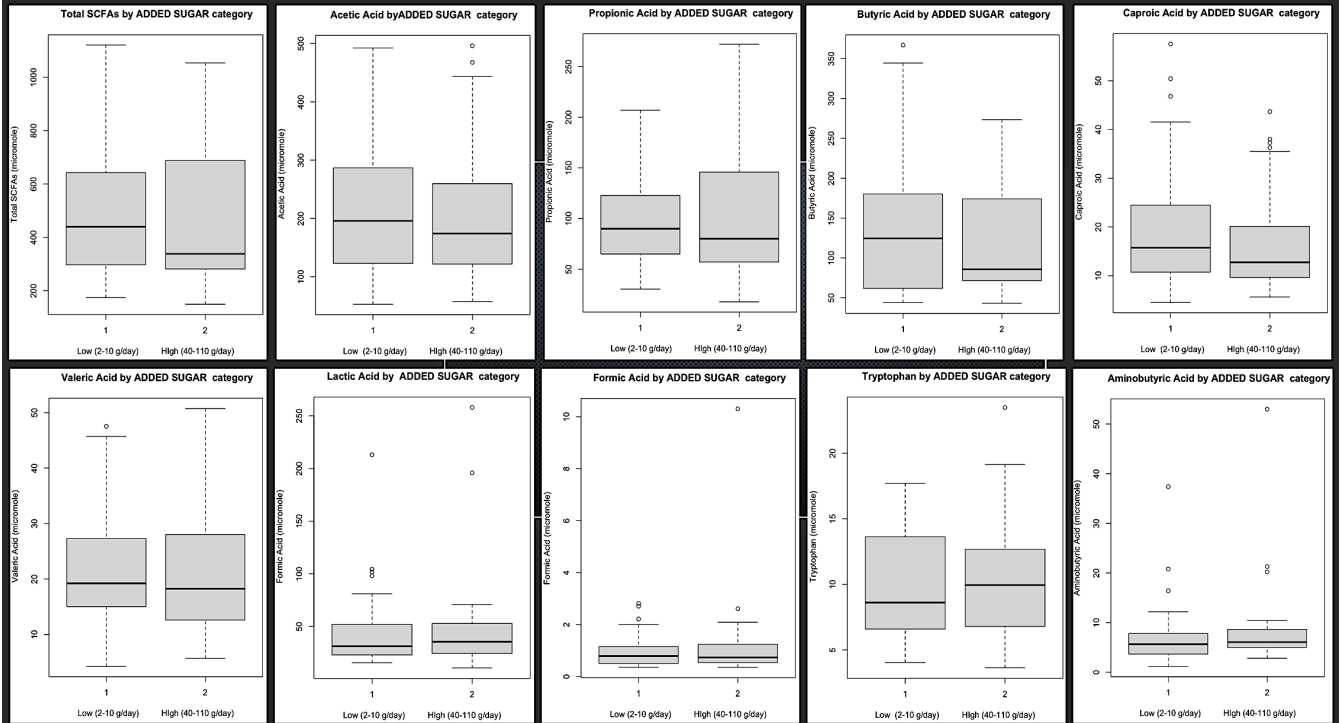
RELATIONSHIPS OF INDIVIDUAL METABOLITE AND SOLUBLE FIBER INTAKE (LOW VS. HIGH)



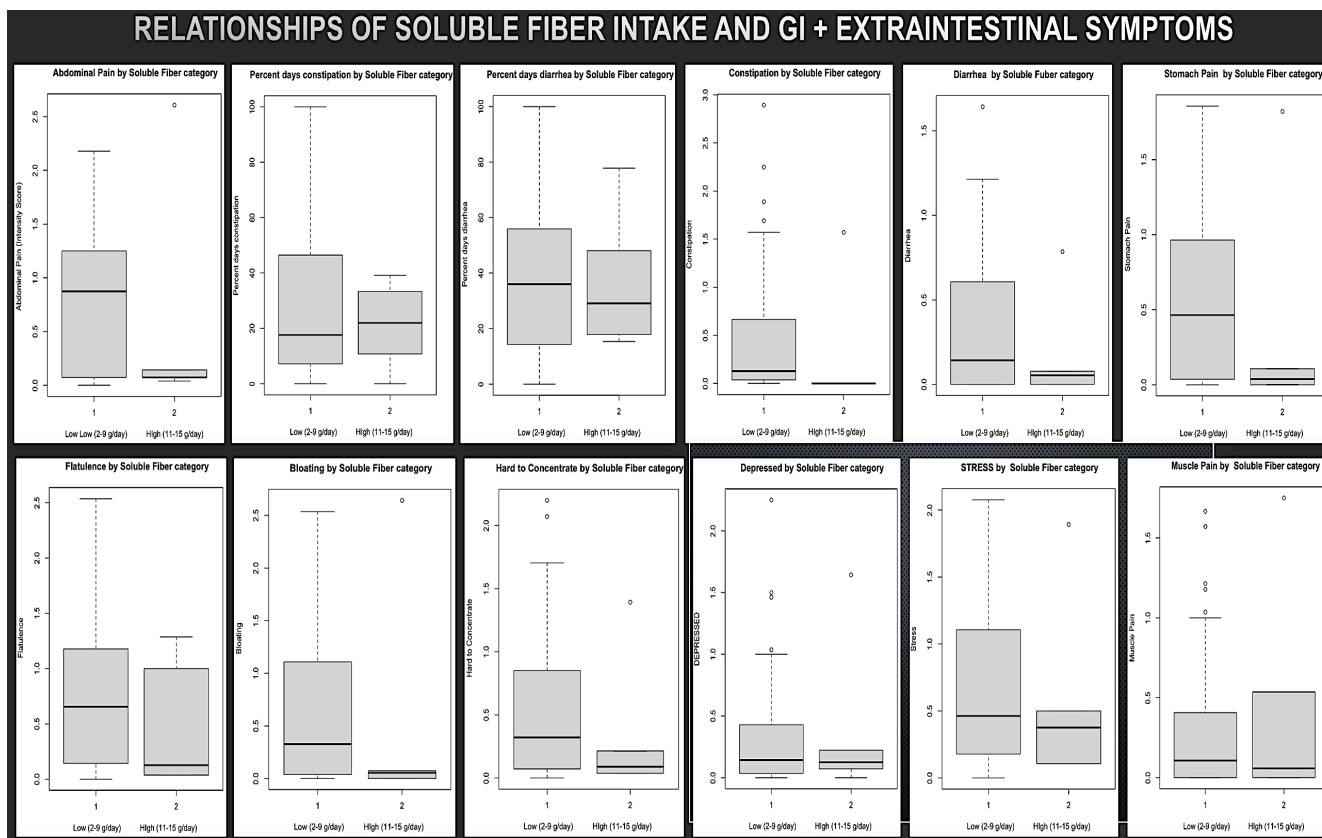
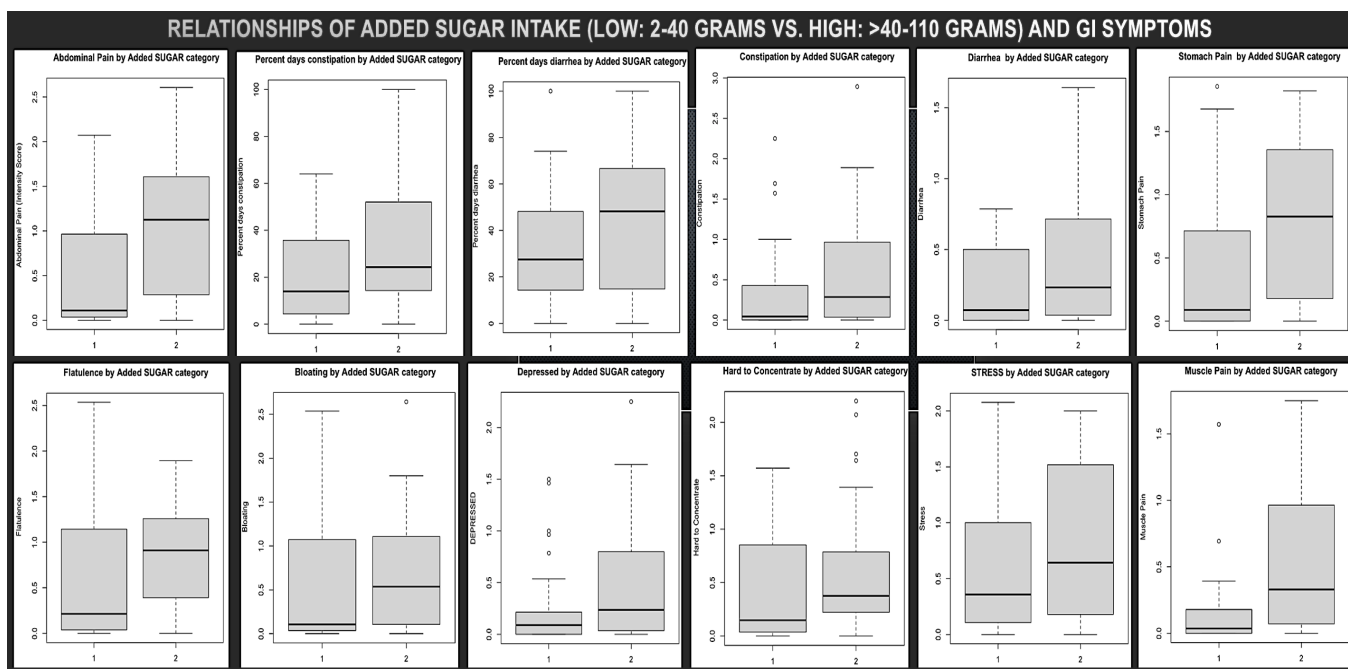
RELATIONSHIPS OF INDIVIDUAL METABOLITE & GLUTEN INTAKE



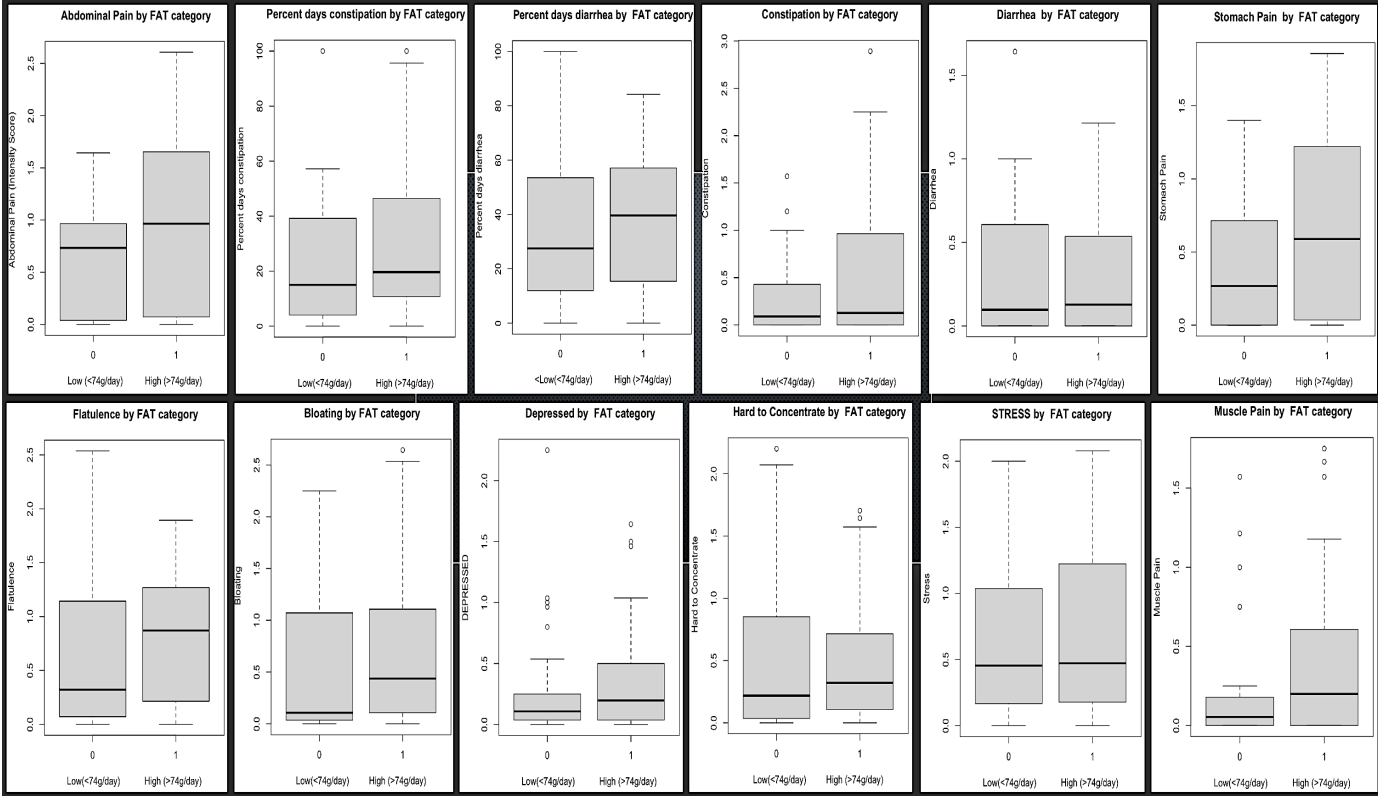
RELATIONSHIPS OF INDIVIDUAL METABOLITE & ADDED SUGAR INTAKE



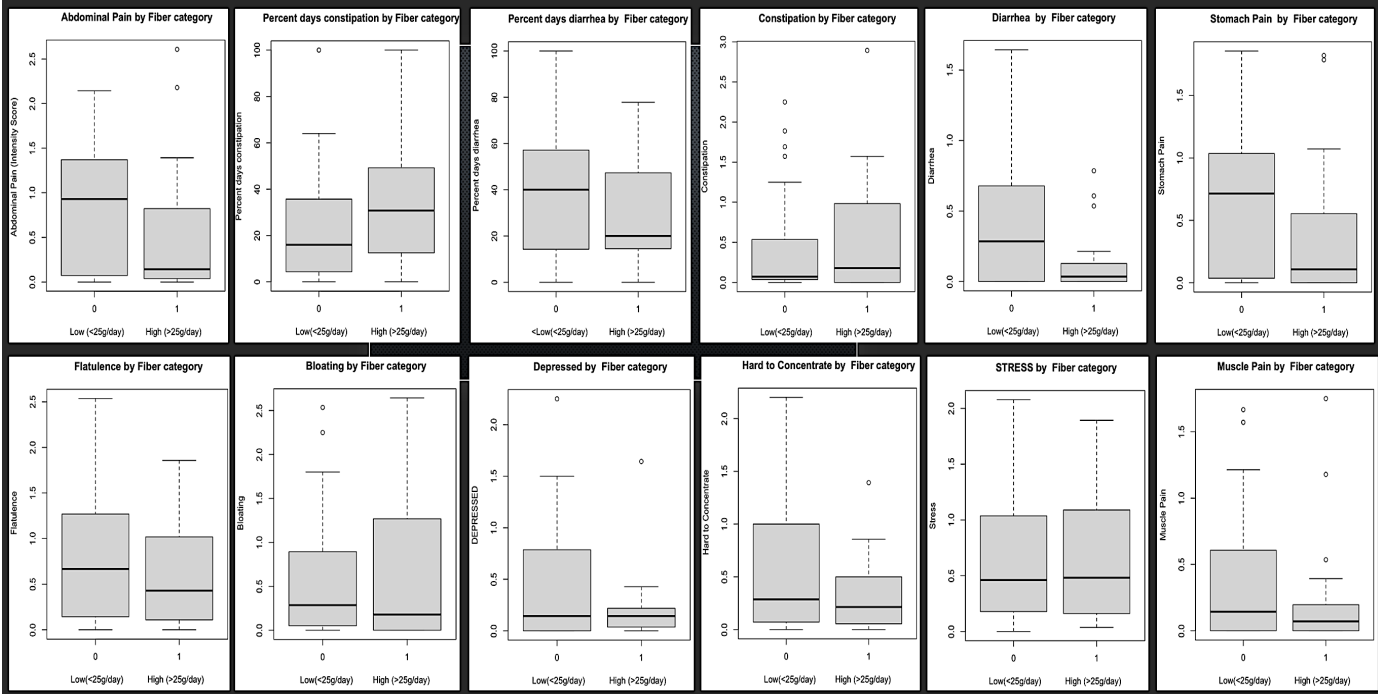
AIM 2c: DIET and GI SYMPTOMS



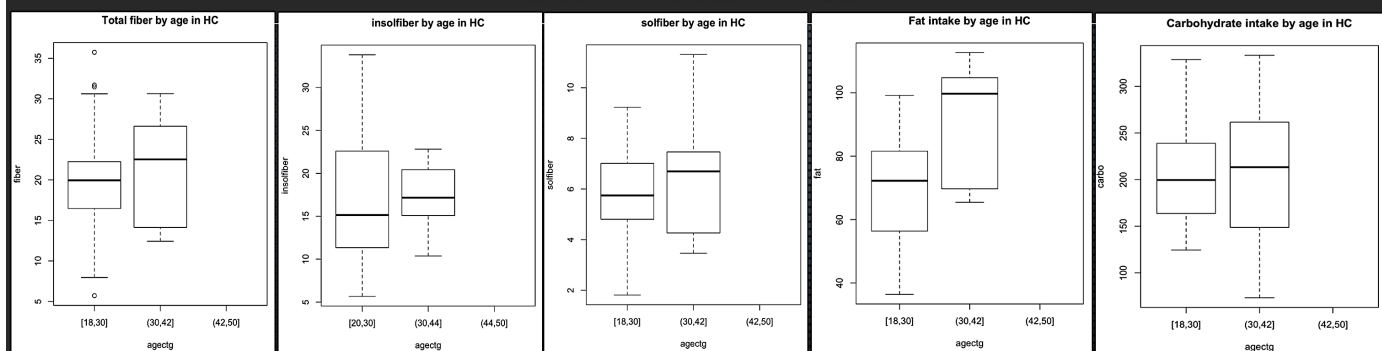
RELATIONSHIPS OF FAT INTAKE AND GI SYMPTOMS



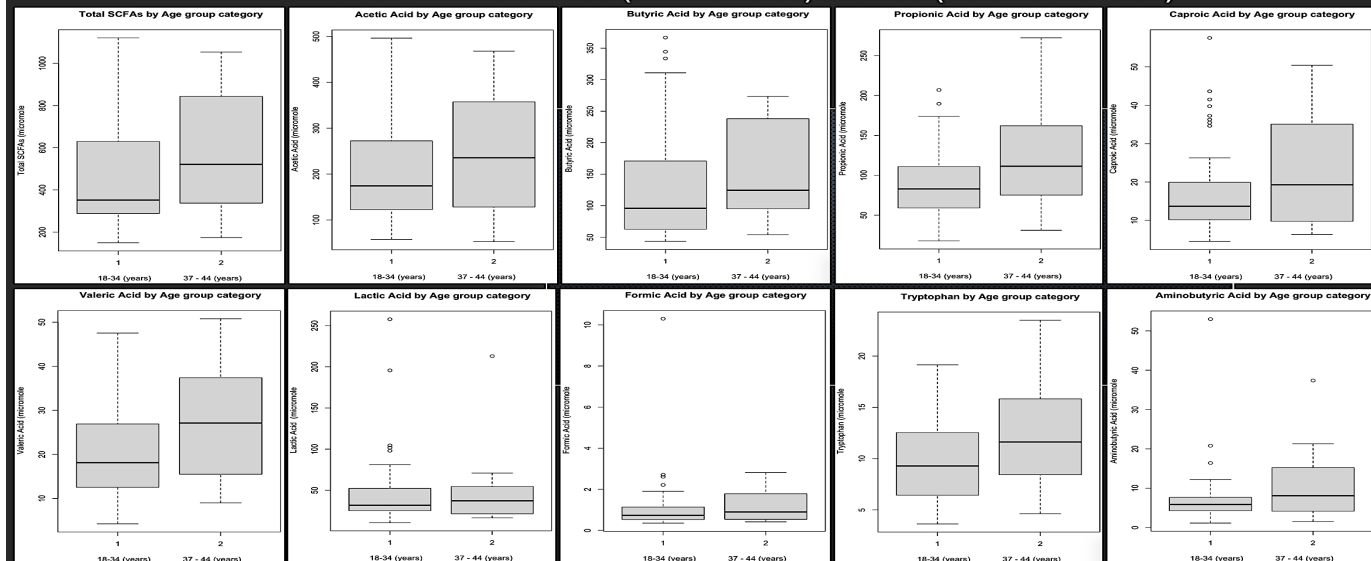
RELATIONSHIPS OF TOTAL DIETARY FIBER AND GI + EXTRAEINTESTINAL SYMPTOMS



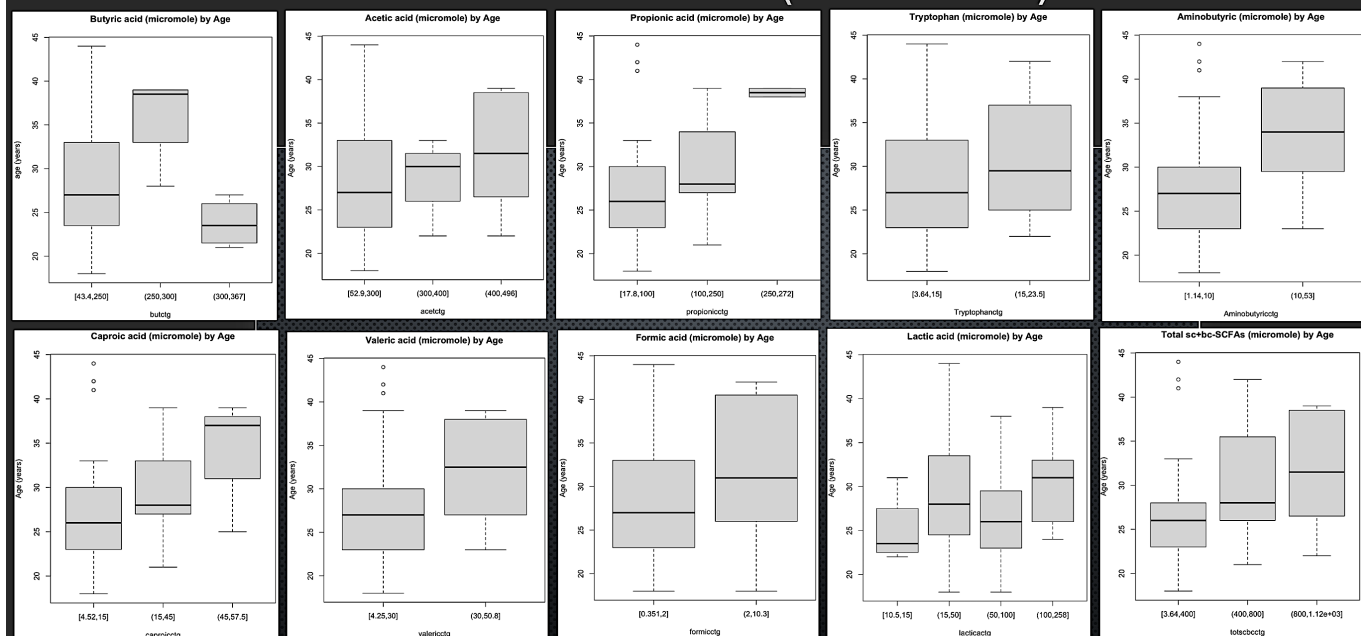
DIETARY INTAKE BY AGE



RELATIONSHIPS OF METABOLITES (MICROMOLE) BY AGE (YOUNG VS. OLD)



RELATIONSHIPS OF METABOLITES (MICROMOLE) BY AGE



RELATIONSHIPS OF METABOLITES (MICROMOLE) BY BMI (LOW VS. HIGH)

