Diet and inflammatory bowel disease; a metabolomic approach

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

Introduction:

Inflammatory bowel disease (IBD), which is consisted of ulcerative colitis (UC) and Crohn's disease (CD), is a relapsing-remitting inflammatory condition of gastrointestinal tract. Although the exact pathophysiology of IBD is not known yet, it has been suggested that a combination of various genetic, microbial, immunological and environmental factors play a role in IBD developments. Previous epidemiological and experimental studies have suggested that dietary factors are among the major environmental contributors of IBD development. Furthermore, many IBD patients attribute their disease onset or relapse to dietary factors. In addition, only a limited number of clinical trials have been carried out to investigate the beneficial effects of dietary modifications for management of IBD symptoms or prevention of disease relapse. However, understanding the role of diet in IBD is challenging due to its multi-faceted effects on host and microbial factors. It has recently been suggested that metabolomics which is the science of studying metabolites in different biological samples in a comprehensive way has the potential to be used for unraveling the role of diet in chronic diseases.

Objectives: In the present thesis, our aim was to investigate the role of diet in the development or management of IBD using a metabolomic approach.

Methods: This thesis is consisted of four sub-projects. In the first study, urinary metabolome was compared between a group of CD patients who developed CD recurrence after ileocolonic resection (n=28) and CD patients who were still in remission after ileocolonic resection (n=10). In the second study the usefulness of urinary metabolomic profiling was assessed to

differentiate between UC patients (n=53) and irritable bowel syndrome (IBS) patients (n=39). In the third project which was a prospective cohort study in UC patients in clinical remission (n=20), the dietary, clinical and metabolomic factors at baseline were compared between patients who presented with UC clinical relapse during the 1-year follow-up and patients who were still in clinical remission. In the last project which was a 6-month randomized controlled trial, 53 adults UC patients were randomized to either an anti-inflammatory diet or a control diet (Canada's Food Guide). The effects of the anti-inflammatory diet for maintenance of remission and prevention of colonic inflammation and the underlying mechanisms were assessed using a metabolomic approach.

Results:

Endoscopic recurrence was associated with increased concentration of urinary levoglucosan which is a diet-related metabolite. In addition, urinary metabolomic profiles of UC patients was significantly different from urinary metabolomic profiles of IBS patients. Decreased amino acids were characteristics of metabolome in UC patients. Furthermore, we found that metabolites in urine and serum could be related to clinical relapse in UC patients. Finally, we should that following an anti-inflammatory diet for 6 months could prevent increases in fecal calprotectin; a major biomarker of colonic inflammation in UC patients. We also identified a number of host and diet-related metabolites in urine and serum that significantly changed from baseline to the end of the study in patients randomized to the anti-inflammatory diet.

Conclusions:

These findings indicate that metabolomics can be used in IBD settings to explore the role of diet in the pathophysiology or management of the disease. These findings provide a basis for further research in this field.

Preface

This thesis is an original work by Ammar Hassanzadeh Keshteli. Ethical approval for all projects was obtained from the Health Research Ethics Board-Biomedical Panel, University of Alberta: The microbiology and immunology of Post-Operative Crohn's Disease Recurrence: Pro00028147, Diets and inflammatory bowel disease - Pilot Study: Pro00032213, and Quality Food Grant - IBD and Diet Study: Pro00035413. All experimental works and recruitment of patients were conducted under my PhD supervisors Dr. Karen Madsen and Dr. Levinus Dieleman.

Section 1.1.4.5 of the first chapter and chapter 5 have been published as Keshteli AH, Madsen KL, Dieleman LA. Diet in the Pathogenesis and Management of Ulcerative Colitis; A Review of Randomized Controlled Dietary Interventions. Nutrients. 2019;11(7). pii: E1498. I was involved In study conceptualization, did the literature review, drafted of the manuscript, and revised it with Dr. Madsen and Dr. Dieleman.

Chapter 2 of this thesis has been published as Keshteli AH, Tso R, Dieleman LA, Park H, Kroeker KI, Jovel J, Gillevet PM, Sikaroodi M, Mandal R, Fedorak RN, Madsen KL. A Distinctive Urinary Metabolomic Fingerprint Is Linked With Endoscopic Postoperative Disease Recurrence in Crohn's Disease Patients. Inflamm Bowel Dis. 2018;24(4):861-870. I was responsible for doing chart reviews, statistical analysis, metabolomic analysis and drafting the manuscript.

Chapter 3 of this thesis has been published as Keshteli AH, Madsen KL, Mandal R, Boeckxstaens GE, Bercik P, De Palma G, Reed DE, Wishart D, Vanner S, Dieleman LA. Comparison of the metabolomic profiles of irritable bowel syndrome patients with ulcerative colitis patients and

healthy controls: new insights into pathophysiology and potential biomarkers. Aliment Pharmacol Ther. 2019;49(6):723-732. I was involved in study design and recruitment of UC patients. I performed all microbial, metabolomic, and statistical analyses and prepared the first draft of the manuscript.

Chapter 4 of this thesis has been published as Keshteli AH, van den Brand FF, Madsen KL, Mandal R, Valcheva R, Kroeker KI, Han B, Bell RC, Cole J, Hoevers T, Wishart DS, Fedorak RN, Dieleman LA. Dietary and metabolomic determinants of relapse in ulcerative colitis patients: A pilot prospective cohort study. World J Gastroenterol. 2017;23(21):3890-3899. I was involved in study design and patients recruitment, did the statistical analysis, metabolomic analysis, and drafted the paper.

Chapter 5 of this manuscript is a collaborative work with Mrs. Cheryl Nickurak, Dr. Rosica Valcheva, Dr. Karen Kroeker, Dr. Rupasri Mandal, Dr. David S Wishart, Dr. Sander Veldhuyzen van Zanten, Dr. Brendan P Halloran, Dr. Richard N Fedorak, Dr. Karen Madsen and Dr. Levinus A Dieleman. I was involved in study design and concepts, responsible to recruit participants, do chart reviews, follow-ups, perform statistical analysis, microbial analysis, metabolomic analysis and draft the manuscript for submission to a journal in the field of gastroenterology.

The remainder of this work is original, performed by myself in consultation with my supervisory committee.

This thesis is dedicated to my wife, Samaneh and my parents, Moloud and NourAllah

For their endless love, support and encouragement.

Acknowledgments

First and foremost, I would like to thank my co-supervisors, Dr. Karen Madsen and Dr. Levinus A. Dieleman for their endless support, patience and encouragement during the years of my Ph.D. It has been an honor for me to be their student. I am grateful to them for trusting me and providing me with numerous opportunities to experience and learn. My PhD thesis would not have been accomplished without their invaluable support and guidance.

I would also like to thank my PhD committee member and career advisor Dr. Leah Gramlich for her thoughtful and constructive comments and feedback.

Finally, I would like to thank my sisters Maedeh and Samaneh for their love and support.

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1. Introduction

1.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract characterized by a relapsing and remitting course (1). The worldwide incidence of IBD has increased dramatically during the past few decades. The onset of IBD symptoms is at young adulthood which continues throughout a patient's lifespan (2). Patients with IBD experience different gastrointestinal symptoms including diarrhea that may contain blood, weight loss, abdominal pain and fatigue (1, 2). Up to 40% of IBD patients may experience extra-intestinal manifestations of the disease such as musculoskeletal, dermatologic, hepato-pancreato-biliary, ocular, renal and pulmonary-related conditions (3). IBD is associated with morbidity, mortality, and substantial costs to the health-care system. In Canada, the direct healthcare cost of IBD is suggested to be higher than CAD $2 billion and its total indirect health-related cost is estimated to be CAD $1.29 billion in 2018 (4, 5).

1.1.2 Ulcerative colitis and Crohn's disease

Ulcerative colitis (UC) and Crohn's disease (CD) are the major subtypes of IBD. Although UC and CD patients may experience similar symptoms, they differ in the anatomical location of involvement, pattern of inflammation and the layers of the gastrointestinal tract which are involved. While in UC inflammation is limited to the mucosal layer, there is a transmural involvement in CD which means all layers of the gut can be affected. Furthermore, while UC

only affects colon, CD can involve any part of the gastrointestinal tract (most commonly the terminal ileum or the perianal region). In addition, UC extends proximally in a continuous manner starting from the anus whereas CD involvement may not be continuous which is known as skip lesions (1, 2).

1.1.3 Epidemiology of IBD

The incidence of IBD has increased substantially in the western world since the recognition of UC in 1875 and CD in 1932. However, a recent systematic review (6) of 147 population-based studies on the incidence or prevalence IBD published from 1990 to 2016 reported that the IBD in most western countries has begun to stabilise or decrease. However, the prevalence of IBD has increased to more than 0·3% of the population in North America, Australia, and many countries in Europe. In addition, the incidence of IBD is increasing rapidly in newly industrialised countries and Asian immigrants to the west due to rapid socioeconomic development and westernisation (Figure 1.1 and Figure 1.2). The incidence and prevalence of IBD in Canada are among the highest in the world. The prevalence of IBD in 2018 was 0.7% of the Canadian population which is estimated to rise to 1.0% of the population by 2030 (403,000 people) (7). There is a relatively equal gender distributions of IBD rate across multiple studies. The incidence of IBD has been found to be the highest between the second and fourth decade of life. The median age of disease onset is slightly higher in UC patients than CD patients (8).

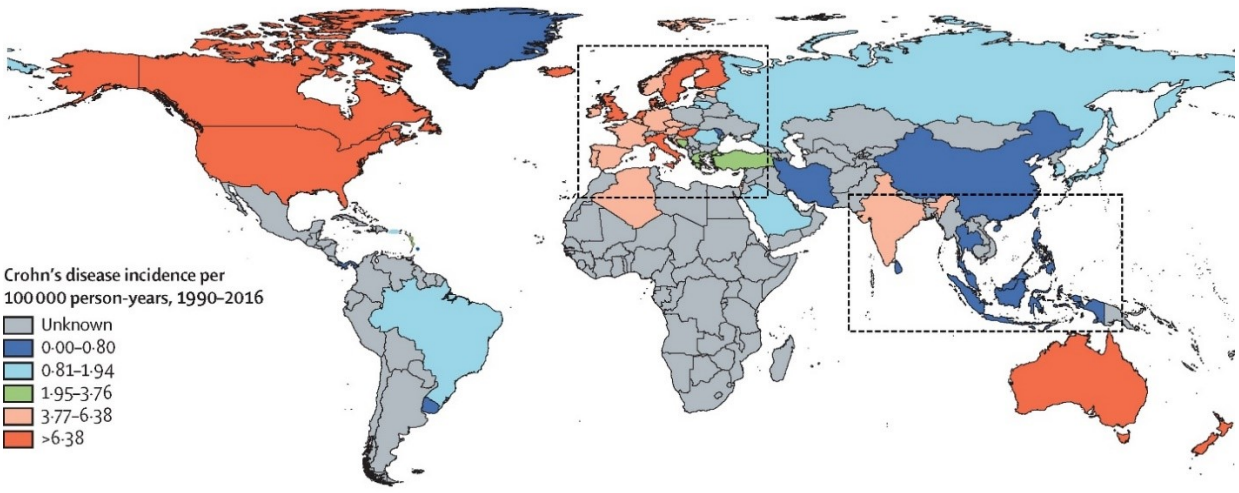


Figure 1.1. Worldwide incidence of Crohn's disease [Adapted with permission (6)].

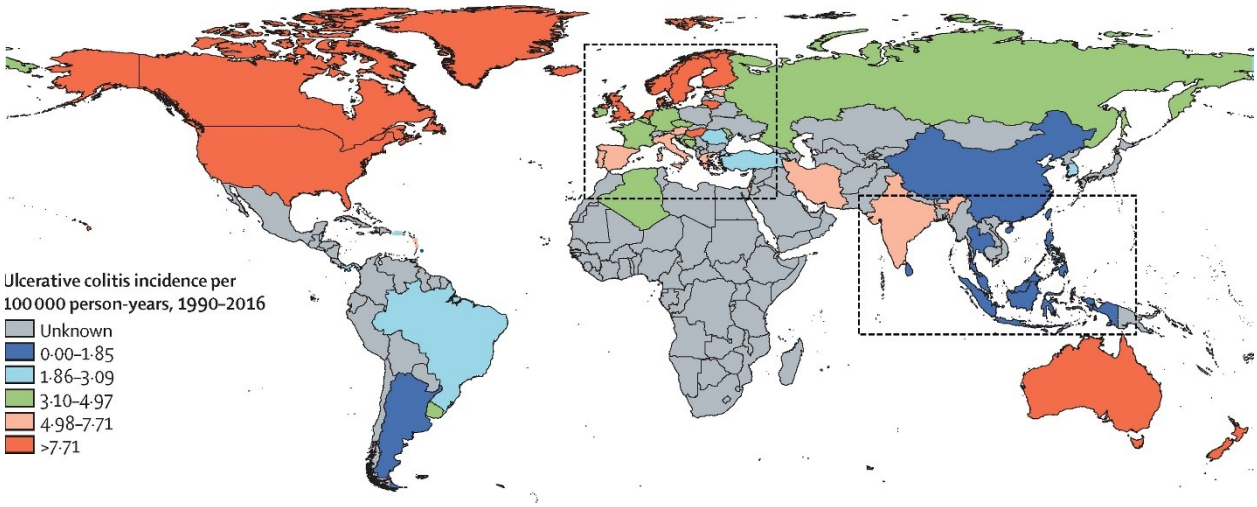


Figure 1.2. Worldwide incidence of ulcerative colitis [Adapted with permission (6)].

1.1.4 Pathophysiology of IBD

The exact pathophysiological mechanisms of IBD development remain unknown. However, it has been suggested that IBD etiology involves a combination of different environmental and microbial risk factors that leads to dysregulated immunological response against the intestinal wall in genetically susceptible individuals (1, 2).

1.1.4.1 Genetic risk factors of IBD development

The role of genetic factors in the etiology of IBD development has been shown in several studies. It has been shown that a child has a 26-fold increased risk for developing CD when another sibling already has it, and the risk is 9-fold increased in the case of UC. In addition, twin studies in CD have shown a 50% concordance in monozygotic twins compared to <10% in dizygotic individuals (9, 10).

Many susceptibility loci responsible for IBD development have been identified in genome-wide association studies. These susceptibility loci are related to several pathways that are crucial for barrier function, epithelial restitution, microbial defence, innate immune regulation, reactive oxygen species generation, autophagy, regulation of adaptive immunity, endoplasmic reticulum stress and metabolic pathways associated with cellular homeostasis and microbial recognition (11). However, genetic factors account for only a small proportion of the disease variance: 13.1% for CD and 8.2% for UC which highlight the important role of microbial and environmental factors (9).

1.1.4.2 Immune factors in IBD

In the human intestines, the first layer of defence against the “outside” is provided by the epithelial layer of gastrointestinal tract which creates a physical barrier against infections. The mucus layer which makes the second defence layer, is consisted of a complex web of mucin and antimicrobial proteins that cover the epithelial layer. It inhibits microorganisms from reaching epithelial cells. The third defence layer is provided by the many immune cells that reside in the gastrointestinal tract, either in organized structures (e.g. Peyer's patches and mesenteric lymph nodes) or scattered throughout the intestinal epithelium and lamina propria. Therefore, the intestinal mucosal barrier which is comprised of mucus layer, epithelial cells and immune cells makes a barrier against the commensal microorganisms, pathobionts, antigens, chemicals, etc (12).

The growing body of evidence suggest that dysfunctions of both innate and adaptive immune pathways contribute to the dysregulated inflammatory response as seen in patients with IBD. The adaptive immune response has classically been considered to play a major role in the pathogenesis of IBD. While CD has long been considered to be driven by a T helper cell type (Th) 1 response, UC has been rather associated with a Th2 response. In addition to the classical Th1 and Th2 responses, a role for Th17 cells has also been suggested in IBD development. Although T cells are key drivers of intestinal inflammation in IBD pathogenesis, the specific contribution of different T cell subsets to CD and UC lesions needs still to be fully explored. Recent advances from immunological and genome-wide association studies have moved the focus of IBD pathogenesis to mucosal innate immune responses. Therefore, it has been suggested that an altered epithelial barrier function contributes to intestinal inflammation in

patients with UC. In CD patients a dysregulation in antimicrobial peptide production, innate microbial sensing and autophagy contribute to disease pathogenesis (13, 14).

1.1.4.3 Gut microbiota in IBD

In human gut, there are a large number of microorganisms including bacteria, viruses, fungi, and protozoa that are collectively referred to as the gut microbiota. Bacteria constitute a major part of our microbiota. The human gut microbiota has physiological functions associated with nutrition (e.g. fermentation of complex undigested polysaccharide polymers, production of short-chain fatty acids (SCFAs), synthesis of certain vitamins, energy production), the immune system (e.g. Th17/T_{reg} balance), and defense of the host. Bacteria are the major constituent of gut microbiota. More than 90% of healthy human gut bacterial species belong to four major phyla: Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria. Within the gastrointestinal tract, 70% of the bacteria colonize in the colon (15, 16).

Findings from genome wide association studies have identified some IBD associated-susceptible genes (e.g. nucleotide-binding, oligomerization-domain-containing protein 2 (NOD2)), that are related to mediating host response to gut microbiota. These findings have been the basis to investigate the microbial contributors of IBD development in other studies. Microbial dysbiosis which is defined as the imbalance in the structures and/or functions of gut microbiota is a key characteristic of gut microbiota in patients with IBD. Microbial dysbiosis in IBD patients is associated with several abnormalities found in IBD patients such as increased epithelial

permeability, epithelial dysfunction, and dysregulated immune response. However, it is still not clear if microbial dysbiosis is the cause of these abnormalities or their consequence (16-19).

Although a core microbiome associated with IBD has not been defined yet (16), several changes in bacterial composition have been reported in IBD patients. For instance, decreased richness and diversity of the fecal intestinal microbiome and decreased abundance of phylum *Firmicutes* was reported in CD patients (20). In another large-scale study on CD children with new onset disease, CD was characterized by increased abundance of *Enterobacteriaceae*, *Pasteurellacaea*, *Veillonellaceae* and *Fusobacteriaceae* and a decreased abundance of *Erysipelotrichales*, *Bacteroidales* and *Clostridiales* (21). In UC patients decreased bacterial diversity with a lower proportion of *Firmicutes* and increased *Gamma-proteobacteria* and *Enterobacteriaceae*, has been reported. Furthermore, UC patients may have increased sulphite-reducing *Deltaproteobacteria* in their colon (22).

Recent advances on the role of gut microbiota in IBD pathogenesis have led to the development of novel therapeutic options based on the modulation of gut microbiota in IBD patients. These emerging microbiota alterations treatments include the use of antibiotics, prebiotics, probiotics, synbiotics and fecal microbiota transplantation. However, these therapeutic approaches are still under investigation and further research studies such as clinical trials are required to elucidate their position in the treatment of IBD (16, 23).

1.1.4.4 Environmental factors in IBD

The role of environmental factors in the development of IBD has been shown in many animal studies and observational studies (24). In spite of shared pathophysiological mechanisms between CD and UC, the effect of some of the environmental risk factors appears to differ between these two IBD subtypes which suggest the existence of some unique mechanisms in their development. A recent meta-analysis study (25) identified nine factors that increased risk of IBD: smoking (CD), urban living (CD and IBD), appendectomy (CD), tonsillectomy (CD), antibiotic exposure (IBD), oral contraceptive use (IBD), consumption of soft drinks (UC), vitamin D deficiency (IBD), and non-*Helicobacter pylori*-like enterohepatic *Helicobacter* species (IBD). The authors also identified seven factors that reduced risk of IBD: physical activity (CD), breastfeeding (IBD), bed sharing (CD), tea consumption (UC), high levels of folate (IBD), high levels of vitamin D (CD), and *H pylori* infection (CD, UC, and IBD). Epidemiologic evidence for all of these associations was of high to moderate strength (25). It should be noted that environmental triggers of disease relapse in IBD patients have not been studied extensively yet.

1.1.4.5 Dietary factors in IBD

One of the major environmental factors that contribute to the development of IBD is dietary factors. Significant changes in dietary intake during the past decades have been associated with the increase in incidence of UC. The relationship between diet and IBD development has been indicated in several epidemiological studies (26). Two recent meta-analysis studies showed that soft drink consumption and sucrose intake was associated with increased risk of UC development, respectively (27, 28). Consumption of fruits and vegetables was related to

decreased odds of UC and CD development in another meta-analysis study (29). A significant association between meat intake (red meat in particular) and IBD risk was found in another meta-analysis study (30). Furthermore, while n-3 polyunsaturated fatty acids (PUFAs) content of diet was related to decreased odds of UC development (31), dietary arachidonic acid (an n-6 PUFA) as measured in adipose tissue increased risk of UC development in a large prospective cohort study among Danish adults (32). Although the exact pathophysiological mechanisms in which diet plays a role in IBD development remain unknown, several plausible explanations including its effects on composition of gut microbiota, production of microbial metabolites, alterations in mucosal immunity and mucosal barrier function have been proposed (33) (**Figure 1.3**).

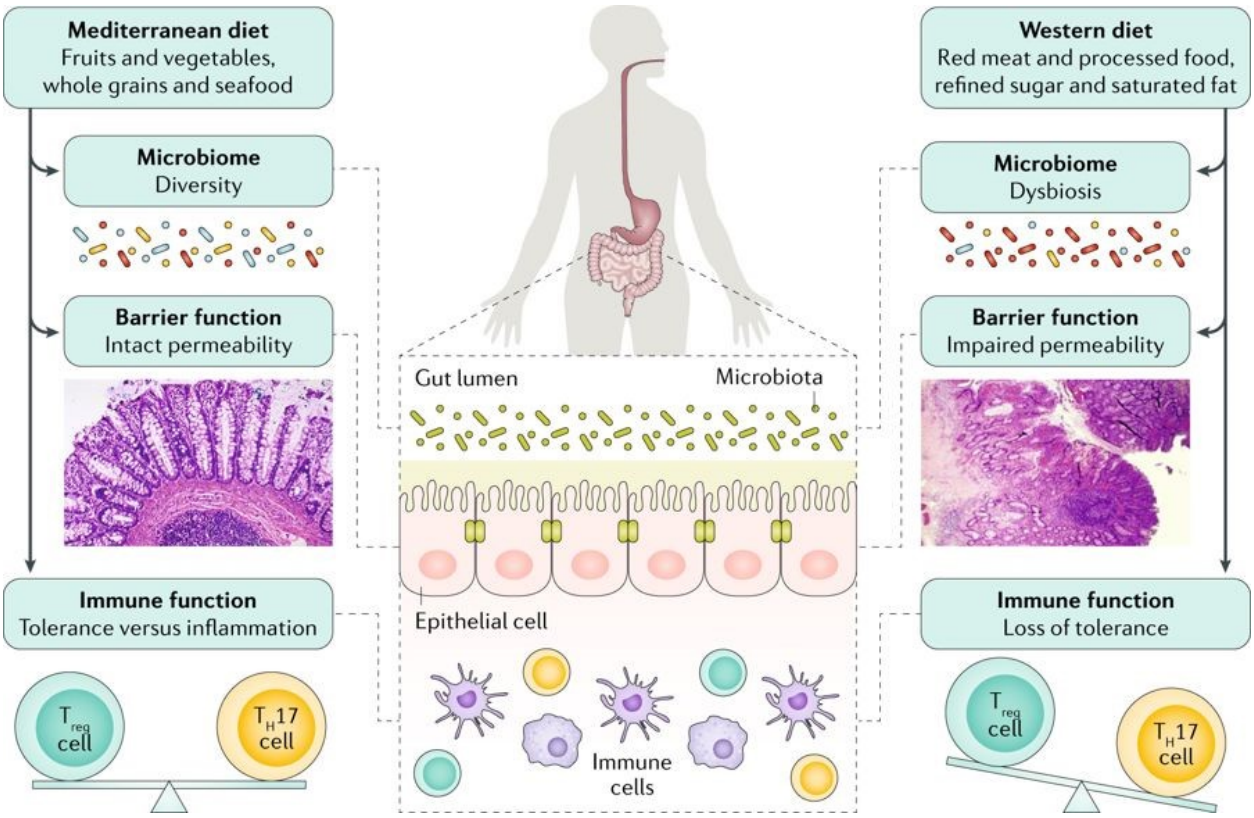


Figure 1.3. Although the exact mechanisms responsible for the association between diet and development of inflammatory bowel disease is unknown, several mechanisms have been suggested. An unhealthy dietary pattern such as a Western diet has been linked to changes in the gut microbiome and epithelial barrier function and seems to have a direct influence on immune function, triggering a pro-inflammatory environment characterized by an imbalance in the T helper 17 (TH17) cell to regulatory T (Treg) cell ratio [Adapted with permission (33)].

Dietary factors can be related to IBD pathogenesis or disease course through direct effects on the host or indirect effects through modulations of composition or function of gut microbiota. Diet has a major role in shaping gut microbial composition (34). For instance, increased

Bacteroidetes and decreased *Firmicutes* and *Enterobacteriaceae* in rural African children in comparison to European children were mainly attributed to differences in dietary patterns between the two populations (35). Therefore, it has been suggested that diet-induced changes in microbiota may transform healthy gut microbiota into a disease-inducing entity that could either initiate or perpetuate inflammation in patients with IBD (34). Agus et al. (36) indicated that a high fat/high sugar diet resulted in intestinal mucosal dysbiosis characterized by an overgrowth of pro-inflammatory proteobacteria and a decrease in protective bacteria. In addition, they showed that the transplantation of feces from high fat/high sugar fed mice to germ-free mice increased susceptibility to adherent-invasive *E. coli* infection.

In addition to their significant effects on microbial composition, dietary factors can also affect the metabolic functions of gut microbiota. Short chain fatty acids (SCFA) which are defined as the groups of fatty acids with fewer than six carbons, including formic acid (C1), acetic acid (C2), propionic acid (C3), butyric acid (C4) and valeric acid (C5) are derived from commensal bacterial fermentation of indigestible dietary fibers in both the small and large intestine (37, 38). Acetate, propionate and butyrate account for more than 95 % of all the SCFA content in the gut (37). Acetate and butyrate in particular have an essential role in maintaining mucosal barrier function and modulating immune function (39, 40). SCFA regulate the functions of epithelial and/or immune cells through altering gene expression, cellular differentiation, chemotaxis, proliferation and apoptosis (37). The number of SCFA-producing bacteria such as *Faecalibacterium prausnitzii* are decreased in some UC patients and are inversely correlated with disease activity (41). Moreover, a western diet characterized by high intake of sugar (36,

42] and fat (36) and decreased amount of dietary fiber was associated with decreased SCFAs and increased susceptibility to colitis in experimental studies.

Dietary factors can have direct effects on host cells. For instance, it was shown that luminal iron may directly affect function of intestinal epithelial cells and T cells in addition to triggering epithelial cell stress-associated apoptosis (43). Zinc is an important co-factor for various intestinal metalloproteinases and zinc deficiency has been associated with reduced barrier integrity and increased permeability in IBD patients (44). There is also increasing evidence for a role of vitamin D in strengthening the innate immune system and reducing inflammation in experimental and human IBD (45). Relationships between PUFAs content of diet and inflammatory processes in IBD have also been shown (46, 47). Eicosapentaenoic acid and docosahexaenoic acid that are long chain dietary n-3 PUFAs inhibit genes that are involved in inflammatory process and alter the composition of cell membranes by displacing n-6 PUFAs, influencing lipid raft formation in cell signalling (47). Dietary amino acids act as key regulatory factors in cellular and microbial metabolic pathways and play important roles in gut homeostasis. Intestinal inflammation as seen in IBD affects several metabolic pathways related to metabolism of amino acids (40). It has been reported that several food additives, such as maltodextrin, emulsifying agents or thickeners, such as carboxymethyl cellulose, carrageenan, and xanthan gum, may also have detrimental effects on intestinal homeostasis as well (48).

Currently, dietary recommendations for management of IBD-related symptoms are scarce and non evidence-based, mainly due to the limited number of dietary interventions in this population.

1.2. Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder, characterized by abdominal pain or discomfort and alteration in bowel habits. The complex and multifactorial pathophysiology of IBS includes genetic predisposition, visceral hypersensitivity, autonomic nervous system dysregulation, abnormalities in gastrointestinal motility, secretory function, increased intestinal permeability, dysregulated immune activation, and abnormal gut microbial composition and function (49). The role of dietary factors in the prevalence of IBS have also been reported (50). IBS affects as many as 11% of individuals worldwide. It is more prevalent in women than men and is more commonly diagnosed in patients younger than 50 years of age (51).

IBS imposes a significant burden on patients and healthcare systems due to its prevalence and lack of successful treatments (52). It is one of the most common outpatient diagnoses in primary care and gastroenterology (53). In addition, it is associated with an increased number of unnecessary medical tests and procedures, including abdominal surgeries (54). In-patient costs associated with IBS contribute significantly to the total healthcare bill (55).

Currently diagnosis of IBS is based on Rome IV criteria, which are symptom-based diagnostic criteria (56). According to Rome IV criteria, IBS is defined as recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with two or more of the followings: i) related to defecation, ii) associated with a change in frequency of stool, iii) associated with a change in form (appearance) of stool. Criteria should be fulfilled for the last 3 months with symptom onset at least 6 months before the diagnosis. According to the Rome IV criteria, IBS is categorized into the following four subtypes based on the predominant bowel

habit: IBS with predominant constipation (IBS-C), IBS with predominant diarrhoea (IBS-D), IBS with mixed bowel habits (IBS-M), and unclassified IBS (IBS-U). However, these criteria have been criticized for being complex and performing modestly in differentiating organic diseases from functional gastrointestinal disorders (57).

1.3 Metabolomics

Omics are defined as the study of related sets of biological molecules in a comprehensive manner which comprise genomics, proteomics, transcriptomics and metabolomics. Metabolomics is one of the newest fields of omics in biology which is defined as the systemic and non-biased identification and quantitation of all metabolites in a given organism or set of biological samples. Metabolites are the final downstream products of the genome and proteome and are defined as the small molecules that are the chemical products of various metabolic and enzymatic reactions that occur in the body. A metabolome is defined as the cumulative composition of specific metabolites present and synthesized within a specific eco-system. It is an extremely complex mixture of chemically diverse compound classes (e.g., amino acids, lipids,), with different chemical properties (e.g., hydrophilic versus hydrophobic) and a dynamic range of molecular concentrations (58, 59).

While a combination of genomics, transcriptomics and proteomics provide extensive information about the genotype of an organism, it does not provide enough information about the phenotype of a given organism. In other words, while genomics, transcriptomics and proteomics reflect the potential for specific metabolic function, metabolomics integrates the

effects of several downstream regulatory mechanisms such as gene regulation, post-transcriptional regulation and different pathway interactions. The metabolomic profile provides a direct functional readout of a physiological or pathological state of an organism. Therefore, metabolomics tools hold great potential in clinical applications including identification of disease biomarkers, investigation of disease pathophysiology and drug discovery (60, 61).

1.3.1 Gut microbiota and metabolome

Several metabolites are produced by intestinal bacteria during the metabolism of nutrients and xenobiotics. The gut microbiota has numerous genes that can be expressed to breakdown substrates, synthetize proteins, produce anti-microbial compounds and signaling molecules which are of physiological importance to the host (e.g. short chain fatty acids) and can be reflected in the metabolome (62). Some of these molecules (metabolites) are excreted in feces whereas others are absorbed through the colonic mucosa and enter the systemic circulation where they can be further modified by human metabolism and excreted into urine. On the other hand, a number of host-derived metabolites such as bile acids are returned to the gut via biliary excretion where they can be further metabolized by the microbiota. These host-microbiota metabolic interactions complicate the interpretation of metabolic profiles (62).

The contribution of the microbial metabolism is more likely reflected in the fecal metabolome than in urinary, serum or breath profiles. Urinary profiles contain human and human-microbial co-metabolites. Although the general belief is that serum metabolomic profiles are less influenced by bacterial metabolism, recent studies have shown that serum metabolomic profiling can be used to assess the role of gut microbiota in the development of different diseases. For instance, Wikoff et al. (63) compared plasma extracts from germ-free mice with

samples from conventional animals by using various mass spectrometry-based methods and found significant different metabolome in these two groups of animals. For example, they reported that the bacterial-mediated production of bioactive indole-containing metabolites derived from tryptophan was impacted based on the presence or absence of certain microbes. In another well-designed study, the serum metabolome of non-diabetic insulin-resistant individuals which is characterized by increased levels of branched-chain amino acids was shown to be correlated with *Prevotella copri* and *Bacteroides vulgatus* in stool samples obtained from Danish non-diabetic individuals (64).

1.3.2 Dietary factors and metabolome

As indicated by several studies in various patients' settings, dietary intake can alter the metabolomic profile of a biofluid (65). As mentioned earlier, gut microbes are able to use different dietary contents as substrates to produce various metabolites that can be identified and quantified using metabolomic techniques. Among these metabolites are short chain fatty acids which can be measured in different types of samples such as fecal samples.

In the past decade, a number of studies in the field of nutrition have incorporated metabolomics data. These studies have focused on short-term or long-term intake of nutrients, foods and food groups such as fruits and vegetables or overall dietary patterns (66, 67). For instance, plasma 2,6-dihydroxybenzoic acid and 2-aminophenol sulfate were reported to increase following a high-fiber diet in a 5-week randomized controlled crossover intervention (68). In another 6-month randomized clinical trial study, concentration of urinary formate could

discriminate between high glycemic index and low glycemic index diets among a sample of overweight individuals. In another study, 77 overweight, non-diabetic subjects followed an 8-week low-calorie diet and were then randomly assigned to a high protein or low protein diet for 6 months. The authors found that while urinary creatine was increased by the high protein diet, citric acid was increased by the low protein diet (69). Distinctive metabolomic profiles have been related to different dietary patterns. For example, several metabolites in various biological samples have been identified in relation to a Mediterranean dietary pattern or its major components (67).

In addition to identification of metabolites in biological samples that can be used as objective markers of dietary intake versus the commonly used dietary assessment tools (e.g. food frequency questionnaires, 24h dietary recalls), metabolomics have been used to deepen our understanding of the underlying mechanisms responsible for beneficial effects of different dietary interventions. For example, using a metabolomic approach, McIntosh et al (70), reported that the beneficial role of following a low fermentable oligosaccharides, disaccharides and monosaccharides and polyols diet in reduction of gastrointestinal symptoms in IBS patients can partly be explained by its role on reduction of histamine levels.

1.3.3 Metabolomic techniques

There are several metabolomic platforms to identify and quantify metabolites in different biological samples. Analytical tools that are used in most metabolomic studies include nuclear magnetic resonance (NMR) spectrometers, mass spectrometers (MS), gas chromatography

(GC), liquid chromatography (LC) systems, ion mobility systems (IMS), capillary electrophoresis (CE) systems, integrated liquid chromatography-mass spectrometry (LC-MS), integrated capillary electrophoresis-mass spectrometry (CE-MS), integrated ion mobility spectrometry-mass spectrometry (IMS-MS), gas chromatography-mass spectrometry (GC-MS), or LC-MS/NMR. Targeted metabolomics and untargeted metabolomics are the two major types of metabolomics experiments. The selection of metabolomic experiments in a study is based on the research questions being tested and the instrumental resources of the laboratory that is responsible for conducting the metabolomic assays. Targeted metabolomics is focused on identifying and quantifying a small subset (~50–500) of metabolites in the metabolome. This approach is useful when a study is aimed to detect specific set of metabolites or assess certain metabolic pathways to test a hypothesis. In contrast to targeted metabolomics, an untargeted metabolomic approach is applied to characterize as many metabolites as possible in the metabolome. Although untargeted metabolomics is often very labor intensive, it is useful to discover new biomarkers and generate hypothesis for future studies (59).

1.3.4 Metabolomics in IBD research

Metabolomic profiling is a powerful exploratory tool for understanding interactions between nutrients, the intestinal metabolism and the microbiota composition in health and disease status. During the past few years, several metabolomic studies have been conducted in IBD settings mostly to identify diagnostic biomarkers (71) to differentiate UC and CD patients from each other or other related conditions.

A recent study that used an untargeted metabolomic profiling on serum samples, reported that pediatric patients with CD and UC could be differentiated from each other and from healthy controls (72). In another study, Ahmed et al. (73) showed that volatile organic metabolites in stool samples could be used to separate diarrhea predominant IBS patients from UC or CD patients.

Although most previous metabolomic studies in IBD settings were aimed to identify diagnostic biomarkers, there have been a few studies to identify metabolites related to disease course in these patients. For instance, Probert et al (74) analyzed plasma NMR spectra from 40 patients with UC who were followed prospectively over 6 months and found that metabolomic profiling could accurately discriminates between high and low endoscopic disease activity and predict progression of UC.

Metabolomic approaches have also been used to predict response to therapeutic interventions in IBD patients. In a randomized controlled trial of fecal microbiota transplantation (FMT) in patients with active, Paramsothy et al. (75) found that UC patients who were remission after FMT had increased levels of short-chain fatty acid biosynthesis and secondary bile acids in comparison to patients who did not respond the FMT.

1.4 Objectives and hypothesis

The objectives of the current project were to investigate if metabolomics could be used to identify biomarkers in different biological samples to 1) discriminate between post-operative recurrence and non-recurrence of CD, 2) differentiate IBD patients from IBS patients, 3) predict clinical relapse in UC patients in remission, and 4) explain the mechanisms in which a dietary

intervention can prevent colonic inflammation in UC patients. Our hypothesis was that metabolomic techniques have the potential to elaborate the role of diet in the development or management of IBD.

1.5 References

1. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019;94(1):155-165.

2. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol. 2014;20(1):91-9.

3. Levine JS, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. Gastroenterol Hepatol (N Y). 2011;7(4):235-41.

4. Kuenzig ME, Benchimol EI, Lee L, Targownik LE, Singh H, Kaplan GG, Bernstein CN, Bitton A, Nguyen GC, Lee K, Cooke-Lauder J. The impact of inflammatory bowel disease in Canada 2018: Direct costs and health services utilization. J Can Assoc Gastroenterol. 2019; 2(Suppl 1):S17-S33.

5. Kuenzig ME, Lee L, El-Matary W, Weizman AV, Benchimol EI, Kaplan GG, Nguyen GC, Bernstein CN, Bitton A, Lee K, Cooke-Lauder J. The Impact of Inflammatory Bowel Disease in Canada 2018: Indirect Costs of IBD Care. J Can Assoc Gastroenterol. 2019; 2(Suppl 1):S34-S41.

6. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JC, Chan FK, Sung JJ. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. The Lancet. 2017;390(10114):2769-78.

7. Kaplan GG, Bernstein CN, Coward S, Bitton A, Murthy SK, Nguyen GC, Lee K, Cooke-Lauder J, Benchimol EI. The Impact of Inflammatory Bowel Disease in Canada 2018: Epidemiology. J Can Assoc Gastroenterol. 2019;2(Suppl 1):S6-S16.

8. Ponder A, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin Epidemiol. 2013;5:237-47.

9. Loddo I, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. Front Immunol. 2015;6:551.

10. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140(6):1704-12.

11. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474(7351):307-17.

12. Perez-Lopez A, Behnsen J, Nuccio SP, Raffatellu M. Mucosal immunity to pathogenic intestinal bacteria. Nat Rev Immunol. 2016;16(3):135-48.

13. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014;13(1):3-10.

14. Wallace KL, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. World J Gastroenterol. 2014;20(1):6-21.

15. Morgan XC, Segata N, Huttenhower C. Biodiversity and functional genomics in the human microbiome. Trends Genet. 2013;29(1):51-8.

16. Khan I, Ullah N, Zha L, Bai Y, Khan A, Zhao T, Che T, Zhang C. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome. Pathogens. 2019;8(3). pii: E126.

17. Yu LC. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. J Biomed Sci. 2018;25(1):79.

18. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11(1):1-10.

19. Miyoshi J, Chang EB. The gut microbiota and inflammatory bowel diseases. Transl Res. 2017;179:38-48.

20. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut. 2006;55(2):205-11.

21. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC. The treatment-naive microbiome in new-onset Crohn’s disease. Cell Host Microbe. 2014;15(3):382-392.

22. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-1770.

23. Aggeletopoulou I, Konstantakis C, Assimakopoulos SF, Triantos C. The role of the gut microbiota in the treatment of inflammatory bowel diseases. Microb Pathog. 2019;137:103774.

24. Ananthakrishnan AN. Debate session: So what causes inflammatory bowel disease? It's all in the environment. J Gastroenterol Hepatol. 2018;33 Suppl 3:24.

25. Piovani D, Danese S, Peyrin-Biroulet L, Nikolopoulos GK, Lytras T, Bonovas S. Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. Gastroenterology. 2019;157(3):647-659.e4.

26. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol. 2011;106(4):563–573.

27. Nie JY, Zhao Q. Beverage consumption and risk of ulcerative colitis: Systematic review and meta-analysis of epidemiological studies. Medicine (Baltimore). 2017;96(49):e9070.

28. Wang F, Feng J, Gao Q, Ma M, Lin X, Liu J, Li J, Zhao Q. Carbohydrate and protein intake and risk of ulcerative colitis: Systematic review and dose-response meta-analysis of epidemiological studies. Clin Nutr. 2017;36(5):1259-1265.

29. Li F, Liu X, Wang W, Zhang D. Consumption of vegetables and fruit and the risk of inflammatory bowel disease: a meta-analysis. Eur J Gastroenterol Hepatol. 2015;27(6):623-30.

30. Ge J, Han TJ, Liu J, Li JS, Zhang XH, Wang Y, Li QY, Zhu Q, Yang CM. Meat intake and risk of inflammatory bowel disease: A meta-analysis. Turk J Gastroenterol. 2015;26(6):492-7.

31. John S, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. Eur J Gastroenterol Hepatol. 2010;22(5):602-6.

32. de Silva PS, Olsen A, Christensen J, Schmidt EB, Overvaad K, Tjonneland A, Hart AR. An association between dietary arachidonic acid, measured in adipose tissue, and ulcerative colitis. Gastroenterology. 2010;139(6):1912-7.

33. Khalili H, Chan SSM, Lochhead P, Ananthakrishnan AN, Hart AR, Chan AT. The role of diet in the aetiopathogenesis of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2018;15(9):525-535.

34. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients. 2012;4(8):1095-119.

35. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107(33):14691-6.

36. Agus A, Denizot J, Thévenot J, Martinez-Medina M, Massier S, Sauvanet P, Bernalier-Donadille A, Denis S, Hofman P, Bonnet R, Billard E, Barnich N. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. Sci Rep. 2016;6:19032.

37. Sun M, Wu W, Liu Z, Cong Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J Gastroenterol. 2017;52(1):1-8.

38. Neis EP, van Eijk HM, Lenaerts K, Olde Damink SW, Blaak EE, Dejong CH, Rensen SS. Distal versus proximal intestinal short-chain fatty acid release in man. Gut. 2019;68(4):764-765.

39. Reddavide R, Rotolo O, Caruso MG, Stasi E, Notarnicola M, Miraglia C, Nouvenne A, Meschi T, De' Angelis GL, Di Mario F, Leandro G. The role of diet in the prevention and treatment of Inflammatory Bowel Diseases. Acta Biomed. 2018;89(9-S):60-75.

40. Sugihara K, Morhardt TL, Kamada N. The Role of Dietary Nutrients in Inflammatory Bowel Disease. Front Immunol. 2019;9:3183.

41. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, Ballet V, Claes K, Van Immerseel F, Verbeke K, Ferrante M, Verhaegen J, Rutgeerts P, Vermeire S. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut. 2014;63(8):1275-83.

42. Koleva P, Ketabi A, Valcheva R, Gänzle MG, Dieleman LA. Chemically defined diet alters the protective properties of fructo-oligosaccharides and isomalto-oligosaccharides in HLA-B27 transgenic rats. PLoS One. 2014;9(11):e111717.

43. Werner T, Wagner SJ, Martínez I, Walter J, Chang JS, Clavel T, Kisling S, Schuemann K, Haller D. Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. Gut. 2011;60(3):325-33.

44. Sturniolo GC, Di Leo V, Ferronato A, D'Odorico A, D'Incà R. Zinc supplementation tightens "leaky gut" in Crohn's disease. Inflamm Bowel Dis. 2001;7(2):94-8.

45. Reich KM, Fedorak RN, Madsen K, Kroeker KI. Vitamin D improves inflammatory bowel disease outcomes: basic science and clinical review. World J Gastroenterol. 2014;20(17):4934-47.

46. Scoville EA, Allaman MM, Adams DW, Motley AK, Peyton SC, Ferguson SL, Horst SN, Williams CS, Beaulieu DB, Schwartz DA, Wilson KT, Coburn LA. Serum Polyunsaturated Fatty Acids Correlate with Serum Cytokines and Clinical Disease Activity in Crohn's Disease. Sci Rep. 2019;9(1):2882.

47. Scaioli E, Liverani E, Belluzzi A. The Imbalance between n-6/n-3 Polyunsaturated Fatty Acids and Inflammatory Bowel Disease: A Comprehensive Review and Future Therapeutic Perspectives. Int J Mol Sci. 2017;18(12).

48. Ruemmele FM. Role of Diet in Inflammatory Bowel Disease. Ann Nutr Metab. 2016;68 Suppl 1:33-41.

49. Lucak S, Chang L, Halpert A, Harris LA. Current and emergent pharmacologic treatments for irritable bowel syndrome with diarrhea: evidence-based treatment in practice. Therap Adv Gastroenterol. 2017;10(2):253-275.

50. Zaribaf F, Keshteli AH, Esmaillzadeh A, Saneei P, Feizi A, Daghaghzadeh H, Feinle-Bisset C, Adibi P. Empirically derived dietary habits are associated with irritable bowel syndrome. Eur J Clin Nutr. 2018;72(11):1537-1547.

51. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10(7):712-721.e4.

52. Jung HK, Kim YH, Park JY, Jang BH, Park SY, Nam MH, Choi MG. Estimating the burden of irritable bowel syndrome: analysis of a nationwide korean database. J Neurogastroenterol Motil. 2014;20(2):242.

53. Nellesen D, Yee K, Chawla A, Lewis BE, Carson RT. A systematic review of the economic and humanistic burden of illness in irritable bowel syndrome and chronic constipation. J Manag Care Pharm. 2013;19(9):755-64.

54. Longstreth GF, Yao JF. Irritable bowel syndrome and surgery: a multivariable analysis. Gastroenterology. 2004;126(7):1665-73.

55. Sethi S, Wadhwa V, Leclair J, Mikami S, Park R, Jones M, Sethi N, Brown A, Lembo A. In-patient discharge rates for the irritable bowel syndrome–an analysis of national trends in the United States from 1997 to 2010. Aliment Pharmacol Ther. 2013 Dec;38(11-12):1338-46.

56. Mearin F, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. Gastroenterology. 2016. pii: S0016-5085(16)00222-5.

57. Sood R, Ford AC. Diagnosis: Rome IV criteria for FGIDs - an improvement or more of the same? Nat Rev Gastroenterol Hepatol. 2016;13(9):501-2.

58. Roberts LD, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. Curr Protoc Mol Biol. 2012;Chapter 30:Unit 30.2.1-24.

59. Wishart DS. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol Rev. 2019;99(4):1819-1875.

60. Horgan RP, Kenny LC. 'Omic'technologies: genomics, transcriptomics, proteomics and metabolomics. The Obstetrician & Gynaecologist. 2011;13(3):189-95.

61. Sun YV, Hu YJ. Integrative Analysis of Multi-omics Data for Discovery and Functional Studies of Complex Human Diseases. Adv Genet. 2016;93:147-90.

62. Daliri EB, Wei S, Oh DH, Lee BH. The human microbiome and metabolomics: Current concepts and applications. Crit Rev Food Sci Nutr. 2017;57(16):3565-3576.

63. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A. 2009;106(10):3698-703.

64. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Doré J, Mattila I, Plichta DR, Pöhö P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trošt K; MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376-81.

65. Brennan L, Gibbons H. Sex matters: a focus on the impact of biological sex on metabolomic profiles and dietary interventions. Proc Nutr Soc. 2019 Jul 31:1-5. doi: 10.1017/S002966511900106X.

66. Guasch-Ferré M, Bhupathiraju SN, Hu FB. Use of Metabolomics in Improving Assessment of Dietary Intake. Clin Chem. 2018;64(1):82-98.

67. Serra-Majem L, Roman-Vinas B, Sanchez-Villegas A, Guasch-Ferre M, Corella D, La Vecchia C. Benefits of the Mediterranean diet: Epidemiological and molecular aspects. Mol Aspects Med. 2019 Jun;67:1-55. doi: 10.1016/j.mam.2019.06.001.

68. Johansson-Persson A, Barri T, Ulmius M, Onning G, Dragsted LO. LC-QTOF/MS metabolomic profiles in human plasma after a 5-week high dietary fiber intake. Anal Bioanal Chem. 2013;405(14):4799-809.

69. Rasmussen LG, Winning H, Savorani F, Toft H, Larsen TM, Dragsted LO, Astrup A, Engelsen SB. Assessment of the effect of high or low protein diet on the human urine metabolome as measured by NMR. Nutrients. 2012;4(2):112-31.

70. McIntosh K, Reed DE, Schneider T, Dang F, Keshteli AH, De Palma G, Madsen K, Bercik P, Vanner S. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut. 2017;66(7):1241-51.

71. De Preter V, Verbeke K. Metabolomics as a diagnostic tool in gastroenterology. World J Gastrointest Pharmacol Ther. 2013;4(4):97-107.

72. Daniluk U, Daniluk J, Kucharski R, Kowalczyk T, Pietrowska K, Samczuk P, Filimoniuk A, Kretowski A, Lebensztejn D, Ciborowski M. Untargeted Metabolomics and Inflammatory Markers Profiling in Children With Crohn's Disease and Ulcerative Colitis—A Preliminary Study. Inflamm Bowel Dis. 2019;25(7):1120-1128.

73. Ahmed I, Greenwood R, Costello Bde L, Ratcliffe NM, Probert CS. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. PLoS One. 2013;8(3):e58204.

74. Probert F, Walsh A, Jagielowicz M, Yeo T, Claridge TD, Simmons A, Travis S, Anthony DC. Plasma nuclear magnetic resonance metabolomics discriminates between high and low

endoscopic activity and predicts progression in a prospective cohort of patients with ulcerative colitis. J Crohns Colitis. 2018;12(11):1326-1337.

75. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, Paramsothy R, Walsh AJ, van den Bogaerde J, Samuel D, Leong RW. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. Gastroenterology. 2019;156(5):1440-54.

Chapter 2. A Distinctive Urinary Metabolomic Fingerprint Is Linked With Endoscopic Postoperative Disease Recurrence in Crohn's Disease Patients.

2.1 ABSTRACT

Background: Crohn's disease patients who undergo ileocolonic resection frequently have disease recurrence. The aim of this preliminary study was to identify urinary metabolomic profiles associated with disease recurrence in order to identify underlying mechanisms of recurrence and possible disease biomarkers.

Methods: Biopsies from neoterminal ileum were collected from CD patients (n=38) following ileocolonic resection in order to assess mucosa-associated microbiota using 16S rRNA multitag pyrosequencing. Urine samples were collected and metabolomic profiling was done using high-resolution nuclear magnetic resolution spectroscopy and a combined direct infusion liquid chromatography tandem mass spectrometry. Rutgeerts scoring system was used to assess endoscopic postoperative recurrence of CD.

Results: There were 28 (73.7%) patients with endoscopic CD recurrence. CD patients who were in endoscopic remission had higher abundance of Bacteroidetes and lower abundance of Fusobacteria and Proteobacteria in comparison to CD patients who had endoscopic recurrence. In addition, metabolomic profiling could also discriminate between these two groups of patients. Endoscopic recurrence was associated with increased concentration of urinary levoglucosan. Rutgeerts score was positively correlated with levoglucosan and propylene glycol levels.

Conclusions: CD patients who present with endoscopic disease recurrence after surgery have unique urinary metabolomic fingerprint that can differentiate them from CD patients who are in endoscopic remission following ileocolonic resection. In addition, mucosal-associated microbiota in CD patients with or without disease recurrence after surgery differs and correlates with some urinary metabolites.

2.2 Introduction

It has been estimated that approximately 50% of Crohn's disease (CD) patients require surgery within 10 years after diagnosis mainly due to failure of conventional medical therapy or disease complications such as strictures or fistulae (1). Ileocolonic end-to-end anastomoses and side-to-side anastomoses are the standard surgical treatments for most CD patients who require ileocecal resection (2). However, after bowel resection, a high proportion of these patients will experience CD recurrence at the site of anastomosis that may require repeated surgeries 3.

Although a few clinical, demographic, and lifestyle-related factors have been related to increased risk of CD recurrence after surgery (3-7), its pathophysiological mechanisms are not fully understood. Currently, the best prognostic factor for clinical relapse appears to be endoscopic disease activity at the anastomosis and in the neo-terminal ileum at an early stage after surgery (8). However, endoscopy is expensive, labor intensive, inconvenient for the patient, and carries some risk (9). Therefore, there is a need for non-invasive and novel biomarkers to identify disease recurrence following surgery (9-12). Fecal calprotectin (FC) has recently been shown to have some correlation with endoscopic postoperative CD recurrence and its severity (11). However, challenges in stool sample collection, modest specificity for assessment of endoscopic recurrence, and variations in FC concentrations due to sampling errors by patients (10) advocates for identification of other potential biomarkers in easily accessible biological samples such as urine.

Metabolomics is defined as a non-biased identification and quantification of all metabolites in a biological system (13). Most previous studies in the area of inflammatory bowel disease (IBD) and metabolomics have focused on identification of metabolites that could differentiate IBD

cohorts from healthy individuals (14). However, it has recently been suggested that metabolomic profiling can also be used as a prognostic tool of relapse in ulcerative colitis (UC) (15). To date, no study has been done to evaluate the role of metabolomic profiling in the identification of metabolites that may be associated with CD recurrence after ileocolonic resection.

The identification of a unique urinary metabolomic fingerprint in IBD would be useful for both monitoring disease progression and assessing response to treatment. Therefore, the aim of the present study was to determine if a urinary metabolic fingerprint differentiated endoscopic disease relapse from remission in CD patients after ileocolonic resection. We also aimed to identify mucosal-associated microbiota that were related to disease recurrence and urinary metabolites after surgery.

2.3 Materials and Methods

2.3.1 Patients and clinical assessments

In this single centre study, 38 adult CD patients who had previously undergone an ileocolonic resection were identified from the clinical database of Centre of Excellence for Gastrointestinal Inflammation and Immunity Research (CEGIIR) affiliated to University of Alberta, Edmonton, Canada. There were no exclusion criteria. All CD patients which had undergone ileocolonic resection and provided urine and biopsy samples to CEGIIR biobank were included. Patients were assessed by expert gastroenterologists six to twelve months after surgery for endoscopic postoperative recurrence of CD based on the Rutgeerts scoring system (16), which is a well-established validated endoscopic scoring system based on examination of a 10-cm segment of ileal segment mucosa proximal to the ileocolonic anastomosis. The definitions were as follows:

i0, no lesions; i1, up to five aphthous lesions; i2, more than five aphthous lesions with normal mucosa between the lesions, or skip areas of larger lesions or lesions confined to the ileocolonic anastomosis; i3, diffuse aphthous ileitis with diffusely inflamed mucosa; and i4, diffuse inflammation with larger ulcers, nodules, and/or narrowing. Endoscopic recurrence was defined as Rutgeerts score of i2 or higher.

Biopsies obtained from macroscopically healthy areas of the neo-terminal ileum and urine samples were obtained from these patients and stored in -80°C freezer.

2.3.2 Sample analysis

DNA was extracted from ileal biopsies using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. The microbial composition of the biopsies was determined using the 16S rRNA gene pyrosequencing. Ten ng of extracted DNA was amplified by PCR using fluorescently labeled forward primer 27F (5'-(6FAM) AGAGTTTGATCCTGGCTCA G-3') and unlabeled reverse primer 355R' (5'-GCTGCCTCCCGTAGGAGT-3'), then the products were diluted and separated on an ABI 3130xl fluorescent capillary sequencer. Finally, peak areas were calculated and OTU's that made up less than 1% of the total composition were eliminated from the analysis to eliminate the variable low abundance components within the communities. DNA was used for PCR at three different dilutions and then run on LH-PCR fingerprinting to check for PCR artifacts. The most reproducible PCRs were used for multitag pyrosequencing (MTPS) at the Microbiome Analysis Center at George Mason University (Manassas, VA) on the GS-Junior FLX platform (Roche, Branford, CT) using tagged fusion primers (17). In total, 494,424 high quality reads were obtained with an average of 3,507 reads per sample. Samples with less than 1,000 reads were removed from downstream analyses. The

average length of the reads was ~350 bases. The QIIME pipeline (18) was used with default parameters for taxonomic classification of samples. Sequences were clustered into OTUs with Uclust (19) and the Greengenes database from August 2013 (20). Taxonomy was assigned to clusters using the RDP classifier (21). Taxa that were present at abundance < 1% were disregarded. Principal coordinates were calculated with the QIIME script 'principal_coordinates.py' using the Bray Curtis' metric, which was plotted with Emperor (22). Additional plots were generated with R. Data is publicly available on our Galaxy portal (http://mbac.gmu.edu:8080/u/masi/h/edmontonset4pgm035080417).

To identify and quantify metabolites in urine samples we used high-resolution nuclear magnetic resolution (NMR) spectroscopy and a combined direct infusion (DI-)/liquid chromatography (LC-) tandem mass spectrometry (MS/MS) (AbsolutIDQ p180 kit, Biocrates Life Sciences AG, Innsbruck, Austria). A detailed protocol of DI-/LC- MS/MS metabolomic assay has previously been described (23). For NMR assay, 720µl of the sample was added to 80µl of DSS (4, 4-dimethyl-4-silapentane-1-sulfonic acid) Chenomx Standard (IS2; 4.6485mM DSS) and then the samples were pH corrected to a pH of between 6.7-6.8. Solutions of 1.0M, 0.1M and 0.01M NaOH and HCl were used to obtain the consistent pH. Finally, the 700µl of each sample was transferred into 4-inch long 5mm diameter NMR tubes and capped. The samples were analyzed using an Oxford 600Hz NMR spectrometer with a Varian VNMRS two channel console and running VNMRJ software version 2.2C on a RHEL 4 host computer. Shims were optimized until an optimal line width value was obtained at relative peaks heights of 50% (< 1.0 Hz), 0.55% (< 12.0 Hz), and 0.11% (< 20.0 Hz) was achieved. All sample handling was done with a Varian 768 AS sample handling robot. Any spectra that did not meet acceptable line height values were

discarded and the sample was re-run manually. The analysis of the NMR spectra was done with Chenomx Inc. NMR suite software version 7.6. Spectra were pre-processes where the phasing of the spectra was aligned, the water peak was eliminated, the baseline was corrected and a final shim correction was done. Identification of the metabolites was done using the Chenomx chemical compound library and concentrations were quantified using the Chenomx standard that was added to all the samples.

2.3.3 Statistical analysis

Quantitative variables are presented as mean ± SD and range. Independent sample t-test was used to compare normally distributed data in two groups of CD patients (remission vs. CD recurrence). Mann-Whitney U-test was used when quantitative variables were not normally distributed (assessed by Kolmogorov-Smirnov test). Qualitative variables were expressed as number of participants and percentage and compared by Chi-square or Fisher's exact tests where appropriate. To test correlation between microbial taxa, metabolites and Rutgeerts scores, Spearman's rank correlation was used. These analyses were performed using SPSS version 20.0 software (IBM, Armonk, NY, USA). P-values smaller than 0.05 were considered statistically significant.

Principal coordinates analysis (PCoA) of weighted UniFrac distances was used to visualise potential clustering of samples in the two CD groups by bacterial composition using the QIIME pipeline. Microbial taxonomic units that were significantly associated to each group of CD patients were determined using the linear discriminant analysis effect size (LEfSe) algorithm (24). We also tested for differences in the relative abundance of the taxonomic units identified by LEfSe algorithm between the two groups using Metastats, which controls for multiple

comparisons. Only taxa with average abundance of >1%, P-value < 0.05, and false discovery rate <0.05 were considered significant.

For the metabolomic analysis, concentrations of urinary metabolites (µmol/L) were normalized to creatinine (mmol/L) and reported as the ratio (µmol/mmol). Also, identified metabolites were normalized using logarithmic transformation and pareto scaling. Metabolites with a P-value less than 0.2 (using Mann-Whitney U test) were selected for generating the logistic regression model. Multivariate statistical analysis was performed using partial least squares discriminant analysis (PLS-DA). A 10-fold cross-validation technique was used to ensure that the logistic regression models were robust. Permutation analysis using random resampling (n=1000) of the two groups of patients (i.e. CD recurrence versus remission) was conducted to determine if the probability of the observed separation was a result of chance or not and a P-value that represents the probability of a random finding was generated. We also generated receiver operating characteristic (ROC) curve plotting sensitivity against false-positive rate values for prediction of CD recurrence by Monte-Carlo cross validation (MCCV). To identify the major metabolites responsible for the discrimination between patients with and without CD variable importance in projection (VIP) scores were used. The VIP score indicates the contribution of each feature to the regression model. Metabolites with VIP scores >1 were considered to play an important role in the discrimination between the two groups. Correction for multiple comparisons was performed by testing the false discovery rate (25) and q-value was reported. For metabolomic-related statistical analysis MetaboAnalyst 3.0 (26), which is a web-based analytical pipeline was used.

2.3.4 Ethical Considerations

This study was approved by the Health Research Ethics Board-Biomedical Panel, University of Alberta, Edmonton, Canada (Pro00028147).

2.4 Results

2.4.1 Subject Demographics

Demographic and clinical characteristics of patients based on their disease status are presented in Table 2.7.1. Thirty-eight CD patients (females: 65.8%) who had previously undergone ileocolonic resection were included in this study. Their mean age was 44.5±12.5 years (range: 18.6-66.0 years). Six patients (15.8%) were smokers at the time of endoscopy. Twenty-eight patients (73.7%) had CD endoscopic recurrence based on their Rutgeerts score. There was no statistically significant difference in gender, age, time since last surgery, and smoking status between two groups of patients. Patients' medications at the time of endoscopy were comparable between CD patients in remission and CD patients with endoscopic relapse. The proportion of biologics use was higher in patients with CD recurrence but did not reach statistical significance (32.1.1 vs 10.0 %, P=0.24).

2.4.2 Microbial Analysis

Microbial profile of biopsy samples from CD patients with or without disease recurrence is shown in Supplementary Figure 2.7.1. As seen in Figure 2.7.1A, there was a clear clustering in the unifrac PCoA of biopsies from CD patients with disease recurrence compared to biopsies from those patients in remission. A number of bacterial taxa were found to be significantly different between the two groups of patients (Figure 2.7.1B). CD patients who were in endoscopic remission had higher abundance of Bacteroidetes but lower abundance of

Fusobacteria and Proteobacteria in comparison to patients who had CD endoscopic recurrence (Figure 2.7.1B and Table 2.7.2). In addition, Rutgeerts score was negatively correlated with relative abundance of Bacteroidaceae (rs = - 0.31, P=0.07).

2.4.3 Metabolomic Analysis

Using NMR and DI/LC-MS/MS metabolomic platforms, 65 and 132 metabolites were identified and quantified in urine samples, respectively. Using univariate analysis 47 metabolites were selected for multivariate statistical analysis. In multivariate analysis, urinary metabolomic profile of post-operative CD patients with endoscopic disease recurrence was significantly different from those patients who were in endoscopic remission (R^2=0.76, Q^2=0.34, P=0.01) (Figure 2.7.2). The median values and IQR of four major metabolites that were responsible for discrimination between two groups of CD patients based on their VIP scores are presented in Table 2.7.3. Higher levels of urinary 1,6-anhydro-beta-D-Glucose (levoglucosan), L-3,4-dihydroxyphenylalanine (L-DOPA), ethylmalonate and lower levels of urinary propylene glycol were related to CD recurrence after ileocolonic resection. The associated ROC cure using these four metabolites, had an area under the curve of 0.91 (95% CI: 0.73-1.00) (Figure 2.7.3). The specificity and sensitivity of detecting CD recurrence using concentrations of these four metabolites were 84.6% and 100%, respectively. In addition, Rutgeerts score was positively correlated with levoglucosan (rs = 0.33, P=0.05) and propylene glycol (rs = - 0.31, P=0.06) levels. After excluding patients who were receiving either adalimumab or infliximab, the list of most important metabolites responsible for the discrimination between the two groups of patients did not change except for L-DOPA which was replaced with methionine sulfoxide (Table 2.7.4).

Bacteroidales and *gammaproteobacteria* correlated with urinary levoglucosan level (Figure 2.7.4). However, there was no correlation between major bacterial taxa and levels of L-DOPA, propylene glycol or ethylmalonate in urine.

2.5 Discussion

In the present preliminary study, we have shown that six to twelve months after ileocolonic resection and re-anastomosis, metabolomic profiling can discriminate between CD patients with or without disease recurrence. In addition, we found that these two groups of CD patients had different mucosal bacterial composition at the neo-terminal ileum after surgery.

Surgical treatment is required in approximately 80% of CD patients at some point in their lives (4). Repeated surgery is required in 70-90% of all patients and multiple surgeries in more than 30%1. While endoscopic recurrence appears in up to 90% of patients 1 year after surgery 4, histopathological recurrence occurs within a few weeks after exposure to the luminal content (27). The exact pathogenesis of CD recurrence after surgical resection of diseased bowel is still not well understood. However, demographic (e.g. age at disease onset), lifestyle-related (e.g. active smoking), and clinical (e.g. penetrating disease behavior, perianal disease, extensive small bowel resection) have been related to increased risk of CD recurrence after ileocolonic resection and re-anastomosis (1, 4, 28). In the present study, we did not find any association between gender and disease recurrence which is in agreement with findings from previous studies (4). Smoking is the only modifiable patient-related risk factor of CD recurrence after surgery (28). Although the prevalence of being current smoker at the time of endoscopy was

higher in patients with CD recurrence, the difference was not statistically significant. This can be attributed to the small number of active smokers in each CD group (1 patient in remission group and 5 patients in the recurrence group).

There are sparse data about the role of gut microbial composition in the pathophysiology of CD recurrence after surgery. D'Haens et al. (27) investigated the effects of infusing intestinal luminal contents into excluded ileum in three CD patients who had undergone a curative ileocolonic resection. They reported that exposure of the mucosa in the neoterminal ileum to fecal material resulted in histologic and immunological changes mimicking CD-related early post-operative changes. The authors suggested that CD patients develop an abnormal immune reaction in response to bacteria or dietary agents (27). In addition, usefulness of nitroimidazole antibiotics in prevention of CD endoscopic and clinical recurrence after surgery has previously been shown (28, 29). These reports suggest a significant role for the gut microbial community in development of postoperative CD and for this reason identification of microbial taxa and related mechanisms have been the main focus of a few recent studies (30-34).

In the present study, we showed that postoperative recurrence of CD was related to decreased abundance of *Bacteroidetes* but increased abundance of *Fusobacteria*, and *Proteobacteria*. In a recent study by Mondot et al. (31), mucosal microbiota was analysed by 16S rRNA gene sequencing in 5 patients with CD recurrence and 5 patients with endoscopic remission. They found that while there was no difference in alpha diversity between two groups, remission was characterised by an increased number of *Bacteroides*, *Dorea*, *Ruminococcus* and *Dialister* and increased *Gemmiger formicilis*, *Enterococcus durans* and *Ruminococcus lactaris*. In another study on 12 patients, CD recurrence was associated with increased *Enterococcus* and *Veillonella*

but decreased *Bacteroides*, *Prevotella*, *Parabacteroides* and *Firmicutes* (32). Moreover, it has been reported that changes in alpha or beta diversity were not related to disease recurrence in (34) CD patients (33). However, the authors found that six months after bowel resection, endoscopic recurrence was significantly associated with an increased abundance of *Proteus* compared with remission. In addition, 18 months after surgery, relatively low abundance of *Faecalibacterium* was a risk factor for endoscopic recurrence after controlling for smoking status (33). Gut microbial composition at the time of surgery was related to recurrence of disease in another study by Dey et al. (34) on 6 CD patients. All these findings suggest that alterations in gut microbial profiles are associated with disease recurrence in CD patients after ileocolonic resection.

Ileocolonoscopy is the gold standard to diagnose CD recurrence after surgical resection of bowel and it has been indicated that it is the most sensitive tool to document morphologic disease recurrence (8). However, due to major limitations of endoscopic evaluations, there is a need to develop simple and non-invasive methods for the detection of postoperative recurrence (35). Metabolomics which is the study of small molecule metabolites within biological samples to understand underlying specific cellular metabolic pathways, has the potential to introduce biomarkers that can be used for diagnostic or mechanistic purposes. Metabolomics has recently been used in IBD settings to distinguish IBD patients from non-IBD cohorts or UC patients from CD patients and to identify metabolites that were related to disease activity (36) or UC flare-ups (15).

In the present study, for the first time, we identified a number of metabolites that were related to CD recurrence after ileocolonic resection. We found that in comparison to CD patients

without endoscopic recurrence, patients who had disease recurrence after surgery had increased concentrations of urinary levoglucosan, L-DOPA, ethylmalonate, and methionine sulfoxide but decreased concentrations of propylene glycol. In addition, we found that increased levoglucosan and decreased propylene glycol were correlated with Rutgeerts postoperative endoscopic recurrence scores.

Dietary factors have been suggested to be associated with disease pathogenesis and flares in IBD patients through their effects on mucosal immune system, epithelial function, and the intestinal microbiome (37). A Western-type diet characterized by decreased consumption of fibre and increased consumption of sugars and meat has been hypothesized to play an important role in the rise of IBD in Western countries in the second half of the 20th century (38). In the present study, we found that urinary levoglucosan was significantly increased in patients with endoscopic CD recurrence after adjusting for multiple comparisons. Levoglucosan is a hydrohexose formed from the pyrolysis of dietary carbohydrates, such as starch and cellulose. In addition, dietary intake of certain foods such as caramel is reported to be highly correlated with urinary levoglucosan level (39). Therefore, we speculate that dietary factors may contribute to development of CD recurrence after ileocolonic resection and suggest that this factor needs to be tested in future studies using appropriate tools to assess participants' dietary intake. Furthermore, we found correlations between urinary levoglucosan and some bacterial taxa. This finding may indicate how dietary factors (e.g. carbohydrate intake) can affect bacterial composition or function in the context of CD recurrence postoperatively. It should also be noted that in the presence of ileal inflammation, dietary changes may be reflected in the urinary metabolome which requires further investigations.

In our study, elevated L-Dopa (an immediate precursor of dopamine), ethylmalonate (an organic acid produced by the carboxylation of butyrate), and methionine sulfoxide (an oxidation product of methionine), as well as decreased propylene glycol (a diol alcohol used as a solvent for different pharmaceutical preparations) levels in urine were also associated with postoperative CD recurrence in multivariate analysis. These metabolites were reported to be related to intestinal inflammation (40), alterations in energy metabolism (41, 42), and oxidative stress (43) in previous studies. However, it should also be noted that urinary levels of these metabolites were not significantly different between the two groups of CD patients in this study after adjusting p-values for multiple comparisons which weakens their potential role in the discrimination between the two groups.

Although in the present exploratory study, we used a combination of both microbial and metabolomic fingerprinting in postoperative CD patients, there are major methodological limitations that should be considered while interpreting our findings. Small sample size remains a major limitation of our study. A larger sample size would enable us to perform microbial and metabolomic analysis in different subgroups of patients based on their clinical parameters (e.g. extent of disease, type of medications). Due to this limitation, some comparisons between the two groups of patients did not reach statistical significance. For example, while the proportion of patients on biologic medications was almost three times higher in CD recurrence group this difference was not statistically significant. Therefore, it can be speculated that the observed difference in the urinary metabolomic profile of patients with CD recurrence and disease remission is due to the difference in the biologic use between the two groups. We tried to overcome this limitation by repeating metabolomic analysis after excluding patients on

biologics (n=10) which did not change the main findings. However, due to the small sample size, we could not do similar analysis in patients who were not receiving biologic agents. In addition, since assessment of endoscopic CD recurrence and gut microbial composition and urinary metabolomic profile were performed at the same time, we cannot produce robust evidence for causal relationships between inflammation and microbial/metabolomic factors. Therefore, these findings will need to be explored in future well-designed studies with larger sample size. Lack of assessment of dietary intake, over the counter supplements (except for vitamins), prebiotics and probiotics are other major limitation of the present study as these factors can influence metabolite profiles. It should also be noted that the urinary metabolome may also be affected by different medications. Although there was no statistically significant difference in type of CD medications between the two groups of patients, we were not able to assess use of other medications (e.g. dopaminergic drugs, antidepressants) that could potentially influence some metabolites in urine. Therefore, findings of our study will need to be validated in future studies with larger sample size with comprehensively assessed clinical (e.g. medications, comorbid conditions) and dietary factors.

In conclusion, in this preliminary study, we found that urinary metabolomic profiling has the potential to be used as a diagnostic tool for identification of CD patients that develop endoscopic disease recurrence after ileocolonic resection. These findings also contribute to improvement of our understanding of the pathophysiological mechanisms of CD recurrence after ileocolonic resection and identification of urinary biomarkers related to CD recurrence and its severity.

Acknowledgement

We wish to thank Alberta IBD Consortium for supporting the study. AHK was supported by a graduate studentship from Alberta Innovates-Health Solutions.

2.6 References

1. Peyrin-Biroulet L, Loftus EV Jr, Colombel JF, et al. The natural history of adult Crohn's disease in population-based cohorts. Am J Gastroenterol. 2010;105:289-97.

2. He X, Chen Z, Huang J, Lian L, et al. Stapled side-to-side anastomosis might be better than handsewn end-to-end anastomosis in ileocolic resection for Crohn's disease: a meta-analysis. Dig Dis Sci. 2014; 59:1544-51.

3. Buisson A, Chevaux JB, Allen PB, et al. Review article: the natural history of postoperative Crohn's disease recurrence. Aliment Pharmacol Ther. 2012; 35:625-33.

4. De Cruz P, Kamm MA, Prideaux L, et al. Postoperative recurrent luminal Crohn's disease: a systematic review. Inflamm Bowel Dis. 2012; 18:758-77.

5. Moss AC. Prevention of postoperative recurrence of Crohn's disease: what does the evidence support? Inflamm Bowel Dis. 2013; 19:856-9.

6. Joyce MR, Hannaway CD, Strong SA, et al. Impact of smoking on disease phenotype and postoperative outcomes for Crohn's disease patients undergoing surgery. Langenbecks Arch Surg. 2013; 398:39-45.

7. Vuitton L, Koch S, Peyrin-Biroulet L. Preventing postoperative recurrence in Crohn's disease: what does the future hold? Drugs. 2013; 73:1749-59.

8. Gionchetti P, Dignass A, Danese S, et al. EUROPEAN Evidence-based consensus on the diagnosis and management of Crohn's disease. 2016: Part 2: Surgical management and special situations. J Crohns Colitis. 2017; 11:135-149.

9. Wright EK, De Cruz P, Gearry R, et al. Fecal biomarkers in the diagnosis and monitoring of Crohn's disease. Inflamm Bowel Dis. 2014; 20:1668-77.

10. Qiu Y, Mao R, Chen BL, et al. Fecal calprotectin for evaluating postoperative recurrence of Crohn's disease: a meta-analysis of prospective studies. Inflamm Bowel Dis. 2015; 21:315-22.

11. Garcia-Planella E, Mañosa M, Cabré E, et al. Fecal Calprotectin Levels Are Closely Correlated with the Absence of Relevant Mucosal Lesions in Postoperative Crohn's Disease. Inflamm Bowel Dis. 2016; 22:2879-2885.

12. Wright EK, Kamm MA, De Cruz P, et al. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. Gastroenterology. 2015; 148:938-947.e1.

13. Dunn WB, Ellis DI. Metabolomics: current analytical platforms and methodologies. TrAC Trends in Analytical Chemistry. 2005; 24:285-94.

14. De Preter V, Verbeke K. Metabolomics as a diagnostic tool in gastroenterology. World J Gastrointest Pharmacol Ther. 2013; 4:97-107.

15. Keshteli AH, van den Brand FF, Madsen KL, et al. Dietary and metabolomic determinants of relapse in ulcerative colitis patients: A pilot prospective cohort study. World J Gastroenterol. 2017; 23:3890-3899.

16. Rutgeerts P, Geboes K, Vantrappen G, et al. Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. Gut 1984; 25:665-72.

17. Gillevet P, Sikaroodi M, Keshavarzian A, et al. Quantitative assessment of the human gut microbiome using multitag pyrosequencing. Chem Biodivers. 2010; 7:1065-75.

18. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7:335-6.

19. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26:2460-1.

20. DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006; 72:5069-72.

21. Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007; 73:5261-7.

22. Vázquez-Baeza Y, Pirrung M, Gonzalez A, et al. EMPeror: a tool for visualizing high-throughput microbial community data. Gigascience. 2013; 2:16.

23. Bouatra S, Aziat F, Mandal R, et al. The human urine metabolome. PLoS One. 2013; 8:e73076.

24. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011; 12:R60.

25. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Stat Methodol. 1995; 57:289-300.

26. Xia J, Sinelnikov IV, Han B, et al. MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res. 2015; 43:W251-7.

27. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998; 114:262-7.

28. Ng SC, Kamm MA. Management of postoperative Crohn's disease. Am J Gastroenterol. 2008; 103:1029-35.

29. Doherty GA, Bennett GC, Cheifetz AS, et al. Meta-analysis: targeting the intestinal microbiota in prophylaxis for post-operative Crohn's disease. Aliment Pharmacol Ther. 2010; 31:802-9.

30. Perry T, Jovel J, Patterson J, et al. Fecal Microbial Transplant After Ileocolic Resection Reduces Ileitis but Restores Colitis in IL-10-/- Mice. Inflamm Bowel Dis. 2015; 21:1479-90.

31. Mondot S, Lepage P, Seksik P, et al. Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. Gut. 2016; 65:954-62.

32. De Cruz P, Kang S, Wagner J, et al. Association between specific mucosa-associated microbiota in Crohn's disease at the time of resection and subsequent disease recurrence: a pilot study. J Gastroenterol Hepatol. 2015; 30:268-78.

33. Wright EK, Kamm MA, Wagner J, et al. Microbial Factors Associated with Postoperative Crohn's Disease Recurrence. J Crohns Colitis. 2017; 11:191-203.

34. Dey N, Soergel DA, Repo S, et al. Association of gut microbiota with post-operative clinical course in Crohn's disease. BMC Gastroenterol. 2013; 13:131.

35. Yamamoto T, Shimoyama T, Umegae S, et al. Serial monitoring of faecal calprotectin for the assessment of endoscopic recurrence in asymptomatic patients after ileocolonic resection for Crohn's disease: a long-term prospective study. Therap Adv Gastroenterol. 2016; 9:664-70.

36. Soubières AA, Poullis A. Emerging Biomarkers for the Diagnosis and Monitoring of Inflammatory Bowel Diseases. Inflamm Bowel Dis. 2016; 22:2016-22.

37. Lee D, Albenberg L, Compher C, et al. Diet in the pathogenesis and treatment of inflammatory bowel diseases. Gastroenterology. 2015; 148:1087-106.

38. Ahmed T, Rieder F, Fiocchi C, et al. Pathogenesis of postoperative recurrence in Crohn's disease. Gut. 2011; 60:553-62.

39. Bergauff MA, Ward TJ, Noonan CW, et al. Urinary levoglucosan as a biomarker of wood smoke: results of human exposure studies. J Expo Sci Environ Epidemiol. 2010; 20:385-92.

40. Magro F, Vieira-Coelho MA, Fraga S, et al. Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease. Dig Dis Sci. 2002; 47:216-24.

41. Di Meo I, Lamperti C, Tiranti V. Mitochondrial diseases caused by toxic compound accumulation: from etiopathology to therapeutic approaches. EMBO Mol Med. 2015; 7:1257-66.

42. Mickiewicz B, Tam P, Jenne CN, et al. Integration of metabolic and inflammatory mediator profiles as a potential prognostic approach for septic shock in the intensive care unit. Crit Care. 2015; 19:1.

43. Mashima R, Nakanishi-Ueda T, Yamamoto Y. Simultaneous determination of methionine sulfoxide and methionine in blood plasma using gas chromatography-mass spectrometry. Anal Biochem. 2003;313:28-33.

2.7 Tables and Figures

Table 2.7.1. Comparison of demographic and clinical characteristics of patients with or without Crohn's disease recurrence after ileocolonic resection.

Patients' characteristics		Recurrent Crohn's disease[1]		P-value
		No (n=10)	Yes (n=28)	
Age (years)[2]		43.8±14.5	44.8±12.0	0.83
Age >40 years, n (%)		6 (60.0)	21 (75.0)	0.37
Females, n (%)		70.0	64.3	0.74
Duration of disease (years)[2]		18.2±14.0	17.0±10.7	0.78
Age at diagnosis, n (%)	A1: ≤16 years	2 (20)	5 (17.9)	0.45
	A2: 17-40 years	8 (80)	19 (67.9)	
	A3: >40 years	0 (0)	4 (14.3)	
Disease location, n (%)	L1: Ileal	6 (60)	16 (57.1)	1.00
	L2: Colonic	0 (0)	0 (0)	
	L3: Ileocolonic	4 (40)	12 (42.9)	
Disease phenotype, n (%)	B1: Inflammatory	0 (0)	0 (0)	1.00
	B2: Stricture	6 (60)	18 (64.3)	1.00
	B3: Penetrating	4 (40)	10 (35.7)	1.00
	P: Perianal disease	1 (10)	4 (14.3)	1.00
Smoking status, n (%)	never smoked	7 (70.0)	16 (57.1)	0.75
	former smoker	2 (20.0)	7 (25.0)	
	currently smoking	1 (10.0)	5 (17.9)	

Rutgeerts score[2]		0.3±0.5	3.0±0.9	<0.001
CD medication at the time of endoscopy, n (%)	no medication	5 (50.0)	7 (25.0)	0.24
	5-aminosalicylic acid	2 (20.0)	5 (17.9)	1.00
	azathioprine/6-mercaptopurine	3 (30.0)	10 (35.7)	1.00
	methotrexate	2 (20.0)	3 (10.7)	0.59
	corticosteroids	0 (0.0)	2 (7.1)	1.00
	biologic therapy Adalimumab Infliximab	1 (10.0) 0 (0.0) 1 (10.0)	9 (32.1) 4 (14.3) 5 (17.9)	0.24 0.56 1.00
Any antibiotics use for post-operative CD recurrence		0 (0.0)	1 (3.6)	1.00
Vitamin B12 use, n (%)		1 (10.0)	7 (25.0)	0.65
Other vitamins use, n (%)		1 (10.0)	8 (28.6)	0.40

[1] Recurrent Crohn's disease was defined as Rutgeerts score >1

[2] Data is presented as mean ± standard deviation

Table 2.7.2. Comparison of mucosal bacterial composition between two groups of Crohn's disease patients after ileocolonic resection

	Recurrent Crohn's disease[1]		**P-value**	**Q-value**
Microbial Taxa [2]	No (n=10)	Yes (n=28)		
Phylum Bacteroidetes	50.7 (45.8-63.8)	22.0 (7.4-39.6)	0.002	0.005
Class Bacteroidia	50.7 (45.0-63.5)	21.4 (5.6-39.2)	0.003	0.034
Order Bacteroidales	50.7 (45.0-63.5)	21.4 (6.0-39.2)	0.001	0.010
Family Bacteroidaceae	47.8 (32.4-61.6)	21.3 (5.9-38.0)	0.007	0.039
Phylum Fusobacteria	0.0 (0.0-0.0)	0.2 (0.0-7.0)	0.007	0.006
Class Fusobacteriia	0.0 (0.0-0.0)	0.2 (0.0-7.0)	0.005	0.037
Order Fusobacteriales	0.0 (0.0-0.0)	0.2 (0.0-7.0)	0.003	0.015
Family Fusobacteriaceae	0.0 (0.0-0.0)	0.2 (0.0-7.0)	0.004	0.034
Phylum Proteobacteria	6.4 (3.4-8.5)	13.2 (4.8-23.1)	0.006	0.006
Class Gammaproteobacteria	3.6 (2.9-6.3)	11.0 (4.2-21.6)	0.002	0.034
Order Enterobacteriales	1.3 (0.8-1.7)	3.0 (0.9-4.7)	0.012	0.031
Family Enterobacteriaceae	1.3 (0.8-1.7)	3.0 (0.9-4.7)	0.004	0.034
Order Pseudomonadales	2.0 (1.7-4.6)	6.5 (3.4-16.1)	0.011	0.031
Phylum Firmicutes	41.8 (24.7-45.8)	46.1 (38.9-62.7)	0.032	0.021
Phylum Actinobacteria	0.4 (0.0-0.9)	0.3 (0.1-0.7)	0.779	0.349

[1] Recurrent Crohn's disease was defined as Rutgeerts score >1

[2] Data is presented as median (interquartile range) of relative abundance

Table 2.7.3. Comparison of major urinary metabolites that discriminated between Crohn's disease patients with and without post-operative disease recurrence. [1]

	Recurrent Crohn's disease		VIP [2] score	P-value [3]	q-value
	No	Yes			
Levoglucosan, µmol/mmol	0.22±0.28	10.43±19.58	2.4	<0.001	0.02
L-DOPA, µmol/mmol	0.02±0.03	0.05±0.05	1.9	0.11	0.18
Propylene glycol, µmol/mmol	38.86±98.91	6.61±15.09	1.7	0.09	0.18
Ethylmalonate, µmol/mmol	2.83±3.88	4.52±4.33	1.4	0.04	0.18

[1] concentrations are presented as mean ± standard deviation

[2] variable importance in projection

[3] obtained from Mann–Whitney U test

Table 2.7.4. Comparison of major urinary metabolites that discriminated between Crohn's disease patients on no biologic medications with and without post-operative disease recurrence. [1]

	Recurrent Crohn's disease		VIP [2] score	P-value [3]	q-value
	No	Yes			
Levoglucosan, µmol/mmol	0.14±0.09	8.37±15.72	2.7	<0.001	0.02
Propylene glycol, µmol/mmol	42.40±104.24	4.07±8.40	1.9	0.03	0.17
Methionine sulfoxide	0.18±0.13	0.27±0.18	1.7	0.18	0.20
Ethylmalonate, µmol/mmol	3.04±4.06	4.79±5.06	1.3	0.10	0.17

[1] concentrations are presented as mean ± standard deviation

[2] variable importance in projection

[3] obtained from Mann–Whitney U test

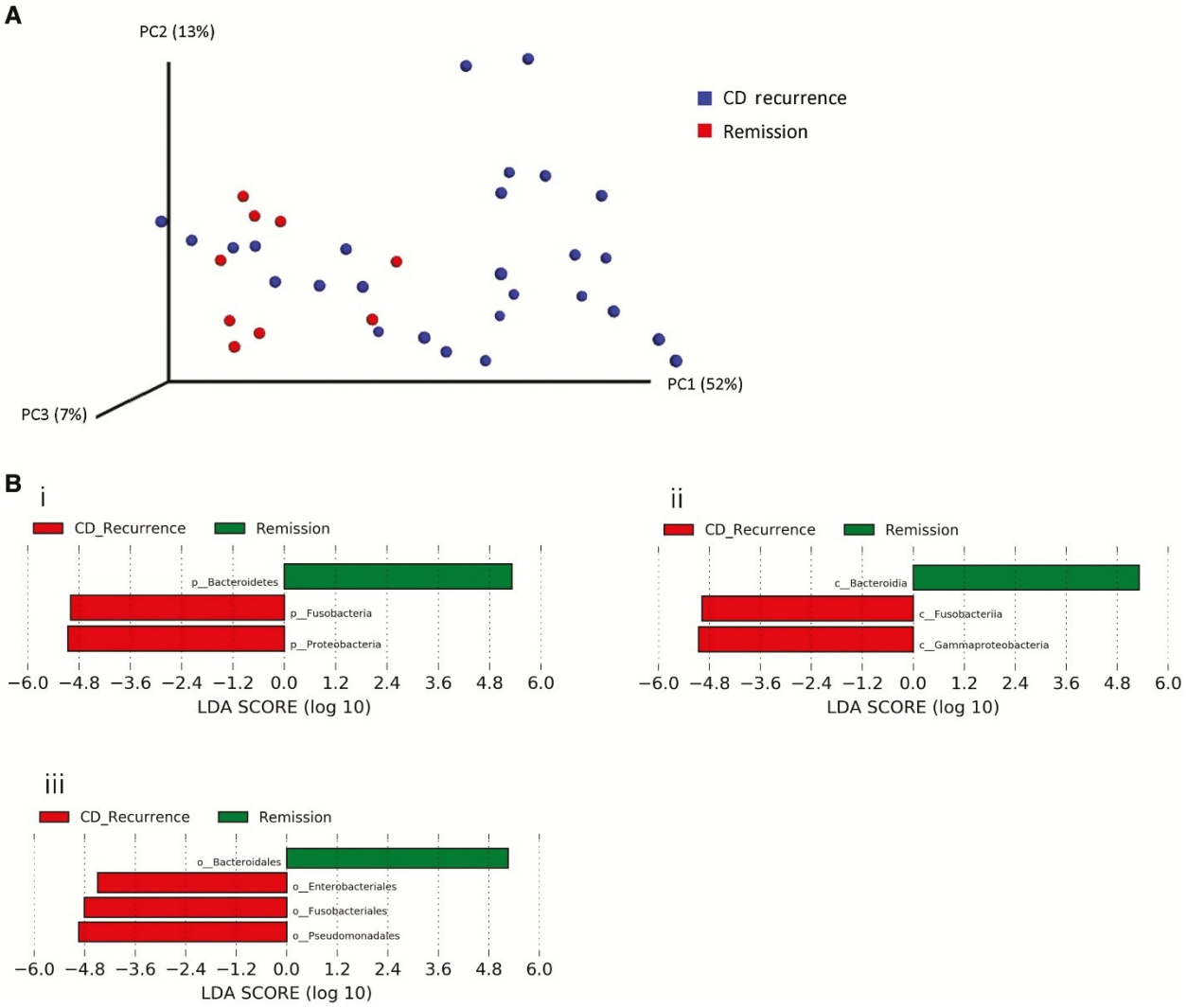


Figure 2.7.1. A) Principal coordinates analysis (PCoA) plot of UniFrac distances showing that Crohn's disease patients with or without disease relapse have distinctive mucosa-associated microbial profiles; **B)** Differentially abundant bacterial taxa at phylum (i), class (ii), and order (iii) levels between the two groups of Crohn's disease patients based on linear discriminant analysis effect size (LEfSe) algorithm.

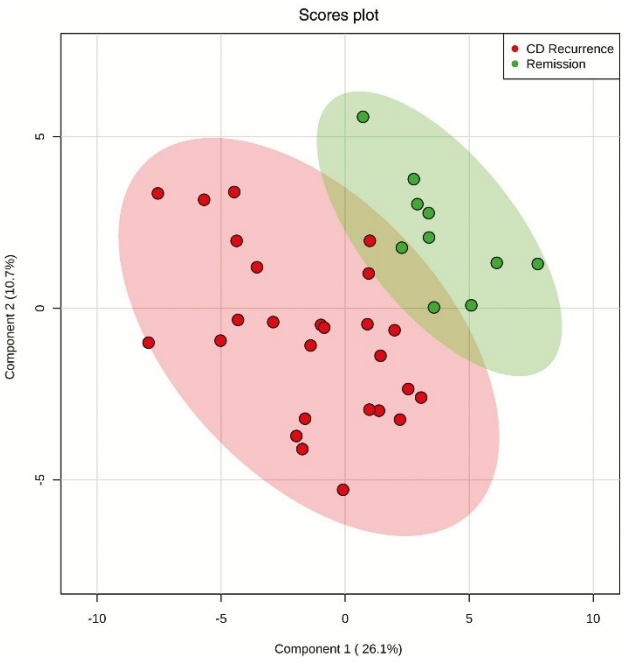


Figure 2.7.2. Partial least squares discriminant analysis plot showing a clear separation of the urinary metabolomic profiles of Crohn's disease patients with or without endoscopic disease recurrence after ileocolonic resection (R^2=0.76, Q^2=0.34, P=0.01).

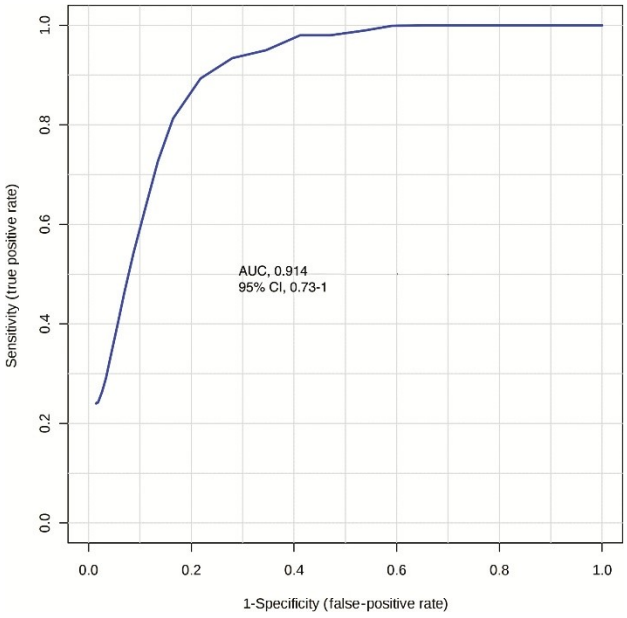


Figure 2.7.3. Receiver-operator characteristic (ROC) curve for detection of postoperative recurrence of Crohn's disease using urinary concentration of four metabolites.

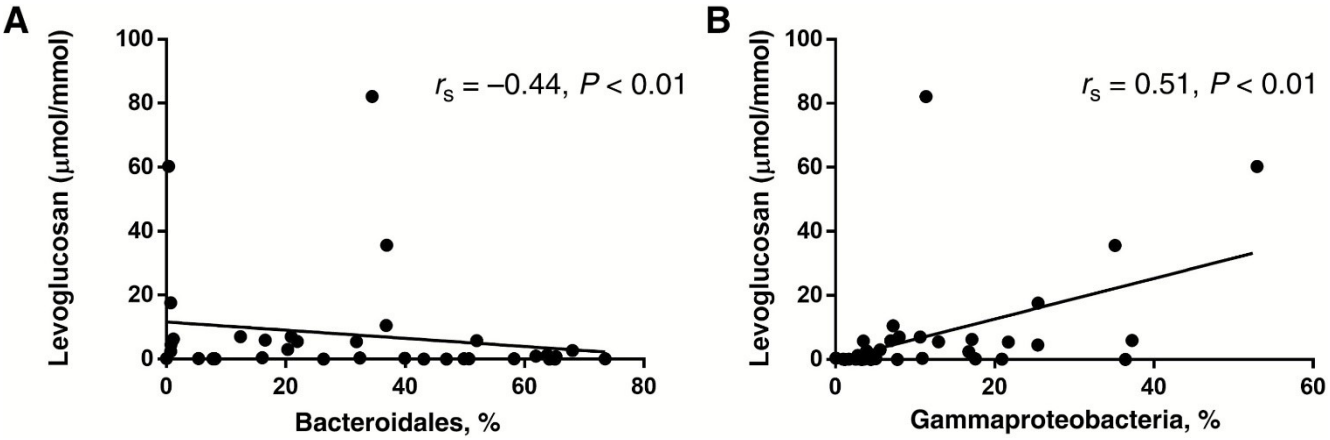
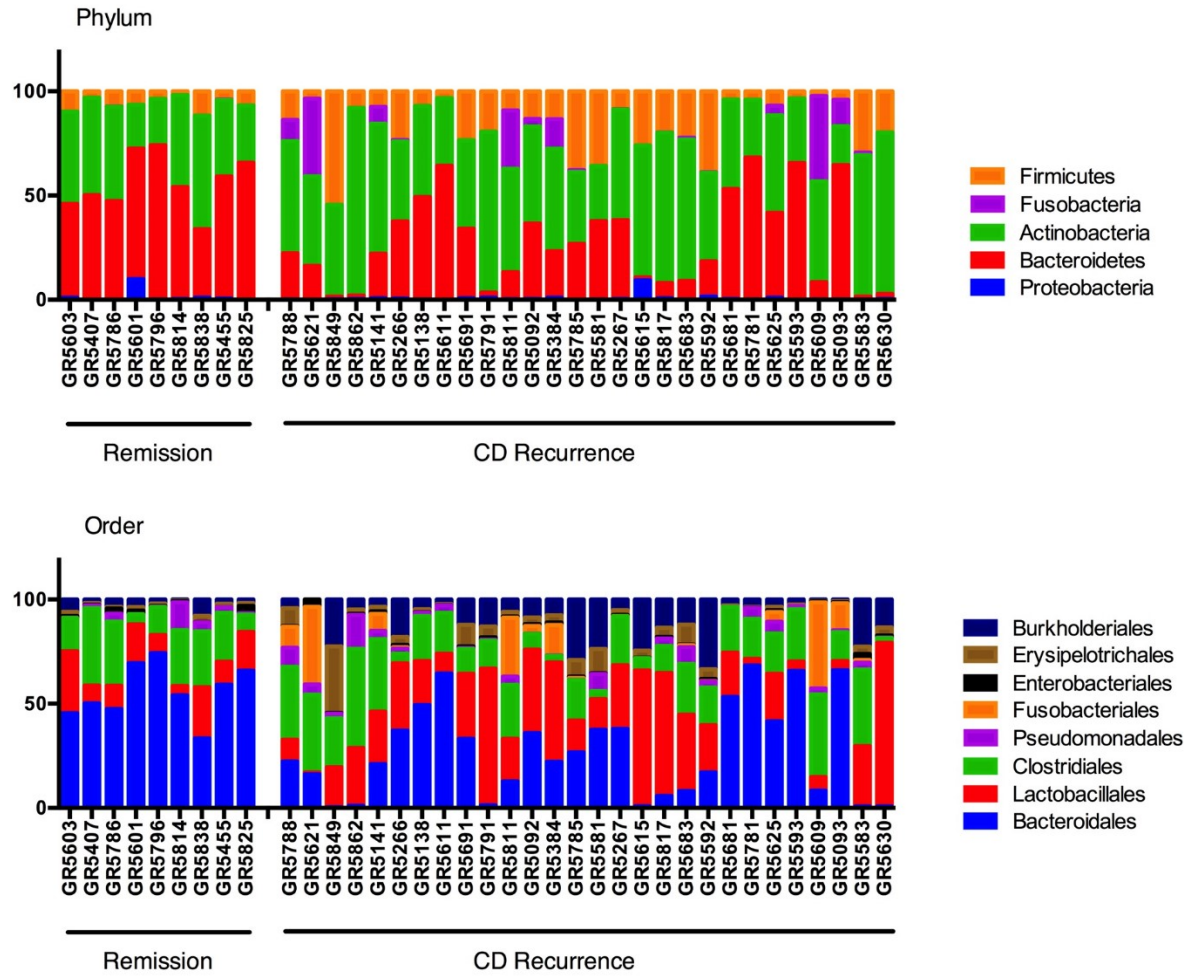


Figure 2.7.4. Correlation of urinary levoglucosan levels with abundance of *bacteroidales* (A) and *gammaproteobacteria* (B) in neoterminal ileum biopsies of post-operative Crohn's disease patients.



Supplementary Figure 2.7.1. Microbial composition at phylum and order levels on neoterminal ileum biopsies from Crohn's disease patients following ileocolonic resection.

Chapter 3. Comparison of the metabolomic profiles of irritable bowel syndrome patients with ulcerative colitis patients and healthy controls: new insights into pathophysiology and potential biomarkers

3.1 ABSTRACT

Background: The evaluation of the metabolomic profile of IBS patients offers an opportunity to identify novel pathophysiological targets and biomarkers that could discriminate this disorder from related conditions.

Aims: to identify potential urinary biomarkers that discriminate irritable bowel syndrome patients from ulcerative colitis patients in remission and healthy controls and explore the pathophysiology of IBS using a metabolomic approach.

Methods: Urine samples were collected from 39 irritable bowel syndrome patients, 53 ulcerative colitis patients in clinical remission and 21 healthy controls. Urinary metabolites were identified and quantified using direct infusion /liquid chromatography tandem mass spectrometry and gas-chromatography mass spectrometry.

Results: irritable bowel syndrome patients had a unique urinary metabolome that could separate them from ulcerative colitis patients with an area under the curve=0.99 (95% confidence interval 0.95-1.00). The most important metabolites for this separation were a group of amino acids and organic acids. In addition, subjects with irritable bowel syndrome could be discriminated from healthy controls using their metabolic fingerprints. Irritable bowel syndrome patients had lower urinary PC ae C38:6, dopamine, and p-hydroxybenzoic acid than

healthy controls. Levels of some urinary metabolites including histamine were correlated significantly with irritable bowel syndrome symptom severity score.

Conclusions: irritable bowel syndrome patients have a unique urinary metabolomic profile compared to ulcerative colitis patients in clinical remission or healthy subjects. These data suggest that metabolomic profiling may provide important insights into pathophysiology and testable biomarkers to discriminate IBS from other disorders that can mimic this condition and can be used to assess its severity and identify potential novel pathophysiological pathways.

3.2 Introduction

Irritable bowel syndrome (IBS) is the most commonly diagnosed gastrointestinal condition. It is a chronic functional gastrointestinal disorder (FGID) characterized by recurring abdominal pain, bloating, loose or frequent stools and/or constipation in the absence of structural or major inflammatory and biochemical abnormalities (1). IBS is associated with impaired health-related quality of life and increased healthcare expenditures (2). Globally, the pooled prevalence of IBS is 11% (3). The complex and multifactorial pathophysiology of IBS includes genetic predisposition and visceral hypersensitivity along with abnormalities in gut motility, secretion, immune activity, autonomic nervous system function, permeability, and microbial composition and function 4.

Currently there is no biological marker or a distinct observable intestinal pathophysiology that is specific for IBS; thus diagnosis remains challenging (5). At the present time, diagnosis of IBS is based on Rome IV criteria, which are symptom-based (6). However, these criteria have been criticized for being complex and performing modestly in differentiating organic diseases from FGIDs (7). Many IBS patients undergo unnecessary endoscopic evaluations to rule out organic conditions such as inflammatory bowel disease (IBD) due to overlapping symptoms (8). Therefore, identification of biomarkers to discriminate IBS patients from healthy individuals and organic diseases is highly desirable.

Metabolomics, defined as the study of all metabolites in biological samples comprehensively, has the potential to identify biomarkers for diagnosis or prognosis of diseases as well as helping to elucidate the pathophysiology of diseases (9). Recently, a limited number of studies have used a metabolomic approach to identify metabolites in breath (10) or fecal (5, 11) samples to

discriminate between IBS patients and healthy volunteers or IBD patients. However, there is a need to perform further metabolomic studies in IBS settings (12) to validate previous findings and identify metabolites in other easily accessible biological samples such as urine.

The primary aim of this study was to identify urinary metabolite profiles that could be used to differentiate IBS patients from healthy controls and IBD patients, and secondarily, to determine if specific urinary biomarkers could distinguish IBS subtypes. We also aimed to investigate if metabolomics could provide new insight into the complex pathophysiology of IBS.

3.3 Materials and Methods

3.3.1 Participants

This cross-sectional study examined adult patients with IBS, ulcerative colitis (UC) in clinical remission and healthy controls. All participants gave informed consent and the study was approved by the Health Research Ethics Board-Biomedical Panel, University of Alberta (Pro00035413) and the Health Sciences Research Ethics Board at Queen's University (DMED-1443-11).

Thirty-nine IBS patients meeting the Rome III criteria (13) were recruited from adult outpatient clinics at a single-centre academic teaching hospital in Kingston, Ontario, Canada. This cohort was previously reported in a diet intervention study (14) and only baseline urine samples were used for the current study.

Fifty-three UC patients in clinical remission were recruited from the IBD clinic at the University of Alberta, Edmonton, Canada. UC diagnosis was confirmed by previously established clinical,

radiological and endoscopic criteria as well as histological findings. Patients were included if they were in clinical remission at the time of enrollment (partial Mayo score <3 (15)). Subjects were excluded if they had used oral corticosteroids or antibiotics in the previous four weeks, or had a history of colectomy.

A group of healthy adult volunteers (n= 21) with no evidence of coronary artery disease, diabetes mellitus, inflammatory or autoimmune conditions was also examined.

3.3.2 Collection of clinical information

A self-administered questionnaire was used to collect demographic information. Clinical information was collected through face-to-face interview and reviewing medical files of participants. IBS severity was assessed using the previously validated IBS symptom severity system (IBS-SSS) (16). The IBS-SSS contains five questions that are rated on a 100-point visual analog scale, including the severity of abdominal pain, the frequency of abdominal pain, the severity of abdominal distention, dissatisfaction with bowel habits, and interference with the quality of life. The range total IBS-SSS score varies from 0 to 500, with a higher score indicating a worse condition.

3.3.3 Sample collection

First morning urine samples were collected from participants in sterile urine specimen cups. After being centrifuged at 4000 rpm for 10 min to remove particulate matter, urine aliquots were stored at −80°C until analysis.

3.3.4 Metabolomic assays

Urine samples were assayed using a combined direct infusion (DI-)/liquid chromatography (LC-) tandem mass spectrometry (MS/MS) and gas-chromatography (GC-) MS assay. All metabolomic measurements were conducted at the Metabolomics Innovation Center (Edmonton, AB, Canada) following a previously described protocol (17). List of identified and quantified metabolites is presented in Supplementary Table 3.7.1.

3.3.5 Gut microbial composition

Genomic DNA was extracted from stool samples from UC and IBS patients for sequencing. Details of 16S rRNA sequencing in IBS patients were presented previously (14). In UC patients, genomic DNA was extracted from stool samples using FastDNA Spin Kit for feces (MP Biomedicals, Lachine, QC, Canada) and quantified using PicoGreen DNA quantification kit (Invitrogen, Carlsbad, CA, USA). Microbial composition was assessed using Illumina's established 16S rRNA amplicon sequencing method and the MiSeq sequencing platform. No deviations from the manufacturer's protocol were used. Briefly, a segment of the V3 and V4 region of the 16S gene was amplified with gene specific primers (aligning to 341bp and 805bp in the gene) that also include an adapter sequence overhang: Bact_16s_ILL1_341mF 5-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3, Bact_16s_ILL1_805mR 5- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3. This PCR reaction was cycled 25 times and the resulting reaction was purified using bead-based clean-up followed by an 8 cycle PCR reaction using Illumina's proprietary bar-coding primers that also align to the adapter sequence. After a second clean-up the bar-coded libraries were diluted, denatured, pooled and run using a V3 300bp reagent

cartridge on the MiSeq system. Bacterial composition was estimated from the data using Quantitative Insights into Microbial Ecology (QIIME 1.9.1) pipelines (18). In brief, QIIME was used to analyze for phylogenetic and operational taxonomic unit (OTU). It was used to de-multiplex the barcoded reads and perform chimera filtering. Filtered sequence reads were grouped into OTUs at a sequence similarity level of 97%, which approximates species-level phylotypes. Taxonomy of the OTUs was assigned and sequences were aligned with RDP classifier and Pynast.

3.3.6 Other laboratory tests

In UC patients, fecal calprotectin (FCP) was measured in stool samples using an enzyme-linked immunosorbent assay with monoclonal antibodies specific to calprotectin (Bühlmann Laboratories AG, Basel, Switzerland). In addition, C-reactive protein (CRP) was measured in serum samples of UC patients.

3.3.7 Statistical analysis

Continuous and categorical variables are presented as mean ± SD or median (interquartile range) and number (%), respectively. Kolmogorov-Smirnov test was used to test the normality of the distribution for continuous variables. For normally distributed variables, one-way analysis of variance or Student's t-test were used. For non-normally distributed variables Mann-Whitney U tests was applied. Categorical variables were compared between two groups using Chi-square test. To explore the correlation between levels of metabolites and severity of IBS, Spearman's rank correlation was used. SPSS version 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis and P-values less than 0.05 were considered statistically significant. To investigate and visualize the correlations between metabolites and gut bacterial composition

(order level), we used debiased sparse partial correlation (DSPC) algorithm option of the Metscape v3.1.3 (19) which is a plug-in for Cytoscape (20). DSPC is an extension of Gaussian graphical model. Under the assumption that the number of true connections among the metabolites is much smaller than the available sample size, DSPC reconstructs a graphical model and provides partial correlation coefficients and P-values for every pair of metabolic features in the dataset. The results can be visualized as weighted networks where nodes represent metabolites and edges represent partial correlation coefficients or the associated P-values19. In the present study, the correlations between gut microbial composition and metabolites were filtered using $|r| > 0.3$ and subsequently correlation network was built.

For metabolomic analysis, metabolites with at least 50% missing values were removed from analysis. Otherwise, missing values were replaced by half of the minimum positive values in the original dataset. Concentrations of urinary metabolites (µmol/L) were normalized to creatinine (mmol/L) and reported as the ratio (µmol/mmol). Multivariate statistical analysis was performed using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). For multivariate analysis, identified metabolites were normalized using logarithmic transformation and pareto scaling. Permutation analysis using random resampling (n=2000) of the two groups of subjects (i.e. IBS versus UC, IBS versus controls, and mixed IBS (IBS-M) versus diarrhea predominant IBS (IBS-D)) was conducted to determine if the probability of the observed separation was a result of chance or not and a P-value was reported. Since age and gender was significantly different between the three groups of participants, we used age and gender adjusted values of metabolites (The residuals of metabolites were computed from linear models with sex and age as independent variables). To identify major metabolites

responsible for the discrimination between different groups of participants, variable importance in projection (VIP) plots were generated. The VIP score indicates the contribution of each feature to the regression model. Higher values of VIP scores indicate greater contribution of the metabolites to the group separation. Correction for multiple comparisons was performed by testing the false discovery rate (21) and Q-value was reported. Metabolites with VIP scores above 1.5 and false discovery rate (Q-value) less than 0.05 were considered to play major roles for the discrimination between two groups of participants. To perform additional validation of metabolomic algorithms, we split the dataset randomly into a discovery (training) set (two thirds of samples) and a validation (test) set (one third of samples). The Least Absolute Shrinkage and Selection Operator technique using 10-fold cross validation was used for variable selection in the regression. Stepwise variable selection using 10-fold cross validation was used to optimize the logistic regression model. To determine the performance of logistical regression models, area under the receiver operating characteristics (ROC) curve (AUC) as well as sensitivity and specificity values were calculated validation set. For metabolomic-related multivariate statistical analysis MetaboAnalyst 3.0 (22) was used.

3.4 Results

In the IBS cohort, most patients were either IBS-M (64.1%) or IBS-D (25.6%). The mean IBS-SSS was ~285, indicating that most patients were in the moderate to severe symptom range 16. The majority of UC patients had either left-sided colitis or pancolitis and their partial Mayo score was <3 (range: 0-2). Their median (interquartile range) values of FCP and CRP was 144.0 µg/g (77.0-343.4 µg/g) and 1.1 mg/L (0.6-3.1 mg/L), respectively. Comparisons of the demographic data (Table 3.7.1) show that the IBS patients were older and predominantly female relative to

the UC patients and healthy controls. Ten (25.6%) IBS patients reported to be on antidepressant medications.

3.4.1 Metabolomic profile of IBS versus UC patients

PLS-DA models showed that urinary metabolomic profiles differentiated IBS patients from patients with UC using metabolites identified by a combination of DI- LC- MS/MS and GC-MS assays ($P<0.001$, Figure 3.7.1). The VIP score of fourteen metabolites were above 1.5, indicating their significant role in the discrimination between IBS and UC patients (Supplementary Figure 3.7.1). The mean levels of these metabolites, which were either amino acids or organic acids, are presented in Table 3.7.2. Based on logistic regression analysis, a model for the prediction of IBS from UC patients was developed which included histidine, lactic acid, proline, and Sumiki's acid. The associated ROC curve using these metabolites had an AUC of 0.99 (95% confidence interval (CI), 0.95-1.00) in the validation sets. In the validation set, the specificity and sensitivity of the model for prediction of IBS was 99.9% and 99.8%, respectively.

3.4.2 Metabolomic profile of IBS versus healthy controls

IBS patients had different urinary metabolic profiles compared with healthy controls using metabolites identified by DI- LC- MS/MS and GC-MS assays ($P=0.01$, Figure 3.7.2). Metabolites that were the most useful in discriminating IBS patients from healthy controls are presented in Table 3.7.3 and Supplementary Figure 3.7.2. Four metabolites including 2-methylsuccinic acid, PC ae C38:6, palmitic acid, and PC aa C34:4 were used to generate a model to differentiate IBS patients from healthy controls using regression analysis. The associated ROC curve had an AUC

of 0.89 (95% CI, 0.72-1.00) in the validation set. The specificity and sensitivity of the model to predict IBS was 57.1% and 100% in the validation set, respectively.

3.4.3 Metabolomic profile of IBS-M versus IBS-D patients

In this study, 35/39 (89.7%) IBS patients were classified as either IBS-M or IBS-D based on Rome III criteria and these patients were further analyzed. They were well matched for age and gender (age= 49.9±16.7 vs. 49.1±15.5 years, P=0.84; females = 88.0 vs. 80.0%, P=0.61, respectively). As shown in Figure 3.7.3, urinary metabolomic profiles of IBS-M and IBS-D patients were not different from each other (P=0.49). However, as shown in Table 3.7.4 patients with IBS-M had higher 3-methyladipic acid and lower palmitic acid levels in comparison to IBS-D patients.

3.4.4 Metabolomic profiling and severity of IBS

Total IBS-SSS score correlated significantly with a number of urinary metabolites including histamine, aspartic acid, methylmalonic acid, phosphatidylcholine diacyl C38:4 (PC aa C38:4), PC ae C36:2 (Table 3.7.5). These metabolites especially correlated with abdominal pain and/or abdominal distention domains of IBS-SSS.

3.4.5 Correlation of urinary metabolites with gut microbes in UC and IBS patients

Microbial composition of IBS and UC patients is presented in Supplementary Figure 3.7.3. In UC patients, lysine level was correlated negatively with abundance of *Clostridiales* (corrected r= -0.50) and proline level was correlated positively with abundance of *Erysipelotrichales* (corrected r= 0.39) (Supplementary Figure 3.7.4). In IBS patients, *oxoglutaric* acid was correlated

negatively with *Bifidobacteriales* (corrected r= -0.48) and palmitic acid was correlated positively with *Bacteroidales* (corrected r= 0.41) (Supplementary Figure 3.7.5).

3.5 Discussion

In this study we demonstrate that the urinary metabolome differs significantly between IBS patients and healthy controls, and also between UC patients in clinical remission and IBS patients. We also identified a few metabolites in urine that were correlated with severity of IBS symptoms. Together, these findings demonstrate proof-of-principle that urinary metabolic profiles have the potential to be useful in the diagnosis of IBS as well as furthering an understanding of disease pathogenesis.

Many factors, including abnormalities in gastrointestinal motility, visceral sensation, brain-gut interaction, psychosocial distress, gut immune activation, intestinal permeability, and intestinal and colonic microbiome have been suggested to play a role in the pathogenesis of IBS (1). However, currently there are no clear IBS related diagnostic biomarkers, making the diagnosis rely solely on the identification of related symptoms and the exclusion of other organic conditions (1). In addition, many IBS patients may present with IBD-like symptoms, which may require further invasive and expensive investigations including colonoscopy (8). Therefore, the development of non-invasive biomarkers may provide more cost-effective tools to screen for IBS in suspected cases.

Metabolomics has increasingly been applied for identification of mechanistic, diagnostic and/or prognostic metabolites in patients with digestive diseases, including inflammatory bowel disease (9). Not surprisingly, these studies have identified significant abnormalities in lipid and

amino acid metabolism, energy-related metabolism, and membrane metabolites in patients with IBD. However, to date very limited work has been performed on metabolomic profiling of IBS patients (12).

In the present study, we have demonstrated for the first time that urinary metabolites can discriminate between IBS patients and UC patients in clinical remission. Previously, ^{1}H-NMR metabolite profiling of fecal extracts was shown to separate IBS from UC patients with decreased choline in IBS being an important discriminator (23). Some (24), but not all (25), studies have found that IBS-D subjects could also be differentiated from UC patients with active disease using fecal volatile organic metabolites. Here we demonstrate that IBS patients have higher urinary levels of amino acids such as histidine, lysine and glutamine but lower levels of proline and glutamic acid in comparison to UC patients. Earlier studies comparing IBD patients with healthy controls or IBS patients also demonstrated altered levels of urinary and/or plasma amino acids (26-28). In the current study, we also found that UC patients had higher lactic acid and ethylmalonic acid than IBS patients. Although increased lactate in urine due to the presence of inflammation in colonic mucosa was previously shown in animal models of IBD (29), the UC patients in this study were all in clinical remission based on partial Mayo scores. However, this does not preclude the possibility of colonic sub-inflammation in UC patients influencing epithelial metabolism and resulting in increased lactate levels. Methylmalonic acid is a malonic acid derivative and is a vital intermediate in the metabolism of fat and protein. Elevated ethylmalonic acid in serum or urine has been linked with cobalamin deficiency or malabsorption which may be related to inflammatory processes in the UC patients (30, 31).

Considering the high value of AUC (0.99) for the separation between IBS and UC patients, after being confirmed in future studies, new laboratory assays could be developed.

Metabolomic profiling has also been used to identify biomarkers capable of discriminating between IBS and healthy controls. However, previous studies mostly focused on fecal (5, 11), breath (10) or plasma (32) samples. In the present study, using metabolomic profiling of urine samples, we found that IBS patients had lower PC ae C38:6 and dopamine but higher hexanoylcarnitine (C6 (C4:1-DC)) compared with healthy controls. PC ae C38:6 is a glycerophospholipid involved in several immune signaling pathways and has been associated with fruits and vegetables intake (33). C6 (C4:1-DC) is an acylcarnitine with pro-inflammatory properties and reflects fatty acid oxidation (34). It has also been reported that sleep disturbances can increase acylcarnitine levels (34, 35). Therefore, it can be speculated that higher prevalence of sleep disorders in IBS patients (36) and reduced dietary intake of fruits and vegetables in this cohort (37) may have contributed to the observed alterations in C6 (C4:1-DC) and PC ae C38:6 respectively. It should also be noted that the observed negative correlation between C6 (C4:1-DC) and *Bacteriodales* may indicate a role for microbial changes in IBS patients that should be explored in future studies.

Interestingly, we found that IBS patients had lower urinary dopamine than healthy controls. Dopamine is a neurotransmitter belonging to the catecholamine family that can modulate interactions between the brain and the enteric nervous system (38). Decreased urinary dopamine levels have been related to anxiety and/or depression (39, 40). It is well-established that IBS patients have a higher prevalence of anxiety and depression (41) and our results suggest that dopamine may be involved in this process. It should also be noted that about one-

fourth of the IBS patients in this study were on antidepressant medications which may alter serotonin or dopamine metabolism (42). In future studies, with larger sample sizes the analyses should be adjusted for use of antidepressant medication as an important confounding variable.

We also found that IBS patients had lower urinary p-hydroxybenzoic acid and 2-methylsuccinic acid compared with healthy controls. P-hydroxybenzoic acid is a phenolic derivative of benzoic acid. Significant amounts of benzoic acid have been found in most berries. In addition, benzoic acid is a byproduct of phenylalanine metabolism in bacteria and is produced when gut microbes process polyphenols from plant sources 43. Recently, we showed that improvement in IBS symptoms after following a low fermentable oligosaccharides, disaccharides and monosaccharides and polyols (FODMAP) diet was associated with a relative increase in urinary p-hydroxybenzoic acid (14). We also showed that p-hydroxybenzoic acid level was correlated with Lactococcus levels (14). Therefore, decreased level of this metabolite in our IBS patients may be due to dietary intake and gut microbial composition, making it a potential target for future dietary and microbiota-targeted therapies in these patients. 2-methylsuccinic acid is carboxylic acid related to tricarboxylic acid cycle. Although we found a negative correlation between levels of this metabolite and *Erysipelotrichales*, its role in IBS requires to be explored in future studies.

Based on patients' self-reported stool consistency, IBS patients are classified into different subtypes. It has been suggested that multifactorial pathophysiology of disease may differ between IBS subtypes (44). Differences in serum-derived melatonin/tryptophan (45) or serotonin (46) levels between IBS-D and IBS-C patients have been reported previously. In the present study, we aimed to investigate the underlying pathophysiological mechanisms of IBS

subtypes through a metabolomic approach. Although we did not find a distinctive metabolic profile in IBS-M and IBS-D patients, we found that 3-methyladipic acid and palmitic acid were had significantly higher levels in IBS-M and IBS-D cases, respectively. 3-methyladipic acid is a medium chain fatty acid that is produced after degradation of phytanic acid from dietary sources such as dairy fat (47). Palmitic acid is one of the most common saturated fatty acids found in animals and plants. It has been suggested that a high fat diet containing palmitate, may chronically enhance the neuronal Toll-like receptor 4 signaling in the colonic myenteric plexus, and ultimately result in apoptosis of myenteric neurons that can result into motility disorders (48).

Metabolomic profiles involve both host and microbial-produced metabolites and can be significantly impacted by dietary intake and alterations in gut microbial composition. Some studies have shown differences in gut microbial composition in UC and IBS patients (49), and it is possible that the differences in urinary metabolic profiles we observed could be related to altered gut microbiota. Indeed, gut microbiota are involved in the production of a range of neurotransmitters such as dopamine (50) and it has been shown that metabolic activity of the gut microbiota may also influence the fatty acid composition of different host tissues (51). In the present study, we also showed that levels of some metabolites in urine were correlated with abundance of bacteria in stool samples. Therefore, differences in gut microbial composition between IBS and UC patients, as well as differences between IBS subtypes (52) could have contributed to the altered metabolic profiles.

In the present study, we identified several urinary metabolites to have a positive correlation with IBS-SSS scores as an indicator of IBS severity. In particular, histamine levels were related to

the severity of abdominal pain, while aspartic acid, methylmalonic acid, PC aa C38:4 and PC ae C36:2 were related to the severity of abdominal distention. Histamine produced by mast cells, at least in part, can play an important role in the pathogenesis of IBS through increasing visceral hypersensitivity, abnormal intestinal barrier function, motility and secretion (53). It has recently been reported that visceral hypersensitivity, symptoms and abdominal pain in IBS patients were reduced following administration of a histamine receptor H1 antagonist (54). In addition, improvement in IBS severity following a low FODMAP diet was associated with reduction of urinary histamine (14). These exciting findings suggest that histamine is a potential biomarker for assessment of IBS severity as well as a potential therapeutic target as part of a low FODMAP diet. In another study, IBS patients with high levels of short chain fatty acids as colonic bacteria-derived fermentation products, presented with significantly worse gastrointestinal symptoms (11). The identification of biomarkers which correlate with disease severity would be very useful as markers of efficacy of different therapeutic interventions in IBS patients.

Limitations of our study include the relatively small sample sizes and significant differences in age and gender between the three groups of participants. However, we addressed these limitations by using robust multivariate analysis while adjusting for age and gender. In addition, no assessment of lifestyle factors such as differences in specific dietary intake which may have affected the observed differences of urinary metabolites between three groups of subjects is another weakness of our study. None of the patients however were on a specific diet such as a gluten free, paleolithic or low FODMAP diet. It should also be noted that the urinary metabolome may also be affected by different medications (e.g. antidepressant medications that may interfere with dopamine or serotonin metabolisms). Therefore, some of the observed

differences in the metabolome between the groups could be explained by differences in the medications or supplements that our participants were taking and this needs to be taken into account in future studies. Recruiting few IBS-C and IBS-U patients was another limitation of the present study. In addition, definition of UC remission in our study was based on partial Mayo scoring not endoscopic assessment. Considering the values of FCP, it can be speculated that not all UC patients in this study were in endoscopic remission and some of them had subclinical inflammation which could have affected their gut microbial composition and urinary metabolome. Furthermore, we could not validate findings from previous metabolomic studies. While only urine samples were available for metabolomic assays in the present study in all three groups of participants, findings from previous studies were driven from metabolomic measurements in other biological samples such as breath, stool, or serum. Although previous studies that applied metabolomic assays on other biological samples identified some IBS-related metabolites, it has been suggested that using urine samples has some advantages that have resulted in the extensive use of urine in most metabolomic studies. It can easily be collected in multiple time points and large quantities, and needs less complex sample pre-treatment due to lower protein content and lower sample complexity (55). Therefore, performing further studies focused on metabolic profiling using urinary samples are required.

In conclusion, in this exploratory study we identified specific novel metabolites in urine that could be used for discriminating IBS patients from healthy controls and UC patients in clinical remission. We also found that some urinary metabolites were associated with severity of abdominal pain and distention in IBS patients. Some of these metabolites were possibly originating from microbial metabolism, thus evidencing the microbiome as a plausible

therapeutic target. Further investigation into these metabolites in larger prospective and clinical trial studies will help to determine the prognostic value of these metabolic profiles and their potential role in pathogenesis.

3.6 References

1. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. JAMA. 2015;313(9):949-58.

2. Agarwal N, Spiegel BM. The effect of irritable bowel syndrome on health-related quality of life and health care expenditures. Gastroenterol Clin North Am. 2011;40(1):11-9.

3. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10(7):712-721.e4.

4. Lucak S, Chang L, Halpert A, Harris LA. Current and emergent pharmacologic treatments for irritable bowel syndrome with diarrhea: evidence-based treatment in practice. Therap Adv Gastroenterol. 2017;10(2):253-275.

5. Shankar V, Reo NV, Paliy O. Simultaneous fecal microbial and metabolite profiling enables accurate classification of pediatric irritable bowel syndrome. Microbiome. 2015;3:73.

6. Mearin F, Lacy BE, Chang L, et al. Bowel Disorders. Gastroenterology. 2016. pii: S0016-5085(16)00222-5.

7. Sood R, Ford AC. Diagnosis: Rome IV criteria for FGIDs - an improvement or more of the same? Nat Rev Gastroenterol Hepatol. 2016;13(9):501-2.

8. Sood R, Law GR, Ford AC. Diagnosis of IBS: symptoms, symptom-based criteria, biomarkers or 'psychomarkers'? Nat Rev Gastroenterol Hepatol. 2014;11(11):683-91.

9. De Preter V, Verbeke K. Metabolomics as a diagnostic tool in gastroenterology. World J Gastrointest Pharmacol Ther. 2013;4(4):97-107.

10. Baranska A, Mujagic Z, Smolinska A, et al. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. Aliment Pharmacol Ther. 2016;44(1):45-56.

11. Tana C, Umesaki Y, Imaoka A, et al. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. Neurogastroenterol Motil. 2010;22(5):512-9, e114-5.

12. Camilleri M, Halawi H, Oduyebo I. Biomarkers as a diagnostic tool for irritable bowel syndrome: where are we? Expert Rev Gastroenterol Hepatol. 2017;11(4):303-316.

13. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterology. 2006;130(5):1480-91

14. McIntosh K, Reed DE, Schneider T, et al. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut. 2017;66(7):1241-1251.

15. De Vos M, Louis EJ, Jahnsen J, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis. 2013;19(10):2111-7.

16. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997;11(2):395-402.

17. Bouatra S, Aziat F, Mandal R, et al. The human urine metabolome. PLoS One. 2013;8(9):e73076.

18. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6.

19. Basu S, Duren W, Evans CR, et al. Sparse network modeling and metscape-based visualization methods for the analysis of large-scale metabolomics data. Bioinformatics. 2017;33(10):1545-1553.

20. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.

21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Stat Methodol. 1995; 57:289-300.

22. Xia J, Sinelnikov IV, Han B, et al. MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res. 2015;43(W1):W251-7.

23. Le Gall G, Noor SO, Ridgway K, et al. Metabolomics of fecal extracts detects altered metabolic activity of gut microbiota in ulcerative colitis and irritable bowel syndrome. J Proteome Res. 2011;10(9):4208-18.

24. Ahmed I, Greenwood R, Costello Bde L, et al. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. PLoS One. 2013;8(3):e58204.

25. Walton C, Fowler DP, Turner C, et al. Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases. Inflamm Bowel Dis. 2013;19(10):2069-78.

26. Stephens NS, Siffledeen J, Su X, et al. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. J Crohns Colitis. 2013;7(2):e42-8.

27. Ooi M, Nishiumi S, Yoshie T, et al. GC/MS-based profiling of amino acids and TCA cycle-related molecules in ulcerative colitis. Inflamm Res. 2011;60(9):831-40.

28. Dawiskiba T, Deja S, Mulak A, et al. Serum and urine metabolomic fingerprinting in diagnostics of inflammatory bowel diseases. World J Gastroenterol. 2014;20(1):163-74.

29. Schicho R, Nazyrova A, Shaykhutdinov R, et al. Quantitative metabolomic profiling of serum and urine in DSS-induced ulcerative colitis of mice by (1)H NMR spectroscopy. J Proteome Res. 2010;9(12):6265-73.

30. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr. 2011;94(1):348S-358S.

31. Sharabi A, Cohen E, Sulkes J, et al. Replacement therapy for vitamin B12 deficiency: comparison between the sublingual and oral route. Br J Clin Pharmacol. 2003;56(6):635-8.

32. Solakivi T, Kaukinen K, Kunnas T, et al. Serum fatty acid profile in subjects with irritable bowel syndrome. Scand J Gastroenterol. 2011;46(3):299-303.

33. Menni C, Zhai G, Macgregor A, et al. Targeted metabolomics profiles are strongly correlated with nutritional patterns in women. Metabolomics. 2013;9(2):506-514.

34. van den Berg R, Mook-Kanamori DO, Donga E, et al. A single night of sleep curtailment increases plasma acylcarnitines: Novel insights in the relationship between sleep and insulin resistance. Arch Biochem Biophys. 2016;589:145-51.

35. Davies SK, Ang JE, Revell VL, et al. Effect of sleep deprivation on the human metabolome. Proc Natl Acad Sci U S A. 2014;111(29):10761-6.

36. Lee YT, Hu LY, Shen CC, et al. Risk of Psychiatric Disorders following Irritable Bowel Syndrome: A Nationwide Population-Based Cohort Study. PLoS One. 2015;10(7):e0133283.

37. Khayyatzadeh SS, Esmaillzadeh A, Saneei P, et al. Dietary patterns and prevalence of irritable bowel syndrome in Iranian adults. Neurogastroenterol Motil. 2016;28(12):1921-1933.

38. Park JH, Rhee PL, Kim G, et al. Enteroendocrine cell counts correlate with visceral hypersensitivity in patients with diarrhoea-predominant irritable bowel syndrome. Neurogastroenterol Motil. 2006;18(7):539-46.

39. Field T, Diego M, Hernandez-Reif M, et al. Comorbid depression and anxiety effects on pregnancy and neonatal outcome. Infant Behav Dev. 2010;33(1):23-9.

40. Roy A, Pollack S. Are cerebrospinal fluid or urinary monoamine metabolite measures stronger correlates of suicidal behavior in depression? Neuropsychobiology. 1994;29(4):164-7.

41. Lee C, Doo E, Choi JM, et al. The Increased Level of Depression and Anxiety in Irritable Bowel Syndrome Patients Compared with Healthy Controls: Systematic Review and Meta-analysis. J Neurogastroenterol Motil. 2017;23(3):349-362.

42. Wijaya CS, Lee JJZ, Husain SF, et al. Differentiating Medicated Patients Suffering from Major Depressive Disorder from Healthy Controls by Spot Urine Measurement of Monoamines and Steroid Hormones. Int J Environ Res Public Health. 2018;15(5).

43. Williamson G, Clifford MN. Colonic metabolites of berry polyphenols: the missing link to biological activity? Br J Nutr. 2010;104 Suppl 3:S48-66.

44. Spiller R, Aziz Q, Creed F, et al. Guidelines on the irritable bowel syndrome: mechanisms and practical management. Gut. 2007;56(12):1770-98.

45. Heitkemper MM, Han CJ, Jarrett ME, et al. Serum Tryptophan Metabolite Levels During Sleep in Patients With and Without Irritable Bowel Syndrome (IBS). Biol Res Nurs. 2016;18(2):193-8.

46. Dunlop SP, Coleman NS, Blackshaw E, et al. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. Clin Gastroenterol Hepatol. 2005;3(4):349-57.

47. Allen NE, Grace PB, Ginn A, et al. Phytanic acid: measurement of plasma concentrations by gas-liquid chromatography-mass spectrometry analysis and associations with diet and other plasma fatty acids. Br J Nutr. 2008;99(3):653-9.

48. Anitha M, Reichardt F, Tabatabavakili S, et al. Intestinal dysbiosis contributes to the delayed gastrointestinal transit in high-fat diet fed mice. Cell Mol Gastroenterol Hepatol. 2016;2(3):328-339.

49. Lopetuso LR, Petito V, Graziani C, et al. Gut Microbiota in Health, Diverticular Disease, Irritable Bowel Syndrome, and Inflammatory Bowel Diseases: Time for Microbial Marker of Gastrointestinal Disorders. Dig Dis. 2018;36(1):56-65.

50. Moloney RD, Johnson AC, O'Mahony SM, et al. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. CNS Neurosci Ther. 2016;22(2):102-17.

51. Wall R, Ross RP, Shanahan F, et al. Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. Am J Clin Nutr. 2009;89(5):1393-401.

52. Jeffery IB, O'Toole PW, Öhman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut. 2012;61(7):997-1006.

53. Wouters MM, Vicario M, Santos J. The role of mast cells in functional GI disorders. Gut. 2016;65(1):155-68.

54. Wouters MM, Balemans D, Van Wanrooy S, et al. Histamine Receptor H1-Mediated Sensitization of TRPV1 Mediates Visceral Hypersensitivity and Symptoms in Patients With Irritable Bowel Syndrome. Gastroenterology. 2016;150(4):875-87.e9.

55. Ryan D, Robards K, Prenzler PD, et al. Recent and potential developments in the analysis of urine: a review. Anal Chim Acta. 2011;684(1-2):8-20.

3.7 Tables and Figures

Table 3.7.1. Demographic and clinical characteristics of study participants

		IBS (n=39)	UC (n=53)	Control (n=21)	P-value
Age, years, median (interquartile range)		50.0 (37.0-67.0)	39.0 (28.5-55.0)	30.0 (30.0-35.0)	< 0.001
Females, n (%)		34 (87.2)	34 (64.2)	7 (33.3)	< 0.001
IBS-subtype, n (%)	IBS-M	25 (64.1)			
	IBS-D	10 (25.6)			
	IBS-C	3 (7.7)			
	IBS-U	1 (2.6)			
UC subtype, n (%)	Proctitis		6 (11.3)		
	Left-sided		22 (41.5)		
	Pancolitis		25 (47.2)		
UC medications, n (%)	No UC medication		5 (9.4)		
	5-aminosalicylic acid		40 (75.5)		
	Immunosuppressants		19 (35.8)		
	Biologics		13 (24.5)		

IBS: irritable bowel syndrome, UC: ulcerative colitis, IBS-M: mixed irritable bowel syndrome, IBS-D: diarrhea- predominant irritable bowel syndrome, IBS-C: constipation- predominant irritable bowel syndrome, IBS-U: unsubtyped irritable bowel syndrome.

Table 3.7.2. Major urinary metabolites (µmol/mmol of creatinine) responsible for the discrimination between irritable bowel syndrome and ulcerative colitis patients [1]

Metabolites	Irritable bowel syndrome (n=39)	Ulcerative colitis (n=53)	P-value [2]	Q-value
Histidine [3]	35.545 (20.090-56.389)	0.889 (0.716-1.223)	<0.001	<0.001
Lactic acid [4]	3.077 (1.351-7.117)	20.120 (12.985-37.028)	<0.001	<0.001
Lysine [3]	5.479 (3.411-7.784)	0.636 (0.546-0.867)	<0.001	<0.001
Proline [3]	0.968 (0.746-1.246)	24.080 (16.554-30.729)	<0.001	<0.001
Oxoglutaric acid [4]	1.825 (0.877-5.208)	17.581 (8.523-42.701)	<0.001	<0.001
Glutamine [3]	29.546 (22.981-38.898)	3.908 (2.183-8.615)	<0.001	<0.001
Glutamic acid [3]	6.386 (2.700-10.887)	84.074 (61.700-147.195)	<0.001	<0.001
Ethylmalonic acid [4]	0.260 (0.053-1.016)	3.003 (1.746-7.434)	<0.001	<0.001
3-Hydroxyisovaleric acid [4]	17.864 (11.364-38.559)	124.378 (78.083-261.316)	<0.001	<0.001
Phenylalanine [3]	3.106 (2.737-3.540)	0.569 (0.462-0.876)	<0.001	<0.001
Sumiki's acid [4]	3.472 (1.750-5.540)	0.883 (0.352-2.216)	<0.001	<0.001
Citrulline [3]	0.851 (0.467-1.978)	28.879 (18.088-39.151)	<0.001	<0.001
o-hydroxyphenylacetic acid [4]	0.092 (0.046-0.182)	0.470 (0.260-0.943)	<0.001	<0.001
Adipic acid [4]	0.808 (0.219-1.538)	1.826 (0.980-3.267)	<0.001	<0.001

[1] Concentrations are presented as median (interquartile range).

[2] Obtained from Mann–Whitney U test

[3] Identified and quantified by direct infusion /liquid chromatography tandem mass spectrometry assay

[4] Identified and quantified by gas-chromatography mass spectrometry assay

Table 3.7.3. Major urinary metabolites (µmol/mmol of creatinine) responsible for the discrimination between irritable bowel syndrome patients and healthy controls [1]

Metabolites	Irritable bowel syndrome (n=39)	Healthy controls (n=21)	P-value [2]	Q-value
PC ae C38:6 [3]	0.002 (0.001-0.003)	0.005 (0.004-0.009)	<0.001	<0.001
p-hydroxybenzoic acid [4]	0.258 (0.075-0.763)	2.486 (1.718-2.852)	<0.001	<0.001
2-Methylsuccinic acid [4]	0.250 (0.104-0.592)	6.187 (4.056-6.227)	<0.001	<0.001
C6 (C4:1-DC) [3]	0.041 (0.027-0.055)	0.066 (0.052-0.078)	<0.001	0.002
Dopamine [3]	0.226 (0.181-0.295)	0.365 (0.275-0.470)	<0.001	0.009
Palmitic acid [4]	11.592 (5.646-18.580)	17.828 (16.392-19.331)	0.002	0.048
Hippuric acid [4]	97.677 (67.233-123.795)	159.070 (112.50-238.319)	0.006	0.057
Ornithine [3]	0.891 (0.736-1.197)	2.352 (1.756-4.375)	0.009	0.059
Hydroxyphenylacetic acid [4]	1.672 (0.983-4.557)	3.413 (2.572-4.248)	0.007	0.057
PC aa C34:4 [3]	0.001 (0.001-0.002)	0.000 (0.000-0.001)	0.009	0.059

[1] Concentration of metabolites are presented as median (interquartile range).

[2] Obtained from Mann–Whitney U test

[3] Identified and quantified by direct infusion /liquid chromatography tandem mass spectrometry assay

[4] Identified and quantified by gas-chromatography mass spectrometry assay

PC ae: Phosphatidylcholine acyl-alkyl, C6 (C4:1-DC): Hexanoylcarnitine PC aa: Phosphatidylcholine diacyl

Table 3.7.4. Major urinary metabolites (µmol/mmol of creatinine) responsible for the discrimination between IBS-M and IBS-D patients [1]

Metabolites [2]	IBS-M (n=25)	IBS-D (n=10)	VIP [3] score	P-value [4]	Q-value
Palmitic acid [2]	3.383 (0.272-9.228)	8.833 (3.975-15.879)	1.57	0.09	0.19
3-Methyladipic acid	0.392 (0.146-1.099)	0.092 (0.069-0.196)	1.51	0.01	0.15

[1] Concentration of metabolites are presented as median (interquartile range).

[2] Identified and quantified by gas-chromatography mass spectrometry assay

[3] Variable importance in projection

[4] Obtained from Mann–Whitney U test

IBS-M: mixed irritable bowel syndrome, IBS-D: diarrhea predominant irritable bowel syndrome

Table 3.7.5. Correlation of urinary metabolites with severity of irritable bowel syndrome [1]

Metabolites	Total IBS-SSS	Severity of abdominal pain	Frequency of abdominal pain	Severity of abdominal distention
Histamine	0.49 [2]	0.46 [2]	0.31 [4]	0.35 [3]
Aspartic acid	0.39 [3]	-0.03 [4]	0.24 [4]	0.51 [2]
Methylmalonic acid	0.34 [3]	0.15 [4]	0.35 [3]	0.36 [3]
PC aa C38:4	0.36 [3]	0.26 [4]	0.22 [4]	0.38 [3]
PC ae C36:2	0.34 [3]	0.06 [4]	0.34 [3]	0.36 [3]

[1] Values are Spearman's rank correlation coefficients (r_s)

[2] $P<0.05$

[3] $P<0.01$

[4] not significant

PC aa: Phosphatidylcholine diacyl, PC ae: Phosphatidylcholine acyl-alkyl

Supplementary Table 3.7.1. List of analyzed metabolites in urine samples

Metabolites	Full biochemical name	Metabolite class
C0	Carnitine	Carnitine
C2	Acetylcarnitine	Acylcarnitines
C3	Propionylcarnitine	Acylcarnitines
C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	Acylcarnitines
C4	Butyrylcarnitine	Acylcarnitines
C4-OH (C3-DC)	Hydroxybutyrylcarnitine	Acylcarnitines
C4:1	Butenylcarnitine	Acylcarnitines
C5	Valerylcarnitine	Acylcarnitines
C5-DC (C6-OH)	Glutarylcarnitine (Hydroxyhexanoylcarnitine)	Acylcarnitines
C5-M-DC	Methylglutarylcarnitine	Acylcarnitines
C5:1	Tiglylcarnitine	Acylcarnitines
C5:1-DC	Glutaconylcarnitine	Acylcarnitines
C6 (C4:1-DC)	Hexanoylcarnitine (Fumarylcarnitine)	Acylcarnitines
C6:1	Hexenoylcarnitine	Acylcarnitines
C7-DC	Pimelylcarnitine	Acylcarnitines
C8	Octanoylcarnitine	Acylcarnitines
C9	Nonaylcarnitine	Acylcarnitines
C10	Decanoylcarnitine	Acylcarnitines
C10:1	Decenoylcarnitine	Acylcarnitines

C10:2	Decadienylcarnitine	Acylcarnitines
C12	Dodecanoylcarnitine	Acylcarnitines
C12:1	Dodecenoylcarnitine	Acylcarnitines
C14	Tetradecanoylcarnitine	Acylcarnitines
C14:1	Tetradecenoylcarnitine	Acylcarnitines
C14:1-OH	Hydroxytetradecenoylcarnitine	Acylcarnitines
C14:2	Tetradecadienylcarnitine	Acylcarnitines
C14:2-OH	Hydroxytetradecadienylcarnitine	Acylcarnitines
C16:2	Hexadecadienylcarnitine	Acylcarnitines
lysoPC a C18:0	lysoPhosphatidylcholine acyl C18:0	Lyso-phosphatidylcholines
lysoPC a C20:4	lysoPhosphatidylcholine acyl C18:0	Lyso-phosphatidylcholines
PC aa C34:2	Phosphatidylcholine diacyl C34:2	Diacyl-phosphatidylcholines
PC aa C34:4	Phosphatidylcholine diacyl C34:4	Diacyl-phosphatidylcholines
PC aa C36:1	Phosphatidylcholine diacyl C36:1	Diacyl-phosphatidylcholines
PC aa C36:3	Phosphatidylcholine diacyl C36:3	Diacyl-phosphatidylcholines
PC aa C38:4	Phosphatidylcholine diacyl C38:4	Diacyl-phosphatidylcholines

PC aa C38:5	Phosphatidylcholine diacyl C38:5	Diacyl-phosphatidylcholines
PC aa C38:6	Phosphatidylcholine diacyl C38:6	Diacyl-phosphatidylcholines
PC ae C32:1	Phosphatidylcholine acyl-alkyl C32:1	Acyl-alkyl-phosphatidylcholines
PC ae C34:1	Phosphatidylcholine acyl-alkyl C34:1	Acyl-alkyl-phosphatidylcholines
PC ae C36:2	Phosphatidylcholine acyl-alkyl C36:2	Acyl-alkyl-phosphatidylcholines
PC ae C36:3	Phosphatidylcholine acyl-alkyl C36:3	Acyl-alkyl-phosphatidylcholines
PC ae C38:1	Phosphatidylcholine acyl-alkyl C38:1	Acyl-alkyl-phosphatidylcholines
PC ae C38:3	Phosphatidylcholine acyl-alkyl C38:3	Acyl-alkyl-phosphatidylcholines
PC ae C38:4	Phosphatidylcholine acyl-alkyl C38:4	Acyl-alkyl-phosphatidylcholines
PC ae C38:6	Phosphatidylcholine acyl-alkyl C38:6	Acyl-alkyl-phosphatidylcholines
PC ae C40:5	Phosphatidylcholine acyl-alkyl C40:5	Acyl-alkyl-phosphatidylcholines

PC ae C42:2	Phosphatidylcholine acyl-alkyl C42:2	Acyl-alkyl-phosphatidylcholines
PC ae C42:3	Phosphatidylcholine acyl-alkyl C42:3	Acyl-alkyl-phosphatidylcholines
PC ae C44:3	Phosphatidylcholine acyl-alkyl C44:3	Acyl-alkyl-phosphatidylcholines
PC ae C44:4	Phosphatidylcholine acyl-alkyl C44:4	Acyl-alkyl-phosphatidylcholines
SM (OH) C24:1	Hydroxysphingomyeline C24:1	Hydroxysphingomyelins
SM C16:0	Sphingomyeline C16:0	Sphingomyelins
SM C16:1	Sphingomyeline C16:1	Sphingomyelins
SM C18:1	Sphingomyeline C18:1	Sphingomyelins
SM C24:1	Sphingomyeline C24:1	Sphingomyelins
Alanine		Amino acids
Arginine		Amino acids
Asparagine		Amino acids
Aspartic acid		Amino acids
Citrulline		Amino acids
Glutamine		Amino acids
Glutamic acid		Amino acids
Glycine		Amino acids
Histidine		Amino acids
Isoleucine		Amino acids

Leucine		Amino acids
Lysine		Amino acids
Methionine		Amino acids
Ornithine		Amino acids
Phenylalanine		Amino acids
Proline		Amino acids
Serine		Amino acids
Threonine		Amino acids
Tryptophan		Amino acids
Tyrosine		Amino acids
Valine		Amino acids
Acetylornithine		Amino acids
Asymmetric dimethylarginine		Amino acids
Symmetric dimethylarginine		Amino acids
Total dimethylarginine		Amino acids
Alpha-Aminoadipic acid		Amino acids
Methionine sulfoxide		Amino acids
Trans-OH proline	trans-hydroxyproline	Amino acids
Carnosine		Dipeptides
Dopamine		Amines
Kynurenine		Amines

Serotonin		Amines
Histamine		Amines
DOPA	L-3,4-dihydroxyphenylalanine	Amines
Putrescine		Amines
Taurine		Amines
Lactic acid		Alpha hydroxy acids
Beta-lactic acid		Beta hydroxy acids
2-Hydroxy-2-methylbutyric acid		Hydroxy fatty acids
3-hydroxybutyric acid		Beta hydroxy acids
3-Hydroxyisovaleric acid		Beta hydroxy acids
Methylmalonic acid		Dicarboxylic acids
Ethylmalonic acid		Dicarboxylic acids
Succinic acid		Dicarboxylic acids
4-Deoxythreonic acid		Monocarboxylic acids
3-Hydroxymethylglutaric acid		Dicarboxylic acids
2-Methylsuccinic acid		Dicarboxylic acids
Adipic acid		Dicarboxylic acids
Pyroglutamic acid		Amino acids
3-Methyladipic acid		Dicarboxylic acids
Sumiki's acid	5-(Hydroxymethyl)furan-2-carboxylic acid	Monocarboxylic acids

ortho-Hydroxyphenylacetic acid		Monocarboxylic acids
Oxoglutaric acid		Dicarboxylic acids
Pimelic acid		Dicarboxylic acids
p-Hydroxybenzoic acid		Monocarboxylic acids
p-Hydroxyphenylacetic acid		Monocarboxylic acids
Suberic acid		Dicarboxylic acids
Trans-Aconitic acid		Tricarboxylic acids
Homovanillic acid		Monocarboxylic acids
Hippuric acid		Monocarboxylic acids
Azelaic acid		Dicarboxylic acids
Palmitic acid		Monocarboxylic acids
HPHPA	3-(3-hydroxyphenyl)-3-hydroxypropionic acid	Monocarboxylic acids
Vanillylmandelic acid		Monocarboxylic acids
Hydroxyphenylacetic acid		Monocarboxylic acids

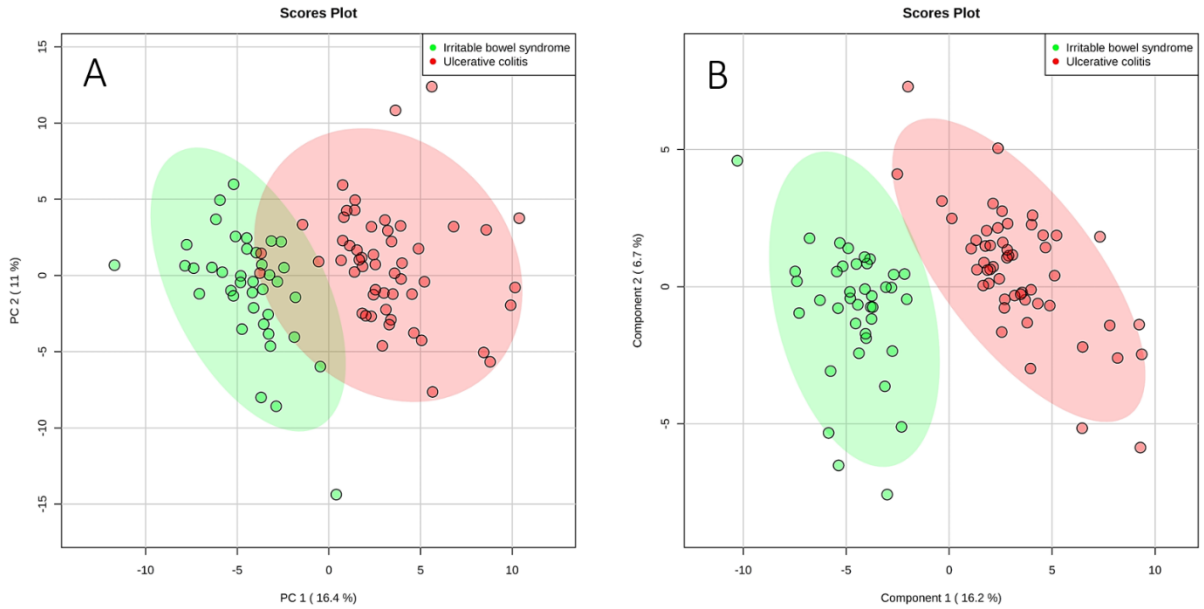


Figure 3.7. 1. A) Principal component analysis plot and B) Partial least squares discriminant analysis plot showing discrimination of patients with irritable bowel syndrome from ulcerative colitis patients in clinical remission using their urinary metabolomic profile assessed by direct infusion/liquid chromatography tandem mass spectrometry and gas-chromatography mass spectrometry assays (R^2=0.85, Q^2=0.76, P<0.001).

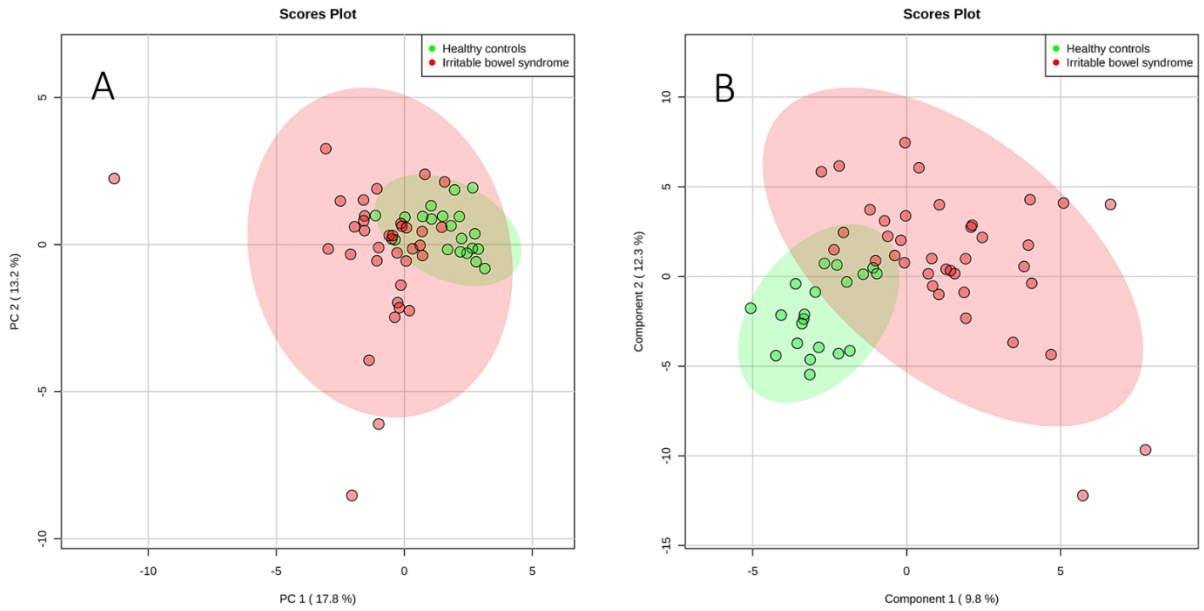


Figure 3.7.2. A) Principal component analysis plot and B) Partial least squares discriminant analysis plot showing discrimination of patients with irritable bowel syndrome from healthy controls using their urinary metabolomic profile assessed by direct infusion/liquid chromatography tandem mass spectrometry and gas-chromatography mass spectrometry assays (R^2=0.79, Q^2=0.45, P=0.008).

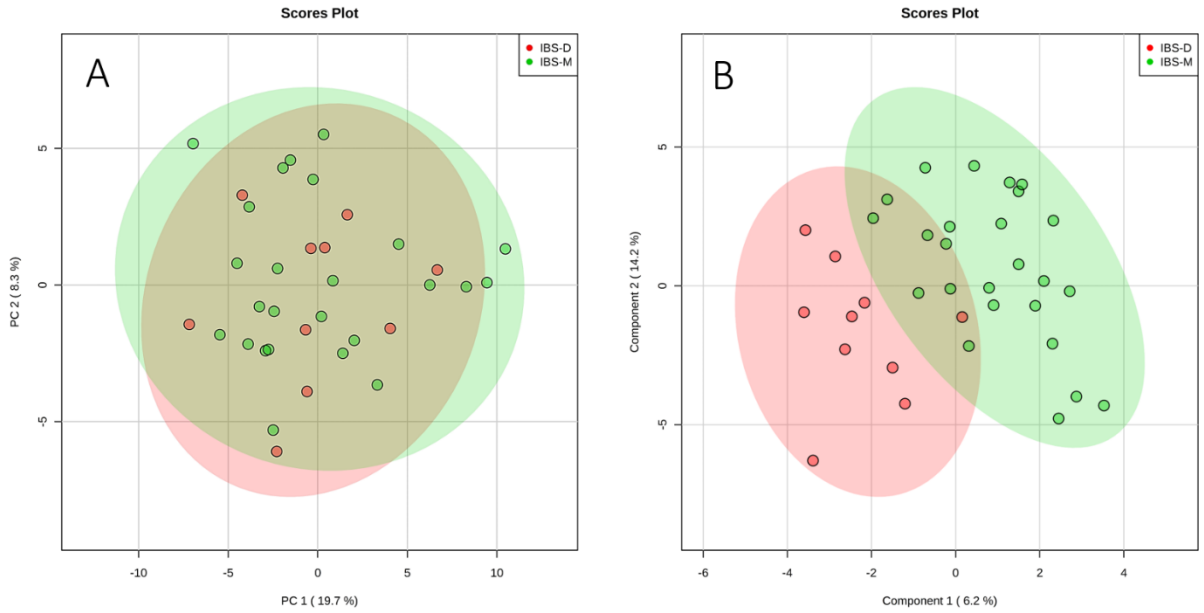
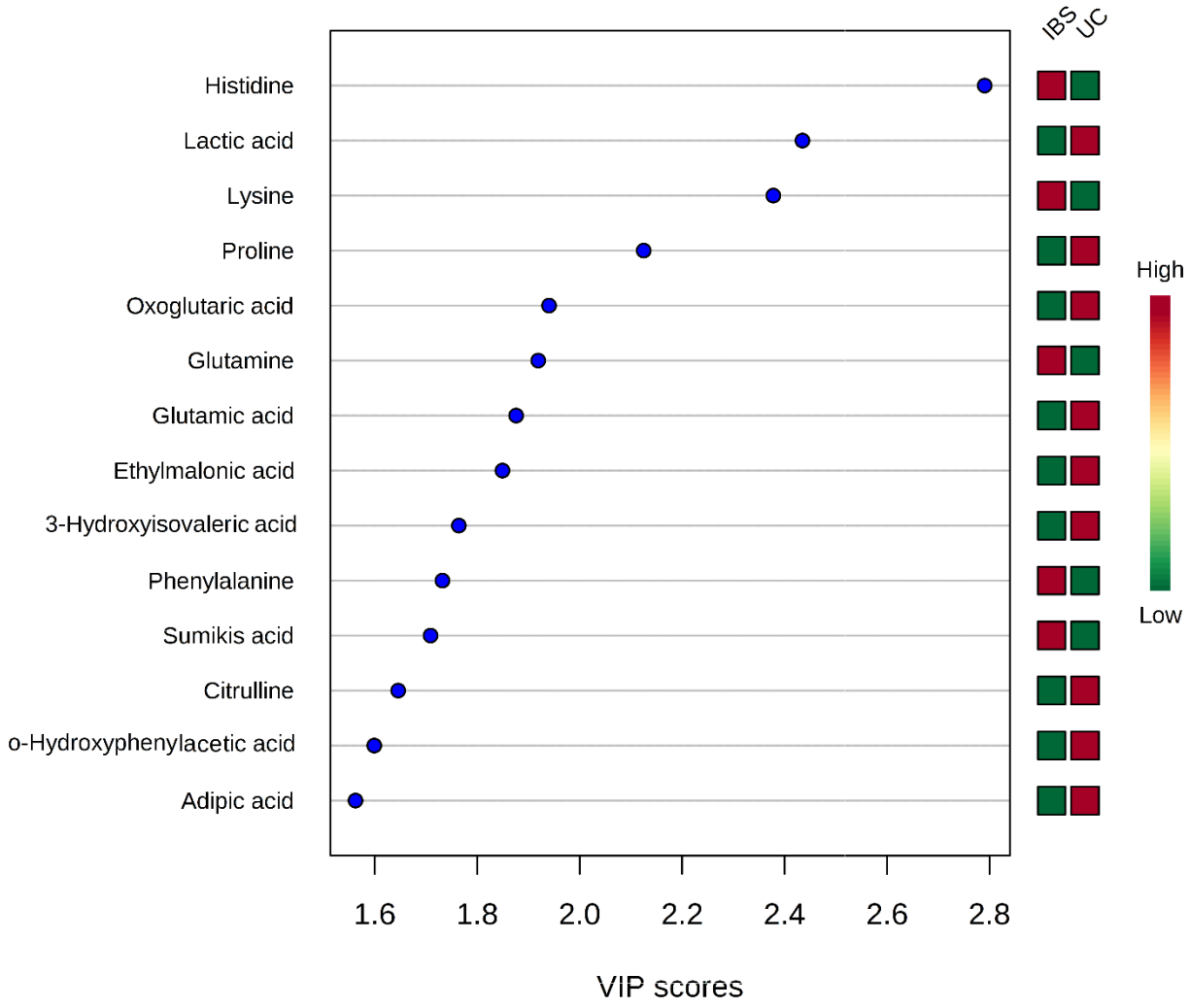
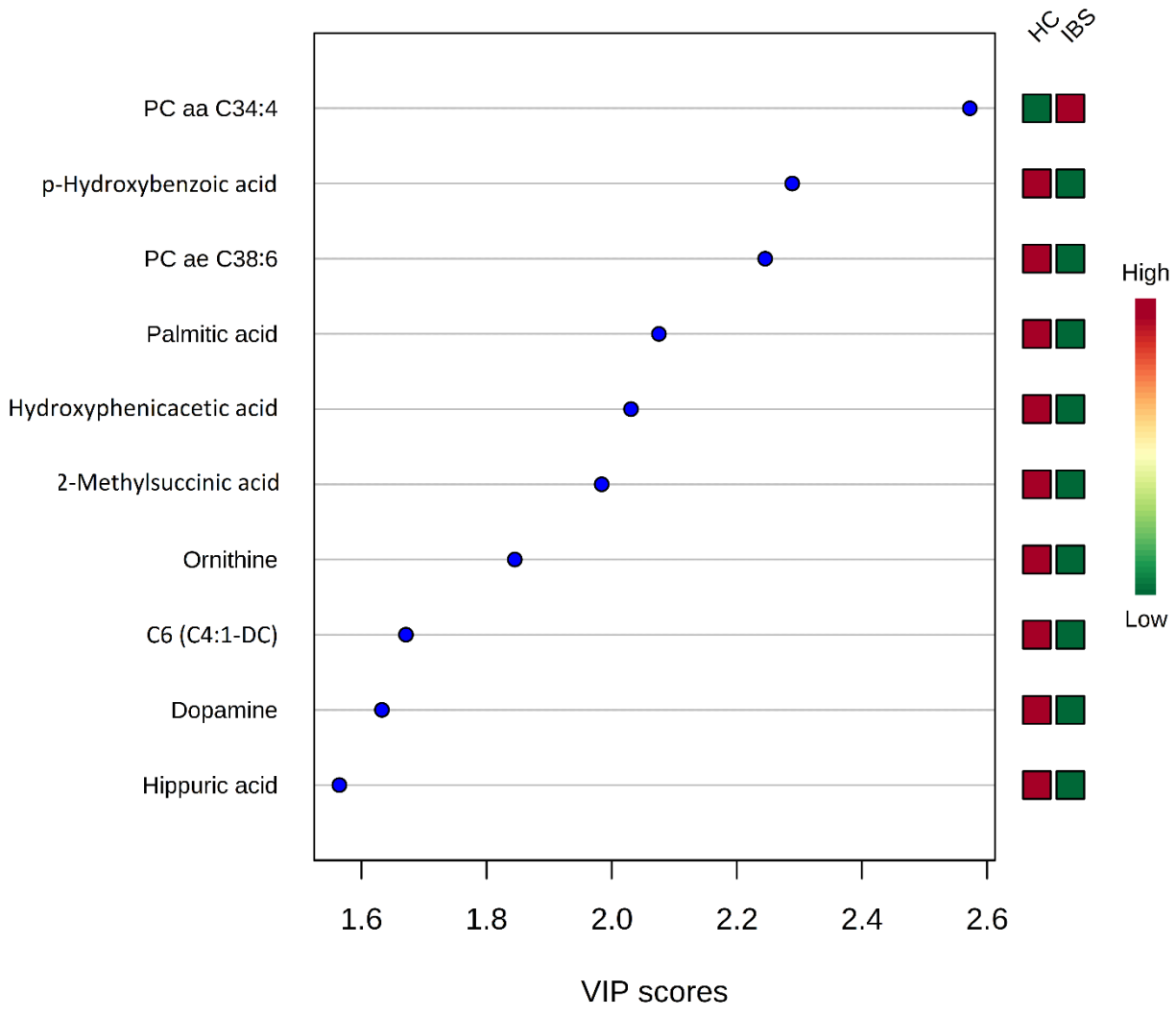


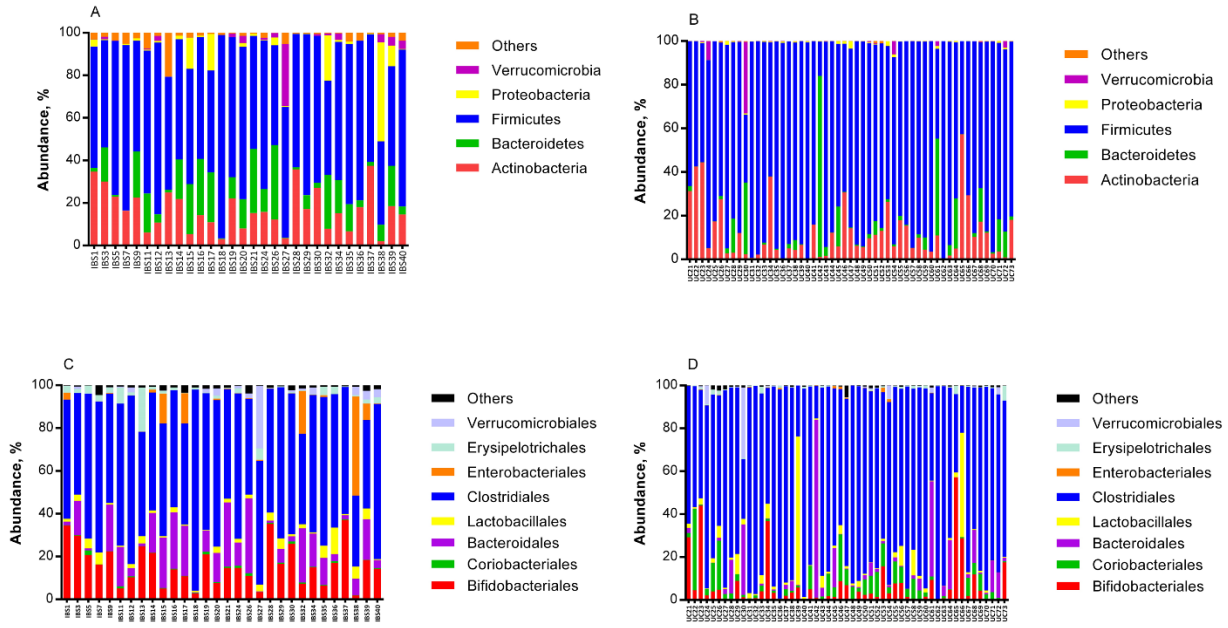
Figure 3.7.3. A) Principal component analysis plot and B) Partial least squares discriminant analysis plot showing no discrimination of patients with diarrhea- predominant irritable bowel syndrome (IBS-D) from mixed irritable bowel syndrome (IBS-M) using their urinary metabolomic profiles (R^2=0.54, Q^2= -0.6, P=0.49).



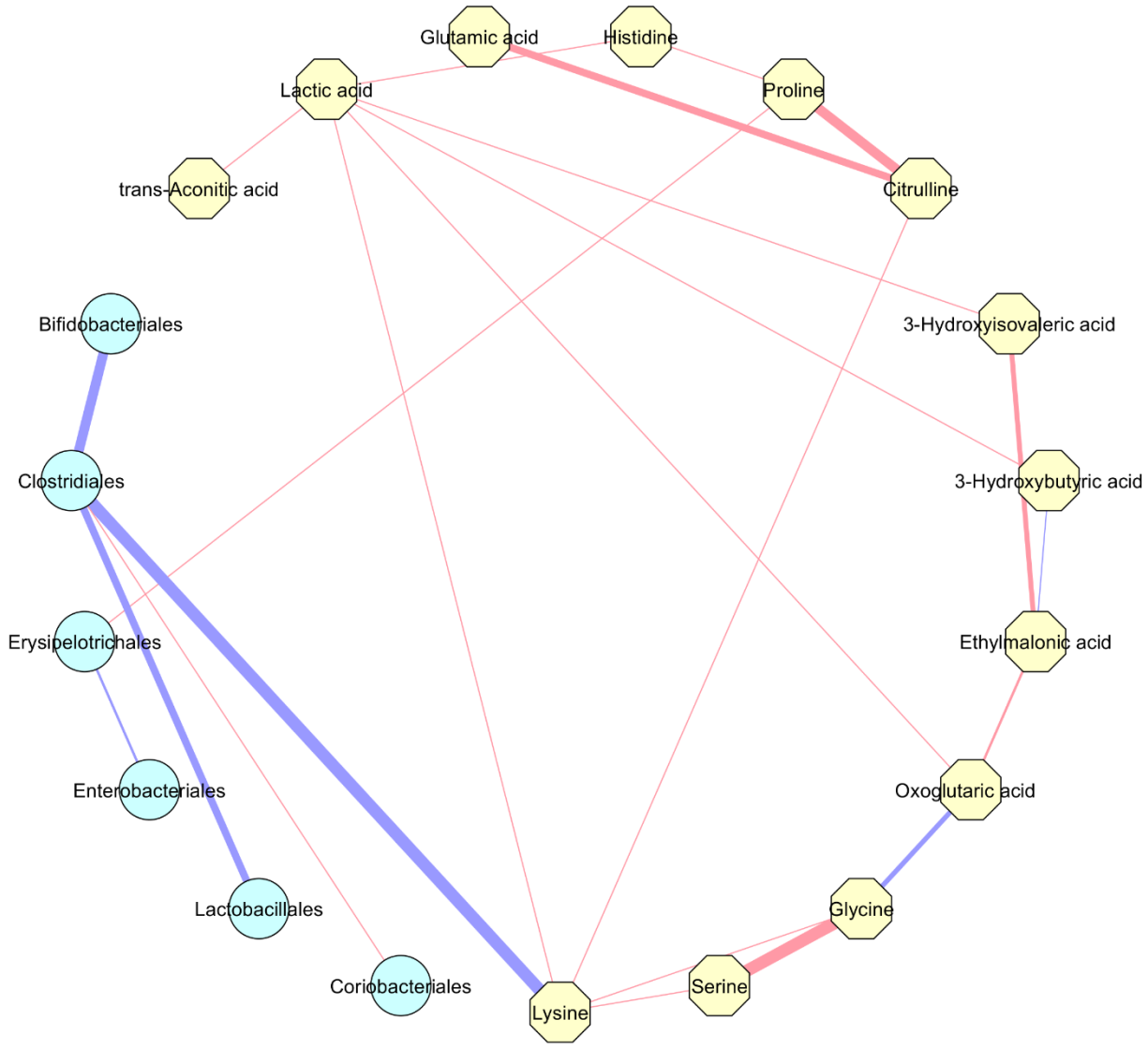
Supplementary Figure 3.7.1. Variable Importance in Projection (VIP) plot of major metabolites in urine responsible for the discrimination between irritable bowel syndrome (IBS) and ulcerative colitis (UC) patients.



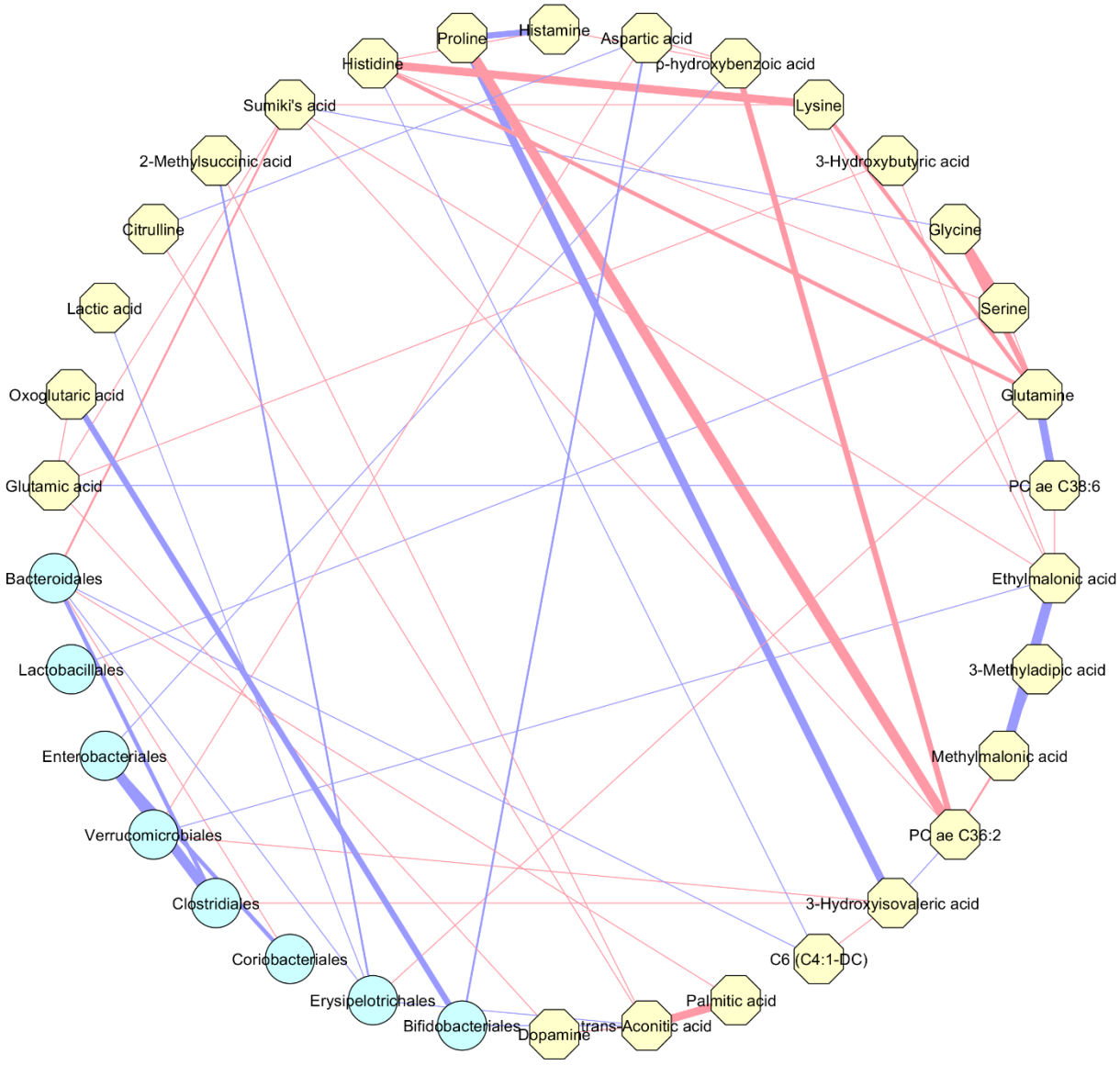
Supplementary Figure 3.7.2. Variable Importance in Projection (VIP) plot of major metabolites in urine responsible for the discrimination between irritable bowel syndrome (IBS) patients and healthy controls (HC).



Supplementary Figure 3.7.3. Microbial composition levels at phylum (A, B) and order levels (C, D) on stool samples in irritable bowel syndrome (A, C) and ulcerative colitis (B, D) patients.



Supplementary Figure 3.7.4. Correlation between urinary metabolites and gut microbial composition in ulcerative colitis (UC) patients. Spearman's rank correlations were calculated between taxonomic data (order level) and urinary metabolites in UC patients that could discriminate them from irritable bowel syndrome patients. The correlations were filtered using $|r| > 0.3$ and subsequently correlation network was built. Blue lines represent negative correlations, and red lines represent positive correlations.



Supplementary Figure 3.7. 5. Correlation between urinary metabolites and gut microbial composition in irritable bowel syndrome patients (IBS). Spearman's rank correlations were calculated between taxonomic data (order level) and urinary metabolites in IBS patients that could discriminate them from ulcerative colitis patients or healthy volunteers, or metabolites responsible for the discrimination between diarrhea- predominant IBS and mixed IBS, or metabolites that were correlated with severity of IBS symptoms. The correlations were filtered using $|r| > 0.3$ and subsequently correlation network was built. Blue lines represent negative correlations, and red lines represent positive correlations.

Chapter 4. Dietary and metabolomic determinants of relapse in ulcerative colitis patients: A pilot prospective cohort study

4.1 ABSTRACT

AIM: To identify demographic, clinical, metabolomic, and lifestyle related predictors of relapse in adult ulcerative colitis patients.

METHODS: In this prospective pilot study, UC patients in clinical remission were recruited and followed-up at 12 months to assess a clinical relapse, or not. At baseline information on demographic and clinical parameters was collected. Serum and urine samples were collected for analysis of metabolomic assays using a combined direct infusion/liquid chromatography tandem mass spectrometry and nuclear magnetic resolution spectroscopy. Stool samples were also collected to measure fecal calprotectin (FCP). Dietary assessment was performed using a validated self-administered food frequency questionnaire.

RESULTS: Twenty patients were included (mean age: 42.7±14.8 years, females: 55%). Seven patients (35%) experienced a clinical relapse during the follow-up period. While 6 patients (66.7%) with normal body weight developed a clinical relapse, 1 UC patient (9.1%) who was overweight/obese relapsed during the follow-up (P=0.02). At baseline, poultry intake was significantly higher in patients who were still in remission during follow-up (0.9 oz vs. 0.2 oz, P= 0.002). Five patients (71.4%) with FCP >150µg/g and 2 patients (15.4%) with normal FCP (≤150µg/g) at baseline relapsed during the follow-up (P=0.02). Interestingly, baseline urinary and serum metabolomic profiling of UC patients with or without clinical relapse within 12

months showed a significant difference. The most important metabolites that were responsible for this discrimination were trans-aconitate, cystine and acetamide in urine, and 3-hydroxybutyrate, acetoacetate and acetone in serum.

CONCLUSION: A combination of baseline dietary intake, fecal calprotectin, and metabolomic factors are associated with risk of UC clinical relapse within 12 months.

4.2. Introduction

Ulcerative colitis (UC), a subtype of the inflammatory bowel diseases (IBD), is a chronic relapse-remitting inflammatory condition that affects the colon in a diffuse, continuous, and superficial pattern. It often presents in young adulthood and is more common in developed countries. UC affects both genders equally and its presenting symptoms are usually rectal bleeding, urgency, and tenesmus, with diarrhea. UC prevalence is estimated at 5-500 people per 100 000 worldwide (1). In addition, the incidence of UC is increasing and its health-care burden is considerable (2). The incidence and prevalence of IBD (including UC and Crohn's disease (CD) in Canada are amongst the highest in the world (3). The pathogenesis of IBD is largely unknown. Current evidence suggests that environmental factors and microbial dysbiosis may interact to trigger a dysregulated immune response which induces chronic intestinal inflammation in genetically susceptible hosts (4).

Patients with UC can experience multiple disease relapses in spite of receiving adequate standard treatment. It has also been shown that poor disease control and multiple relapses result in deteriorated quality of life (5) and an increased probability of colitis-associated colorectal cancer (6). Although determinants of UC relapse have not been fully elucidated, a variety of demographic, clinical, endoscopic, psychosocial, serologic and fecal biomarkers have been investigated in several studies with inconsistent findings (7-13).

Metabolomics is the systemic identification and quantitation of all metabolites in a given organism or set of biological samples (14). Similar to other "omic" approaches that are used to study the pathophysiology of different diseases, metabolomics has the potential to reveal the underlying multifactorial mechanisms of diseases, including IBD (15), especially if measured

before disease relapse occurs. Other investigators have shown that urinary, serum, and fecal metabolomic profiles of IBD patients differ from healthy controls (15,16). In addition, it has been suggested that metabolomics has the potential to identify novel biomarkers that could be useful for surveillance and early detection of IBD relapse (15).

Understanding predictors of UC relapse is of great importance for both patients and healthcare providers and only few prospective studies have been done in this regard. Therefore, the aim of this study was to examine the roles of multiple clinical, demographic, dietary and metabolomic factors that may predict UC relapse.

4.3 Methods

Patient Cohort: This pilot prospective cohort study was performed in Edmonton, Alberta, Canada. Using a convenience non-probability sampling method, adult UC patients who were able to read and write in English were recruited consecutively from the IBD clinic at the University of Alberta. The diagnosis of UC was confirmed using a combination of clinical, endoscopic and histological criteria. All patients were included if they were in clinical remission at the time of enrollment determined by a validated partial Mayo score of less than 3 (17). Subjects were excluded if they had used oral corticosteroids in the previous four weeks, used corticosteroids within the previous two weeks before enrollment, used any biological agents for UC management within 3 months before the enrollment, or had a history of colectomy. Written informed consent was obtained from all participants and the study protocol was approved by the Health Research Ethics Board-Biomedical Panel, University of Alberta, Edmonton, Canada (Pro00032213).

Participants were asked to come to the research clinic for the first visit (Baseline). At baseline visit participants' demographic and clinical information was obtained and participants completed a food frequency questionnaire (FFQ) that assessed their food intake in the past 12 months. Anthropometric assessments (as described below), clinical information, and urine and blood samples were collected for metabolomics analyses, and stool was collected for fecal calprotectin (FCP). Twelve months after the baseline visit, patients were followed-up by a telephone interview and their clinical files were reviewed to determine if they had experienced a clinical UC relapse (partial Mayo score of 3 or more) during these 12 months. Comparisons were made between patients who remained in clinical remission versus those who experienced a clinical relapse.

Demographic and Clinical Information: At baseline, demographic (age, gender), and clinical information was collected. Long-term dietary intake was assessed using the National Cancer Institute's self-administered Diet History Questionnaire II (DHQ) (18, 19). This validated semi-quantitative FFQ included questions about 134 food items and accounts for seasonal intake of a variety of foods, portion size and frequency of intake for each food item. Body weight was measured to the nearest 0.01 kg (Health o Meter Professional 752KL medical scale) and height was measured (HM200P Portstad portable stadiometer, Charder Electronic Co, Ltd) without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the narrowest part of abdomen over light clothing using a non-stretch measuring tape and recorded to the nearest 0.1 cm. Waist to height ratio was calculated as the ratio of waist circumference/height. Body composition (i.e.

total fat mass and body fat percentage) was determined by air displacement plethysmography (BodPod) (COSMED Concord, CA, USA).

Sample Collection and Analysis: Subjects were provided appropriate materials and instructions to collect morning urine and stool samples. In addition, fasting blood samples were collected from each participant at baseline. Urine and serum samples were assayed using a combined direct infusion (DI-)/liquid chromatography (LC-) tandem mass spectrometry (MS/MS) (AbsolutIDQ p180 kit, Biocrates Life Sciences AG, Innsbruck, Austria) and nuclear magnetic resolution (NMR) spectroscopy, using the previously described protocol (20) in order to identify and quantify metabolites. All metabolomic assays were performed at the Metabolomics Innovation Centre (Edmonton, Canada). Fecal calprotectin (FCP) was measured in stool samples using an enzyme-linked immunosorbent assay with monoclonal antibodies specific to calprotectin (Bühlmann Laboratories AG, Basel, Switzerland). FCP levels above 150 µg/g stool were used to define “high FCP” due to its association with increased risk of UC relapse (21).

Metabolomic Analysis: For the metabolomic analysis, concentrations of urinary metabolites (µmol/L) were normalized by creatinine (mmol/L) and reported as a ratio (µmol/mmol). Concentrations of identified metabolites were normalized using logarithmic transformation and pareto scaling. Metabolites with a p-value less than 0.1 in the univariate analyses) were selected for generating the logistic regression model. Multivariate statistical analysis was performed using partial least squares discriminant analysis (PLS-DA). A 10-fold cross-validation technique was used to ensure that the logistic regression models were robust. Permutation analysis using random resampling (n=2000) of the two groups of patients (i.e. clinical relapse versus remission) was conducted to determine the probability that the observed separation

was a result of chance or not, and a p-value that represents the probability of a random finding was generated. To identify the major metabolites that were responsible for the discrimination between patients with clinical relapse and patients in clinical remission variable importance in projection (VIP) values were used. The VIP value indicates the contribution of each feature to the regression model. MetaboAnalyst 3.0 (22) was used for the metabolomic statistical analysis.

Statistical analysis: Categorical and numerical variables are presented as percentage and median (interquartile range (IQR), respectively. Fisher's exact test and Mann-Whitney U test were used to compare categorical and numerical variables between two groups of UC patients (i.e. clinical relapse versus remission), respectively. To test the relationship between overweight/obesity and disease relapse, we used binary logistic regression analysis after adjusting for age and gender. A receiver operating characteristic (ROC) curve was constructed in order to calculate the accuracy of FCP in predicting UC patients who developed a clinical relapse (partial Mayo score>2 (17)) versus those who remained in clinical remission during the 12-month follow-up. Statistical Package for the Social Sciences, version 16.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. A two-tailed P value of less than 0.05 was considered to be statistically significant.

4.4 Results

Subject Demographics: Twenty UC patients in clinical remission were recruited with a mean age of 42.7±14.8 years; 11 (55%) were females. Two (10%) patients were current smokers. Eleven patients (55%) were diagnosed to have pancolitis and 13 (65%) subjects were on either oral or rectal 5-aminosalicylic acid (5-ASA) medications. Twenty three percent of patients were on no UC-related medication. (Table 4.7.1)

UC relapse and demographic, clinical, and anthropometric parameters: Patients were followed for 12.1 ±1.9 months and during this time 7 (35%) patients experienced a clinical relapse. The comparison between different demographic, anthropometric, and clinical characteristics of patients (at the time of recruitment) with clinical relapse and those who were still in clinical remission at the time of follow-up is presented in Table 4.7.1. There was no significant difference between these two groups of patients in terms of age, gender, and UC-related factors (age at diagnosis, months since last relapse, UC subtype, and UC medication) at baseline. However, UC patients who developed a clinical relapse within 12 months had significantly lower BMI, waist circumference, waist to height ratio, and fat mass compared to patients with no clinical relapse. Six out of 9(66.7%) patients with normal BMI (18.5 – 24.9 kg/m2) had a clinical relapse, whereas 1 out of 11(9.1%) patients with overweight/obesity (BMI>25 Kg/m2) at baseline relapsed during the follow-up (relative risk (RR): 7.3, 95% confidence interval (CI): 1.1-50.3, P=0.02) and this was still statistically significant (P=0.03) after adjusting for age and gender.

Effect of dietary intake: There was no statistically significant difference between intake of different macro-, micronutrients as well as food groups at baseline in patients with clinical relapse versus remission within 12 months of follow-up, except for poultry and maltose intake which were significantly higher in patients who remained in remission (Table 4.7.2). There was a positive correlation between maltose intake and total grain (r=0.50, P=0.03) and whole grain intake (r=0.47, P=0.04) suggesting that the main source of maltose in our patients was grain or grain products.

Fecal calprotectin (FCP): The median (IQR) level of FCP at baseline in UC patients with clinical relapse and remission at 12 months of follow-up was 195.9 (41.2-347.9) and 23.3 (12.9-84.5) µg/g, respectively (P=0.05). Five (71.4%) patients with high FCP versus 2 (15.4%) patients with normal FCP at baseline relapsed during the follow-up (RR: 4.6, 95% CI: 1.2-18.1, P=0.02). ROC curves for FCP as a predictor of clinical relapse in UC is presented in Figure 4.7.1. An FCP concentration of 124 µg/g resulted in a sensitivity of 71.4%, a specificity of 84.6%, a positive predictive value (PPV) of 71.4, and a negative predictive value (NPV) of 84.6% in predicting UC clinical relapse.

Metabolomic analysis: Using the described metabolomic assays, we identified and quantified 216 and 247 metabolites in serum and urine samples, respectively. After conducting univariate analysis, 16 candidate metabolites were candidate for further statistical analysis based on the P-value of < 0.1. As presented in Figure 4.7.2, UC patients who experienced clinical relapse or stayed in clinical remission during follow-up could be discriminated in different clusters from each other by their metabolomic profile at baseline. Using the permutation testing, we showed that this separation was statistically significant (P=0.04). The R^2 and Q^2 of the model was 0.84 and 0.59, respectively. VIP values of six metabolites were above 1.0, showing their important role in the discrimination between metabolomic profiles of the two UC groups. The median (IQR) levels and VIP scores of these metabolites are presented in Table 4.7.3. In comparison to UC patients who were still in remission during follow-up, those study patients with clinical relapse had significantly higher levels of trans-aconitate (urine), 3-hydroxybutyrate (serum), acetoacetate (serum), acetone (serum), and lower levels of acetamide (urine) and cystine (urine).

4.5 Discussion

In this small pilot study, we identified potential predictors of clinical relapse in UC patients. We found that a history of higher dietary poultry and maltose intake, and high BMI, body fat mass, and waist circumference at baseline were associated with UC clinical remission during a 12-month follow-up. Of significant interest, we found that the baseline serum and urinary metabolomic profile of patients who relapsed during follow-up was significantly different from those patients who did not develop a relapse.

The clinical course of UC includes periods of remission and relapse. Although mucosal healing (macroscopic or microscopic) as determined by endoscopic and histologic evaluation is shown to be a strong predictor of long-term remission (11), due to the invasive nature of colonoscopy and its burden on healthcare system there has been considerable interest in identifying non-invasive predictors of disease relapse in UC. However, so far, only a limited number of clinical, lifestyle-related factors or biomarkers have been identified in relation to UC relapse (7-13).

Interestingly, we found that baseline serum and urine metabolomic profiles of UC patients who developed UC relapse versus those who did not, were significantly different from each other. Metabolomics which is the science of studying metabolites in the different biological samples, has recently been used in IBD-related research. However, the focus of most previous studies was to identify “biomarkers” in urine, serum, or stool (15, 16) samples of IBD patients that could discriminate them from non-IBD cohorts. To date, only one study has used a metabolomic approach to find metabolites in relation to risk of clinical relapse in UC patients. In a prospective cohort study, Hisamatsu et al. (23) measured plasma levels of nineteen amino acids and found that decreased histidine level in plasma free amino acids was associated with

increased risk of relapse in UC patients during a one-year follow-up. To the best of our knowledge, our study is the first one that used NMR and DI- LC- MS/MS methods to identify metabolites in urine and serum samples that had the potential to predict UC relapse. Although we could identify and quantify specific serum and urine amino acids using NMR, we did not find any statistically significant difference in serum histidine levels between the two UC groups which might be due to sampling from a different population of UC patients or the result of small sample size.

In the present study, higher levels of trans-aconitate (urine), 3-hydroxybutyrate (serum), acetoacetate (serum), and acetone (serum) were found in the patients who relapsed within 12 months. In a previous study by Stephens et al. (24), it was reported that urinary trans-aconitate, which is a tricarboxylic acid, was decreased in IBD patients in comparison to non-IBD controls. Previously, 3-hydroxybutyrate (ketone body) was shown to be higher in serum samples of UC (25) or IBD (26) patients than in controls. Elevated serum levels of acetoacetate (ketone body) were also reported in IBD patients in comparison to controls (26). The large increase in concentration of ketone bodies (acetoacetate, acetone and 3-hydroxybutyrate) was previously reported in DSS-induced colitis mice which may reflect the higher demand of the body for energy (27) and changes in cellular energy metabolism that occur in IBD patients (26), in our population even before disease relapse. In addition, we noticed a negative correlation between some of these ketone bodies with BMI which highlights the role of energy-related metabolic alterations before UC relapse (data not shown).

In our study, we also found that relatively lower levels of cystine and acetamide in urine were associated with increased risk of relapse. Cystine is an oxidized dimeric form of cysteine (a

semi-essential proteinogenic amino acid). Cystine and cysteine are limiting substrates in the biosynthesis of tripeptide glutathione (GSH), which is known to be the most important intracellular antioxidant. It was shown that low plasma cysteine and cystine levels were associated with decreased mucosal synthesis of GSH, increased oxidative damage, and presence of inflammation in UC and CD patients (28). In the present study, decreased cystine levels were associated with disease relapse possibly through reduction in GSH synthesis and increased oxidative damage. Acetamide is the amide of acetic acid. It has been shown that acetamide has antimicrobial, anti-inflammatory, and antibiotic functions (29, 30). Acetamide has dietary sources. A significant increase in urinary acetamide level was reported in rats that were fed a diet enriched with sweet potato residue as dietary fibre (31). In another study, rats on a wheat bran fibre diet had significantly higher urinary acetamide than the control group (32). Interestingly, in the present study a history of high dietary intake of non-whole grain products, and thus less fibre, was inversely correlated with urinary acetamide levels (data not shown). These findings suggest a role for specific dietary components in the pathophysiology of UC relapse and should be examined in future prospective larger cohort studies and clinical trials.

In the present study, we indicated that FCP at baseline could predict UC clinical relapse. We found that UC patients who had elevated FCP levels at baseline had 4.6 times higher risk of developing clinical relapse during follow-up than patients with low FC. This finding is in agreement with several previous studies (9, 17, 21, 33).

Our small pilot study indicated that overweight/obesity at baseline was protective for development of clinical relapse. In addition, waist circumference, waist to height ratio, and fat

mass was also higher in patients who stayed in remission 12 months after the initiation of the study. The relationship between body weight and IBD is controversial. Although obesity is associated with a pro-inflammatory state (34) and increased intestinal permeability (35), increased BMI was not related to incidence of UC in EPIC study (36). In contrast, in a large prospective cohort of US women higher indicators of adiposity were associated with an increased risk of CD, but not UC (37). In a recent study by Flores et al. (38) it was found that obese UC patients were significantly less likely to receive anti-TNF treatment or experience a hospitalization for their UC. The authors concluded that obesity is a marker of less aggressive or less severe UC (38).

Interestingly, a higher intake of poultry and maltose was found to be related to decreased risk of UC clinical relapse in the present study. However, we did not find any association between intake of other macro/micronutrients and development of relapse. So far, there have been only a few prospective cohort studies to investigate the dietary determinants of relapse in IBD patients. In a prospective cohort study by Jowett et al. (13), 191 UC patients in remission where followed for one year. The authors reported that consumption of meat, red meat and processed meat, protein, alcohol, sulphur and sulphate were related to increased risk of relapse. However, similar to our pilot study, they did not find any association between consumption of dairy products, fibre, carbohydrate, and fat and increased risk of UC relapse. In another recent study of 489 UC patients, Brotherton et al. (39) did not find any association between fibre intake and disease relapse. In another recent study, higher intake of lactose, alpha linolenic fatty acid, and myristic fatty acid were related to increased risk of relapse in UC patients (40). In comparison to red meat, poultry has less saturated fat and heme iron, both

being inducers of oxidative stress and DNA damage (41). In addition, poultry consumption was shown to be inversely related to inflammation (42) and is suggested to be a healthier source of animal protein than red meat (43).

We also found maltose consumption decreases risk of UC relapse. Maltose is a disaccharide derived from two units of glucose and is found largely in vegetables, fruits and grains. There are scarce data on the beneficial effects of maltose intake for human health in comparison to other types of sugars. However, maltose is among the preferred carbohydrate energy sources for specific colonic bacteria that have beneficial properties (44, 45). It should also be mentioned that in the present study we also observed a positive correlation between maltose intake and total grain and whole grain intake (data not shown) which suggests that the main source of maltose in our patients could have been grain or grain products. Although fruit and whole grain intake in our study was numerically higher at baseline in patients with clinical remission than patients who developed a UC clinical relapse, this difference was not statistically significant.

Age, gender, UC subtype, UC medication, age at diagnosis, and months since last clinical relapse were not related to UC clinical relapse over a 12-month period in our study. Similarly, Zenlea et al. (46) did not find any relationship between age, gender, type of medication, and increased risk of UC relapse. Also, median duration of remission before study and disease extent was not related to increased risk of UC relapse in another prospective cohort study by Bessissow et al. (47) which is in agreement with our findings. However, younger age, shorter duration of remission before study, and greater number of prior relapses were associated with earlier time to relapse in (7). In addition, relapse was more frequent in females during a 5-year follow-up in IBSEN study (48). Our finding of no relationship between smoking status and UC relapse was

also shown in previous studies (7, 46-48). However, Höie et al. (49) showed that UC patients who were current smokers had a lower relapse rate than nonsmokers during a 10-year follow-up.

Although in our pilot prospective cohort study we tried to investigate several potential contributors of relapse in UC patients which makes the findings valuable, our study has several limitations as well. The major limitation of the study is the small sample size. Thus, for several parameters between two groups of patients our study did not have the enough statistical power to detect true differences. Due to this limitation, we could not perform more complex statistical analysis (e.g. comparison of relapse between tertiles of dietary intake for each nutrient and adjusting for several confounding variables). In addition, since we did not correct our analyses for multiple testing, some of the findings in this study might have been "false positive" findings which needs to be considered before interpreting our results. However, despite this limitation, the separate clusters with significant difference between urinary and serum metabolomics as a predictor for clinical relapse versus remission remains striking. In addition, using a FFQ to assess dietary intake during the past 12 months is subject to recall bias which is another limitation of this epidemiological study. However, we have tried to overcome this limitation by using a validated tool which has been shown to assess long-term dietary intake among adult population in several settings. Since this project is considered to be a pilot study, we believe that these findings should be regarded as hypothesis-generating findings, deserving further evaluation in future studies.

In conclusion, we identified several metabolites, as well as dietary parameters that are related to the development of clinical relapse in UC patients within 12 months. Due to the importance

of this topic in the management of UC patients, we suggest that further well-designed prospective cohort studies studying these parameters with larger sample size should be performed.

Acknowledgements:

We wish to thank all participants of this study for their excellent cooperation. This study was funded by Alberta Innovates-Bio Solutions. AHK was supported by a graduate studentship from Alberta Innovates-Health Solutions.

4.6 References

1. Ford AC, Moayyedi P, Hanauer SB. Ulcerative colitis. BMJ. 2013;346:f432.

2. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol. 2015;12(12):720-7.

3. Rocchi A, Benchimol EI, Bernstein CN, Bitton A, Feagan B, Panaccione R, Glasgow KW, Fernandes A, Ghosh S. Inflammatory bowel disease: a Canadian burden of illness review. Can J Gastroenterol. 2012;26(11):811-7.

4. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066-78.

5. Casellas F, Arenas JI, Baudet JS, Fábregas S, García N, Gelabert J, Medina C, Ochotorena I, Papo M, Rodrigo L, Malagelada JR. Impairment of health-related quality of life in patients with inflammatory bowel disease: a Spanish multicenter study. Inflamm Bowel Dis. 2005;11(5):488-96.

6. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48(4):526-35.

7. Bitton A, Peppercorn MA, Antonioli DA, Niles JL, Shah S, Bousvaros A, Ransil B, Wild G, Cohen A, Edwardes MD, Stevens AC. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. Gastroenterology. 2001;120(1):13-20.

8. Bitton A, Sewitch MJ, Peppercorn MA, deB Edwardes MD, Shah S, Ransil B, Locke SE. Psychosocial determinants of relapse in ulcerative colitis: a longitudinal study. Am J Gastroenterol. 2003;98(10):2203-8.

9. Theede K, Holck S, Ibsen P, Kallemose T, Nordgaard-Lassen I, Nielsen AM. Fecal Calprotectin Predicts Relapse and Histological Mucosal Healing in Ulcerative Colitis. Inflamm Bowel Dis. 2016;22(5):1042-8.

10. Hosseini SV, Safarpour AR, Taghavi SA. Developing a novel risk-scoring system for predicting relapse in patients with ulcerative colitis: A prospective cohort study. Pak J Med Sci. 2015;31(6):1511-6.

11. Liverani E, Scaioli E, Digby RJ, Bellanova M, Belluzzi A. How to predict clinical relapse in inflammatory bowel disease patients. World J Gastroenterol. 2016;22(3):1017-33.

12. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut. 2006;55(3):426-31.

13. Jowett SL, Seal CJ, Pearce MS, Phillips E, Gregory W, Barton JR, Welfare MR. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. Gut. 2004;53(10):1479-84.

14. Idle JR, Gonzalez FJ. Metabolomics. Cell Metab. 2007;6(5):348-51.

15. De Preter V, Verbeke K. Metabolomics as a diagnostic tool in gastroenterology. World J Gastrointest Pharmacol Ther. 2013;4(4):97-107.

16. Bjerrum JT, Wang Y, Hao F, Coskun M, Ludwig C, Günther U, Nielsen OH. Metabonomics of human fecal extracts characterize ulcerative colitis, Crohn's disease and healthy individuals. Metabolomics. 2015;11:122-133.

17. De Vos M, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, D'haens GR, Franchimont D, Baert FJ, Torp RA, Henriksen M, Potvin PM, Van Hootegem PP, Hindryckx PM, Moreels TG, Collard A, Karlsen LN, Kittang E, Lambrecht G, Grimstad T, Koch J, Lygren I, Coche JC, Mana F, Van Gossum A, Belaiche J, Cool MR, Fontaine F, Maisin JM, Muls V, Neuville B, Staessen DA, Van Assche GA, de Lange T, Solberg IC, Vander Cruyssen BJ, Vermeire SA. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis. 2013;19(10):2111-7.

18. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, McIntosh A, Rosenfeld S. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires : the Eating at America's Table Study. Am J Epidemiol. 2001;154(12):1089-99.

19. National Institutes of Health, Epidemiology and Genomics Research Program, National Cancer Institute. Diet History Questionnaire II, Version 2.0. 2010. http://www.epi.grants.cancer.gov/dhq2 (accessed April 2017)

20. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J, Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS. The human urine metabolome. PLoS One. 2013;8(9):e73076.

21. Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut. 2005;54(3):364-8.

22. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res. 2015;43(W1):W251-7.

23. Hisamatsu T, Ono N, Imaizumi A, Mori M, Suzuki H, Uo M, Hashimoto M, Naganuma M, Matsuoka K, Mizuno S, Kitazume MT. Decreased plasma histidine level predicts risk of relapse in patients with ulcerative colitis in remission. PloS one. 2015;10(10):e0140716.

24. Stephens NS, Siffledeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. J Crohns Colitis. 2013;7(2):e42-8.

25. Zhang Y, Lin L, Xu Y, Lin Y, Jin Y, Zheng C. 1H NMR-based spectroscopy detects metabolic alterations in serum of patients with early-stage ulcerative colitis. Biochem Biophys Res Commun. 2013;433(4):547-51.

26. Dawiskiba T, Deja S, Mulak A, Zabek A, Jawien E, Pawelka D, Banasik M, Mastalerz-Migas A, Balcerzak W, Kaliszewski K, Skóra J. Serum and urine metabolomic fingerprinting in diagnostics of inflammatory bowel diseases. World J Gastroenterol. 2014;20(1):163-74.

27. Schicho R, Nazyrova A, Shaykhutdinov R, Duggan G, Vogel HJ, Storr M. Quantitative metabolomic profiling of serum and urine in DSS-induced ulcerative colitis of mice by (1)H NMR spectroscopy. J Proteome Res. 2010;9(12):6265-73.

28. Sido B, Hack V, Hochlehnert A, Lipps H, Herfarth C, Dröge W. Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. Gut. 1998;42(4):485-92.

29. Jawed H, Shah SU, Jamall S, Simjee SU. N-(2-hydroxy phenyl) acetamide inhibits inflammation-related cytokines and ROS in adjuvant-induced arthritic (AIA) rats. Int Immunopharmacol. 2010;10(8):900-5.

30. Muri EM, Williamson JS. Anti-Helicobacter pylori agents. An update. Mini Rev Med Chem. 2004;4(2):201-6.

31. Liu G, Yang G, Fang T, Cai Y, Wu C, Wang J, Huang Z, Chen X. NMR-based metabolomic studies reveal changes in biochemical profile of urine and plasma from rats fed with sweet potato fiber or sweet potato residue. RSC advances. 2014;4(45):23749-58.

32. Liu G, Xiao L, Fang T, Cai Y, Jia G, Zhao H, Wang J, Chen X, Wu C. Pea fiber and wheat bran fiber show distinct metabolic profiles in rats as investigated by a 1H NMR-based metabolomic approach. PLoS One. 2014;9(12):e115561.

33. Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol. 2015;50(1):74-80.

34. Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Gallimore JR, Pepys MB, Campbell LV. Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation. 2004;109(24):3022-8.

35. Gummesson A, Carlsson LM, Storlien LH, Bäckhed F, Lundin P, Löfgren L, Stenlöf K, Lam YY, Fagerberg B, Carlsson B. Intestinal permeability is associated with visceral adiposity in healthy women. Obesity (Silver Spring). 2011;19(11):2280-2.

36. Chan SS, Luben R, Olsen A, Tjonneland A, Kaaks R, Teucher B, Lindgren S, Grip O, Key T, Crowe FL, Bergmann MM. Body mass index and the risk for Crohn's disease and ulcerative colitis: data from a European Prospective Cohort Study (The IBD in EPIC Study). Am J Gastroenterol. 2013;108(4):575-82.

37. Khalili H, Ananthakrishnan AN, Konijeti GG, Higuchi LM, Fuchs CS, Richter JM, Chan AT. Measures of obesity and risk of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis. 2015;21(2):361-8.

38. Flores A, Burstein E, Cipher DJ, Feagins LA. Obesity in inflammatory bowel disease: a marker of less severe disease. Dig Dis Sci. 2015;60(8):2436-45.

39. Brotherton CS, Martin CA, Long MD, Kappelman MD, Sandler RS. Avoidance of fiber is associated with greater risk of Crohn's Disease flare in a 6-month period. Clin Gastroenterol Hepatol. 2016;14(8):1130-6.

40. Barnes EL, Nestor MA, Onyewadume L, De Silva PS, Korzenik JR. A prospective study: the role of diet in exacerbations of patients with ulcerative colitis in remission on monotherapy with mesalamine. Gastroenterology. 2016;150(4):S5-6.

41. Tappel A. Heme of consumed red meat can act as a catalyst of oxidative damage and could initiate colon, breast and prostate cancers, heart disease and other diseases. Med Hypotheses. 2007;68(3):562-4.

42. van Bussel BC, Henry RM, Ferreira I, van Greevenbroek MM, van der Kallen CJ, Twisk JW, Feskens EJ, Schalkwijk CG, Stehouwer CD. A healthy diet is associated with less endothelial

dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular disease. J Nutr. 2015;145(3):532-40.

43. Ley SH, Sun Q, Willett WC, Eliassen AH, Wu K, Pan A, Grodstein F, Hu FB. Associations between red meat intake and biomarkers of inflammation and glucose metabolism in women. Am J Clin Nutr. 2014;99(2):352-60.

44. Scott KP, Martin JC, Duncan SH, Flint HJ. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. FEMS Microbiol Ecol. 2014;87(1):30-40.

45. Charalampopoulos D, Pandiella SS, Webb C. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. Int J Food Microbiol. 2003;82(2):133-41.

46. Zenlea T, Yee EU, Rosenberg L, Boyle M, Nanda KS, Wolf JL, Falchuk KR, Cheifetz AS, Goldsmith JD, Moss AC. Histology grade is independently associated with relapse risk in patients with ulcerative colitis in clinical remission: a prospective study. Am J Gastroenterol. 2016;111(5):685-90.

47. Bessissow T, Lemmens B, Ferrante M, Bisschops R, Van Steen K, Geboes K, Van Assche G, Vermeire S, Rutgeerts P, De Hertogh G. Prognostic value of serologic and histologic markers on clinical relapse in ulcerative colitis patients with mucosal healing. Am J Gastroenterol. 2012;107(11):1684-92.

48. Henriksen M, Jahnsen J, Lygren I, Sauar J, Kjellevold Ø, Schulz T, Vatn MH, Moum B; IBSEN Study Group. Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). Inflamm Bowel Dis. 2006;12(7):543-50.

49. Höie O, Wolters F, Riis L, Aamodt G, Solberg C, Bernklev T, Odes S, Mouzas IA, Beltrami M, Langholz E, Stockbrügger R. Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. Am J Gastroenterol. 2007;102(8):1692-701.

4.7 Tables and Figures

Table 4.7.1. Comparison of demographic, anthropometric, body composition, and clinical characteristics of ulcerative colitis patients at baseline according to their relapse status after 12 months.

		Remission (n=13)	Relapse (n=7)	P
Age, years		46.0 (32.5-56.5)	33.0 (28.0-52.0)	0.18
Females, n (%)		7 (53.8)	4 (57.1)	1.00
Current smoker, n (%)		2 (15.4)	0 (0.0)	0.52
Body mass index, Kg/m^2		28.1 (25.3-32.7)	22.0 (20.3-22.8)	<0.01
Overweight/obese, n (%)		10 (76.9)	1 (14.3)	0.02
Waist circumference, cm		99.1 (84.4-105.3)	82.8 (70.1-89.0)	0.03
Waist to height ratio		0.6 (0.5-0.6)	0.5 (0.4-0.5)	0.02
Body fat percentage		35.8 (27.0-47.6)	29 (20.4-34.1)	0.16
Fat mass, Kg		34.1 (20.4-45.5)	20.1 (16.3-24.6)	0.04
Age at diagnosis, years		26.0 (22.5-44.5)	25.0 (17-29)	0.39
Months since last relapse		12.0 (4.5-33)	11.0 (6.0-40)	0.76
UC subtype, n (%)	Proctitis	1 (7.7)	4 (14.3)	
	Left-sided	4 (30.8)	3 (42.9)	0.71
	Pancolitis	8 (61.5)	3 (42.9)	
Medication, n (%)	5-ASA	9 (69.2)	4 (57.1)	0.65
	Immunosuppressants	4 (30.8)	2 (28.6)	1.00
	No medication	3 (23.1)	1 (14.3)	1.00

UC: ulcerative colitis

Numerical variables are presented as median (interquartile range)

Table 4.7.2. Dietary intake of nutrients and food groups at baseline in ulcerative colitis patients with and without clinical relapse [1, 2].

		Patient groups		P [3]
		Remission (n=13)	Relapse (n=7)	
Energy (Kcal/d)		1530.8 (1258.3-2121.4)	1820.2 (1430.8-2337.1)	0.70
Nutrients				
	Carbohydrate (g/d)	229.6 (217.0-253.4)	201.5 (156.4-237.1)	0.21
	Protein (g/d)	61.6 (55.0-74.9)	62.0 (40.6-70.0)	0.70
	Fat (g/d)	59.0 (50.7-61.6)	62.3 (46.2-76.0)	0.21
	Cholesterol (g/d)	198.0 (155.9-275.4)	166.8 (117.6-207.4)	0.18
	SFA (g/d)	18.5 (16.4-20.3)	20.4 (11.9-23.8)	0.52
	MUFA (g/d)	21.3 (17.3-23.1)	21.7 (15.0-30.4)	0.64
	PUFA (g/d)	14.4 (12.9-14.8)	12.9 (9.1-15.5)	0.70
	TFA (g/d)	3.3 (2.8-3.5)	3.5 (1.7-4.5)	0.97
	Vitamin C (mg/d)	112.3 (101.2-166.7)	100.1 (93.6-124.3)	
	Vitamin B6 (mg/d)	1.8 (1.5-2.5)	1.7 (1.3-1.9)	0.52
	Total folate (mcg/d)	401.3 (289.5-540.9)	402.3 (222.8-474.9)	0.77
	Vitamin B12 (mcg/d)	3.8 (3.3-5.7)	4.6 (2.3-5.7)	0.90
	Vitamin E (IU)	14.8 (8.7-20.8)	10.2 (4.4-14.4)	0.13
	Calcium (mg/d)	832.0 (730.8-1234.0)	719.9 (551.7-924.0)	0.21
	Iron (mg/d)	13.4 (10.4-25.6)	11.7 (7.4-15.0)	0.42

	Zinc (mg/d)	9.7 (9.4-16.2)	9.8 (6.9-10.2)	0.64
	Sodium (mg/d)	2558.6 (2175.3-2900.6)	2521.9 (1818.8-2810.8)	0.77
	Potassium (mg/d)	2948.8 (2216.0-3624.5)	2951.9 (1890.8-3330.1)	0.83
	Fibre (g/d)	16.1 (13.5-26.0)	14.1 (9.7-22.2)	0.32
	Sucrose (g/d)	40.2 (29.9-47.3)	27.6 (20.6-40.4)	0.11
	Fructose (g/d)	31.1 (18.4-42.3)	25.5 (11.7-38.5)	0.64
	Lactose (g/d)	13.6 (9.4-19.0)	9.8 (1.8-17.5)	0.32
	Maltose (g/d)	3.9 (3.1-4.3)	1.6 (1.1-3.0)	<0.01
Food groups				
	Fruit (cup equivalents/d)	2.1 (0.8-2.6)	1.4 (0.7-2.0)	0.28
	Vegetable (cup equivalents/d)	1.8 (1.5-2.4)	1.8 (1.0-3.1)	0.77
	Whole grain (oz equivalents/d)	1.0 (0.5-1.3)	0.7 (0.2-1.1)	0.32
	Meat (oz/d)	1.0 (0.6-1.3)	1.0 (0.3-1.8)	1.00
	Processed meat (oz/d)	0.3 (0.1-0.5)	0.2 (0.0-1.0)	0.83
	Fish (oz/d)	0.3 (0.2-0.8)	0.5 (0.2-2.2)	0.64
	Poultry (oz/d)	0.9 (0.1-1.3)	0.2 (0.1-0.3)	<0.01

	Dairy (cup equivalents/d)	1.4 (1.0-1.8)	1.2 (0.3-2.0)	0.52
	Eggs (oz equivalents/d)	0.4 (0.2-0.8)	0.2 (0.2-0.4)	0.42
	Alcohol (drink/d)	0.6 (0.0-0.8)	0.6 (0.0-3.5)	0.90

[1] Values are presented as median (interquartile range).

[2] Intake of different nutrients and foods were adjusted for total energy intake using the residual method.

[3] Obtained from Mann–Whitney U test

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, polyunsaturated fatty acids, TFA: trans fatty acids

Table 4.7.3. Comparison of major serum and urinary metabolites responsible for the separation between two groups of ulcerative colitis patients [1, 2].

	UC groups		VIP score	Fold change (remission/relapse)	P [3]
	Remission	Relapse			
Trans-aconitate (urine)	0.5 (0.5-1.6)	3.6 (1.9-5.5)	1.9	0.2	0.02
3-hydroxybutyrate (serum)	21.0 (15.0-33.9)	127.8 (37.5-232.0)	1.4	0.2	<0.01
Cystine (urine)	5.3 (4.0-8.6)	3.3 (1.4-5.9)	1.4	2.7	0.07
Acetamide (urine)	4.9 (2.7-7.1)	2.6 (0.8-3.8)	1.3	2.4	0.05
Acetoacetate (serum)	7.8 (5.8-13.0)	25.6 (10.5-60.2)	1.2	0.3	0.02
Acetone (serum)	5.3 (4.2-8.7)	11.9 (6.0-24.7)	1.1	0.4	0.06

[1] concentrations are presented as median (interquartile range).

[2] unit of concentration for the metabolites in serum and urine is µmol and µmol/mmol, respectively.

[3] obtained from Mann–Whitney U test

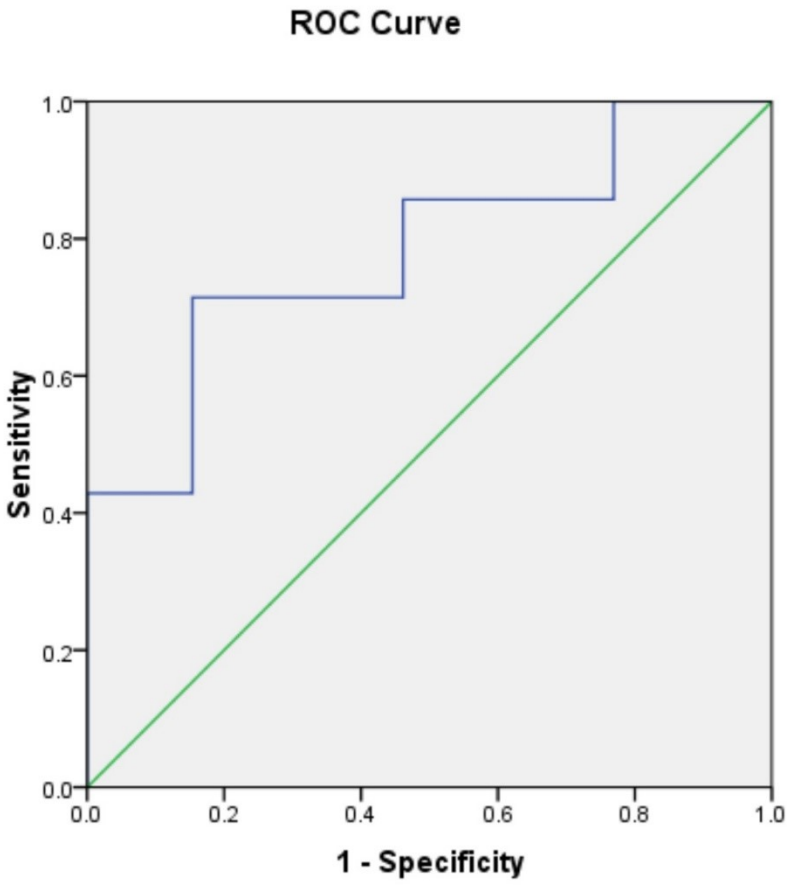


Figure 4.7.1. Receiver operating characteristic (ROC) curve for fecal calprotectin concentration in predicting ulcerative colitis relapse. The area under the curve was 0.78 (95% confidence interval: 0.55-1.0).

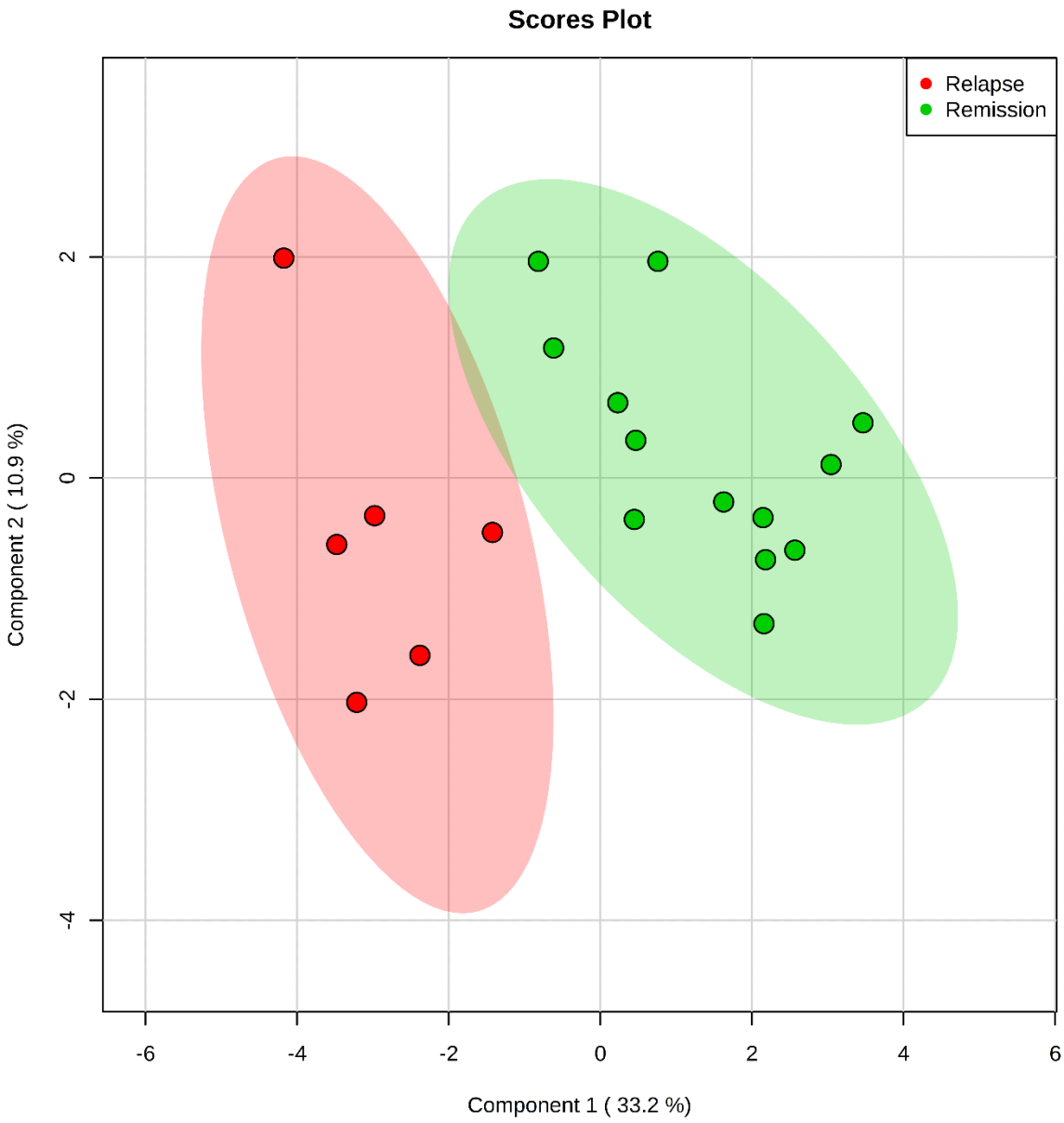


Figure 4.7.2. Partial least squares discriminant analysis plot showing a clear separation of the metabolomic fingerprints of ulcerative colitis patients in clinical remission who developed a Relapse or stayed in Remission within 12 months of follow-up (R^2=0.84, Q^2=0.59, P=0.04).

Chapter 5. A review of randomized controlled dietary interventions in ulcerative colitis patients

5.1 ABSTRACT

Epidemiological and experimental studies have suggested that diet is one of the environmental factors that contributes to the onset and pathophysiology of ulcerative colitis. Although many patients suffering from ulcerative colitis attribute their symptoms or disease relapse to dietary factors, only a few well-designed randomized controlled trials have been done to investigate the role of diet in the management of ulcerative colitis. Here, we summarize randomized controlled dietary interventions that have been conducted in ulcerative colitis patients.

5.2 Introduction

Ulcerative colitis (UC) -a subtype of inflammatory bowel disease (IBD)- is a chronic, idiopathic inflammatory disease that affects the colon and is characterised by relapsing and remitting mucosal inflammation (1). UC patients mostly present with blood in the stool and diarrhea (1). UC is associated with major morbidity in Western countries and its incidence is increasing in developing countries (2). The multifactorial pathophysiology of UC includes genetic predisposition, epithelial barrier defects, dysregulated immune responses, microbial dysbiosis and environmental factors (1, 2).

It has been suggested that environmental factors play a major role in the pathogenesis of IBD. Early-life events such as mode of birth, breastfeeding and exposure to antibiotics, and other factors such as air pollution, smoking, psychological state, exercise, and diet are among the potential environmental contributors of IBD development or disease activity (3).

Significant changes in dietary intake during the past decades have been associated with the increase in incidence of UC. The relationship between diet and UC development has been indicated in several epidemiological studies (4). Although the exact pathophysiological mechanisms in which diet plays a role in IBD development remain unknown, several plausible explanations including its effects on composition of gut microbiota, production of microbial metabolites, alterations in mucosal immunity and mucosal barrier function have been proposed (5).

Currently, dietary recommendations for management of IBD-related symptoms are scarce and non evidence-based, mainly due to the limited number of dietary interventions in this

population. In the present review article, we summarize findings from previously conducted dietary interventions in UC patients.

5.3 Methods

An electronic search in MEDLINE (Ovid) from inception to April 1, 2019 was conducted in order to identify any dietary intervention studies on UC subjects). Reference list of included studies were also checked to identify relevant studies that might have been missed during initial search in MEDLINE. Studies that only focused on nutritional supplements, enteral or parenteral nutrition or were published in languages other than English were not included. Comprehensive full-text review of identified studies was conducted after the title screening and ABSTRACT screening of potentially relevant articles. Collected data included journal name, publication year, design of the study, age, sex, sample size, disease condition, intervention and comparator(s) of interest, outcome(s), outcome measures, and main findings. The MEDLINE search strategy was as follows:

1. randomized controlled trial/

2. clinical trial.pt.

3. randomi?ed.ti,ab.

4. placebo.ti,ab.

5. randomly.ti,ab.

6. trial.ti,ab.

7. 1 or 2 or 3 or 4 or 5 or 6

8. Inflammatory Bowel Diseases/

9. inflammatory bowel disease.tw.

10. ibd.tw.

11. ulcerative colitis.tw.

12. colitis.tw.

13. 8 or 9 or 10 or 11 or 12

14. Diet/

15. diet*.tw.

16. food.tw.

17. 14 or 15 or 16

18. 7 and 13 and 17

5.4 Results

Our primary electronic search yielded 424 unique references. After title and ABSTRACT screening 9 studies were selected for full-text review. Following the full-text review, 7 randomized controlled trials that met the inclusion and exclusion criteria were selected for this review (6-12). The general characteristics of the included studies are summarized in Table 5.7.1.

Wright et al. (6) randomly allocated UC patients with disease relapse into a milk-free diet (n=26) (All milk and milk products, whether in the form of dairy products such as fresh milk and cheese

or as powdered milk, were excluded. Butter was permitted.) a gluten-free plus milk-free diet (n=27), or a control group (n=24). Patients were asked to follow the diets for one year after the induction of remission and they were followed monthly to assess if they experienced disease relapse which was defined as diarrhea with an average of four or more stools a day for at least a week and with macroscopic blood present, together with sigmoidoscopic evidence of inflammation. Although the relapse rate was higher in patients randomized to the control group in comparison to those on a milk-free diet (79.2 %vs. 61.5%) it did not reach statistical significance (P=0.2). In addition, the relapse rate in the gluten-free plus milk-free diet was 70.4%, which was comparable to that in the other two groups.

In a small randomized controlled trial for 6 weeks (7), 18 adult UC patients with mild to moderate disease activity were randomized to a symptoms-guided elimination diet (n=11) or a control group (n=7). Patients in the control group were asked to document but not alter their dietary intake. However, patients in the experimental group were instructed to exclude foods that appeared to provoke their symptoms. Fried foods were prohibited. In addition, refined sugars, additives and preservatives, all condiments and spices other than salt, and beverages other than boiled water were prohibited during the 6-week trial for patients randomized to the elimination diet group. In the first week, dairy products were excluded from the diet, but were introduced over the next weeks in the following order: skim milk, yogurt, skim-milk cheese, full-cream milk, cream, and full-cream cheese. Each week, subjects in the intervention group were interviewed in person and their symptoms were reviewed in relation to the foods eaten during the previous week. The food menu was expanded over the 6-week trial to include as more variety of foods that each participant could tolerate. The induction of clinical remission rate

(the passage of normal stools with absence of rectal bleeding) 6 weeks after the baseline visit was significantly higher in patients who received the symptoms-guided diet (36.3% vs. 0.0%). However, the endoscopic and histologic improvement was comparable between the two groups.

In another study (8), children with newly diagnosed UC were randomly assigned to a cow's milk protein (CMP) elimination diet (n=14) or a normal diet as the control group ((n=15). The study aimed to compare the clinical remission rate between the two groups following the IBD induction therapy and the rate of clinical relapse (defined as the occurrence or worsening of symptoms accompanied by an increase of Pediatric Ulcerative Colitis Activity Index >10 points which required treatment with corticosteroids, immunosuppressive agents, or surgery) between the two groups during the one-year trial. The authors reported that the clinical response rate 4 weeks after the initiation of the induction therapy was not different between the two groups (92.8% in CMP elimination diet vs. 80.0% in the control group, P=0.6). In addition, clinical relapse rate was comparable between the two groups (53.8% in CMP elimination diet group vs. 53.3% in the control group). In addition, they found no significant changes in serum C-reactive protein (CRP), erythrocyte sedimentation rate or fecal calprotectin (FCP) in the two diet groups from baseline to the last visit.

Kyaw et al. (9) recruited 112 adult UC patients and randomly assigned them to a dietary intervention and a control group. Patients in the intervention group were given an educational booklet that contained dietary recommendations to eat little and often (four to six times a day), drink adequate fluids, decrease excess intake of fat, decrease simple carbohydrates and decrease high-fibre foods during flare. Patients were also advised to increase intake of "good-

quality protein" during flare and eliminate dairy products if they were lactose intolerant. Patients randomized to the control group were provided a booklet which included general recommendations on healthy eating (e.g. to choose higher fibre or whole grain carbohydrates, to eat lots of fruits and vegetables) and were assigned to follow their usual diet. At 24 weeks, there was a significant reduction in the Simple Clinical Colitis Activity Index (SCCAI) score in the intervention group compared with an increase in the score in the control group. However, there was no statistically significant change in quality of life scores from baseline to week 24 in the two groups.

Bhattacharyya et al. (10) conducted a small randomized, double-blind, placebo-controlled, multicenter, clinical trial on UC patients to investigate the effect of the common food additive carrageenan on clinical relapse rates. The authors recruited UC patients over the age of 18 in clinical remission (SCCAI ≤2). Patients randomized to the carrageenan group (n=5) received the carrageenan-containing capsules (200 mg/day). Patients randomized to the placebo group received similar-appearing dextrose-containing capsules (n=7). The study duration was 12 months and participants were instructed to follow a carrageenan-free diet during that period. The primary outcome measure was occurrence of clinical relapse, which was defined as an increase of two (or more) points on the SCCAI in association with an increase in treatment. The Short Inflammatory Bowel Disease Questionnaire was used to assess changes in quality of life. In addition, blood and stool samples were collected to measure inflammatory markers. They found that UC patients who were on a carrageenan-free diet plus placebo had a lower relapse rate in comparison to patients who were on a similar diet plus two oral capsules of carrageenan per day (0.0% vs. 60.0%, P=0.05).In addition, they reported that carrageenan consumption

aggravated disease activity as indicated by increase in FCP (P=0.06) and interleukin-6 (P=0.02). However, there was no statistically significant difference between the two groups in terms of changes in quality of life scores.

Pedersen et al. (11) conducted an open-label trial of patients with IBD (61 UC and 28 CD) in remission or with mild-to-moderate disease and coexisting IBS-like symptoms. Patients were randomly assigned to a low Fermentable, Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAP) diet (n=44) or a normal diet (n=45) for 6 weeks. In UC patients, there was a significant decrease in severity of IBS-related symptoms (assessed by IBS symptom Severity System) in both diet groups and this response was not different between the two groups. However, the authors reported a significant decrease in disease activity assessed by SCCAI but only in patients randomized to the low-FODMAP diet. In addition, low-FODMAP diet increased quality of life of IBD patients (assessed by SIBDQ). However, low-FODMAP diet did not change CRP and FCP levels significantly.

In a recent open-label, stratified study, Jian et (12) randomly allocated 97 UC patients who were in remission or had mild to moderate disease activity to a food exclusion group versus a sham diet group. At baseline, the presence of blood IgG antibodies specific to egg, wheat, milk, corn, tomato, crab, rice, soybean, cod, shrimp, mushrooms, beef, chicken, and pork antigens were tested. Based of IgG antibody titers, patients randomized to the exclusion diet group were instructed to stop or reduce taking specific food items. Patients in the control group were asked to follow their routine diet. The duration of the trial was 6 months. They reported that in comparison to the control diet, following the exclusion diet was associated with a significant decrease in Mayo scores and improvement in quality of life.

5.5 Discussion

It has been suggested that environmental factors including diet play an important role in the pathophysiology of IBD and especially in UC, a chronic colonic inflammation. In the present article, after a brief overview of potential mechanisms in which diet plays a role in the pathogenesis of IBD, we then reviewed dietary intervention studies in UC patients.

The three randomized controlled trials that have been performed to assess the efficacy of dietary interventions for maintenance of remission in UC (6, 8, 10) were all focused on complete exclusion of one or more food items that were hypothesized to trigger IBD symptoms. Two of these studies (6, 8) aimed to eliminate milk or dairy products, however they failed to show a significant decrease in relapse rate in patients randomized to the elimination diet group in comparison to those randomized to the control diet. This finding is important as unnecessary dietary restrictions that lack supporting scientific evidence may result in several nutritional deficiencies (e.g. calcium due to exclusion of milk and dairy products) in IBD patients (13). Therefore, patients should be informed by their health care team about the possible harmful effects of food elimination diets.

In the present review, the only elimination diet that was associated with a reduction in clinical relapse rate in UC patients who were in remission at baseline was a carrageenan-free diet (10). Carrageenan belongs to a family of sulfated polysaccharides and are extracted from seaweeds. It is approved as Generally Recognized as Safe by the United States Food and Drug Administration and is used in the food industry for its gelling, thickening, and stabilizing properties. It has been suggested that carrageenan may reduce protein and peptide bioaccessibility, disrupt normal epithelial function, and promote intestinal inflammation (14).

However, others have been skeptical about these findings, which are mainly derived from experimental animal studies (15). The results from the randomized clinical trial in which a carrageenan-free diet was found to be related to lower relapse rate and decreased inflammation (as assessed by decreased serum interleukin-6 and FCP) should be interpreted with caution as the sample size of this multi-center trial was very small (n=12) and the reported P-values obtained from parametric tests were marginally significant. Therefore, these interesting findings need to be confirmed in future well-powered randomized controlled studies.

We identified only 2 studies (7, 8) that tested the efficacy of diet for induction of remission in UC patients. In the first study, exclusion of food items that were found to trigger UC-related symptoms was associated with higher clinical remission rate in comparison to a normal diet (7). Although the elimination of foods was based on each participant's self-reported food intolerance, there were some general recommendations regarding specific food groups/items such as dairy products, refined sugar and beverages. However, the study was performed on a small number of patients (n=18) and the duration of follow-up was short (6 weeks). In addition, the intervention did not result in endoscopic or histologic improvement in that time period. Furthermore, patients in the intervention group experienced a mean weight loss of 2.5 kg that was not explained in the study. The authors also reported that there was no food that triggered symptoms in all patients. However, spicy and curried foods and fruits (specially grapes, melon and citruses) were commonly reported to provoke symptoms. In the second study, which was performed in pediatric UC patients with active disease, elimination of cow milk protein from diet was not beneficial neither for induction or for maintenance of remission during a one-year

follow-up in comparison to a control diet (8). As mentioned by the authors, the dietary restrictions that many IBD patients follow often are not supported by scientific evidence. These inappropriate diets reduce caloric intake and may contribute to malnutrition and micronutrient deficiencies, especially in pediatric patients. Whether a subgroup of patients with UC (e.g. patients with lactose intolerance or atopy) will benefit from elimination diets or not needs to be explored in future clinical trials.

In this review, we also included 3 other studies that recruited patients with active disease and UC remission concurrently. They reported the effectiveness of comprehensive dietary advices (9), low FODMAP (11) or IgG-guided exclusion diets (12) in reduction of disease activity in UC patients. Although these findings are encouraging, one of the major limitations of these studies is that they did not report their findings for patients with active disease versus patients in UC remission separately to allow meaningful interpretations (16). Therefore, we suggest that in future studies the dietary interventions be focused on clearly specified groups of patients (e.g. active disease or in remission) or study outcomes to be reported for different groups of participants separately.

Diet is of major interest for IBD patients and they use a variety of dietary strategies to manage their underlying disease and related symptoms (17). Despite the significant role of diet in the development of IBD or management of gastrointestinal symptoms in these patients, we could identify only a few randomized controlled trials that assessed the efficacy of diet for induction of remission, maintenance of remission or improvement of gastrointestinal symptoms in UC patients. In addition, in the previous studies the underlying mechanisms in which diet may prevent increases in colonic or systemic inflammation and ultimately help patients to maintain

remission have not been investigated. There are many -omics field involved to study pathogenesis of IBD such as genomics, metagenomics, transcriptomics, proteomics, and metabolomics (18). As dietary factors have a significant impact on some of these key players of IBD development, investigating the changes in this multi-omic network of IBD during a controlled dietary intervention has the potential to elucidate the underlying mechanisms of diet-IBD interactions. High quality, well-powered human dietary intervention studies for management of IBD may include the following: quantification of baseline habitual diet using appropriate tools such as food frequency questionnaires, monitoring of adherence to the diet using food recalls/records, large long-term controlled trials, use of a control diet to determine the specificity of observed effects to the intervention, use of a variety of subjective and objective endpoints (e.g. symptoms, quality of life, clinical biomarkers, endoscopic and histological evaluations) to monitor response to dietary interventions (17), and consider the use of omic-based assessments of serum, urine, stool and/or intestinal biopsies to investigate underlying protective mechanisms. Considering findings from previous observational studies and clinical trials, investigating the potential benefits of following a healthy dietary pattern such as experimental anti-inflammatory diets that incorporate several dietary recommendations, is of great value in the management of UC-related symptoms and inflammation. Furthermore, as indicated in elimination diet studies, food intolerances are individual-based and not all patients will benefit from excluding certain food items/groups. Therefore, personalized dietary recommendations that take into account each patient's food intolerances and food preferences should be the subject of future well-designed dietary trials in IBD patients.

In conclusion, we found that there have been few well-designed and/or adequately powered randomized clinical trials to investigate the role of diet in maintenance of remission in UC patients. As suggested in a recent Cochrane systematic review (16), consensus on the composition of evidence-based dietary interventions in IBD patients is required and there is a need for more high-quality, well-powered, randomized controlled trials to assess the efficacy of these interventions.

5.6 References

1. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-1770.

2. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019;94(1):155-165.

3. Ananthakrishnan AN. Debate session: So what causes inflammatory bowel disease? It's all in the environment. J Gastroenterol Hepatol. 2018;33 Suppl 3:24.

4. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol. 2011;106(4):563–573.

5. Khalili H, Chan SSM, Lochhead P, Ananthakrishnan AN, Hart AR, Chan AT. The role of diet in the aetiopathogenesis of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2018;15(9):525-535.

6. Wright R, Truelove SC. A controlled therapeutic trial of various diets in ulcerative colitis. Br Med J. 1965;2(5454):138-41.

7. Candy S, Borok G, Wright JP, Boniface V, Goodman R. The value of an elimination diet in the management of patients with ulcerative colitis. S Afr Med J. 1995;85(11):1176-9.

8. Strisciuglio C, Giannetti E, Martinelli M, Sciorio E, Staiano A, Miele E. Does cow's milk protein elimination diet have a role on induction and maintenance of remission in children with ulcerative colitis? Acta Paediatr. 2013;102(6):e273-8.

9. Kyaw MH, Moshkovska T, Mayberry J. A prospective, randomized, controlled, exploratory study of comprehensive dietary advice in ulcerative colitis: impact on disease activity and quality of life. Eur J Gastroenterol Hepatol. 2014;26(8):910-7.

10. Bhattacharyya S, Shumard T, Xie H, Dodda A, Varady KA, Feferman L, Halline AG, Goldstein JL, Hanauer SB, Tobacman JK. A randomized trial of the effects of the no-carrageenan diet on ulcerative colitis disease activity. Nutr Healthy Aging. 2017;4(2):181-192.

11. Pedersen N, Ankersen DV, Felding M, Wachmann H, Végh Z, Molzen L, Burisch J, Andersen JR, Munkholm P. Low-FODMAP diet reduces irritable bowel symptoms in patients with inflammatory bowel disease. World J Gastroenterol. 2017;23(18):3356-3366.

12. Jian L, Anqi H, Gang L, Litian W, Yanyan X, Mengdi W, Tong L. Food Exclusion Based on IgG Antibodies Alleviates Symptoms in Ulcerative Colitis: A Prospective Study. Inflamm Bowel Dis. 2018. doi: 10.1093/ibd/izy110.

13. Lim HS, Kim SK, Hong SJ. Food Elimination Diet and Nutritional Deficiency in Patients with Inflammatory Bowel Disease. Clin Nutr Res. 2018;7(1):48-55.

14. Fahoum L, Moscovici A, David S, Shaoul R, Rozen G, Meyron-Holtz EG, Lesmes U. Digestive fate of dietary carrageenan: Evidence of interference with digestive proteolysis and disruption of gut epithelial function. Mol Nutr Food Res. 2017;61(3).

15. Weiner ML, McKim JM. Comment on "Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods?". Food Funct. 2019;10(3):1760-1762.

16. Limketkai BN, Iheozor-Ejiofor Z, Gjuladin-Hellon T, Parian A, Matarese LE, Bracewell K, MacDonald JK, Gordon M, Mullin GE. Dietary interventions for induction and maintenance of remission in inflammatory bowel disease. Cochrane Database Syst Rev. 2019;2:CD012.

17. Haskey N, Gibson DL. An Examination of Diet for the Maintenance of Remission in Inflammatory Bowel Disease. Nutrients. 2017;9(3).

18. Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR, Fiocchi C. Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol. 2018;15(1):39-49.

5.7 Tables

Table 5.7.1. General characteristics of studies examining the role of diet for maintenance of remission in ulcerative colitis patients

First Author (Year)	Country	Study Design	Population	Intervention/Comparator(s) (Sample Size [1])	Duration	Outcomes and Assessment Tools
Wright (1965) [35]	UK	Randomized controlled clinical trial	Adult UC patients in clinical remission after induction of remission	Milk-free diet (n = 26)/gluten-free plus milk-free diet (n = 27)/"dummy diet" as control (n = 24)	12 months	Relapse: Symptoms + sigmoidoscopy, biopsy, dietary adherence: interview
Candy (1995) [36]	South Africa	Randomized, controlled clinical trial	Adult UC patients with mild to moderate disease activity	Symptoms-guided elimination diet (n = 11)/normal diet as control (n = 7)	6 weeks	Induction of clinical remission, sigmoidoscopy, histopathology, dietary adherence: interview
Strisciuglio (2013) [37]	Italy	Single-center, randomized, controlled clinical trial	Pediatric newly diagnosed UC patients	Cow's milk protein elimination diet (n = 14)/normal diet as control (n = 15)	12 months	Induction of clinical remission, clinical relapse: PUCAI, Physician global assessment, serum C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, endoscopic evaluation, histological evaluation, dietary adherence: food diaries
Kyaw (2014) [38]	UK	Randomized, controlled clinical trial	Adult UC patients	Comprehensive dietary advices (n = 61)/general dietary recommendations +normal diet as control(n = 51)	24 weeks	Disease activity: SCCAI, quality of life: IBDQ, dietary adherence: food frequency questionnaire
Bhattacharyya (2017) [39]	USA	Randomized, double-blind, placebo-controlled, multicenter, clinical trial	Adult UC patients in clinical remission	No-carrageenan diet + carrageenan-containing capsules (200 mg/d) (n = 7)/no-carrageenan diet + placebo (dextrose) (n = 7)	12 months	Clinical relapse: SCCAI, quality of life: SIBDQ, serum cytokines, fecal calprotectin, dietary adherence: 24 h dietary recalls
Pedersen (2017) [40]	Denmark	Randomized, open-label, controlled clinical trial	Adult UC patients in remission, or mild to moderate disease activity and coexisting IBS-like symptoms	Low FODMAP diet (n = 44)/normal habitual diet as control (n = 45)	6 weeks	Disease activity: SCCAI, Severity of IBS symptoms: IBS-SSS, quality of life: SIBDQ, C-reactive protein, fecal calprotectin, dietary adherence: food frequency questionnaire
Jian (2018) [41]	China	Randomized, open-label, stratified clinical trial	Adult UC patients in remission, or mild to moderate disease activity	Immunoglobulin G-guided exclusion diet (n = 49)/normal diet as control (n = 48)	6 months	Disease activity: Mayo score, quality of life: IBDQ, body mass index, albumin, transferrin, prealbumin, extraintestinal manifestation of the disease, food-specific IgG antibodies, dietary adherence: food diaries

UC: ulcerative colitis; PUCAI: Pediatric Ulcerative Colitis Activity Index; SCCAI: Simple Clinical Colitis Activity Index; SIBDQ: Short Inflammatory Bowel Disease Questionnaire; IBS: Irritable bowel syndrome; FODMAP: Fermentable, Oligosaccharides, Disaccharides, Monosaccharides and Polyols; IBS-SSS: IBS symptom severity system; IBDQ: Inflammatory Bowel Disease Questionnaire. [1] Number of patients used for statistical analysis.

Chapter 6. Following an anti-inflammatory diet prevents increases of fecal calprotectin and alters metabolomic profile of ulcerative colitis patients, a randomized controlled trial

6.1 ABSTRACT

Background: A relationship between ulcerative colitis (UC) and diet has been shown in epidemiological and experimental studies, but few clinical trials have been done in this regard. The aim of this study was to investigate the effectiveness of an anti-inflammatory diet for maintenance of remission in UC patients.

Methods: In a 6-month randomized control trial, adult UC patients in clinical remission (partial Mayo score <3) with a clinical disease relapse within the previous 18 months were randomized to either an "Anti-inflammatory Diet (AID)" or "Canada's Food Guide (CFG)" as controls. A dietitian provided dietary recommendations to all patients in monthly face-to-face and telephone sessions. Menu plans in the AID group were designed to increase dietary intake of fiber, prebiotics, probiotics, anti-oxidants and omega-3 fatty acids and to decrease intake of red meat, refined sugar and alcohol. Monthly 24h dietary recalls were used to assess dietary adherence. Stool was collected for fecal calprotectin (FCP) and microbial analysis by 16S rDNA sequencing using an Illumina platform at baseline and at month 6, or clinical relapse. Metabolomic analysis was performed on urine (GC-MS, DI- LC MS/MS), serum (NMR, DI-LC MS/MS) and stool (NMR) samples collected at baseline and month 6, or at clinical relapse.

Results: Fifty-three patients were randomized to the two diet groups (mean age: 41.4±14.7 years, 64.2% females). Five (19.2%) patients in the AID group and 8 (29.6%) patients in the CFG group relapsed during the trial (P=0.38). Patients in the AID group significantly increased their intake of fiber, zinc, phosphorus, selenium, yogurt and seafood versus the control group.

Interestingly, over the 6-month intervention, the AID group showed no significant increase in FCP, whereas patients following CFG had a statistically significant increase ($P<0.05$). In regression analysis, changes in FCP levels from baseline to the end of the trial was positively correlated with changes in *Bacteroidaceae*. While metabolomic profiles of the two groups were similar at baseline, there was a clear separation at month 6, or at relapse. Increased urinary carnosine level was the major factor responsible for the separation of the metabolomes between the two diet groups at the end of the study. In comparison to the CFG group, patients in the AID group had a significant decrease in their dietary inflammatory index score.

Conclusion:

Dietary modifications to increase intake of anti-inflammatory foods and decrease inflammatory-type foods was effective in limiting the onset of subclinical inflammation and associated microbial and metabolic changes in UC patients in remission.

6.2 Introduction

Ulcerative colitis (UC) is a subtype of inflammatory bowel disease (IBD) characterized by chronic relapsing and remitting inflammation of the colonic mucosa (1). Diarrhoea and presence of blood in the stool are the most common symptoms of UC. UC prevalence and incidence have been increasing worldwide (2). Although the exact pathophysiological mechanisms of UC development still remain unknown, it has been suggested that a combination of several factors including genetic predisposition, epithelial barrier defects, dysregulated immune responses, microbial dysbiosis, and environmental factors play a major role (1, 2).

As shown in several experimental and epidemiological studies, dietary factors are among the potential environmental contributors of UC development. Among them are high intake of soft drinks and sucrose, n-6 polyunsaturated fatty acids (PUFAs), and low intake of fruits, vegetables, and n-3 PUFAs (3-7). Although the exact mechanisms responsible for the association between diet and development of UC is unknown, several mechanisms have been suggested. An unhealthy dietary pattern such as a Western diet has been linked to changes in the gut microbiome, epithelial barrier, and immune function which may ultimately trigger increased chronic colonic inflammation (4).

It has been shown that multiple disease relapse result in impaired quality of life in IBD patients (8) and an increased probability of colitis-associated colorectal cancer in these patients (9). On the other hand, many UC patients attribute their disease relapse to diet, and several dietary intake factors have been related to increased risk of UC relapse (10, 11). However, according to a recent Cochrane systematic review, consensus on the composition of evidence-based dietary interventions in UC patients is required and there is a need for more high-quality, well-

powered, randomized, controlled trials to assess the efficacy of these interventions (12). In the present randomized controlled trial, we aimed to assess the role of an anti-inflammatory diet and its potential protective mechanisms for maintenance of remission in adult UC patients.

6.3 Methods

6.3.1 Study design and patients

This study was a controlled, randomized, parallel trial conducted from September 2014 to September 2016 in Edmonton, Alberta, Canada. Women and men UC patients aged 18 to 75 years and able to communicate in English were recruited into this 6-month dietary intervention study. We recruited patients who were in clinical remission (partial Mayo score<3 (13) but had a confirmed history of a UC clinical relapse (partial Mayo score≥3) in the past 18 months. Participants could be on any UC-related medications as long as they were on stable dosage of oral 5-aminosalicylic acid (5-ASA) for at least 2 weeks and on stable dosage of immunosuppressants or anti-tumor necrosis factor (TNF)-α for at least 2 months. We excluded patients who were on steroids or antibiotics within 2 weeks of enrollment. In addition, they were excluded if they were pregnant or lactating, had a significant comorbidity, or a history of colectomy.

After stratification for age and use of anti-TNF medications, subjects were randomized 1:1 into the AID or control group using the randomization module of REDCap (Research Electronic Data Capture) (14). The study protocol was approved by the Health Research Ethics Board, University of Alberta (Pro00035413) and written informed consent was obtained from all participants.

6.3.2 Intervention

Participants randomized to the AID were provided with 45 to 60-minute face-to-face dietary counselling by a registered dietitian at baseline, months 1, 3, and 6. At month 2, 4, and 5 the dietary recommendations were delivered by the same dietitian through 30-minute telephone counselling sessions. Patients were instructed to follow a structured four-week menu plan that included recipes and nutrition tips. The menu plan was a modified version of the previously developed menu plan for management and prevention of type 2 diabetes which has been described elsewhere (15). The menu plan used in the current study emphasized specific foods that have been shown in the literature to improve IBD-related symptoms or prevent IBD relapse. The menus followed the food group servings outlined in the Eating Well with Canada's Food Guide 2007 (CFG) (16) and on average they provided 2000 kcal with 54%, 19% and 27% of the energy from carbohydrates, protein and fat, respectively. Each recipe included ways to increase or reduce kcal by 200 kcal. Menu plans and nutrition tips were designed to increase participants' intake of antioxidants, prebiotics, probiotics, and n-3 PUFA and decrease consumption of red meat, processed meat and added sugar. For example, foods high in antioxidants such as berries have been incorporated daily into the menu plan. Other foods high in antioxidants such as legumes or pulses were incorporated into several days of each of the four weeks of menus. Probiotics from such foods as plain yogurt with probiotics noted on product were included. In addition, a significant number of recipes included foods with high prebiotics content such as onion, garlic, and asparagus. Each week of the intervention menu plan ensured participants were consuming 2 servings of fish weekly. Furthermore, at least 50% of the weekly fish recipes involved high n-3 PUFA containing fish such as salmon. In order to

facilitate adherence to the menus, several recipes, cooking tips, weekly grocery lists and a list of Alberta-produced foods and places to obtain them were also provided to participants. Furthermore, participants randomized to the AID were provided with a food list from which they could choose their preferred food items. This could help the dietitian direct an individualized dietary plan for participants to incorporate their daily dietary requirements.

Patients who were randomized to the control arm, received dietary recommendations to comply with the CFG (16) with respect to daily recommended food group servings. CFG has been primarily developed to help Canadians achieve a healthy, balanced diet and to reduce risk of obesity, type 2 diabetes, heart disease, certain types of cancer and osteoporosis. The dietary recommendations were provided by the same dietitian through 30-minute face-to-face visits at baseline, month 1, 3 and 6 as well as telephone communications at months 2, 4, ad 5.

6.3.3 Assessments

Demographic and clinical information was collected at baseline. At baseline and at the end of the study weight and height were measured and body mass index (BMI) was calculated. We used the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) (17) for assessment of health related quality of life in patients at baseline and at month 6 or clinical relapse.

To assess adherence to dietary recommendations and changes in dietary intake from baseline to the end of the trial, monthly self administered 24h dietary recalls were used. Dietary intake data for 24-hour recalls were collected and analyzed using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, 2014, developed by the National Cancer Institute, Bethesda, MD (18). Dietary inflammatory index (DII) scores which assess the inflammatory

potential of diet were calculated using the method proposed by Shivappa et al (19). A higher DII score (more positive values) indicates a more inflammatory diet and a lower DII score (more negative values) indicates a less inflammatory diet.

Disease activity was assessed at baseline, clinic visits and telephone counselling (month 2, 4 and 5) using partial Mayo score (Mayo score without the endoscopy subscore).

Blood, urine and stool samples were collected from all patients during the clinic visits. Serum C-reactive protein (CRP) and hemoglobin levels were measured at baseline, month 3 and 6 (or at the time of relapse). Fecal calprotectin (FCP) was measured in stool samples at baseline, month 1, 3 and 6 (or at the time of relapse) using an enzyme-linked immunosorbent assay with monoclonal antibodies specific to calprotectin (Bühlmann Laboratories AG, Basel, Switzerland).

Targeted metabolomic analysis was conducted using urine, serum and stool samples at baseline and at the end of the trial (month 6 or at relapse). Urine samples were assayed using a combined direct infusion (DI-)/liquid chromatography (LC-) tandem mass spectrometry (MS/MS) and gas-chromatography (GC-) MS assay. DI- LC MS/MS and nuclear magnetic resonance (NMR) spectroscopy were used to identify and quantify metabolites in serum samples. NMR was used to identify and quantify metabolites in stool samples. All metabolomic assays were conducted at the Metabolomics Innovation Center (Edmonton, AB, Canada) following previously described protocols (20-23).

Fecal genomic DNA was extracted using a commercial DNA extraction kit and 16s rRNA sequencing was used to assess microbial composition (21).

6.3.4 Outcomes

The primary outcome was clinical relapse of UC, defined as partial Mayo score greater than 2. The secondary outcomes were changes in FCP, CRP, and health related quality of life scores from baseline to end of the trial.

6.3.5 Statistical Analysis

An intention to treat approach was used to analyze data, such that data from all patients were analyzed according to the diet that they were randomized to. Kolmogorov-Smirnov test and histograms were used to assess the normality of data distribution. Continuous and categorical variables are presented as mean ± SD or median (interquartile range) and number (%) respectively. Logarithmic transformation was used to normalize data that were not normally distributed. Chi-square tests or Fisher's exact test were used to compare qualitative variables between groups. Student's t-test or Mann-Whitney U test were used to compare quantitative variables between groups where appropriate. Baseline values and endpoint measures were compared within each group using paired t-test or Wilcoxon signed-rank test. FCP changes from baseline to months 1, 3 and 6 within each group was assessed using analysis of variance with the Friedman test. To examine the effect of intervention on outcome variables, split-plot repeated measures ANOVA (split-plot rANOVA) was used, in which the effect of time, intervention (between groups effects) and time × intervention interactions were assessed. In this analysis, we controlled for the baseline levels of outcome variables and potential confounding variables. SPSS version 20.0 (IBM, Armonk, NY, USA) was used for statistical analysis and $P < 0.05$ were considered statistically significant.

For metabolomic analysis, metabolites with at least 50% missing values were removed from analysis. Missing values were replaced by half of the minimum positive values in the original dataset. Concentrations of urinary metabolites (µmol/L) were normalised to creatinine (mmol/L) and reported as the ratio (µmol/mmol). Concentrations of fecal metabolites were normalized to the weight of stool sample and reported as µmol/gr. Multivariate statistical analysis was performed using partial least squares discriminant analysis (PLS-DA) using metabolites that their levels were different ($P<0.2$) between two diet groups in univariate analysis. Permutation analysis using random resampling ($n = 2000$) of the two groups of patients (i.e. AID baseline vs CFG baseline and AID end of trial vs CFG end of trial) was conducted and a P value was reported. Variable importance in projection (VIP) scores were used to identify major metabolites responsible for the discrimination between the metabolomic profiles of patients between the two groups. The VIP score indicates the contribution of each feature to the regression model. Higher values of VIP scores indicate greater contribution of the metabolites to the group separation. The MetaboAnalyst 4.0 (24) was used for metabolomic-related statistical analysis.

Taxa with an average abundance of >1% were included for microbial analysis. Principal component analysis (PCA) plots were generated using ClustVis (25). GraphPad Prism (v.5.03) was employed for bar graph generation. Differential abundance analysis of taxonomic units was performed between groups using t-test after log transformation and Pareto scaling. We used paired t-test to compare the abundance of taxonomic units in each diet group before and after the trial. P-values were corrected for multiple inference using the Benjamini-Hochberg false discovery rate. To explore the correlation between changes in microbial composition and

changes in FCP levels from baseline to month 6/time of relapse Spearman's rank correlation was used.

6.4 Results

In total, 53 UC patients in clinical remission were randomized to the two dietary intervention groups; 26 to the AID and 27 to the CFG group. The flow of participants through the trial is presented in Figure 6.7.1. Two patients left the study just before the clinic visit at month 3, however, their data were used for statistical analysis. Mean age of participants was 41.4±14.7 years, 34 (64.2%) participants were female, 22 (41.5%) had left-sided colitis, and 13 (24.5%) were on anti-TNF medications. Baseline demographic and clinical characteristics of participants across the two diet groups are summarized in Table 6.7.1.

In total, 13 (24.5%) patients experienced a clinical relapse. Five (19.2%) patients in the AIG group and 8 (29.6%) patients in the CFG diet group had a UC clinical relapse which was not statistically significant (P=0.38).

Changes in FCP from baseline to month 6 in the two diet groups are presented in Table 6.7.2 and Figure 6.7.2. While there was no statistically significant change in FCP from baseline to the end of the trial in patients randomized to the AID, FCP increased significantly in patients randomized to the CFG group from baseline to month 6. In addition, comparison of these changes between the two diet groups using split-plot rANOVA was statistically significant (P=0.02).

A microbial profile of stool samples from patients in the two diet groups at baseline and at the last visit (either month 6 or time of relapse) is shown in Supplementary Figure 6.7.1. As presented in the PCA plots (Figure 6.7.3), there were no significant changes in gut bacterial composition of patients in the two diet groups from baseline to month 6 or time of relapse. However, changes in FCP levels from baseline to the end of the trial was positively correlated with changes in *Bacteroidaceae* (rs=0.38, P=0.006).

Using different metabolomic platforms, we could identify and quantify 184, 122, and 49 metabolites in serum, urine, and stool samples, respectively. After performing univariate analysis, 46 candidate metabolites were selected for further analysis using multivariate analysis. Metabolomic profiles of patients between the two diet groups at baseline were not significantly different from each other. However, there was a statistically significant separation in the metabolome of patients randomized to the two diet groups using metabolites levels at month 6 or at the time of relapse samples (Figure 6.7.4). Using VIP values, we could identify 3 metabolites with most significant roles in the discrimination between metabolomic profiles of subjects in the two diet groups after the dietary intervention. The VIP values of these three metabolites as well as their mean levels in each diet group before and after the intervention are presented in Table 6.7.3. In the AID group there was a significant increase in urinary carnosine and PC ae C38:5 levels from baseline versus the end of the trial. Patients randomized to the ICFG group had a significant increase in their serum PC aa C38:1 level from baseline to month 6/relapse.

While there were no significant changes in dietary intake of patients randomized to the CFG group, patients in the AID group significantly increased their intake of fiber, zinc, phosphorus,

selenium, yogurt and seafood (Supplementary Table 6.7.1). Furthermore, there was a significant decrease in total DII score of patients in AIG group following the 6-month dietary intervention (Figure 6.7.5). In addition, changes in FCP were significantly correlated with changes in intake of yogurt (rs=-0.39, P=0.01), poultry (rs=-0.34, P=0.01), seafood (rs=-0.29, P=0.05), fruit juices (rs=0.38, P=0.01), cured meat (rs=0.29, P=0.04), saturated fatty acids (rs=0.28, P=0.05) from baseline to the end of the trial.

There were no significant changes in SIBDQ scores and CRP levels from baseline to the end of trial between the two diet groups.

6.5 Discussion

Our study is the first real life 6 months dietary intervention study to assess prevention of relapses in UC. In this 6-month RCT on UC patients in clinical remission, we found that adherence to the AID diet could prevent increases in FCP levels in comparison to following the dietary recommendations based on CFG as the control diet. Importantly, we also found that significant changes in the metabolome following the AID in comparison to CFG dietary recommendations.

The role of diet in the pathogenesis of IBD has been indicated in several animal and epidemiological studies. It has been suggested that a Western diet characterized by high content of refined carbohydrates, saturated fatty acids, red meat and processed meat, and low content of fruits, vegetables, legumes and fibers increases the risk of IBD development through significant proinflammatory impacts on host immune system and microbial composition or

function (4, 26). In addition, prospective cohort studies have reported several dietary factors such as red meat and processed meat intake to be associated with increased risk of disease relapse in UC patients (27). Many IBD patients also believe that dietary factors are responsible for their disease development or relapse of symptoms. According to a large cross-sectional study in UK (28) about half of IBD patients believed that diet could be the initiating factor in IBD and or could trigger a flare. In addition, Limdi et al. reported about IBD 66% of patients deprived themselves of their favorite foods in order to prevent relapse and such dietary restrictions may eventually lead to various nutritional deficiencies (28). However, there are limited evidence based dietary recommendations for prevention of relapse in IBD patients partly due to lack of well-designed randomized dietary interventions.

The AID used in the present study was a dietary pattern characterized by increased intake of antioxidants, fibers, prebiotics, probiotics, and n-3 PUFA and decreased consumption of red meat, processed meat and added sugar. AID development was based on our current understanding of the role of dietary factors in the pathogenesis or disease course in IBD patients. For instance, red and processed meat intake was shown to be related to increased risk of UC relapse (27). Furthermore, consumption of fruits and vegetables as two major sources of antioxidants and fibers was related to decreased odds of UC development in a meta-analysis study (29). Another recent meta-analysis study found a negative association between long-chain n-3 PUFAs intake (EPA + DHA) and UC development (30).

In the present study, we did not find a significant difference in clinical relapse rate between the two dietary interventions. It should be noted that our study was not statistically powered enough to detect a difference in clinical relapse rate between the AID and CFG diets. However,

following the AID could prevent the increase in FCP which is an important objective marker of colonic inflammation and a strong predictor of future disease relapse. Therefore, a larger sample size and/or longer duration of follow-up could indicate a statistically significant difference in relapse rate between the two groups.

The association between dietary factors and changes in gut microbial composition in UC patients have been shown recently (31). In a recent prospective cohort study, Godny et al (32) reported that fruit consumption was associated with changes in microbial composition, and lower consumption of fruit was correlated with the development of pouchitis in a group of UC patients who underwent proctocolectomy. Although it has been suggested that modulation of gut microbiota through dietary manipulations may result in favorable outcomes in IBD patients, the impacts of dietary interventions on gut microbial composition in these patients have been evaluated in few RCTs in UC patients (33). A recent high-quality study demonstrated that diet accounted for a small proportion (3%) of taxonomic variation between subjects, and 0.7% of taxonomic variation longitudinally in IBD patients (34). In our study, we did not find any significant changes in the overall gut bacterial composition in stool samples collected before and after the intervention between the two diet groups. However, increases in FCP levels were significantly correlated with increases in Gram-negative bacteria belonging to the family Bacteroidaceae. This finding is in agreement with previous reports in other IBD-related studies. For example, exclusive enteral nutrition resulted in a significant decrease in disease activity and relative abundance of Bacteroidaceae in children with active Crohn's disease (35). Furthermore, progression of colitis was associated with significant expansion of Bacteroidaceae in mice (36).

These findings highlight the role of Bacteroidaceae in the development of inflammation in IBD which requires further investigations.

Metabolomics, defined as the study of all metabolites in biological samples comprehensively, has been shown to have diagnostic (21, 37) or prognostic (38, 39) potentials in different IBD settings. In addition, metabolomic approaches have been used recently to elucidate the mechanisms of response to different therapeutic interventions in IBD patients (40, 41). To the best of our knowledge, our study is the first one investigating the modifications in the metabolomic profiles of UC patients following a dietary intervention. In this study, we found a significant change in the metabolome of patients randomized to the AID following the 6-month trial. The most important metabolite responsible for the separation of the metabolome between the two diet groups was carnosine levels in urine which was significantly higher in patients randomized to the AIG group. Carnosine (β-alanyl-l-histidine) is a histidine-containing dipeptide which is present in meat and fish. It has several biological roles such as pH buffering, calcium regulation, anti-glycation, and antioxidant activity (42). Although, carnosine levels in urine has been suggested to be a biomarker of meat intake in healthy individuals (43), a recent randomized trial study showed that carnosine homeostasis was unaffected by a 6-month vegetarian diet (44). In the present study we found a positive correlation between changes in seafood intake and changes in urinary carnosine levels (r_s=0.28, P=0.04). This finding necessitates performing further studies for identification of dietary intake-related metabolites that are specific for IBD patients as bioavailability of dietary contents may be different in these individuals in comparison to healthy people due to altered absorption and microbial composition in these patients.

Although our study is among the first studies investigating the potential benefits of a dietary intervention for maintenance of remission in UC patients, it has some limitations that should be considered while interpreting the findings. Small sample size is the major limiting factor in the current study which may be an important factor in not reaching the statistical significance for the comparison of clinical relapse rate between the two diet groups. However, the significant role the AID in prevention of the increase in FCP levels in AID in comparison to the control diet is encouraging and advocates performing a larger study to evaluate the contribution of the AID or other types of dietary manipulation (e.g. a Mediterranean diet) for prevention of disease relapse in UC patients. Furthermore, assessment of dietary intake changes from baseline to the end of the trial was based on self-reported 24h dietary recalls which is subject to recall bias and inaccurate reporting. Lack of valid dietary intake biomarkers in IBD patients advocates for performing further well-designed clinical trials to introduce objective measures of dietary intake in these patients.

In conclusion, we showed that adherence to an ant-inflammatory type diet can prevent increases in FCP levels in UC patients who are in clinical remission which was accompanied by significant changes in the metabolomic profiles of subjects. We suggest conducting further well-designed RCTs with larger sample size to assess the benefits of following such diets for maintenance of remission in UC patients.

Acknowledgments:

We wish to thank all participants of the study and their families for supporting them in following the dietary recommendations for six months.

Funding information:

The study was funded by Alberta Agriculture Funding Consortium and Alberta Innovates-Bio Solutions and. LAD and KLM took part in the IMAGINE study funded by the Canadian Institute of Health and Research (CIHR). LAD and KLM were funded by Alberta Innovates-Bio Solution. KLM was funded through a CIHR operating grant. AHK was supported by a graduate studentship from Alberta Innovates-Health Solutions.

6.6 References

1. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-1770.

2. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019;94(1):155-165.

3. Keshteli AH, Madsen KL, Dieleman L. Diet in the Pathogenesis and Management of Ulcerative Colitis; A Review of Randomized Controlled Dietary Interventions. Nutrients. 2019;11(7). pii: E1498.

4. Khalili H, Chan SSM, Lochhead P, Ananthakrishnan AN, Hart AR, Chan AT. The role of diet in the aetiopathogenesis of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2018;15(9):525-535.

5. Reddavide R, Rotolo O, Caruso MG, Stasi E, Notarnicola M, Miraglia C, Nouvenne A, Meschi T, De' Angelis GL, Di Mario F, Leandro G. The role of diet in the prevention and treatment of Inflammatory Bowel Diseases. Acta Biomed. 2018;89(9-S):60-75.

6. Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR, Fiocchi C. Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol. 2018;15(1):39-49.

7. Levine A, Sigall Boneh R, Wine E. Evolving role of diet in the pathogenesis and treatment of inflammatory bowel diseases. Gut. 2018;67(9):1726-1738.

8. Casellas F, Arenas JI, Baudet JS, Fábregas S, García N, Gelabert J, Medina C, Ochotorena I, Papo M, Rodrigo L, Malagelada JR. Impairment of health-related quality of life in patients with inflammatory bowel disease: a Spanish multicenter study. Inflamm Bowel Dis. 2005;11(5):488-96.

9. Choi CR, Bakir IA, Hart AL, Graham TA. Clonal evolution of colorectal cancer in IBD. Nat Rev Gastroenterol Hepatol. 2017;14(4):218-229.

10. Vedamurthy A, Ananthakrishnan AN. Influence of Environmental Factors in the Development and Outcomes of Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y). 2019;15(2):72-82.

11. Martin TD, Chan SS, Hart AR. Environmental factors in the relapse and recurrence of inflammatory bowel disease: a review of the literature. Dig Dis Sci. 2015;60(5):1396-405.

12. Limketkai BN, Iheozor-Ejiofor Z, Gjuladin-Hellon T, Parian A, Matarese LE, Bracewell K, MacDonald JK, Gordon M, Mullin GE. Dietary interventions for induction and maintenance of remission in inflammatory bowel disease. Cochrane Database Syst Rev. 2019;2:CD012839.

13. De Vos M, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, D'haens GR, Franchimont D, Baert FJ, Torp RA, Henriksen M, Potvin PM, Van Hootegem PP, Hindryckx PM, Moreels TG, Collard A, Karlsen LN, Kittang E, Lambrecht G, Grimstad T, Koch J, Lygren I, Coche JC, Mana F, Van Gossum A, Belaiche J, Cool MR, Fontaine F, Maisin JM, Muls V, Neuville B, Staessen DA, Van Assche GA, de Lange T, Solberg IC, Vander Cruyssen BJ, Vermeire SA. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis. 2013;19(10):2111-7.

14. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.

15. Soria-Contreras DC, Bell RC, McCargar LJ, Chan CB. Feasibility and efficacy of menu planning combined with individual counselling to improve health outcomes and dietary adherence in people with type 2 diabetes: a pilot study. Can J Diabetes. 2014;38(5):320-5.

16. Health Canada. Eating Well With Canada's Food Guide. 2007. Ottawa, Ont., Canada. Available from https://www.canada.ca/en/health-canada/services/canada-food-guide/about/history-food-guide/eating-well-with-canada-food-guide-2007.html [Accessed October 8, 2019].

17. Irvine EJ, Zhou Q, Thompson AK. The Short Inflammatory Bowel Disease Questionnaire: a quality of life instrument for community physicians managing inflammatory bowel disease. CCRPT Investigators. Canadian Crohn's Relapse Prevention Trial. Am J Gastroenterol. 1996;91(8):1571-8.

18. National Cancer Institute. Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool. https://epi.grants.cancer.gov/asa24/. [Accessed September 5, 2019]

19. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr. 2014;17(8):1689-96.

20. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J, Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS. The human urine metabolome. PLoS One. 2013;8(9):e73076.

21. Keshteli AH, Madsen KL, Mandal R, Boeckxstaens GE, Bercik P, De Palma G, Reed DE, Wishart D, Vanner S, Dieleman LA. Comparison of the metabolomic profiles of irritable bowel syndrome patients with ulcerative colitis patients and healthy controls: new insights into pathophysiology and potential biomarkers. Aliment Pharmacol Ther. 2019;49(6):723-732.

22. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS. The human serum metabolome. PLoS One. 2011;6(2):e16957.

23. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorndahl TC, Bouatra S, Anderson T, Oudit GY, Wishart DS, Dyck JR; Alberta HEART. Metabolomic fingerprint of heart failure with preserved ejection fraction. PLoS One. 2015 May;10(5):e0124844.

24. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486-W494.

25. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 2015;43:W566–70.

26. Rizzello F, Spisni E, Giovanardi E, Imbesi V, Salice M, Alvisi P, Valerii MC, Gionchetti P. Implications of the Westernized Diet in the Onset and Progression of IBD. Nutrients. 2019;11(5). pii: E1033.

27. Jowett SL, Seal CJ, Pearce MS, Phillips E, Gregory W, Barton JR, Welfare MR. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. Gut. 2004;53(10):1479-84.

28. Limdi JK, Aggarwal D, McLaughlin JT. Dietary Practices and Beliefs in Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis. 2016;22(1):164-70.

29. Li F, Liu X, Wang W, Zhang D. Consumption of vegetables and fruit and the risk of inflammatory bowel disease: a meta-analysis. Eur J Gastroenterol Hepatol. 2015;27(6):623-30.

30. Mozaffari H, Daneshzad E, Larijani B, Bellissimo N, Azadbakht L. Dietary intake of fish, n-3 polyunsaturated fatty acids, and risk of inflammatory bowel disease: a systematic review and meta-analysis of observational studies. Eur J Nutr. 2019. doi: 10.1007/s00394-019-01901-0.

31. Weng YJ, Gan HY, Li X, Huang Y, Li ZC, Deng HM, Chen SZ, Zhou Y, Wang LS, Han YP, Tan YF, Song YJ, Du ZM, Liu YY, Wang Y, Qin N, Bai Y, Yang RF, Bi YJ, Zhi FC. Correlation of diet, microbiota and metabolite networks in inflammatory bowel disease. J Dig Dis. 2019;20(9):447-459.

32. Godny L, Maharshak N, Reshef L, Goren I, Yahav L, Fliss-Isakov N, Gophna U, Tulchinsky H, Dotan I. Fruit Consumption is Associated with Alterations in Microbial Composition and Lower Rates of Pouchitis. J Crohns Colitis. 2019;13(10):1265-1272.

33. McIlroy J, Ianiro G, Mukhopadhya I, Hansen R, Hold GL. Review article: the gut microbiome in inflammatory bowel disease-avenues for microbial management. Aliment Pharmacol Ther. 2018;47(1):26-42.

34. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ, Casero D, Courtney H, Gonzalez A, Graeber TG, Hall AB, Lake K, Landers CJ, Mallick H, Plichta DR, Prasad M, Rahnavard G, Sauk J, Shungin D, Vázquez-Baeza Y, White RA 3rd; IBDMDB Investigators, Braun J, Denson LA, Jansson JK, Knight R, Kugathasan S, McGovern DPB, Petrosino JF, Stappenbeck TS, Winter HS, Clish CB, Franzosa EA, Vlamakis H, Xavier RJ, Huttenhower C. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;569(7758):655-662.

35. Schwerd T, Frivolt K, Clavel T, Lagkouvardos I, Katona G, Mayr D, Uhlig HH, Haller D, Koletzko S, Bufler P. Exclusive enteral nutrition in active pediatric Crohn disease: Effects on intestinal microbiota and immune regulation. J Allergy Clin Immunol. 2016;138(2):592-6.

36. Osaka T, Moriyama E, Arai S, Date Y, Yagi J, Kikuchi J, Tsuneda S. Meta-Analysis of Fecal Microbiota and Metabolites in Experimental Colitic Mice during the Inflammatory and Healing Phases. Nutrients. 2017;9(12). pii: E1329.

37. De Preter V. Metabolomics in the Clinical Diagnosis of Inflammatory Bowel Disease. Dig Dis. 2015;33 Suppl 1:2-10.

38. Probert F, Walsh A, Jagielowicz M, Yeo T, Claridge TDW, Simmons A, Travis S, Anthony DC. Plasma Nuclear Magnetic Resonance Metabolomics Discriminates Between High and Low

Endoscopic Activity and Predicts Progression in a Prospective Cohort of Patients With Ulcerative Colitis. J Crohns Colitis. 2018;12(11):1326-1337.

39. Keshteli AH, Tso R, Dieleman LA, Park H, Kroeker KI, Jovel J, Gillevet PM, Sikaroodi M, Mandal R, Fedorak RN, Madsen KL. A Distinctive Urinary Metabolomic Fingerprint Is Linked With Endoscopic Postoperative Disease Recurrence in Crohn's Disease Patients. Inflamm Bowel Dis. 2018;24(4):861-870.

40. Nakanishi M, Matz A, Klemashevich C, Rosenberg DW. Dietary Walnut Supplementation Alters Mucosal Metabolite Profiles During DSS-Induced Colonic Ulceration. Nutrients. 2019;11(5). pii: E1118.

41. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, Paramsothy R, Walsh AJ, van den Bogaerde J, Samuel D, Leong RWL, Connor S, Ng W, Lin E, Borody TJ, Wilkins MR, Colombel JF, Mitchell HM, Kaakoush NO. Specific Bacteria and Metabolites Associated With Response to Fecal Microbiota Transplantation in Patients With Ulcerative Colitis. Gastroenterology. 2019;156(5):1440-1454.e2.

42. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine.Physiol Rev. 2013;93(4):1803-45.

43. Cheung W, Keski-Rahkonen P, Assi N, Ferrari P, Freisling H, Rinaldi S, Slimani N, Zamora-Ros R, Rundle M, Frost G, Gibbons H, Carr E, Brennan L, Cross AJ, Pala V, Panico S, Sacerdote C, Palli D, Tumino R, Kühn T, Kaaks R, Boeing H, Floegel A, Mancini F, Boutron-Ruault MC, Baglietto L, Trichopoulou A, Naska A, Orfanos P, Scalbert A. A metabolomic study of biomarkers of meat and fish intake. Am J Clin Nutr. 2017;105(3):600-608.

44. Blancquaert L, Baguet A, Bex T, Volkaert A, Everaert I, Delanghe J, Petrovic M, Vervaet C, De Henauw S, Constantin-Teodosiu D, Greenhaff P, Derave W. Changing to a vegetarian diet reduces the body creatine pool in omnivorous women, but appears not to affect carnitine and carnosine homeostasis: a randomised trial. Br J Nutr. 2018;119(7):759-770.

6.7 Tables and Figures

Table 6.7.1. Demographic and clinical characteristics of study participants at baseline

		AID (n=26)	CFG (n=27)	P-value
Age, years		36.5 (30.0-55.5)	43.0 (25.0-54.0)	0.64
Females, n (%)		15 (57.7)	19 (70.4)	0.34
Current smoker, n (%)		1 (3.8)	1 (3.7)	1.00
University degree, n (%)		16 (61.5)	10 (37.0)	0.07
Body mass index, Kg/m^2		25.2 (22.1-29.2)	24.2 (22.6-27.6)	0.78
Years since diagnosis, years		9.0 (5.5-12.8)	6.0 (3.0-13.0)	0.35
Duration of remission, months		6.0 (3.0-9.5)	6.0 (4.0-8.0)	0.96
UC subtype, n (%)	Proctitis	3 (11.5)	3 (11.1)	
	Left-sided	12 (46.2)	10 937.0)	0.77
	Pancolitis	11 (42.3)	14 (51.9)	
UC medications, n (%)	No UC medication	2 (7.7)	3 (11.1)	0.67
	5-aminosalicylic acid	18 (69.2)	22 (81.5)	0.30
	Immunosuppressants	9 (34.6)	7 (25.9)	0.49
	Biologics	7 (26.9)	6 (22.2)	0.69
C-reactive protein		1.1 (0.7-2.0)	1.2 (0.5-3.7)	0.67
Fecal calprotectin		129.4 (70.5-266.0)	184.1 (85.5-458)	0.43

Table 6.7.2. Percentage of changes in fecal calprotectin levels from baseline to month 6 or at relapse in patients randomized to anti-inflammatory diet (AID) versus Canada's Food Guide (CFG) [1]

	AID [2]	**CFG [3]**
Month 1 (%change from Baseline)	-17.6 ± 51.4	71.2 ± 236.3
Month 3 (%change from Baseline)	9.6 ± 93.3	204.9 ± 476.5
Month 6 (% change from Baseline)	63.1 ± 220.2	384.5 ± 904.3

[1] Values are presented as mean ± standard deviation

[2] P=0.92 for within group comparison using Friedman test

[3] P<0.001 for within group comparison using Friedman test

Table 6.7.3. Concentration of major metabolites responsible for the discrimination of metabolome between the two diets at month 6 or clinical relapse.

	AID		**P-value[1]**	**CFG**		**P-value[1]**	**P-value[2]**	**VIP score**
	Baseline	Month6		Baseline	Month6			
Carnosine (urine)	0.47(0.23-1.60)	1.12(0.39-2.69)	0.026	0.35 (0.10-1.27)	0.42(0.23-0.84)	0.755	0.018	2.39
PC ae C38:5 (urine)	0.002(0.001-0.006)	0.006(0.002-0.016)	0.025	0.002(0.001-0.006)	0.003(0.001-0.006)	0.889	0.047	1.88
PC aa C38:1 (serum)	1.46(0.91-1.92)	1.25(0.92-1.76)	0.517	1.44(1.19-2.38)	1.71(1.33-1.96)	0.004	0.034	1.34

[1] within group comparison

[2] between groups comparison

AID: Anti-inflammatory diet; CFG: Canada's Food Guide diet; VIP: Variable importance in projection

Supplementary Table 6.7.1. Comparison of changes in dietary intake of foods and nutrients from baseline to month 6 between the two diet groups.

	AID		P-value[1]	CFG		P-value[2]	P-value[3]
	Baseline	Month 1-6		Baseline	Month 1-6		
Energy (kcal/d)	1882.4±524.7	1958.9±551.9	0.29	1950.2±616.4	1911.5±568.5	0.45	0.26
Protein (%E)	16.2±4.3	17.3±3.3	0.32	19.9±5.7	18.7±4.4	0.11	0.21
Carbohydrate (%E)	49.4±8.4	49.8±8.9	0.78	47.1±11.5	45.3±9.3	0.30	0.31
Fat (%E)	35.8±9.6	33.4±6.8	0.15	33.5±7.5	34.5±8.3	0.37	0.21
Fiber (g/d)	20.2±6.6	22.8±6.7	0.04	22.5±11.8	22.3±8.3	0.61	0.09
Vitamin A (mcg/d)	1018.1±794.1	1134.9±731.7	0.13	1198.5±696.9	900.7±440.2	0.03	0.01
Niacin (mg/d)	24.8±17.5	27.2±14.7	0.23	29.2±10.0	27.8±11.1	0.27	0.06
Zinc (mg/d)	10.6±5.3	11.7±6.1	0.05	17.7±11.7	16.1±8.0	0.45	0.00
Phosphorus (mg/d)	1408.6±626.6	1561.4±548.8	0.05	1571.9±637.3	1472.5±435.0	0.36	0.02
Selenium (mcg/d)	110.4±61.8	122.2±39.6	0.04	128.4±36.6	121.6±25.7	0.34	0.01
Choline (mg/d)	297.8±130.8	334.5±118.3	0.07	367.6±176.3	332.1±132.3	0.46	0.05
Seafood (ounce equivalents/d)	0.7±1.2	1.4±1.5	0.01	1.2±2.1	1.1±1.3	0.82	0.07
Yogurt (cup equivalents/d)	0.3±0.3	0.5±0.3	0.00	0.1±0.2	0.2±0.2	0.28	0.06

[1] for comparison of baseline vs month 6/relapse levels in the anti-inflammatory diet (AID) group

[2] for comparison of baseline vs month 6/relapse levels in the Canada's Food Guide (CFG) group

[3] for comparison of changes from baseline to month 6/relapse between AID and CFG groups

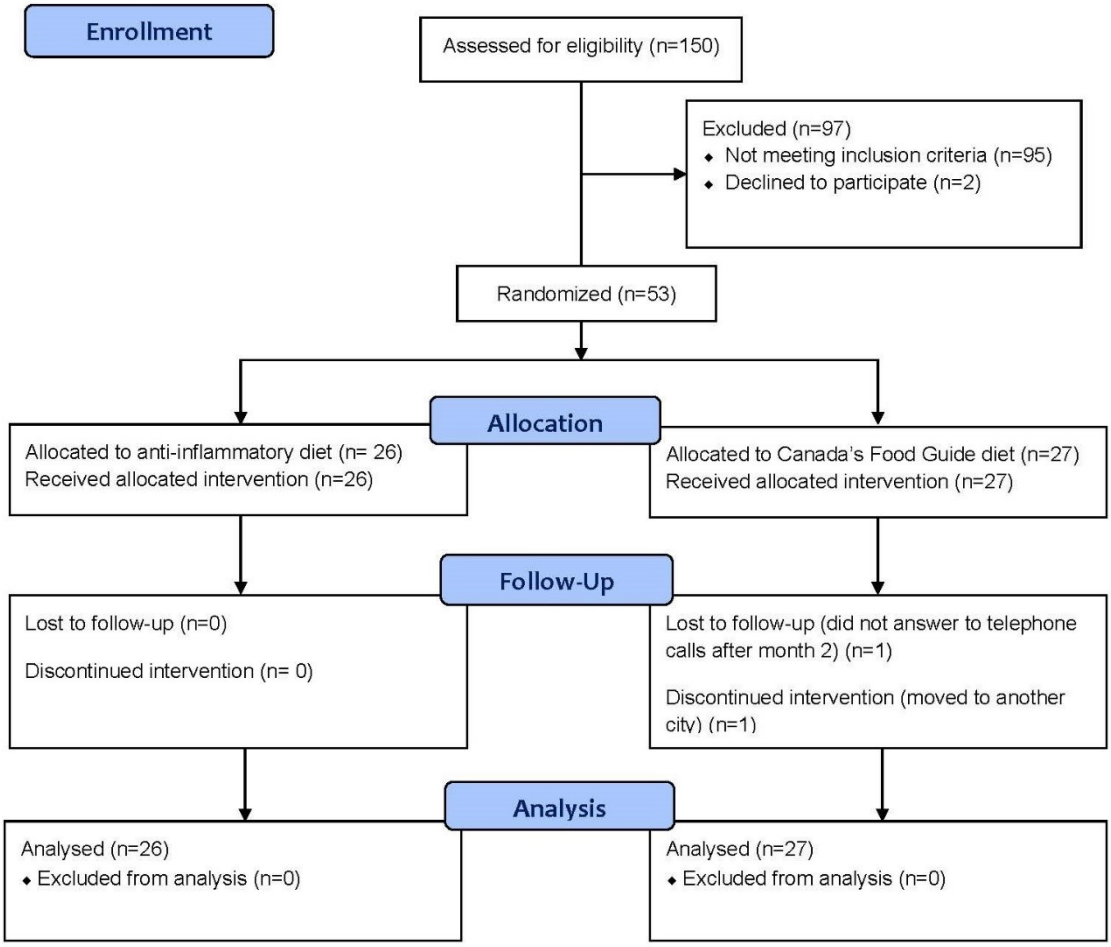


Figure 6.7.1. CONSORT flow diagram

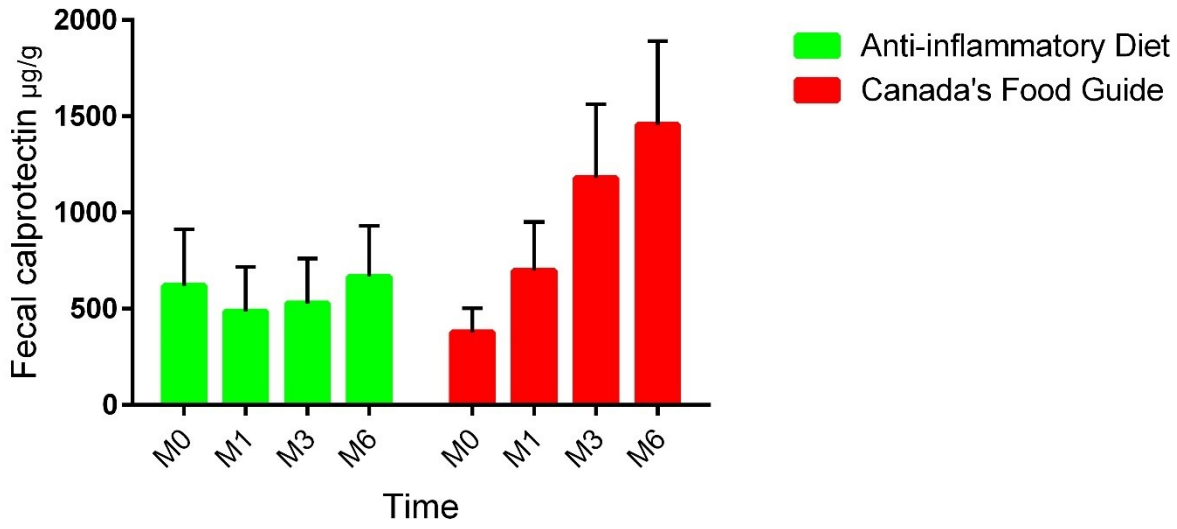


Figure 6.7.2. Changes in fecal calprotectin levels from baseline to the end of the trial. While there was no significant change in fecal calprotectin from baseline to month 6 in patients randomized to the anti-inflammatory diet (P=0.73), fecal calprotectin levels increased significantly from baseline to the end of the intervention (P=0.01).

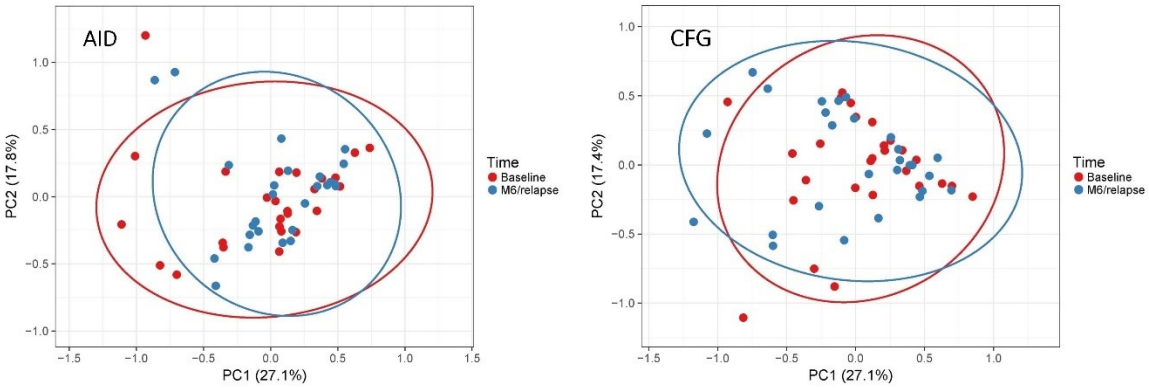


Figure 6.7.3. Principal components analysis plot showing no significant changes in gut microbial composition in the anti-inflammatory (AID) and Canada's Food Guide (CFG) groups following the intervention.

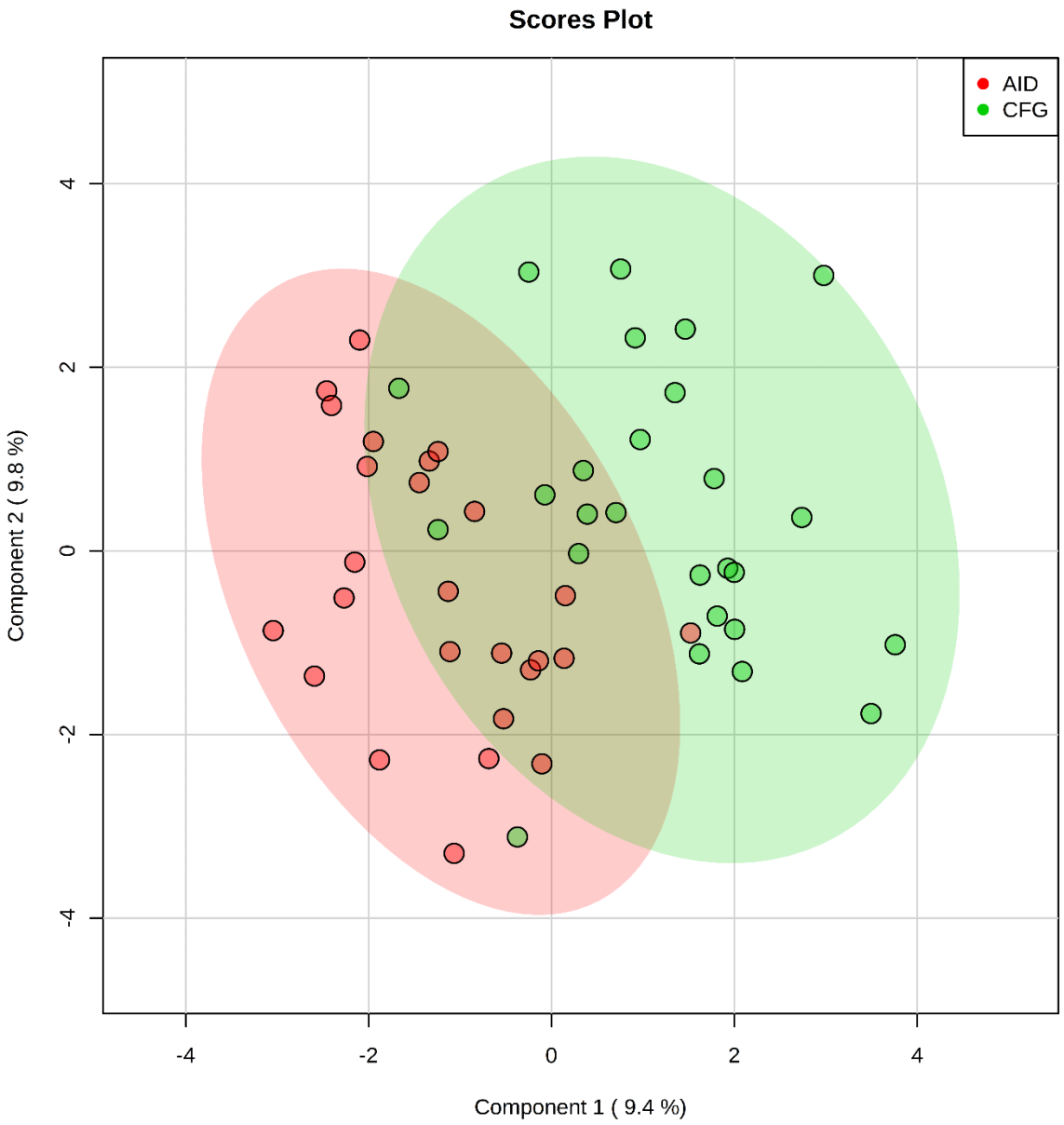


Figure 6.7.4. Partial least squares discriminant analysis plot showing a significant separation of the metabolome in patients randomized to the anti-inflammatory diet (AID) compared with patients on Canada's Food Guide diet (p=0.007, R^2=0.57, Q^2=0.30).

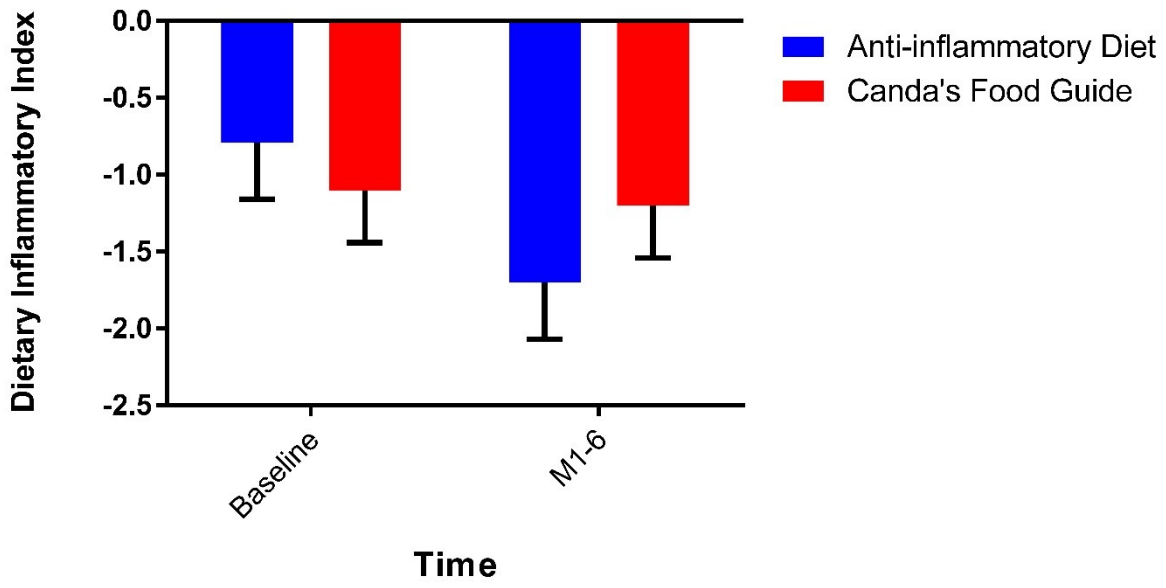
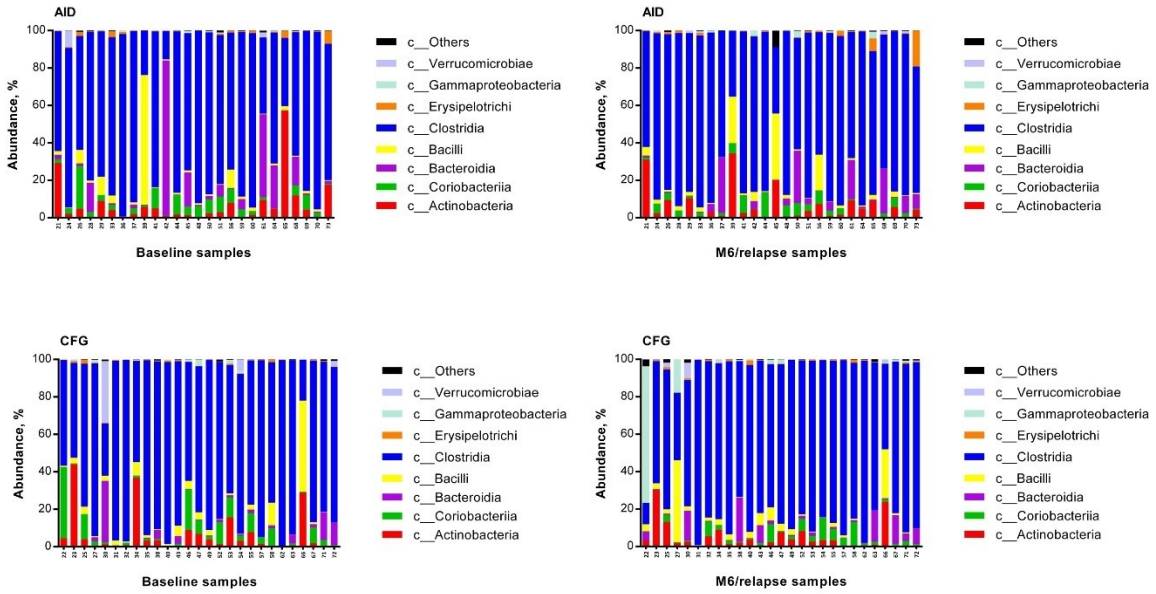


Figure 6.7.5. Comparison of dietary inflammatory index (DII) scores from baseline to the end of the trial between the two intervention groups. There was a significant decrease in DII score in patients randomized to the anti-inflammatory diet (P=0.01).



Supplementary Figure 6.7.1. Gut bacterial composition (class level) in stool samples collected from patients randomized to the anti-inflammatory diet (AID) and Canada's Food Guide (CFG) groups before and after the dietary intervention.

Chapter 7. Conclusions and Future Directions

7.1 Conclusions

In my thesis project we performed a few projects to examine if metabolomic profiling can be used to explore the relationship between diet and pathophysiology or management of IBD. Here I presented findings from four different projects that all used a metabolomic approach in various IBD settings.

The first project was a cross-sectional study on a group of adult CD patients who had undergone ileocolectomy due to uncontrolled CD-related symptoms or complications. Rutgeerts scoring system was used to assess endoscopic postoperative recurrence of CD. In addition, we collected biopsies from neoterminal ileum to assess mucosa-associated microbiota using. Urine samples were collected and metabolomic profiling was done using NMR and DI- LC-MS/MS. We found that urinary metabolomic profile of patients with recurrence of CD after ileocolectomy was distinctive from patients who were still in remission after bowel resection. Interestingly, the most important metabolite responsible for this separation was levoglucosan which is diet-related metabolite (e.g. carbohydrates metabolism) based on previous studies. In addition, we found that there were significant correlations between levels of this metabolite and some bacteria taxa (in mucus layer) which may indicate how dietary factors (e.g. carbohydrate intake) can affect bacterial composition or function in the context of CD recurrence postoperatively.

In the second study, we collected urine samples from patients with UC or IBS and healthy individuals to investigate if metabolomic profiling could distinguish between IBS patient and UC patients. Such an investigation is of great value as currently there are only few easy to use

biomarkers that can be applied to distinguish between IBS patients and IBD patients who sometime present with similar gastrointestinal symptoms. In this study, we used DI- LC- MS/MS and GC-MS methods to identify and quantify metabolites. After performing robust multivariate analysis, we found that a panel of metabolites in urine could significantly distinct IBS patients from UC patients. The most important metabolites for this separation were a group of amino acids and organic acids of them many had dietary or microbial sources.

In the third study, we designed a pilot prospective cohort study which aimed to identify metabolomic and dietary determinants of relapse in adult UC patient in clinical remission. At baseline we collected clinical and dietary information (by means of a food frequency questionnaire) of patients. We also collected urine and serum samples from these patients to perform metabolomic assays. Then we followed these patients for one year to record any episodes of clinical relapse. In this study, we found that in addition to laboratory (e.g. elevated fecal calprotectin) and dietary factors (e.g. low intake of poultry), there were some metabolites in baseline urine (trans-aconitate, cystine and acetamide) and serum (3-hydroxybutyrate, acetoacetate and acetone) samples which could be used to predict future disease relapse. Interestingly, as shown in previous studies some of these metabolites had dietary sources (e.g. acetamide).

In the last study, we designed a randomized controlled trial on adult UC patients in clinical remission to assess the effectiveness of flowing an anti-inflammatory diet for 6 months in maintenance of remission and prevention of colonic inflammation in comparison to a control diet. In this study, stool, blood and urine samples were collected for metabolomic assessment. In addition, changes in gut microbial composition and fecal calprotectin were assessed in stool

samples. In this study, which is one of the first randomized dietary trials in UC setting, we found that adherence to the anti-inflammatory diet prevented the increase in fecal calprotectin from baseline to the end of the trial. We found that patients who were randomized to the anti-inflammatory diet group has distinctive metabolomic profiles at the end of the trial when compared to the end of trial metabolomic profiles of subjects in the control group. Interestingly, the major metabolite responsible for this separation was carnosine level in urine which was related to changes in dietary intake of seafood in the present study.

Overall, these findings support our hypothesis that metabolomics can be applied to understand the complex relationship between dietary factors and IBD. Our finding of diagnostic or prognostic diet-related biomarkers in IBD patients highlight the role of diet in the pathophysiology of this multifactorial gastrointestinal condition. Furthermore, we indicated that metabolomic techniques can be used to understand the underlying mechanisms of response to dietary modifications in IBD patients.

7.2 Future directions

Although findings from our studies on the robust properties of metabolomics in nutrition research in IBD settings, some important points should be considered while designing future studies.

1) Further well-designed prospective cohort studies or randomized controlled trials with larger sample size will help researchers apply more sophisticated bioinformatics data analysis and perform subgroup analysis based on different clinical or demographic factors that may have profound effects on disease outcomes and metabolomes.

2) Use of other metabolomic platforms to identify and quantify important metabolites such as bile acids that may play an important role in the interaction between diet, gut microbiota and host factors in the context of IBD should be considered.
3) There is a wide person to person variability in clinical, microbial and immune factors in IBD patients. These variabilities may affect IBD patients' response to different therapeutic interventions such as a dietary intervention. In other words, response to these treatments can vary dramatically from one person to another person. In future studies, using robust analytical techniques and large sample sizes, the usefulness of metabolomic profiling to identify prognostic biomarkers of response to dietary interventions should be investigated. Findings from these studies, can provide the basis for future personalized dietary recommendations in IBD patients.

Bibliography

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066-78.
2. Agarwal N, Spiegel BM. The effect of irritable bowel syndrome on health-related quality of life and health care expenditures. Gastroenterol Clin North Am. 2011;40(1):11-9.
3. Aggeletopoulou I, Konstantakis C, Assimakopoulos SF, Triantos C. The role of the gut microbiota in the treatment of inflammatory bowel diseases. Microb Pathog. 2019;137:103774.
4. Agus A, Denizot J, Thévenot J, Martinez-Medina M, Massier S, Sauvanet P, Bernalier-Donadille A, Denis S, Hofman P, Bonnet R, Billard E, Barnich N. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. Sci Rep. 2016;6:19032.
5. Ahmed I, Greenwood R, Costello Bde L, Ratcliffe NM, Probert CS. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. PLoS One. 2013;8(3):e58204.
6. Ahmed T, Rieder F, Fiocchi C, et al. Pathogenesis of postoperative recurrence in Crohn's disease. Gut. 2011; 60:553-62.
7. Allen NE, Grace PB, Ginn A, et al. Phytanic acid: measurement of plasma concentrations by gas-liquid chromatography-mass spectrometry analysis and associations with diet and other plasma fatty acids. Br J Nutr. 2008;99(3):653-9.
8. Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR, Fiocchi C. Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol. 2018;15(1):39-49.

9. Ananthakrishnan AN. Debate session: So what causes inflammatory bowel disease? It's all in the environment. J Gastroenterol Hepatol. 2018;33 Suppl 3:24.
10. Anitha M, Reichardt F, Tabatabavakili S, et al. Intestinal dysbiosis contributes to the delayed gastrointestinal transit in high-fat diet fed mice. Cell Mol Gastroenterol Hepatol. 2016;2(3):328-339.
11. Baranska A, Mujagic Z, Smolinska A, et al. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. Aliment Pharmacol Ther. 2016;44(1):45-56.
12. Barnes EL, Nestor MA, Onyewadume L, De Silva PS, Korzenik JR. A prospective study: the role of diet in exacerbations of patients with ulcerative colitis in remission on monotherapy with mesalamine. Gastroenterology. 2016;150(4):S5-6.
13. Basu S, Duren W, Evans CR, et al. Sparse network modeling and metscape-based visualization methods for the analysis of large-scale metabolomics data. Bioinformatics. 2017;33(10):1545-1553.
14. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Stat Methodol. 1995; 57:289-300.
15. Bergauff MA, Ward TJ, Noonan CW, et al. Urinary levoglucosan as a biomarker of wood smoke: results of human exposure studies. J Expo Sci Environ Epidemiol. 2010; 20:385-92.
16. Bessissow T, Lemmens B, Ferrante M, Bisschops R, Van Steen K, Geboes K, Van Assche G, Vermeire S, Rutgeerts P, De Hertogh G. Prognostic value of serologic and histologic

markers on clinical relapse in ulcerative colitis patients with mucosal healing. Am J Gastroenterol. 2012;107(11):1684-92.

17. Bhattacharyya S, Shumard T, Xie H, Dodda A, Varady KA, Feferman L, Halline AG, Goldstein JL, Hanauer SB, Tobacman JK. A randomized trial of the effects of the no-carrageenan diet on ulcerative colitis disease activity. Nutr Healthy Aging. 2017;4(2):181-192.
18. Bitton A, Peppercorn MA, Antonioli DA, Niles JL, Shah S, Bousvaros A, Ransil B, Wild G, Cohen A, Edwardes MD, Stevens AC. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. Gastroenterology. 2001;120(1):13-20.
19. Bitton A, Sewitch MJ, Peppercorn MA, deB Edwardes MD, Shah S, Ransil B, Locke SE. Psychosocial determinants of relapse in ulcerative colitis: a longitudinal study. Am J Gastroenterol. 2003;98(10):2203-8.
20. Bjerrum JT, Wang Y, Hao F, Coskun M, Ludwig C, Günther U, Nielsen OH. Metabonomics of human fecal extracts characterize ulcerative colitis, Crohn's disease and healthy individuals. Metabolomics. 2015;11:122-133.
21. Blancquaert L, Baguet A, Bex T, Volkaert A, Everaert I, Delanghe J, Petrovic M, Vervaet C, De Henauw S, Constantin-Teodosiu D, Greenhaff P, Derave W. Changing to a vegetarian diet reduces the body creatine pool in omnivorous women, but appears not to affect carnitine and carnosine homeostasis: a randomised trial. Br J Nutr. 2018;119(7):759-770.
22. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine.Physiol Rev. 2013;93(4):1803-45.

23. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J, Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS. The human urine metabolome. PLoS One. 2013;8(9):e73076.

24. Brennan L, Gibbons H. Sex matters: a focus on the impact of biological sex on metabolomic profiles and dietary interventions. Proc Nutr Soc. 2019 Jul 31:1-5. doi: 10.1017/S002966511900106X.

25. Brotherton CS, Martin CA, Long MD, Kappelman MD, Sandler RS. Avoidance of fiber is associated with greater risk of Crohn's Disease flare in a 6-month period. Clin Gastroenterol Hepatol. 2016;14(8):1130-6.

26. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients. 2012;4(8):1095-119.

27. Buisson A, Chevaux JB, Allen PB, et al. Review article: the natural history of postoperative Crohn's disease recurrence. Aliment Pharmacol Ther. 2012; 35:625-33.

28. Camilleri M, Halawi H, Oduyebo I. Biomarkers as a diagnostic tool for irritable bowel syndrome: where are we? Expert Rev Gastroenterol Hepatol. 2017;11(4):303-316.

29. Candy S, Borok G, Wright JP, Boniface V, Goodman R. The value of an elimination diet in the management of patients with ulcerative colitis. S Afr Med J. 1995;85(11):1176-9.

30. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6.

31. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr. 2011;94(1):348S-358S.

32. Casellas F, Arenas JI, Baudet JS, Fábregas S, García N, Gelabert J, Medina C, Ochotorena I, Papo M, Rodrigo L, Malagelada JR. Impairment of health-related quality of life in patients with inflammatory bowel disease: a Spanish multicenter study. Inflamm Bowel Dis. 2005;11(5):488-96.

33. Chan SS, Luben R, Olsen A, Tjonneland A, Kaaks R, Teucher B, Lindgren S, Grip O, Key T, Crowe FL, Bergmann MM. Body mass index and the risk for Crohn's disease and ulcerative colitis: data from a European Prospective Cohort Study (The IBD in EPIC Study). Am J Gastroenterol. 2013;108(4):575-82.

34. Charalampopoulos D, Pandiella SS, Webb C. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. Int J Food Microbiol. 2003;82(2):133-41.

35. Cheung W, Keski-Rahkonen P, Assi N, Ferrari P, Freisling H, Rinaldi S, Slimani N, Zamora-Ros R, Rundle M, Frost G, Gibbons H, Carr E, Brennan L, Cross AJ, Pala V, Panico S, Sacerdote C, Palli D, Tumino R, Kühn T, Kaaks R, Boeing H, Floegel A, Mancini F, Boutron-Ruault MC, Baglietto L, Trichopoulou A, Naska A, Orfanos P, Scalbert A. A metabolomic study of biomarkers of meat and fish intake. Am J Clin Nutr. 2017;105(3):600-608.

36. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. JAMA. 2015;313(9):949-58.

37. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140(6):1704-12.

38. Choi CR, Bakir IA, Hart AL, Graham TA. Clonal evolution of colorectal cancer in IBD. Nat Rev Gastroenterol Hepatol. 2017;14(4):218-229.

39. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486-W494.

40. Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut. 2005;54(3):364-8.

41. Daliri EB, Wei S, Oh DH, Lee BH. The human microbiome and metabolomics: Current concepts and applications. Crit Rev Food Sci Nutr. 2017;57(16):3565-3576.

42. Daniluk U, Daniluk J, Kucharski R, Kowalczyk T, Pietrowska K, Samczuk P, Filimoniuk A, Kretowski A, Lebensztejn D, Ciborowski M. Untargeted Metabolomics and Inflammatory Markers Profiling in Children With Crohn's Disease and Ulcerative Colitis—A Preliminary Study. Inflamm Bowel Dis. 2019;25(7):1120-1128.

43. Davies SK, Ang JE, Revell VL, et al. Effect of sleep deprivation on the human metabolome. Proc Natl Acad Sci U S A. 2014;111(29):10761-6.

44. Dawiskiba T, Deja S, Mulak A, Zabek A, Jawien E, Pawelka D, Banasik M, Mastalerz-Migas A, Balcerzak W, Kaliszewski K, Skóra J. Serum and urine metabolomic fingerprinting in diagnostics of inflammatory bowel diseases. World J Gastroenterol. 2014;20(1):163-74.

45. De Cruz P, Kamm MA, Prideaux L, Allen PB, Desmond PV. Postoperative recurrent luminal Crohn's disease: a systematic review. Inflamm Bowel Dis. 2012; 18:758-77.

46. De Cruz P, Kang S, Wagner J, Buckley M, Sim WH, Prideaux L, Lockett T, McSweeney C, Morrison M, Kirkwood CD, Kamm MA. Association between specific mucosa-associated

microbiota in Crohn's disease at the time of resection and subsequent disease recurrence: a pilot study. J Gastroenterol Hepatol. 2015; 30:268-78.

47. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107(33):14691-6.

48. De Preter V, Verbeke K. Metabolomics as a diagnostic tool in gastroenterology. World J Gastrointest Pharmacol Ther. 2013;4(4):97-107.

49. De Preter V. Metabolomics in the Clinical Diagnosis of Inflammatory Bowel Disease. Dig Dis. 2015;33 Suppl 1:2-10.

50. de Silva PS, Olsen A, Christensen J, Schmidt EB, Overvaad K, Tjonneland A, Hart AR. An association between dietary arachidonic acid, measured in adipose tissue, and ulcerative colitis. Gastroenterology. 2010;139(6):1912-7.

51. De Vos M, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, D'haens GR, Franchimont D, Baert FJ, Torp RA, Henriksen M, Potvin PM, Van Hootegem PP, Hindryckx PM, Moreels TG, Collard A, Karlsen LN, Kittang E, Lambrecht G, Grimstad T, Koch J, Lygren I, Coche JC, Mana F, Van Gossum A, Belaiche J, Cool MR, Fontaine F, Maisin JM, Muls V, Neuville B, Staessen DA, Van Assche GA, de Lange T, Solberg IC, Vander Cruyssen BJ, Vermeire SA. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis. 2013;19(10):2111-7.

52. DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006; 72:5069-72.

53. Dey N, Soergel DA, Repo S, et al. Association of gut microbiota with post-operative clinical course in Crohn's disease. BMC Gastroenterol. 2013; 13:131.

54. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998; 114:262-7.

55. Di Meo I, Lamperti C, Tiranti V. Mitochondrial diseases caused by toxic compound accumulation: from etiopathology to therapeutic approaches. EMBO Mol Med. 2015; 7:1257-66.

56. Doherty GA, Bennett GC, Cheifetz AS, et al. Meta-analysis: targeting the intestinal microbiota in prophylaxis for post-operative Crohn's disease. Aliment Pharmacol Ther. 2010; 31:802-9.

57. Dunlop SP, Coleman NS, Blackshaw E, et al. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. Clin Gastroenterol Hepatol. 2005;3(4):349-57.

58. Dunn WB, Ellis DI. Metabolomics: current analytical platforms and methodologies. TrAC Trends in Analytical Chemistry. 2005; 24:285-94.

59. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48(4):526-35.

60. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26:2460-1.

61. Fahoum L, Moscovici A, David S, Shaoul R, Rozen G, Meyron-Holtz EG, Lesmes U. Digestive fate of dietary carrageenan: Evidence of interference with digestive proteolysis and disruption of gut epithelial function. Mol Nutr Food Res. 2017;61(3).

62. Field T, Diego M, Hernandez-Reif M, et al. Comorbid depression and anxiety effects on pregnancy and neonatal outcome. Infant Behav Dev. 2010;33(1):23-9.

63. Flores A, Burstein E, Cipher DJ, Feagins LA. Obesity in inflammatory bowel disease: a marker of less severe disease. Dig Dis Sci. 2015;60(8):2436-45.

64. Ford AC, Moayyedi P, Hanauer SB. Ulcerative colitis. BMJ. 2013;346:f432.

65. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997;11(2):395-402.

66. Garcia-Planella E, Mañosa M, Cabré E, et al. Fecal Calprotectin Levels Are Closely Correlated with the Absence of Relevant Mucosal Lesions in Postoperative Crohn's Disease. Inflamm Bowel Dis. 2016; 22:2879-2885.

67. Ge J, Han TJ, Liu J, Li JS, Zhang XH, Wang Y, Li QY, Zhu Q, Yang CM. Meat intake and risk of inflammatory bowel disease: A meta-analysis. Turk J Gastroenterol. 2015;26(6):492-7.

68. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014;13(1):3-10.

69. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC. The treatment-naive microbiome in new-onset Crohn’s disease. Cell Host Microbe. 2014;15(3):382-392.

70. Gillevet P, Sikaroodi M, Keshavarzian A, et al. Quantitative assessment of the human gut microbiome using multitag pyrosequencing. Chem Biodivers. 2010; 7:1065-75.

71. Gionchetti P, Dignass A, Danese S, et al. EUROPEAN Evidence-based consensus on the diagnosis and management of Crohn's disease. 2016: Part 2: Surgical management and special situations. J Crohns Colitis. 2017; 11:135-149.

72. Godny L, Maharshak N, Reshef L, Goren I, Yahav L, Fliss-Isakov N, Gophna U, Tulchinsky H, Dotan I. Fruit Consumption is Associated with Alterations in Microbial Composition and Lower Rates of Pouchitis. J Crohns Colitis. 2019;13(10):1265-1272.

73. Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Gallimore JR, Pepys MB, Campbell LV. Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation. 2004;109(24):3022-8.

74. Guasch-Ferré M, Bhupathiraju SN, Hu FB. Use of Metabolomics in Improving Assessment of Dietary Intake. Clin Chem. 2018;64(1):82-98.

75. Gummesson A, Carlsson LM, Storlien LH, Bäckhed F, Lundin P, Löfgren L, Stenlöf K, Lam YY, Fagerberg B, Carlsson B. Intestinal permeability is associated with visceral adiposity in healthy women. Obesity (Silver Spring). 2011;19(11):2280-2.

76. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.

77. Haskey N, Gibson DL. An Examination of Diet for the Maintenance of Remission in Inflammatory Bowel Disease. Nutrients. 2017;9(3).

78. He X, Chen Z, Huang J, Lian L, et al. Stapled side-to-side anastomosis might be better than handsewn end-to-end anastomosis in ileocolic resection for Crohn's disease: a meta-analysis. Dig Dis Sci. 2014; 59:1544-51.

79. Health Canada. Eating Well With Canada's Food Guide. 2007. Ottawa, Ont., Canada. Available from https://www.canada.ca/en/health-canada/services/canada-food-guide/about/history-food-guide/eating-well-with-canada-food-guide-2007.html [Accessed October 8, 2019].

80. Heitkemper MM, Han CJ, Jarrett ME, et al. Serum Tryptophan Metabolite Levels During Sleep in Patients With and Without Irritable Bowel Syndrome (IBS). Biol Res Nurs. 2016;18(2):193-8.

81. Henriksen M, Jahnsen J, Lygren I, Sauar J, Kjellevold Ø, Schulz T, Vatn MH, Moum B; IBSEN Study Group. Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). Inflamm Bowel Dis. 2006;12(7):543-50.

82. Hisamatsu T, Ono N, Imaizumi A, Mori M, Suzuki H, Uo M, Hashimoto M, Naganuma M, Matsuoka K, Mizuno S, Kitazume MT. Decreased plasma histidine level predicts risk of relapse in patients with ulcerative colitis in remission. PloS one. 2015;10(10):e0140716.

83. Höie O, Wolters F, Riis L, Aamodt G, Solberg C, Bernklev T, Odes S, Mouzas IA, Beltrami M, Langholz E, Stockbrügger R. Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. Am J Gastroenterol. 2007;102(8):1692-701.

84. Horgan RP, Kenny LC. 'Omic'technologies: genomics, transcriptomics, proteomics and metabolomics. The Obstetrician & Gynaecologist. 2011;13(3):189-95.

85. Hosseini SV, Safarpour AR, Taghavi SA. Developing a novel risk-scoring system for predicting relapse in patients with ulcerative colitis: A prospective cohort study. Pak J Med Sci. 2015;31(6):1511-6.

86. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol. 2011;106(4):563–573.

87. Idle JR, Gonzalez FJ. Metabolomics. Cell Metab. 2007;6(5):348-51.

88. Irvine EJ, Zhou Q, Thompson AK. The Short Inflammatory Bowel Disease Questionnaire: a quality of life instrument for community physicians managing inflammatory bowel disease. CCRPT Investigators. Canadian Crohn's Relapse Prevention Trial. Am J Gastroenterol. 1996;91(8):1571-8.

89. Jawed H, Shah SU, Jamall S, Simjee SU. N-(2-hydroxy phenyl) acetamide inhibits inflammation-related cytokines and ROS in adjuvant-induced arthritic (AIA) rats. Int Immunopharmacol. 2010;10(8):900-5.

90. Jeffery IB, O'Toole PW, Öhman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut. 2012;61(7):997-1006.

91. Jian L, Anqi H, Gang L, Litian W, Yanyan X, Mengdi W, Tong L. Food Exclusion Based on IgG Antibodies Alleviates Symptoms in Ulcerative Colitis: A Prospective Study. Inflamm Bowel Dis. 2018. doi: 10.1093/ibd/izy110.

92. Johansson-Persson A, Barri T, Ulmius M, Onning G, Dragsted LO. LC-QTOF/MS metabolomic profiles in human plasma after a 5-week high dietary fiber intake. Anal Bioanal Chem. 2013;405(14):4799-809.

93. John S, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. Eur J Gastroenterol Hepatol. 2010;22(5):602-6.

94. Jowett SL, Seal CJ, Pearce MS, Phillips E, Gregory W, Barton JR, Welfare MR. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. Gut. 2004;53(10):1479-84.

95. Joyce MR, Hannaway CD, Strong SA, et al. Impact of smoking on disease phenotype and postoperative outcomes for Crohn's disease patients undergoing surgery. Langenbecks Arch Surg. 2013; 398:39-45.

96. Jung HK, Kim YH, Park JY, Jang BH, Park SY, Nam MH, Choi MG. Estimating the burden of irritable bowel syndrome: analysis of a nationwide korean database. J Neurogastroenterol Motil. 2014;20(2):242.

97. Kaplan GG, Bernstein CN, Coward S, Bitton A, Murthy SK, Nguyen GC, Lee K, Cooke-Lauder J, Benchimol EI. The Impact of Inflammatory Bowel Disease in Canada 2018: Epidemiology. J Can Assoc Gastroenterol. 2019;2(Suppl 1):S6-S16.

98. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol. 2015;12(12):720-7.

99. Keshteli AH, Madsen KL, Dieleman L. Diet in the Pathogenesis and Management of Ulcerative Colitis; A Review of Randomized Controlled Dietary Interventions. Nutrients. 2019;11(7). pii: E1498.

100. Keshteli AH, Madsen KL, Mandal R, Boeckxstaens GE, Bercik P, De Palma G, Reed DE, Wishart D, Vanner S, Dieleman LA. Comparison of the metabolomic profiles of irritable

bowel syndrome patients with ulcerative colitis patients and healthy controls: new insights into pathophysiology and potential biomarkers. Aliment Pharmacol Ther. 2019;49(6):723-732.

101. Keshteli AH, Tso R, Dieleman LA, Park H, Kroeker KI, Jovel J, Gillevet PM, Sikaroodi M, Mandal R, Fedorak RN, Madsen KL. A Distinctive Urinary Metabolomic Fingerprint Is Linked With Endoscopic Postoperative Disease Recurrence in Crohn's Disease Patients. Inflamm Bowel Dis. 2018;24(4):861-870.
102. Keshteli AH, van den Brand FF, Madsen KL, et al. Dietary and metabolomic determinants of relapse in ulcerative colitis patients: A pilot prospective cohort study. World J Gastroenterol. 2017; 23:3890-3899.
103. Khalili H, Ananthakrishnan AN, Konijeti GG, Higuchi LM, Fuchs CS, Richter JM, Chan AT. Measures of obesity and risk of Crohn’s disease and ulcerative colitis. Inflamm Bowel Dis. 2015;21(2):361-8.
104. Khalili H, Chan SSM, Lochhead P, Ananthakrishnan AN, Hart AR, Chan AT. The role of diet in the aetiopathogenesis of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2018;15(9):525-535.
105. Khan I, Ullah N, Zha L, Bai Y, Khan A, Zhao T, Che T, Zhang C. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome. Pathogens. 2019;8(3). pii: E126.
106. Khayyatzadeh SS, Esmaillzadeh A, Saneei P, et al. Dietary patterns and prevalence of irritable bowel syndrome in Iranian adults. Neurogastroenterol Motil. 2016;28(12):1921-1933.

107. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474(7351):307-17.

108. Koleva P, Ketabi A, Valcheva R, Gänzle MG, Dieleman LA. Chemically defined diet alters the protective properties of fructo-oligosaccharides and isomalto-oligosaccharides in HLA-B27 transgenic rats. PLoS One. 2014;9(11):e111717.

109. Kuenzig ME, Benchimol EI, Lee L, Targownik LE, Singh H, Kaplan GG, Bernstein CN, Bitton A, Nguyen GC, Lee K, Cooke-Lauder J. The impact of inflammatory bowel disease in Canada 2018: Direct costs and health services utilization. J Can Assoc Gastroenterol. 2019; 2(Suppl 1):S17-S33.

110. Kuenzig ME, Lee L, El-Matary W, Weizman AV, Benchimol EI, Kaplan GG, Nguyen GC, Bernstein CN, Bitton A, Lee K, Cooke-Lauder J. The Impact of Inflammatory Bowel Disease in Canada 2018: Indirect Costs of IBD Care. J Can Assoc Gastroenterol. 2019; 2(Suppl 1):S34-S41.

111. Kyaw MH, Moshkovska T, Mayberry J. A prospective, randomized, controlled, exploratory study of comprehensive dietary advice in ulcerative colitis: impact on disease activity and quality of life. Eur J Gastroenterol Hepatol. 2014;26(8):910-7.

112. Le Gall G, Noor SO, Ridgway K, et al. Metabolomics of fecal extracts detects altered metabolic activity of gut microbiota in ulcerative colitis and irritable bowel syndrome. J Proteome Res. 2011;10(9):4208-18.

113. Lee C, Doo E, Choi JM, et al. The Increased Level of Depression and Anxiety in Irritable Bowel Syndrome Patients Compared with Healthy Controls: Systematic Review and Meta-analysis. J Neurogastroenterol Motil. 2017;23(3):349-362.

114. Lee D, Albenberg L, Compher C, et al. Diet in the pathogenesis and treatment of inflammatory bowel diseases. Gastroenterology. 2015; 148:1087-106.

115. Lee YT, Hu LY, Shen CC, et al. Risk of Psychiatric Disorders following Irritable Bowel Syndrome: A Nationwide Population-Based Cohort Study. PLoS One. 2015;10(7):e0133283.

116. Levine A, Sigall Boneh R, Wine E. Evolving role of diet in the pathogenesis and treatment of inflammatory bowel diseases. Gut. 2018;67(9):1726-1738.

117. Levine JS, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. Gastroenterol Hepatol (N Y). 2011;7(4):235-41.

118. Ley SH, Sun Q, Willett WC, Eliassen AH, Wu K, Pan A, Grodstein F, Hu FB. Associations between red meat intake and biomarkers of inflammation and glucose metabolism in women. Am J Clin Nutr. 2014;99(2):352-60.

119. Li F, Liu X, Wang W, Zhang D. Consumption of vegetables and fruit and the risk of inflammatory bowel disease: a meta-analysis. Eur J Gastroenterol Hepatol. 2015;27(6):623-30.

120. Lim HS, Kim SK, Hong SJ. Food Elimination Diet and Nutritional Deficiency in Patients with Inflammatory Bowel Disease. Clin Nutr Res. 2018;7(1):48-55.

121. Limdi JK, Aggarwal D, McLaughlin JT. Dietary Practices and Beliefs in Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis. 2016;22(1):164-70.

122. Limketkai BN, Iheozor-Ejiofor Z, Gjuladin-Hellon T, Parian A, Matarese LE, Bracewell K, MacDonald JK, Gordon M, Mullin GE. Dietary interventions for induction and

maintenance of remission in inflammatory bowel disease. Cochrane Database Syst Rev. 2019;2:CD012.

123. Liu G, Xiao L, Fang T, Cai Y, Jia G, Zhao H, Wang J, Chen X, Wu C. Pea fiber and wheat bran fiber show distinct metabolic profiles in rats as investigated by a 1H NMR-based metabolomic approach. PLoS One. 2014;9(12):e115561.

124. Liu G, Yang G, Fang T, Cai Y, Wu C, Wang J, Huang Z, Chen X. NMR-based metabolomic studies reveal changes in biochemical profile of urine and plasma from rats fed with sweet potato fiber or sweet potato residue. RSC advances. 2014;4(45):23749-58.

125. Liverani E, Scaioli E, Digby RJ, Bellanova M, Belluzzi A. How to predict clinical relapse in inflammatory bowel disease patients. World J Gastroenterol. 2016;22(3):1017-33.

126. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ, Casero D, Courtney H, Gonzalez A, Graeber TG, Hall AB, Lake K, Landers CJ, Mallick H, Plichta DR, Prasad M, Rahnavard G, Sauk J, Shungin D, Vázquez-Baeza Y, White RA 3rd; IBDMDB Investigators, Braun J, Denson LA, Jansson JK, Knight R, Kugathasan S, McGovern DPB, Petrosino JF, Stappenbeck TS, Winter HS, Clish CB, Franzosa EA, Vlamakis H, Xavier RJ, Huttenhower C. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;569(7758):655-662.

127. Loddo I, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. Front Immunol. 2015;6:551.

128. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterology. 2006;130(5):1480-91

129. Longstreth GF, Yao JF. Irritable bowel syndrome and surgery: a multivariable analysis. Gastroenterology. 2004;126(7):1665-73.

130. Lopetuso LR, Petito V, Graziani C, et al. Gut Microbiota in Health, Diverticular Disease, Irritable Bowel Syndrome, and Inflammatory Bowel Diseases: Time for Microbial Marker of Gastrointestinal Disorders. Dig Dis. 2018;36(1):56-65.

131. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10(7):712-721.e4.

132. Lucak S, Chang L, Halpert A, Harris LA. Current and emergent pharmacologic treatments for irritable bowel syndrome with diarrhea: evidence-based treatment in practice. Therap Adv Gastroenterol. 2017;10(2):253-275.

133. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, Ballet V, Claes K, Van Immerseel F, Verbeke K, Ferrante M, Verhaegen J, Rutgeerts P, Vermeire S. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut. 2014;63(8):1275-83.

134. Magro F, Vieira-Coelho MA, Fraga S, et al. Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease. Dig Dis Sci. 2002; 47:216-24.

135. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut. 2006;55(2):205-11.

136. Martin TD, Chan SS, Hart AR. Environmental factors in the relapse and recurrence of inflammatory bowel disease: a review of the literature. Dig Dis Sci. 2015;60(5):1396-405.

137. Mashima R, Nakanishi-Ueda T, Yamamoto Y. Simultaneous determination of methionine sulfoxide and methionine in blood plasma using gas chromatography-mass spectrometry. Anal Biochem. 2003;313:28-33.

138. McIlroy J, Ianiro G, Mukhopadhya I, Hansen R, Hold GL. Review article: the gut microbiome in inflammatory bowel disease-avenues for microbial management. Aliment Pharmacol Ther. 2018;47(1):26-42.

139. McIntosh K, Reed DE, Schneider T, Dang F, Keshteli AH, De Palma G, Madsen K, Bercik P, Vanner S. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut. 2017;66(7):1241-51.

140. Mearin F, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. Gastroenterology. 2016. pii: S0016-5085(16)00222-5.

141. Menni C, Zhai G, Macgregor A, et al. Targeted metabolomics profiles are strongly correlated with nutritional patterns in women. Metabolomics. 2013;9(2):506-514.

142. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 2015;43:W566–70.

143. Mickiewicz B, Tam P, Jenne CN, et al. Integration of metabolic and inflammatory mediator profiles as a potential prognostic approach for septic shock in the intensive care unit. Crit Care. 2015; 19:1.

144. Miyoshi J, Chang EB. The gut microbiota and inflammatory bowel diseases. Transl Res. 2017;179:38-48.

145. Moloney RD, Johnson AC, O'Mahony SM, et al. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. CNS Neurosci Ther. 2016;22(2):102-17.

146. Mondot S, Lepage P, Seksik P, et al. Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. Gut. 2016; 65:954-62.

147. Morgan XC, Segata N, Huttenhower C. Biodiversity and functional genomics in the human microbiome. Trends Genet. 2013;29(1):51-8.

148. Moss AC. Prevention of postoperative recurrence of Crohn's disease: what does the evidence support? Inflamm Bowel Dis. 2013; 19:856-9.

149. Mozaffari H, Daneshzad E, Larijani B, Bellissimo N, Azadbakht L. Dietary intake of fish, n-3 polyunsaturated fatty acids, and risk of inflammatory bowel disease: a systematic review and meta-analysis of observational studies. Eur J Nutr. 2019. doi: 10.1007/s00394-019-01901-0.

150. Muri EM, Williamson JS. Anti-Helicobacter pylori agents. An update. Mini Rev Med Chem. 2004;4(2):201-6.

151. Nakanishi M, Matz A, Klemashevich C, Rosenberg DW. Dietary Walnut Supplementation Alters Mucosal Metabolite Profiles During DSS-Induced Colonic Ulceration. Nutrients. 2019;11(5). pii: E1118.

152. National Cancer Institute. Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool. https://epi.grants.cancer.gov/asa24/. [Accessed September 5, 2019]

153. National Institutes of Health, Epidemiology and Genomics Research Program, National Cancer Institute. Diet History Questionnaire II, Version 2.0. 2010. http://www.epi.grants.cancer.gov/dhq2 (accessed April 2017)

154. Neis EP, van Eijk HM, Lenaerts K, Olde Damink SW, Blaak EE, Dejong CH, Rensen SS. Distal versus proximal intestinal short-chain fatty acid release in man. Gut. 2019;68(4):764-765.

155. Nellesen D, Yee K, Chawla A, Lewis BE, Carson RT. A systematic review of the economic and humanistic burden of illness in irritable bowel syndrome and chronic constipation. J Manag Care Pharm. 2013;19(9):755-64.

156. Ng SC, Kamm MA. Management of postoperative Crohn's disease. Am J Gastroenterol. 2008; 103:1029-35.

157. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JC, Chan FK, Sung JJ. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. The Lancet. 2017;390(10114):2769-78.

158. Nie JY, Zhao Q. Beverage consumption and risk of ulcerative colitis: Systematic review and meta-analysis of epidemiological studies. Medicine (Baltimore). 2017;96(49):e9070.

159. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11(1):1-10.

160. Ooi M, Nishiumi S, Yoshie T, et al. GC/MS-based profiling of amino acids and TCA cycle-related molecules in ulcerative colitis. Inflamm Res. 2011;60(9):831-40.

161. Osaka T, Moriyama E, Arai S, Date Y, Yagi J, Kikuchi J, Tsuneda S. Meta-Analysis of Fecal Microbiota and Metabolites in Experimental Colitic Mice during the Inflammatory and Healing Phases. Nutrients. 2017;9(12). pii: E1329.

162. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, Paramsothy R, Walsh AJ, van den Bogaerde J, Samuel D, Leong RWL, Connor S, Ng W, Lin E, Borody TJ, Wilkins MR, Colombel JF, Mitchell HM, Kaakoush NO. Specific Bacteria and Metabolites Associated With Response to Fecal Microbiota Transplantation in Patients With Ulcerative Colitis. Gastroenterology. 2019;156(5):1440-1454.e2.

163. Park JH, Rhee PL, Kim G, Lee JH, Kim YH, Kim JJ, Rhee JC, Song SY. Enteroendocrine cell counts correlate with visceral hypersensitivity in patients with diarrhoea-predominant irritable bowel syndrome. Neurogastroenterol Motil. 2006;18(7):539-46.

164. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Doré J, Mattila I, Plichta DR, Pöhö P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trošt K; MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376-81.

165. Pedersen N, Ankersen DV, Felding M, Wachmann H, Végh Z, Molzen L, Burisch J, Andersen JR, Munkholm P. Low-FODMAP diet reduces irritable bowel symptoms in patients with inflammatory bowel disease. World J Gastroenterol. 2017;23(18):3356-3366.

166. Perez-Lopez A, Behnsen J, Nuccio SP, Raffatellu M. Mucosal immunity to pathogenic intestinal bacteria. Nat Rev Immunol. 2016;16(3):135-48.

167. Perry T, Jovel J, Patterson J, et al. Fecal Microbial Transplant After Ileocolic Resection Reduces Ileitis but Restores Colitis in IL-10-/- Mice. Inflamm Bowel Dis. 2015; 21:1479-90.

168. Peyrin-Biroulet L, Loftus EV Jr, Colombel JF, et al. The natural history of adult Crohn's disease in population-based cohorts. Am J Gastroenterol. 2010;105:289-97.

169. Piovani D, Danese S, Peyrin-Biroulet L, Nikolopoulos GK, Lytras T, Bonovas S. Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. Gastroenterology. 2019;157(3):647-659.e4.

170. Ponder A, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin Epidemiol. 2013;5:237-47.

171. Probert F, Walsh A, Jagielowicz M, Yeo T, Claridge TDW, Simmons A, Travis S, Anthony DC. Plasma Nuclear Magnetic Resonance Metabolomics Discriminates Between High and Low Endoscopic Activity and Predicts Progression in a Prospective Cohort of Patients With Ulcerative Colitis. J Crohns Colitis. 2018;12(11):1326-1337.

172. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS. The human serum metabolome. PLoS One. 2011;6(2):e16957.

173. Qiu Y, Mao R, Chen BL, et al. Fecal calprotectin for evaluating postoperative recurrence of Crohn's disease: a meta-analysis of prospective studies. Inflamm Bowel Dis. 2015; 21:315-22.

174. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019;94(1):155-165.

175. Rasmussen LG, Winning H, Savorani F, Toft H, Larsen TM, Dragsted LO, Astrup A, Engelsen SB. Assessment of the effect of high or low protein diet on the human urine metabolome as measured by NMR. Nutrients. 2012;4(2):112-31.

176. Reddavide R, Rotolo O, Caruso MG, Stasi E, Notarnicola M, Miraglia C, Nouvenne A, Meschi T, De' Angelis GL, Di Mario F, Leandro G. The role of diet in the prevention and treatment of Inflammatory Bowel Diseases. Acta Biomed. 2018;89(9-S):60-75.

177. Reich KM, Fedorak RN, Madsen K, Kroeker KI. Vitamin D improves inflammatory bowel disease outcomes: basic science and clinical review. World J Gastroenterol. 2014;20(17):4934-47.

178. Rizzello F, Spisni E, Giovanardi E, Imbesi V, Salice M, Alvisi P, Valerii MC, Gionchetti P. Implications of the Westernized Diet in the Onset and Progression of IBD. Nutrients. 2019;11(5). pii: E1033.

179. Roberts LD, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. Curr Protoc Mol Biol. 2012;Chapter 30:Unit 30.2.1-24.

180. Rocchi A, Benchimol EI, Bernstein CN, Bitton A, Feagan B, Panaccione R, Glasgow KW, Fernandes A, Ghosh S. Inflammatory bowel disease: a Canadian burden of illness review. Can J Gastroenterol. 2012;26(11):811-7.

181. Roy A, Pollack S. Are cerebrospinal fluid or urinary monoamine metabolite measures stronger correlates of suicidal behavior in depression? Neuropsychobiology. 1994;29(4):164-7.

182. Ruemmele FM. Role of Diet in Inflammatory Bowel Disease. Ann Nutr Metab. 2016;68 Suppl 1:33-41.

183. Rutgeerts P, Geboes K, Vantrappen G, et al. Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. Gut 1984; 25:665-72.

184. Ryan D, Robards K, Prenzler PD, et al. Recent and potential developments in the analysis of urine: a review. Anal Chim Acta. 2011;684(1-2):8-20.

185. Scaioli E, Liverani E, Belluzzi A. The Imbalance between n-6/n-3 Polyunsaturated Fatty Acids and Inflammatory Bowel Disease: A Comprehensive Review and Future Therapeutic Perspectives. Int J Mol Sci. 2017;18(12).

186. Schicho R, Nazyrova A, Shaykhutdinov R, Duggan G, Vogel HJ, Storr M. Quantitative metabolomic profiling of serum and urine in DSS-induced ulcerative colitis of mice by (1)H NMR spectroscopy. J Proteome Res. 2010;9(12):6265-73.

187. Schwerd T, Frivolt K, Clavel T, Lagkouvardos I, Katona G, Mayr D, Uhlig HH, Haller D, Koletzko S, Bufler P. Exclusive enteral nutrition in active pediatric Crohn disease: Effects on intestinal microbiota and immune regulation. J Allergy Clin Immunol. 2016;138(2):592-6.

188. Scott KP, Martin JC, Duncan SH, Flint HJ. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. FEMS Microbiol Ecol. 2014;87(1):30-40.

189. Scoville EA, Allaman MM, Adams DW, Motley AK, Peyton SC, Ferguson SL, Horst SN, Williams CS, Beaulieu DB, Schwartz DA, Wilson KT, Coburn LA. Serum Polyunsaturated Fatty Acids Correlate with Serum Cytokines and Clinical Disease Activity in Crohn's Disease. Sci Rep. 2019;9(1):2882.

190. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011; 12:R60.

191. Serra-Majem L, Roman-Vinas B, Sanchez-Villegas A, Guasch-Ferre M, Corella D, La Vecchia C. Benefits of the Mediterranean diet: Epidemiological and molecular aspects. Mol Aspects Med. 2019 Jun;67:1-55. doi: 10.1016/j.mam.2019.06.001.

192. Sethi S, Wadhwa V, Leclair J, Mikami S, Park R, Jones M, Sethi N, Brown A, Lembo A. In-patient discharge rates for the irritable bowel syndrome–an analysis of national trends in the United States from 1997 to 2010. Aliment Pharmacol Ther. 2013 Dec;38(11-12):1338-46.

193. Shankar V, Reo NV, Paliy O. Simultaneous fecal microbial and metabolite profiling enables accurate classification of pediatric irritable bowel syndrome. Microbiome. 2015;3:73.

194. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.

195. Sharabi A, Cohen E, Sulkes J, et al. Replacement therapy for vitamin B12 deficiency: comparison between the sublingual and oral route. Br J Clin Pharmacol. 2003;56(6):635-8.

196. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr. 2014;17(8):1689-96.

197. Sido B, Hack V, Hochlehnert A, Lipps H, Herfarth C, Dröge W. Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. Gut. 1998;42(4):485-92.

198. Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol. 2015;50(1):74-80.

199. Solakivi T, Kaukinen K, Kunnas T, et al. Serum fatty acid profile in subjects with irritable bowel syndrome. Scand J Gastroenterol. 2011;46(3):299-303.

200. Sood R, Ford AC. Diagnosis: Rome IV criteria for FGIDs - an improvement or more of the same? Nat Rev Gastroenterol Hepatol. 2016;13(9):501-2.

201. Sood R, Law GR, Ford AC. Diagnosis of IBS: symptoms, symptom-based criteria, biomarkers or 'psychomarkers'? Nat Rev Gastroenterol Hepatol. 2014;11(11):683-91.

202. Soria-Contreras DC, Bell RC, McCargar LJ, Chan CB. Feasibility and efficacy of menu planning combined with individual counselling to improve health outcomes and dietary adherence in people with type 2 diabetes: a pilot study. Can J Diabetes. 2014;38(5):320-5.

203. Soubières AA, Poullis A. Emerging Biomarkers for the Diagnosis and Monitoring of Inflammatory Bowel Diseases. Inflamm Bowel Dis. 2016; 22:2016-22.

204. Spiller R, Aziz Q, Creed F, et al. Guidelines on the irritable bowel syndrome: mechanisms and practical management. Gut. 2007;56(12):1770-98.

205. Stephens NS, Siffledeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. J Crohns Colitis. 2013;7(2):e42-8.

206. Strisciuglio C, Giannetti E, Martinelli M, Sciorio E, Staiano A, Miele E. Does cow's milk protein elimination diet have a role on induction and maintenance of remission in children with ulcerative colitis? Acta Paediatr. 2013;102(6):e273-8.

207. Sturniolo GC, Di Leo V, Ferronato A, D'Odorico A, D'Incà R. Zinc supplementation tightens "leaky gut" in Crohn's disease. Inflamm Bowel Dis. 2001;7(2):94-8.

208. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, McIntosh A, Rosenfeld S. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires : the Eating at America's Table Study. Am J Epidemiol. 2001;154(12):1089-99.

209. Sugihara K, Morhardt TL, Kamada N. The Role of Dietary Nutrients in Inflammatory Bowel Disease. Front Immunol. 2019;9:3183.

210. Sun M, Wu W, Liu Z, Cong Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J Gastroenterol. 2017;52(1):1-8.

211. Sun YV, Hu YJ. Integrative Analysis of Multi-omics Data for Discovery and Functional Studies of Complex Human Diseases. Adv Genet. 2016;93:147-90.

212. Tana C, Umesaki Y, Imaoka A, et al. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. Neurogastroenterol Motil. 2010;22(5):512-9, e114-5.

213. Tappel A. Heme of consumed red meat can act as a catalyst of oxidative damage and could initiate colon, breast and prostate cancers, heart disease and other diseases. Med Hypotheses. 2007;68(3):562-4.

214. Theede K, Holck S, Ibsen P, Kallemose T, Nordgaard-Lassen I, Nielsen AM. Fecal Calprotectin Predicts Relapse and Histological Mucosal Healing in Ulcerative Colitis. Inflamm Bowel Dis. 2016;22(5):1042-8.

215. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-1770.

216. van Bussel BC, Henry RM, Ferreira I, van Greevenbroek MM, van der Kallen CJ, Twisk JW, Feskens EJ, Schalkwijk CG, Stehouwer CD. A healthy diet is associated with less endothelial dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular disease. J Nutr. 2015;145(3):532-40.

217. van den Berg R, Mook-Kanamori DO, Donga E, et al. A single night of sleep curtailment increases plasma acylcarnitines: Novel insights in the relationship between sleep and insulin resistance. Arch Biochem Biophys. 2016;589:145-51.

218. Vázquez-Baeza Y, Pirrung M, Gonzalez A, et al. EMPeror: a tool for visualizing high-throughput microbial community data. Gigascience. 2013; 2:16.

219. Vedamurthy A, Ananthakrishnan AN. Influence of Environmental Factors in the Development and Outcomes of Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y). 2019;15(2):72-82.

220. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut. 2006;55(3):426-31.

221. Vuitton L, Koch S, Peyrin-Biroulet L. Preventing postoperative recurrence in Crohn's disease: what does the future hold? Drugs. 2013; 73:1749-59.

222. Wall R, Ross RP, Shanahan F, et al. Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. Am J Clin Nutr. 2009;89(5):1393-401.

223. Wallace KL, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. World J Gastroenterol. 2014;20(1):6-21.

224. Walton C, Fowler DP, Turner C, et al. Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases. Inflamm Bowel Dis. 2013;19(10):2069-78.

225. Wang F, Feng J, Gao Q, Ma M, Lin X, Liu J, Li J, Zhao Q. Carbohydrate and protein intake and risk of ulcerative colitis: Systematic review and dose-response meta-analysis of epidemiological studies. Clin Nutr. 2017;36(5):1259-1265.

226. Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007; 73:5261-7.

227. Weiner ML, McKim JM. Comment on "Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods?". Food Funct. 2019;10(3):1760-1762.

228. Weng YJ, Gan HY, Li X, Huang Y, Li ZC, Deng HM, Chen SZ, Zhou Y, Wang LS, Han YP, Tan YF, Song YJ, Du ZM, Liu YY, Wang Y, Qin N, Bai Y, Yang RF, Bi YJ, Zhi FC. Correlation of diet, microbiota and metabolite networks in inflammatory bowel disease. J Dig Dis. 2019;20(9):447-459.

229. Werner T, Wagner SJ, Martínez I, Walter J, Chang JS, Clavel T, Kisling S, Schuemann K, Haller D. Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. Gut. 2011;60(3):325-33.

230. Wijaya CS, Lee JJZ, Husain SF, et al. Differentiating Medicated Patients Suffering from Major Depressive Disorder from Healthy Controls by Spot Urine Measurement of Monoamines and Steroid Hormones. Int J Environ Res Public Health. 2018;15(5).

231. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A. 2009;106(10):3698-703.

232. Williamson G, Clifford MN. Colonic metabolites of berry polyphenols: the missing link to biological activity? Br J Nutr. 2010;104 Suppl 3:S48-66.

233. Wishart DS. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol Rev. 2019;99(4):1819-1875.

234. Wouters MM, Balemans D, Van Wanrooy S, Dooley J, Cibert-Goton V, Alpizar YA, Valdez-Morales EE, Nasser Y, Van Veldhoven PP, Vanbrabant W, Van der Merwe S. Histamine Receptor H1-Mediated Sensitization of TRPV1 Mediates Visceral Hypersensitivity and Symptoms in Patients With Irritable Bowel Syndrome. Gastroenterology. 2016;150(4):875-87.e9.

235. Wouters MM, Vicario M, Santos J. The role of mast cells in functional GI disorders. Gut. 2016;65(1):155-68.

236. Wright EK, De Cruz P, Gearry R, Day AS, Kamm MA. Fecal biomarkers in the diagnosis and monitoring of Crohn's disease. Inflamm Bowel Dis. 2014; 20:1668-77.

237. Wright EK, Kamm MA, De Cruz P, Hamilton AL, Ritchie KJ, Krejany EO, Leach S, Gorelik A, Liew D, Prideaux L, Lawrance IC. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. Gastroenterology. 2015; 148:938-947.e1.

238. Wright EK, Kamm MA, Wagner J, Teo SM, Cruz P, Hamilton AL, Ritchie KJ, Inouye M, Kirkwood CD. Microbial Factors Associated with Postoperative Crohn's Disease Recurrence. J Crohns Colitis. 2017; 11:191-203.

239. Wright R, Truelove SC. A controlled therapeutic trial of various diets in ulcerative colitis. Br Med J. 1965;2(5454):138-41.

240. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res. 2015;43(W1):W251-7.

241. Yamamoto T, Shimoyama T, Umegae S, Matsumoto K. Serial monitoring of faecal calprotectin for the assessment of endoscopic recurrence in asymptomatic patients after ileocolonic resection for Crohn's disease: a long-term prospective study. Therap Adv Gastroenterol. 2016; 9:664-70.

242. Yu LC. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. J Biomed Sci. 2018;25(1):79.

243. Zaribaf F, Keshteli AH, Esmaillzadeh A, Saneei P, Feizi A, Daghaghzadeh H, Feinle-Bisset C, Adibi P. Empirically derived dietary habits are associated with irritable bowel syndrome. Eur J Clin Nutr. 2018;72(11):1537-1547.

244. Zenlea T, Yee EU, Rosenberg L, Boyle M, Nanda KS, Wolf JL, Falchuk KR, Cheifetz AS, Goldsmith JD, Moss AC. Histology grade is independently associated with relapse risk in

patients with ulcerative colitis in clinical remission: a prospective study. Am J Gastroenterol. 2016;111(5):685-90.

245. Zhang Y, Lin L, Xu Y, Lin Y, Jin Y, Zheng C. 1H NMR-based spectroscopy detects metabolic alterations in serum of patients with early-stage ulcerative colitis. Biochem Biophys Res Commun. 2013;433(4):547-51.

246. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol. 2014;20(1):91-9.

247. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorndahl TC, Bouatra S, Anderson T, Oudit GY, Wishart DS, Dyck JR; Alberta HEART. Metabolomic fingerprint of heart failure with preserved ejection fraction. PLoS One. 2015 May;10(5):e0124844.