Evaluation of Select Food Additive Exposures in Children with Crohn's Disease

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Abstract

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Epidemiology

Crohn's Disease (CD) is a chronic, debilitating inflammatory bowel disease and recent research has revealed the possibility that environmental exposures such as diet may play a role in CD etiology and disease activity. Several food additives (e.g. carboxymethylcellulose, carrageenan, maltodextrin) have been shown to cause intestinal inflammation, mucosal barrier alterations, and microbiome dysbiosis in animal models, therefore it has been hypothesized that these additives may be associated with disease activity in children with CD. This project evaluated food additive exposure in children with CD in an effort to understand additive intake among this population and to establish a methodology for future studies. A cohort of 135 children, ages 8-21, from Philadelphia Children's Hospital enrolled in a trial evaluating bone health completed 24-hour dietary recalls at baseline, 6, 12, and 24 months. All recorded foods from the baseline visit were compiled into a database, organized into 31 distinct food groupings, and 4,965 unique foods were examined for the presence of soy lecithin, carboxymethylcellulose, xanthan gum, maltodextrin, titanium dioxide, carrageenan, aluminosilicates, and polysorbate-80 by evaluating ingredient labels. The additives with the highest

number of mean exposures per day were soy lecithin (2.71 ± 1.34), xanthan gum (0.96 ± 0.72), maltodextrin (0.95 ± 0.77), and carrageenan (0.58 ± 0.63). The foods with the fewest mean exposures per day included titanium dioxide (0.09 ± 0.21), polysorbate-80 (0.07 ± 0.16) and carboxymethylcellulose (0.05 ± 0.13), while no exposures to aluminosilicates were found. Of the eight additives of interest, participants were exposed to an average of 2.7 different additives per recall day and experienced an average of 5.4 total additive exposures per recall day. In evaluating the 24-hour dietary recall results, grouping foods, and determining food additive content, unique recommendations were created for future studies assessing food additive exposure and the relationship to CD disease activity, including more precise 24-hour recall intake and utilizing available ingredient databases.

Background

Crohn's Disease (CD) is a debilitating, chronic autoimmune disease that adversely affects quality of life of and is associated with increased morbidity.[1] A 2008 study estimated the financial burden of medical care for patients with CD in the United States at $3.6 billion per year.[1] Costly medications and complex surgeries comprise the majority of current treatments, however possible dietary triggers of CD flare-ups are gaining interest and one possible trigger may be food additives. Removing specific foods or additives from an individual's diet may be a cheaper, more effective form of treatment with fewer side effects or may be effective adjunctive therapy.

Research in animal and ex-vivo models has revealed that some common food additives, such as carrageenan and maltodextrin, adversely affect the gastrointestinal (GI) tract by promoting inflammation, altering the gut microbiome, and/or disrupting the mucosal barrier. It has therefore been hypothesized that certain food additives may cause disease activity in individuals with CD. Focusing on eight specific food additives (Table 1), this paper aims to clarify which of those additives were commonly consumed among a cohort of pediatric CD patients in an effort to direct future studies in this area.

Table 1: Food additives of interest

Emulsifiers
• Polysorbate-80
• Carboxymethylcellulose
• Xanthan gum
• Soy lecithin
Microparticles
• Titanium dioxide
• Aluminosilicates (i.e. sodium/calcium aluminosilicate)
Thickeners
• Carrageenan
• Maltodextrin (used as both an additive and an ingredient)

Crohn's Disease

CD is a chronic autoimmune disease causing inflammation in the GI tract and is marked by periods of inflammation and remission. During a flare-up, symptoms range in severity and can include diarrhea, abdominal pain, bloating, weight loss, fatigue, nausea, loss of appetite, fever, anemia, joint pain, eye irritation and dermatological problems.[2] Although the exact etiology of CD is unknown, it is generally believed that genetics, environmental factors, and a dysregulated immune response all play a role (i.e., in a genetically susceptible individual an environmental trigger promotes uncontrolled inflammation in response to particular bacteria).[3] Although the specific environmental factors are still unknown, the rapid rise in CD prevalence has led researchers to study disruptions in host-microbiome symbiosis and it has been shown that patients with inflammatory bowel disease (IBD) experience changes to their microbiome, both in composition and organization, as well as a diminished mucosal barrier.[3] Common therapies for CD include medications (aminosalicylates, corticosteroids, immunomodulators, biologics), bowel rest, and surgery to remove damaged portions of the intestine.[2]

Alternative therapies

The majority of patients with CD receive immunosuppressive medications, however recently diet-based therapies for the treatment of CD have garnered interest from patients, clinicians and researchers.[4] Although the mechanisms of dietary-associated gastrointestinal inflammation are still being studied, some studies suggest that the inflammatory response may be related to the alteration of the intestinal microbiota, the triggering of immune responses due to exposures to dietary antigens, and modifications of inflammatory cytokine profiles.[5] Intensive nutrition-based therapies such as exclusive enteral nutrition (EEN) and the Specific Carbohydrate Diet (SCD) have been shown to induce clinical remission and mucosal healing in children with CD.[6] A 2000 and a 2007 meta-

analysis independently concluded that EEN was as effective as corticosteroids in inducing remission in children with CD with the added benefit of a decrease in side effects.[7,8] The SCD was developed in the 1920's to treat celiac disease and was expanded as a therapy for other GI disorders in the 1980's.[5] The diet restricts complex carbohydrates such as wheat, rice, potatoes, and corn, under the premise that the undigested remains of these carbohydrates ferment in the colon leading to inflammatory byproducts and bacterial overgrowth.[5] Processed meats, dairy products with lactose, processed foods, and added sugars are also eliminated from the diet while unprocessed meats, lactose-free cheeses, fruits, vegetables, nuts and honey are encouraged.[5] Early results from studies on the SCD diet are promising; pediatric studies have shown patients may be able to control their CD flare-ups with a strict SCD diet, maintaining proper growth and development.[5] Although the bulk of research in dietary therapies has focused mainly on EEN, table-food diets such as the SCD have shown promising results, however further research is needed in this area.[9-12]

The gut microbiome

The gut "microbiome" refers to diverse and numerous microbes that are a part of the normal community that inhabits the GI tract and aid in metabolism regulation, colonization resistance, immunity and the synthesis of vitamins.[13-15] Inflammation can occur if the balance of these microbes within the host is altered due to influences of the environment, genetics, diet, or antibiotics.[15] This inflammation may be associated with inflammatory and autoimmune diseases such as allergies, intestinal infections, obesity, metabolic syndrome and IBD, which includes both CD and ulcerative colitis.[13,15]

Mouse models have revealed that germ-free mice possess underdeveloped immune systems, likely related to the lack of a microbiome.[15] The importance and contributions of the intestinal microbial ecosystem are becoming apparent as emerging studies are revealing how an alteration in composition may be linked to disease pathogenesis.[16] Individuals with diseases such as obesity, metabolic

syndrome, atherosclerosis, diabetes and IBD have been shown to have altered intestinal microbiome function and/or structure.[16] Microbiome samples taken from CD patients have shown an increase in total numbers of bacteria, a reduction in bacterial species diversity, and alterations in microbiome organization.[16] In one study that used metagenomics to investigate intestinal bacterial diversity, participants with CD had 30 fewer ribotypes of the bacteria phylum Firmicutes compared to healthy controls.[17]

Intestinal mucosal barrier

The mucosal barrier is a multi-layered structure covering the surface of the intestinal tract that includes a mucus layer and an epithelial cell layer and works to protect against potential threats such as mechanical stress, bile acids, hydrochloric acid, proteolytic enzymes, and bacteria.[18] Made up of proteins called mucins, the thin mucus layer functions to trap and move luminal content through the GI tract and acts as an antimicrobial matrix that protects the epithelial layer from bacteria.[18] As a first-line defense against these potential threats, defects in the mucus layer may lead to bacterial penetration (bacteria reaching and moving through epithelial cell junctions) stimulating an immune system response and leading to intestinal inflammation.[18] The epithelial cell layer does not allow hydrophilic solutes to pass through (in the absence of transporters) and damage to this membrane by mucosal irritants or cytotoxic products results in a loss of barrier function.[19] The tight junction, which seals the paracellular space between the epithelial cells, is made up of proteins called claudin and occludin and is regulated by cytokines.[19] It has been shown that individuals with CD have altered claudin expression, which may affect barrier function.[19] Some evidence suggests that intestinal barrier dysfunction, such as a defective mucosal layer or damage to the epithelial membrane, may play a role in the pathogenesis of CD due to increased intestinal permeability, and may contribute to disease progression.[19] Mucosal damage, loss or dysfunction alone is insufficient to cause chronic disease in healthy individuals, however in individuals who are genetically susceptible,

such as those with CD, robust immunoregulatory mechanisms are induced and an inappropriate inflammatory response occurs.[19]

Food additives

Food additives are legally described as "any substance the intended use of which results or may reasonably be expected to result—directly or indirectly—in its becoming a component or otherwise affecting the characteristics of any food" and have concentration limits or are generally recognized as safe (GRAS) by the Food and Drug Administration.[20] It has been hypothesized that certain food additives play a role in intestinal inflammation, possibly leading to flare-ups in individuals with CD, and numerous dietary therapies for CD have stipulated the avoidance of processed foods and food additives.[4,21] Commonly used food additives such as emulsifiers, microparticles, thickeners and stabilizers are cleared for usage by the FDA in certain amounts, however it is unclear what physiological effects may occur when these additives are consumed in high quantities or multiple times per day and when they comingle in the gut. In our retrospective study we evaluate exposure to emulsifiers (used for stabilization and texture), microparticles (used for visual appeal and texture), and thickeners (used for texture and viscosity). The additives that we focused on, which may belong to more than one grouping, are listed in Table 1.

Emulsifiers are generally used to preserve the texture of foods, prevent the separation of ingredients and maintain shelf stability. Detergent-like in molecular structure, it is thought that emulsifiers may disrupt the intestinal mucous layer and alter the host-microbiota relationship, allowing for bacteria to reach the epithelial layer and promote inflammation.[13] In one study, mice exposed to relatively low concentrations of carboxymethylcellulose or polysorbate-80 had increased rates of low-grade intestinal inflammation, obesity, metabolic syndrome and colitis.[13] The mice with emulsifier-induced metabolic syndrome showed altered microbiota species composition and microbiota

encroachment leading to low-grade inflammation and, subsequently, metabolic syndrome.[13] It has yet to be determined how emulsifier-induced microbiota alterations and subsequent low-grade inflammation may affect individuals with sensitive or compromised GI systems, such as those with CD. One major area of concern surrounding xanthan gum is the association with necrotizing enterocolitis (NEC) in premature infants who were fed milk or formula thickened with a xanthan gum product.[22] In a rat model, xanthan gum was found to increase sugars by 150% and intraluminal water by 400% in the intestinal tract, which may cause injury to a premature infant's immature gut.[22,23] In rat and human models, it has been shown that an increase of luminal sugars leads to an increase in fecal bacterial fermentation of xanthan gum which increases the production of H_2 and short-chain fatty acids (SCFA) in the colon.[22] It is theorized these excess SCFAs lead to colonic mucosal injury and may play a role in the pathogenesis of NEC.[22] Additionally, in rat models fecal bile acid excretion is increased in the presence of xanthan gum; an accumulation of cytotoxic bile acids in the ileal lumen promotes the development of NEC.[22] With underdeveloped, highly immunoreactive intestinal mucosa, premature infants are at risk of inflammation and intestinal injury in response to intestinal stimuli that xanthan gum may cause such as the increases in water, sugars, SCFA, bile acid accumulation and macrophage/lymphocyte activation.[22]

It has been hypothesized that hypersensitivity reactions in the gut may be caused by a GI allergy to particular foods—the triggering of immune responses due to exposures of dietary antigens—however this is difficult to diagnose due to the similarity of the symptoms to GI disorders such as CD. [24] It has been shown that individuals with CD have elevated histamine content and secretion in the mucosa of the GI tract, and it is possible there is an association between GI allergy and CD.[24,25] Soy is a common allergen, and soy products such as the food additive soy lecithin may play a role in these hypersensitivity reactions, however the relationship between food-allergy-related-enteropathy and IBD must be studied further.[24,25] Conversely, soy lecithin is commonly found in formulas used for

EEN, with a single study suggesting a beneficial effect of lecithin supplementation in the setting of ulcerative colitis.[26]

Microparticles are submicron-sized particles resistant to degradation and have been shown to increase inflammation in the GI tract; it has been hypothesized that CD flare-ups may be triggered by these food additives.[27] Originally assumed to be inert material of no physiological significance, microparticles have been shown to cause inflammation and fibrosis in tissues such as the lungs.[28] Titanium dioxide (100-200nm) and aluminosilicates (<100-400nm) are bacteria-sized microparticles added to foods for their brightening/whitening and anti-caking effects, respectively. In ex-vivo models, however, these additives have been identified in human gut associated lymphoid (GALT) tissue which, in susceptible individuals, may cause chronic inflammation.[28] Although diet-derived microparticles are generally found in the Peyer's patches (lymphoid tissue) of the small intestine, resection specimens from patients with CD revealed microparticles in the dilated lymphatics of the intestinal mucosa, mesenteric lymph nodes, and in some transmural inflammatory aggregates—areas where the first signs of CD manifest.[29] [28] The results of one ex vivo study revealed that microparticles were associated with impaired macrophage phagocytic activity and may act as adjuvant immune response modulators when exposed to bacterial antigens, aggravating the chronic inflammatory response in those with CD .[29] The results of a second ex vivo study showed titanium dioxide particles acting as pro-inflammatory modulators, concluding that in vivo research must be done to determine if microparticles may impact the immune system.[29] In examining titanium dioxide effects on microbiota isolates, results indicated titanium dioxide may affect SCFA production, hydrophobicity, extracellular matrix sugar content and electrophoretic mobility.[15] Alternatively, a multi-center clinical trial in Europe evaluated adults with active CD placed on a steroid taper and randomized to a low vs normal microparticle diet and found that no differences were observed in the

primary outcome (clinical disease activity) at 16 weeks.[30] Although the authors concluded that a low microparticle diet does not offer benefit in addition to corticosteroid treatment in individuals with active CD, the use of steroids may have eclipsed the effect of the low microparticle dietary intervention.[30]

Thickeners such as carrageenan and maltodextrin are thought to adversely affect the intestinal epithelial border and/or the mucus layer. Carrageenan, an extract of red seaweed, is most often used in foods as a thickener, stabilizer, or emulsifier and is commonly found in infant formula, soymilk, dairy products and processed meats. The estimated daily average consumption in the U.S. is 250 mg.[31] Although the FDA acknowledges evidence revealing the possibility of carrageenan having detrimental effects on the GI epithelium at high doses, it has concluded that no evidence exists to demonstrate a hazard to the public when carrageenan is used at current levels.[32] Carrageenan has been used in thousands of cell and animal studies to induce inflammation in an effort to study anti-inflammatory medications.[31] Further, it has been used in numerous mammalian studies to induce inflammation, ulcerations, polyps, colitis and colorectal tumors.[31] In human colonic epithelial cells, carrageenan has been shown to induce the activation of NFkB and interleukin 8 (inflammatory cytokines), thus inducing inflammation.[33] A 2001, 45-study review of the association between carrageenan exposure and deleterious effects in animal models found that there was sufficient evidence that carrageenan may be a factor in colorectal malignancy and IBD.[34] The additive/ingredient maltodextrin, a polysaccharide polymer derived from starch hydrolysis, is generally recognized as safe by the FDA and has been commonly used as a filler, coating agent, texturizer and thickener since the mid-1950's.[35] It is so common, in fact, that a survey found 60% of all packaged food items in a grocery store contained maltodextrin or a similar product, and a food frequency questionnaire-based study indicated that 98.6% of participants consume an average of 2.6 maltodextrin-containing foods per day.[3] CD is associated with thick biofilms formed on the

epithelium—evidence of microbiome dysbiosis—and findings from a 2012 ex-vivo study demonstrated maltodextrin enhances the adhesion of adherent-invasive *E. coli* strain LF82 (a disease-associated strain of *E. coli*), resulting in biofilm formation.[16] This study concluded that maltodextrin metabolism may promote colonization of *E.coli* strains in new areas of the intestine (i.e. the ileum rather than the colon). [16] Maltodextrin has also been shown to impair the anti-bacterial response of cells and suppress the anti-microbial defense mechanisms present in the intestine.[3] In one in vivo mouse study, mice supplemented with maltodextrin were found to have commensal bacteria uncharacteristically within the mucosal barrier and in contact with the epithelium.[3] It is thought that individuals with CD may be more sensitive to maltodextrin and the epithelial damage it has been shown to cause. In those individuals, exposure to maltodextrin may result in a limited ability to effectively react to bacteria passing through the intestinal protective barrier.[3]

The cause of the striking increase in CD incidence in the U.S. is still unknown, however there is strong evidence that the "western diet" may play a role. With increasing consumption rates of prepackaged foods, food additive exposures also rise. Studies of several food additives, including soy lecithin, maltodextrin, carrageenan, polysorbate-80, carboxymethylcellulose, aluminosilicates xanthan gum and titanium dioxide have revealed negative physiological effects, including alterations to the intestinal microbiota,[16] alterations to the mucosal barrier, and the promotion of inflammation. With that information in mind, this study aimed to evaluate food additive exposure in children with CD.

Methods

Study participants

Participants in a randomized controlled trial (RCT) of low magnitude mechanical stimuli (LMMS) on bone density and structure in pediatric CD were recruited from 2007-2010 from the Children's Hospital of Philadelphia, PA (CHOP), from gastroenterology clinics in the greater Delaware Valley, during the annual CHOP Inflammatory Bowel Disease Center Family Day, and through advertisements on the Crohn's and Colitis Foundation website.[36] Children 8-21 years were eligible for study entry 6 months after a diagnosis of CD and if bone density Z-scores were below the 25th percentile for age, sex and race compared with healthy controls. Exclusion criteria included pregnancy, weight >250 lbs., medical illness unrelated to CD that could influence bone or body composition, extended travel during the study period, a sibling/acquaintance enrolled in the trial, and developmental disorders that could affect completion of the study procedures. The resulting 135 participants were followed over the course of 24 months with assessments at baseline, 6, 12, and 24 months. This RCT did not show an effect of LMMS on bone mineral density or body composition outcomes.[36] Data collected from this bone density study included detailed dietary intake information, which was evaluated in our study.

Dietary assessment

Three 24-hour dietary recalls were obtained at each of four points in the study (0, 6, 12, and 24 months), with two recalls on weekdays and one recall on a weekend day. For our study, data from the baseline visit (0 month) visit were utilized. The 24-hour recalls were obtained via phone call using a validated multi-pass method with prompting from a trained bionutritionist. Participants were given reference pamphlets to help determine portion size, and both children and their parents responded to the recall questions. The dietary recall data were processed using the University of

Minnesota Nutrition Data Systems for Research (NDSR) which allows for evaluation of macronutrient and micronutrient composition of foods—but not food additive content.

Evaluation of food additive content across product categories

To begin to characterize which food products contain the eight food additives of interest (Table 1), we conducted a preliminary evaluation of food categories and the likelihood of food additive content at a Safeway grocery store in Seattle, Washington. Safeway was chosen because of its generalizability; it is a national chain belonging to one of the largest food and drug retailers in the U.S. (Albertsons--with over 2,200 stores across 33 states).[37] Broad food categories included: dairy/egg products, cereals (dry), cereals (other), beverages, frozen foods, sweet snacks, savory snacks, baked goods and baking products, sauces/dressings, imitation meats and tofu, prepared foods/soups. Multiple items in each category were examined with ingredient labels evaluated for our additives of interest. Food manufacturers are mandated to include all ingredients on their labels, however quantities are not required; ingredients are listed in descending order of weight while those that make up less than 2% weight of the product do not need to be listed in descending weight order. Most additives are listed in the ingredients label under the "Contains 2% or less of:" section.

Ingredient Data Collection

We sorted all food items captured from the baseline visit 24-hour recalls in an Excel spreadsheet by alphabetical order and repeated food items were deleted, thus creating a database of all unique foods in our dataset. We then created a spreadsheet of the unique food items and the eight additives of interest to evaluate ingredients and additive content. We were granted permission from the Safeway grocery store manager to obtain ingredient label information over multiple days. For each of the 4,965 Excel spreadsheet food items available in the grocery store, we read the ingredient label information and marked whether any of the eight additives of interest were found in the ingredients

list. For food items not found in the Safeway grocery store, the Walmart.com website was utilized to evaluate ingredients and complete the Excel spreadsheet. In analyzing restaurant foods, we screened restaurant websites for ingredient information and we evaluated home-made and non-specified food items for possible additive-containing components, marking "likely" if that food item likely contained an additive of interest (e.g., we extrapolated that homemade bread pudding would likely contain soy lecithin because most bread products contain soy lecithin). The completed list of gathered additive information was then transferred to the electronic version of the Excel spreadsheet. The electronic database of unique foods with newly collected data on food additive content was merged back into the original 24-hour dietary recall dataset containing all dietary recall data. This protocol was established in collaboration with the Nutrition Assessment Shared Resource (NASR) at the Fred Hutchinson Cancer Research Center.

Food Groupings

In the original Philadelphia RCT, foods from the dietary recalls were grouped based on the NDSR-assigned Nutrition Coordinating Center (NCC) Food Group IDs (Appendix 1). The NCC distributes and supports the NDSR dietary analysis software and maintains a research-quality food and nutrient database. Here, we reduced the food groups from the original 135 NCC Food Group ID subgroups under 16 group headings to 135 food subgroups under 31 more descriptive food group headings (Appendix 2).

Statistical Analysis

Results were analyzed using STATA 12.1 software. Three participants were excluded because they did not complete dietary recalls at baseline. To generate food additive exposure data, results from the baseline dietary recall information were averaged for the three recall days and descriptive statistics were produced for the 132 participants.

Results

Baseline study participant characteristics showed a majority of participants were Caucasian with an average age of 14.1 years and mean ± SD Pediatric Crohn's Disease Activity Index (PCDAI) scores of 11.0 ± 10.0 (Table 2). Males and females participated almost equally with no significant additive exposure difference between the sexes (Table 3).

Table 2: Baseline Participant and Crohn's Disease Characteristics

Variable	**(n=135)**
Age (years)	14.1 ± 2.8
Range	(8.0, 21.7)
Male sex, n(%)	64(47)
Race, % Black	6
Height Z score	-0.76 ± 1.0
Range	(-3.5, 1.4)
BMI Z score	-0.25 ± 1.1
Range	(-3.6, 2.2)
Duration since diagnosis (yr)	3.2 ± 2.7
PCDAI, mean ± SD	11.0 ± 10.0
PCDAI categories	
No active disease (<=10), n (%)	82 (61)
Mild (11-30), n (%)	46 (34)
Moderate to severe (>30), n (%)	7 (5)
Albumin (g/dL), median (range)	4.4 (2.7 to 5.3)
ESR (mm/h), median (range)	15 (0 to 100)
Site of disease, n (%)	
Ileal**	13 (10)
Colononic	22 (16)
Ileocolonic	92 (68)
Isolated upper tract disease	103 (76)
Perianal involvement	8 (6)

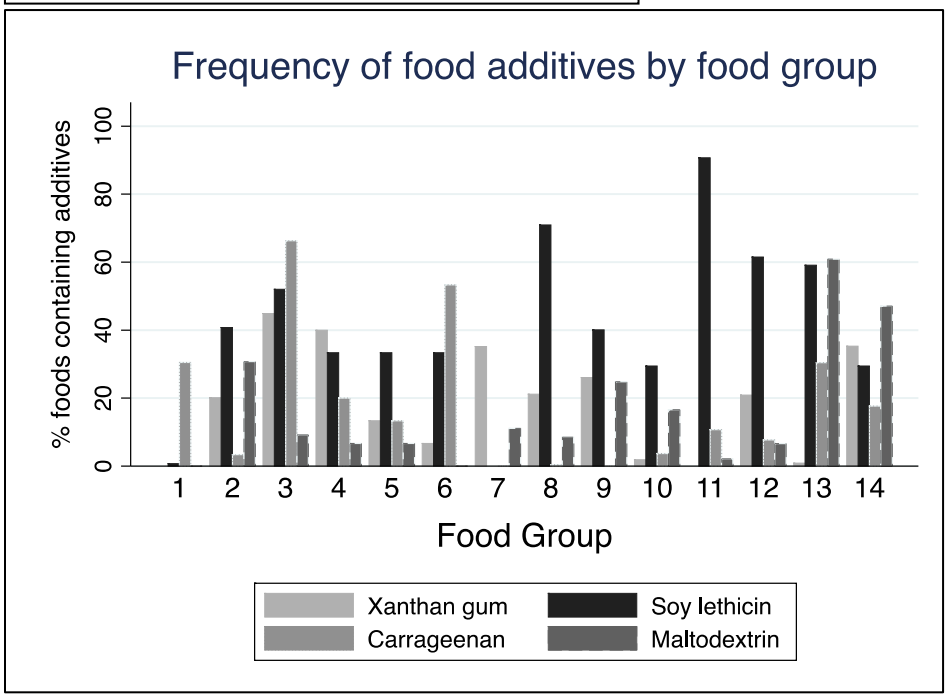
Table 3: Mean Food Additive Exposures by Sex (mean frequency/day)

Additive	Male (n=64)	Female (n=71)	P-value
Polysorbate-80	0.06 ± 0.17	0.08 ± 0.16	0.53
Carboxymethylcellulose	0.05 ± 0.14	0.06 ± 0.13	0.37
Xanthan gum	0.88 ± 0.82	1.04 ± 0.62	0.03
Soy Lecithin	2.76 ± 1.34	2.67 ± 1.35	0.84
Titanium Dioxide	0.08 ± 0.20	0.10 ± 0.23	0.84
Aluminosilicates	--	--	--
Carrageenan	0.52 ± 0.58	0.64 ± 0.68	0.24
Maltodextrin	1.01 ± 0.87	0.91 ± 0.67	0.69

The preliminary assessment of food categories for food additive content revealed that with the exception of most dry and hot cereal and most soda and juice, the majority of packaged items in the remaining food groups contained one or more additive of interest.

In evaluating across food groups (Figure 1) the frozen desserts group included a large number of items containing carrageenan (66%), soy lecithin (52%), and xanthan gum (45%). Of the meal replacement beverages, 61% contained maltodextrin 59% contained soy lecithin, and 30% contained carrageenan. 91% of cookies and 72% of grain and bread items contained soy lecithin. Commercial entrees also frequently contained additives with 47% containing maltodextrin, 35% containing xanthan gum, and 29% containing soy lecithin (see Appendix 3 for more detailed information).

Figure 1: Frequency of Food Additives by Food Group



KEY:
1. Processed meat
2. Meat/poultry/fish
3. Frozen desserts
4. Cocoa/milk beverages
5. Milk/cheese
6. Alternative dairy products
7. Salad dressings
8. Grain and bread items
9. Pasta/rice/grain recipes
10. Savory snacks
11. Cookies
12. Non-cookie desserts
13. Meal replacement beverages
14. Commercial entrees

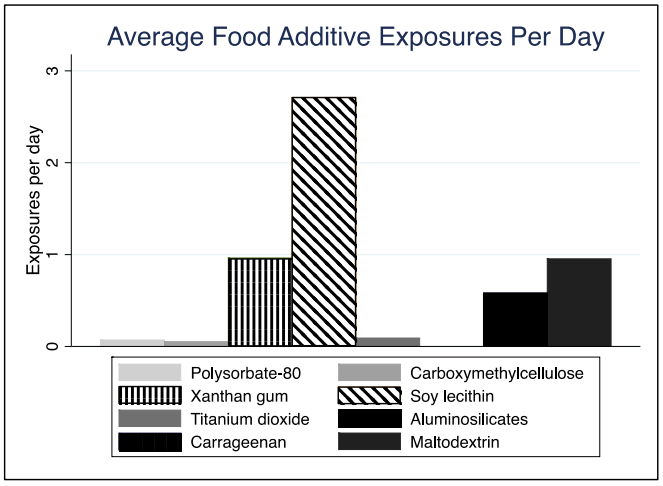
Out of a total of 4,965 food items 1,098 (22.1%) contained soy lecithin, 386 (0.08%) contained maltodextrin, 235 (0.05%) contained carrageenan, 37 (0.01%) contained titanium dioxide, 29 (0.01%) contained carboxymethylcellulose and 28 (0.01%) contained polysorbate-80.

Additive exposure frequency and variety

In evaluating the eight additives of interest, participants were exposed to an average of 2.7 different additives per recall day and experienced an average of 5.4 total additive exposures per recall day. In Examining individual additives, Figure 2 illustrates how many exposures of each additive were observed on average.

The additives with the highest mean exposures per day were soy lecithin (2.71 ± 1.34), xanthan gum (0.96 ± 0.72), maltodextrin (0.95 ± 0.77), and carrageenan (0.58 ± 0.63). The foods with the fewest mean exposures per day included titanium dioxide (0.09 ± 0.21), polysorbate-80 (0.07 ± 0.16) and carboxymethylcellulose (0.05 ± 0.13), while no exposures to aluminosilicates were found.

Figure 2: Average Food Additive Exposures Per Day



Discussion

Whole foods such as fresh fruits and vegetables contain no food additives, however the majority of pre-packaged foods contain one or more additives for shelf stability, color, texture, flavor, or emulsification. Although there are nearly 4,000 FDA-approved food additives,[20] several additives have been reported to increase inflammation, affect the GI mucosal layer, and/or alter the intestinal microbiome. The aim of this study was to evaluate which of the 8 food additives of interest were found in foods commonly eaten by pediatric CD patients. We found that participants in this study were exposed to multiple additives each recall day and experienced an average of more than five total additive exposures per recall day.

Of the eight additives of interest, soy lecithin was the most prevalent, commonly found in many packaged products across multiple food groups. Generally when a product involves bread or baked goods (bread/breading, crust, baked desserts, etc), soy lecithin is found in the ingredient list. Conversely, aluminosilicates were not noted in any foods evaluated in this study, however can be found in powdered or granulated foods such as powdered hot cocoa and powdered coffee creamer.

Interestingly, similar additives to our additives of interest were found in some food items (polysorbate-60 rather than polysorbate-80 and sunflower lecithin rather than soy lecithin) and future studies will need to determine whether these similar additives have biologic effects analogous to those previously studied and whether they should be included in future studies.

With the development of crowdsourced websites such as Fooducate.com and with websites that provide food product content such as Walmart.com, food ingredient information is increasingly available and easy to access. Tools such as phone and computer apps make tracking food intake and CD symptoms fast and convenient. These resources may play a role in future research and may be helpful in patient education if certain foods or additives must be avoided to prevent disease activity in individuals with CD.

Strengths and Limitations

The strengths of this study include the uniqueness of the focus (food additives and CD have not been previously studied in humans), the use of a 24 hour dietary recall (rather than the less-accurate food frequency questionnaire), and the ability to characterize additive content given ingredient label information and apply this information to 24-hour recall data from a population of individuals with CD. Study limitations include a lack of information on the exact quantity of additives in each product (not required on labeling), potential for subject recall bias (with regards to 24-hour recalls), the need for extrapolating food additive content based on the dietary recall description, and the imprecise nature of food groupings. When grouping similar foods, there are often varying factors that complicate the process such as foods that do not fit into any specific category, foods that could fit into multiple categories, or a lack of clarity on the exact recipe of homemade foods. For these

reasons, this study expanded the food group headings for more precise results, however some grouping issues remained.

Future studies

Creation of a detailed database and/or the utilization of crowd-sourced database of all commonly consumed foods and their ingredients may be a resource for further study of the role of food additives in disease. Through this project, we determined what information is needed for more precise data collection. These revisions to recall questions and/or methods will enable future studies to delineate more detailed data by capturing more accurate food intake, thus increasing accuracy in exposure rates. The following 24-hour recall intake modifications and resource information may prove useful:

1. Determine whether a product is homemade, from a restaurant or store-bought
2. If a food item is homemade, determine whether any pre-packaged/canned foods were used in the recipe
3. For each described pre-packaged/canned food item, determine what brand was used
4. If a food item is from a restaurant, determine which restaurant and include any substitutions, condiments or alterations to that food item
5. Determine quantity/servings of each food item recorded
6. Fooducate.com is a website including a crowdsourced database of pre-packaged food ingredient information.
7. Walmart.com can be utilized to evaluate ingredients for many pre-packaged foods.

Another recommendation for future studies includes the use of a daily food and symptom journal (participants would need to be trained in keeping an accurate journal) that includes foods eaten each day alongside CD symptoms, or they could download CD symptom-tracking phone or computer apps

such as “GI Buddy,” “My IBD,” or “GI Monitor,” to log meals, GI symptoms, pain level, and other patterns. This more detailed and descriptive information may allow researchers to find correlations between particular additives and CD flare-ups. Although a daily journal or app may be able to capture large quantities of data in real time, this modality of data capture has limitations as well, including the potential for reporting bias.

Conclusion

Environmental exposures play an integral role in CD pathogenesis and studying diet is important but difficult due to the complexity of eating behaviors and food composition. In this study, seven out of our eight food additives of interest were identified in many foods commonly eaten by a pediatric CD population. Although this intake is likely well within the FDA’s established safety guidelines, it remains unclear what additive quantity is consumed, what the interaction of additives may result in, and what effect these additives may have on individuals with compromised immune systems or sensitive GI tracts. The new information presented in this study allows a low-food-additive diet to be further assessed in CD therapy. In examining the food database derived from study participants’ 24 hour recalls, new methodologies have also been generated to capture additive consumption frequency more thoroughly. Future studies will elucidate whether food additives play a role in CD flare-ups.

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Appendix 1

NCC Food Group File

NCC Food Group ID	Name	NCC Food Group Code
	Meat, fish, and poultry	
2	Beef	010400
3	Lamb	010800
4	Pork	011200
5	Veal	011600
6	Game - meat	012000
7	Game - fowl	012001
8	Cold cuts and sausage - beef	012400
9	Cold cuts and sausage - lamb	012401
10	Cold cuts and sausage - pork	012402
11	Cold cuts and sausage - game	012403
12	Cold cuts and sausage - poultry	012404
13	Cold cuts and sausage - combinations	012405
14	Poultry	012800
15	Organ meats - beef	013200
16	Organ meats - lamb	013201
17	Organ meats - pork	013202
18	Organ meats - game	013203
19	Organ meats - poultry	013204
20	Fish and fish roe	013600
21	Shellfish	014000
22	Meat, poultry, and fish recipes	014400
	Milk, cream, cheese, and related products	
24	Milk	050400
25	Cream	050800
26	Cheese	051200
27	Ice cream, ice milk, sherbet, nondairy frozen dessert, and milkshakes	051600
28	Frozen treats	051700
29	Yogurt	051800
30	Yogurt - frozen	051801
31	Cocoa and milk type beverages	052000
32	Baby formula	052001
33	Milk and cheese recipes	052400

NCC Food Group ID	Name	NCC Food Group Code
	Imitation milk, cream, and related products	
35	Polyunsaturated vegetable fat - filled milk	100400
36	Polyunsaturated vegetable fat - ice cream	100401
37	Polyunsaturated vegetable fat - creamers	100402
38	Polyunsaturated vegetable fat - whipped toppings	100403
39	Polyunsaturated vegetable fat - cheese	100404
40	Saturated vegetable fat - filled milk	100800
41	Saturated vegetable fat - ice cream	100801
42	Saturated vegetable fat - creamers	100802
43	Saturated vegetable fat - whipped toppings	100803
44	Saturated vegetable fat - cheese	100804
45	Saturated vegetable fat - cream	100805
	Eggs and related products	
47	Eggs	150400
48	Imitation eggs	150800
49	Egg recipes	151200
	Fats, oils, and nuts	
51	Animal fat	200400
52	Stick, soft or squeeze margarines	200800
53	Diet margarines	200900
54	Whipped margarines	201000
55	Table spreads	201100
56	Oil	201200
57	Shortening, household	201600
58	Shortening, commercial	202000
59	Salad dressing	202400
60	Nuts and nut butters	202800
	Fruits and fruit products	
62	Fruit juices and drinks	250800
63	Fruits, fresh and unsweetened	251200
64	Fruits, sweetened	251600
65	Fruits, dried	252000
66	Fruit recipes	252100
	Vegetables and vegetable products	
68	Raw vegetables	300400
69	Cooked vegetables, fresh, frozen or canned	300800
70	Mature dried beans and peas	301200
71	Vegetarian meat substitutes and related products	301600
72	Vegetable recipes	302000

NCC Food Group ID	Name	NCC Food Group Code
	Grain products	
74	Breads, rolls, biscuits, and other related products (i.e., non-sweet breads)	350400
75	Sweet rolls, fruit breads, doughnuts, muffins, and other related products (i.e., sweet breads)	350800
76	French toast, pancakes, and waffles	351000
77	Ready-to-eat cereals	351200
78	Cooked cereals, prepared and unprepared	351201
79	Grains and flour	351202
80	Baby food cereals	351203
81	Pasta and rice (includes recipes)	351600
82	Cheese snack chips	352001
83	Corn chips	352002
84	Popcorn	352003
85	Potato chips	352004
86	Pretzels	352005
87	Tortilla chips	352006
88	Diet, fruit and granola bars	352007
89	Miscellaneous snacks	352008
90	Crackers	352009
91	Miscellaneous grain recipes	352400
	Soups, gravy, and sauces	
93	Soups	400400
94	Gravy and sauces	400800
	Desserts	
96	Cookies	450400
97	Cakes	451200
98	Cakes from mixes	451400
99	Frostings, fillings and toppings for cakes and pies	451600
100	Pie crusts, pastry and meringue shells	452000
101	Pie fillings	452400
102	Pies	452600
103	Puddings	452800
104	Miscellaneous desserts	453200
105	Commercial snack cakes and muffins	453201
106	Commercial snack pies	453202
107	Commercial snack doughnuts	453203
108	Commercial snack sweet rolls and coffee cakes	453204
109	Commercial snack - miscellaneous desserts	453205
110	Baby food deserts	453210

NCC Food Group ID	Name	NCC Food Group Code
	Candy, sugar, and sweets	
112	Chocolate candy	500401
113	Non-chocolate candy	500402
114	Sugar, syrup, preserves and jelly	500800
	Beverages	
116	Nonalcoholic beverages (includes soda & bottled water)	550400
117	Alcoholic beverages	550800
118	Coffee	551200
119	Tea	551201
120	Milk-based meal replacement/supplement beverages	551400
121	Soy-based meal replacement/supplement beverages	551600
122	Other-based meal replacement/supplement beverages	551800
	Miscellaneous	
124	Spices, flavorings, and miscellaneous recipe ingredients	600300
125	Condiments, pickles, and olives	600400
	Supplements and drugs	
127	Vitamin-mineral supplements	700400
128	Drugs	700800
	Commercial entrees and dinners	
130	Commercial entrees and dinners - Single item	800100
131	Commercial entrees and dinners - Two items	800200
132	Commercial entrees and dinners - Three items	800300
133	Commercial entrees and dinners - Four or more items	800400
	Commercial ingredients	
135	Commercial ingredients	900100

Appendix 2

Food Groupings

New Group Number	NCC Food Group ID number(s)	New Group Name
1	2-7, 14-21	Whole meat, fish, and poultry
2	8-13	Processed Meat
3	22	Meat, poultry, fish recipes
4	24, 25 ,26 ,29	Dairy
5	27,28 ,30	Frozen Dessert
6	31	Cocoa and milk-type beverages
7	33	Milk and cheese recipes
8	35-45	Alternative Dairy Products
9	47-49	Eggs and Egg Recipes
10	51-58, 60	Fats, Oils and Nuts
11	59	Salad Dressing
12	62-66	Fruit and Fruit Products
13	68-70	Vegetables and Legumes
14	71-72	Vegetable products and recipes
15	74-76, 79	Grains and Breads
16	77-78	Hot and Cold Cereals
17	81, 91	Pasta, Rice and Grain Recipes
18	82-90	Savory Snacks
19	93-94	Soups, Gravy and Sauces
20	96	Cookies
21	97-110	Non-Cookie Desserts
22	112-114	Candy, Sugar and Sweets

23	116	Nonalcoholic Beverages (includes soda and bottled water)
24	117	Alcoholic Beverages
25	118-119	Coffee and Tea
26	120-122	Meal Replacement Beverages
27	124	Miscellaneous
28	125	Condiments, Pickles and Olives
29	127-128	Supplements and Drugs
30	130-133	Commercial Entrees
31	135	Commercial Ingredients

Appendix 3

Detailed reporting of food groups in which 30% or more of all included food items contain a specific food additive:

Additive	Food group	% of items in food groups that contain additive
Soy lecithin	Cookies	91%
	Grain and bread items	72%
	Non-cookie desserts	62%
	Meal replacement beverages	59%
	Frozen desserts	52%
	Meat/poultry/fish recipes (specivically meat pizza and meat sandwiches, which contain soy lecithin-containing bread products)	41%
	Pasta/rice/grain recipes	40%
	Cocoa/milk beverages	33%
	Milk/cheese recipes	33%
	Alternative dairy products	33%
	Savory snacks	30%
Xanthan gum	Frozen desserts	45%
	Cocoa/milk beverages	40%
	Salad dressings	35%
	Commercial entrees	35%
Maltodextrin	Meal replacement beverages	61%
	Commercial entrees	47%
	Meat/poultry/fish recipes (predominantly meat pizza and meat sandwiches)	31%

Carrageenan	Frozen desserts	66%
	Alternative dairy products	53%
	Processed meat	31%
	Meal replacement beverages	30%
Titanium dioxide	Found in very few items, primarily grains and breads, cookies, non-cookie desserts and candy/sugar/sweets though not in significant numbers.	
Polysorbate-80	Found in meat/poultry/fish recipes, frozen desserts, cookies, and condiments, though not in significant numbers.	
Carboxymethyl cellulose	Found primarily in meat/poultry/fish recipes, frozen desserts, pasta/rice/grain recipes, savory snacks and commercial entrees, though not in significant numbers.	
Aluminosilicates	Found in no evaluated food items	