Proposal: A Cross-Over Trial Assessing the Impact of a Gluten Free Diet on Crohn's Disease Activity

by

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Submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Dietetics
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December, 2015

Abstract

The role of diet has become very important in the management of Crohn's Disease (CD), however, there is a general lack of rigorous scientific evidence that demonstrates which diet is best for certain patients. This project is a research proposal for a study to assess the effectiveness of a gluten-free diet on CD activity. The hypothesis is that the gluten-free diet will be effective in improving symptoms and inflammation for some subjects, particularly those with the HLA-DQ2/8 genotype, which has recently been linked to CD outside of celiac disease. It has also been postulated that gluten might have a direct impact on intestinal barrier function and the mucosal immune system in patients with CD with the HLA-DQ2/8 genotype. Studying dietary interventions, such as a gluten-free diet, which has the potential to improve symptoms and inflammation in CD patients, can make it possible for a greater number of patients to receive evidence-based dietary recommendations that can potentially improve their disease course and downscale their medication regimen.

Acknowledgements

This project wouldn't have been possible without the unparalleled support of Dr. Megan Baumler. Thank you for all of your feedback and guidance throughout this paper. I don't what I would have done without your encouragement and support. Thanks also to Dr. Tara LaRowe for your feedback and support. Lastly, I'd also like to thank my family for loving and supporting me throughout this process. I would have not survived without you.

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Chapter 1: Introduction

Crohn's disease (CD) is a lifelong, chronic disorder of the gastrointestinal (GI) tract characterized by destructive inflammation and frequent disease relapses and remissions. Currently, CD can be treated but not cured, and there is no standard treatment for managing all individuals with CD. Approximately 780,000 Americans currently have CD and the total number of new cases diagnosed in the United States each year is 10.7 per 100,000 people, or approximately 33,000 new cases per year (Loftus et al., 2014). When CD is active, it can have significant impact on quality of life for patients due to flare-ups and complications.

The exact cause of CD is unknown, but multiple factors play a role in its pathogenesis, including immunologic factors, environmental factors, genetic predisposition, and alteration of the intestinal microbiome. Diet can affect symptoms of the disease and has also long been implicated as a contributing factor for CD flare-ups (Herfarth et al., 2014). Furthermore, diet may play a role in the underlying inflammatory process through several biologically plausible mechanisms, including antigen presentation, change in prostaglandin balance, and alteration of the microflora (Hou et al., 2011). Dietary whole food recommendations for CD are poorly developed and the investigation of nutritional approaches in treating CD has been largely limited to the use of enteral and total parental nutrition with the aim of providing bowel rest (Olendzki et al., 2014).

With a lack of specific dietary recommendations to offer CD patients, more research investigating diet's impact on CD should be a priority. This study proposal will examine the effect of a gluten free (GF) diet on CD activity and symptoms. A validated tool, the Harvey Bradshaw Index (HBI), will be used to measure changes in disease activity, while a subjective assessment of symptoms, including abdominal pain, bloating, diarrhea, constipation, urgency, bleeding, and fatigue will be used to measure changes in symptoms. Additionally, C-reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR) will serve as measures to assess changes in inflammation and fecal samples will be collected for fecal analysis to assess changes in the gut microbiota. Finally, genetic testing for the HLA-DQ2/8 alleles will be done to determine if this genotype is necessary for the gluten free diet to be impactful. These haplotypes are better known for their gluten dependent relationship with celiac disease; however, recent research has shown that the presence of these alleles by themselves, outside of celiac disease, is associated with CD (DiGiacomo et al., 2013). Several studies have shown that patients with diarrhea predominant Irritable Bowel Syndrome (IBS) who are carriers of HLA-DQ2/8, (but do not have celiac disease) respond favorably to a gluten free diet (Wahnschaffe et al., 2007; Vazquez-Roque et al., 2012; Vazquez-Roque et al., 2013). It has also been postulated that gluten might have a direct impact on intestinal barrier function and the mucosal immune system in patients with CD with the HLA-DQ2/8 genotype (Verdu et al., 2009).

This study could potentially provide clinicians with evidence-based recommendations to either favor or disregard trialing a GF diet in patients with CD. This study may also help determine if the HLA-DQ2/8 genotype is necessary for a gluten free diet to be impactful in individuals with CD. Additionally, this study could potentially

open up new research avenues examining diet's impact on CD symptoms, changes in the microbiota, and possibly the mechanism of action in which diet can worsen or improve intestinal inflammation.

Hypotheses

A GF diet will improve symptoms and inflammation associated with CD compared to standard diet.

Subproblems

1. Is the genotype HLA-DQ2/8 necessary for GF diet to be effective?
2. Will a GF diet change the composition and complexity of the gut microbiota in patients with CD?
3. Will a GF diet decrease the need for medications, whether it be total elimination of certain medications and/or decrease in dosages?

Limitations

1. Changes in symptoms will be based on subjective assessment.
2. The Harvey Bradshaw Index (HBI) does not correlate with the extent of ielocolonic inflammation.

Delimitations

1. This study will only include patients with mild to moderate CD.
2. The study will only examine the effect of gluten on CD, and no other dietary components.

Assumptions

1. Methodology is appropriate for the research question being addressed.

2. The intervention (GF diet) will result in improved outcomes for the population group (CD subjects).
3. This topic of study is a common issue of concern to dietetics practice, as well as gastroenterology practice.
4. The intervention is feasible and subjects will be compliant to diet assigned.
5. Results of this study will be meaningful and valuable to the fields of dietetics and gastroenterology.

Definition of Terms

Abscess: An enclosed collection of liquefied tissue, known as pus, somewhere in the body. It is the result of the body's defensive reaction to foreign material.

Adhesions: Fibrous bands of scar tissue that form between internal organs and tissues, joining them together abnormally.

Allergenicity: Causing allergic sensitization.

Anal fissure: A small tear in the thin, moist tissue (mucosa) that lines the anus.

Anastomosis: A connection made surgically between adjacent blood vessels, parts of the intestine, or other channels of the body, or the operation in which this is constructed.

Ankylosing spondylitis: Inflammation of the joints in the spine.

Antigen: A toxin or other foreign substance that induces an immune response in the body, especially the production of antibodies.

Aphthous ulcer: A small sensitive painful ulcer crater in the lining of the mouth. Commonly called a canker sore.

Autophagy: A normal physiological process in the body that deals with destruction of cells in the body. It maintains homeostasis or normal functioning by protein degradation and turnover of the destroyed cell organelles for new cell formation.

Biological therapy: Treatment to stimulate or restore the ability of the immune (defense) system to fight infection and disease.

Calprotectin: An abundant neutrophil protein found in both plasma and stool that is markedly elevated in infectious and inflammatory conditions, including inflammatory bowel disease (IBD).

Colectomy: surgical removal of all or part of the colon.

Colonoscopy: A medical procedure where a long, flexible, tubular instrument called the colonoscope is used to view the entire inner lining of the colon (large intestine) and the rectum.

Computed topography (CT): Radiography in which a three-dimensional image of a body structure is constructed by computer from a series of plane cross-sectional images made along an axis.

Conjunctivitis: Inflammation or infection of the outer membrane of the eyeball and the inner eyelid.

C-reactive protein (CRP): A blood test that measures the amount of a protein called C-reactive protein in your blood. C-reactive protein measures general levels of inflammation in your body.

Crohn's (granulomatous) colitis: Crohn's disease of the colon.

Cutaneous: Of, relating to, or affecting the skin.

Cytokine: Any of a number of substances, such as interferon, interleukin, and growth factors, that are secreted by certain cells of the immune system and have an effect on other cells.

Dysbiosis: An unhealthy change in the normal bacterial ecology of a part of body, e.g., of the intestines or the oral cavity.

Dysplasia: An abnormality of development; in pathology, alteration in size, shape, and organization of adult cell.

Edema: A condition characterized by an excess of watery fluid collecting in the cavities or tissues of the body.

Elemental formula: A formula that contains individual amino acids, is low in fat, especially long chain triglycerides, and as such, is thought to require minimal digestive function.

Endoscopy: Examination of the inside of the body by using a lighted, flexible instrument called an endoscope.

Episcleritis: Inflammation of the episclera, a thin membrane that covers the white of the eye (sclera).

Erythema nodosum: An inflammatory condition characterized by inflammation of the fat cells under the skin, resulting in tender red nodules or lumps that are usually seen on both shins.

Erythrocyte sedimentation rate (ESR): A blood test that detects and monitors inflammation in the body. It measures the rate at which red blood cells (RBCs) in a test tube separate from blood serum over time, becoming sediment in the bottom of the test tube. The sedimentation rate increases with more inflammation. Also commonly called the sed rate.

Extra-intestinal: Situated or occurring outside the intestines.

Fibrosis: The formation of excessive fibrous tissue, as in a reparative or reactive process.

Flare: An exacerbation of a chronic disease. Sometimes referred to as a flare-up, a flare occurs when symptoms of a disease that has been present for a time suddenly worsen.

Flora: The bacteria and fungi, both normally occurring and pathological, found in or on an organ.

Fistula: A permanent abnormal passageway between two organs in the body or between an organ and the exterior of the body.

FODMAPs: Stands for "**F**ermentable **O**ligo-, **D**i-, **M**ono-saccharides and **P**olyols." Short-chain carbohydrates that some individuals cannot digest.

Gastroduodenal Crohn's Disease: Crohn's disease that affects the stomach and the first part of the duodenum.

Genome: An organism's complete set of DNA, including all of its genes.

Glossitis: Inflammation of the tongue.

Granuloma: A mass of granulation tissue, typically produced in response to infection, inflammation, or the presence of a foreign substance.

Hematochezia: Bright red blood in the stool.

Hygiene hypothesis: A hypothesis that states that exposure to allergens in the environment early in life reduces the risk of developing allergies by boosting immune system activity. Conversely, relatively clean environment in early life would sway the immune system towards allergy-promoting responses.

Hyperemia: An excess of blood in the vessels supplying an organ or other part of the body.

Hyperhomocysteinemia: condition with plasma levels of homocysteine associated with atherosclerosis, and with venous and arterial thrombosis, apparently by damaging endothelial cells.

Ileitis: Crohn's disease affecting only the ileum; inflammation of the ileum.

Ileocolitis: Crohn's disease involving the ileum and the colon.

Ileocolonoscopy: Endoscopic examination of the distal gastrointestinal tract, including the rectum, colon, and terminal ileum.

Ileostomy: A surgical operation in which a piece of the ileum is diverted to an artificial opening in the abdominal wall.

Immunoglobulin G (IgG): A group of antibodies against certain viral infections that circulate in the bloodstream.

Immunomodulator: A chemical agent that modifies the immune response or the functioning of the immune system.

Immunoregulation: The control of specific immune responses and interactions between B and T lymphocytes and macrophages.

Innate immunity: Immunity that occurs naturally as a result of a person's genetic constitution or physiology and does not arise from a previous infection or vaccination.

Intestinal permeability: The result of damage to the intestinal lining, making it less able to protect the internal environment as well as to filter needed nutrients and other biological substances. Also called leaky gut syndrome.

Ischemic: A decrease in the blood supply to a bodily organ, tissue, or part caused by constriction or obstruction of the blood vessels.

Jejunoileitis: Crohn's diseae of the jejunum and ileum; inflammation of the jejunum and ileum.

Lactoferrin: An iron-binding protein found in the granules of neutrophils where it apparently exerts an antimicrobial activity by withholding iron from ingested bacteria and fungi; it also occurs in many secretions and exudates, such as milk, tears, mucus, saliva, and bile.

Lumen: The inner open space or cavity of a tubular organ, as of a blood vessel or an intestine.

Magnetic resonance imaging (MRI): A technique that uses a magnetic field and radio waves to create detailed images of the organs and tissues within the body.

Macrophages: A large white blood cell, occurring principally in connective tissue and in the bloodstream that ingests foreign particles and infectious microorganisms by phagocytosis.

Megaloblastic anemia: A type of anemia characterized by very large red blood cells. In addition to the cells being large, the inner contents of each cell are not completely developed.

Mesentery: A fold of the peritoneum that attaches the stomach, small intestine, pancreas, spleen, and other organs to the posterior wall of the abdomen.

Microbiome: The totality of microorganisms and their collective genetic material present in or on the human body or in another environment.

Microbiota: The microorganisms of a particular site, habitat, or geological period. Also called microbial flora.

Mucosa: Moist tissue that lines certain parts of the inside of the body, including the nose, mouth, lungs, and urinary and digestive tracts.

Nuclear factor-kappa beta (NF-κB): A protein complex that controls transcription of DNA, cytokine production and cell survival.

Peptide: A molecule consisting of 2 or more amino acids.

Peripheral arthritis: Arthritis that affects the large joints of the arms and legs, including the elbows, wrists, knees, and ankles.

Phenotype: Whereas the "genotype" is the genetic makeup of an organism, the phenotype is how genetic and environmental influences come together to create an organism's physical appearance and behavior.

Polymeric formula: A formula that contains intact proteins, complex carbohydrates and mainly long chain triglycerides.

Pouchitis: Acute inflammation of the mucosa of an ileal reservoir or pouch that has been surgically created, usually following total colectomy for inflammatory bowel disease or multiple polyposis.

Primary sclerosing cholangitis: A disease process in which the bile ducts in the liver become inflamed, narrow and prevent bile from flowing properly.

Proctocolectomy: Surgical removal of the rectum together with part or all of the colon.

Prostaglandin: One of a number of hormone-like substances that participate in a wide range of body functions such as the contraction and relaxation of smooth muscle, the dilation and constriction of blood vessels, control of blood pressure, and modulation of inflammation.

Pyoderma gangrenosum: A rare condition that causes large, painful sores (ulcers) to develop on your skin, most often on your legs.

Sacroiliitis: Inflammation of one or both of your sacroiliac joints — the places where your lower spine and pelvis connect.

Semi-elemental formula: A formula that contains peptides of varying chain length, simple sugars, glucose polymers or starch and fat, primarily as medium chain triglycerides.

Serologic: The science that deals with the properties and reactions of serums, especially blood serum.

Serosa: The outermost coat or serous layer of a visceral structure that lies in the body cavities of the abdomen.

Short-chain fatty acids (SCFA): Fatty acids having a chain length up to roughly 6 carbon atoms long. They are produced by bacterial anaerobic fermentation, particularly of dietary carbohydrates, in the large intestine. They are readily absorbed and are metabolized in the liver and muscle tissues, producing energy.

Small bowel follow through (SBFT): An x-ray with contrast dye (usually barium) performed to identify abnormalities in the small bowel.

Small intestinal bacterial overgrowth (SIBO): A condition in which abnormally large numbers of bacteria are present in the small intestine, while the types of bacteria found in the small intestine are more like the bacteria found in the colon.

Spondylarthritis: An umbrella term for inflammatory diseases that involve both the joints and the entheses (the sites where the ligaments and tendons attach to the bones).

Stoma: An opening into the body from the outside that is created by a surgeon.

Stomatitis: Inflammation of the mucous membrane of the mouth.

Stricture: An abnormal narrowing of a body passage, especially a tube or a canal. Stricture refers to both the process of narrowing and the narrowed part itself.

Strictureplasty: A surgical technique for treating or opening a bowel blockage used to spare the intestines from surgical removal.

Surrogate marker: A measure of effect of a specific treatment that may correlate with a real clinical endpoint but does not necessarily have a guaranteed relationship.

T helper (TH) cells: A type of T cell that provides help to other cells in the immune response by recognizing foreign antigens and secreting substances called cytokines that activate T and B cells.

Transforming Growth Factor-Beta (TGF-β): A regulatory cytokine that has multifunctional properties that can enhance or inhibit many cellular functions, including interfering with the production of other cytokines and enhancing collagen deposition. It exists in multiple subtypes and is produced by platelets and macrophages but can be made by many other cell types.

Transmural: Extending through or affecting the entire thickness of the wall of an organ or cavity.

Toxic megacolon: A potentially lethal complication of inflammatory bowel disease (IBD) or infectious colitis that is characterized by total or segmental nonobstructive colonic dilatation plus systemic toxicity.

Tumor necrosis factor (TNF): A protein that is produced chiefly by monocytes and macrophages in response especially to endotoxins, that mediates inflammation, and that induces the destruction of some tumor cells and the activation of white blood cells.

Uveitis: A form of eye inflammation that affects the middle layer of tissue in the eye wall (uvea).

Villous blunting: The villi are often blunted or flattened due to intestinal damage or injury.

CHAPTER 2: The Literature Review

Introduction

Crohn's disease (CD) is an idiopathic, chronic, and recurrent immune-mediated inflammatory bowel disease (IBD) that can affect any part of the gastrointestinal tract from the mouth to the anus (Basson, 2012). In CD, segments of inflamed bowel may be separated by healthy segments of bowel, whereas in Ulcerative Colitis (UC), the other major form of IBD, the disease process is continuous. Involvement of the intestinal mucosal in CD is transmural, meaning that it affects all layers of the mucosa. The exact etiology of this autoimmune condition is unknown, however, factors that most likely promote the onset and continuation of intestinal inflammation include genetic predisposition, intestinal dysbiosis, and unidentified environmental triggers (Herfath et al., 2014). Even though CD is treatable, it can significantly impact quality of life, may have a high financial burden, has a slightly higher overall mortality rate compared to the general healthy population, and significantly increases the risk for malabsorption and malnutrition. Nutrition interventions are lacking and can likely help improve disease outcomes.

CD affects as many as 700,000 Americans and can occur at any age, but is more prevalent among those between the ages of fifteen and 35 (CCFA, 2015). The annual incidence of CD ranges from one to ten cases per 100,000, with peak age-specific

incidence occurring between ten and 20 years of age. A second smaller peak occurs near the age of 50, and is thought to be due to more common diseases in this age group such as diverticulitis and ischemic disease. CD prevalence ranges from ten to 70 per 100,000 people, but some North American studies have shown prevalence as high as 200 per 100,000 people (Cleveland Clinic, 2014). Genetic influences seem to be more prominent in the younger subgroup of patients than those who present with CD after the age of 40 (Johns Hopkins, 2013). Geography is also a factor with an apparent north-south gradient worldwide, where populations in higher latitudes such as Scandinavia, Canada, and Australia have higher incidence than populations in lower latitudes such as southern United States, Spain, and Italy (Cleveland Clinic, 2014). Additionally, migrants who move from a low-risk region to a high-risk region have a risk of developing CD that is similar to that in the high-risk region within one generation (Cleveland Clinic, 2014). Males and females in the United States are equally affected; however, both whites and Ashkenazi Jews are at much higher risk compared to the rest of the population. Lastly, it has been reported that active smokers are more than twice as likely as nonsmokers to develop CD (Cosnes, 2008).

Nutritional approaches for the management of CD have traditionally been limited to enteral nutrition (EN) and total parental nutrition (TPN) to provide bowel rest (Olendzki et al., 2014). Many studies support that clinical remission and mucosal healing can be achieved in CD with a switch from a normal diet to EN, but the mechanisms for this response remain unexplained (Richman, 2013). Theories include changes in nature or quantity of gut bacteria, improved nutritional status, reduced allergenicity of gut contents,

avoidance of food additives, and provision of an anti-inflammatory factor such as transforming growth factor-beta (TGF-β) (Richman, 2013).

The therapeutic effect of EN on CD and the epidemiological associations between diet and CD implicate diet as an environmental trigger (Richman, 2013). However, evidenced based therapeutic dietary interventions are largely absent for CD. Due to insufficient evidence, clinicians have traditionally taken a more simplified approach, focusing on a well-balanced diet to ensure adequate nutrition and avoidance of any specific dietary items that trigger symptoms. Lack of dietary advice can potentially lead patients to seek nutritional advice from non-credible sources and adopt overly restrictive diets. Therefore, it is the clinician's responsibility to provide 'best evidence-based dietary advice' to inform patients of the current state of knowledge and help prevent them from adopting unnecessary dietary restrictions (Richman, 2013).

The purpose of this literature review is to analyze the existing evidence on existing therapeutic dietary interventions for CD. A greater understanding of effective medical nutrition therapy for CD could significantly improve the quality of life for people with CD by decreasing their risk for malnutrition and nutrient deficiencies, alleviating or eliminating symptoms caused by inflammation in the digestive tract, and improving medical and surgical therapy outcomes.

Background

Five different types of CD have been identified, each with its own set of symptoms and complications. Affecting approximately 50 percent of CD patients, ileocolitis is the most common type of CD, and affects the ileum and colon. Ileitis is limited to the ileum and affects 30 percent of CD patients, while Crohn's

(granulomatous) colitis is limited to the colon and affects 20 percent of CD patients (Lichtenstein, 2008). Jejunoileitis and gastroduodenal CD are less common types of CD, affecting only the jejunum, and the stomach and beginning of the duodenum, respectively. Jejunoileitis has been documented to affect less than 5% of CD patients, whereas gastroduodenal CD has been reported to occur in 4% or less of all patients with CD (Johns Hopkins, 2013; Kefalas, 2003). Despite the classification of five different types of CD, some individuals have more than one area of the digestive tract that is affected, and there can be overlap between the different types of CD.

Pathophysiology and Etiology

The cause of CD is not completely understood, but it is known to involve the interaction between genes, the GI immunologic system, and environmental factors. While the immune system of a healthy individual will typically attack and kill foreign invaders, such as bacteria, viruses, and other microorganisms, the immune system of an individual with CD mounts an inappropriate inflammatory response to the intestinal tract (Beyer, 2008).

Microscopically, the initial lesion begins as a focal inflammatory infiltrate around the crypts, followed by an ulceration of superficial mucosa. Inflammatory cells invade the deep mucosal layers and begin to organize into non-caseating granulomas, which extend through all layers of the intestinal wall and into the mesentery and the regional lymph nodes. Neutrophil infiltration into the crypts forms crypt abscesses, leading to destruction of the crypt and atrophy of the colon. Additionally, chronic damage may be seen in the form of villous blunting in the small intestine. Furthermore, ulcerations are common, often seen on a background of normal mucosa. Interestingly, the absence of granuloma

formation does not exclude the diagnosis of CD, and in fact, is not detected in about half of patients with CD (Walfish, 2015).

Macroscopically, the initial abnormality consists of hyperemia and edema of the involved mucosa. Discrete, superficial ulcers will then form over lymphoid aggregates and are seen as mucosal lesions, which may develop into deep longitudinal and transverse ulcers over an inflamed mucosa, creating a characteristic cobblestone appearance to the bowel. The lesions are often segmental and referred to as skip lesions, because they are separated by healthy areas of mucosa (Walfish, 2015).

Transmural spread of inflammation leads to thickening of the bowel wall and narrowing of the lumen. As CD progresses, it can be complicated by obstruction or deep ulceration leading to fistulization by way of the sinus tracts penetrating the serosa, causing microperforation, abscess formation, adhesions, and malabsorption (Walfish, 2015). Bowel obstruction is caused initially by significant edema of the mucosa and associated spasm of the bowel. It is intermittent and can often be reversed by means of conservative measures and anti-inflammatory agents, however, with further disease progression, the obstruction can become chronic due to fibrotic scarring, luminal narrowing, and stricture formation. The inflammation extending through the bowel may also involve the mesentery and surrounding lymph nodes and creeping fat may be seen when the mesentery wraps around the bowel surface (Walfish, 2015).

Since the mesentery and serosa are inflamed, a characteristic feature of CD is the tendency for involved bowel loops to be firmly matted together by fibrotic bands. This adhesive process is often associated with fistula formation. Fistulas begin as ulcerations and gradually burrow through the serosa into adjacent organs. Such fistulas communicate

between the loops of small bowel themselves, as well as between loops of small bowel and colon, skin, perineum, bladder or vagina, or they may end blindly in indolent abscess cavities located within the peritoneal cavity, mesentery or retroperitoneal structures (Walfish, 2015).

Immunology

Defects in both the innate and adaptive immune systems are present in CD. Barrier function, which is the first line of innate defense, is impaired by an inadequate mucous layer and abnormally low levels of protective antimicrobial peptides (Akbariqomi, 2014). Dendritic cells control tolerogenicity, and are the key link between the innate and adaptive immune systems. Dendritic cell distribution, expression of toll-like receptors, co-stimulatory markers and homing markers, as well as secretion of cytokines, are all altered in CD (Akbariqomi, 2014). The role of the adaptive immune system in CD is characterized by an imbalance between pro-inflammatory effector cells and regulatory cells.

Chronic inflammation from T-cell activation leading to tissue injury is implicated in the pathogenesis of CD. After activation by antigen presentation, unrestrained responses of T-helper (TH) cells predominate in CD as a consequence of defective regulation. TH cytokines such as interleukin (IL)-12 and tumor necrosis factor (TNF)- α then stimulate the inflammatory response. Inflammatory cells recruited by these cytokines release nonspecific inflammatory substances, including arachidonic acid metabolites, proteases, platelet activating factor, and free radicals, which result in direct injury to the intestine (Walfish, 2015).

Genetics

Scientific evidence clearly points to the role of genetics in the development of CD, however, having genetic predisposition for CD is not sufficient to develop the disease. It is probable that CD patients have a genetic predisposition for the development of the disease coupled with disturbances in immunoregulation, triggered by environmental factors (Tsianos, 2012). Early epidemiologic evidence for the role of genetic factors in the pathogenesis of CD came from studies demonstrating higher rates of CD among individuals of Caucasian and Jewish ethnicity, familial aggregation of CD, and higher concordance rates of both monozygotic twins developing CD compared with dizygotic twins (Tsianaos, 2012). Studies have shown that five to twenty percent of individuals affected by CD have a first-degree relative with IBD (Russell, 2008).

Numerous genes and genetic mutations correlating with CD have been identified, however, genetic susceptibility is now recognized to be diverse, with a number of possible gene mutations that affect risk and characteristics of the disease (Beyer, 2008). The diversity in the genetic alterations among affected individuals may help explain the wide differences in the onset, aggressiveness, complications, location, and responsiveness to different therapies as seen in the clinical setting. Over 160 susceptibility loci/genes for IBD have been discovered to date. These genes are related to innate pattern recognition receptors, epithelial barrier homeostasis and maintenance of epithelial barrier integrity, autophagy, and lymphocyte differentiation. Thus far, the strongest and most replicated associations with CD have been done with NOD2/CARD15, IL23R, and ATG16L1 genes (Tsiani, 2012). While genetic testing for patients is possible, it currently remains a research tool that is not yet proven to be of clinical benefit for the general assessment of

diagnosis, guidance of patient care, or prediction of response to specific medical therapies (Lichtenstein, 2008).

Microbiome

The enteric microbiota is now accepted as an important etiologic factor in the pathogenesis of CD and immune-mediated chronic experimental intestinal inflammation (Denson et al., 2013). Dramatic advances have been made in understanding the fundamental composition and community structure of the intestinal microbiota and how these enteric bacterial species and their metabolic products interact with the host to mediate mucosal homeostasis versus chronic intestinal inflammation. Evidence suggests that host genes affect microbiota profiles and that specific commensal microbes (either viruses or bacteria) selectively interact with host genes to influence intestinal inflammation (Denson et al., 2013). Four broad mechanisms explain the complex relationship between the commensal microbiota and CD: dysbiosis of conventional microbiota; induction of intestinal inflammation by pathogens and functionally altered commensal bacteria; host genetic defects in containing commensal microbiota; and defective host immunoregulation. These mechanisms all increase the exposure of bacterial antigens to effector T cells and innate immune cells resident in the intestinal mucosa and/or alter the host immune response to commensal bacteria (Sartor, 2012).

Environmental Triggers

A large number of potential environmental risk factors for the development of CD have been investigated, including smoking, psychological stress, use of non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and oral contraceptives, appendectomy, breastfeeding, infections and other events related to the “hygiene hypothesis” in

childhood, as well as dietary factors. In addition to their putative effects on the development of CD, some environmental factors can also play a role in modulating the clinical course of CD (Latella, 2012).

Smoking is the best-established environmental factor increasing the susceptibility to CD (Latella, 2012). Active smokers are more than twice as likely as nonsmokers to develop CD (Cosnes, 2008). Beyond the higher incidence of CD among smokers, several studies suggest that continuing to smoke leads to worse clinical outcomes, however the underlying mechanisms of this effect are not well understood (Latella, 2012). Additionally, smoking has also been associated with a higher risk of relapse and increased need for immunomodulators. Research has demonstrated beneficial consequences of smoking cessation on CD. In a study done by Cosnes et al. in 2001, CD patients who stopped smoking for at least six months were found to have a lower risk of relapse in the following twelve to eighteen months compared to those who continued to smoke (Latella, 2012).

Due to the established role of intestinal flora in the pathogenesis of CD, it is possible that unrelated use of antibiotics can influence risk of development of CD through their effect on the microbiome (Ananthakrishnan, 2013). Shaw et al. found that antibiotic use within the first year of life was more common among pediatric IBD cases compared to matched controls (Ananthakrishnan, 2013).

Psychological stress has long been hypothesized to play a role in the etiology and pathogenesis of CD, and to mediate a role in disease flares (Ananthakrishnan, 2013). Mood components of stress including depression and anxiety may play a strong role in mediating exacerbation of disease, however most of the literature analyzing this role on

CD incidence has been restricted to major life events or retrospective ascertainment of exposures (Ananthakrishnan, 2013). Observational studies have produced mixed results on the relationship between psychological stress and exacerbation of CD (Molodecky, 2010).

The hygiene hypothesis is the most predominant theory to explain the abnormal inflammatory response of the intestinal microflora triggered by unknown environmental factors (Molodecky, 2010). This hypothesis proposes that the rising frequency of immunologic disorders can be attributed to a lack of pathogen exposure in childhood. Research suggests that improved sanitation and hygiene, along with decreased exposure to enteric organisms during early childhood, may lead to a greater susceptibility to develop an inappropriate immunologic response upon exposure to new antigens later in life (Molodecky, 2010). Helminthic infection, *Helicobacter pylori (H. pylori)* exposure, antibiotic use, breastfeeding, and sibship represent the most promising factors supporting the hygiene hypothesis in IBD, but more carefully designed prospective evaluation is needed (Koloski et al., 2008).

Diet: A Probable Environmental Trigger

The role of diet in the development of CD has been extensively studied but has been disappointing with inconsistent findings. Along with microbiota, dietary products are the most common luminal antigens in the bowel and may influence intestinal inflammation (Cabre, 2012). Proposed mechanisms of action include a direct antigenic effect, alteration in gene expression, modulation of inflammatory mediators, changes in the composition of the enteric flora, and effects on gut permeability (Cabre, 2012).

Although it is currently believed that diet cannot cause or cure CD, the spread of the Western diet, which is high in processed foods, saturated fat, and sugar, but low in fiber, fruits, and vegetables, has been proposed as a possible explanation for the recent increase in CD incidence (Hou et al., 2011). Hou et al. performed a systematic review examining the possible association between pre-diagnosis dietary intake and the risk of developing IBD. They identified nineteen studies (eighteen case-controls and one cohort) with a total of 2,609 IBD patients (1,340 with UC and 1,269 with CD). These studies reported increased risk of CD with high intake of PUFAs, omega-6 fatty acids, saturated fats, and meat, while decreased risk of CD was associated with high intake of dietary fiber and fruits. Hou et al.'s systematic review observed that high intake of fruits was associated with a 73-80 percent decreased risk of CD, however, this association was confounded by dietary fiber intake and the fact that a diet high in fruits may conversely be low in fats and meats (Hou et al., 2011). Studies conflicted regarding vegetable intake, with no studies demonstrating statistically significant results.

Recent studies have demonstrated that a diet high in fiber is beneficial to patients with CD and decreases the incidence of the disease (Ananthakrishnan et al., 2013; Brotherton and Taylor, 2013). Hou et al.'s review also indicated a consistent association between high dietary fiber and decreased risk of CD, with the protective effect observed to be statistically significant in those consuming more than 22.1 grams per day compared to those consuming less than 13.8 grams per day (Hou et al., 2011). Dietary fiber has been investigated as a means of increasing short-chain fatty acid (SCFA) production and CD has been linked with impaired SCFA production. SCFAs are mainly produced by the anaerobic bacterial fermentation of undigested carbohydrates and

fiber polysaccharides. In 1995, Galvez et al. reviewed a number of studies that concluded that dietary fiber confers clinical benefits in patients with IBD because it maintains remission and reduces colonic damage. This is thought to occur by increasing SCFA production and by altering the gut flora towards predominantly non-pathogenic bacteria (Rajendran et al., 2010).

Signs and Symptoms

Signs and symptoms of CD typically begin with indications of inflammation, such as diarrhea, abdominal pain, fever, fatigue, stomatitis, anal fissures, and/or weight loss. Hematochezia may occur when the inflammatory process affects the large bowel, but bleeding is much less common in CD than in UC (Cleveland Clinic, 2014). Extra-intestinal manifestations of CD related to inflammation might include spondylarthritis (ankylosing spondylitis and sacroiliitis), peripheral arthritis, cutaneous manifestations (erythema nodosum and pyoderma gangrenosum), ocular inflammation (uveitis, episcleritis, or sclera-conjunctivitis), primary sclerosing cholangitis, and hypercoagulability (Lichtenstein, 2008). Strictures and fistulas may also develop as CD advances and children with extensive small bowel involvement can present with growth retardation and delayed puberty (Cleveland Clinic, 2015).

Symptoms of ileocolitis typically include significant weight loss, diarrhea and cramping, and pain in the middle or lower right part of the abdomen. Ileitis usually presents with the same symptoms as ileocolitis, but may also include the formation of fistulas or inflammatory abscesses in the right lower quadrant of the abdomen. Jejunoileitis typically presents with post-prandial cramps, formation of fistulas, diarrhea,

and intense abdominal pain. Symptoms of Crohn's (granulomatous) colitis include skin lesions, joint pains, diarrhea, rectal bleeding, as well as the formation of ulcers, fistulas, and abscesses around the anus (Bandzar, 2013). Lastly, gastroduodenal CD typically causes nausea, weight loss, loss of appetite, obstructions, and vomiting.

Natural History of CD

The clinical course of CD may be mild and episodic or severe and unremitting (Beyer, 2008). CD typically has a chronic relapsing course with approximately half of all patients being in clinical remission at any particular time. If a patient is in remission for one year, there is an 80 percent chance that they will remain in remission over the course of the subsequent year. For a patient with active disease in the past year, there is a 70 percent chance that this patient's CD will be active in the forthcoming year, and a 50 percent chance of being in remission within the ensuing three years (Lichtenstein, 2008). Overall, thirteen percent of patients will have a relapse-free course, 20 percent will have relapses every year, and 67 percent will have a combination of years in relapse and years in remission within the first eight years after initial diagnosis. It has also been estimated that less than five percent of patients with CD will have a continuous course of active disease (Lichtenstein, 2008).

Complications

There are a number of disease complications that may occur with CD. Intestinal complications of CD tend to occur when intestinal inflammation is severe, chronic, widespread, and extends beyond the inner lining of the intestines (BIDMC, 2015). The most common complication of CD is small bowel obstruction (SBO), which can occur

due to swelling and the formation of scar tissue. The result is thickening of the bowel wall and a narrowed intestinal passage. These narrowed areas, called strictures, can be mild or severe, depending on how obstructive they are. Surgery may be indicated if the obstruction is severe and does not respond to medical treatment, or if the blockage recurs frequently (BIDMC, 2015).

Abscesses may form in the intestinal wall, which are localized pockets of pus caused by bacterial infection. CD may also cause deep sores or ulcers within the intestinal tract that can turn into fistulas, connecting different parts of the intestine. Fistulas can also tunnel into the surrounding tissues of the bladder, vagina, or skin. These abnormal passages often become infected and affect about 30 percent of CD patients (Johns Hopkins, 2013). Large or multiple fistulas might require surgery, while smaller fistulas may only require antibiotics. Another complication known as fissures, or tears or cracks in the lining of the anus can cause mild to severe rectal pain and bleeding, particularly during bowel movements.

Patients with Crohn's colitis have an increased risk of colon cancer, while anal cancer is a rare complication of long-standing perianal CD. There is also a clear increased risk of small bowel adenocarcinoma in patients with CD, but remains exceedingly rare (BIDMC, 2015). Small intestinal bacterial overgrowth (SIBO) is another potential complication of CD in which excessive amounts of bacteria are present in the small intestine, producing gas, abdominal pain, bloating, and diarrhea.

Patients with CD are also considered to be at significant nutritional risk. Factors that play a role in altering nutritional status in CD include: decreased nutrient intake due to anorexia or fear of eating, nausea, vomiting, diarrhea, abdominal pain, restrictive diets,

side effects of medications, oral aphthous ulcerations, appetite suppression or taste changes, protein losses from inflamed ulcerated mucosa, increased needs for healing, intestinal resections, increased vitamin and mineral needs, bacterial overgrowth, malabsorption, and blood loss (Donnellan, 2013).

Nutrition-Related Complications: Malnutrition

The rate of malnutrition in CD is difficult to accurately establish due to widely differing definitions, but malnutrition is thought to affect 20 to 85 percent of CD patients, particularly those with disease activity in the small bowel (Donnellan, 2013). The degree of malnutrition depends upon the extent, severity and duration of the disease, with a higher incidence of protein-energy malnutrition occurring when disease is active in the small bowel compared to colonic disease (Basson, 2012). Patients suffer from malnutrition due to a combination of factors including modified diets, medication side effects, altered intestinal absorption due to inflammation, and intestinal resection. Malnutrition is also associated with increased severity of CD in patients requiring hospitalization (Naik, 2012).

Nutrition-Related Complications: **Micronutrient Deficiencies**

Micronutrient deficiencies are relatively common in CD, particularly in patients with active small bowel disease and/or multiple resections. Micronutrient deficiencies are associated with several extra-intestinal complications of CD, however patients with micronutrient deficiencies can also present with nonspecific symptoms such as fatigue and depression (Donnellan, 2013). Iron deficiency is the most common extra-intestinal manifestation of IBD, with prevalence rates ranging from 36 to 88 percent.

Malabsorption, decreased dietary intake of iron rich foods due to food aversions and inflammation, and chronic intestinal blood loss are all factors that can contribute to iron deficiency anemia (Basson, 2012).

Vitamin B12 deficiency occurs in approximately one-fifth of CD patients, and is more prominent in those with intestinal resections, but can also occur in the setting of inflammation of the native terminal ileum (Basson, 2012; Naik, 2012). Although deficiency is thought to occur in all patients after resection of more than 60 centimeters of terminal ileum (Mowat et al., 2011), a small study of 42 patients with resections of between 20 and 60 centimeters of bowel demonstrated abnormal B12 absorption in 52 percent, highlighting the need to monitor these patients carefully (Basson, 2012).

Historically, IBD cohort studies have demonstrated that folate deficiency affects 51 to 80% of patients, particularly those with CD, but more recent studies have shown the risk of folate deficiency to be much lower in CD patients, ranging from four to 28.8% (Donnellan, 2013; Hwang, 2012). Folate deficiency can result from poor intake, malabsorption, and competitive inhibition from sulfasalazine or methotrexate use. Folate deficiency is associated with multiple systemic side effects including megaloblastic anemia, glossitis, angular stomatitis, and depression (Hwang, 2012). Folate deficiency may increase colorectal cancer in patients with colonic CD, possibly secondary to hyperhomocysteinemia, which leads to DNA methylation and DNA instability (Phelip et al., 2008).

As many as 30 to 60% of patients with CD have bone density considered to be lower than average, and it has been estimated that osteoporosis occurs in 30 to 50% of CD patients (Basson, 2012). Causes of bone loss in CD include long-term steroid use,

inadequate calcium intake, vitamin D deficiency, malabsorption, malnutrition, hypogonadism, and systemic inflammation (Ardon, 2002).

Several studies have reported a higher prevalence of vitamin D deficiency in patients with IBD, although it has not been universally established that this rate is higher than in other chronic illnesses, inflammatory diseases, or even healthy individuals in certain regions. There are several factors contributing to vitamin D deficiency in patients with CD, some causes specifically related to underlying bowel disease, while others are in common with the non-IBD population. These include inadequate exposure to sunlight, inadequate dietary intake, impaired absorption, impaired conversion of vitamin D to its active products, increased catabolism, and increased excretion. The Nurses' Health Study cohort examined the association between vitamin D status and incident IBD directly, and found that over a 22 year follow-up, higher predicted plasma 25(OH) D levels were associated with a significant reduction of incident CD (Moule, 2013). Additionally, Moule et al. found that plasma 25(OH) D <20 ng/mL was associated with an increased risk of surgery and hospitalization compared with those with sufficient levels. Furthermore, CD patients who normalized their plasma 25(OH) D had a reduced likelihood of IBD-related surgery compared with those who remained deficient (Moule, 2013).

Zinc deficiency is most common in CD patients with short gut syndrome; however, patients with excessive stool losses or high output fistulas may also be deficient (Naik, 2012; Eiden, 2003). IBD patients also appear to be at increased risk of magnesium deficiency, with reported rates ranging from thirteen to 88%, likely due to a combination

of decreased dietary intake, losses from chronic diarrhea and fistula output, and malabsorption (Hwang, 2012).

Clinical Relevance

Diagnosis

No single test is used to diagnose CD, but rather an initial diagnosis is based on a composite of endoscopic, radiographic, and pathological findings. Laboratory study results are generally nonspecific, but may help support a diagnosis. Serologic studies can provide adjunctive support for the diagnosis of CD, especially to differentiate between CD and UC, but are not sufficiently sensitive or specific to be recommended for use as a screening tool (Lichtenstein, 2008). Even though evidence suggests that CD is a combination of genetic predisposition and potential environmental triggers, the use of genetic testing remains a research tool and is currently not recommended for the general assessment of diagnosis in CD (Lichtenstein, 2008).

Endoscopic visualization is essential in the diagnosis of CD. Ileocolonoscopy is a highly sensitive and specific tool used for CD diagnosis and for management of patients with established CD. This procedure allows for the obtainment of biopsy tissue, evaluation of mass lesions, assessment of mucosal healing, and evaluation of surgical anastomoses as a means of predicting the likelihood of clinical relapse and response to postoperative therapy (Llano, 2010). Ileocolonoscopy has a sensitivity of 74% and a

specificity of 100% in the assessment of CD, leading to a positive predictive value of 100% as a diagnostic test. When combined with small bowel imaging, the sensitivity of these two diagnostic tests is increased to 78%, with a continued positive predictive value of 100% (Llano, 2010). Colonoscopy may also play an important role in cancer surveillance considering the increased risk of colorectal cancer in CD patients (Bandza, 2013). If patients present with upper GI symptoms, upper endoscopy may be used to examine the esophagus, stomach, and duodenum, and is used to diagnose suspected gastroduodenal CD.

Endoscopic biopsy is also essential in the diagnosis of CD. Biopsied tissues obtained during endoscopy are analyzed in pathology to determine the presence of disease. They can establish a diagnosis, differentiate between CD and UC, differentiate from other inflammatory, infectious, or acute conditions, and/or identify dysplasia or cancer (Llano, 2010).

There are a number of radiologic tests that are used to evaluate the small intestine, because endoscopy and colonoscopy can only see the beginning and end of the small intestine. The test most commonly used to evaluate the small intestine is the small bowel follow through (SBFT). If present, SBFT will demonstrate inflammation, fistulas, and strictures of the small intestine. More recently, many gastroenterologists have been using computed topography (CT) and magnetic resonance imaging (MRI) for the evaluation of small bowel pathology in CD. These modalities are replacing SBFT studies due to their ability to better distinguish location of inflammation and diagnose serious complications such as fistulas or abscesses (Lichtenstein, 2008). CT is also the preferred technique to assess extramural complications, as well as hepatobiliary and renal complications.

Laboratory results for CD are nonspecific and are used for facilitating disease management. They may also be used as surrogate markers for inflammation and nutritional status. A complete blood count (CBC) may show anemia, or may demonstrate an elevated white blood cell (WBC) or platelet count (PLT), the latter of which are both markers of inflammation (Bandza, 2010). Acute inflammatory markers include C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), which may correlate with disease activity in some patients, however, normal values should not deter further evaluation if CD is suspected (Vermeire, 2006). Furthermore, these tests can confirm active inflammation, but do not diagnose specifically the cause of inflammation.

Stool samples are often collected and examined for blood and signs of inflammation or infection, including parasites that could cause similar symptoms. Clostridium difficile, e. coli, campylobacter, salmonella, and shigella, all mimic symptoms of CD and can be identified by testing stool samples. Stool biomarkers, calprotectin and lactoferrin, can also be useful to detect inflammation (Bandza, 2013).

Determining Disease Activity

A gold standard to measure disease activity in CD is lacking; therefore, severity of disease is established based on clinical parameters, systemic manifestations, and the global impact of the disease on the individual's quality of life (Lichtenstein, 2008).

The most widely used clinical disease activity index for CD was developed in the 1970s by investigators from the National Cooperative Crohn's Study and is known as the Crohn's Disease Activity Index (CDAI). CDAI scores range from zero to approximately 600 as determined by an equation that contains eight variables that have been found to best reflect the impact CD has on the patient as a whole, rather than just bowel

symptoms. The equation is generated using multiple logistic regression, and is based on the average of scores over the course of an entire week. A score ≤150 indicates remission while a score ≥450 is associated with very active disease. In most studies, the CDAI is used as the primary index for evaluation of disease activity at inclusion of the study and to assess therapeutic success of the therapy (Herfarth, 2013).

Due to the validation work that went into the development of the CDAI and its long history of use, it has been considered the gold standard clinical activity index for CD. However, there has been criticism of the CDAI because several variables are based on subjective responses, it employs a seven-day patient diary that is inconvenient to use in clinical practice, and there is inconsistency in how investigators score the CDAI (Sostegni et al., 2003). Furthermore, the CDAI has been demonstrated to lack correlation with the extent of ileocolonic inflammation. For example, patients could be in remission as defined by a CDAI score less than 150 points, but still have endoscopically significant colonic inflammation, and vice versa (Herfarth, 2013). Findings from a study done by Modigliani et al. in 1990 have indicated that abdominal pain, general well-being, and amount of diarrhea (the most heavily weighted symptoms of the index), are not specific features of intestinal inflammation. Thus, patients with IBS can easily achieve a CDAI score of 450 and higher without evident intestinal inflammation. If significant numbers of these "non-inflammatory" CD patients are included in clinical trials investigating novel anti-inflammatory therapies using only the CDAI as a criterion for study entry and outcome, these clinical studies might produce inaccurate results (Herfarth, 2013).

The Harvey Bradshaw Index (HBI) is another activity index that was created to simplify the CDAI. This index utilizes one-day measurements and excludes the variables

body weight, hematocrit, and use of antidiarrheals, but also suffers from subjective assessment. Two other activity indices that have been proposed include the Organization Mondiale de Gastroenterologie (OMGE) index and the Cape Town Index. All three of these clinical indices are validated and correlate with each other and the CDAI and can be surrogate measures (Sostegni et al., 2003).

Quality of life is another parameter that is widely used in clinical trials as a secondary endpoint to monitor disease activity. Both the Rating Form of Inflammatory Bowel Disease Patient Concerns and the Inflammatory Bowel Disease Questionnaire (IBDQ) are validated tools used to measure quality of life in CD, and have been shown to correlate with CDAI (Sostegni et al., 2003). The IBDQ is a 32-item questionnaire with four dimensions (bowel function, emotional status, systemic symptoms, and social function) with higher scores indicating better quality of life, while the Rating Form of IBD Patient Concerns quantifies disease-related patients' concerns (Sostegni et al., 2003).

The achievement of clinical remission alone has long been the therapeutic target in CD; however, the assessment of disease activity and the definition of remission in CD has evolved in such a way than many physicians now look beyond symptomatic remission alone as a treatment target for CD patients (Osterman, 2013). The use of mucosal healing as a treatment endpoint is in its early stages and presents two major limitations. These limitations include lack of a clear definition for mucosal healing in CD patients, and the inability to achieve mucosal healing in many CD patients despite combination immunosuppressive therapy (Osterman, 2013). The latter begs the question if mucosal healing is even a reasonable and/or attainable goal for the majority of CD patients.

Treatment and Management

CD is a chronic inflammatory disorder that is neither medically or surgically curable. The main goals of treatment are to induce remission, maintain remission, improve quality of life, and minimize toxicity (Lichtenstein, 2008). Management of CD is multifactorial and includes dietary, pharmacotherapy, and surgical approaches, with treatment options varying depending on disease stage, location, and severity. In more recent years, treatment goals of CD have evolved and expanded to include: mucosal healing, preventing complications such as fistulae, abscesses, and cancer, preventing hospitalization, and preventing surgery (Bandza, 2013).

Pharmacology

Anti-inflammatories, immunosuppressants, antibiotics, biologic therapies, and drugs for symptomatic relief are the five main categories of medications used to treat CD (Triantafillidis, 2011). Anti-inflammatory drugs used for CD include aminosalicylates and corticosteroids. Aminosalicylates are compounds that contain 5-aminosalicylic acid (5-ASA) and act to decrease inflammation at the wall of the intestine. Examples are sulfasalazine, balsalazide, mesalamine, and olsalazine. They are effective in treating mild-to-moderate episodes of CD, preventing relapses, and maintaining remission. Mesalamine might also act as a chemopreventive agent of colorectal cancer in the context of CD (Triantafillidis, 2011). Corticosteroids include prednisone, prednisolone, and budesonide. They suppress inflammation by blocking the early manifestations of inflammation, and they also influence immunological responses such as T-responses to antigens, down regulate production of inflammatory cytokines, and interfere with nuclear factor-kappa beta (NF-kB) production, thereby blunting inflammatory response

(Triantafillidis, 2011). Corticosteroids are highly effective for induction of remission, but are only recommended for short-term control of flare-ups due to the side effects they produce and their inability to achieve mucosal healing.

Immunosuppressants include azathioprine, 6-mercaptopurine (6-MP), and methotrexate. They inhibit proliferation and activation of lymphocytes, thereby modifying the activity of the immune system so that it cannot cause ongoing inflammation. They are effective for inducing remission in patients with active CD, as well as for the maintenance of remission, especially in those who have not responded to other medications or who have only responded to steroids (Triantafillidis, 2011).

Antibiotics, ciprofloxacin and metronidazole, provide modest benefit for patients with CD that affects the colon or the area around the anus. Researchers believe that antibiotics can help control symptoms of CD by reducing intestinal bacteria and by directly suppressing the intestine's immune system. Antibiotics are effective as long-term therapy in some CD patients, particularly those with fistulas or recurrent abscesses near the anus.

The development of biologic agents and their use in CD has improved patient quality of life by modifying disease course and preventing complications and surgery. Biologic therapies are indicated for patients with moderately to severely active disease who have not responded well to conventional therapy (Triantafillidis, 2011). Anti-tumor necrosis factor-alpha (TNF-α) agents are designed to bind to, and block the effects of TNF-α, an inflammatory protein or cytokine that is seen in high levels in patients with CD. Infliximab, adalimumab, and certolixumab pegol are the three anti-TNF agents that are currently approved for the treatment of moderate-to-severe CD (Triantafillidis, 2011).

All three of these agents are effective in both induction and maintenance of remission, while infliximab and adalimumab are also effective for fistula healing. Anti-adhesion molecules, natalizumab and vedolizumab, are humanized IgG4 monoclonal antibodies that work by blocking certain inflammatory cells from travelling from the bloodstream to the intestine, thereby decreasing intestinal inflammation. These drugs have been shown to be effective for both induction and maintenance of remission of CD. They are effective therapeutic alternatives for patients with moderate-to-severe CD who don't respond to, or have failed, anti-TNF therapy (Triantafillidis, 2011).

Surgery

About 70% of patients with CD will require surgery during their lifetime, and as many as many as 39% of that 70% will require repeated surgery (Lewis, 2010). Approximately 30% of patients who have surgery for CD experience recurrence of their symptoms within three years and up to 60% will have recurrence within ten years (Lewis, 2010). The most common indication for surgical intervention is refractory disease despite medical treatment (Parray et al. 2011). Other indications for surgery include intestinal obstruction, abscesses and inflammatory masses, perforation of the bowel, toxic megacolon, dysplasia or cancer, and growth restriction in children (Lewis, 2010). Gastrointestinal hemorrhage is a rare complication of CD and accounts for only one percent of operations (Lewis, 2010).

Different types of surgical procedures may be performed for CD, depending on the reason for surgery, severity of illness, and location of the disease. Intestinal resection is the most common surgical procedure performed for CD. The removal of the diseased portion of the bowel may provide relief from symptoms for many years, but is not

curative (Lewis, 2010). Removal of part of the colon (partial colectomy) or removal of the entire colon (total colectomy) may be indicated for patients with severe CD in the colon. Colon re-sectioning is another option, which involves removing a part of the colon and reattaching the remaining sections to restore bowel function. If CD does not affect the ileum and rectum, it may be possible to connect the rectum to the small intestine. Otherwise, an ileostomy or stoma is necessary, in which the end of the small intestine is brought out through an opening in the wall of the abdomen, and an external bag is fitted onto the opening to collect waste. If both the rectum and colon are affected, both are removed with a surgery called a proctocolectomy. This procedure is performed along with an ileostomy.

Strictureplasty is a surgical procedure that can treat strictures and blockages in the small intestine without removing any part of the intestine. The narrowed section of the intestine is opened up with a lengthwise cut, and is then reshaped by sewing it up the opposite way, allowing food to pass freely through the reshaped section of the intestine. The rationale for the use of this technique is that it corrects obstructive strictures while preserving functional intestinal length (Lichtenstein, 2008). Furthermore, there is growing use of laparoscopic surgery in patients with CD. Goals of this treatment include potential decrease of adhesion formation, decrease in postoperative pain, and decreased length of hospital stay (Lichtenstein, 2008).

Medical Nutrition Therapy

There is no question that nutrition plays an important role in the management of patients with CD. Unfortunately, there is no clear nutrition prescription that works best for all patients. Enteral nutrition (EN) has a role in support for the malnourished patient,

as well as in primary therapy to induce and maintain remission. The use of parenteral nutrition (PN) in CD is mainly limited to the preoperative setting or for patients with intestinal failure, but does not offer any additional advantage over EN in disease control. Dietary modifications may improve symptoms, but there currently is very limited data to suggest that these approaches play any role in the induction or maintenance of remission (Donnellan, 2013).

Exclusive Enteral Nutrition

Enteral nutrition (EN) has been used for the treatment of CD since Voitk et al. demonstrated a reduction in CD activity in a small series of patients on an elemental diet in 1973 (Donnellan, 2013). Since then, multiple clinical trials and meta-analyses have analyzed and assessed EN's role in the management of active CD. Generally, exclusive enteral nutrition (EEN) is used for the induction of remission and is achieved by a period of six to eight weeks of exclusive liquid feeding with either elemental or polymeric formula (Kansal et al., 2013). Evidence has consistently supported EEN as an effective therapy for the induction of remission in pediatric CD; however, a much smaller body of evidence examining the efficacy of EEN in adult CD has been much less conclusive, with multiple confounding factors (Levine and Wine, 2013; Critch et al., 2013). Furthermore, this practice is uncommon in adults with CD in most countries apart from Japan (Levine, 2013; Wall, 2013).

Two meta-analyses have concluded that EN is just as effective as corticosteroids in inducing remission (Heuschkel et al., 2000; Dziechciarz et al., 2007). Pediatric studies have indicated that approximately 50 to 80 percent of children fed an exclusive liquid diet with no exposure to other food, and irrespective of the specific type of liquid diet, will

enter complete remission (Levine, 2013). Even though there are no studies comparing EN to placebo due to ethical considerations, the benefits of EN have been demonstrated in clinical trials by comparing EN response rates (53 to 80 percent) to usual placebo response rates (18 to 42 percent) (Gurram, 2012). Additionally, both Borelli et al. and Yamamoto et al. reported mucosal healing rates between 44 and 74 percent on EEN, while Banerjee et al. reported a rapid reduction in pro-inflammatory cytokines in children receiving EEN for active CD (Gurram, 2012). To date, there is no conclusive evidence for the use of EN to maintain remission in pediatric CD.

Partial Enteral Nutrition

Several small-randomized controlled trials (mean age 30) have shown an effect of partial enteral nutrition (PEN) on the maintenance of nutrition (Levine and Wine, 2013). Most of these studies found that patients consuming 50 percent of daily calories from elemental or semi-elemental formulas for a year had fewer relapses compared to free diet (Levine and Wine, 2013). Yamomoto et al. evaluated postoperative occurrence in patients on free diet compared to patients receiving 50 percent of daily requirements using an elemental formula and a low-fat diet for one year. Clinical recurrence occurred in five percent of the PEN group compared to 35 percent recurrence in the free diet group. Furthermore, endoscopic recurrence occurred in 30 percent of PEN patients compared to 70 percent recurrence in the free diet group (Yamamoto et al., 2007). In another study using the same methodology, Yamamoto et al. investigated 40 patients in clinical remission. Half of these patients received PEN and a low-fat diet while the other half received a free diet for one year. Patients on the free diet had a 65 percent relapse rate

compared to a 25 percent relapse rate in the PEN group. Additionally, mucosal cytokines were significantly lower at one year in the PEN group (Yamamoto et al., 2007).

Enteral Nutrition as Maintenance Therapy

There have only been two randomized controlled trials assessing EN as maintenance therapy in CD. In a study done by Verma et al. published in 2001, 33 patients with steroid-dependent CD were given either an elemental or polymeric diet to provide 30 to 50 percent of their energy requirements. Response was defined as successful withdrawal of steroids without a CDAI increase of more than 100 points to a score greater than 200, and avoidance of surgery. This was achieved in fourteen of the 27 patients who tolerated the feed. Subsequently, an open-label extension allowed the fourteen responders to choose whether or not to remain on enteral supplements. All seven patients who chose to return to an unrestricted diet relapsed within four months, requiring further steroid treatment, while six of the seven patients who continued on enteral supplements remained in remission for 24 months (Verma et al., 2001). Despite these positive findings, there are concerns as to whether long-term EN for CD maintenance may impair quality of life because of the restrictive nature and possible need for repeated nasogastric tube intubations (Donnellan, 2013).

The mechanism of action by which remission is induced by EN is unclear. Suggested mechanisms of action include direct anti-inflammatory effects, improved epithelial barrier function, and modulation of the gut microbiota (Kaakoush et al., 2015). The alteration of intestinal microbiota and its relationship to the development of IBD has been given more attention lately. According to the "dysbiosis" hypothesis, a breakdown between the balance of good bacteria and harmful bacteria significantly contributes to the

development of IBD, and it has been suggested that EN has an anti-inflammatory effect by modification of the gut microbiota (Kansal, 2013). Research has also shown EN to have a beneficial effect on growth and nutritional status, as well as markers of inflammation and the promotion of mucosal healing (Critch et al., 2012). It is important to keep in mind that the disease remission criteria used by researchers can have a profound impact on study results. Comparison of disease remission rates between clinical studies is challenging when disease remission is not universally defined, as is the case in CD.

Dietary Modifications

Many CD patients can consume a normal diet during times of disease remission, but may need to alter their diet during flares, meaning the simple act of eating can no longer be taken for granted. Because CD affects digestion and absorption, diet and nutrition are impacted in a variety of ways. Typically, certain foods that may worsen or trigger symptoms need to be avoided, which can make maintaining a well-balanced and nutrient rich diet challenging. Dietary recommendations are currently aimed at easing symptoms during flares and ensuring overall adequate intake and optimal absorption of nutrients, vitamins, and minerals (NCM, 2015). During acute and severe exacerbations of CD, the diet should be individualized based on: symptoms, disease state (remission versus flare), location of disease, presence of strictures, history of surgery, and whether there are any existing nutritional deficiencies.

Food allergies and other immunologic reactions to specific foods have been considered in the pathogenesis of IBD and its symptoms, however, the incidence of documented food allergies compared with food intolerances, is relatively small. The

permeability of food and cell fragments is likely increased in inflammatory states, allowing the potential for increased interaction of antigens with the host immune system (Rajendran, 2010). Certain foods or beverages may irritate the digestive tract and aggravate symptoms during a flare, and may need to be avoided. Food intolerances occur more often in persons with CD than in the population at large, but the patterns are not consistent among individuals or even between exposures from one time to the next. In one prospective study, 65 percent of patients with IBD reported some sort of food intolerance compared to fourteen percent of controls (Cabre, 2012). Reasons for specific food intolerances vary and are typically related to the severity, location, and complications associated with the disease process (Rajendron, 2010).

Dietary guidelines vary in scope and complexity, but most indicate that limiting lactose, excess fat, excess carbohydrate, and reducing fiber in the diet is necessary, particularly during flares of the disease (Olendzki et al., 2014). Patients with stricturing small bowel CD are typically advised to consume a low residue, low fiber diet to decrease the risk of bowel obstruction. Traditionally, CD patients experiencing flare symptoms have been recommended to follow a low residue, low fiber diet; however, this recommendation is not supported by controlled studies (Naik, 2012). Lactose intolerance may also be of concern for those with CD, particularly during disease flares. In a large cohort of patients with IBD, 51 percent of participants avoided milk and milk products because they believed that dairy worsened their symptoms, which could reflect a component of lactose intolerance. In 2011, Eadala and colleagues found that lactose sensitivity was demonstrated across all IBD subtypes, and is thought to be due to lactase

gene downregulation. However, it shouldn't be assumed that all CD patients have lactose intolerance (Eadala et al., 2011).

A variety of dietary modifications and measures have been evaluated in achieving and maintaining CD remission. Fish oil has been shown to have specific anti-inflammatory properties in a wide variety of diseases, however, a Cochrane review of six trials failed to demonstrate a clear clinical benefit of using fish oils in CD maintenance (Turner, 2009). Probiotics may aid in maintaining CD remission by modifying the microbial flora that is incriminated by CD and by suppressing the inflammatory response (Beyer, 2008). While the research supporting probiotic use for pouchitis in UC is strong, the research for probiotic use in CD is inconsistent and lacks sufficiently rigorous studies. A Cochrane systematic review published in 2006 and a meta-analysis published in 2008 that included eight randomized controlled trials examined the efficacy of probiotics for the maintenance of remission in CD. Both found insignificant results, concluding that probiotic use cannot be recommended as effective therapy for the maintenance of remission in CD (Basson, 2012).

It has been hypothesized that emulsifiers, which are ubiquitous in processed foods, might play a role in the increased incidence of IBD. In a recent study, Chassaing et al. suggest that the relatively low use of emulsifying agents, particularly carboxymethylcellulose and polysorbate-80, can disturb the host-microbiota relationship resulting in a microbiota with enhanced mucolytic and pro-inflammatory activity that promotes inflammation (Chassaing et al., 2015). Other studies have suggested links between CD and food additives such as maltodextrin, sucralose, and food dyes.

To date, it is unclear whether a therapeutic diet has the ability to induce and maintain remission, or is predominantly of symptomatic benefit (Donnellan, 2013). An elimination-reintroduction diet was one of the earliest described modification diets evaluated for disease maintenance in CD (Donnellan, 2013). Elimination entails remission induced via elemental feeds, followed by the reintroduction of single food types in an effort to identify those that trigger symptoms. Unfortunately, this approach is complicated, lengthy, and can be costly due to professional support needed. Other diets that have been used in the current treatment of CD are discussed in more detail below.

Current Research on Dietary Treatment of CD

The investigation of nutritional approaches in treating CD has largely been limited to the use of EN and PN with the goal of providing bowel rest and inducing remission. Dietary whole food recommendations are poorly developed even though there is enough evidence to indicate that CD is impacted by diet. Furthermore, an alteration in the intestinal microbiome is believed to provide a possible pathway for dietary manipulations that may reduce inflammation in CD (Olendzki et al., 2014). Below is the critical analysis of researched dietary recommendations currently available for CD.

Anti-inflammatory Diet for IBD

The Anti-Inflammatory Diet for IBD (IBD-AID), developed at the UMass Medical School, is the first evidenced-based diet for IBD, with a goal to assist with a decreased frequency and severity of flares, as well as to obtain and maintain remission in IBD (Olendzki et al., 2014). IBD-AID is made up of five basic components, and focuses on delivering a dietary therapeutic approach that not only addresses nutrient adequacy, malabsorption issues and symptom relief, but also facilitates remission (Gray, 2014,

quote by Olendzki). The first component is the modification of certain carbohydrates, including lactose and refined or processed complex carbohydrates. The second component emphasizes the ingestion of pre- and probiotics to help restore intestinal flora balance, while the third component distinguishes between saturated, trans, mono- and polyunsaturated fats. The fourth component encourages a review of the overall dietary pattern, detection of missing nutrients, and identification of intolerances. The final and fifth component modifies the textures of foods as needed per patient symptomology to minimize intact fiber and to improve absorption of nutrients (Olendzki et al., 2014). The IBD-AID consists of lean meats, poultry, fish, omega-3 eggs, particular sources of carbohydrate (including lactose, and refined or processed complex carbohydrates), select fruits and vegetables, nut and legume flours, limited aged cheeses made with active cultures and enzymes, fresh cultured yogurt, kefir, miso and other cultured products rich with certain probiotics, and honey. Prebiotics in the form of soluble fiber containing beta-glucans and inulin are suggested. Additionally, patients are advised to begin at a texture phase of the diet that matches their symptomology, starting with phase one if in an active flare (Olendzki et al., 2014). Phase four is appropriate for those in remission with no strictures. With improvement in symptoms, patients are able to advance phases to more whole foods, but should do so cautiously.

Olendzki et al. offered the IBD-AID to 40 patients with IBD, ranging from ages seventeen to 70, who were retrospectively reviewed. Only eleven of these patients met eligibility by IRB for further medical review and had complete data. Eligibility for this further review included seeing an IRB-approved gastroenterologist and dietitian in clinic, endoscopically-diagnosed IBD and failure of drug treatment, persistent symptoms, or

reluctance to proceed with other options (Olendzki, 2014). Medical record review included clinic notes, lab values, drug concentration levels, imaging studies, and endoscopic evaluation. Eight of these patients had CD, while three had UC. Their progress was assessed before and one to three months after initiating IBD-AID using validated tools to assess disease activity. The Harvey Bradshaw Index (HBI) was used for CD patients while the Modified TrueLove and Witts Severity Index (MTLWSI) scoring system was used for UC patients. HBI remission was defined as an HBI score of less than or equal to four points, while HBI response was defined as a decrease in HBI score of greater than or equal to three points. Clinical remission criteria for UC was determined by a MTWSI of less than or equal to two, while clinical response was defined as a reduction from baseline in the MTWSI of greater than or equal to two points (Olendzki et al., 2014).

All patients used the diet for at least four weeks and kept detailed food records, along with time and recording of symptoms on a scale of zero to five. Symptoms included a subjective assessment of bloating, pain, diarrhea, urgency, bleeding, and fatigue. After using the IBD-AID, 100 percent of the eleven patients worked with their gastroenterologists to downscale their medication regimen and 100 percent also reported a reduction in their baseline IBD symptoms, including bowel frequency (Olendzki et al., 2014). The mean baseline HBI for CD patients averaged eleven, but decreased to 1.5 post dietary intervention. The mean baseline score of the MTLWSI for UC patients was seven, and mean follow-up score was zero. Furthermore, mean decrease in HBI was 9.5, while mean decrease in MTLWSI was seven (Olendzki et al., 2014). Additionally, of the original 40 patients, 24 patients had a good or very good response to the diet, as measured

by self-reported symptoms and greater than 70 percent dietary compliance (Olendzki et al., 2014).

The dietary pattern of the IBD-AID is carefully oriented to decrease inflammation, improve nutritional status, and is thought to maintain a beneficial intestinal bacteria balance; however, this has yet to be proven by rigorous scientific experimentation (Olendzki et al., 2014). Olendzki et al. concluded that their data suggests that at least some patients with IBD can benefit from the use of IBD-AID, particularly in terms of reducing symptomatology and consequently, a reduction in the use of medication (Olendzki et al., 2014).

Weaknesses of this study include small sample size and case series study design, as well as a difficult and restrictive diet to follow. Additionally, a comparison group was lacking, and results were partially based on self-reported food records and subjective assessment of symptoms. Furthermore, Olendzki et al. were unable to confirm their hypothesized mechanisms of action by examining the impact of IBD-AID on the intestinal microbiome. While this case series is promising and provides valuable information on a therapeutic diet for IBD, the study of IBD-AID could benefit from a more rigorous analysis provided by a randomized controlled clinical trial, including evaluation of mucosal healing and assessment of change in gut flora to examine the exact mechanisms of benefit (Olendzki et al., 2014).

Gluten-Free Diet and IBD

The use of a gluten-free diet (GFD) in clinical practice among patients with significant intestinal symptoms (which can’t be solely explained by the degree of

intestinal inflammation) has the potential to be a safe and highly efficient therapeutic approach, even after celiac disease has been ruled out (Herfarth, 2014).

In an internet-based cohort study of patients with IBD, part of the ongoing Crohn's and Colitis Foundation of American (CCFA) Partners, Herfarth et al. performed a cross-sectional study using a twelve-question survey on GFD. A baseline survey and the specific GFD questionnaire was completed by a total of 1,647 patients. Ten (0.6 percent) and 81 (4.9 percent) patients had been diagnosed with celiac disease and gluten sensitivity by their health care provider, respectively (Herfarth, 2014). Three hundred fourteen (19.1 percent) participants reported ever having tried a GFD, and 135 (8.2 percent) reported current use of a GFD. Sixty-one percent of these patients had CD and 39 percent had UC. Baseline characteristics between GFD and non-GFD, including disease type, duration of disease, concomitant medication, educational status, disease activity, quality of life, and BMI, were not statistically significant. Furthermore, those who completed the GFD questionnaire were compared to individuals within the CCFA Partners cohort who were not asked to complete a GFD questionnaire. In this sub-analysis, no clinical significant difference in disease or baseline characteristics was found between those completing the GFD questionnaire and those that did not (Herfarth, 2014).

Of the 314 patients who had ever followed or were still following a GFD, 206 (65.6 percent) reported that they experienced an improvement in at least one clinical symptom that has been associated with gluten exposure (Herfarth, 2014). These clinical symptoms included bloating, diarrhea, abdominal pain, nausea, and fatigue. Additionally, 38.3 percent of the 314 recounted fewer and less severe IBD flares while on a GFD, and 23.6 percent reported requirement of fewer medications to control their disease (Herfarth,

2014). Of the 135 patients still following a GFD, adherence was excellent in 41.5 percent, average in 34.1 percent, and fair/poor in 24.4 percent. The only clinical symptom that was statistically reduced with excellent GFD adherence was fatigue when compared to fair/poor adherence ($p<0.03$) (Herfarth, 2014).

Weaknesses of this study include self-reported data, which is highly subjective, and participation bias, as subjects were a volunteer sample of patients, which may not be representative of the diet habits in patients with IBD collectively. This study was based solely on a questionnaire without collection of blood samples, meaning occult celiac disease with serological tissue transglutaminase testing could not be ruled out, and HLA-DQ2 and HLA-DQ8 status could not be determined. Strengths include no significant differences in baseline characteristics between the GFD and non-GFD groups, as well as no significant difference in baseline characteristics between those who completed the GFD questionnaire and those within the CCFA Partners cohort who were not asked to complete a GFD questionnaire, ruling out selection bias. Herfarth et al. concluded that the high prevalence of a GFD in the CCFA Partners cohort strongly suggests a potential role of this diet in the adjunctive therapeutic management of subgroups of patients with IBD (Herfarth et al., 2014). They also concluded that further research investigating possible mechanisms of gluten-mediated worsening of intestinal inflammation in susceptible patients with IBD is warranted (Herfarth et al., 2014).

FODMAPs and IBD

Carbohydrate malabsorption has long been thought to induce GI symptoms and lactose malabsorption has been identified as a potential trigger of GI symptoms due to the rapid fermentability and high osmotic activity of malabsorbed lactose (Gearry, 2008).

Lactose malabsorption is prevalent in 70 percent of patients with small intestinal Crohn's disease compared to fifteen percent of those with colonic Crohn's disease and the general population (Gearry, 2008). Fructose has also been identified as a possible cause of functional GI symptoms in those who incompletely absorb it (Gearry, 2008). The prevalence of fructose malabsorption in IBD is unknown, but several factors might explain its possible role in malabsorption. These include small bacterial overgrowth, small intestinal mucosal disease and shortened bowel length, together with alterations in transit time and exposure to cytokines that might reduce small intestinal absorption of fructose (Gearry, 2008). Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAPs) are also thought to induce GI symptoms via rapidity of gas production from their quick fermentation by intestinal bacteria, and by increased fluid delivery to the large bowel due to their osmotic effect. (Gearry, 2008). FODMAPs include fructo-oligosaccharides, lactose, fructose, galactans, and sorbitol. Limiting their intake has been shown to improve symptoms in greater than 50 percent of patients with IBS or other functional GI disorders (Gearry, 2008). Therefore, low FODMAP diets are thought to likely reduce functional symptoms in patients with CD by reducing the osmotic load and bacterial fermentation, rather than primarily having an anti-inflammatory effect (Gibson and Shepherd, 2010).

Gearry et al. set out to address the hypothesis that FODMAPs are clinically significant dietary triggers of functional abdominal symptoms, specifically in patients with IBD. Gearry et al. also measured factors that related to the success and implementation of the diet, including factors associated with adherence, as well as barriers to use (Gearry, 2008). Patients with IBD from Victoria, Australia were included

in the study if they received dietary intervention aimed at reducing gastrointestinal symptoms, but were excluded if they saw a dietitian for weight loss and caloric supplementation, or if they had celiac disease. Patients received a brief one-on-one low FODMAP diet instruction, and were provided literature on the diet and food lists as additional resources. Recipe books were available for purchase and patients were given the option to meet with the dietitian again if necessary. Repeat consultations included general dietary review, confirmation of understanding dietary principles, request for strategies and advice regarding purchasing and preparing low FODMAP foods, or further dietary evaluation and advice. Fifty-two patients with CD and twenty patients with UC underwent a retrospective telephone questionnaire. The structured telephone interview was performed by an investigator who was not a dietitian involved in giving the dietary intervention. Patients were encouraged to answer honestly and were informed that the results were confidential. A wide range of data was collected including the following: patient characteristics and demographics; family and domestic demographics, IBD phenotype and treatment history; results of fructose and lactose breath tests if available; recall of dietary advice given; adherence to dietary advice including questioning concerning ingestion of individual FODMAPs and specific questioning concerning FODMAP-containing foods in order to validate the patient's responses concerning overall adherence; opinions regarding the palatability and ease of following the diet as well as its cost and availability of specialty foods in shops; and change in GI symptoms following dietary intervention. GI symptoms were recorded on a scale from -10 (change to worst symptoms possible) to +10 (change to complete absence of symptoms), in relation to a baseline score of 0 that represented the patient's symptoms at the time of

dietary intervention. Symptoms were scored at least three months following the dietary intervention and a significant change in symptoms was arbitrarily judged as +5 for improvement and -5 for deterioration in keeping with previous research performed by this group (Gearry, 2008). Descriptive data analysis was done using chi-squared or Fisher's exact testing to determine significance or differences between groups, as appropriate.

Up to 70 percent of patients were adherent to the diet. Overall, abdominal symptoms, abdominal pain, bloating, wind and diarrhea improved in patients with CD and UC ($p<0.02$ for all), but not for constipation. The characteristics of those who did and those who did not respond to overall or individual symptoms were compared for the CD group, but not for the UC group. For CD, efficacy was associated with dietary adherence ($p=0.033$) and inefficacy with non-adherence ($p=0.013$). Sustained response was associated with post-secondary education and working 35 hours or less per week ($p<0.03$) (Gearry et al., 2008).

Cause and effect are extremely difficult to prove in this retrospective cohort. Other weaknesses of this study include lack of a detailed analysis of disease activity over the period of study, and no formal evaluation of medication changes and possible resultant changes in disease activity, symptoms, and/or inflammation. This study was also subject to recall bias and failed to blind the investigator. Furthermore, this diet is complicated, time consuming, and seems to favor those with a higher level of education and more time for grocery shopping and food preparation. Lastly, a diet of this nature might produce more favorable results with more frequent visits and closer follow-up with the Registered Dietitian.

Gearry et al. concluded that their study of patients' impression of the low FODMAP approach indicates that such dietary change might play a significant role in the control of abdominal symptoms in patients with IBD. They also concluded that a randomized controlled clinical trial is warranted in this patient group in order to truly test their observations and to define whether this therapeutic dietary approach deserves wider application (Gearry et al., 2008).

The Specific Carbohydrate Diet for Inflammatory Bowel Disease

The Specific Carbohydrate Diet (SCD), developed by gastroenterologist, Sidney Haas in 1951, is a diet that claims to induce and maintain drug-free remission in patients with IBD, however, neither the characteristics of patients who follow this diet nor the benefits of the diet have been well described in the literature (Kakodkar et al., 2015). The SCD predominantly consists of monosaccharides, solid proteins, fats, a high ratio of amylose to amylopectin vegetables, fruits, and nuts. Disaccharides and most polysaccharides are excluded. Elaine Gottschall, author of *Breaking the Vicious Cycle: Intestinal Health Through Diet*, hypothesized that individuals with IBD can only optimally absorb monosaccharides and that disaccharides and high amylopectin foods are both poorly digested and absorbed by patients with IBD. There is thought to be a dysfunction of the host's disaccharidases, which has been posited to arise from excessive mucus production, ultimately causing maldigestion. Additionally, it has been posited that the SCD can optimally nourish a patient with IBD and lower the amounts of disaccharide

sugars entering the colon, preventing and reversing a significantly altered and dysfunctional microbiota thought to be present in the GI tract of patients with IBD (Kakodkar et al., 2015).

Kakodkar et al. collected survey data from 50 subjects with IBD following the SCD in the United States. Thirty-six subjects had CD, nine had UC, and five had ID. All subjects were in remission and had Gastrointestinal Symptom Severity Checklist (GSSC) scores that reflected mild gastrointestinal symptoms. Intervention results included a high quality of life with a mean Short Quality of Life in Inflammatory Bowel Disease Questionnaire (SIBDQ) score of 60.9 (range=35 to 70). Out of 22 patients who were taking no medications at all, sixteen had discontinued all steroids, three had discontinued TNF inhibitors, and five discontinued 6-MP and had remained in remission (Kakodkar et al., 2015). The mean time the SCD was followed was 35.4 months (range=1 to 216 months) and self-rating of dietary compliance had a mean adherence rating of 95.2% (range=71%-100%). Sixteen subjects (32.1%) reported occasional intake of "forbidden" foods, but seven (43.7%) of these patients were also taking some type of maintenance medication.

Kakodkar et al. concluded that SCD can potentially be an effective tool in the management of some patients with IBD, particularly those with colonic and ileocolonic CD. Their results also suggest that some patients with moderate to severe IBD who follow SCD might be able to discontinue immunosuppressants (Kakodkar et al., 2015). Despite this being the largest series of patients with IBD following SCD to date, this study is not representative of the IBD population as a whole due to a highly educated pool of subjects, all of whom were in remission. Additionally, meal preparation time

(reported average time of 10.8 hours/week) as well as length of time to feel physical improvements (reported average time of 29.2 days) may be a barrier for individuals to adhere to a SCD. While this might not seem like a great deal of time for clinicians considering potential benefit, most patients will likely find this diet challenging, time consuming, and unsustainable. Furthermore, study results are not likely accurate considering data was reported retrospectively.

Even though this study design is fairly weak, the results are promising, showing that at least a subgroup of patients with IBD may notably improve as a result of following the SCD and/or other dietary interventions in general (Kakodkar et al., 2015). If the SCD, or other dietary interventions for that matter, have the ability to change the microbiome of patients with IBD and/or reverse dysbiosis in patients with IBD (as suggested), this could be a low-cost intervention to induce and maintain remission with little or no adverse reactions (Kakodkar et al., 2015). Regardless, further studies examining the impact of SCD and other diet therapies for IBD are warranted.

Analysis of Gut Microbiome and Diet Modification in Patients with CD

The relationship between the gut microbiota and mucosal health is of great importance in CD and diet modification is thought to have the ability to alter the gut microbial balance. It has been estimated that over 100 trillion microbes belonging to over 500 species co-exist in the human colon, and that changes in the composition of this intestinal microbiota have been observed in disease involving infectious and non-infectious etiologies (Walters et al., 2014). Analysis of the bacterial communities in human feces is widely utilized to determine these changes, as human feces can provide a

complex microbial niche and reflect the microbiota that are present in the large intestine (Walters et al., 2014).

Walters et al. aimed to investigate diet's effect on restoring the gut microbial complexity in patients with CD. They also investigated the ability of DNA extraction methods to detect changes in the complexity of fecal microbiome in healthy controls and patients with CD (Walters et al., 2014). Only eight participants, aged 16-50 years, were enrolled in this study, two of which were healthy controls and six with a diagnosis of CD. Inclusion criteria included: confirmed diagnosis of CD, in clinical remission, no probiotics use, and willing to sign consent for enrollment into the trial. Exclusion criteria included: failure to meet any of the inclusion criteria, poor compliance with the diet during the study phase, failure to submit stool samples as indicated at each phase of the study, and need for antibiotic use during the study. Patients and the IBD care team were blinded to either a Low Residue Diet (LRD) or the Specific Carbohydrate Diet (SCD). Patients followed the diet for 30 days, followed by a "washout" phase for 30 days in which they resumed their normal pre-study diet. Stool samples were collected and evaluated on days one and 30 for both the study diet and "washout" pre-study diet. Standard IBD questionnaires (IBDQ) were collected from all subjects at the beginning and end of the study to assess clinical outcomes. Those who were noncompliant or experienced worsening of clinical disease state were automatically excluded from the study. As previously mentioned, two healthy participant's sans gastrointestinal symptoms or other chronic illnesses were also enrolled in the study to provide a baseline control fecal sample (Walters et al., 2014). For the duration of the study, CD medications and dosages were unchanged.

Two patients withdrew from the study due to dietary noncompliance, however one of these patients provided a pre-diet modification fecal sample. In total, seventeen fecal samples were obtained from five patients, of which sixteen samples were used for the analysis of dietary associated changes in the fecal microbiome. Five samples were used for the comparison of CD versus controls (Walters et al., 2014). Dietary compliance was approximately 80 percent. DNA extraction from fecal samples using a column based method provided consistent results. A marked decrease in the overall microbial diversity was observed in fecal samples from five CD participants at the pre-diet modification time point compared to the negative controls. There also was a significant overlap of bacterial classes that were increased or decreased in the presence of CD. The temporal response of gut microbiome to the SCD resulted in an increased microbial diversity while the LRD diet was associated with reduced diversity of the microbial communities (Walters et al., 2014). Additionally, the microbiome during the diet was retained during the washout period. Lastly and unfortunately, increased microbial diversity was not associated with any change in clinical status.

Weaknesses of this study include a very small sample size, limited data, and inability to show a significant clinical improvement with increased microbial diversity. Also, gut inflammation was assumed, rather than characterized. Strengths include double blinding and longitudinal samples that provided critical evidence of the effects of diet modification on the fecal microbiota. Walters et al. concluded that the SCD was associated with restructuring of the gut microbial communities, however, more research is warranted to explore specific diet regimens for clinical improvement in CD patients, using the restructured gut microbial diversity a s a correlate (Walters et al., 2014).

Furthermore, Walters et al. believe future studies can help delineate the host-microbe interactions in the gut that help maintain intestinal health.

Research investigating dietary whole food recommendations for CD is limited and weak, however, it suggests that certain dietary modifications can improve the course of CD. Additionally, the role of diet and nutrition is a major concern for this patient population. The role of dietary interventions in the management of CD still needs to be tested vigorously. There is a need for large prospective, controlled trials to provide the appropriate dietary recommendations that patients are seeking, rather than leaving them to seek nonmedical resources for dietary guidance and adopt overly restrictive diets, unnecessarily.

Chapter 3: Proposal

Crohn's disease (CD) currently has no standard dietary approach or medical cure. The purpose of this study will be to investigate the effects of a gluten free (GF) diet on CD activity, inflammation, and symptoms.

Study Design and Objectives

This will be a controlled, cross-over trial (sans randomization) to investigate whether a gluten free diet is an effective adjunct nutritional therapy for the treatment of CD. Several dependent measures will be examined since CD is a multifactorial disease. The primary aim of this study is to identify whether a gluten free diet for eight weeks has an effect on CD activity (measured by the HBI), symptoms associated with CD (measured by subjective assessment), and inflammation (measured by ESR and CRP). Secondary aims are to examine the impact of GF diet on the gut microbiota and to determine whether the genetic mutation HLA-DQ2/HLA-DQ8 is necessary for the impact of the gluten free diet on management of CD. IRB approval will be obtained prior to data collection.

Recruitment & Sample Size

Subjects will be recruited from outpatient clinics at Group Health Cooperative and UW Health (Gastroenterology clinics and Digestive Health Center) in Madison, Wisconsin. It is estimated that these clinics manage about 500 patients with CD. The study will be limited to subjects 18-65 years of age with mild to moderate CD as defined by a HBI score of 5-16. Subjects with a score lower than 5, but who are experiencing symptoms including abdominal pain, bloating, diarrhea, constipation, urgency, bleeding, and fatigue will also be eligible to participate in the study. Exclusion criteria includes people who smoke, use NSAIDs, are pregnant, have been diagnosed with celiac disease, are underweight, use or have used probiotics or antibiotics within the last month, and patients who are already on a strict, gluten-free diet. A target of 60 subjects will be recruited for this study to allow for attrition. Power analysis was used to determine that 40 subjects would be adequate to detect significant differences in the primary outcome measure.

Subjects eligible for the study will be evaluated by their gastroenterologist at a routine follow-up or consultation appointment. Informed consent will be obtained at that time.

Intervention

All subjects will first be on a standard diet for eight weeks, followed by an eight-week gluten-free diet period. All subjects will provide a three-day food record and complete a Food Frequency Questionnaire (FFQ) prior to the start of the study in order to assess typical intake. Subjects will be provided with all meals and snacks, which will be prepared in a test kitchen. A 3- to 4-day supply of food will be delivered to subjects twice

per week. Subjects are allowed to eat food that is not provided, but must adhere to assigned diet and record all foods eaten that were not provided by the study. Calories provided will be individualized and determined by a baseline of 28 kcals/kg of body weight with additional kcals provided based on activity level. The research registered dietitian (RD) will provide a GF diet instruction in a 60-minute one-on-one session prior to the beginning of the study. Subjects will be given literature on the diet, food lists, and recipes to assist. Subjects will follow-up with the RD in person at weeks four and eight for each diet period, and by phone weekly. Further consultations with the dietitian will also be offered if desired. Dietary adherence will be assessed by two unannounced 24-hour dietary recalls for each diet period, requested at any point in time.

Crohn's Assessment

All subjects will be evaluated at baseline, 4 weeks, and 8 weeks of each diet period for an HBI score and report of symptoms. Response to the intervention will be classified as nonresponse, clinical response, or clinical remission. Clinical response will be defined as reduction in HBI score ≥ 3 points, while clinical remission will be defined by an asymptomatic patient with an HBI score ≤ 4 points. Gastrointestinal symptoms will be recorded on a scale from 0 (complete absence of symptoms) to 5 (worst symptoms possible) weekly.

Genotyping

Subjects will also undergo genetic testing. Blood will be collected at the initial assessment appointment and DNA will be extracted from peripheral blood for HLA typing of DQ alleles. HLA-DQ2 and 8 will be determined using six HLA-tagging single-nucleotide polymorphisms (SNPs).

Gut Microbiome Characterization

Stool will be collected and analyzed for composition and complexity of the gut microbiota at the beginning and end of each diet period. An initial stool sample will be used to test for efficacy and reliability of the bacterial DNA extraction method. Two DNA extraction methods, P1 (Phenol Chloroform: P) and Q1 (Qiagen Stool Kit: Q), will be used and compared. Two separate 250 μL stool samples will be used for the Q1 test, whereas for P1 500 μL of sample will be digested overnight, and then split into two 250 μL samples for P1 extraction. The four resulting DNA extracts will be diluted to test for optimal PCR template concentration. 4 ng, 20 ng, and 100 ng of each of the four templates will be individually amplified by PCR for bacterial 16S rRNA gene (16S) and for archaeal 16S. Each dilution will be amplified independently eight times on a temperature gradient of 48°C to 58°C. PCR products will be visualized on 1% agarose gels containing ethidium bromide. 20 ng of DNA template will be consistently amplified well, and the amplifications from 48°C-58°C will show larger bands. All subsequent PCRs will be done with 20 ng total DNA templates on a gradient of 48°C to 58°C to decrease the likelihood of missing sensitive bacterial populations. Variations to the starting materials will include: P2: 250 mg of feces instead of 250 uL. P3: Same as P1 but using 250 uL of feces in Trizol. Q1: 250 uL of frozen stool sample. Extract used will be Qia Amp DNA Stool Miniprep Kit as described by manufacturers. Elute in 50 uL Buffer AE. Q2: Same as Q1 but use 250 mg of frozen feces. Q3: Same as Q1 but use 250 uL of fecec in Trizol.

DNA from a second stool sample will be extracted with both the P2 and Q2 methods to test extraction comparability again while standardizing for mass. The sample

will then be extracted using the P3 and Q3 methods to test whether extracting from stool samples in Trizol (Invitrogen) will give different results than performing extraction from the original fecal sample.

Bacterial DNA amplification will be done on a Master cycler gradient thermocycler (Eppendorf). The 50 µL reaction mixture will contain 21.75 µL of sterile water, 5 µL of 10X PCR Buffer (Takara), 0.25 µL of 5U/µL ExTaq (Takara), 4 µL of 2.5 mM dNTP, 5 µL of 10 mg/mL BSA (New England Biolabs), 5µL each of 3µM forward and reverse primers, and 4 µL of template DNA. Bacterial primers will be 27°F (5'-agagtttgatcctggctcag-3') and 1492R (5'- ggttaccttgttacgactt-3'). Archaeal primers were 4Fa (5'-tccggttgatcctgccrg-3') and 1492R (5'- ggttaccttgttacgactt-3'). PCR products will be hybridized to the Phylochips as previously described by Yergeau and colleagues (Yergeau et al., 2009). Briefly, the hybridization method is similar to the standard Affymetrix hybridization protocol. Control oligo used will be a DOE 213 primer (5' biotin-7 TCCTGAACGGTAGCATCTTGACGAC 3'), and a custom spike-in control of known bacterial DNAs will be added to the fragmentation mix during DNA fragmentation.

Other Clinical Data

Blood will be collected at baseline, four weeks, and eight weeks of each diet period to measure inflammatory markers, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Subjects will also report medications and dosages at the beginning and end of each diet period.

Data Analysis/Statistical Tests

All data will be kept in a central database with a high level of security, protection, and quality control. All data will be analyzed using SPSS. Findings will be considered to be statistically significant if the p value is less than or equal to 0.05. All subjects will serve as their own control. Dependent t-tests will be used to assess primary outcomes (changes in HBI scores, symptoms, and inflammatory markers). The secondary outcome, association of gluten free diet effectiveness with HLA-DQ2/8 genotype, will be assessed using Wilcoxon's rank-sum test. For the secondary outcome (changes in microbiota) cell files will be analyzed with PhyloTrac (Institute for Genome Sciences, Baltimore, USA) and dCHIP (Harvard School of Public Health, Boston, USA). PhyloTrac implements background subtraction, normalization and probe scoring algorithms as previously described by Schatz and colleagues (Schatz et al., 2010). DChip will be used to do a basic comparison of samples as previously described by Li et al. and Sheddden et al. (Li et al., 2001; Shedden et al., 2005). Only scores with a pf score >0.9 will be analyzed.

Chapter 4: Discussion

More research is needed examining the role of diet in CD pathogenesis. Further investigation of the effects of diet on CD disease course could potentially lead to novel, dietary therapeutic interventions. Findings could influence CD treatment options and may even play a role in CD prevention.

Potential problems

There are several anticipated problems that may accompany the proposed study. Being a 16-week study, subjects may grow tired of adhering to the diet interventions and drop out, failing to complete the study, which will make within-subject comparison impossible. A potential solution to this problem would be to eliminate these subjects from the study; however, this would result in a decrease or loss of statistical power due to fewer subjects. Alternatively, these subjects could be kept in the study and included in an unpaired data analysis, however, this makes it more difficult to find significant results. If the rate of attrition results in a power of less than 80%, the validity of a negative

conclusion is weakened, but this would not be of concern in the event of findings that support the hypothesis. Providing food should be strong incentive for most subjects to stay in the study, and cushioning the number of subjects (recruiting 60) to allow for some attrition will hopefully allow the power to be at least 80%. Additionally, there is uncertainty in the length of time necessary for a GF diet to be effective in this population. Eight week dietary interventions were determined based on the length of time previous research studies have shown it takes CD subjects to respond to nutrition support. While it is assumed that this duration of time is adequate for response, response rate remains unknown. The only solution for this problem is the replication of this study for a longer period of time. Lastly, it is anticipated that some subjects will come into the study with self-imposed dietary restrictions and may be not be entirely willing to consume what is provided to them. The dietitian and cooks will accommodate all subjects, adapting the GF and standard diets based on individual food preferences, with the exception of minimizing or eliminating gluten-containing foods for the standard diet.

Anticipated Results

Improvement of CD symptoms on the GF diet compared to control with statistical significance is anticipated, based on previous studies (Herfarth et al., 2014; Gearry et al., 2014). Furthermore, a stronger response to GF diet for those with HLA-DQ2/8 is anticipated, but a positive response in at least some subjects without this genotype is also anticipated. The expected prevalence of this genotype in the CD population is about 40% (DiGiacomo et al., 2013). Other anticipated results include improved subjective gastrointestinal symptoms and changes in fecal microbiota following diet modification. The GI microbiota of healthy individuals is dominated by four major bacterial phyla:

Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Wright et al., 2015). Research has confirmed that the overall microbial diversity is decreased in CD patients. A decreased representation of several taxa in the Firmicutes phylum and Bacteroidetes has been found in CD patients, as well as an increase in potentially harmful bacteria, Gammaproteobacteria and Enterobacteriaceae (Wright et al., 2015). Similar findings are anticipated in this study, followed by a change in microbial diversity post GF dietary intervention. Whether this change is beneficial or not remains to be seen.

Clinical Implications & Application

Typically, GF diet is recommended solely for those with celiac disease. This will be the first randomized crossover trial examining a GF diet for subjects with CD. Furthermore, if a link between positive response to GF diet and HLA-DQ2/8 carriers is statistically significant, this could warrant routine testing for this genotype in clinical practice for this population. GF diet is likely not appropriate for the entire CD population, hence should only be recommended to those who are likely to benefit from it. A gluten free diet can be a challenging diet to adopt and sustain. Additionally, a GF diet, when done incorrectly, can also put patients at risk for nutritional deficiencies. For a subset of the CD population, diet modification could be a more economical, safer and more effective means of reducing symptoms and flare-ups compared to pharmacological therapy.

Future Studies

Results supporting a GF diet as a therapeutic approach for CD would warrant further investigation through larger clinical trials. New findings from The Crohn's & Colitis Foundation of America's (CCFA) Microbiome Research Initiative reveal that the microbiome might hold the key to curing or preventing IBD, thus dietary changes to adjust the makeup of the microbiome deserve further investigation. Additionally, further research investigating possible mechanisms of gluten-mediated worsening of intestinal inflammation in patients with CD would be helpful (Herfarth, 2014).

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Appendix A

Mount Mary University
Institutional Review Board (IRB)
for the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN
UNTIL THE IRB HAS APPROVED THIS PROJECT

Directions:

- Faculty and student researchers, as well as student research advisors, should **read all relevant information on the College IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html
- The IRB application must be filed and approved by the IRB **prior** to any Mount Mary College faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
- If there is a cooperating institution, attach copy of their IRB approval.
- In the case of a student research project, the student may complete the IRB application but the student's research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g. informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as **Exempt** from full IRB review, the research advisor may request the student to complete and submit a full IRB application.

- All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org
- Complete this application on-line; click on boxes to enter your responses, print it out and obtain signatures. (**Handwritten applications will not be accepted**.) For your benefit, save the completed application in case it needs to be revised and resubmitted.
- Submit the completed application, with required signatures and attachments, to Marmy Clason, IRB Chair, Communications Department. **(Emailed applications will not be accepted.)**
- This is a professional document; please check spelling, grammar and punctuation.
- Allow a **minimum of 10 working days** to process your application. Make sure this time frame is accounted for when considering initiation of data collection and due dates for student projects.
- For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
- Upon receipt of the IRB letter of approval, data collection may begin.

I. Required Documentation (No action will be taken without these attachments.)

Are the following attached to the IRB application?		
Consent application	☒ Yes	Applications should include explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix.A.
Questionnaire/Survey Instrument(s)	☒ Yes	If survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question should be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	☒ Yes	Copy of transcript, certificate or other evidence .
Copy of cooperating institution's IRB approval.	☒ Yes	Not required if there is no cooperating institution.

If student, list Research Advisor and complete Section II. Research Advisor must provide requested information and verify.

Department: Dietetics

Research Advisor's Name: Megan Baumler
Email: baumlerm@mtmary.edu Phone: 414-443-3659
Research Advisor: Have you completed Human Subject's Training? ☒ Yes ☐ No

Research advisor's signature indicates responsibility for student compliance with all IRB requirements.

Signature: _____ Date:
Research Advisor

II. Investigator(s):

Name: Julie Crow Phone: 414-736-1737
Affiliation with Mount Mary College (e.g. faculty, student, etc):
Student
Email: julie.heling@gmail.com

Signature: Julie Crow Date: 08/01/2015

Name: Phone:
Affiliation with Mount Mary College:
Email:

Signature: _____ Date:

III. Project Description

Instructions: Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):
The purpose of this project is to determine if a gluten free diet has an impact on Crohn's disease activity, inflammation, and symptoms.

2) Relevance to practice/body of knowledge:
There currently is no standard dietary approach or medical cure for Crohn's disease. Dietary whole food recommendations are poorly developed even though there is enough evidence to indicate that Crohn's disease is impacted by diet. Lack of evidence-based recommendations leads patients to adopt restrictive

diets unnecessarily and can result in nutritional deficiencies. Going gluten free is particularly popular in society and can be especially appealing to individuals with Crohn's disease seeking symptomatic relief. To date, there is only one cross-sectional study examining the impact of a gluten free diet on symptoms in patients with inflammatory bowel disease. More research examining the impact of a gluten free diet on Crohn's disease symptoms is needed. Additionally, investigating whether gluten plays a role in inflammation and changes in the microbiota (which is now accepted as an important etiologic factor in the pathogenesis of Crohn's Disease) is also needed. Clarifying the role a gluten free diet plays in Crohn's disease will be invaluable to this population.

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):
This is a randomized, controlled, cross-over trial recruiting a target of 40 subjects. Subjects will be initially randomized to either the gluten free diet or control diet. Each of the two intervention periods will be eight weeks long, separated by a minimum washout period of six weeks. After the washout period, subjects will cross over to the other arm of the study. All meals and snacks will be provided. Subjects will be recruited from outpatient gastroenterology clinics in Madison, Milwaukee, and Chicago. The study will be limited to subjects 18-65 years of age with mild to moderate Crohn's disease as defined by a CDAI score of 150-220. Subjects with a score lower than 150, but who are experiencing functional gut symptoms as measured by change in gastrointestinal symptoms will also be eligible to participate in the study. Exclusion criteria include individuals who smoke, use NSAIDs, are pregnant, have been diagnosed with celiac disease, are underweight, use or have used probiotics or antibiotics within the last month, and patients who are already on a strict, gluten-free diet. Baseline data on diet will be collected from all patients by a detailed diet history in a thorough interview with the dietitian. All subjects will also complete a Food Frequency Questionnaire (FFQ). Subjects on the GF diet will be instructed to follow the diet for eight weeks. The Registered Dietitian will provide a GF diet instruction for those on the GF diet in a 60-minute one-on-one session prior to the beginning of the study. Subjects will be given literature on the diet, food lists, and recipes to assist. Subjects who eat meals out or eat food outside of what is provided will be asked to record this intake to monitor compliance. Subjects will follow-up with the RD in person at weeks four and eight for each diet period, and by phone weekly. Further consultations with the dietitian will also be offered if desired. Dietary adherence will be assessed by two unannounced 24-hour dietary recalls. All subjects will be evaluated at baseline, 4 weeks, and 8 weeks for each diet period for a CDAI score and report of gastrointestinal symptoms. Response to the intervention will be classified as nonresponse, clinical response, or clinical remission. Clinical response will be defined as reduction in CDAI score $\geq$ 70-100 points, while clinical remission will be defined by an asymptomatic patient with a CDAI score less than 150 points. Gastrointestinal symptoms will be recorded on a scale from -10 (change to worst symptoms possible) to +10 (change to complete absence of symptoms) in relation to a baseline score of 0 that represents the patient's symptoms at the time of intervention as previously described (Gearry et al., 2008). Blood will be collected at baseline, four weeks, and eight weeks of each diet period to measure inflammatory markers, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Subjects will report medications and dosages at baseline and at the end of each diet period.
Subjects will also undergo genetic testing. Blood will be collected at the initial assessment appointment and DNA will be extracted from peripheral blood for HLA typing of DQ alleles. HLA-DQ2 and 8 will be determined using six HLA-tagging single-nucleotide polymorphisms (SNPs). Additionally, stool will be collected and analyzed for composition and complexity of the gut microbiota at baseline, and at the end of each diet period. All data will be analyzed using SPS. Findings will be considered to be statistically significant if the p value is less than or equal to 0.05. The Wilcoxon signed rank test will be used to determine whether or not symptoms had changed significantly from baseline. Differential diet effects depending on HLA status will be assessed using ANCOVA models by also including an overall HLA term and an HLA by diet interaction term. Cell files will be analyzed with PhyloTrac (Institute for Genome Sciences, Baltimore, USA) and dCHIP (Harvard School of Public Health, Boston, USA).

4) What measurement/data collection tools are being used?
Detailed diet history, Food Frequency Questionnaire, CDAI scores, Subjective assessment of gastrointestinal symptoms, Genetic testing for HLA-DQ2/8 alleles (blood sample), ESR and CRP (blood sample), and Stool Analysis using PhyloTrac (Institute for Genome Sciences, Baltimore, USA) and dCHIP (Harvard School of Public Health, Boston, USA).

Is the proposed project "research" as defined by Institutional Review Board requirements?

Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

Does the research involve human subjects or official records about human subjects?

☒ Yes
☐ No

***If NO STOP here and SUBMIT application*.**

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box: ☒ Yes ☐ No

If the YES box is CHECKED, proceed to SECTION IV.

***If the NO box is CHECKED, STOP here and SUBMIT application*.**

IV. Exemptions

Are you requesting exemption from IRB review in one of the federally approved categories? If yes, please reference OHRP website http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html and continue with application.

1) Does the research meet the criteria for exempt category 1 (education)? [45 CFR 46.101 (b) (1)]

Is the research conducted in established or commonly accepted educational settings (e.g. schools, colleges or other sites where educational activities regularly occur)? ☐ Yes ☒ No

Does the research study involve only normal education practices (e.g. instructional strategies, techniques, curricula, or classroom management techniques)? ☐ Yes ☒ No

If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.

2) Does the research meet the criteria for exempt category 2 (specific procedures)? [45 CFR 46.101 (b) (2)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior? ☐ Yes ☒ No

Is the information obtained recorded in such a manner that human subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B) ☒ Yes ☐ No

If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.

3) Does the research meet the criteria for exempt category 3 (public officials)? [45 CFR 46.101 (b) (3)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?
☐ Yes
☒ No

Are the human subjects elected or appointed public officials or candidates for public office? **If no, proceed to Category 4.**
☐ Yes
☒ No

Does any federal statute require without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter? (See Appendix B)
☐ Yes
☐ No

If **all** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.

4) Does the research meet the criteria for exempt category 4 (existing data/specimens)? [45 CFR 46.101 (b) (4)]

Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens?
☐ Yes
☒ No

Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)
☒ Yes
☐ No

If **both** questions are answered **yes,** stop here, proceed to **Section I Required Documentation**, and **submit** application.

5) Does the research meet the criteria for exempt category 5 (federal program research)? [45 CFR 46.101 (b) (5)]

Does the research involve studying, evaluating or examining federal public benefit or service programs?
☐ Yes
☒ No

Is the research conducted through a federal agency?
☐ Yes
☒ No

If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.

6) Does the research meet the criteria for exempt category 6 (taste and food quality)? [45 CFR 46.101 (b) (6)]

Does the research involve a taste and food quality evaluation or consumer acceptance study?
☐ Yes
☒ No

Does the food consumed contain no additives, or a limited amount of food additives at or below a level approved by the FDA or EPA or the Food Safety and Inspection Service of the U.S. Department of Agriculture?
☐ Yes
☒ No

If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.

If no exemptions apply, continue with application.

V. Additional Project Information

1) What human subjects training has the researcher completed (e.g. course work, online certification)?
Online certification

2) What process is used for obtaining informed consent (attach the informed consent application)? See Appendix for consent application.
Informed consent application

3) Does the research include special populations?

Minors under 18 years of age?	☐ Yes	☒ No
Persons legally incompetent?	☐ Yes	☒ No
Prisoners?	☐ Yes	☒ No
Pregnant women, if affected by research?	☐ Yes	☒ No
Persons institutionalized?	☐ Yes	☒ No
Persons mentally incapacitated?	☐ Yes	☒ No

4) If **YES,** describe additional precautions included in the research procedures.

5) Does the research involve any of the following procedures?

False or misleading information to subjects?	☐ Yes	☒ No
Withholds information such that their informed consent might be questioned?	☐ Yes	☒ No
Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects?	☐ Yes	☒ No

6) If **YES**, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

7) Does the research involve measurement in any of the following areas?

Sexual behaviors?	☐ Yes	☒ No
Drug use?	☐ Yes	☒ No
Illegal conduct?	☐ Yes	☒ No
Use of alcohol?	☐ Yes	☒ No

8) If **YES**, describe additional precautions included in the research procedures.

9) Are any portions of the research being conducted online?

Survey posted on a website?	☐ Yes	☒ No	If yes, assure anonymity
URL for survey includes information that could identify participants?	☐ Yes	☒ No	If yes, assure anonymity
Invitation to participate sent by email?	☐ Yes	☒ No	If yes, assure anonymity
Items use drop-down box?	☐ Yes	☒ No	If yes, assure that items allow choice of "no response"

10) If **YES**, describe additional procedures.

11) Describe the methods used to ensure confidentiality of data obtained.

Study codes will be used on data documents instead of recording identifying information and a separate document will be kept that links the study code to subjects' identifying information locked in a separate location with restricted access to this document (e.g., only allowing primary investigators access). Identifiable data will be encrypted. Information obtained from data/documents will be properly disposed, destroyed, and deleted.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)
There are no risks associated with this study proposal.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)
Currently, there are no known benefits that might result from this research, however, it may help us to understand the role a gluten free diet may play in Crohn's disease activity, inflammation, and symptoms.

Appendix A: Required Elements of Informed Consent

Informed consent is the process of communicating to a prospective participant, in easy-to-understand language (usually sixth- to eighth-grade level), all that he or she needs to know about participating in a research project, and then obtaining the prospective participant's agreement to participate. The following ten elements of consent are widely recognized and, except under certain specific conditions, **must be included in all consent processes and forms**:

1. An explanation of the study, including goals, procedure, and a statement that the study is research.
2. A description of what participants are expected to do and expected length of participation.
3. A description of any likely risks or discomforts for the participants. Potential harm should be explained in language that participants can understand and that relate to everyday life.
4. A description of any likely benefits to the participant or to others.
5. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant.
6. A statement describing the level of privacy assured for collected information (anonymous, confidential) and how private information and information security will be managed.
7. An explanation of whom to contact for answers to questions about the research. When a Mount Mary student is the principal investigator, the name and phone number of a supervising faculty member is required.
8. An explanation of whom to contact for concerns about the participant's privacy and rights, which for Mount Mary College is its IRB Chair.
9. For research involving more than minimal risk, a statement describing any compensation for injuries and contact information. (Minimal risk is a risk of harm to the participant that is no greater than the risk encountered in normal, day-to-day activities or during routine physical or psychological examinations.)
10. A statement that research participation is voluntary and the participant may withdraw from participation at any time, without penalty or loss of benefits to which the participant is otherwise entitled. If the participant is a patient or client receiving medical, psychological, counseling, or other treatment services, there should be a statement that withdrawal from the study will not jeopardize or

otherwise affect any treatment or services the participant is currently receiving or may receive in the future. Participants also should be told whether their data will be destroyed should they withdraw from the study. If a survey instrument or interview questions are used and some questions deal with sensitive issues, the participants should be told they may refuse to answer individual questions.

Appendix B: IRB De-Identification Standard for Information

Protecting the privacy of research participants is a general concern in the vast majority of research projects. The degree to which privacy needs to be ensured or maintained depends on the nature of the particular research, its setting, and the research participants. Researchers share a general obligation to design their research to reduce the risks of disclosure of collected information about individual research participants. Thus, the present standard for de-identification of information is useful as a guide to protecting privacy even when it is not required or fully required. In this regard, the researcher should consider the following question when collecting and handling data.

Does the information I am accessing, recording, and/or disclosing contain identifiers? Simple access to information may be without concern, for example when the researcher is an employee who routinely handles the records in carrying out his or her position. But, the presence of identifiers in any **recorded or disclosed** information in the research means the information is not anonymous and so does not meet the IRB de-identification standard, which in some cases may also disqualify the research from exemption from IRB review. The IRB de-identification standard includes all 18 direct identifiers specified in the HIPAA Privacy Rule de-identification standard—*45 CFR 164.514(b)*. Below are listed specific direct and indirect identifiers that lead to information not being anonymous.

Identifiers: Direct; Indirect

One way to distinguish between information that is truly anonymous and information that is simply being kept confidential is to determine whether the data set contains direct or indirect identifiers. Information in a data set with either direct or indirect identifiers is not anonymous.

Direct Identifiers include:
- Names
- Addresses
- Telephone and fax numbers
- Email addresses, IP addresses, and URLs
- Social Security numbers
- Medical record numbers
- Account numbers, such as those associated with bank accounts or health plans
- License or certificate numbers, including driver's license numbers
- License plate numbers and other vehicle identifiers
- Fingerprints, voiceprints, or full-face photographic images
- Other unique characteristics or identification numbers (example student ID numbers)

Indirect Identifiers can be combined with publicly available information to identify individuals. The determination of indirect identifiers depends on the nature of the research participants. For example, in a study of residents of the state of Wisconsin, the information that someone graduated from one of the UW system schools probably would not be a unique identifier. However, in a study of small business leaders in Racine, WI, the same information might well apply to only one individual. In general, if any single variable in a data set applies to fewer than five participants, it is considered a potential indirect identifier.

Examples of indirect identifiers include:
- Detailed geographical information, such as state, county, or census tract of residence
- Organizations to which participants belong
- Educational institutions from which participants graduated
- Exact occupations
- Places where participants grew up
- Many dates, e.g. birth dates, hospital admission dates, high school or college graduation dates, etc.

- Detailed income information
- Offices or posts held by participants.

Appendix B

Consent Form for Participation in a Research Study
Mount Mary University

A Randomized Cross-Over Trial Assessing the Impact of a Gluten Free Diet Compared to Control on Crohn's Disease Activity, Inflammation, and Symptoms

Description of the research and your participation

You are invited to participate in a research study conducted by Julie Crow. The purpose of this research is to determine if a gluten free diet impacts Crohn's disease activity, inflammation, and/or symptoms.

Your participation will require you to follow 2 diet interventions, each 8 weeks long. All food and snacks will be provided. You will also be required to meet with a Registered Dietitian at the beginning of the study in which he or she will obtain a detailed diet history from you. If you have been randomized to the intervention group, a detailed gluten free diet instruction will be provided at this time. For those initially in the control group, a detailed gluten free diet instruction will be provided prior to beginning the second diet intervention. You will be required to follow-up with the dietitian at weeks 4 and 8 for each diet period, and by phone weekly. You will also be evaluated by your gastroenterologist at baseline, 4 weeks, and 8 weeks of each diet period for a CDAI score, inflammatory blood markers ESR and CRP, and report of gastrointestinal symptoms. This will require a blood draw each time. Blood will also be collected at the initial

assessment appointment for genetic testing. Additionally, you will be required to provide stool samples at baseline and at the end of each diet period.

Risks and discomforts

There are no known risks associated with this research.

Potential benefits

Currently, there are no known benefits to you that would result from your participation in this research. This research may help us to understand the role a gluten free diet may play in Crohn's disease activity, inflammation, and symptoms.

Protection of confidentiality

Every effort will be made to maintain the confidentiality of your participation in this project. Confidentiality will be maintained within legal limits. Your identity will not be revealed in any publication resulting from this study.

Voluntary participation

Your participation in this research study is voluntary. You may choose not to participate and you may withdraw your consent to participate at any time. You will not be penalized in any way should you decide not to participate or to withdraw from this study.

Contact information

If you have any questions or concerns about this study or if any problems arise, please contact Julie Crow of Mount Mary University at 414.736.1737. If you have any questions or concerns about your rights as a research participant, please contact the Mount Mary University Institutional Review Board at 414.258.4810.

Consent

I have read this consent form and have been given the opportunity to ask questions. I give my consent to participate in this study.

Participant's signature_____ Date:_____

A copy of this consent form should be given to you.