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The impact of a whole-food, plant-based diet on intestinal inflammation

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THE IMPACT OF A WHOLE-FOOD, PLANT-BASED DIET ON INTESTINAL INFLAMMATION

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ABSTRACT

There is substantial evidence that the "Western" dietary pattern, a diet defined as being relatively high in red and processed meat, total fat, refined/processed foods, and relatively lacking in vegetables and fruits, is associated with increased intestinal inflammation, which in turn is implicated in the pathophysiology of disease states such as inflammatory bowel disease (IBD) and colorectal cancer (CRC). Conversely, there is accumulating evidence suggesting that plant-based foods that contain whole grains, dietary fiber, antioxidant vitamins, and phytochemicals, have anti-inflammatory effects in the gut. However, there is a paucity of research investigating the anti-inflammatory effects of a dietary pattern that eliminates all animal products (typical of a Western dietary pattern) and is instead entirely plant-based. Such a pattern, which eliminates all animal products (including meat, eggs, and dairy), eliminates the foods associated with intestinal inflammation and instead replaces them with plant-based foods, many of which have been found to be anti-inflammatory.

The proposed study is a prospective study that will use fecal calprotectin to quantify the levels of intestinal inflammation in healthy participants before and after shifting them from a predominantly Western dietary pattern to an entirely plant-based dietary pattern. This study will help determine whether substituting a plant-based dietary pattern for Western dietary pattern decreases intestinal inflammation, thereby supporting

its use as a potential treatment modality for those with IBD (in conjunction with or in place of pharmaceutical treatment regimens) and as an intervention for primary prevention of IBD and CRC.

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LIST OF ABBREVIATIONS

APC……....antigen presenting cell

CD……...Crohn disease

CRC..... colorectal cancer

DII……..dietary inflammatory index

FFQ…….food frequency questionnaire

GALT……gut-associated lymphoid tissue

GI……...gastrointestinal

H_2S……..hydrogen sulfide

IBD..... inflammatory bowel disease

Neu5Gc……...*N*-glycolylneuraminic acid

PUFA……polyunsaturated fatty acid

ROI…….reactive oxygen intermediates

ROS……..reactive oxygen species

SCFA……short chain fatty acid

UC.....ulcerative colitis

INTRODUCTION

Background

Inflammation is the result of an immunological response to substances perceived by the intricate components of the body's innate immune system to be foreign or harmful. Through numerous response pathways aimed at ridding the body of harmful substances, cells of the innate immune system (such as leukocytes), cytokines and other inflammatory mediators, and inflammation-related serum proteins are recruited to the site of antigenic attack, producing a local inflammatory state characterized by the cardinal signs of tumor, rubor, calor, and dolor (swelling, redness, heat, and pain).[1]

The immune response, and subsequent inflammatory state, are regulated by a complex interplay of host components and the host microbiome (resident microbes). Dysregulation of the immune response and dysbiosis (an imbalance in the host's microbiome) either alone or in combination, can lead to an inappropriate inflammatory response.[2] Dysbiosis is believed to play a role in diseases of multiple organ systems, including the gut.[1] Alteration in the balance of the gut's resident microbes has been shown to be impacted by environmental factors such as components of an individual host's diet, and has been implicated as a significant cause of intestinal inflammation.[3,4]

In the gut, microscopic manifestations of the inflammatory state include local release and recruitment of factors such as the numerous cells of the innate and adaptive immune system, cytokines, complement, and prostaglandins.[2] Inappropriate continuation of the inflammatory state may lead to macroscopic manifestations including mucosal lesions/ulcerations in the intestinal lumen, scarring and stricture formation with

subsequent narrowing of the bowel lumen, and, in cases of severe Crohn disease, transmural defects leading to fistula formation. This in turn produces patient symptoms such as abdominal pain, diarrhea, and hematochezia.[5,6]

Under the umbrella of inappropriate intestinal inflammation lie the disease states of inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn disease (CD), and colorectal cancer (CRC). Chronic intestinal inflammation (as is seen in longstanding IBD) has been identified as an important factor in colorectal carcinogenesis.[7] Additionally, the prevailing hypothesis of IBD etiology is that, in addition to dysregulation of the host immune response, gut dysbiosis can "contribute to the initiation and perpetuation of inflammation associated with IBD,"[2] although the exact pathophysiology remains somewhat unclear.

The public health burden of both IBD and CRC is not insignificant. In the North America, it is estimated that the incidence ranges of UC and CD are 2.2 – 19.2 cases per 100,000 person-years and 3.1 – 20.2 cases per 100,00 person-years, respectively.[8] CRC, as a common and deadly disease, carries a remarkable public health burden. Globally, it is the third most commonly diagnosed cancer in males and the second in females. Rates in North America are among the highest worldwide. In the United States specifically, it is estimated that approximately 135,430 cases of large bowel cancer are diagnosed each year. Incidence and mortality rates have been increasing in the US, despite our screening efforts; approximately 50,260 Americans die of CRC annually, making it responsible for about 8 percent of total cancer deaths.[9]

Statement of the Problem

Given the significant global burden of IBD and CRC, and the shared association of each with intestinal inflammation, further study on modifiable risk factors for intestinal inflammation is prudent and necessary. As mentioned above, gut dysbiosis has been shown to play a significant role in intestinal inflammation.[3,4,10–13] In turn, there is evidence that gut dysbiosis is impacted by components of the individual host's diet, with research primarily focusing on the differing effects of individual foods or micronutrients, which can be broadly categorized as either animal-based or plant-based. Consumption of animal-derived dietary components is associated with an alteration of the gut microbiome to favor a pro-inflammatory state, while plant-derived dietary components are associated with an altered gut microbiome favoring an anti-inflammatory state.[13,14]

Animal proteins and fats have additionally been shown to indirectly favor the creation of a pro-inflammatory state through downstream effects of pathways activated by hyperinsulinemia and COX-2 upregulation.[15] Conversely, many plant-derived factors including fiber and phytochemicals have been shown to have a direct anti-inflammatory effect on the bowel.[16–18]

Existing research has focused primarily on individual food items or specific micro-/macronutrients rather than the study of a dietary pattern as a whole. Isolation of the effect of a specific dietary factor on intestinal inflammation has proven to be problematic, as humans do not exclusively consume specific food items or micronutrients, but instead consume a dietary pattern with a mixture of components interacting to give synergist and additive (or even multiplicative) effects on bowel

inflammation. Cho et al. states that "the analysis of individual foods or nutrients does not allow for consideration of the complicated interactions or high inter-correlations between dietary factors, and the effect of any single nutrient may be too small to detect."[15] To avoid this phenomenon of multicollinearity that is inherent in nutritional epidemiology[19], further research should focus on composite dietary patterns and disease.

The existing evidence can be characterized by the broad dichotomy of either animal-derived or plant-derived dietary factors, with data suggesting the association of animal-based foods with a pro-inflammatory state and the association of plant-based foods with an anti-inflammatory state. Conveniently, the dietary patterns of a "Western" style diet and a plant-based dietary pattern (often referred to as a "vegan" dietary pattern) each embody the extremes of this dichotomy. A Western diet is typified by relatively high consumption of animal fats and proteins (specifically red and processed meats), high-fat dairy products, and refined grains, with relatively low consumption of fruits, vegetables, whole grains, fish, and poultry.[20] A vegan diet by definition excludes all animal products and is instead entirely plant-based.[21] Research focusing specifically on a vegan diet with emphasis on whole-foods rather than refined or processed foods would be of particular benefit.

While studying a whole-foods, plant-based diet would not elucidate the contributions of individual dietary components to intestinal inflammation, given the existing evidence, it is not unreasonable to extrapolate that the potentially additive effects of exclusion of animal-derived foods and increased consumption of whole grains, dietary fiber, vegetables, and fruit may contribute to a decrease in intestinal inflammation, with

this in turn having a protective effect on disease states in the gut associated with inflammation, namely IBD and CRC.

Hypothesis

Shifting subjects from a Western dietary pattern to a whole-food, plant-based dietary pattern will result in a quantifiable decrease in intestinal inflammation, as measured by the intestine-specific inflammatory marker fecal calprotectin.

Objectives and specific aims

Based on the hypothesis that consumption of animal-derived dietary components is associated with a pro-inflammatory state in the gut, while plant-derived dietary components are associated with an anti-inflammatory state in the gut, the objective of this study is to quantify a decrease in intestinal inflammation resulting from strict consumption of a whole-food, plant-based diet. These findings will have potential implications for both the primary prevention of CRC, modifiable risk factors for IBD, and dietary modifications in the management of IBD.

Specific aims include:

- To provide evidence that a Western dietary pattern plays a role in intestinal inflammation.
- To provide evidence that a whole-food, plant-based diet has utility in decreasing intestinal inflammation.

- To lay the foundation for future study on the utility of a whole-food, plant-based diet in the management of IBD and primary prevention of CRC.

REVIEW OF THE LITERATURE

Overview

Intestinal Inflammation in Disease

Intestinal inflammation, in its broadest sense, is a result of abnormal host-microbe interactions in the gut.[22] It is a manifestation of the immune response to substances in the gut perceived by the host to be foreign or harmful. In the process of digestion of consumed foods and absorption of nutrients, the human intestine is required to distinguish between harmless food antigens and infectious or toxic substances or organisms. For protection of the host against substances perceived to be harmful, the intestine is equipped with an effective barrier, i.e., an intact intestinal epithelium with mucus and other secreted protective factors, in addition to the numerous cells and molecules of the innate and adaptive immune systems.[2]

The innate immune system is composed of cells including neutrophils, monocytes, dendritic cells, macrophages, and natural killer cells as well as molecules such as complement, C-reactive protein, kinins, and cytokines. Together, these are involved in initiation of the immune response to either a pathogenic or commensal (resident) microorganism and trigger the response pathways ultimately leading to inflammation.[2]

The adaptive immune system is composed mainly of B and T lymphocytes, which are involved in the immune response to specific foreign antigens. CD4+ T helper cells produce and secrete cytokines involved in inflammation and are themselves critically important in regulating the inflammatory response at mucosal surfaces such as in the gut.[2]

Immune cells residing in the gut are referred to as gut-associated lymphoid tissue (GALT). Epithelial cells, antigen presenting cells (APCs), and other leukocytes in the GALT also secrete a number of cytokines which play a role in regulation of responses to foreign antigens as well as influencing gut immune homeostasis. Through a complex interplay of immune cells such as neutrophils, macrophages, and T cells, pro-inflammatory cytokines and chemokines are released at the site of insult in the gut, leading to an acute inflammatory state.[2]

This acute inflammatory state is normally a self-resolving process due to intestinal immune system mechanisms which control disproportionate responses to microbes in the intestine, whether they be commensal or pathogenic. Proper balance of the components of the immune response is key, as either an excessive response or an insufficient response will lead to inappropriate intestinal inflammation.[2]

In addition to immune dysregulation, composition and quantity of gut microbiota has been implicated in inappropriate intestinal inflammation. Studies of animal models have shown that intestinal microbiota can play a large role in the initiation of intestinal inflammation as well as inappropriate prolongation of the inflammatory response leading to chronic inflammation. For example, studies by Hammer et al.[3] and Rath et al.[4] demonstrated the involvement of intestinal microbiota in the development of inflammation using transgenic germ-free mice, which, when exposed to a defined set of bacteria, developed colitis and gastritis. Further studies using mice with null mutations in the T-cell receptor expressing a phenotype of spontaneous IBD with cytokine imbalance and autoantibody production showed that intestinal inflammation can be initiated by

specific organisms or groups of organisms that are normally present in the intestinal microbiota.[23–25]

In humans, the role of intestinal microbiota in inflammation has been demonstrated in clinical experiments in IBD in which diversion of the fecal stream improved symptoms in patients with Crohn disease (CD). Clinical experiments have also shown that post-operative exposure of the terminal ileum of CD patients to contents of the lumen directly lead to increased inflammation. In addition, similar to findings in animal models, experiments using cells taken from actively inflamed tissues of IBD patients showed that these cells were "activated by exposure to samples of autologous or heterologous GI microflora" while cells from normal controls responded only to heterologous microflora, indicating that intestinal inflammation might be due to impaired host tolerance to antigens normally found in autologous microflora.[22]

In addition to abnormal host-microbial interactions causing intestinal inflammation, the composition of gut microflora has been shown to differ between IBD patients and healthy controls. This implicates both the quantity and content of the gut microbiome in disruption of immune homeostasis in the gut and initiation or continuation of inappropriate intestinal inflammation. The dysbiosis in IBD patients (when compared with healthy controls) has been shown to favor an increase in quantity of bacteria with pro-inflammatory properties, a decrease in quantity of bacteria with anti-inflammatory properties, and a decrease in complexity of commensal bacteria.[3,4]

Specifically, several studies have shown that members of the phyla *Bacteroidetes* and *Firmicutes* were reduced in patients with IBD.[10–12] These human commensal

microbes have been shown to have anti-inflammatory effects in cell systems and animal models;[16] a reduction in quantity of these microorganisms could therefore contribute to inappropriate intestinal inflammation. Additionally, pro-inflammatory microbes such as entero-adherent and invasive *E. coli* are found in greater relative abundance and in greater frequency in patients with IBD than in healthy individuals, further implicating the role of microbes in IBD.

However, it is unclear whether dysbiosis is a cause of the inappropriate inflammatory response seen in IBD or instead an effect of the disease.[22] In support of the former, studies suggest that diet has a significant effect on alteration of gut microbiota, independent of any disease states in the gut. Song et al. notes that switching subjects from a "low-fat, plant-rich diet to a Western diet changed microbial composition, metabolic pathways, and gene expression in the gut microbiome."[17] Studies of specific dietary components have shown that a diet high in animal fats and proteins decreases the quantity of *Firmicutes*[13], which, as mentioned above has members with anti-inflammatory properties. *Firmicutes* also play a role in metabolism of dietary plant polysaccharides such as dietary fiber.[26] Studies of dietary fiber have shown a beneficial effect on the structure of gut microbiota via its fermentation product butyrate, a short chain fatty acid (SCFA) which is known to have anti-inflammatory, anti-carcinogenic, and anti-oxidant properties.[17,26]

This interplay suggests that dietary patterns are likely to play a role in maintaining a balance of commensal intestinal microbes, which in turn are important players in immune and inflammatory responses. Therefore dysbiosis due to an imbalance in dietary

components may play a role in initiation of inflammation,[17] and consequently, could play a role in pathogenesis of disease states such as IBD and CRC, both of which share inflammation as an etiologic mechanism.

Patient Presentation

The symptomatic presentation of intestinal inflammation can have a wide range. In its mildest form, as in an acute gastrointestinal (GI) infection, patients can present with self-limiting symptoms of mild to moderate abdominal discomfort plus or minus nausea, vomiting, and/or diarrhea. On the moderate to severe side of the spectrum are the inflammatory bowel diseases, Crohn disease and ulcerative colitis, which are chronic inflammatory diseases of the intestinal tract with intermittent acute flares of symptoms.

Crohn disease is characterized by transmural inflammation (inflammation occurring through the entire wall of the intestine). Any portion of the GI tract can be involved, with lesions skipping from mouth to anus. Symptoms include crampy abdominal pain, diarrhea with or without blood, weight loss, and frank blood per rectum. Severe disease can also cause malabsorption resulting in nutrient deficiencies and/or weight loss, fistula formation, and luminal narrowing due to fibrotic strictures which can result in constipation and in some cases bowel obstruction.[5]

Ulcerative colitis, as its name implies, is limited to the colon and is characterized by recurrent episodes of inflammation limited only to the mucosal layer. It commonly involves the rectum and may extend proximally in a continuous fashion to other regions of the colon. Patients most frequently present with diarrhea, which may or may not be

bloody. Associated symptoms include colicky abdominal pain, urgency, tenesmus, and incontinence. Severe cases may result in anemia due to profound blood loss or bowel perforation as a consequence of toxic megacolon.[6]

Intestinal Inflammation and IBD Pathogenesis

Although the exact pathogenesis of IBD remains unclear, the prevailing hypothesis at this time is that a dysregulated host immune system due to underlying genetic defects coupled with alterations in host intestinal bacteria contribute to the initial development and/or continuation of intestinal inflammation.[2] In short, this dysregulated immune response involves genetic defects (such as in CARD15, which is involved in innate immunity)[27] as well as impaired and/or incorrect migration ("homing" of B and T cells to the gut in response to a microbial or dietary antigen).[2] In the overall picture of gut dysbiosis, no single microorganism has yet been identified to have a consistent association with IBD. However, specific microbial components, such as the polysaccharide A tail of the commensal microbe *Bacterioides fragilis,* and products of microbial fermentation, such as SCFAs, are means through which the gut microbiome can influence both the intestinal immune response and intestinal inflammation.[2]

Aside from genetic susceptibility, environmental factors have been implicated to play a role in the development of IBD, in particular diet. Andersen et al. state that the high rate of both symptomatic and endoscopic remission induced by enteral nutritional therapy "suggests a major effect of diet on intestinal inflammation." They propose several biologically plausible mechanisms through which diet might affect risk of IBD,

namely through the effect of dietary components on gut homeostasis via oxidative stress, alteration of transcription factors involved in regulating intestinal inflammation, and by modulating mediators of the inflammatory response.[16]

In addition, incidence of IBD has been increasing in both developed and developing countries, in parallel with adoption of a "Western" diet, which is high in fat and protein but low in fruits and vegetables.[28,29] For example, the increased incidence of IBD in Japan in recent decades has been strongly correlated with increased intake of total animal and milk protein, total fat, animal fat, N-6 PUFAs (predominantly found in animal food sources), and relatively decreased intake of N-3 PUFAs (predominantly found in fish and plant food sources) in the Japanese population.[16,28] Additionally, research has shown increasing incidence of IBD in populations who emigrate from the developing world to developed countries.[30] These findings have not been explained by the recent genetic discoveries in IBD pathogenesis[28] and indicate the importance of modifiable environmental risk factors involved in the development of IBD.

In particular, diet-related intestinal inflammation has been suggested to play a role in IBD pathogenesis via mechanisms other than alteration of the gut microbiome. Animal models of IBD have shown that fatty acids, dietary fibers, and phytochemicals lessen intestinal inflammation.[16] Chapman-Kiddell et al. note that the components of a standard "Western" diet could contribute to intestinal inflammation via mechanisms such as the effects of insulin resistance and modification of intestinal permeability.[28] Furthermore, these authors note that a Western diet is associated with obesity, which has been

recognized as a chronic inflammatory state and could potentially increase risk of IBD in genetically susceptible individuals.[28]

In a systematic review of studies on pre-illness diet and subsequent risk of developing IBD, Hou et al. revealed that high intake of total fats, poly unsaturated fatty acids (PUFAs), omega-6 fatty acids, and meat (standard components of a Western style diet) were consistently associated with increased risk of developing both UC and CD; high vegetable intake was consistently associated with decreased risk of UC; and fiber and fruit intake were consistently associated with reduced risk of CD. These authors propose that diet could modulate intestinal inflammation via mechanisms such as antigen presentation and alteration in prostaglandin balance.[29]

Intestinal Inflammation and CRC Pathogenesis

Chronic intestinal inflammation, whether as a result of longstanding IBD or of other factors, has long been implicated in the pathogenesis of CRC.[15] Indeed, Coussens et al. states that the "strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in individuals with IBD."[7] Inflammatory cells predominate the neoplastic microenvironment. Tumor cells take over specific molecules of the innate immune system, including selectins, chemokines, and their receptors (factors involved in the inflammatory response) for invasion, migration, and metastasis. Furthermore, disruption of normal cell death and repair occurs in chronically inflamed tissues, leading to DNA replication and proliferation of cells that are no longer under host

control of normal growth. An environment abundant in inflammatory cells therefore supports uncontrolled cell proliferation and neoplastic growth.[7]

While genetic factors are an additional known risk factor for CRC, accumulating evidence suggests that a considerable proportion of CRC cases are due to environmental factors, most notably diet-induced intestinal inflammation.[15,31,32] Similar to incidence of IBD, incidence of CRC is highest in high-income, industrialized, developed countries; is increasing in developing countries; and is greater among individuals who emigrate to developed countries which have high incidences of CRC as compared to populations who remain in lower income, less industrialized countries, implicating a significant influence of dietary and lifestyle factors.[31,33] Chan et al. states that CRC incidence increases "in parallel with economic development and adoption of a Western lifestyle,"[33] with a "Western lifestyle" typified by a diet high in animal proteins and fats and processed food, in addition to a relatively sedentary activity level.[31]

Fung et al. estimates that the majority (at least 80%) of CRC cases are "inducible and could be prevented with changes in diet and lifestyle."[31] There is also epidemiological evidence indicating that 30% to 70% of all CRC cases "attributable to diet, with red and processed meat intakes implicated as important dietary factors."[32] Moreover, an estimate by Sansbury et al. states that "patient-specific differences in diet are responsible for more variation in CRC than any other factor and could account for up to 90% of CRC deaths in the US."[34]

A case-control study by Cho et al. on dietary inflammatory index (DII), which assesses the inflammatory potential of a dietary pattern, and risk of CRC demonstrated

that higher dietary inflammatory index scores were associated with an increased incidence of CRC, suggesting that a pro-inflammatory diet is involved in colorectal carcinogenesis. This is in line with several studies showing an association between higher DII scores and CRC, including the Iowa Women's Health Study[35] and the Women's Health Initiative[36] reporting that individuals in the highest quintile of DII were at a 20% increased risk of CRC. Another US cohort study demonstrated a 40% increased CRC risk associated with the highest quartile of DII scores.[37] Two case-control studies conducted in Italy[38] and Spain[39] similarly demonstrated increased CRC risk associated with increased DII scores.

There are several biologically plausible mechanisms in which diet-related inflammation can induce colorectal carcinogenesis. Inflammation can induce insulin resistance and subsequent increased circulating levels of insulin, glucose, triglycerides, and non-esterified fatty acids. These growth-promoting factors provide a proliferative environment to epithelial cells in the colon and also expose them to reactive oxygen intermediates, ultimately resulting in the promotion of CRC.[15,40,7]

Activation of the COX-2 pathway can cause local cell proliferation, angiogenesis, and mutagenesis via activation of inflammatory cells which generate reactive oxygen intermediates (ROIs) that are mutagenic and mitogenic.[15,40] The COX-2 pathway can be activated by inflammatory cytokines (e.g., IL-6) and growth factors. It should be noted that higher DII scores have been found to be associated with inflammatory cytokines, specifically IL-6 and C-reactive protein. COX-2 can conversely be downregulated by dietary components such as vitamin D, antioxidants, and n-3 fatty acids.[15]

Dietary components themselves can have a direct effect on colorectal carcinogenesis via inflammation. Pro-inflammatory diets high in red and processed meats are often high in N-nitroso compounds, which potentially promote DNA damage. Anti-inflammatory diets high in fruits and vegetables contain antioxidants and micronutrients which possess anti-neoplastic properties. Diets high in fiber also lessen intestinal transit time of consumed food, therefore resulting in decreased contact time between carcinogens present in food and the colonic epithelial cells.[15]

Diet-induced inflammation, therefore, is a significant and modifiable risk factor for colorectal carcinogenesis, as dietary components affecting CRC risk are consistently associated with their pro-inflammatory potential. Evidence suggests that specific dietary components play a role in inflammation. Fruit, vegetable, fiber, and moderate alcohol intake may decrease inflammation, while red meat, processed meat, and fat may increase inflammation.[15]

Furthermore, Song et al. proposes that "diet likely influences colorectal carcinogenesis through several interacting mechanisms" including "direct effects on immune responsiveness and inflammation and indirect effects of over-nutrition and obesity." These authors also cite emerging evidence that demonstrates involvement of the intestinal microbiome in the relationship between diet and cancer.[17] It is important to note that immune dysregulation, intestinal inflammation, obesity, and gut dysbiosis are all players in IBD pathogenesis as well. Therefore, studies focusing on the effect of diet-induced intestinal inflammation will have impacts on prevention of both CRC and IBD.

The following section will focus on specific dietary components with either pro- or anti-inflammatory effects on the gut.

Existing research

The Relationship Between Diet and Intestinal Inflammation

Much of the existing research on intestinal inflammation associated with diet has focused on specific dietary components and association with risk of IBD or CRC. As research has tended to focus on the study of an individual food item or macronutrient, many studies have yielded inconclusive results, likely due to interacting and potentially confounding effects of individual food items on each other. There are, however, specific macronutrients or food items that have been found to be either positively or negatively associated with inflammation when results are adjusted for potential confounders.

Pro-inflammatory Foods/Food Components

Red and processed meat

Red meat "refers to any unprocessed mammalian muscle meat that has a red color when raw,"[41] and usually includes beef, veal, lamb, mutton and pork.[33,42] Heme iron, a component of red meat, has been shown to induce oxidative stress,[17] which is defined as "an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination protective mechanisms, referred to as antioxidants."[43] Oxidative stress can in turn can induce inflammation. This is supported clinically by a prospective cohort study of UC patients in remission by Jowett et al. which showed that meat intake, particularly red and processed meat, was

associated with increased risk of relapse (acute symptomatic flares of intestinal inflammation).[44]

Another pro-inflammatory compound found in red meat is *N*-glycolylneuraminic acid (Neu5Gc), a silica acid found in lamb, pork, and beef, as well as cow milk. Since humans do not possess the gene encoding the enzyme required for synthesis of Neu5Gc, dietary intake is our only source of this compound. Unlike the immune systems of many other vertebrates in whom synthesis of Neu5Gc naturally occurs, the human immune system does not recognize this compound, thus treating it as a foreign substance and subsequently triggering an immune response.[41] It is thought to promote chronic inflammation in the gut via anti-Neu5Gc antibodies.[45] Although there is a biologically plausible role for Neu5Gc for chronic intestinal inflammation and therefore a potential contribution to development of IBD and CRC, there is currently a lack of epidemiological data.

Red meat has also been deemed "probably carcinogenic" to humans for cancers including CRC by the 2015 International Agency for Research on Cancer's World Cancer Report.[46] This is in line with findings from the 2011 Colorectal Cancer Report stating that there is convincing evidence that red and processed meat increases risk of CRC.[47] However, it is unclear whether the mechanism is directly through inflammation.

Processed meat is defined by Jeyakumar et al. as "meat that has been modified to enhance flavor or improve preservation through methods such as salting, curing, fermentation, and smoking."[41] Meats fitting these criteria usually include ham; sausages; hamburgers; smoked, cured, and salted meat (e.g., bacon); and canned meat.[42] Inorganic

sulfur is commonly used as a preservative in processed meat; sulfur-containing amino acids are also found in red meat and animal proteins in general. In the gut, sulfur is metabolized by hydrogen sulfide (H_2S) by resident bacteria. H_2S has been implicated in intestinal inflammation in UC and has also been implicated in the pathogenesis of CRC, as excess chronic H_2S exposure in the colon is associated with inflammation and modification of immune function.[17] Additionally, a 2010 case-control study of pre-illness diet and risk of IBD by Maconi et al. demonstrated that increased intake of processed meat is associated with increased risk of CD.[48]

Excess protein/meat

Increased consumption of meat in general, whether it be red or white meat, is associated with increased sulfide levels, indicating that meat is a critical substrate for sulfide-producing bacteria in the colon. As mentioned above, sulfide is associated with an increase in intestinal inflammation.

Epidemiological data and clinical studies have shown a positive association of increased animal protein/meat intake with increased risk of IBD. For example, there was a strong correlation between increased CD incidence and increased consumption of animal and milk protein in Japan.[28] A prospective cohort study by Jowett et al., demonstrated that increased meat and protein intake increased the likelihood of relapse of UC in patients in remission.[44] A large French prospective study showed that high total protein intake, specifically from animal sources, was associated with increased risk of IBD.[49] Despite a relatively small number of studies, there is some data supporting a

relationship between excess protein/meat intake and intestinal inflammation (with IBD as the studied outcome), with the proposed mechanism being via the action of gut bacteria and their products.

In this same vein, Andersen et al. suggests that meat has the potential to deliver bacteria such as *Yersinia* which have been implicated in the pathogenesis of CD. These authors also suggest that antibiotics, which are used in extensive amounts in animal agriculture, may still be present in meat products. These antibiotics could theoretically alter the gut microflora and thus have the potential to be involved in inappropriate inflammation.[16]

Fats

A high-fat diet is implicated in intestinal inflammation via its induction of changes in the gut microbiome with subsequent changes in immune and inflammatory cells in the gut. Individual fatty acids have specific effects on the inflammatory response.[28] Experimental data have shown that, in particular, omega-6 PUFAs have pro-inflammatory effects, which are likely due to antagonism of the anti-inflammatory effects of omega-3 PUFAs.[17] Furthermore, omega-6 PUFAs, including linoleic acid and arachidonic acid, are metabolized in the gut to pro-inflammatory eicosanoids (like prostaglandins, leukotrienes, and thromboxane). Arachidonic acid in particular specifically stimulates inflammation by disruption of intestinal tight junction molecules, thus leading to disruption of the intestinal barrier, as well as induction of the inflammatory response via inflammatory

cytokines and free radicals. It is important to note that arachidonic acid has been found in high levels in the colonic mucosa of IBD patients.[16]

Dietary sources of omega-6 PUFAs include poultry, eggs, mayonnaise, dairy, processed pork products (e.g., sausage, ham, bacon, etc.) and fatty cuts of beef and chicken, in addition to many oils from plant sources. In support of this experimental data is epidemiological data from Japan indicating a strong correlation between the recent increased incidence of CD and increased intake of total fat, animal fat, and omega-6 PUFAs.[28] Studies of pre-illness diets and IBD have also shown that high intakes of PUFAs were associated with an increased risk of both UC and CD.[50,51] Furthermore, analyses of the fatty acid makeup of adipose tissue demonstrated that a high baseline level of arachidonic acid is associated with a four-fold increased risk of UC development in a statistically significant dose-response manner, thus suggesting a causal effect of this fatty acid on intestinal inflammation.[16]

Anti-inflammatory Foods/Food Components

Dietary fiber and whole grains

Sources of dietary fiber are exclusively plant-based foods. Foods containing the highest amount of dietary fiber per weight include cereals; beans; legumes such as split peas, chickpeas, and lentils; pumpkin seeds; and fruits and vegetables such as artichokes, pears, avocados, and apples.[52] Resident bacteria in the gut ferment resistant starch in dietary fiber to short-chain fatty acids (SCFAs). Butyrate is the major SCFA produced by bacterial fermentation in the colon. It has been shown to have anti-inflammatory effects

via its inhibition of NFκβ, which subsequently prevents transcription of pro-inflammatory cytokines.[28] SCFAs additionally have modulatory effects on the immune system, which Song et al. proposes influences GI and potentially systemic health.[17]

Furthermore, high-fiber diets as a whole are known to favorably modulate the gut microbial community, therefore playing a role in the balance between homeostasis and inflammation. Studies have also demonstrated a role for dietary fiber in preventing translocation across the intestinal mucosa. Translocation of *E. coli* isolates from CD patients and healthy controls was inhibited by certain soluble dietary fibers, which may prove beneficial since one of the pathogenic mechanisms of IBD is thought to involve disruption of the intestinal barrier. Moreover, experimental models of high fiber diets in animals have been shown to improve inflammation in colitis.[16]

Grains which are refined or processed retain only the endosperm. In contrast, whole grains contain the components of germ and bran, which are significant sources of various substances such as fiber, antioxidants, and phytochemicals which have a potentially anti-inflammatory effect. In addition, dietary consumption of whole grains is associated with decreased insulin resistance and subsequent decreased fasting levels of insulin.[17] As described above, hyperinsulinemia and its resultant increase in circulating growth factors has been associated with increased inflammation.

Vitamins

Vitamin D

Vitamin D, in its active form ($1,25(OH)_2D$), is involved in attenuation of inflammation via its effects on the innate immune system. Vitamin D stimulates the synthesis of cathelicidin and some defensins, which are antimicrobial peptides involved in the innate immune system located on the epithelial surface of the GI tract. These molecules have antibacterial, antiviral, and antifungal effects and also have functions in chemotaxis and cytokine and chemokine regulation. Their main function is to protect the host from microbial growth and inflammation, supporting that a critical action of vitamin D is immune regulation. This is supported by studies suggesting that dysregulated induction of defensins and cathelicidin occurs in CD.[53] Andersen et al. further report that the higher incidence of CD seen in northern regions of the world could be due to vitamin D deficiency caused by less sunlight exposure.[16] Experimental animal models using mice with colitis have also shown improvement in intestinal inflammation following consumption of a diet high in vitamin D.[17]

There are very few foods which are naturally high in vitamin D. It is possible for human keratinocytes to synthesize necessary vitamin D with adequate sun exposure. Fatty fish such as salmon, tuna, and mackerel are among the best dietary sources of naturally occurring vitamin D. The majority of vitamin D in the American diet comes from fortified foods such as dairy and non-dairy milk as well as ready-to-eat breakfast cereals.[54]

Vitamin A

There are two forms of vitamin A in the human diet: preformed vitamin A (retinol) and the provitamin A carotenoids. Preformed vitamin A is found mainly in animal sources; its concentrations are highest in liver and fish oils. The majority of provitamin A sources are plant foods such as leafy green vegetables like spinach; orange and yellow vegetables such as sweet potatoes, squash, and carrots; tomatoes and tomato products; fruits such as mangos and apricots, and some vegetable oils. Both preformed and provitamin A must be intracellularly metabolized to the active forms of retinal and retinoic acid in order for the biological functions of vitamin A to be supported.[55]

Retinoic acid is important in the appropriate induction of B and T cells in the immune response to consumption of microbial and dietary antigens. In the presence of retinoic acid, dendritic cells of the innate immune system are signaled to induce regulatory T cells. However, in an environment lacking retinoic acid, dendritic cells instead induce Th17 cells, thus triggering an inflammatory response via the cytokine IL-17. Retinoic acid also plays an important role in the appropriate translocation, or homing of antigen-presenting B and T cells to the gut, as evidenced by impaired migration of B and T cells to the gut in rats deficient in vitamin A.[16] As described above, impaired homing of B and T lymphocytes is a mechanism postulated to be involved in IBD pathogenesis.[2]

The provitamin A carotenoid beta carotene is referred to as an antioxidant nutrient due to its protective effects against oxidative stress caused by reactive oxygen species. Its antioxidant effects therefore ultimately protect cells against inflammation as well.[17]

Vitamin C

Vitamin C is also considered an antioxidant and anti-inflammatory nutrient.[17] In addition to its direct antioxidant effects, vitamin C is also capable of regenerating antioxidants in the human body, including vitamin E.[56] Case-control studies have also demonstrated an inverse association between vitamin C intake and many cancers, including CRC.[57,58] It is thought that this is due to its protective effects against oxidative damage that could potentially play a role in carcinogenesis. However, evidence for a protective effect from prospective studies and randomized clinical trials against cancer risk has been inconsistent.[56]

Fruits and vegetables are the best dietary sources of vitamin C; those with the highest concentrations of vitamin C per mg are bell peppers, oranges and orange juice, grapefruit juice, kiwis, broccoli, strawberries, brussel sprouts, tomato juice, and cantaloupe.[56]

Vitamin E

Another antioxidant and anti-inflammatory nutrient is vitamin E, or alpha-tocopherol.[17] It specifically halts the production of ROS formed from oxidation of dietary fat. Its role in prevention of chronic diseases associated with oxidative stress is currently under investigation. Plant foods such as nuts, seeds, vegetable oils, and green leafy vegetables are some of the best dietary sources of vitamin E.[59]

Omega-3 polyunsaturated fatty acids

Omega-3 PUFAs are found in fish and fish oils as well as plant oils such as flaxseed, soybean, and canola. Dietary omega-3 PUFAs are metabolized to anti-inflammatory molecules including prostacyclins, lipoxins, and epoxy-eicosatrienoic acids in the gut.[16] Experimental data has shown that omega-3 PUFAs attenuate the inflammatory response via inhibition of the conversion of arachidonic acid to pro-inflammatory eicosanoids, suppression of inflammatory cytokines, and downregulation of genes involved in inflammation.[28] Prospective studies of UC have found a non-significant protective effect on consumption of omega-3 PUFAs on UC development. Andersen et al. cite evidence of decreased risk of cardiovascular disease associated with omega-3 PUFAs to extrapolate that omega-3 PUFAs could have anti-inflammatory effects in other inflammatory disease states such as IBD.[16]

Allyl sulfur and sulfur-containing glycosides

Sulfur-containing glycosides (mainly glucosinolates) found in cruciferous vegetables like cabbage, brussel sprouts, and broccoli are known to have anti-inflammatory effects in the gut, in contrast to inorganic sulfur and sulfur-containing amino acids described above. Allyl sulfur, found in garlic, also has anti-inflammatory effects in the gut via reduction of oxidative stress.

Phytochemicals obtained from whole fruits and vegetables

Phytochemicals are defined as the non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods.[18] Under this umbrella are compounds including carotenoids, polyphenolic acids, flavonoids, and stilbenes/lignans. These plant-based compounds are known to have antioxidant and anti-inflammatory effects. Diets rich in fruits and vegetables are thus hypothesized to play a major role in protection against numerous chronic disease states which are induced by oxidative stress and subsequent inflammation.[18,60]

Eberhardt et al. propose that the additive and synergistic effects of the complex mixtures of phytochemicals in whole fruits and vegetables, as opposed to isolated phytochemicals, are responsible for their antioxidant properties,[61] as individual antioxidants studied as supplements in clinical trials have shown inconsistent results. Indeed, the hypothesis that dietary antioxidants decrease the risk of certain chronic diseases was formed from epidemiological studies of whole fruits and vegetables.[18] For example, studies have shown that consumption of green and yellow vegetables and fruit is associated with decreased cancer risk. These fruits and vegetables are rich in beta-carotene; when studied in isolation however, beta-carotene has not shown the same protective effects.[18]

As an additional example, an experimental model by Liu et al. estimates that the vitamin C in apples with skin is responsible for only 0.4% of the total antioxidant activity of this fruit, indicating that it is the combination of phytochemicals in fruits and

vegetables, as opposed to an isolated antioxidant such as vitamin C, that is responsible for the majority of their antioxidant effects.[18]

As the estimated 8,000 different phytochemicals present in whole foods each differ in molecular size, polarity, and solubility, it is plausible that each differs in bioavailability and effect on different cells, organs, and tissues. Liu et al. states that "pills or tablets simply cannot mimic this balanced natural combination of phytochemicals present in fruit and vegetables," as the bioavailability of the isolated compound may be attenuated or lost.[18]

Studies have suggested biologically plausible synergistic mechanisms of action of phytochemicals such as scavenging of oxidative agents and alteration of the immune response.[18] A postulated protective mechanism of phytochemicals specific to IBD development is that flavonoids may be involved in the intestinal immune system's first line of defense, i.e., an effective barrier.[2] Flavonoids seem to play a role in the preservation of intestinal intercellular tight junctions,[16] the disruption of which is hypothesized to be involved in the impairment of the intestinal barrier function implicated in IBD pathogenesis.[2]

Studying the whole dietary pattern

In line with Liu et al.'s rationale to study whole fruits and vegetables in order to accurately ascertain the effects of synergistic phytochemicals, accumulating evidence supports the utility of studying dietary patterns as a whole, as opposed to individual dietary components. Nutritional epidemiology is inherently subject to multicollinearity, a

statistical phenomenon which can occur when two or more independent variables are highly correlated, and which often leads to difficulty elucidating the independent effects of an individual dietary component. This often occurs in studies of foods and nutrients, as "diet is a complex mixture of foods, nutrients, and other dietary constituents" with potentially additive or even multiplicative effects.[19] Song et al. suggest that studying a combination of nutrients and foods may demonstrate more robust results associated with disease pathogenesis, specifically noting that different nutrients with either pro- or anti-inflammatory effects may work together to influence inflammation through overlapping pathways.[17] These authors also state that the substitution of favorable dietary components for detrimental foods, not merely the addition of beneficial foods, is of critical importance. Therefore, shifting to a new dietary pattern overall is likely necessary for significant effects on human health.

In their study of eating patterns and risk of CRC, Slattery et al. revealed that analysis of entire dietary patterns allowed significantly stronger characterization of whole eating patterns with risk of disease than individual dietary components alone. Their results showed that the consumption of meat had a minimal effect on CRC risk. However, when considering an eating pattern as a whole, they found that a Western diet is associated with increased risk beyond that seen in the individual food components that comprise a Western diet. The risk associated with a composite eating pattern was more consistent and robust than risk associated for individual food items. This indicates that "the overall pattern of a diet may have a greater effect on health than any one food."[20]

Cho et al. agree that "the analysis of individual foods or nutrients does not allow for consideration of the complicated interactions or high inter-correlations between dietary factors, and the effect of any single nutrient may be too small to detect. Combining dietary factors, such as dietary patterns, may overcome these limitations."[15] Their study design using the dietary inflammatory index integrates individual dietary components to analyze the inflammatory effects of an entire diet. These authors note that the study of a whole dietary pattern could "be used without concern regarding dietary intercorrelations and the particular dietary culture of the study population."[15]

Similarly, Wirfalt et al. contend that "the true effect of diet may only be observed when all components are considered simultaneously," as the dietary components of foods consumed together are subsequently metabolized together and therefore are likely to act synergistically in their effects on the host's health. They state that because of this, studies of individual foods or micronutrients are an "inefficient approach in nutrition epidemiology" and suggest cluster analysis, a pattern methodology similar to the methods utilized by Slattery et al., may "turn the analytical difficulties into an advantage" by taking into account the composite effects of dietary components which comprise a certain dietary pattern. These authors specifically note this advantageous effect when the hypothesized association between diet and disease includes the effects of non-energy contributing plant foods, as their "use of density variables based on consumption frequency and standardized to have the same variance allowed food patterns characterized by low energy foods to emerge"[62] This would be especially useful in the study of phytochemicals, in line with Liu et al.'s proposition that the effects of these

compounds are greatly attenuated when analyzed individually as opposed to as components of whole plant foods, described above.[18]

Furthermore, Flood et al. also note the advantage of a diet patterns approach to nutritional epidemiology in order to "capture the totality of dietary experience, including all the nutrient interactions, in a manner that studies of single nutrients or individual foods cannot." Their 2008 study of diet and CRC risk identified two dietary patterns ubiquitous in several dietary pattern-CRC studies conducted in numerous countries throughout the world prior to theirs: the fruit and vegetable pattern and the meat and potatoes pattern. Their findings were consistent with the results of the majority of these studies and indicated that the fruit and vegetable pattern was associated with a reduced risk of CRC while the meat and starch pattern was associated with increased risk of CRC. This data is of particular importance as the common observations of these studies remained highly stable over time and despite different geographical locations and cultures and the use of different food frequency questionnaires (FFQs) among studies.[63]

When considering the existing research on the effect of individual dietary components on intestinal inflammation, it becomes apparent that pro-inflammatory foods are largely animal-based and are foods typified by a Western dietary pattern, while anti-inflammatory foods are almost exclusively plant-based. Therefore, a Western diet and a vegan (plant-based) diet conveniently embody the extremes of this animal-food versus plant-food dichotomy and present a novel area of study. In particular, the quantifiable effects on intestinal inflammation after shifting individuals from a primarily Western type dietary pattern to a whole-food, plant-based dietary pattern would be of utility, as

intestinal inflammation is a common pathogenic mechanism in the disease states of IBD and CRC, both of which carry a large public health burden. A comparison between these two dietary patterns is found in Table 1 below.

Table 1: Western Diet vs Vegan/Plant-Based Diet

Western Diet	Vegan/Plant-Based Diet
Processed meat	Excludes all animal products, i.e., all meat, dairy, and egg products and replaces them instead with increased consumption of:
Red meat	Legumes
Eggs	Total fruit and vegetables (and therefore their associated phytochemicals)
Butter and margarine	Whole grains
High-fat dairy foods	Antioxidant nutrients
Potatoes	
Refined grains	
Added sugar	
High sugar drinks and desserts	

Markers of Inflammation

Currently, the most sensitive and specific marker of intestinal inflammation is fecal calprotectin, a calcium- and zinc-binding protein, which is able to provide a quantitative measure of intestinal inflammation. It is considered specific to neutrophils and is inflammation- rather than disease-specific; an elevated measurement is indicative of intestinal inflammation due to any cause. The amount of calprotectin detected reflects the amount of neutrophils involved in inflammation, thus providing a noninvasive

measurement of neutrophil recruitment to the intestine in the inflammatory process.[64] Calprotectin is therefore significantly associated with IBD and CRC.[65]

It is currently used as a prognostic and diagnostic tool for IBD and carries a 93% sensitivity and 94% specificity for IBD.[66] Most studies report the normal range to be between 10-60 mcg/g. Values over 50-60 mcg/mg are generally considered abnormal. Values over 200 mcg/g have a higher predictive value for pathology and values in the range of 500-600 mcg/g nearly guarantee pathological findings.[64]

As a non-invasive marker of inflammation, fecal calprotectin minimizes the number of patients having to undergo an invasive endoscopy in the work up of suspected IBD, as a large proportion of patients with suspected IBD have no findings on endoscopy.[65] It also reliably predicts clinical relapse with an 80% sensitivity in patients with an established diagnosis of IBD.[64] Its role in screening, diagnosis, and prognosis of CRC is currently under investigation.[67]

METHODS

Study design

This study will be a prospective cohort study quantitatively measuring intestinal inflammation using fecal calprotectin in healthy participants before and after shifting from a primarily Western dietary pattern to a whole-food, plant-based diet.

Study population and sampling

Healthy participants will be recruited over a period of twelve months from outpatient primary care clinics of Boston Medical Center. Inclusion and exclusion criteria are shown in Table 2. Eligible participants will be identified as primarily consuming a Western dietary pattern by completion of a semi-quantitative food frequency questionnaire (FFQ), described below. We define a Western dietary pattern as relatively high intake of red and processed meat, high-fat dairy products, eggs, refined grains, processed foods, and high-sugar snacks and beverages, and relatively low intake of fruits, vegetables, legumes, whole grains, seafood, and poultry.

After identifying eligible participants, a sample size of 268 individuals will be included in the study using sample size calculations that assume a 10% decrease in fecal calprotectin given 90% power and alpha level 0.05.

Table 2: Inclusion and Exclusion Criteria for Healthy Participants

Inclusion	Exclusion
1. All participants will be over the age of 18 and able to provide informed consent. 2. All participants will be	1. Individuals with a diagnosis of ulcerative colitis, Crohn disease, irritable bowel syndrome, or individuals with recent or current

identified as consuming a primarily Western dietary pattern.	symptoms of IBD or IBS. 2. Individuals with diagnosis of celiac disease. 3. Individuals with diagnosis of cystic fibrosis. 4. Individuals with history of or current diagnosis of colorectal cancer. 5. Individuals with a family history or personal history of Lynch syndrome or FAP. 6. Current smokers. 7. Excessive use of ethanol (>1 drink per day for women, >2 drinks per day for men). 8. Chronic use of NSAIDs or aspirin. 9. Individuals with a GI infection within 4 weeks of beginning the study period. 10. Recent use of antibiotics within 4 weeks of beginning the study period. 11. Individuals who exercise greater than 5 times per week. 12. Use of dietary supplements including vitamins, minerals, and fish oil. 13. Pregnant women (due to recommendations for folic acid supplementation during pregnancy).

Intervention

Participants will be asked to abstain from eating all animal products including meat, eggs, and dairy and will be switched to a whole-food, plant-based diet for a study period of twelve weeks. All participants will be provided three whole-food, plant-based meals per day, free of charge, for the entirety of the study period.

After the study period, participants will have the option of purchasing whole-food, plant-based meals to continue this dietary pattern or resuming their pre-intervention diet. This potentially provides the opportunity for a follow-up study to evaluate whether intestinal inflammation, as quantified by fecal calprotectin, increases after participants resume their baseline dietary pattern.

Study variables and measures

The primary outcome will be amount of decrease in intestinal inflammation, as quantified by fecal calprotectin levels. Fecal calprotectin concentrations less than or equal to 50 mcg/g indicate absence of active intestinal inflammation, concentrations between 50.1 and 120.0 mcg/g are considered borderline and may represent a mild inflammatory process in the gastrointestinal tract, and concentrations greater than or equal to 120.1 mcg/g are suggestive of significant active intestinal inflammation. The mean value in healthy adults is approximately 25 mcg/g with a standard deviation of 6 mcg/g.[68]

Recruitment

Healthy participants who meet the inclusion and exclusion criteria will be recruited from the outpatient primary care clinics of Boston Medical Center. Fliers providing details of the study and contact information for the research team will be placed at the front desk and waiting areas of participating clinics. Healthcare providers at these clinics will also be informed and educated about the study and its inclusion and exclusion criteria in order to facilitate patient participation. Interested patients will be instructed to contact the research team and will then be provided further details of the study, including risks and benefits, as well as the FFQ described below to determine eligibility in the study.

Our FFQ will include 131 food items with specified serving sizes as previously described by Hu et al.[69] Food items and food groupings used in the FFQ can be found in the appendix. For each food item, participants will indicate the average frequency of consumption over the past one year, with options including "never," "almost never," "1-2 times per month," "1-2 times per week," "4-6 times per week," "once per day," "1-2 times per day," "4-6 times per day," ">6 times per day."

At the end of the twelve-month recruitment period, a complete list of patients identified as primarily consuming a Western dietary pattern by their FFQ will be put through a randomization software program to randomly select the sample size calculated above. These patients will be contacted by the research team and asked to provide informed consent for participation in the study.

Data collection

Prior to beginning the whole-food, plant-based dietary intervention, participants will provide a stool sample for analysis of baseline fecal calprotectin. Additional stool samples from each participant will be collected at weeks six and twelve of the study period for repeat analyses of fecal calprotectin. Per Mayo Medical Laboratory recommendations,[68] stool samples of a minimum volume of 1g will be analyzed or frozen within 18 hours of collection.

Data analysis

To analyze the primary outcome, a paired t-test will be used to compare pre- and post-dietary intervention levels of fecal calprotectin. Appropriate mean, standard deviation, and ranges will be calculated for fecal calprotectin as well. Statistical significance will

additionally be calculated using McNemar Chi-Square analysis. Absolute and relative risk will be calculated for pre- and post-exposure level of inflammation as well as number needed to treat. Multivariate analysis, specifically analysis of covariance, will be used to adjust for possible confounders such as age, gender, and ethnicity.

Timeline and resources

Table 3: Study Timeline

Fall 2017	IRB submission and approval
January 2018 – January 2019	Patient recruitment
February 2019 – April 2019	Dietary intervention
Summer 2019	Data analysis Manuscript submitted for peer review

The primary and co-investigators will be responsible for patient education, oversight of the study, data collection, and data entry. A statistician will perform data analysis. We will need access to a laboratory assay for measurement of fecal calprotectin levels.

Institutional Review Board

The design of the study will be submitted for full IRB review to the Boston University Medical Campus IRB under INSPIR II criteria, as well as to the corresponding IRB of Boston Medical Center.

CONCLUSION

Discussion

The proposed study has several notable strengths when compared to prior studies of nutrition and intestinal pathology. First, the prospective study design will avoid recall bias that can confound data in retrospective studies. Our study design would also eliminate the ambiguity often seen in defining a vegetarian diet, which can be broken into several patterns including lacto-ovo vegetarian, pescatarian, and semi-vegetarian, making it difficult to meaningfully compare the health benefits of a vegetarian diet and a dietary pattern that includes animal products. Studying a vegan diet would eliminate this variability because of its rigid definition.

Prior studies have also noted an attenuation of results associated with participants' self-definition of their dietary pattern as vegetarian.[70] Our study rigidly and quite simply defines the dietary pattern being investigated as one that eliminates all animal products, including eggs and dairy. Additionally, prior investigators report difficulty in establishing a classification system to characterize typical dietary patterns.[20] Instead of analyzing the baseline dietary patterns of participants and grouping them into patterns that emerge within the study population, our study clearly defines the two dietary patterns we will investigate in order to facilitate useful comparison. It also encourages compliance with the intervention by supplying participants with three plant-based meals per day for the duration of the study period, free of charge.

Furthermore, previous studies have noted that those identifying as vegetarians likely abide by other lifestyle factors which have beneficial health effects, thus making

the effects of diet difficult to isolate.[70] This possible confounding factor would be eliminated by our study design because we are not studying individuals who adopt a plant-based diet of their own volition and are likely to be more health-conscious at baseline, which will conceivably yield more robust results.

Another limitation of prior vegetarian studies has been the narrow range of intake of fruits and vegetables between the lowest and highest quintiles, giving a limited capacity to compare extremes of intake.[70] Our study looks at the extremes of the plant-food vs animal-food dichotomy and will likely have a wide range of fruit and vegetable intake when comparing pre-intervention diet to post-intervention diet.

Despite these strengths, our study is not without limitations, the most prominent of which being recruitment from a relatively small geographical area. As all participants will likely be from the Northeast region of the united states, the generalizability of the study to other regions of the country is limited, as it is possible that environmental factors other than diet play a role in modulation of intestinal microflora.

Summary

There is substantial evidence regarding the inflammatory potential of diet. However, the data on effects of individual dietary components on risk of IBD and CRC has remained largely inconsistent, likely due to the additive and overlapping effects of individual micro- and macro-nutrients on both intestinal inflammation and carcinogenesis. Studies of an overall dietary pattern on intestinal inflammation have yielded more robust results than studies focusing on individual dietary components. By studying dietary patterns at the extreme ends of the inflammatory potential spectrum, we will both eliminate foods

that have been shown to have a pro-inflammatory effect on the gut and replace them with foods having anti-inflammatory effects, leaving little room for variability.

Clinical and/or public health significance

IBD and CRC are both common diseases with rising incidence rates worldwide. As they share the common pathogenic factor of inappropriate intestinal inflammation, an easily modifiable risk factor such as diet would have implications on primary prevention of both diseases. Data on quantifiably decreasing intestinal inflammation with dietary modification would also help clinicians provide evidence-based dietary recommendations to patients with IBD in order to reduce frequency of flares and lessen severity of symptoms. Dietary modification in IBD may be used in conjunction with, or even in place of, currently used pharmacological agents, providing an inexpensive and safe treatment option for control of the disease.

APPENDIX

Food groupings used in the FFQ, adapted from Hu et al.[69]

Foods or food groups	Food items
Processed meats	Processed meats, bacon, hot dogs, sausage
Red meats	Beef, pork, lamb, hamburger
Organ meats	Beef, calf, pork, chicken, and turkey liver
Fish and other seafood	Canned tuna fish, dark-meat fish, other fish, shrimp, lobster, scallops
Poultry	Chicken or turkey with or without skin
Eggs	Eggs
Butter	Butter
Margarine	Margarine
Low-fat dairy products	Skim or low-fat milk, sherbet, yogurt
High-fat dairy products	Whole milk, cream, half and half, sour cream, ice cream, cream cheese, other cheese
Liquor	Liquor
Wine	Red wine, white wine
Beer	Beer
Tea	Tea
Coffee	Coffee
Fruit	Grapes or raisins, avocado, bananas, cantaloupe, watermelon, fresh apples or pears, oranges, grapefruit, strawberries, blueberries, peaches, apricots, plums
Fruit juices	Apple juice, orange juice, grapefruit juice, other fruit juice
Cruciferous vegetables	Broccoli, cabbage, cauliflower, Brussel sprouts, kale, mustard greens, sauerkraut
Dark-yellow vegetables	

Tomatoes	Carrots, yellow squash, yams
	Tomatoes, tomato juice, tomato sauce
Green, leafy vegetables	Spinach, iceberg or head lettuce, romaine or leaf lettuce
Legumes	String beans, peas or lima beans, beans or lentils, tofu or soybeans, alfalfa sprouts
Other vegetables	Celery, mushrooms, green pepper, corn, mixed vegetables, eggplant, summer squash
Garlic	Garlic
Potatoes	Potatoes
French fries	French fries
Whole grains	Cooked oatmeal, other cooked breakfast cereal, dark bread, brown rice, other grains, bran, wheat germ
Cold breakfast cereal	Cold breakfast cereal
Refined grains	White bread, English muffins, bagels, rolls, muffins, biscuits, white rice, pasta, pancakes, waffles
Pizza	Pizza
Snacks	Potato chips, corn chips, crackers, popcorn, rice cakes
Nuts	Peanuts, almonds, cashews, macadamia nuts, pistachios, other nuts, peanut butter and other nut butters
High-energy drinks	Soda with sugar, other carbonated beverages with sugar, fruit drinks
Low-energy drinks	Diet soda, other diet or low-sugar carbonated beverages
Oil and vinegar salad dressing	

Mayonnaise and other creamy salad dressing	Oil and vinegar salad dressing Mayonnaise and other creamy salad dressings
Chowder or cream soup	Chowder or cream soup
Other soup	Homemade soup, ready-made soup
Sweets and desserts	Chocolate bars or pieces, candy bars, cookies, brownies, doughnuts, cake, pie, pastries, coffee cake
Condiments	Ketchup, red chili sauce, mustard, soy sauce, Worcestershire sauce, jam, jelly, syrup, honey

LIST OF JOURNAL ABBREVIATIONS

Am J Clin Nutr	American Journal of Clinical Nutrition
Am J Epidemiol	American Journal of Epidemiology
Am J Gastroenterol	American Journal of Gastroenterology
Am J Pathology	American Journal of Pathology
BMC Cancer	BMC Cancer
Curr Opin Gastroent	Current Opinion in Gastroenterology
Dig Liver Dis	Digestive and Liver Disease
Eur J Clin Nutr	European Journal of Clinical Nutrition
Gastroent Hepatol	Gastroenterology and Hepatology
Genes Nutr	Genes and Nutrition
Inflamm Bowel Dis	Inflammatory Bowel Disease
Int J Cancer	International Journal of Cancer
Int J Mol Sci	International Journal of Molecular Science
J Gastroenterol	Journal of Gastroenterology
J Clin Invest	Journal of Clinical Investigation
Nutr Cancer	Nutrition and Cancer
Nutr Clin Care	Nutrition in Clinical Care
PLoS	Public Library of Science
Proc Natl Acad Sci	Proceedings of the National Academy of Sciences
Q J Med	Quarterly Journal of Medicine
World J Gastroenterol	World Journal of Gastroenterology

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