5-2021

Food Sensitivity in Individuals with Altered and Unaltered Digestive Tracts

Walker Carson

FOOD SENSITIVITY IN INDIVIDUALS WITH ALTERED AND UNALTERED DIGESTIVE TRACTS.

By

Walker K Carson

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Food Science and Technology

Under the Supervision of Professor Jacques Izard

Lincoln, Nebraska

May 2021

FOOD SENSITIVITY IN INDIVIDUALS WITH ALTERED AND UNALTERED DIGESTIVE TRACTS.

Walker Carson, MS

University of Nebraska, 2021

Advisor: Jacques Izard

Immunoglobulin G (IgG) against food proteins has become the subject of much discussion with regards to its role in adverse food reactions. An estimated 20% of the population suffers from some type of food intolerance. Food sensitivity can present with a vast range of symptoms and severities. Diet can have a substantial impact on the wellbeing of individuals with diseases of the gastrointestinal tract, such as Crohn's disease or ulcerative colitis. Incidentally, these diseases have been associated with elevated levels of food-specific IgG as well an increased likelihood of food sensitivity. The presence of food-specific IgG and food sensitivity in individuals with digestive tracts that have been altered by ostomy surgery has not been previously evaluated. Understanding the relationship between various disease states and the presence of food-specific IgG could open the door to better understanding of food sensitivity and the underlying mechanisms. Ostomates provide a particularly useful insight into the development of food-specific IgG by illuminating the impact of different regions of the digestive tract on oral tolerance and therefore the generation of food-specific IgG.

In this thesis, by assessing the presence of food-specific IgG in individuals with altered digestive tracts, we have further explored the relationship between disease status, intestinal permeation, and food sensitivity.

ACKNOWLEDGMENTS

I would like to extend my deep thanks to my advisor Dr. Jacques Izard for his patience and guidance throughout the duration of this project, as well as the opportunity to work in his laboratory. I would also like to express my gratitude to my committee members, Dr. Jennifer Auchtung, Dr. Joseph Baumert, and Dr. Peter Angeletti for their input and encouragement as this project has developed. I would like to give a special thanks to Dr. Jennifer Clarke for her immense contribution to the statistical analysis of this project. I would additionally like to thank the participants of the Nebraska Biobank for their contribution to this project by making their samples available for research use.

I would like to thank our laboratory manager, Rebecca Cederberg, for her constant support and encouragement, as well as her friendship, which have had such a positive impact on me during the difficulties precipitated by the COVID-19 pandemic. I would also like to acknowledge the aid of Lisa Whisenhunt for her work in the clinical laboratory. Dr. Xiaoben Jiang, has also been a great source of help as my time at UNL has come to a close.

Thank you to my fellow graduate students, namely Minho Kim, Brooke Parrish, Onay Dogan, Constanza Avello, and Ashley Toney for their kindness, friendship, and persistent encouragement which have allowed me to make the most of my graduate experience at UNL. An additional thank you to all the Food Science and Technology graduate students, faculty, and staff who have made me feel at home here at UNL.

Finally, I would like to thank my family for being my rocks, without whom none of this would have been possible.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 2

LIST OF TABLES

CHAPTER 2

CHAPTER 1. OSTOMY, INFLAMMATORY CONDITIONS OF THE GASTROINTESTINAL TRACT, AND FOOD-SPECIFIC IgG: A REVIEW

1.1 INTRODUCTION

The majority of individuals show very little consideration when making decisions regarding dietary composition (1). Often, any food will suffice so long as it is cheap, fast, and available. Individuals with ailments related to the digestive tract, however, must spend a significant amount of time to ensure their diets are properly managed. This is frequently done to maintain health and avoid adverse symptoms that can be experienced upon the consumption of specific foods.

Ostomates are individuals that undergo a bowel resection and must discharge bodily waste from an artificial opening placed in or on the abdomen. Ostomates can have tremendous difficulty adjusting their diets to ensure adequate nutrition and optimize comfort (2, 3). Additionally, certain diseases of the gastrointestinal tract, such as Crohn's disease, ulcerative colitis, and eosinophilic esophagitis are linked to dietary intake (4, 5). These diseases can cause significant distress if mismanaged. Unfortunately, the exact way that these diseases develop is usually difficult to determine. One suggested mechanism is immunoglobulin G (IgG) against specific food proteins.

In this study, I have compared levels of IgG to foods from ostomates to levels from individuals with inflammatory diseases along the digestive tract. I will introduce background regarding the different components of this project, which compares IgG levels of ostomates to those of individuals with inflammatory diseases of the digestive tract. In this review, I will describe ostomy-based gastrointestinal alteration, the role of IgG in hypersensitivity, associations of food-specific IgG with food sensitivity, and

recent findings of the efficacy of IgG-based elimination diets. Additionally, the review will discuss background knowledge of biomarkers regarding immunocompetency via the detection of total IgG and total IgA, as well as systemic inflammation via the detection of serum calprotectin. This chapter will conclude by describing multiple inflammatory diseases of the digestive tract. These diseases are examined in the following study, along with current knowledge about their coincidence with food sensitivity to serve as a comparative basis to those with ostomies.

1.2 OSTOMY

In the United States, between ~750,000 and ~1,000,000 individuals are living with ostomies (6, 7). An ostomy is a surgical procedure that reroutes bodily waste from its usual path toward an external collection system. The need for ostomy surgery can occur for various reasons, including but not limited to cancer (colon cancer, cancer of the cervix or endometrium, etc.), damage from radiation due to cancer treatment, the escalation of a gastrointestinal disorder such as Crohn's disease or ulcerative colitis, or traumatic abdominal injury, such as gunshot (8-10).

Ostomates must learn to manage their diet and fecal output systems in order to maintain a normal lifestyle. This is made difficult by the fact that ostomates suffer from high rates of surgical complication, with reports ranging from 21-70% of ostomates experiencing different types of complication including electrolyte abnormalities, renal dysfunction, short bowel syndrome, parastomal hernia, dehydration, skin irritation, and stomal prolapse, to name a few (11, 12).

One of the largest challenges presented to post-surgery ostomates is the readaptation to food intake (13). At the time of the procedure, the majority of ostomates

are malnourished due to the diseases that made ostomy surgery necessary (13). Adequate nourishment must also be carefully monitored after surgery, as significant portions of the digestive tract are often removed. As such, the diet of ostomates often needs to be substantially altered in order to manage nutrition and fecal output. These alterations, in conjunction with food sensitivity, can limit the dietary options available to some ostomates (12).

1.3 IGG AND OTHER BIOMARKERS OF FOOD SENSITIVITY

1.3.1 IgG and hypersensitivity

IgG is the most abundant antibody in the immune system and is the body's main defense against infection and disease (14). Of the four subclasses of IgG produced (IgG1, IgG2, IgG3, IgG4), IgG1, IgG2, and IgG3 are the three most prevalent and they are all involved in complement activation, effector cell recruitment, and opsonization (14). Deficiencies in IgG can result in increased susceptibility to bacterial infection, especially when accompanied by deficiency of another class of immunoglobulin (15). Additionally, IgG can also play a role in immune hypersensitivity reactions.

Four types of immune hypersensitivity reactions are generally recognized. These are denoted as reaction types I-IV and are differentiated based on their mechanism of action. Type I hypersensitivity reactions are mediated by allergen-specific IgE and always involve the degranulation of mast cells and basophils (16). These reactions are immediate and often life-threatening. Type II hypersensitivity reactions are mediated by IgG and IgM antibodies against cell antigens, which leads to cell destruction through complement, cell degranulation, or phagocytosis (16). Type III hypersensitivity reactions are also mediated by IgG and IgM, antibodies that can form complexes around both self-

and non-self-antigens and accumulate in tissue. They can then cause damage by directing immune responses inappropriately (16). Type IV hypersensitivity reactions are primarily T-cell driven and are typically delayed from the time of exposure (16). Type III hypersensitivity reactions are of particular interest to this review, as IgG complexes can initiate complement cascades leading to inflammation (14).

Symptoms of hypersensitivity reactions vary substantially, although some are commonly shared (17). These include tissue damage and inflammation. For many hypersensitivity reactions, the exact mechanism of action remains debated or unknown, which makes the task of disentangling symptoms and causes of the illness quite difficult. Importantly, there is frequently contradicting ideas about the status of diseases with poorly understood pathophysiology. This can make the classification of some hypersensitivity reactions a subject of intense debate.

1.3.2 Food Sensitivity and IgG

It is estimated that 20% of the of people living in industrialized countries may suffer from food intolerance or food allergies (18). In certain groups of people, this number could be much higher. A cross-sectional study of 11,078 individuals indicated that up to 70% of those affected with irritable bowel syndrome had food-related symptoms (19). Because of the challenges associated with nutrition management, food-related immune interactions that can be managed by diet are of significant interest to those investigating ways to improve wellness.

Typically, when food is consumed, proteins will be broken down into oligopeptides and amino acids in the digestive tract. When contact with immune cells occurs, these oligopeptides and amino acids may trigger either an immunotolerant effect

or an inflammatory effect. The immunization response varies from food to food and from person to person. The exact causes of the type of response created are currently not well understood, however implicated factors include but are not limited to the intestinal microbiota, intestinal inflammation, intestinal barrier disfunction, and pathogenic infection (20-22). The lining of the gastrointestinal tract provides a barrier between ingested antigens and the immune system. However, several mechanisms exist and allow antigen sampling to take place. These mechanisms include goblet cell associated passages, trans-epithelial dendrites, M cell sampling, and paracellular leak (23). When antigens from food pass through the mucosa, the process of antigen uptake has an impact on the immune response (23). Increased permeability of the gastrointestinal tract can increase the number of antigens sampled, thereby increasing the likelihood of an altered immune response (23, 24). Inflammation can increase the permeability of the digestive tract lining (25). Tordesillas and Berin suggest that the inflammation can impact the generation of a tolerance response to consumed antigens (26). In 2004, Aljada et al. reported the evidence that food consumption is associated with a pro-inflammatory immune response (27). The immunization response generated will have a substantial impact on the severity of the reaction to the food. One possible pathway of food sensitivity pathogenesis occurs by way of food-specific IgG.

Juchnowicz, et al. demonstrated that individuals with major depressive disorder have significantly higher serum levels of food-specific IgG antibodies' than healthy controls, and that higher levels of food-specific IgG are correlated with gastric complaints (28). Many studies have been done on Crohn's disease and ulcerative colitis, suggesting higher levels of food-specific IgG in Crohn's disease patients than in controls

(29-31). Frehn et al. described distinct IgG and IgA profiles against food and microbial antigens when comparing inflammatory bowel disease patients to controls (32). Hvatum et al. also found that IgG titers against food proteins were elevated in rheumatoid arthritis patients (33). Additionally, Wilders-Trusching et al. reported elevated levels of food-specific IgG associated with increased intima thickness and inflammation in obese juveniles (34).

Food sensitivity is a topic that is subject to much discussion. However, the growing body of literature continues to suggest that non-IgE mediated food sensitivity may be at play.

1.3.3 IgG-guided elimination diets

Further evidence of the connection between food sensitivity and non-IgE mediated hypersensitivity is supported by the growing amount of data on the utility of elimination diets. One study found that a food elimination diet based on serum IgG against food-specific proteins was effective in reducing symptoms of irritable bowel syndrome in affected individuals (35). Another study by Xie et al. demonstrated that an IgG elimination diet reduced migraines and irritable bowel syndrome symptoms in 60 affected individuals (36). In individuals with Crohn's disease, stool frequency decreased, and overall wellbeing increased when dietary interventions were taken based on food-specific IgG testing results (31). An IgG-guided exclusion diet has also relieved symptoms and improved quality of life for individuals with ulcerative colitis (37).

1.3.4 IgA and food sensitivity

IgA is the second most abundant immunoglobulin produced by the human immune system. IgA is found primarily on the mucosal surfaces of the body, such as the

nostrils, mouth, and digestive tract lining (38). On mucosal surfaces, secretory IgA (SIgA) are secreted as dimers and transported to the mucosal surface through epithelial cells (39). Like IgG, IgA plays a role in protection against infection. Unlike IgG, IgA also plays a role in preventing antigens from coming into contact with the immune system. This protective mechanism of IgA occurs through a unique process known as immune exclusion, whereby SIgA prevents antigens from coming into contact with the immune system by transporting antigens out of the lamina propria to the enterocyte surface (40). Immune exclusion is also critical for the active maintenance of the commensal intestinal microbiome (41). When bound to an antigen, IgA can downregulate immune system functions such as chemotaxis, cytokine release, and IgG-mediated phagocytosis (42-44). With these protective functions, it is not surprising that SIgA is frequently associated with the presence of immune tolerance to orally acquired antigens, namely food. This also led us to ask the question: how is oral tolerance impacted by the absence of SIgA?

Selective IgA deficiency occurs when an individual has drastically reduced, or even undetectable levels of serum IgA (45). Most frequently, levels of other circulating antibodies remain unaffected. Selective IgA deficiency is the most common primary immunodeficiency, with an estimated prevalence varying geographically from 1:143 in Saudi Arabia to 1:18,500 in Japan (45). It frequently remains undiagnosed, as up to 75% of those infected may remain asymptomatic (46). The most common symptoms of selective IgA deficiency are recurrent respiratory infections, presence of autoimmune diseases, the development of gastrointestinal disorders such as ulcerative colitis and Celiac disease, and food allergy (47). For many, the occurrence of these symptoms alone is not indicative of a greater problem, thus many individuals remain undiagnosed.

Previous studies have indicated an association between respiratory and food allergies, and selective IgA deficiency. An Egyptian study found that of 100 individuals with food allergy, 67% were IgA deficient (48). A study found that of 126 children in Brazil with selective IgA deficiency, 46% suffered from either respiratory or atopic allergy (49). Additionally, it was observed in Italy that 2.6% of individuals with selective IgA deficiency were also diagnosed with Celiac disease (50). Two studies have been done examining the presence of food-specific antibodies in individuals with selective IgA deficiency— both of which indicated that IgA deficiency is associated with increased levels of circulating IgG against foods (51, 52).

1.3.5 Calprotectin as a marker of inflammation

As mentioned above, it is often difficult to assess the extent and severity of disease in individuals with inflammatory bowel diseases. Because the level of inflammation is a key indicator to the severity of the disease, researchers have searched for an indirect method by which to measure inflammation. Many have looked to calprotectin.

When infection occurs, the host immune system will initiate an inflammatory response to recruit immune cells for host defense. As part of the innate immune response, neutrophils and macrophages are often the first cells recruited to the site of infection, where they release calprotectin (53). Calprotectin is a calcium and zinc-binding protein found in neutrophils and macrophages in the body. When released, calprotectin has antibacterial properties, induces apoptosis, and aids in chemotaxis (54, 55). In individuals with dysregulated inflammatory responses of the digestive tract, such as ulcerative colitis and Crohn’s disease, levels of neutrophil recruitment are elevated (56). The calprotectin

is released into the digestive tract and measured in feces. Today, fecal calprotectin is a widely used biomarker to assess the severity of inflammatory bowel diseases (57, 58). Fecal calprotectin is typically used for disease assessment in inflammatory bowel diseases, as it can indicate inflammation by way of neutrophilic migration to the intestinal lining.

In diseases such as rheumatoid arthritis and systemic lupus erythematosus inflammation may not be localized to one specific region. In individuals with these diseases, it has been found that elevated levels of serum calprotectin can be observed (59, 60). Serum calprotectin has also been shown to be a useful diagnostic tool for assessing disease burden in patients with inflammatory bowel disease, as well as an indicator of systemic inflammation (61-63).

1.4 INFLAMMATORY CONDITIONS OF THE GASTROINTESTINAL TRACT

In order to establish a basis of the effects of inflammation on the generation of food-specific IgG, we will introduce the following inflammatory conditions of the digestive tract: periodontitis, eosinophilic esophagitis, duodenitis, appendicitis, Crohn's disease, and ulcerative colitis. In the following sections, a brief introduction of these conditions will be given, and the present knowledge of food sensitivity in each disease will be evaluated.

1.4.1 Periodontitis

Periodontitis is chronic inflammation of the periodontium due to microbial interactions in the host oral cavity (64). Periodontitis is the sixth most common human disease, with an estimated 45-50% of the global population being affected. (65). Periodontitis can begin in

childhood but is most common in adult populations. There are currently four recognized types of periodontitis: necrotizing, chronic, aggressive, and periodontitis as a manifestation of systemic diseases (66). Symptoms of periodontitis can vary; however, the effects frequently include the gums becoming red and bleeding. In later stages, the gums can also pull away from the teeth and is associated with bone loss (67). One of the primary causes is the buildup of microbial biofilms, known as plaque, on the surface of the tooth. In individuals with periodontitis, a shift in the oral microbiota has been observed from a gram-positive dominated population to a gram-negative dominated population (68). This shift leads to changes in host immune responses to the oral microbiota, which contributes to periodontitis symptoms including bone loss (69). Periodontitis has been associated with several comorbidities, including diabetes, cardiovascular disease, pancreatic cancer, and chronic obstructive pulmonary disease (70-74). Treatments for periodontitis usually include the removal of plaque and buildup from the tooth surface and require home-management of tooth health, i.e. brushing (75). In recurring disease, additional therapeutic measures used include local or systemic antibiotics and surgical intervention to aid in periodontium regeneration (76-78).

Several studies have shown that periodontitis can have an effect of IgE mediated allergy, however none has been done so far on the incidence of IgG-mediated food sensitivity (79, 80).

1.4.2 Eosinophilic esophagitis

Eosinophilic esophagitis is a recently recognized disease, which was first described in the 1990s (81). Since then, work has been done has been done to better characterize the precise mechanisms of disease and clinical outcomes. Eosinophilic esophagitis is

typically characterized by inflammation of the esophagus leading to difficulty swallowing and food impaction. Other symptoms include chest pain, heartburn, nausea, and vomiting (82). These symptoms are similar to those of gastroesophageal reflux disease (GERD) and can be difficult to diagnose. Because of this, diagnosis of eosinophilic esophagitis must be confirmed by observation of at least 15 eosinophils per high-power field in esophageal biopsy tissue (83). The exact pathogenesis has been the subject of some dispute. Environmental factors have been indicated in symptom development, and the role of diet in eosinophilic esophagitis has been investigated since at least 1995, when it was observed that an amino-acid diet managed symptoms until the reintroduction of food (84-86). There have also been studies done which suggest that the esophageal microbiome may have a role to play (87, 88).

In 15-43% of individuals, IgE mediated allergies have been observed, which has led to the conclusion that IgE plays a crucial role in the development of eosinophilic esophagitis (89). However, additional studies have shown elevated levels of IgG4 in serum of patients, leading to the hypothesis that eosinophilic esophagitis is an IgG4 related disease (90). Moreover, in the same study, no beneficial response was seen when anti-IgE therapy was introduced. In some cases, eosinophilic esophagitis has also been shown to develop during food oral immunotherapy used to treat food allergies. It is well established that specific foods are one of the most prevalent triggers in individuals with eosinophilic esophagitis. (91, 92). The role of T_H2 cells in pathogenesis has also been investigated, with one research group finding elevated CD4+ T_H2 cells in blood of eosinophilic esophagitis patients when compared to controls after consumption of milk (93). Methods of treating eosinophilic esophagitis currently include the administration of

proton pump inhibitors, the implementation of diets which avoid food that trigger symptoms, administration of corticosteroids, and esophageal dilation using endoscopic balloons (94, 95).

1.4.3 Duodenitis

The duodenum is the first portion of the small intestine located immediately following the stomach and is approximately 25-38 cm in length. When inflammation occurs in this region, it is known as duodenitis. There are several diseases and incidents related to the symptoms of duodenitis, including celiac disease, peptic duodenitis, inflammatory bowel disease, autoimmune disease, allergy to soy and cow's milk, and bacterial overgrowth (96-100). Additionally, long term use of non-steroidal anti-inflammatory drugs can also induce duodenal inflammation (101). Current treatments for duodenitis can vary greatly and often hinge on the resolution of the primary disease.

No specific studies have been focused on the presence of food sensitivity in individuals with duodenitis. Several of the conditions such as Celiac disease, which can lead to duodenitis have been associated with food sensitivity (96).

1.4.4 Appendicitis

Inflammation of the appendix, or appendicitis, is one of the most common gastrointestinal emergencies requiring surgery worldwide, with an estimated 7% of individuals affected at some point in their lives (102). The exact cause of appendicitis is unknown; however, it has been attributed to obstruction, infection, and environmental triggers (103, 104). Studies have also confirmed that individuals with family members affected by appendicitis have a three times higher risk of contracting the illness themselves (105). Additionally, a study conducted on the appendicular microbiome of

people with appendicitis identified that bacterial species not generally associated with the human intestine were present (106). Treatment of appendicitis may vary, although surgical removal of the appendix is the most common and effective management strategy. Recently, antibiotic treatment of appendicitis has been studied, implying that it may be an effective treatment (107).

At this time, no studies have been done on the relationship between appendicitis and food sensitivity.

1.4.5 Inflammatory bowel disease

Inflammatory bowel diseases are chronic inflammatory conditions that are mediated by T-cell disfunction in the gastrointestinal mucosa (108). The two major types of inflammatory bowel disease are Crohn's disease and ulcerative colitis. The development of inflammatory bowel diseases has been linked to disruptions in the intestinal mucosa and the reduction in gastrointestinal microbial diversity (109, 110). Inflammatory bowel diseases are known to impact mucosal permeability in the gastrointestinal tract and they have also been associated with an increased risk of colorectal cancer (7, 111). Given the similarity in symptoms across different inflammatory bowel diseases, it can often be difficult to ascertain the exact diagnosis of individuals in a non-invasive way. Endoscopy or histologic samples are usually needed to verify the disease in question.

A meta-analysis by Kappelman, et al., 2007, analyzed 9 million insurance health claims and determined that the overall prevalence of irritable bowel diseases, Crohn's disease and ulcerative colitis, in US adults is 201 and 238 per 100,000, respectively (112). This study also indicated that the prevalence of both Crohn's disease and

ulcerative colitis increase with age. Incidence of inflammatory bowel disease is higher in western countries, despite the increasing frequency observed in areas of the developing world such as Latin America (113).

Multiple studies have been conducted to evaluate the impact of diet on inflammatory bowel diseases, but few have evaluated the factors associated with symptom resolution in successful trials (114). Additionally, many of these studies conducted do not include a precise way to determine which foods are problematic during symptom development.

1.4.5.1 Crohn's disease

Crohn's disease is a chronic disease that can affect any part of the digestive tract. Diagnosis of Crohn's disease is quite difficult due to its similarity to other diseases of the gastrointestinal tract. Clinically, a diagnosis is normally given after endoscopic observation of the presence of skip lesions on the digestive mucosa that appear alongside normal-appearing tissue. These lesions affect all layers of the gastrointestinal wall (115). Common symptoms of Crohn's disease include weight loss, diarrhea, iron deficiency, nausea, and vomiting (109). Often, the symptoms of Crohn's disease can flare and retreat, causing more difficulty in the diagnosis of the disease (108).

The exact pathogenesis of Crohn's disease remains unknown, but it has been demonstrated that individuals with Crohn's disease have reduced tolerance to commensal intestinal microorganisms (116). Crohn's disease is characterized primarily by a T_H1 immune response, with cytokines IFNγ and IL-2 being elevated in Chron's disease patients (117). Tumor necrosis factors (TNF) are cytokines released by multiple immune cells that recruit immune cells and stimulate an inflammatory response (118). Success in

using anti-TNF therapy to treat Crohn's disease has implicated a role of the TNF protein family in the pathogenesis of Crohn's disease (119).

Dietary management of Crohn's has been suggested as a possible course of treatment for some time now, and recent studies have indicated a prevalence of food sensitivity in individuals with Crohn's disease as well (120-122). It has also been shown that individuals who demonstrate long term intake of dietary fiber from fruits are at reduced risk for Crohn's disease (4). A retrospective study performed in 2020 examined food-specific IgG in 355 patients diagnosed with Crohn's disease and found that that over 50% had IgG against corn (61.10%), egg (59.45%), rice (59.18%), tomato (56.16%) and soybean (51.23%) (120). Another trial by Riordan et al. found that individuals with Crohn's disease who participated in an exclusion diet could effectively manage their symptoms, with 84% of individuals who adhered to an elemental diet displaying reduced symptoms after two weeks (121).

1.4.5.2 Ulcerative colitis

Ulcerative colitis is similar to Crohn's disease in symptom presentation, but they differ in extent and pathology. Ulcerative colitis is characterized by relapsing inflammation confined to the colonic mucosa, and patients often have bloody stool and abdominal tenderness (123). Endoscopic evaluation is the necessary method to confirm suspected diagnosis and extent of ulcerative colitis. Unlike in Crohn's disease, the area surrounding the ulcerations characteristic of ulcerative colitis often appear inflamed, despite the fact that the depth of inflammation in ulcerative colitis is limited to the mucosa and submucosa (124). In addition, differing from the T_H1 immune response present in Crohn's disease, ulcerative colitis has been associated with a unique T_H2 immune

response. The response is mediated by natural killer cells that release IL-13 and have cytotoxic effects on epithelial cells of the colon (125). Treatments for ulcerative colitis include 5-aminosalicylic acid, a nonsteroidal anti-inflammatory drug, as well as multiple corticosteroids used to reduce inflammation. In severe cases, anti-TNF therapy has also shown effectiveness in treating ulcerative colitis (123).

Diet has been suggested as a mediator for ulcerative colitis since at least the 1960s (5). A study conducted by Candy et. al. asked subjects to avoid foods that seemed to induce symptoms, and a significant difference in remission rate was seen when compared to control subjects (126). Evidence suggests that individuals consuming diets rich in fat and sugar are at higher risk of acquiring ulcerative colitis (127, 128).

1.6 CONCLUSION

The relationship between food sensitivity and food-specific IgG is complex, and further investigation into the mechanisms of IgG-mediated food sensitivity is needed. That said, the studies discussed above indicate a link between the presence of food-specific IgG and adverse gastrointestinal symptoms. In the following chapter, the relationship between food-specific IgG, digestive tract alterations, and inflammation will be further explored.

1.5 REFERENCES

1. Gallup I. Most Americans Overlook Restaurants' Nutrition Labels2018.

2. Popek S, Grant M, Gemmill R, Wendel CS, Mohler MJ, Rawl SM, Baldwin CM, Ko CY, Schmidt CM, Krouse RS. Overcoming challenges: life with an ostomy. Am J Surg. 2010;200(5):640-5. Epub 2010/11/09. doi: 10.1016/j.amjsurg.2010.07.009. PubMed PMID: 21056145.

3. Turnbull GB, Arnold A, Aronson L, Hawke G, LeBlanc K, Parslow N, Phillips D, St-Cyr D, Steeves C, Tremblay L, Wells C, Zorzes SM. The role of industry in improving quality of life for persons with an ostomy: a Canadian consensus. Ostomy Wound Manage. 2004;50(9):78-85. Epub 2004/10/19. PubMed PMID: 15487040.

4. Ananthakrishnan AN, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Korzenik JR, Fuchs CS, Willett WC, Richter JM, Chan AT. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. Gastroenterology. 2013;145(5):970-7. Epub 2013/08/06. doi: 10.1053/j.gastro.2013.07.050. PubMed PMID: 23912083; PMCID: PMC3805714.

5. Wright R, Truelove SC. A Controlled Therapeutic Trial of Various Diets in Ulcerative Colitis. Br Med J. 1965;2(5454):138-41. Epub 1965/07/17. doi: 10.1136/bmj.2.5454.138. PubMed PMID: 14304053; PMCID: PMC1845668.

6. United Ostomy Associations of America I. Our mission 2020. Available from: https://www.ostomy.org/our-mission-history/.

7. Söderholm JD, Peterson KH, Olaison G, Franzén LE, Weström B, Magnusson KE, Sjödahl R. Epithelial permeability to proteins in the noninflamed ileum of Crohn's

disease? Gastroenterology. 1999;117(1):65-72. doi: 10.1016/s0016-5085(99)70551-2. PubMed PMID: 10381911.

8. Hashmi ZG, Dalton MK, Sheikh SS, McCarty JC, Salim A, Haider AH. National estimates of intestinal ostomy creation and reversal for trauma. J Trauma Acute Care Surg. 2021;90(3):459-65. Epub 2021/02/23. doi: 10.1097/TA.0000000000003022. PubMed PMID: 33617196.

9. Ambe PC, Kurz NR, Nitschke C, Odeh SF, Moslein G, Zirngibl H. Intestinal Ostomy. Dtsch Arztebl Int. 2018;115(11):182-7. Epub 2018/04/03. doi: 10.3238/arztebl.2018.0182. PubMed PMID: 29607805; PMCID: PMC5913578.

10. Hope JM, Pothuri B. The role of palliative surgery in gynecologic cancer cases. Oncologist. 2013;18(1):73-9. Epub 2013/01/10. doi: 10.1634/theoncologist.2012-0328. PubMed PMID: 23299775; PMCID: PMC3556259.

11. Shabbir J, Britton DC. Stoma complications: a literature overview. Colorectal Dis. 2010;12(10):958-64. Epub 2009/07/17. doi: 10.1111/j.1463-1318.2009.02006.x. PubMed PMID: 19604288.

12. Carmel J, Colwell J, Goldberg MT, Wound Ostomy and Continence Nurses Society. Wound, Ostomy and Continence Nurses Society core curriculum. Ostomy management. Philadelphia: Wolters Kluwer; 2016. p. p.

13. Burgess-Stocks J. Eating with an ostomy; A comprehensive nutrition guide for those living with an ostomy. United Ostomy Associations of America; 2020.

14. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520. Epub 2014/10/20. doi: 10.3389/fimmu.2014.00520. PubMed PMID: 25368619; PMCID: PMC4202688.

15. Demirdag YY, Gupta S. Update on Infections in Primary Antibody Deficiencies. Front Immunol. 2021;12:634181. Epub 2021/02/11. doi: 10.3389/fimmu.2021.634181. PubMed PMID: 33643318; PMCID: PMC7905085.

16. Dispenza MC. Classification of hypersensitivity reactions. Allergy Asthma Proc. 2019;40(6):470-3. doi: 10.2500/aap.2019.40.4274. PubMed PMID: 31690397.

17. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, Fiocchi A, Chiang W, Beyer K, Wood R, Hourihane J, Jones SM, Lack G, Sampson HA. ICON: food allergy. J Allergy Clin Immunol. 2012;129(4):906-20. Epub 2012/03/01. doi: 10.1016/j.jaci.2012.02.001. PubMed PMID: 22365653.

18. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet. 1994;343(8906):1127-30. doi: 10.1016/s0140-6736(94)90234-8. PubMed PMID: 7910231.

19. Monsbakken KW, Vandvik PO, Farup PG. Perceived food intolerance in subjects with irritable bowel syndrome-- etiology, prevalence and consequences. Eur J Clin Nutr. 2006;60(5):667-72. Epub 2006/01/05. doi: 10.1038/sj.ejcn.1602367. PubMed PMID: 16391571.

20. Berni Canani R, Paparo L, Nocerino R, Di Scala C, Della Gatta G, Maddalena Y, Buono A, Bruno C, Voto L, Ercolini D. Gut Microbiome as Target for Innovative Strategies Against Food Allergy. Front Immunol. 2019;10:191. Epub 2019/02/15. doi: 10.3389/fimmu.2019.00191. PubMed PMID: 30828329; PMCID: PMC6384262.

21. Ménard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol. 2010;3(3):247-59. Epub 2010/03/10. doi: 10.1038/mi.2010.5. PubMed PMID: 20404811.

22. Bouziat R, Hinterleitner R, Brown JJ, Stencel-Baerenwald JE, Ikizler M, Mayassi T, Meisel M, Kim SM, Discepolo V, Pruijssers AJ, Ernest JD, Iskarpatyoti JA, Costes LM, Lawrence I, Palanski BA, Varma M, Zurenski MA, Khomandiak S, McAllister N, Aravamudhan P, Boehme KW, Hu F, Samsom JN, Reinecker HC, Kupfer SS, Guandalini S, Semrad CE, Abadie V, Khosla C, Barreiro LB, Xavier RJ, Ng A, Dermody TS, Jabri B. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science. 2017;356(6333):44-50. doi: 10.1126/science.aah5298. PubMed PMID: 28386004; PMCID: PMC5506690.

23. Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, Floyd AN, Hogan SP, Hsieh CS, Newberry RD. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. Mucosal Immunol. 2020;13(2):271-82. Epub 2019/12/09. doi: 10.1038/s41385-019-0240-7. PubMed PMID: 31819172; PMCID: PMC7044050.

24. Zhou Q, Zhang B, Verne GN. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain. 2009;146(1-2):41-6. doi: 10.1016/j.pain.2009.06.017. PubMed PMID: 19595511; PMCID: PMC2763174.

25. Sundqvist T, Magnusson KE, Sjodahl R, Stjernstrom I, Tagesson C. Passage of molecules through the wall of the gastrointestinal tract. II. Application of low-molecular weight polyethyleneglycol and a deterministic mathematical model for determining intestinal permeability in man. Gut. 1980;21(3):208-14. Epub 1980/03/01. doi: 10.1136/gut.21.3.208. PubMed PMID: 7399321; PMCID: PMC1420346.

26. Tordesillas L, Berin MC. Mechanisms of Oral Tolerance. Clin Rev Allergy Immunol. 2018;55(2):107-17. doi: 10.1007/s12016-018-8680-5. PubMed PMID: 29488131; PMCID: PMC6110983.

27. Aljada A, Mohanty P, Ghanim H, Abdo T, Tripathy D, Chaudhuri A, Dandona P. Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. Am J Clin Nutr. 2004;79(4):682-90. Epub 2004/03/31. doi: 10.1093/ajcn/79.4.682. PubMed PMID: 15051615.

28. Karakula-Juchnowicz H, Galecka M, Rog J, Bartnicka A, Lukaszewicz Z, Krukow P, Morylowska-Topolska J, Skonieczna-Zydecka K, Krajka T, Jonak K, Juchnowicz D. The Food-Specific Serum IgG Reactivity in Major Depressive Disorder Patients, Irritable Bowel Syndrome Patients and Healthy Controls. Nutrients. 2018;10(5). Epub 2018/05/02. doi: 10.3390/nu10050548. PubMed PMID: 29710769; PMCID: PMC5986428.

29. Zuo XL, Li YQ, Li WJ, Guo YT, Lu XF, Li JM, Desmond PV. Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia. Clin Exp Allergy. 2007;37(6):823-30. doi: 10.1111/j.1365-2222.2007.02727.x. PubMed PMID: 17517095.

30. Cai C, Shen J, Zhao D, Qiao Y, Xu A, Jin S, Ran Z, Zheng Q. Serological investigation of food specific immunoglobulin G antibodies in patients with inflammatory bowel diseases. PLoS One. 2014;9(11):e112154. Epub 2014/11/14. doi: 10.1371/journal.pone.0112154. PubMed PMID: 25393003; PMCID: PMC4230978.

31. Bentz S, Hausmann M, Piberger H, Kellermeier S, Paul S, Held L, Falk W, Obermeier F, Fried M, Scholmerich J, Rogler G. Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention study. Digestion. 2010;81(4):252-64. Epub 2010/02/05. doi: 10.1159/000264649. PubMed PMID: 20130407.

32. Frehn L, Jansen A, Bennek E, Mandic AD, Temizel I, Tischendorf S, Verdier J, Tacke F, Streetz K, Trautwein C, Sellge G. Distinct patterns of IgG and IgA against food and microbial antigens in serum and feces of patients with inflammatory bowel diseases. PLoS One. 2014;9(9):e106750. Epub 2014/09/13. doi: 10.1371/journal.pone.0106750. PubMed PMID: 25215528; PMCID: PMC4162554.

33. Hvatum M, Kanerud L, Hällgren R, Brandtzaeg P. The gut-joint axis: cross reactive food antibodies in rheumatoid arthritis. Gut. 2006;55(9):1240-7. Epub 2006/02/16. doi: 10.1136/gut.2005.076901. PubMed PMID: 16484508; PMCID: PMC1860040.

34. Wilders-Truschnig M, Mangge H, Lieners C, Gruber H, Mayer C, März W. IgG antibodies against food antigens are correlated with inflammation and intima media thickness in obese juveniles. Exp Clin Endocrinol Diabetes. 2008;116(4):241-5. Epub 2007/12/10. doi: 10.1055/s-2007-993165. PubMed PMID: 18072008.

35. Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. Gut. 2004;53(10):1459-64. Epub 2004/09/14. doi: 10.1136/gut.2003.037697. PubMed PMID: 15361495; PMCID: PMC1774223.

36. Xie Y, Zhou G, Xu Y, He B, Wang Y, Ma R, Chang Y, He D, Xu C, Xiao Z. Effects of Diet Based on IgG Elimination Combined with Probiotics on Migraine Plus Irritable Bowel Syndrome. Pain Res Manag. 2019;2019:7890461. Epub 2019/08/21. doi: 10.1155/2019/7890461. PubMed PMID: 31531150; PMCID: PMC6721378.

37. Jian L, Anqi H, Gang L, Litian W, Yanyan X, Mengdi W, Tong L. Food Exclusion Based on IgG Antibodies Alleviates Symptoms in Ulcerative Colitis: A Prospective Study. Inflamm Bowel Dis. 2018;24(9):1918-25. doi: 10.1093/ibd/izy110. PubMed PMID: 29788288.

38. Kerr MA. The structure and function of human IgA. Biochem J. 1990;271(2):285-96. Epub 1990/10/15. doi: 10.1042/bj2710285. PubMed PMID: 2241915; PMCID: PMC1149552.

39. Phalipon A, Corthesy B. Novel functions of the polymeric Ig receptor: well beyond transport of immunoglobulins. Trends Immunol. 2003;24(2):55-8. Epub 2003/01/28. doi: 10.1016/s1471-4906(02)00031-5. PubMed PMID: 12547499.

40. Robinson JK, Blanchard TG, Levine AD, Emancipator SN, Lamm ME. A mucosal IgA-mediated excretory immune system in vivo. J Immunol. 2001;166(6):3688-92. Epub 2001/03/10. doi: 10.4049/jimmunol.166.6.3688. PubMed PMID: 11238608.

41. Li Y, Jin L, Chen T. The Effects of Secretory IgA in the Mucosal Immune System. Biomed Res Int. 2020;2020:2032057. Epub 2020/01/31. doi: 10.1155/2020/2032057. PubMed PMID: 31998782; PMCID: PMC6970489.

42. Van Epps DE, Williams RC, Jr. Suppression of leukocyte chemotaxis by human IgA myeloma components. J Exp Med. 1976;144(5):1227-42. Epub 1976/11/02. doi: 10.1084/jem.144.5.1227. PubMed PMID: 825608; PMCID: PMC2190463.

43. Wolf HM, Fischer MB, Puhringer H, Samstag A, Vogel E, Eibl MM. Human serum IgA downregulates the release of inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6) in human monocytes. Blood. 1994;83(5):1278-88. Epub 1994/03/01. PubMed PMID: 8118031.

44. Wilton JM. Suppression by IgA of IgG-mediated phagocytosis by human polymorphonuclear leucocytes. Clin Exp Immunol. 1978;34(3):423-8. Epub 1978/12/01. PubMed PMID: 369752; PMCID: PMC1537540.

45. Yel L. Selective IgA deficiency. J Clin Immunol. 2010;30(1):10-6. Epub 2010/01/27. doi: 10.1007/s10875-009-9357-x. PubMed PMID: 20101521; PMCID: PMC2821513.

46. Jorgensen GH, Gardulf A, Sigurdsson MI, Sigurdardottir ST, Thorsteinsdottir I, Gudmundsson S, Hammarstrom L, Ludviksson BR. Clinical symptoms in adults with selective IgA deficiency: a case-control study. J Clin Immunol. 2013;33(4):742-7. Epub 2013/02/08. doi: 10.1007/s10875-012-9858-x. PubMed PMID: 23389234.

47. Cunningham-Rundles C. Physiology of IgA and IgA deficiency. J Clin Immunol. 2001;21(5):303-9. Epub 2001/11/27. doi: 10.1023/a:1012241117984. PubMed PMID: 11720003.

48. Shahin RY, Ali FHA, Melek NAN, Elateef IAA, Attia MY. Study of selective immunoglobulin A deficiency among Egyptian patients with food allergy. Cent Eur J Immunol. 2020;45(2):184-8. Epub 2021/01/19. doi: 10.5114/ceji.2020.97907. PubMed PMID: 33456329; PMCID: PMC7792446.

49. Jacob CM, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC. Autoimmunity in IgA deficiency: revisiting the role of IgA as a silent housekeeper. J Clin

Immunol. 2008;28 Suppl 1:S56-61. Epub 2008/01/19. doi: 10.1007/s10875-007-9163-2. PubMed PMID: 18202833.

50. Cataldo F, Marino V, Ventura A, Bottaro G, Corazza GR. Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP) and "Club del Tenue" Working Groups on Coeliac Disease. Gut. 1998;42(3):362-5. Epub 1998/05/13. doi: 10.1136/gut.42.3.362. PubMed PMID: 9577342; PMCID: PMC1727042.

51. Cunningham-Rundles C, Brandeis WE, Good RA, Day NK. Milk precipitins, circulating immune complexes, and IgA deficiency. Proc Natl Acad Sci U S A. 1978;75(7):3387-9. Epub 1978/07/01. doi: 10.1073/pnas.75.7.3387. PubMed PMID: 277938; PMCID: PMC392781.

52. Husby S, Oxelius VA, Svehag SE. IgG subclass antibodies to dietary antigens in IgA deficiency quantification and correlation with serum IgG subclass levels. Clin Immunol Immunopathol. 1992;62(1 Pt 1):85-90. Epub 1992/01/01. doi: 10.1016/0090-1229(92)90026-k. PubMed PMID: 1728983.

53. Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in Inflammation. Front Immunol. 2018;9:1298. Epub 2018/06/27. doi: 10.3389/fimmu.2018.01298. PubMed PMID: 29942307; PMCID: PMC6004386.

54. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biol Pharm Bull. 2003;26(6):753-60. doi: 10.1248/bpb.26.753. PubMed PMID: 12808281.

55. Steinbakk M, Naess-Andresen CF, Lingaas E, Dale I, Brandtzaeg P, Fagerhol MK. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. Lancet. 1990;336(8718):763-5. doi: 10.1016/0140-6736(90)93237-j. PubMed PMID: 1976144.

56. Eberhardson M, Levine YA, Tarnawski L, Olofsson PS. The brain-gut axis, inflammatory bowel disease, and bioelectronic medicine. Int Immunol. 2021. Epub 2021/04/30. doi: 10.1093/intimm/dxab018. PubMed PMID: 33912906.

57. Cypers H, Varkas G, Beeckman S, Debusschere K, Vogl T, Roth J, Drennan MB, Lavric M, Foell D, Cuvelier CA, De Vos M, Delanghe J, Van den Bosch F, Elewaut D. Elevated calprotectin levels reveal bowel inflammation in spondyloarthritis. Ann Rheum Dis. 2016;75(7):1357-62. Epub 2015/12/23. doi: 10.1136/annrheumdis-2015-208025. PubMed PMID: 26698844; PMCID: PMC4941173.

58. Fu Y, Wang L, Xie C, Zou K, Tu L, Yan W, Hou X. Comparison of non-invasive biomarkers faecal BAFF, calprotectin and FOBT in discriminating IBS from IBD and evaluation of intestinal inflammation. Sci Rep. 2017;7(1):2669. Epub 2017/06/01. doi: 10.1038/s41598-017-02835-5. PubMed PMID: 28572616; PMCID: PMC5453945.

59. Haga HJ, Brun JG, Berntzen HB, Cervera R, Khamashta M, Hughes GR. Calprotectin in patients with systemic lupus erythematosus: relation to clinical and laboratory parameters of disease activity. Lupus. 1993;2(1):47-50. Epub 1993/02/01. doi: 10.1177/096120339300200108. PubMed PMID: 8485559.

60. De Rycke L, Baeten D, Foell D, Kruithof E, Veys EM, Roth J, De Keyser F. Differential expression and response to anti-TNFalpha treatment of infiltrating versus resident tissue macrophage subsets in autoimmune arthritis. J Pathol. 2005;206(1):17-27. Epub 2005/04/06. doi: 10.1002/path.1758. PubMed PMID: 15809977.

61. Pathirana WGW, Chubb SP, Gillett MJ, Vasikaran SD. Faecal Calprotectin. Clin Biochem Rev. 2018;39(3):77-90. PubMed PMID: 30828114; PMCID: PMC6370282.

62. Fukunaga S, Kuwaki K, Mitsuyama K, Takedatsu H, Yoshioka S, Yamasaki H, Yamauchi R, Mori A, Kakuma T, Tsuruta O, Torimura T. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and immunohistochemical localization. Int J Mol Med. 2018;41(1):107-18. Epub 2017/11/07. doi: 10.3892/ijmm.2017.3244. PubMed PMID: 29115397; PMCID: PMC5746327.

63. Kalla R, Kennedy NA, Ventham NT, Boyapati RK, Adams AT, Nimmo ER, Visconti MR, Drummond H, Ho GT, Pattenden RJ, Wilson DC, Satsangi J. Serum Calprotectin: A Novel Diagnostic and Prognostic Marker in Inflammatory Bowel Diseases. Am J Gastroenterol. 2016;111(12):1796-805. Epub 2016/09/06. doi: 10.1038/ajg.2016.342. PubMed PMID: 27596694.

64. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44. Epub 1998/03/12. doi: 10.1111/j.1600-051x.1998.tb02419.x. PubMed PMID: 9495612.

65. Kassebaum NJ, Smith AGC, Bernabe E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marcenes W, Collaborators GBDOH. Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. J Dent Res. 2017;96(4):380-7. Epub 2017/08/10. doi: 10.1177/0022034517693566. PubMed PMID: 28792274; PMCID: PMC5912207.

66. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4(1):1-6. Epub 2000/06/23. doi: 10.1902/annals.1999.4.1.1. PubMed PMID: 10863370.

67. Stephens MB, Wiedemer JP, Kushner GM. Dental Problems in Primary Care. Am Fam Physician. 2018;98(11):654-60. Epub 2018/11/30. PubMed PMID: 30485039.

68. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res. 1994;8(2):263-71. Epub 1994/07/01. doi: 10.1177/08959374940080022001. PubMed PMID: 7865085.

69. Cochran DL. Inflammation and bone loss in periodontal disease. J Periodontol. 2008;79(8 Suppl):1569-76. Epub 2008/09/04. doi: 10.1902/jop.2008.080233. PubMed PMID: 18673012.

70. Sanz M, Marco Del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, Chapple I, Dietrich T, Gotsman I, Graziani F, Herrera D, Loos B, Madianos P, Michel JB, Perel P, Pieske B, Shapira L, Shechter M, Tonetti M, Vlachopoulos C, Wimmer G. Periodontitis and cardiovascular diseases: Consensus report. J Clin Periodontol. 2020;47(3):268-88. Epub 2020/02/06. doi: 10.1111/jcpe.13189. PubMed PMID: 32011025; PMCID: PMC7027895.

71. Spiropoulou A, Zareifopoulos N, Bellou A, Spiropoulos K, Tsalikis L. Review of the association between periodontitis and chronic obstructive pulmonary disease in smokers. Monaldi Arch Chest Dis. 2019;89(1). Epub 2019/04/11. doi: 10.4081/monaldi.2019.1018. PubMed PMID: 30968666.

72. Preshaw PM, Bissett SM. Periodontitis and diabetes. Br Dent J. 2019;227(7):577-84. Epub 2019/10/13. doi: 10.1038/s41415-019-0794-5. PubMed PMID: 31605062.

73. Maisonneuve P, Amar S, Lowenfels AB. Periodontal disease, edentulism, and pancreatic cancer: a meta-analysis. Ann Oncol. 2017;28(5):985-95. Epub 2017/04/30. doi: 10.1093/annonc/mdx019. PubMed PMID: 28453689.

74. Michaud DS, Izard J, Wilhelm-Benartzi CS, You DH, Grote VA, Tjonneland A, Dahm CC, Overvad K, Jenab M, Fedirko V, Boutron-Ruault MC, Clavel-Chapelon F, Racine A, Kaaks R, Boeing H, Foerster J, Trichopoulou A, Lagiou P, Trichopoulos D, Sacerdote C, Sieri S, Palli D, Tumino R, Panico S, Siersema PD, Peeters PH, Lund E, Barricarte A, Huerta JM, Molina-Montes E, Dorronsoro M, Quiros JR, Duell EJ, Ye W, Sund M, Lindkvist B, Johansen D, Khaw KT, Wareham N, Travis RC, Vineis P, Bueno-de-Mesquita HB, Riboli E. Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. Gut. 2013;62(12):1764-70. Epub 2012/09/20. doi: 10.1136/gutjnl-2012-303006. PubMed PMID: 22990306; PMCID: PMC3815505.

75. Apatzidou DA, Kinane DF. Nonsurgical mechanical treatment strategies for periodontal disease. Dent Clin North Am. 2010;54(1):1-12. Epub 2010/01/28. doi: 10.1016/j.cden.2009.08.006. PubMed PMID: 20103469.

76. Larsson L, Decker AM, Nibali L, Pilipchuk SP, Berglundh T, Giannobile WV. Regenerative Medicine for Periodontal and Peri-implant Diseases. J Dent Res. 2016;95(3):255-66. Epub 2015/11/27. doi: 10.1177/0022034515618887. PubMed PMID: 26608580; PMCID: PMC4766955.

77. Feres M, Figueiredo LC, Soares GM, Faveri M. Systemic antibiotics in the treatment of periodontitis. Periodontol 2000. 2015;67(1):131-86. Epub 2014/12/17. doi: 10.1111/prd.12075. PubMed PMID: 25494600.

78. Matesanz-Perez P, Garcia-Gargallo M, Figuero E, Bascones-Martinez A, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. J Clin Periodontol. 2013;40(3):227-41. Epub 2013/01/17. doi: 10.1111/jcpe.12026. PubMed PMID: 23320860.

79. Laurikainen K, Kuusisto P. Comparison of the oral health status and salivary flow rate of asthmatic patients with those of nonasthmatic adults--results of a pilot study. Allergy. 1998;53(3):316-9. doi: 10.1111/j.1398-9995.1998.tb03894.x. PubMed PMID: 9542614.

80. Friedrich N, Völzke H, Schwahn C, Kramer A, Jünger M, Schäfer T, John U, Kocher T. Inverse association between periodontitis and respiratory allergies. Clin Exp Allergy. 2006;36(4):495-502. doi: 10.1111/j.1365-2222.2006.02455.x. PubMed PMID: 16630155.

81. Attwood SE, Smyrk TC, Demeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. Dig Dis Sci. 1993;38(1):109-16. Epub 1993/01/01. doi: 10.1007/BF01296781. PubMed PMID: 8420741.

82. Dellon ES, Kim HP, Sperry SL, Rybnicek DA, Woosley JT, Shaheen NJ. A phenotypic analysis shows that eosinophilic esophagitis is a progressive fibrostenotic disease. Gastrointest Endosc. 2014;79(4):577-85 e4. Epub 2013/11/28. doi: 10.1016/j.gie.2013.10.027. PubMed PMID: 24275329; PMCID: PMC4599711.

83. Dellon ES, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA, American College of G. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis

(EoE). Am J Gastroenterol. 2013;108(5):679-92; quiz 93. Epub 2013/04/10. doi: 10.1038/ajg.2013.71. PubMed PMID: 23567357.

84. Markowitz JE, Spergel JM, Ruchelli E, Liacouras CA. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. Am J Gastroenterol. 2003;98(4):777-82. Epub 2003/05/10. doi: 10.1111/j.1572-0241.2003.07390.x. PubMed PMID: 12738455.

85. van Rhijn BD, van Ree R, Versteeg SA, Vlieg-Boerstra BJ, Sprikkelman AB, Terreehorst I, Smout AJ, Bredenoord AJ. Birch pollen sensitization with cross-reactivity to food allergens predominates in adults with eosinophilic esophagitis. Allergy. 2013;68(11):1475-81. Epub 2013/12/20. doi: 10.1111/all.12257. PubMed PMID: 24351068.

86. Kelly KJ, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology. 1995;109(5):1503-12. Epub 1995/11/01. doi: 10.1016/0016-5085(95)90637-1. PubMed PMID: 7557132.

87. Harris JK, Fang R, Wagner BD, Choe HN, Kelly CJ, Schroeder S, Moore W, Stevens MJ, Yeckes A, Amsden K, Kagalwalla AF, Zalewski A, Hirano I, Gonsalves N, Henry LN, Masterson JC, Robertson CE, Leung DY, Pace NR, Ackerman SJ, Furuta GT, Fillon SA. Esophageal microbiome in eosinophilic esophagitis. PLoS One. 2015;10(5):e0128346. Epub 2015/05/28. doi: 10.1371/journal.pone.0128346. PubMed PMID: 26020633; PMCID: PMC4447451.

88. Benitez AJ, Hoffmann C, Muir AB, Dods KK, Spergel JM, Bushman FD, Wang ML. Inflammation-associated microbiota in pediatric eosinophilic esophagitis.

Microbiome. 2015;3:23. Epub 2015/06/01. doi: 10.1186/s40168-015-0085-6. PubMed PMID: 26034601; PMCID: PMC4450515.

89. Maggadottir SM, Hill DA, Ruymann K, Brown-Whitehorn TF, Cianferoni A, Shuker M, Wang ML, Chikwava K, Verma R, Liacouras CA, Spergel JM. Resolution of acute IgE-mediated allergy with development of eosinophilic esophagitis triggered by the same food. J Allergy Clin Immunol. 2014;133(5):1487-9, 9 e1. Epub 2014/03/19. doi: 10.1016/j.jaci.2014.02.004. PubMed PMID: 24636092.

90. Clayton F, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, Lowichik A, Chen X, Emerson L, Cox K, O'Gorman MA, Peterson KA. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. Gastroenterology. 2014;147(3):602-9. Epub 2014/06/04. doi: 10.1053/j.gastro.2014.05.036. PubMed PMID: 24907494.

91. Cianferoni A. Eosinophilic Esophagitis as a Side Effect of Food Oral Immunotherapy. Medicina (Kaunas). 2020;56(11). Epub 2020/11/20. doi: 10.3390/medicina56110618. PubMed PMID: 33207848; PMCID: PMC7697667.

92. Arias A, Gonzalez-Cervera J, Tenias JM, Lucendo AJ. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: a systematic review and meta-analysis. Gastroenterology. 2014;146(7):1639-48. Epub 2014/02/19. doi: 10.1053/j.gastro.2014.02.006. PubMed PMID: 24534634.

93. Cianferoni A, Ruffner MA, Guzek R, Guan S, Brown-Whitehorn T, Muir A, Spergel JM. Elevated expression of activated TH2 cells and milk-specific TH2 cells in milk-induced eosinophilic esophagitis. Ann Allergy Asthma Immunol. 2018;120(2):177-

83 e2. Epub 2018/01/01. doi: 10.1016/j.anai.2017.11.006. PubMed PMID: 29289462; PMCID: PMC5875940.

94. Peterson KA, Byrne KR, Vinson LA, Ying J, Boynton KK, Fang JC, Gleich GJ, Adler DG, Clayton F. Elemental diet induces histologic response in adult eosinophilic esophagitis. Am J Gastroenterol. 2013;108(5):759-66. Epub 2013/02/06. doi: 10.1038/ajg.2012.468. PubMed PMID: 23381017.

95. Straumann A, Katzka DA. Diagnosis and Treatment of Eosinophilic Esophagitis. Gastroenterology. 2018;154(2):346-59. Epub 2017/08/02. doi: 10.1053/j.gastro.2017.05.066. PubMed PMID: 28756235.

96. Walker MM, Murray JA. An update in the diagnosis of coeliac disease. Histopathology. 2011;59(2):166-79. Epub 2010/11/09. doi: 10.1111/j.1365-2559.2010.03680.x. PubMed PMID: 21054494.

97. Memeo L, Jhang J, Hibshoosh H, Green PH, Rotterdam H, Bhagat G. Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in H. pylori gastritis. Mod Pathol. 2005;18(8):1134-44. Epub 2005/04/02. doi: 10.1038/modpathol.3800404. PubMed PMID: 15803187.

98. Wright BL, Kulis M, Guo R, Orgel KA, Wolf WA, Burks AW, Vickery BP, Dellon ES. Food-specific IgG. J Allergy Clin Immunol. 2016;138(4):1190-2.e3. Epub 2016/04/06. doi: 10.1016/j.jaci.2016.02.024. PubMed PMID: 27130859; PMCID: PMC5053831.

99. Garcia-Careaga M, Jr., Kerner JA, Jr. Gastrointestinal manifestations of food allergies in pediatric patients. Nutr Clin Pract. 2005;20(5):526-35. Epub 2005/10/07. doi: 10.1177/0115426505020005526. PubMed PMID: 16207693.

100. Jonas A, Krishnan C, Forstner G. Pathogenesis of mucosal injury in the blind loop syndrome. Gastroenterology. 1978;75(5):791-5. Epub 1978/11/01. PubMed PMID: 359400.

101. Lanas A, Sopena F. Nonsteroidal anti-inflammatory drugs and lower gastrointestinal complications. Gastroenterol Clin North Am. 2009;38(2):333-52. Epub 2009/05/19. doi: 10.1016/j.gtc.2009.03.007. PubMed PMID: 19446262.

102. Stewart B, Khanduri P, McCord C, Ohene-Yeboah M, Uranues S, Vega Rivera F, Mock C. Global disease burden of conditions requiring emergency surgery. Br J Surg. 2014;101(1):e9-22. Epub 2013/11/26. doi: 10.1002/bjs.9329. PubMed PMID: 24272924.

103. Carr NJ. The pathology of acute appendicitis. Ann Diagn Pathol. 2000;4(1):46-58. Epub 2000/02/23. doi: 10.1016/s1092-9134(00)90011-x. PubMed PMID: 10684382.

104. Lamps LW. Infectious causes of appendicitis. Infect Dis Clin North Am. 2010;24(4):995-1018, ix-x. Epub 2010/10/13. doi: 10.1016/j.idc.2010.07.012. PubMed PMID: 20937462.

105. Ergul E. Heredity and familial tendency of acute appendicitis. Scand J Surg. 2007;96(4):290-2. Epub 2008/02/13. doi: 10.1177/145749690709600405. PubMed PMID: 18265855.

106. Guinane CM, Tadrous A, Fouhy F, Ryan CA, Dempsey EM, Murphy B, Andrews E, Cotter PD, Stanton C, Ross RP. Microbial composition of human appendices from patients following appendectomy. mBio. 2013;4(1). Epub 2013/01/17. doi: 10.1128/mBio.00366-12. PubMed PMID: 23322636; PMCID: PMC3551545.

107. Salminen P, Paajanen H, Rautio T, Nordstrom P, Aarnio M, Rantanen T, Tuominen R, Hurme S, Virtanen J, Mecklin JP, Sand J, Jartti A, Rinta-Kiikka I, Gronroos

JM. Antibiotic Therapy vs Appendectomy for Treatment of Uncomplicated Acute Appendicitis: The APPAC Randomized Clinical Trial. JAMA. 2015;313(23):2340-8. Epub 2015/06/17. doi: 10.1001/jama.2015.6154. PubMed PMID: 26080338.

108. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology. 1998;115(1):182-205. Epub 1998/07/03. doi: 10.1016/s0016-5085(98)70381-6. PubMed PMID: 9649475.

109. Loftus EV, Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology. 2004;126(6):1504-17. Epub 2004/05/29. doi: 10.1053/j.gastro.2004.01.063. PubMed PMID: 15168363.

110. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology. 2014;146(6):1489-99. Epub 2014/02/25. doi: 10.1053/j.gastro.2014.02.009. PubMed PMID: 24560869; PMCID: PMC4034132.

111. Ullman TA, Itzkowitz SH. Intestinal inflammation and cancer. Gastroenterology. 2011;140(6):1807-16. Epub 2011/05/03. doi: 10.1053/j.gastro.2011.01.057. PubMed PMID: 21530747.

112. Kappelman MD, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clin Gastroenterol Hepatol. 2007;5(12):1424-9. Epub 2007/10/02. doi: 10.1016/j.cgh.2007.07.012. PubMed PMID: 17904915.

113. Ng SC, Bernstein CN, Vatn MH, Lakatos PL, Loftus EV, Jr., Tysk C, O'Morain C, Moum B, Colombel JF, Epidemiology, Natural History Task Force of the International Organization of Inflammatory Bowel D. Geographical variability and environmental risk

factors in inflammatory bowel disease. Gut. 2013;62(4):630-49. Epub 2013/01/22. doi: 10.1136/gutjnl-2012-303661. PubMed PMID: 23335431.

114. Wight N, Scott BB. Dietary treatment of active Crohn's disease. BMJ. 1997;314(7079):454-5. Epub 1997/02/15. doi: 10.1136/bmj.314.7079.454. PubMed PMID: 9056785; PMCID: PMC2126013.

115. Sturm A, Leite AZ, Danese S, Krivacic KA, West GA, Mohr S, Jacobberger JW, Fiocchi C. Divergent cell cycle kinetics underlie the distinct functional capacity of mucosal T cells in Crohn's disease and ulcerative colitis. Gut. 2004;53(11):1624-31. Epub 2004/10/14. doi: 10.1136/gut.2003.033613. PubMed PMID: 15479683; PMCID: PMC1774268.

116. Mow WS, Vasiliauskas EA, Lin YC, Fleshner PR, Papadakis KA, Taylor KD, Landers CJ, Abreu-Martin MT, Rotter JI, Yang H, Targan SR. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. Gastroenterology. 2004;126(2):414-24. Epub 2004/02/06. doi: 10.1053/j.gastro.2003.11.015. PubMed PMID: 14762777.

117. Fais S, Capobianchi MR, Pallone F, Di Marco P, Boirivant M, Dianzani F, Torsoli A. Spontaneous release of interferon gamma by intestinal lamina propria lymphocytes in Crohn's disease. Kinetics of in vitro response to interferon gamma inducers. Gut. 1991;32(4):403-7. Epub 1991/04/01. doi: 10.1136/gut.32.4.403. PubMed PMID: 1902808; PMCID: PMC1379080.

118. Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc Res Tech. 2000;50(3):184-95. Epub 2000/07/13. doi:

10.1002/1097-0029(20000801)50:3<184::AID-JEMT2>3.0.CO;2-H. PubMed PMID: 10891884.

119. van Dullemen HM, van Deventer SJ, Hommes DW, Bijl HA, Jansen J, Tytgat GN, Woody J. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology. 1995;109(1):129-35. Epub 1995/07/01. doi: 10.1016/0016-5085(95)90277-5. PubMed PMID: 7797011.

120. Pan J, Fu D, Li Y, Wang Y, Lian G, Liu X. Body weight, serum albumin and food intolerance were linked to upper gastrointestinal Crohn's disease: a 7-year retrospective analysis. Ann Transl Med. 2020;8(21):1370. Epub 2020/12/15. doi: 10.21037/atm-20-2212. PubMed PMID: 33313115; PMCID: PMC7723648.

121. Riordan AM, Hunter JO, Cowan RE, Crampton JR, Davidson AR, Dickinson RJ, Dronfield MW, Fellows IW, Hishon S, Kerrigan GN, et al. Treatment of active Crohn's disease by exclusion diet: East Anglian multicentre controlled trial. Lancet. 1993;342(8880):1131-4. Epub 1993/11/06. doi: 10.1016/0140-6736(93)92121-9. PubMed PMID: 7901473.

122. King TS, Woolner JT, Hunter JO. Review article: the dietary management of Crohn's disease. Aliment Pharmacol Ther. 1997;11(1):17-31. Epub 1997/02/01. doi: 10.1046/j.1365-2036.1997.90262000.x. PubMed PMID: 9042971.

123. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-70. Epub 2016/12/05. doi: 10.1016/S0140-6736(16)32126-2. PubMed PMID: 27914657; PMCID: PMC6487890.

124. Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of G. Ulcerative colitis practice guidelines in adults: American College Of

Gastroenterology, Practice Parameters Committee. Am J Gastroenterol. 2010;105(3):501-23; quiz 24. Epub 2010/01/14. doi: 10.1038/ajg.2009.727. PubMed PMID: 20068560.

125. Fuss IJ, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S, Yang Z, Exley M, Kitani A, Blumberg RS, Mannon P, Strober W. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. J Clin Invest. 2004;113(10):1490-7. Epub 2004/05/18. doi: 10.1172/JCI19836. PubMed PMID: 15146247; PMCID: PMC406524.

126. Candy S, Borok G, Wright JP, Boniface V, Goodman R. The value of an elimination diet in the management of patients with ulcerative colitis. S Afr Med J. 1995;85(11):1176-9. Epub 1995/11/01. PubMed PMID: 8597010.

127. Ananthakrishnan AN, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Fuchs CS, Willett WC, Richter JM, Chan AT. Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. Gut. 2014;63(5):776-84. Epub 2013/07/06. doi: 10.1136/gutjnl-2013-305304. PubMed PMID: 23828881; PMCID: PMC3915038.

128. Investigators IBDiES, Tjonneland A, Overvad K, Bergmann MM, Nagel G, Linseisen J, Hallmans G, Palmqvist R, Sjodin H, Hagglund G, Berglund G, Lindgren S, Grip O, Palli D, Day NE, Khaw KT, Bingham S, Riboli E, Kennedy H, Hart A. Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. Gut. 2009;58(12):1606-11. Epub 2009/07/25. doi: 10.1136/gut.2008.169078. PubMed PMID: 19628674.

CHAPTER 2. DIFFERENCES IN LEVELS OF FOOD-SPECIFIC IGG BETWEEN INDIVIDUALS WITH ALTERED AND UNALTERED DIGESTIVE TRACTS

2.1 INTRODUCTION

It is currently estimated that 20% of the of people living in industrialized countries may suffer from some type of food sensitivity or food allergy (1). Symptoms of food sensitivity can vary widely between individuals, from atopic rash to severe abdominal pain, and can have a substantial impact on individual wellness. Often, these sensitivities are present in individuals who do not possess any traditional IgE-mediated allergies.

While the mechanisms leading to the development of food sensitivity are not fully understood, more evidence suggests that immunoglobulin G may contribute to the development of food sensitivity, but underlying mechanisms are not fully understood. IgG is an antibody that is crucial in the immune system processes of infection management and inflammation regulation (2). When repeated exposure to partially digested food proteins over time occurs, the body will begin to create IgG antibodies against these proteins. This can occur more frequently when intestinal permeation is increased due to disease (3). Food-specific IgG has been associated with adverse food sensitivity reactions in individuals who do not have laboratory confirmed food allergies (4). The American Academy of Allergy, Asthma, and Immunology (AAAAI) does not currently recommend testing for IgG against food proteins for the diagnosis of food allergy or intolerance (5).

Although there is some debate about the diagnostic utility of food-specific IgG in recognizing food sensitivities, several studies have shown positive results in using food-specific IgG testing to reduce disease burden (6, 7). IgG mediated exclusion diets have been identified as a promising way to manage food sensitivity symptoms including but not limited to migraine, symptoms of irritable bowel syndrome, symptoms of Crohn's disease, and symptoms of ulcerative colitis (6, 8, 9). Elevated levels of food-specific IgG have been linked with inflammatory conditions such as irritable bowel syndrome, Crohn's disease, ulcerative colitis, obesity, rheumatoid arthritis, and major depressive disorder (7, 10-14). In extreme cases of gastrointestinal disorders, such as Crohn's disease or ulcerative colitis, progression of the disease can require surgical intervention, sometimes in the form of an ostomy.

In the United States, there are between 750,000 and 1,000,000 individuals living with ostomies (15). An ostomy is a surgical procedure that reroutes bodily waste from its usual path toward an external collection system. This can happen for various reasons, including but not limited to cancer, damage from radiation due to cancer treatment, the escalation of a gastrointestinal disorder such as Crohn's disease or ulcerative colitis, or traumatic abdominal injury. Ostomates must learn to manage their diet and fecal output system in order to maintain a normal lifestyle and can often achieve this. Many ostomates continue to live healthy and fulfilling lives.

However, one of the largest challenges presented to ostomates post-surgery is the readaptation to food intake (16). At the time of the procedure, many ostomates are malnourished due to the diseases which caused the ostomy to be necessary (16). Adequate nourishment must also be carefully monitored after surgery, as significant

portions of digestive tract are often removed. Because of this, the diet of ostomates often needs to be substantially altered in order to manage nutrient acquisition, absorption, and fecal output. This can limit the dietary options available to some ostomates (17). Furthermore, the presence of a food sensitivity can make this challenge particularly difficult. Unfortunately, there is no current body of research evaluating food sensitivity in ostomates.

Several methods can be used to measure serum IgG. Clinical facilities and laboratories frequently utilize nephelometry for measuring total IgG, which analyzes scattering of light passed through a sample. This is a popular method due to its usability and automation. Another widely used method is microarrays, where antigens are printed onto a small chip that are treated and analyzed to detect IgG. Microarrays are an effective method for analyzing IgG against many antigens. However, microarrays can be quite expensive and are therefore often not practical for use. Enzyme linked immunosorbent assays (ELISA) are also commonly used. Antigens are bound to a microplate to which the desired standard and sample is added. Changes in color are measured via optical density and a standard curve is generated to predict sample concentrations.

In this study, we evaluated the presence of IgG antibodies against food-specific antigens using ELISA techniques. This was done in individuals with altered digestive tracts, specifically those with ostomies, alongside samples from individuals with inflammatory conditions located along the digestive tract. This study was performed in an effort to determine the impact of digestive tract alterations on food sensitivity. We have also measured biomarkers of systemic inflammation and immune competency in order to evaluate their impact on food sensitivity. The aim was to evaluate the impact of disease

status on their presence. Our primary hypothesis was that the presence of food-specific IgG would be indicative of events altering the gastrointestinal tract. Our study found that individuals with some types of ostomies and inflammatory conditions of the gastrointestinal tract are more likely to develop food-specific IgG, and that they can have stronger responses to food antigens than those with different conditions.

2.2 MATERIALS AND METHODS

2.2.1 Acquisition of serum samples from the Nebraska Biobank

Serum samples used in this study were acquired from the Nebraska Biobank (RRID: SCR_021024; University of Nebraska Medical Center, Omaha, NE). The program is partially funded by the Nebraska Research Initiative (NRI) and the Center for Clinical and Translational Research. The samples were acquired by Dr. Jacques Izard. Under project ID 19490, the Institutional Review Board (IRB) of the University of Nebraska-Lincoln made the determination that this project and the use of samples did not meet the definitions of human subject research under regulatory requirements at 45 CFR 46.102 and the project did not require IRB approval.

Biobank samples were originally collected with the consent of Nebraska Medicine patients and consist of remaining donated blood-samples from scheduled laboratory tests. Serum, plasma, and DNA are recovered from the samples and stored for future research studies. De-identified data from the Electronic Health Record (EHR) such as age, race, BMI, diagnoses, laboratory results and medications are linked to the stored samples. The inclusion criteria for the request were for de-identified sera from individuals over the age of 19 with specific medical diagnoses affecting the digestive tract. Diagnosis requests are made using the tenth revision of the International Classification of Diseases (ICD-10).

The exclusion criteria were limited to the presence of a urostomy in ostomate samples and that no two samples from the same individual were to be included.

A total of 198 de-identified samples were selected for sampling (Table 2.1). The received samples were from individuals with the following diagnoses: jejunostomy (n=22), colostomy (n=18), ileostomy (n=31), Crohn's disease (n=18), ulcerative colitis (n=15), appendicitis (n=18), duodenitis (n=25), eosinophilic esophagitis (n=15), food intolerance (n=18), and periodontitis (n=18). At the source, all samples were temporarily stored at 4 ºC for 5 days, followed by long-term storage at -80 ºC at the biobank. All samples were collected from July 2014 through September 2019.

Table 2.1. Description of samples acquired from Nebraska Biobank

[1]International Classification of Diseases, Tenth Revision (ICD10). Asterisks represent that the preceding alphanumeric sequence may be further broken down into subcategories of the selected ICD10 code. Here, these codes represent the individual diagnostic groups.

ICD10 code requested[1]	ICD10 Code Description (Diagnostic group)	Number of samples completed[2]
Z93.4	Jejunostomy present (HCC)	**22**
Z93.3	Colostomy in place (HCC)	**18**
Z93.2	Ileostomy present (HCC)	**31**
K50.0*	Crohn's Disease	**18**
K50.00	Crohn's disease of small intestine without complication (HCC)	7
K50.012	Crohn's disease of small intestine with intestinal obstruction (HCC)	3
K50.013	Crohn's disease of small intestine with fistula (HCC)	1
K50.018	Crohn's disease of small intestine with other complication (HCC)	3
K50.019	Terminal ileitis with complication (HCC)	4
K35.*	Acute appendicitis	**18**
K35.20	Acute appendicitis with generalized peritonitis	1
K35.32	Acute appendicitis with rupture	2
K35.80	Acute appendicitis, unspecified acute appendicitis type	8
K35.30	Acute appendicitis with localized peritonitis	7
K29.80	Duodenitis	**25**
K51.0*	Ulcerative colitis	**15**
K51.00	Ulcerative pancolitis without complication (HCC)	12
K51.011	Ulcerative pancolitis with rectal bleeding (HCC)	3
K20.0	Eosinophilic esophagitis	**15**
K90.49	Food Intolerance	**18**
K05.30	Periodontitis	**18**

[2]Bolded numbers refer to the total number of samples received in each diagnostic category. Non-bolded numbers are the breakdown of the number of samples received from each subcategory.

2.2.2 Food sensitivity ELISA based testing

Serum samples were analyzed using the 109 Foods Mediterranean Food Allergy IgG ELISA kit (Catalog number: CNS14M; Eagle Biosciences, Amherst, NH) to measure the level of IgG against 109 different foods. This was a 96 well-based ELISA kit with a few related foods pooled in a single well, such as lemon and lime. The list of the food targets

of the IgG detection methodology is detailed in Table 2.2. For further analysis and clarity, tested foods were placed into 16 groups according to the United States Department of Agriculture Food Data Central database (18).

Table 2.2. Food-specific IgG tested by 109 foods IgG ELISA

Category	Food	FDC ID	Category	Food	FDC ID
Beverages	Coffee	171890	**Nuts and seeds**	Pistachio	170184
	Tea	174155		Almond	170567
Cereal grains and pasta	Wheat	169725		Hazelnut	170581
	Gluten	168147		Chestnut	170164
	Buckwheat	170286		Cocoa Bean	169593
	Corn (maize)	170288		Cola Nut	169588
	Barley	170284		Pine Seed	170591
	Rice	168931		Sesame Seed	170150
	Rye	168884		Sunflower Seed	170562
	Durum Wheat	169721		Walnut	170187
	Oat	169705	**Other vegetables**	Chilli	170108
Dark green vegetables	Broccoli	787465		Courgette (Zucchini)	169291
	Spinach	787373		Onion	170000
Eggs	Egg White	172183		Garlic	169230
	Egg Yolk	172184		Artichoke	169205
Finfish	Cod	171955		Aubergine (Eggplant)	169228
	Salmon	175138		Cauliflower/Cabbage	169986, 169975
	Sarind/Anchovy	175139, 174182		Chicory	169992
	Sea Bass	175142		Cucumber	168409
	Sole	174196		Fennel	169385
	Trout/Hake	175153		Lettuce	169249
	Tuna	173706		Mushroom	169251
Fruit	Lemon/Lime	167746, 168155		Parsley	170416
	Melon	169092		Potato	170026
	Apricot/Peach	171697, 169928		String Bean	169961
	Orange/Tangerine	169919, 169105	**Poultry**	Chicken	171116
	Pineapple	169124		Turkey	171505
	Cherry	171719	**Red and orange vegetables**	Tomato	170457
	Olive	169094		Carrot	170393
	Apple	171689		Peppers/Capiscum	787810
	Banana	173944		Pumpkin	168448
	Fig	173021	**Red meat**	Rabbit	174347
	Grape Black/White	174682		Beef	168608
	Kiwifruit	168153		Lamb	174370
	Pear	169118		Pork	167902
	Plum	169949	**Shellfish**	Oyster/Clam	171978, 782757
	Strawberry	167762		Sepia/Calamar/Octopus	174215, 782743, 174218
	Watermelon	167765		Crab/Lobster	174204, 174208
Legumes and legume products	Peanut	172430		Mussel	174216
	Soya Bean	174270		Prawn/Shrimp	175179
	Pea	170419	**Spices, herbs, and sweets**	Mustard	172234
	Lentil	172420		Basil	172232
	Chickpea	173756		Black/White Pepper	170931, 170933
	Haricot/Kideny Bean	175193		Caper	172238
Milk	Cow's Milk	781084		Honey	169640
	Goat's/Sheep's Milk	171278, 170882	**Yeasts**	Yeast (beer)	788564
				Yeast (bread)	175043

ELISA manufacturer instructions were as follows; 25 µl of selected serum was diluted into 10 ml of supplied sample diluent and 100 µl was added to the food antigen coated ELISA plate. The plate was incubated for 30 minutes. Next, the plate wells were aspirated and washed 3 times with 425 µl of provided wash buffer with a BioTek 405 TS plate washer (BioTek, Winooski, VT) before the addition of 100 µl of horseradish

peroxidase conjugated goat anti-human IgG to each well. This was followed by an additional 30-minute incubation. The plate was then aspirated and washed 4 additional times with 425 µl of wash buffer. After washing, 100 µl of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was added to the wells and allowed to incubate for 10 minutes. After 10 minutes, 100 µl of stop solution was added, and the ELISA plate was immediately read at wavelengths of 450 nm and 620 nm (BioTek Synergy H1, Winooski, VT). Optical densities from the 620 nm reading were considered as background values and were subtracted from the 450 nm optical densities prior to analysis. Once the ELISAs were performed, the relative abundance of food-specific IgG was calculated using the standard curve provided by the ELISA manufacturer which consisted of a 0 AU/ml standard, a 25 AU/ml standard, and a positive control. Individual responses to foods were then extrapolated from this curve and categorically graded as 0 (negative), 1 (borderline), 2 (positive), or 3 (strong positive) based on a manufacturer supplied ranges for use in interpretation Table 2.3. Grades of 1, 2, and 3 taken from the 109 food IgG ELISA were summed for each individual. This number, the categorical sum, was used to assess overall reactivity for each individual. Additionally, the number of grades of 1, 2, and 3 per individual were included to determine the total number of food-specific IgG present in each serum sample.

Table 2.3. Manufacturer supplied chart for assigning categorical grade to samples.

Response	Range (AU[1]/ml)	Grade
Negative	<8	0
Borderline	8-12.5	1 (Equivocal)
Positive	12.5-25.0	2
Strong positive	>25.0	3

[1]AU; absorbance units

The purpose of this protocol was to examine whether the presence of an ostomy increased the amount of food-specific IgG present or the relative abundance of all food-

specific IgG tested in serum. Using the table provided by the manufacturer, categorical sums were generated by adding all calculated grades of 1, 2, and 3 for each food for every individual. In this way, the grades have been treated as Likert scale data to estimate overall level of food-specific IgG in sample serum.

2.2.3 Quantification of total IgG in serum

Reduced levels of total serum IgG can be indicative of an immune deficiency. To evaluate the relationship between immune-competency and food-specific IgG, we chose to evaluate all samples for levels of total IgG to determine any associations with food-specific IgG that may be present with immune competency.

Total serum IgG was quantified using the commercially available Human IgG ELISA assay (Catalog number: EGG39-K01; Eagle Biosciences, Amherst, NH). The ELISA had a sensitivity of 1.816 ng/ml and a dynamic range: 15.6 ng/ml - 500 ng/ml. To best fit the samples to the curve, serum samples were diluted 80,000-fold by serial dilution prior to analysis. All standards and reagents were appropriately diluted prior to analysis according to manufacturer instruction.

For the analysis, 100 µl of all standards and samples were pipetted into microplate wells in duplicate. The plate was covered and incubated for one hour at room temperature. After incubation, the plate was aspirated and washed four times with 300 µl of provided wash buffer. Next, 100 µl of horseradish peroxidase anti-IgG conjugate was added to each well and allowed to incubate for 20 minutes in the dark. The plate was then washed four additional times. Following washing, 100 µl of TMB solution was added to each well and incubated in the dark for 10 minutes. Finally, 100 µl of stop solution was added to each of the wells.

The optical density of plate was immediately read at 450 nm. The mean absorbance of duplicate standards and samples was calculated, and the average zero-standard optical density was subtracted. IgG levels were categorized into groups for analysis based on previously published data and guidelines from Michigan Health at the University of Michigan (Ann Arbor, MI) (Table 2.4) (19, 20).

Table 2.4 Categorization of Total IgG levels

Total IgG level (mg/dL)	Category
0-299	Profoundly or significantly reduced
300-599	Moderately reduced
600-1600	Normal
>1600	Elevated

2.2.4 Quantification of total IgA in serum

Like total IgG, reduced levels of total serum IgA can indicate the presence of an immune deficiency. We chose to evaluate all samples for levels of total IgA in order to determine any associations with diagnosis or food-specific IgG that may be present. Total serum IgA was quantified using the commercially available Human IgA ELISA assay (Catalog number: HUG39-K01; Eagle Biosciences, Amherst, NH). The ELISA had a sensitivity of 6.477 ng/ml and a dynamic range: 12.5 ng/ml - 800 ng/ml. To best fit the samples to the curve, serum samples were diluted 10,000-fold by serial dilution prior to analysis. All standards and reagents were appropriately diluted prior to analysis according to manufacturer instruction.

For the analysis, 100 µl of all standards and samples were pipetted into microplate wells in duplicate. The plate was covered and allowed to incubate for 30 minutes at room temperature. After incubation, the plate was aspirated and washed four times with 300 µl wash buffer. Next, 100 µl of horseradish peroxidase anti-IgG conjugate was added to each well and allowed to incubate for 30 minutes in the dark. The plate was then washed

four additional times. Following washing, 100 µl of TMB solution was added to each well and incubated in the dark for 10 minutes. Finally, 100 µl of stop solution was added to each of the wells. The optical density of plate was immediately read at 450 nm. The mean absorbance of duplicate standards and samples was calculated, and the average zero-standard optical density was subtracted. IgA levels were categorized into groups based on guidelines from the Mayo Clinic (Rochester, MN) and Merck & Co (Kenilworth, NJ) (Table 2.5) (21, 22).

Table 2.5 Categorization of Total IgA levels

Total IgA level (mg/dL)	Category
0-6	Deficient
7-60	Reduced
61-356	Normal
>356	Elevated

2.2.5 Determination of serum calprotectin levels

Serum calprotectin serves as a biomarker for systemic inflammation. This can be related to the integrity of the intestinal barrier and functionality of the mucosal immune system. For these reasons, our next goal was to evaluate the levels of serum calprotectin present in sampled individuals. Serum calprotectin was quantified using a commercially available Calprotectin ELISA kit (Catalog number: ab267628; Abcam, Cambridge, UK). The ELISA had a sensitivity of 35 pg/ml and a range of 32.77 pg/ml - 8000 pg/ml. In order to best fit the samples to the curve, serum samples were diluted 4,000-fold by serial dilution prior to analysis. All standards and reagents were also appropriately diluted prior to analysis according to manufacturer instruction.

For the analysis, 100 µl of all standards and samples were pipetted into microplate wells in duplicate. The plate was then covered and incubated for 2.5 hours at room

temperature. Following incubation, the plate was washed four times with 300 µl of wash buffer. Next, 100 µl of biotinylated calprotectin antibody was added to the wells and allowed to incubate for one hour while shaking gently. Following incubation, the plate was again washed four times with 300 µl of provided wash buffer. After washing, 100 µl of horseradish peroxidase-streptavidin conjugate was added to the wells and allowed to incubate for 45 minutes while shaking gently. After incubation, the plate was washed four times with 300 µl of wash buffer. After washing, 100 µl of TMB solution was added to each of the wells and allowed to incubate for 30 minutes in the dark while shaking gently. At the conclusion of this incubation, 50 µl of stop solution was added to each well. The optical density of the plate was then read immediately at 450 nm. The mean absorbance of duplicate standards and samples was calculated, and the average zero standard optical density was subtracted.

2.2.6 Statistical Analysis

2.2.6.1 Power analysis

The software Java Applets for Power and Sample Size (University of Iowa, USA) was used to conduct a power analysis for a one-way ANOVA comparing categorical sums between selected diagnostic groups. The effect size was estimated to be equivalent across sample groups and was estimated using the initial 145 samples analyzed. The best estimate for the standard deviation within groups was sigma = 7.50. This effect size estimate was then used in the power analysis along with alpha = 0.05 and power = 0.80.

2.2.6.2 Standard Curves

All standard curves for were calculated using the R package “drc” (https://github.com/DoseResponse/drc) in R version 4.0.3 using RStudio for mac OS (ver.

1.4.1103). The standard curves were plotted on a semi-log graph, with the concentration plotted logarithmically and the optical density plotted linearly. The best-fit line was calculated using a 4-parameter logistics curve.

2.2.6.3 Kruskal-Wallis ANOVA

Statistical differences between sample groups were analyzed using a Kruskal-Wallis ANOVA and Dunn's test. Typically, when performing a Dunn's test, p-values are corrected based on the number of pairwise comparisons made in order to adjust for possible error. These adjustments are quite conservative, and due to the high number of groups being tested, p-values for the Dunn's test presented in the results section are unadjusted unless otherwise specified.

2.2.6.4 Wilcoxon sum-rank test

Additionally, differences between ostomate and non-ostomate groups were assessed. Differences in the number of foods present, categorical sum, total serum IgG, total serum IgA, and total serum calprotectin were analyzed for significant differences using a Wilcoxon rank-sum test.

2.2.6.5 Responders versus non-responders

A chi-squared test of homogeneity was used to evaluate significance in the percentage of subjects with at least one positive value across diagnostic groups to test whether the response frequencies are homogenous. Individuals with food-specific IgG against at least one food have been termed as "responders" and those without "non-responders". The same testing method was also used to determine if this homogeneity exists between ostomy and non-ostomy samples using the same method. To further investigate the possible factors impacting response, a logistic regression model was used to assess the

impact of total serum IgG, total serum IgA, and ICD10 of selection on the presence of food-specific IgG while controlling for age and BMI. The dependent variable which measures the likelihood of food-specific IgG presence is response. Response is equal to 1 if the serum of the individual tests positive for any food-specific IgG, otherwise it is 0. Because the independent variable of ICD10 is discrete, a linear regression analysis is inappropriate. The logistic regression model was used to estimate the degree to which ICD10, total IgG, and total IgA impact the likelihood of response. Because some BMI measurements were missing from the metadata (n=46), values have been imputed for analysis using k-nearest neighbors methodology. All statistical significance was determined at p-value< 0.05.

2.3 RESULTS

2.3.1 Power analysis

A statistical power analysis was performed for sample size estimation using Java Applets for Power and Sample Size (University of Iowa, USA). This was executed at the conclusion of the analysis of 149 samples, with regards to the overall categorical sum. With sigma=7.5, alpha=0.05 and power=80%, the estimated sample size required was approximately n=160 (16 samples per diagnostic category) to observe significant differences in categorical sum between groups 80% of the time. The initial power analysis allowed us to estimate 16 samples would need to be included in each category. For the diagnostic groups of ulcerative colitis and eosinophilic esophagitis, only 15 samples were available.

2.3.2 Food-specific IgG present within sample population at large

Upon completion, 81.31% of all individuals tested were found to have food-specific IgG against at least one food. To break this down further, food-specific antibodies were detected in 93.3% (14 out of 15 participants) of eosinophilic esophagitis patients, 86.7% (13/15) of food intolerance patients, 88.9% (16/18) of ulcerative colitis patients, 83.3% (15/18) of colostomates, 80.6% (25/31) of ileostomates, 80% (20/25) of duodenitis patients, 83.3% (15/18) of appendicitis patients, 77.8% (14/18) of Crohn's disease patients, 77.3% (17/22) of jejunostomates, and 66.7% (12/18) of periodontitis patients. Sampled individuals had food-specific IgG against 54 of the 109 foods tested by ELISA (Table 2.3 from Material and Methods). The top 5 most prevalent food IgG within the population were cow's milk (55%), egg white (50%), wheat (36%), goat's/sheep's milk (35%), and egg yolk (33%), respectively.

Table 2.6. Percent of population with antibodies against food broken down by diagnostic category.

Food	Total	Appendicitis	Colostomy	CD[1]	Duodenitis	EE[2]	FI[3]	Ileostomy	Jejunostomy	Periodontitis	UC[4]
Cow's Milk	55%	61%	61%	39%	56%	73%	78%	61%	41%	33%	47%
Egg White	50%	56%	33%	33%	56%	87%	61%	55%	32%	50%	40%
Wheat	36%	28%	33%	44%	16%	60%	44%	39%	41%	33%	33%
Goat's/Sheep's Milk	35%	17%	33%	11%	28%	67%	72%	45%	32%	28%	20%
Egg Yolk	33%	50%	33%	17%	32%	53%	44%	39%	18%	22%	20%
Yeast (beer)	28%	22%	22%	50%	16%	13%	50%	29%	50%	6%	20%
Peanut	19%	17%	28%	11%	12%	27%	33%	19%	23%	11%	13%
Yeast (bread)	19%	11%	6%	50%	8%	0%	22%	23%	41%	6%	13%
Gluten	15%	6%	11%	6%	4%	53%	17%	19%	14%	17%	7%
Soya Bean	14%	6%	17%	11%	8%	33%	17%	16%	18%	6%	13%
Pistachio	13%	11%	17%	6%	20%	13%	17%	19%	9%	0%	13%
Mustard	13%	11%	17%	17%	8%	20%	28%	6%	5%	6%	20%
Corn (Maize)	10%	0%	11%	22%	4%	13%	0%	10%	32%	0%	0%
Pea	9%	0%	22%	6%	4%	7%	11%	10%	18%	0%	7%
Oyster/Clam	9%	17%	17%	0%	4%	13%	22%	10%	0%	0%	7%
Lemon/Lime	9%	6%	22%	11%	0%	20%	22%	6%	0%	6%	0%
Barley	8%	0%	22%	11%	0%	13%	6%	13%	14%	0%	0%
Almond	8%	6%	6%	6%	4%	20%	17%	10%	5%	6%	7%
Chick Pea	6%	0%	6%	0%	0%	7%	17%	3%	18%	0%	13%
Buckwheat	6%	0%	11%	11%	0%	7%	6%	10%	14%	0%	0%
Hazelnut	6%	0%	11%	6%	0%	13%	6%	3%	9%	6%	7%
Sepia/Calamar/ Octopus	5%	6%	6%	0%	4%	7%	6%	10%	0%	0%	7%
Melon	5%	0%	11%	6%	0%	7%	6%	3%	14%	0%	0%

Haricot/Kidney Bean	4%	0%	6%	6%	0%	0%	11%	3%	14%	0%	0%
Lentil	4%	0%	0%	6%	0%	7%	6%	3%	14%	0%	0%
Rice	3%	0%	11%	0%	0%	13%	0%	0%	9%	0%	0%
Orange/ Tangerine	3%	0%	6%	6%	0%	7%	6%	3%	0%	0%	0%
Coffee	3%	6%	0%	6%	0%	7%	6%	3%	0%	0%	0%
Chestnut	2%	0%	0%	0%	0%	13%	6%	0%	5%	0%	0%
Garlic	2%	0%	0%	0%	0%	0%	6%	3%	5%	0%	0%
Apricot/Peach	2%	0%	6%	0%	0%	7%	6%	0%	0%	0%	0%
Cherry	2%	0%	6%	0%	0%	7%	6%	0%	0%	0%	0%
Pineapple	2%	0%	11%	0%	0%	0%	0%	0%	5%	0%	0%
Rabbit	2%	6%	6%	0%	0%	0%	0%	0%	0%	0%	7%
Tomato	1%	0%	6%	0%	0%	7%	0%	0%	0%	0%	0%
Rye	1%	0%	0%	0%	0%	7%	0%	0%	5%	0%	0%
Cod	1%	0%	6%	0%	0%	0%	0%	0%	0%	6%	0%
Banana	1%	0%	0%	0%	0%	0%	0%	0%	9%	0%	0%
Courgette	1%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%
Onion	1%	0%	0%	0%	0%	0%	0%	0%	5%	0%	0%
Chilli	1%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%
Blakc/White Pepper	1%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%
Olive	1%	0%	0%	0%	0%	7%	0%	0%	0%	0%	0%
Mussel	1%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Tuna	1%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Strawberry	1%	0%	0%	0%	0%	7%	0%	0%	0%	0%	0%

[1]Crohn's Disease, [2]Eosinophilic esophagitis, [3] Food intolerance, [4]Ulcerative Colitis

2.3.3 Food-specific IgG present between ostomates and non-ostomates

Initial analysis by Wilcoxon rank-sum test indicated no significant difference in the number of food-specific IgG present in ostomates versus non-ostomates ($p=0.38$).

Upon the exclusion of milk and eggs, the difference observed between the number of food-specific IgG present in the serum of ostomates and non-ostomates approached significance but remained above $p=0.05$ ($p=0.056$) (Figure 2.1).

Based on three Wilcoxon rank-sum tests, no significant differences were found among non-ostomates and jejunostomates ($p=0.074$), colostomates ($p=0.2$), and ileostomates ($p=0.5$) respectively (Figure 2.2).

Figure 2.1. Wilcoxon sum-rank test on number of food-specific IgG present in ostomates versus non-ostomates.

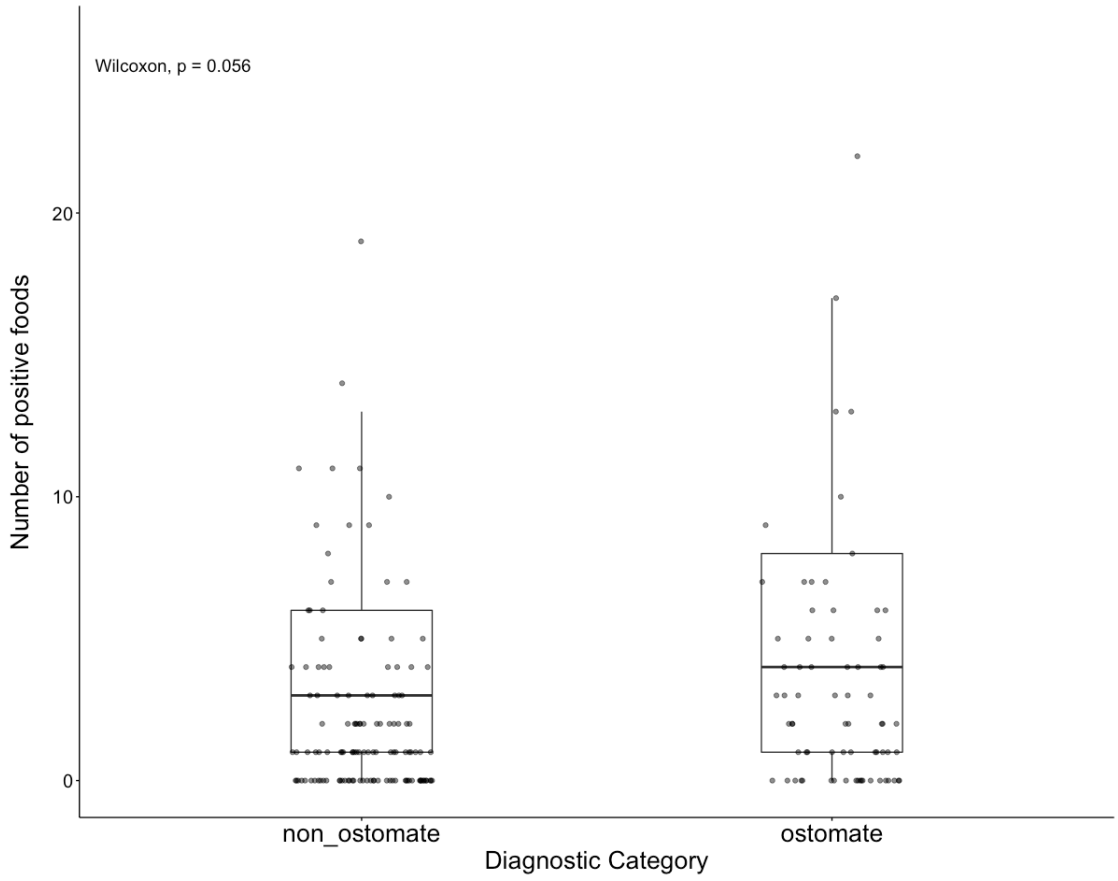
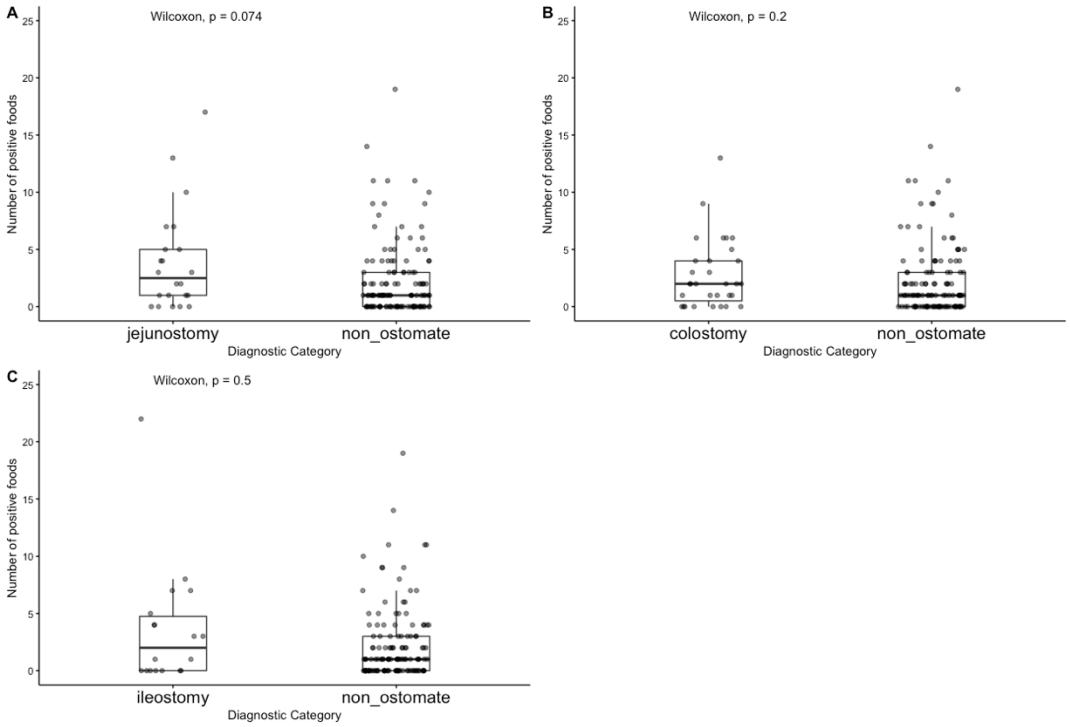


Figure 2.2. Wilcoxon sum rank test on number of food-specific IgG in different ostomy groups compared to non-ostomates. **(A)** Jejunostomates versus non-ostomates. **(B)** Colostomates versus non-ostomates. **(C)** Ileostomates versus non-ostomates.



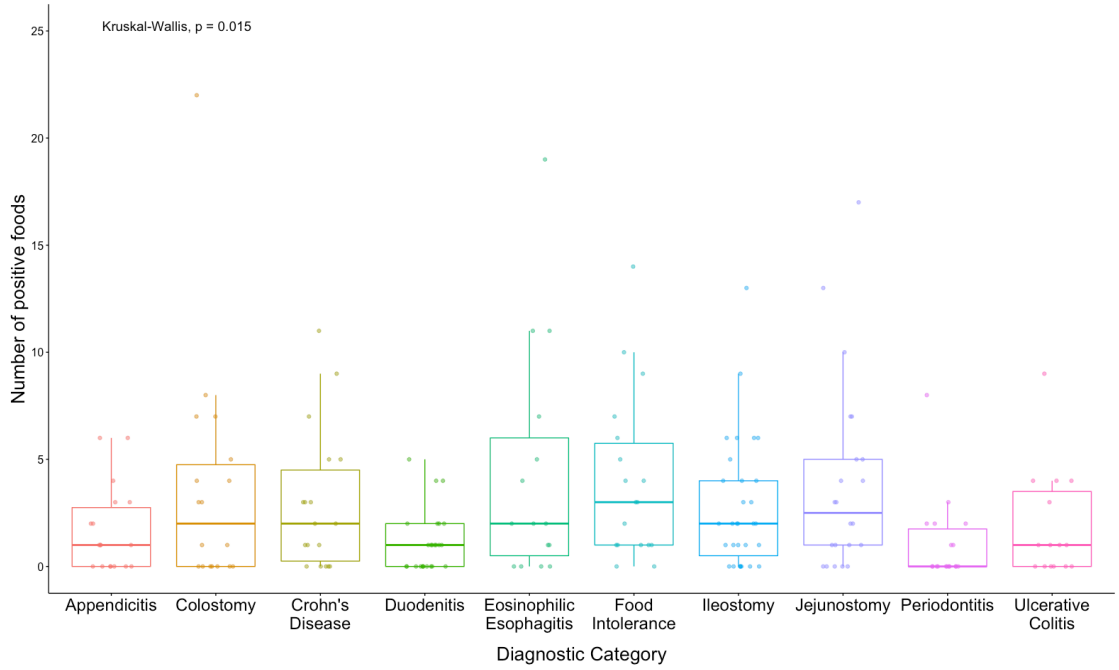
2.3.4 Differences in number of foods positive among diagnostic categories

Although insignificant, the previous analysis indicated a tendency for ostomates to have higher numbers of food-specific IgG than non-ostomates. In order to further investigate the impact of diagnosis on number of food-specific IgG present in serum, the differences in number of foods positive between each diagnostic group were examined. We did this using a Kruskal-Wallis ANOVA, which is a non-parametric one-way ANOVA used to determine differences in group median.

No significant difference was observed using a Kruskal-Wallis test on all sampled diagnoses (p=0.1). However, the presence of a significant difference in the number of foods positive per diagnostic category was observed when excluding egg and milk

categories (p=0.015) (Figure 2.3).

Figure 2.3. Kruskal-Wallis ANOVA on number of positive foods and diagnostic categories.



To confirm this result, a Dunn's test was performed post-hoc. A Dunn's test performs pairwise comparisons on each possible combination of diagnostic groups to determine significant differences between group means. The Dunn's test indicated a significantly larger number of food-specific IgG present in the serum of those with food intolerance versus periodontitis (p=0.001), jejunostomy versus periodontitis (p=0.002), food intolerance versus duodenitis (p=0.002), jejunostomy versus duodenitis (p=0.006), eosinophilic esophagitis versus periodontitis (p=0.007), ileostomy versus periodontitis (p=0.007), Crohn's disease versus periodontitis (p=0.014), eosinophilic esophagitis versus duodenitis (p=0.016), ileostomy versus duodenitis (p=0.017), food intolerance versus appendicitis (p=0.020), colostomy versus periodontitis (p=0.023), food intolerance versus ulcerative colitis (p=0.026), Crohn's disease versus duodenitis (p=0.032), and

jejunostomy versus appendicitis (p=0.048). Significance values for all pairwise Dunn's test comparisons are presented in Table 2.7.

Table 2.7. Dunn's test pairwise comparisons between diagnostic categories of interest and the number of foods positive.

	Appendicitis	Colostomy	CD[1]	Duodenitis	EE[2]	FI[3]	Ileostomy	Jejunostomy	Periodontitis
Colostomy	0.182								
CD[1]	0.135	0.423							
Duodenitis	0.253	0.050	**0.032***						
EE[2]	0.079	0.293	0.359	**0.016***					
FI[3]	**0.020***	0.125	0.169	**0.002***	0.291				
Ileostomy	0.109	0.417	0.496	**0.017***	0.341	0.139			
Jejunostomy	**0.048***	0.237	0.305	**0.006***	0.457	0.311	0.277		
Periodontitis	0.138	**0.023***	**0.014***	0.304	**0.007***	**0.001***	**0.007***	**0.002***	
UC[4]	0.492	0.199	0.151	0.258	0.091	**0.026***	0.127	0.059	0.145

*Indicates $p \leq 0.05$
[1]Crohn's disease
[2]Eosinophilic esophagitis
[3]Food intolerance
[4]Ulcerative colitis

2.3.4 Differences in overall reactivity to food between ostomates and non-ostomates

Based on the Wilcoxon rank-sum test, no significant differences were observed in the categorical sums of ostomates versus non-ostomates (p=0.52). Once milk and eggs were excluded, the difference observed between the number of food-specific IgG present in the serum of ostomates and non-ostomates approached significance but remained above p=0.05 (p=0.074) (Figure 2.4).

Three Wilcoxon rank-sum tests were performed on non-ostomates and jejunostomates (p=0.1), colostomates (p=0.62), and ileostomates (p=0.19) respectively, but no significant differences were observed (Figure 2.5).

Figure 2.4. Wilcoxon Sum Rank on overall level of reactivity in ostomates versus non-ostomates.

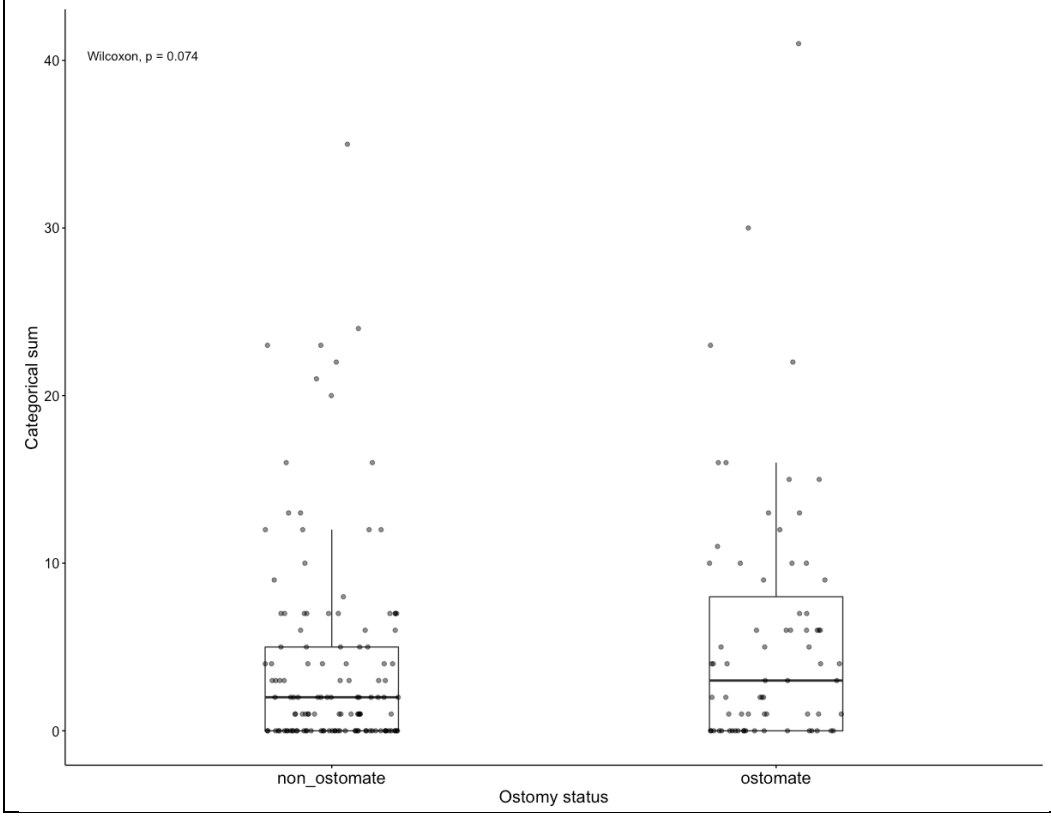
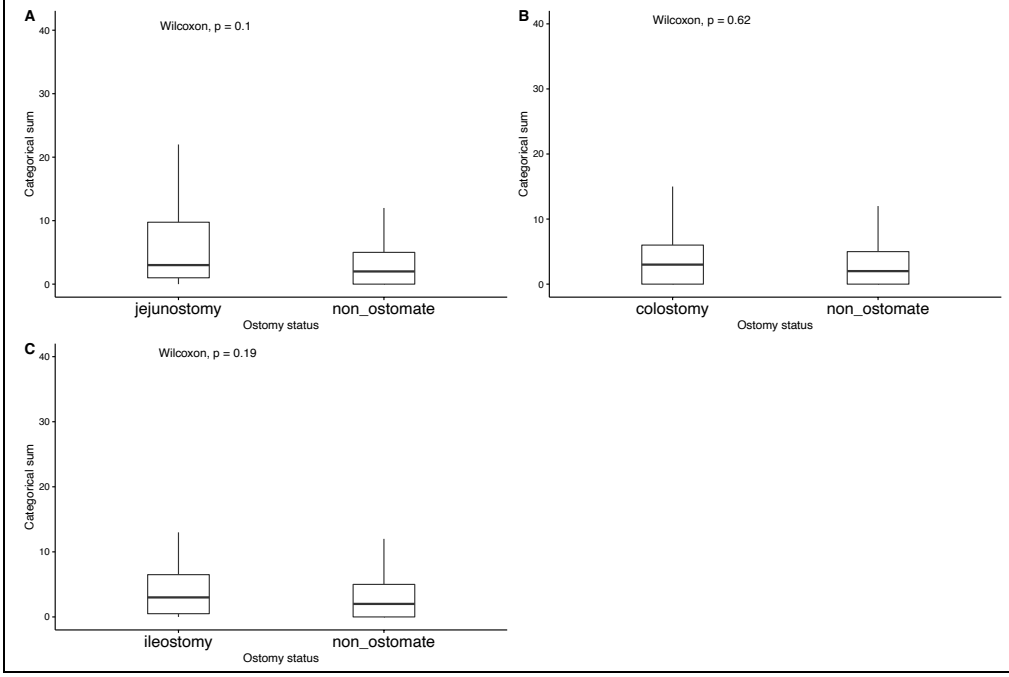


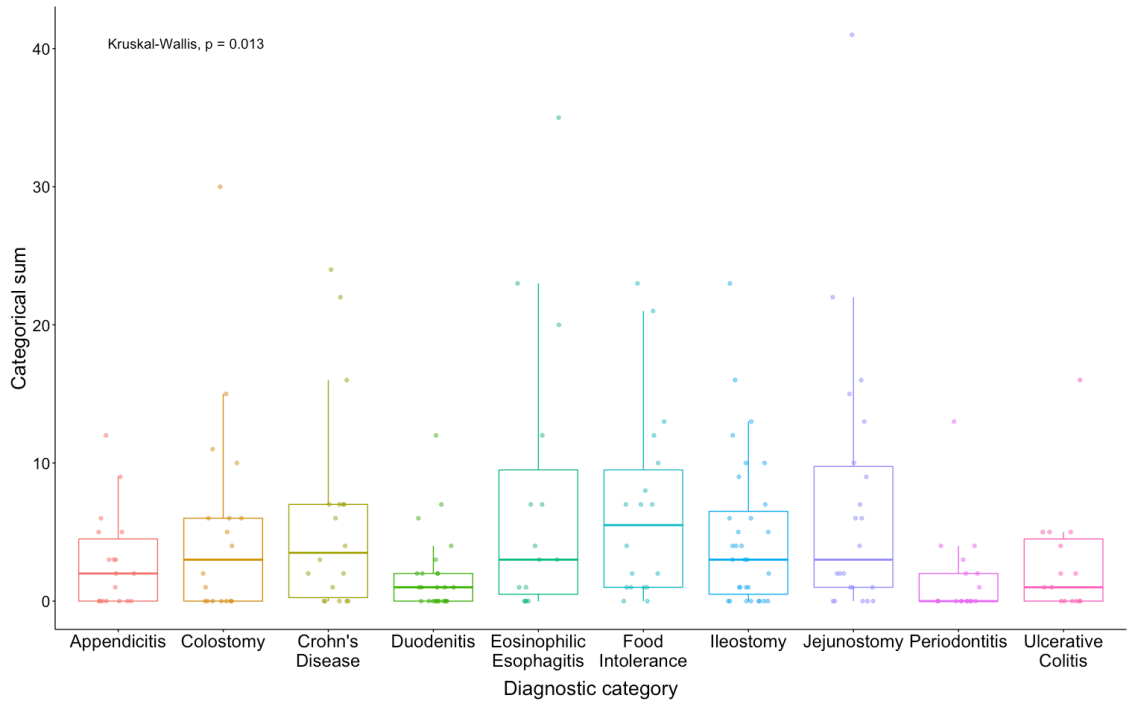
Figure 2.5. Wilcoxon sum rank test on overall level of reactivity in different ostomy groups compared to non-ostomates. **(A)** Jejunostomates versus non-ostomates. **(B)** Colostomates versus non-ostomates. **(C)** Ileostomates versus non-ostomates.



2.3.5 Differences in overall reactivity to food with regards to disease state

Initially, no significant difference was noted between the categorical sums of all diagnostic groups upon analysis by Kruskal-Wallis ANOVA (p=0.072). Upon the exclusion of egg and milk categories, it was indicated that there was a significant difference in the categorical sum per diagnostic category (p=0.013) (Figure 2.6).

Figure 2.6. Kruskal-Wallis ANOVA on food-specific-IgG categorical reactivity and diagnostic categories.



Again, a post-hoc Dunn's test was performed to better evaluate which groups were different. The Dunn's test indicated that there was a significant difference in the categorical sums of those with food intolerance versus periodontitis (p=0.001), food intolerance versus duodenitis (p=0.002), jejunostomy versus periodontitis (p=0.003), ileostomy versus periodontitis (p=0.006), Crohn's disease versus periodontitis (p=0.007), jejunostomy versus duodenitis (p=0.007), eosinophilic esophagitis versus periodontitis (p=0.007), ileostomy versus duodenitis (p=0.014), Crohn's disease versus duodenitis

(p=0.015), eosinophilic esophagitis versus duodenitis (p=0.017), food intolerance versus ulcerative colitis (p=0.022), Colostomy versus periodontitis (p=0.029), and food intolerance versus appendicitis (p=0.031). Significance values for all pairwise Dunn's test comparisons are listed in Table 2.8.

Table 2.8. Dunn's test pairwise comparisons between ICD10 of interest and categorical sum.

	Appendicitis	Colostomy	CD[1]	Duodenitis	EE[2]	FI[3]	Ileostomy	Jejunostomy	Periodontitis
Colostomy	0.261								
CD[1]	0.110	0.278							
Duodenitis	0.200	0.063	**0.015***						
EE[2]	0.107	0.263	0.470	**0.017***					
FI[3]	**0.031***	0.110	0.261	**0.002***	0.296				
Ileostomy	0.131	0.343	0.398	**0.014***	0.372	0.164			
Jejunostomy	0.073	0.216	0.433	**0.007***	0.467	0.307	0.321		
Periodontitis	0.105	**0.029***	**0.007***	0.306	**0.007***	**0.001***	**0.006***	**0.003***	
UC[4]	0.409	0.201	0.081	0.290	0.079	**0.022***	0.095	0.052	0.167

*Indicates $p<0.05$
[1]Crohn's disease
[2]Eosinophilic esophagitis
[3]Food intolerance
[4]Ulcerative colitis

2.3.6 Testing for immunocompetence: Differences in total serum IgG with regards to disease status

Total IgG levels in serum have been quantified and analyzed for all samples. A Kruskal-Wallis test indicated that there were no significant differences in the levels of total serum IgG between groups tested (p=0.66) (Figure 2.7). In addition, ostomate groups were compared with non-ostomates using the Wilcoxon rank-sum test, and no significant differences were observed (p=0.65) (Figure 2.8).

Three Wilcoxon rank-sum tests on non-ostomates and jejunostomates (p=0.54), colostomates (p=0.85), and ileostomates (p=0.90) showed that no significant differences were observed (Figure 2.9).

Figure 2.7. Kruskal-Wallis ANOVA on diagnostic category and total serum IgG.

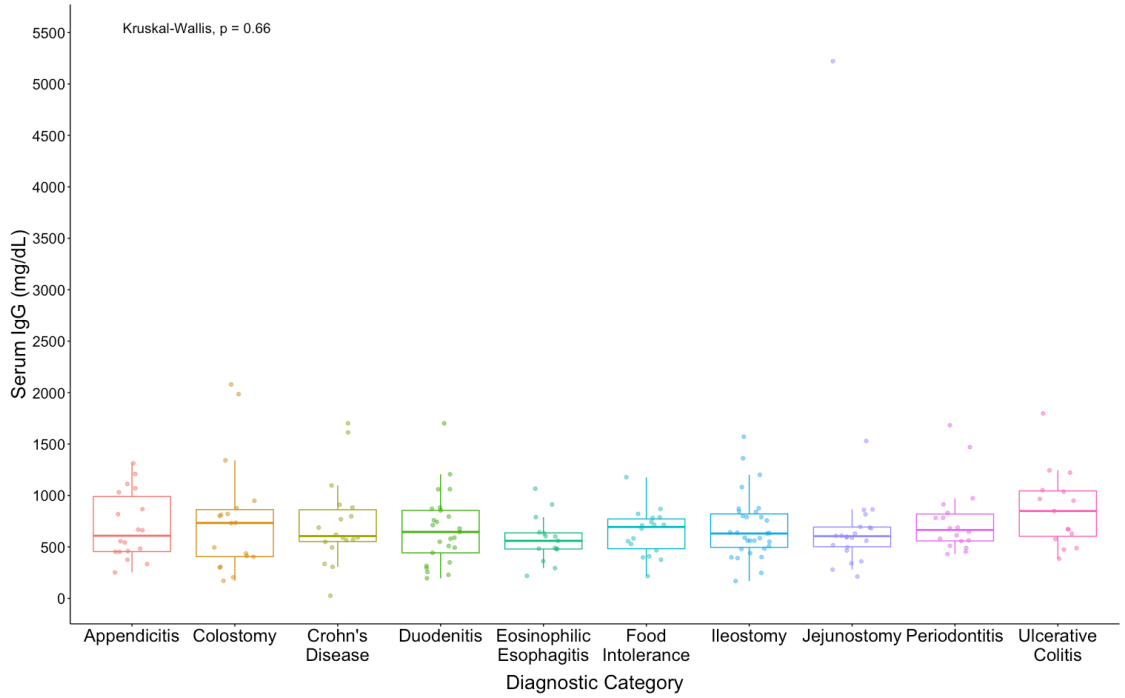


Figure 2.8. Wilcoxon sum-rank test on total serum IgG of ostomates versus non-ostomates.

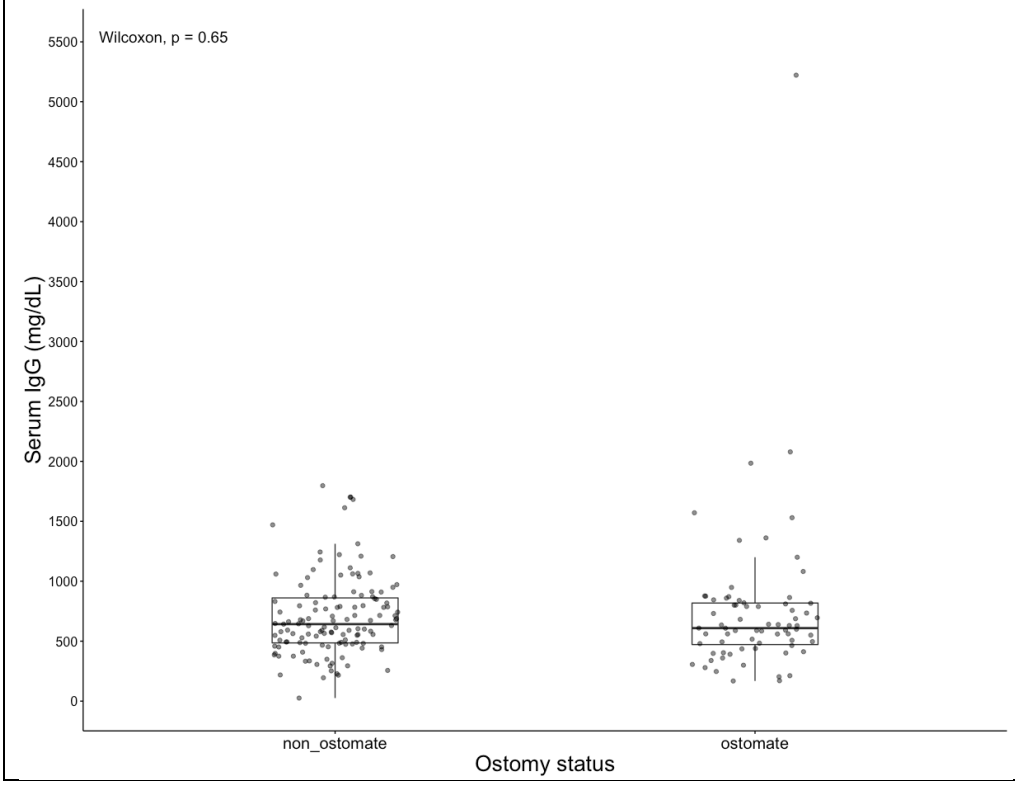
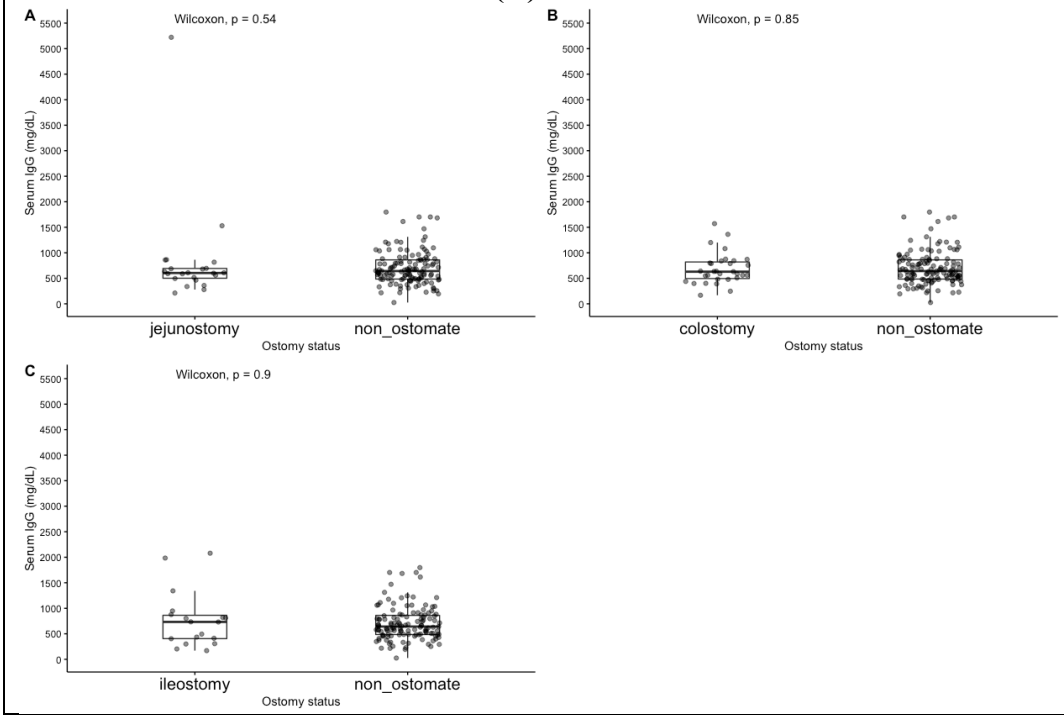


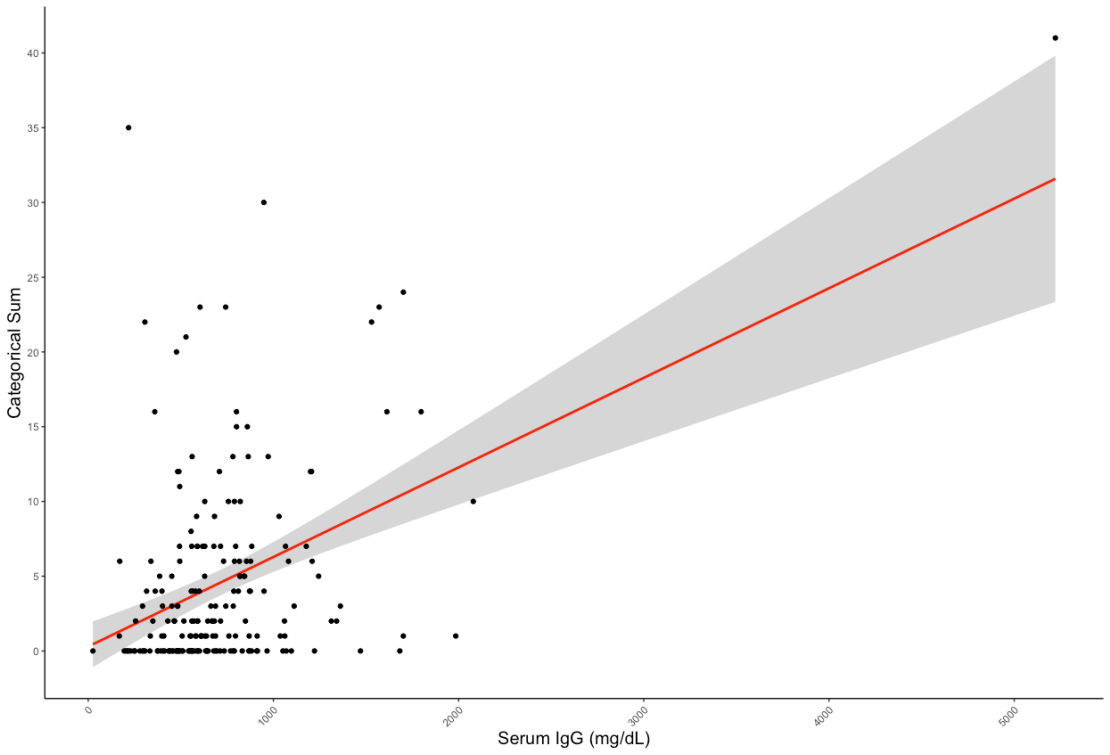
Figure 2.9. Wilcoxon sum rank test on level of total serum IgG in different ostomy groups compared to non-ostomates. **(A)** Jejunostomates versus non-ostomates. **(B)** Colostomates versus non-ostomates. **(C)** Ileostomates versus non-ostomates.



2.3.7 Relationship between total serum IgG and categorical sum

The linear regression indicated a strong positive correlation ($p<0.001$) between total serum IgG and categorical sum, suggesting that elevated levels of total IgG are associated with higher levels of reactivity to food-specific IgG (Figure 2.10). This correlation was maintained even after removing the outlier data point ($p<0.001$).

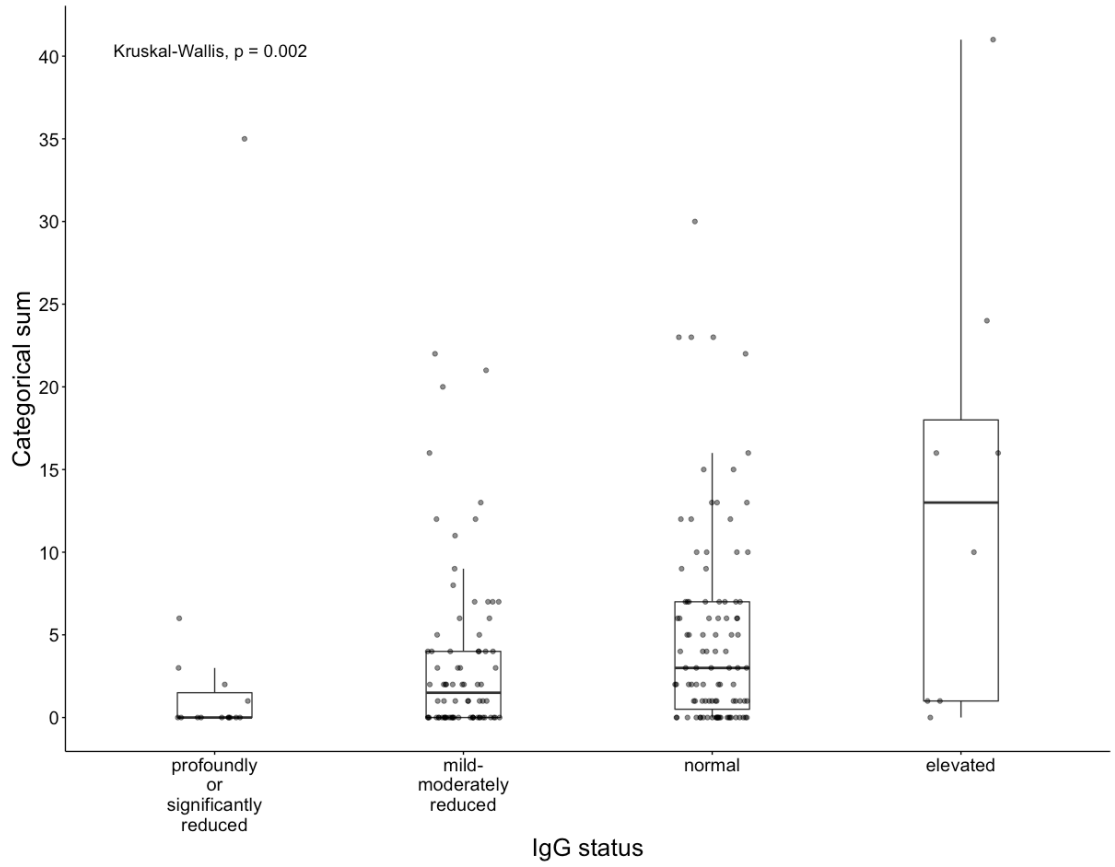
Figure 2.10. Linear regression of total serum IgG versus categorical sum.



The relationship between total IgG and categorical sum was further explored by looking at categorical ranges of IgG levels to evaluate whether specific ranges were statistically different. Individuals were put into four groups to indicate IgG status: "profoundly or significantly reduced", "mild-moderately reduced", "normal", and "elevated" (Figure 2.4) The Kruskal-Wallis ANOVA indicated that there were significant differences between the groups ($p=0.002$) (Figure 2.11). A post-hoc Dunn's test was performed, and significant pairwise differences were observed between elevated and

mild-moderately reduced (p=0.03), mild-moderately reduced and normal (p=0.04), elevated and profoundly or significantly reduced (p=0.014), and normal and profoundly or significantly reduced (p=0.019). P-value adjustments were made using the Benjamini-Yeukateli adjustment.

Figure 2.11. Kruskal-Wallis ANOVA on total serum IgG level versus categorical sum.



2.3.8 Serum calprotectin quantitation in context of disease status

Serum calprotectin levels in samples were quantified and analyzed for all samples. A Wilcoxon rank-sum test indicated that no significant differences were present between ostomates and non-ostomates (p=0.92) (Figure 2.12). Additionally, a Kruskal-Wallis test indicated that there were no significant differences in the levels of serum calprotectin between groups tested (p=0.72) (Figure 2.13).

Figure 2.12. Wilcoxon sum rank test on serum calprotectin in ostomates versus non-ostomates.

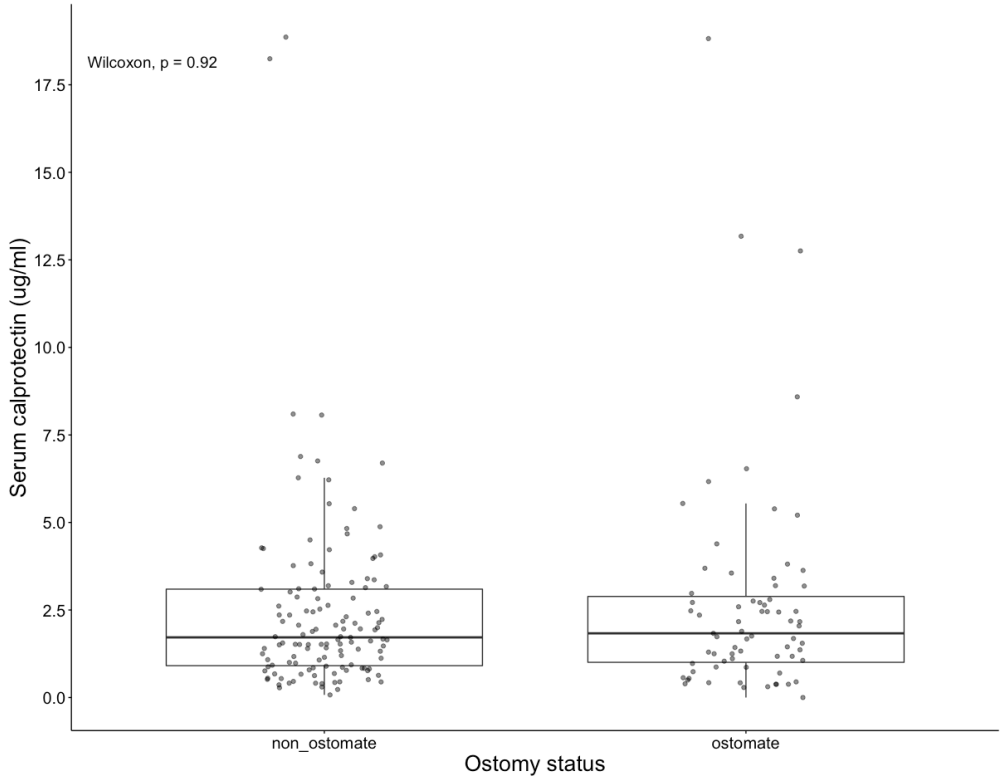
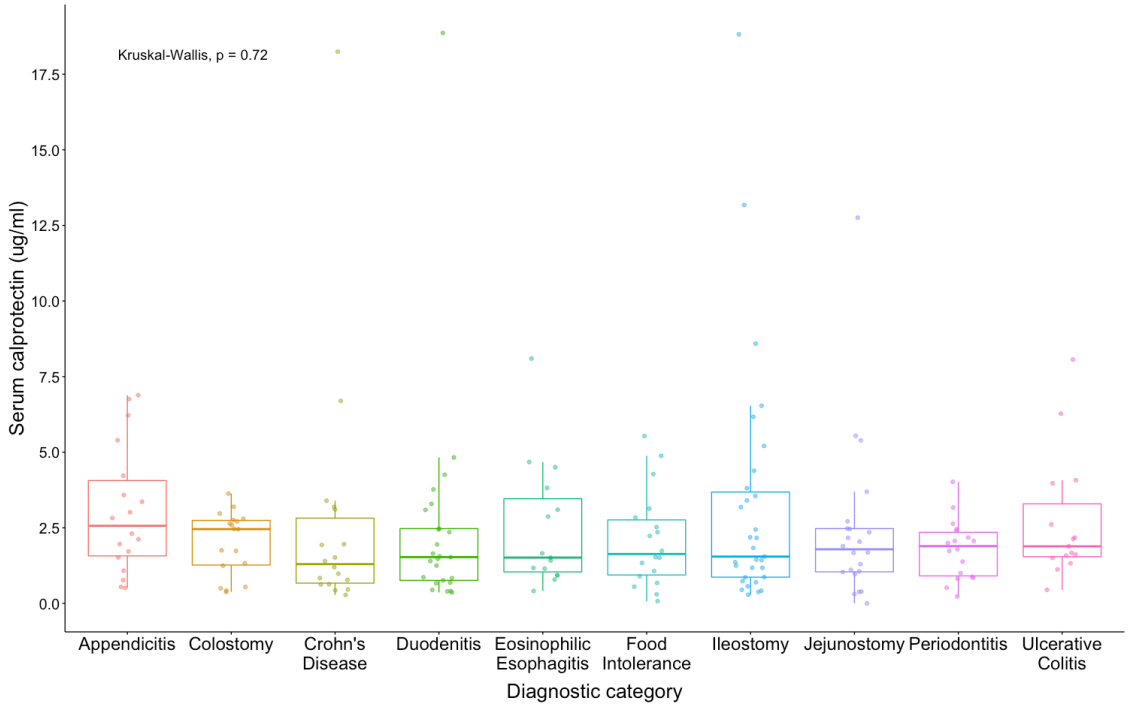


Figure 2.13. Kruskal-Wallis ANOVA on total serum calprotectin.



2.3.9 Testing for immunocompetence: Differences in total serum IgA with regards to disease status

Total IgA levels in serum were quantified and analyzed for all samples. A Kruskal-Wallis test indicated that there were no significant differences in the levels of total serum IgA between groups tested (p=0.74) (Figure 2.14). In addition, ostomate groups were compared with non-ostomates using the Wilcoxon rank-sum test, and no significant differences were observed (p=0.56) (Figure 2.15).

We further refined analysis by comparing each individual ostomate category to non-ostomates (Figure 2.16). Three Wilcoxon rank-sum tests were performed on non-ostomates and jejunostomates (p=0.93), colostomates (p=0.6), and ileostomates (p=0.2) respectively. No significant differences were observed.

Figure 2.14. Kruskal-Wallis ANOVA on diagnostic category and total serum IgA.

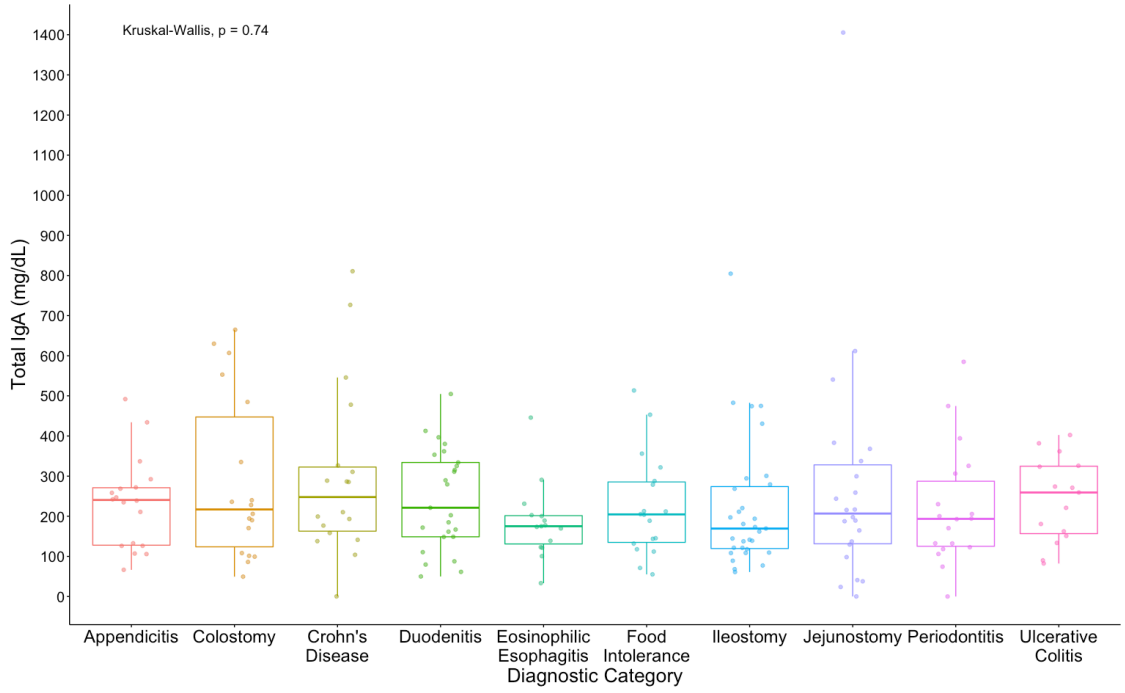


Figure 2.15. Wilcoxon sum-rank test on total serum IgA of ostomates versus non-ostomates.

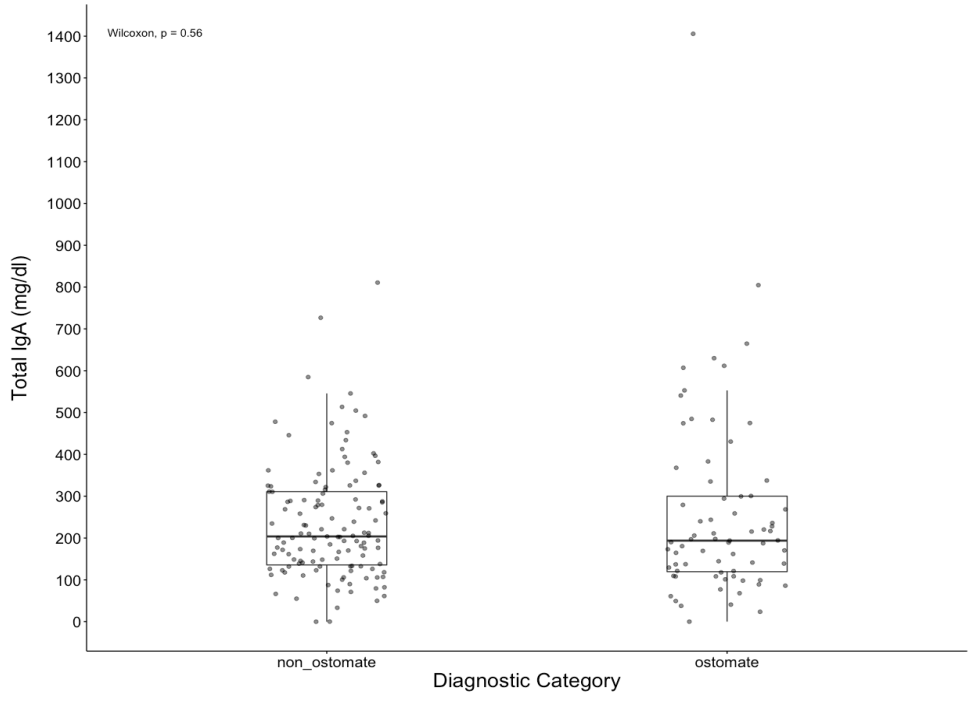
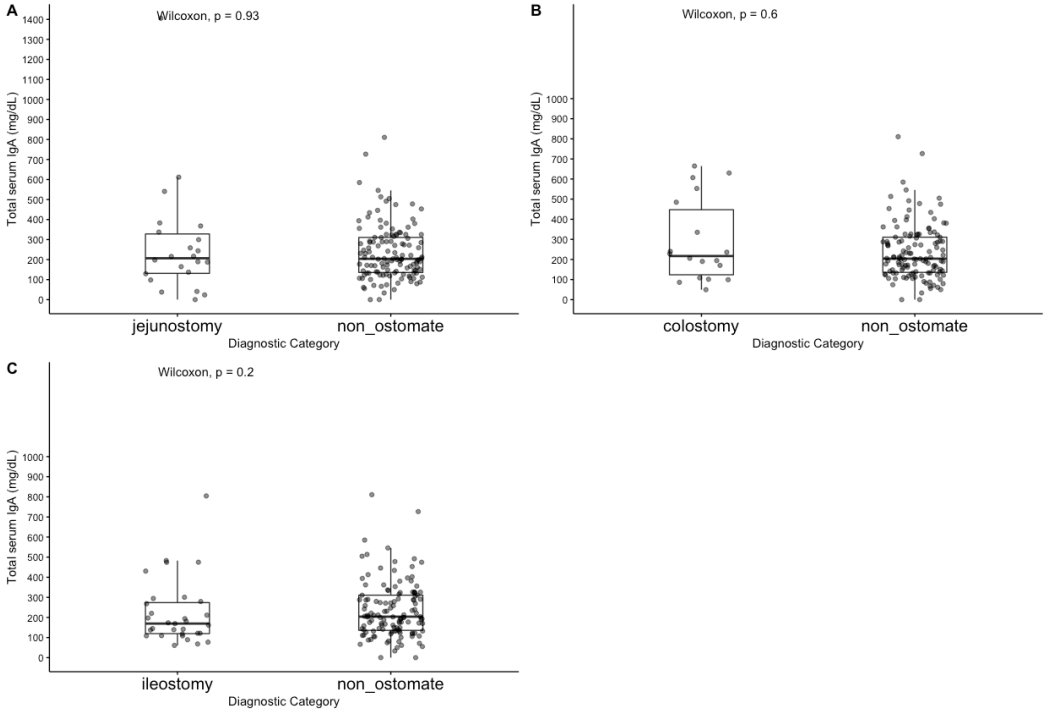


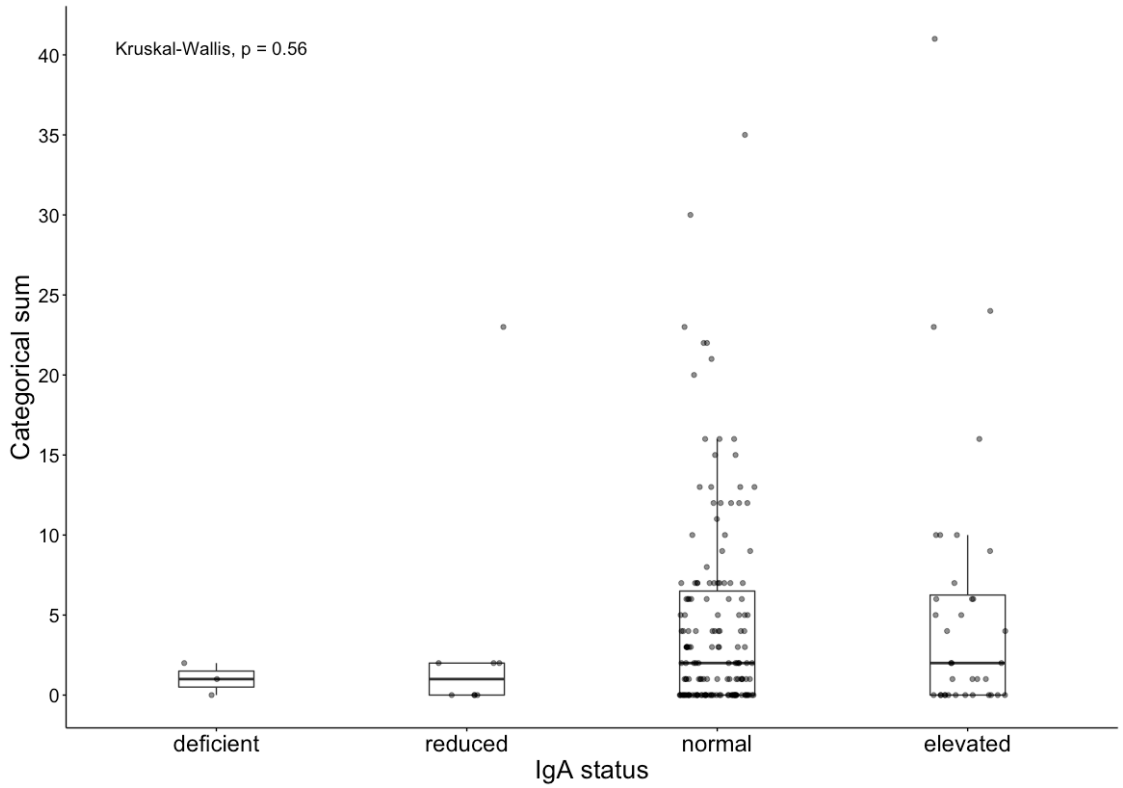
Figure 2.16. Wilcoxon sum rank test on level of total serum IgA in different ostomy groups compared to non-ostomates. **(A)** Jejunostomates versus non-ostomates. **(B)** Colostomates versus non-ostomates. **(C)** Ileostomates versus non-ostomates.



2.3.10 Relationship between total serum IgA and categorical sum

The relationship between total IgA and categorical sum was explored by looking at categorical ranges of IgA levels to evaluate whether specific ranges were statistically different. Individuals were put into four groups to indicate IgA status: "deficient", "reduced", "normal", and "elevated" (Table 2.5). A Kruskal-Wallis ANOVA was performed and indicated that there were no significant differences between the groups (p=0.56) (Figure 2.17).

Figure 2.17. Kruskal-Wallis ANOVA on total serum IgA level versus categorical sum.



2.3.11 Probability of response based on age, BMI, total serum IgG, total serum IgA, and ICD10

Initially, a chi-square test was performed to evaluate the homogeneity of responders versus non-responders between the diagnostic groups, but no significance was observed.

The regression model predicts the probability of a binary dependent variable in terms of the log odds, as a linear combination of a set of independent variables. We have used age of the individual, BMI, total serum IgG, total serum IgA, and ICD10 (Table 2.9). Because no group differences or relationship to categorical sum were seen when evaluating calprotectin levels, it has been excluded from the regression. In this model, the presence of eosinophilic esophagitis, an ileostomy, a jejunostomy, and food intolerance were found to be significantly influential factors in predicting the presence of food-

specific IgG, with odds ratios of 6.68, 5.58, 10.4, and 16.7, respectively. This indicates that ileostomates were 5.88 times as likely to have food-specific IgG than individuals with periodontitis, when controlling for age, BMI, and other diagnoses. In a similar fashion, individuals with eosinophilic esophagitis were 6.68 times more likely to have food-specific IgG than those with periodontitis, jejunostomates were 10.4 times more likely to have food-specific IgG than those with periodontitis, and individuals diagnosed with food intolerance were 16.7 times more likely to have food-specific IgG. Total serum IgG levels were broken into four categories to assess the ranges of values that are associated with the presence of food-specific IgG. Individuals with profoundly or significantly reduced and mild-moderately reduced levels of serum IgG were less likely to develop food-specific IgG, with odds ratios of 0.11 and 0.38, respectively. IgA levels were broken down into categories for the same purpose, however no significance was observed.

Table 2.9 Logistic regression model of predictors of food-specific IgG presence

Characteristic	OR[1]	95% CI[2]	p-value
(Intercept)	0.27	0.03, 2.11	0.22
age_years	1.01	0.99, 1.03	0.24
bmi	1.03	0.97, 1.10	0.39
IgAstatus			
normal	1	—	
deficient	1.81	0.14, 46.5	0.66
reduced	0.38	0.07, 2.06	0.25
elevated	0.42	0.16, 1.05	0.063
IgGstatus			
normal	1	—	
profoundly or significantly reduced	0.11	0.03, 0.38	**<0.001**
mild-moderately reduced	0.38	0.18, 0.79	**0.01**
elevated	4.21	0.64, 83.9	0.2
ICD10			
Periodontitis	1	—	
Ulcerative Colitis	2.04	0.46, 9.74	0.36
Duodenitis	2.4	0.58, 10.5	0.23
Colostomy	2.69	0.59, 13.2	0.21
Appendicitis	2.86	0.69, 12.8	0.15
Crohn's Disease	4.44	1.03, 21.5	0.052
Eosinophilic Esophagitis	6.68	1.34, 39.5	**0.026**
Ileostomy	5.88	1.55, 24.7	**0.011**
Jejunostomy	10.4	2.26, 56.8	**0.004**
Food Intolerance	16.7	2.95, 143	**0.003**

AIC = 249.73
[1]OR = Odds Ratio
[2]CI = Confidence Interval

2.4 DISCUSSION

Sera used for testing were acquired in September of 2019 from the Nebraska Biobank. Of the 198 sera analyzed, 161 exhibited food-specific IgG against at least one food antigen. Previous studies done on the presence of food-specific IgG in serum of individuals with Crohn’s disease, ulcerative colitis, and irritable bowel syndrome have indicated that both healthy controls and diseased individuals have high levels of milk and egg specific IgG

(11, 23, 24). This was further validated in our study, as 55%, 50%, and 36% of our population tested positive for IgG against cow's milk, egg white, and wheat respectively (Table 2.4). Therefore, we excluded categories of milk and eggs in analyses. The food categories with the highest prevalence of food-specific IgG were Eggs, Milk, Cereal Grains and Pasta, Yeast, and Legumes and Legume products. Coincidentally, these categories also include foods known to be common trigger foods in IgE mediated allergy, such as peanut, soybean, wheat, milk, and eggs. Interestingly, only two individuals tested positive for IgG against any type of fish, despite fish being a common IgE mediated allergy.

While all groups had individuals with food-specific IgG, the diagnostic group with the highest prevalence of food-specific IgG was eosinophilic esophagitis. This is in line with studies that have described elevated levels of food-specific IgG in eosinophilic esophagitis (25, 26). Because, most of the antigen sampling that takes place in the digestive system occurs in the large intestine, it is somewhat surprising that eosinophilic esophagitis displayed the highest prevalence of food-specific IgG (27). This also makes it more predictable that individuals with periodontitis exhibited the lowest prevalence of food-specific IgG. Multiple studies have found that increased inflammation in the digestive tract can have a deleterious effect on the barrier provided by the gastrointestinal lining (28-30). Because of this, it is not surprising that we find food-specific IgG in all our inflammatory controls. Despite this, no differences were observed in total calprotectin levels between our sample groups (Figure 2.13).

Because ostomy surgery can be caused by damage to the digestive tract associated with higher levels of intestinal permeation, an increase in exposure of the immune system

to food antigens would occur in ostomates. The data collected suggests that ostomates do tend to exhibit food-specific IgG to higher numbers of foods and display higher levels of overall reactivity than non-ostomates, however the difference was not statistically significant (Figure 2.1, Figure 2.4). Of note, is that diagnoses which occur in the proximal digestive tract, except for eosinophilic esophagitis, tend to display a lower number of foods positive and a lower level of overall reactivity by way of categorical sum than their more distal counterparts further along the digestive tract. Additional studies that examine individual diet, cause of ostomy, and extent of intestinal damage would need to be undertaken to determine if this relationship is causal as opposed to simply correlated.

The observation that ostomates have higher levels of food-specific IgG becomes especially meaningful when the impact of ostomy on the likelihood of response is considered (Table 2.9). The logistic regression analysis indicates that multiple factors influence the likelihood of an individual to generate food-specific IgG. It suggests that certain disease states, such as food intolerance and eosinophilic esophagitis increase the likelihood of an individual to develop food-specific IgG. It also indicates that individuals with certain types of ostomies may be at higher risk of developing food-specific IgG, however additional study taking the cause of the ostomy into consideration is necessary to ascertain the reason for this relationship.

The analysis also indicates that individuals with reduced levels of total IgG are less likely to produce food-specific IgG against the foods tested. One possible reason for this could be that the presence of an immunodeficiency could result in a weaker response to antigens sampled in the lumen of the digestive tract. Our study attempted to address

this concern by examining levels of total IgA in the sample population, however the logistic regression did not indicate that IgA level had a significant impact on the development of food-specific IgG. Additionally, no significant differences were seen in IgA levels between diagnostic groups (Figure 2.14).

2.5 CONCLUSION

This study has shown that individuals with jejunostomies and ileostomies, as well as those with food sensitivity, eosinophilic esophagitis, and Crohn's disease have food-specific IgG against a greater number of foods and at a higher level than individuals with periodontitis, duodenitis and appendicitis. Additionally, it was shown that IgG levels and disease status can have a significant impact on the development of food-specific IgG. In doing so, it has broadened the context for examining food sensitivity in individuals with altered digestive tracts and those with established inflammatory conditions of the digestive system to include additional immune factors that may have a role. Besides simply comparing the differences between antigen and calprotectin levels in ostomates and individuals with inflammatory conditions, it has demonstrated that food-sensitivity is associated with these in a unique way. Further study on the specific roles of IgG, IgA, inflammation, biogeography, and the intestinal microbiome are critical to untangling this complex web of interactions between food proteins, host immune systems, and commensal microorganisms.

2.6 REFERENCES

1. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet. 1994;343(8906):1127-30. doi: 10.1016/s0140-6736(94)90234-8. PubMed PMID: 7910231.

2. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520. Epub 2014/10/20. doi: 10.3389/fimmu.2014.00520. PubMed PMID: 25368619; PMCID: PMC4202688.

3. Ménard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol. 2010;3(3):247-59. Epub 2010/03/10. doi: 10.1038/mi.2010.5. PubMed PMID: 20404811.

4. Shakoor Z, AlFaifi A, AlAmro B, AlTawil LN, AlOhaly RY. Prevalence of IgG-mediated food intolerance among patients with allergic symptoms. Ann Saudi Med. 2016;36(6):386-90. doi: 10.5144/0256-4947.2016.386. PubMed PMID: 27920409; PMCID: PMC6074204.

5. Adverse Reactions to Foods Committee. AAAAI support of the EAACI Position Paper on IgG4. American Academy of Allergy Asthma & Immunology; 2010.

6. Jian L, Anqi H, Gang L, Litian W, Yanyan X, Mengdi W, Tong L. Food Exclusion Based on IgG Antibodies Alleviates Symptoms in Ulcerative Colitis: A Prospective Study. Inflamm Bowel Dis. 2018;24(9):1918-25. doi: 10.1093/ibd/izy110. PubMed PMID: 29788288.

7. Bentz S, Hausmann M, Piberger H, Kellermeier S, Paul S, Held L, Falk W, Obermeier F, Fried M, Scholmerich J, Rogler G. Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention

study. Digestion. 2010;81(4):252-64. Epub 2010/02/05. doi: 10.1159/000264649. PubMed PMID: 20130407.

8. Alpay K, Ertas M, Orhan EK, Ustay DK, Lieners C, Baykan B. Diet restriction in migraine, based on IgG against foods: a clinical double-blind, randomised, cross-over trial. Cephalalgia. 2010;30(7):829-37. Epub 2010/03/10. doi: 10.1177/0333102410361404. PubMed PMID: 20647174; PMCID: PMC2899772.

9. Aydinlar EI, Dikmen PY, Tiftikci A, Saruc M, Aksu M, Gunsoy HG, Tozun N. IgG-based elimination diet in migraine plus irritable bowel syndrome. Headache. 2013;53(3):514-25. Epub 2012/12/06. doi: 10.1111/j.1526-4610.2012.02296.x. PubMed PMID: 23216231.

10. Zuo XL, Li YQ, Li WJ, Guo YT, Lu XF, Li JM, Desmond PV. Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia. Clin Exp Allergy. 2007;37(6):823-30. doi: 10.1111/j.1365-2222.2007.02727.x. PubMed PMID: 17517095.

11. Cai C, Shen J, Zhao D, Qiao Y, Xu A, Jin S, Ran Z, Zheng Q. Serological investigation of food specific immunoglobulin G antibodies in patients with inflammatory bowel diseases. PLoS One. 2014;9(11):e112154. Epub 2014/11/14. doi: 10.1371/journal.pone.0112154. PubMed PMID: 25393003; PMCID: PMC4230978.

12. Wilders-Truschnig M, Mangge H, Lieners C, Gruber H, Mayer C, März W. IgG antibodies against food antigens are correlated with inflammation and intima media thickness in obese juveniles. Exp Clin Endocrinol Diabetes. 2008;116(4):241-5. Epub 2007/12/10. doi: 10.1055/s-2007-993165. PubMed PMID: 18072008.

13. Hvatum M, Kanerud L, Hällgren R, Brandtzaeg P. The gut-joint axis: cross reactive food antibodies in rheumatoid arthritis. Gut. 2006;55(9):1240-7. Epub 2006/02/16. doi: 10.1136/gut.2005.076901. PubMed PMID: 16484508; PMCID: PMC1860040.

14. Karakula-Juchnowicz H, Galecka M, Rog J, Bartnicka A, Lukaszewicz Z, Krukow P, Morylowska-Topolska J, Skonieczna-Zydecka K, Krajka T, Jonak K, Juchnowicz D. The Food-Specific Serum IgG Reactivity in Major Depressive Disorder Patients, Irritable Bowel Syndrome Patients and Healthy Controls. Nutrients. 2018;10(5). Epub 2018/05/02. doi: 10.3390/nu10050548. PubMed PMID: 29710769; PMCID: PMC5986428.

15. United Ostomy Associations of America I. Our mission 2020. Available from: https://www.ostomy.org/our-mission-history/.

16. Burgess-Stocks J. Eating with an ostomy; A comprehensive nutrition guide for those living with an ostomy. United Ostomy Associations of America; 2020.

17. Carmel J, Colwell J, Goldberg MT, Wound Ostomy and Continence Nurses Society. Wound, Ostomy and Continence Nurses Society core curriculum. Ostomy management. Philadelphia: Wolters Kluwer; 2016. p. p.

18. FoodData Central: United States Department of Agriculture; 2021 [updated April 2021; cited 2020]. Available from: https://fdc.nal.usda.gov/.

19. Agarwal S, Cunningham-Rundles C. Assessment and clinical interpretation of reduced IgG values. Ann Allergy Asthma Immunol. 2007;99(3):281-3. Epub 2007/10/04. doi: 10.1016/S1081-1206(10)60665-5. PubMed PMID: 17910333; PMCID: PMC3099256.

20. Medicine UoM. Immunoglobulins 2020 [updated September 23, 2020; cited 2021]. Available from: https://www.uofmhealth.org/health-library/hw41342#hw41354.

21. IMMG - Clinical: Immunoglobulins (IgG, IgA, and IgM), Serum 2021. Available from: https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/8156.

22. Selective IgA Deficiency - Immunology; Allergic Disorders - Merck Manuals Professional Edition: @MerckManualPro; 2021. Available from: https://www.merckmanuals.com/professional/immunology-allergic-disorders/immunodeficiency-disorders/selective-iga-deficiency.

23. Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. Am J Gastroenterol. 2005;100(7):1550-7. doi: 10.1111/j.1572-0241.2005.41348.x. PubMed PMID: 15984980.

24. Wang HY, Li Y, Li JJ, Jiao CH, Zhao XJ, Li XT, Lu MJ, Mao XQ, Zhang HJ. Serological investigation of IgG and IgE antibodies against food antigens in patients with inflammatory bowel disease. World J Clin Cases. 2019;7(16):2189-203. Epub 2019/09/19. doi: 10.12998/wjcc.v7.i16.2189. PubMed PMID: 31531314; PMCID: PMC6718778.

25. Clayton F, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, Lowichik A, Chen X, Emerson L, Cox K, O'Gorman MA, Peterson KA. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. Gastroenterology. 2014;147(3):602-9. Epub 2014/06/04. doi: 10.1053/j.gastro.2014.05.036. PubMed PMID: 24907494.

26. Schuyler AJ, Wilson JM, Tripathi A, Commins SP, Ogbogu PU, Kruzsewski PG, Barnes BH, McGowan EC, Workman LJ, Lidholm J, Rifas-Shiman SL, Oken E, Gold

DR, Platts-Mills TAE, Erwin EA. Specific IgG4 antibodies to cow's milk proteins in pediatric patients with eosinophilic esophagitis. J Allergy Clin Immunol. 2018;142(1):139-48 e12. Epub 2018/04/22. doi: 10.1016/j.jaci.2018.02.049. PubMed PMID: 29678750; PMCID: PMC6245555.

27. Tordesillas L, Berin MC. Mechanisms of Oral Tolerance. Clin Rev Allergy Immunol. 2018;55(2):107-17. doi: 10.1007/s12016-018-8680-5. PubMed PMID: 29488131; PMCID: PMC6110983.

28. Sundqvist T, Magnusson KE, Sjodahl R, Stjernstrom I, Tagesson C. Passage of molecules through the wall of the gastrointestinal tract. II. Application of low-molecular weight polyethyleneglycol and a deterministic mathematical model for determining intestinal permeability in man. Gut. 1980;21(3):208-14. Epub 1980/03/01. doi: 10.1136/gut.21.3.208. PubMed PMID: 7399321; PMCID: PMC1420346.

29. Zhou Q, Zhang B, Verne GN. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain. 2009;146(1-2):41-6. doi: 10.1016/j.pain.2009.06.017. PubMed PMID: 19595511; PMCID: PMC2763174.

30. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med. 1986;105(6):883-5. Epub 1986/12/01. doi: 10.7326/0003-4819-105-6-883. PubMed PMID: 3777713.