Diet Quality and Food Additive Exposure in Children with and without Celiac Disease

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2020

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Program Authorized to Offer Degree:
Nutritional Sciences

University of Washington

Abstract

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Epidemiology

Treatment of celiac disease (CeD) requires adherence to a gluten-free (GF) diet, a restriction that affects food choices and impacts dietary patterns. Pre-clinical research suggests that certain food additives may contribute to ongoing mucosal damage, including increased intestinal permeability and inflammation. The purpose of this study was to quantify the frequency of food additive exposure and assess diet quality in children with and without CeD. Twenty-eight children with (n=15) and without (n=13) CeD between the ages of 6-18 years (mean: 11.29 ± 3.2) were recruited as study participants from Seattle Children's Hospital. Three-day food records and anthropometric data were obtained for all participants. The intake frequency of 9 common food additives (polysorbate 80, carboxymethyl cellulose, xanthan gum, guar gum, soy lecithin, carrageenan, maltodextrin, titanium dioxide, and aluminosilicates) and macro- and micronutrient intake relative to individualized DRI/AI values were analyzed. Results show that children in the celiac group had a higher number of exposures to xanthan gum and fewer met recommendations for thiamin, folate, magnesium, manganese, and potassium compared to their non-celiac counterparts. Secondary analyses revealed that both groups had similar intake of processed foods, however gluten-free products (GFPs) contributed 29% of total energy in the GF diet and a higher proportion of GFPs contained food additives compared to other processed foods. In the celiac group, those with higher consumption of GFPs had s higher frequency of exposure to food additives. This research provides insight into the nuances of the GF diet and the contribution of GPFs. Such an understanding may inform dietary recommendations for children with CeD and highlights a need for additional research to explore the health implications of food additive intake in this population.

Introduction

Celiac disease (CeD) is a chronic immune-mediated condition of the small intestine, induced by exposure to dietary gluten in genetically susceptible persons.[1] Gluten – a protein found in wheat, barley, malt, and rye – is comprised of two main fractions, α-gliadin and glutenin.[2] CeD manifests when α-gliadin peptides pass through the lamina propria of the small intestine where they interact with antigen-presenting cells,[1] resulting in the production of anti-tissue transglutaminase (anti-tTG) and endomysial (EM) antibodies.[2]

The main genetic factor that contributes to CeD are the HLA haplotypes HLA-DQ2 and HLA-DQ8, which are present in 95% of all patients with CeD.[2] These genetic markers are thought to be present in approximately 40% of the total population, yet CeD develops in only 2-3% of those in which they are present.[3] Though non-HLA polymorphisms associated with increased risk of CeD have been identified,[4,5] evidence suggests that environmental factors and genetic-environmental interactions also play a key role in disease development.[4,6,7] Environmental factors may include anything that increases the ability of gliadin peptides to reach the lamina propria and thus trigger an immune response,[1] yet the precise combination of factors contributing to CeD are unknown. Hypotheses include age of initial gluten exposure, length of time breastfeeding, and presence of intestinal infection during infancy[1,2] which may result in increased mucosal permeability.

CeD has been associated with structural abnormalities in the intestinal mucosa, including nonspecific mucosal lesions (celiac sprue),[8] cryptal hyperplasia, and villous blunting.[3] Such abnormalities are common in those with active, untreated celiac disease,[7,9] however such abnormalities have also been observed prior to the onset of CeD[7,10] suggesting that mucosal damage is a predisposing factor for disease development.[11] While the root cause of pre-existing mucosal abnormalities is unknown, it has been suggested that alterations in the gastrointestinal (GI) barrier may be associated with industrial food additives.[12] Pre-clinical trials and epidemiological data suggest that food additives may contribute not only to the development of celiac disease (and other auto-immune diseases),[7] but may also contribute to ongoing mucosal damage.

Currently, the only treatment for CeD is elimination of gluten from the diet and lifelong adherence to a gluten-free (GF) diet[1] which relieves symptoms and contributes to mucosal healing in those with CeD. However, some evidence suggests that despite these improvements, strict adherence to a GF diet may not be sufficient to completely reverse structural damage,[9,13,14] or normalize nutrient levels.[15] This persistent mucosal damage is generally thought to occur due to poor compliance with a GF diet and/or late diagnosis, but it may be, at least in part, a result of the GF diet itself. Adherence to a GF diet changes food options and impacts dietary patterns in a way that has been shown to contribute to nutritional imbalances [16–23] and exacerbate nutrient deficiencies common in those with CeD.[15] Due to their use of alternative, often highly-refined and frequently unenriched flours and starches,[24] commercially-available gluten-free products (GFPs) are often lower in fiber and other vitamins and minerals compared to their gluten-containing counterparts.[16–23] Thus, those with celiac disease often remain at higher risk of anemia, low bone mineral density (BMD) which may lead to osteopenia and osteoporosis,[15] and development of intestinal cancers[16] than the general population.

Considering the frequent use of food additives to improve texture and palatability GFPs[25] it is possible that this diet also leads to increased food additive intake, thus reinforcing the ongoing cycle of mucosal damage and nutrient malabsorption. Promoting a diet that contributes to mucosal healing while also providing adequate nutrition is essential, especially for children who are not only growing, but may also be in the process of healing from damage to the GI tract as a result of previously untreated CeD.[26] Understanding how a GF diet impacts the dietary fiber, nutritional quality, and food additive intake of children with CeD compared to their peers will allow for improved dietary guidance and recommendations by providers.

Though food additives have been present in the food system for decades, their long-term impact on mucosal health, particularly in those genetically predisposed to GI-related autoimmune conditions such as CeD, remains unknown. The goal of this pilot study is to characterize the foods and food additive content of a typical GF diet consumed by children with CeD in the United States (US) compared to a typical diet consumed by similar children without CeD. This study aims to quantify the frequency of exposure to specific food additives (polysorbate 80, methyl cellulose,

xanthan gum, guar gum, soy lecithin, carrageenan, maltodextrin, aluminosilicates, and titanium dioxide), to assess the nutritional adequacy of GF and standard diets, and to evaluate the role of processed foods and GFPs in these diets and their impact on food additive exposure in children with and without CeD.

Background & Literature Review

An electronic search was implemented in the PubMed database using the terms "(celiac disease OR coeliac disease) AND food additives [mesh]". Twenty-eight results were found, 20 of which were excluded due to either "celiac disease" or "food additives" not being a main mesh term. The remaining articles from the initial search were used to identify additional references using a manual search of the cited sources. As this review is narrative rather than systematic, these references were selected according to their relevance and were used to identify additional relevant sources. Other references including characteristic and cornerstone references on CeD were also included. Cited articles were published between 1985 and 2020.

Nutritional Adequacy of the Gluten-Free Diet and Gluten-Free Products

Adherence to a GF diet treats the immunological response that occurs in CeD and is a good predictor of long-term outcomes, with better adherence being associated with increase improvement in mucosal healing,[27] and improved epithelial barrier integrity as measured by zonula occludens 1 (ZO-1) – a protein marker of tight junction integrity.[14] Positive associations have also been found between adherence to a GF diet and tTG IgA lab normalization, improved GI symptoms, and reduced risk of long-term complications[8,16] such as low bone-mineral density[28,29] and intestinal malignancy.[16] In one study, strong compliance with a GF diet was also linked to a decreased risk of mortality.[30]

Despite these positive impacts, the GF diet cannot be touted as a completely perfect solution. Adherence to a GF diet changes food options and impacts dietary patterns raising concerns for nutritional quality. Foods containing gluten – breads, pasta, cereals and processed, packaged snack items such as cookies, cakes, crackers, and chips – are highly prevalent in the standard American diet, and rather than being replaced with alternative, naturally GF foods, they are commonly replaced with similar GFPs. Here, we use the term GFPs to refer to *commercially-prepared products made to replace foods that usually contain wheat, barley, or rye ingredients and are advertised as gluten-free on the label.*

Adherence to a GF diet has been shown to contribute to nutritional imbalances including higher intake of simple carbohydrates, fats, and sugars, and lower intake of complex carbohydrates and fiber compared to a standard diet.[16–23] The GF diet has also been found to increase risk of certain nutrient deficiencies including magnesium, calcium, iron and zinc.[15,31–33] These deficiencies are due to multiple factors including inconsistent fortification of GFPs,[24] avoidance of foods naturally high in fiber and other nutrients like whole grains, relatively lower nutrient content in GF cereal products, the use of starches and highly refined flours in GFPs, malabsorption due to preexisting intestinal inflammation and mucosal damage, and patient choice.[15] Historically, GFPs have also been found to be lower in thiamin, riboflavin, niacin, folate and iron as compared to enriched wheat flour products.[24] Approximately 28-50% of patients on a GF diet continue to experience anemia,[32] and research suggests that adherence to a GF diet may have limited impact on normalizing bone mineral density, particularly in those diagnosed with CeD later in life.[34] Overall, the CeD population has a higher prevalence of osteoporosis than the general population (3.4% vs. 0.2%).[35]

GF grains also inherently lack the viscoelastic properties naturally provided by the gluten proteins found in wheat, barley, or rye. These unique properties provide baked goods with both the elasticity and viscosity necessary to retain gas while maintaining structure, resulting in an agreeable texture. Much research has been conducted on ways to achieve similar texture and consistency in GFPs by using additives such as hydrocolloid agents (methylcellulose, xanthan gum, and guar gum) or enzymes such as transglutaminases.[36] The general consensus is that use of these additives improves texture, consistency, and color of GFPs,[37–41] making it likely that they will be present in higher concentrations in GFPs compared to other processed foods.

Processed Foods & Food Additives

The definition of processed foods, and the value of labeling them as such for the purposes of nutrition research, has become a topic of debate in recent years. While some would argue that the historical research paradigm of evaluating the link between nutrient intake and chronic disease is sufficient,[42] there has been a shift toward consideration of overall diets or dietary patterns and their impact on health and disease. To assess whether diets or dietary patterns

higher in processed foods have an effect on health outcomes, it has been essential to create a single and universal definition of processed foods.[43] The NOVA Food Classification System was developed for that purpose by a team of food scientists and researchers at the Center for Epidemiological Research in Nutrition and Health (NUPENS). It classifies foods into four basic categories: Group 1 – unprocessed or minimally processed foods, Group 2 -- Oils, Fats, Salts, and Sugar, Group 3 – Processed Foods, and Group 4 – Ultra-Processed Foods. According to these definitions, processed foods generally include foods in which group 2 items are combined with group 1 items using various preservation methods including canning, pickling and fermentation. Ultra-processed foods are defined not as modified or recognizable versions of foods from groups 1 and 2, but rather as "formulations made mostly or entirely from substances derived from foods and additives".[44] For the purpose of this study, we have defined processed foods as *any food classified as NOVA Group 3 or 4*.

Since the development of the NOVA classification system, researchers have found that diets higher in processed and ultra-processed foods are associated with poor health outcomes, which may ultimately contribute to increased burden of nutrient deficiencies, obesity, and non-communicable diet-related chronic diseases such as heart disease and metabolic syndrome.[44,45] Processing methods have also come into question with findings that diets high in advanced glycation end products (AGEs), which are a result of high-heat processing methods, are associated with biomarkers of inflammation.[46] However, the impact of specific food additives on human health has not been fully elucidated.

Though food additives are generally subject to premarket approval by the US Food and Drug Administration (FDA), many are granted an exemption to this requirement by qualifying as 'generally recognized as safe (GRAS)'. According to FDA regulation CFR 170.30(b), any substance used in food before 1958 is considered GRAS based on 'common use'. For this status to be reconsidered, the FDA depends on post-market reporting of adverse events.[47] However, intestinal inflammation and mucosal damage are unlikely to cause the types of overt symptoms that would lead to such reporting. Thus, many of these substances have been present in the food system for decades, while their impact on mucosal health remains unknown.

Measuring Food Additive Exposures

Several countries in European Union (EU) have published preliminary studies attempting to estimate exposure to specific food additives using a method proposed in the 2001 Report from the European Commission on Dietary Food Additive Intake. The food additives in question include colors, preservatives, antioxidants, stabilizers, emulsifiers and sweeteners for which an acceptable daily intake (ADI) has been defined by the Scientific Committee on Food in the EU. Data on occurrence of these additives was obtained from the food industry and intake estimates were modeled on population-level diet surveys and assumed food habit scenarios to determine a tier for each additive, with tier 1 denoting little risk of exceeding ADI and tier 3 denoting high risk of exceeding ADI. Additives without a specified ADI (including titanium dioxide, xanthan gum, carboxymethyl cellulose, and guar gum) are considered 'acceptable for specified use' and thus were not considered in these studies. In the UK, 8 and 17 additives or additive groups for adults and children respectively were found to be high risk (tier 3) for exceeding ADIs including carrageenan and aluminosilicates. Preliminary lists of tier 3 additives were also identified by 6 other EU countries and marked for further consideration.[48,49]

In the United States (US), ADI values for food additives have not been established and no additional studies that we are aware of have attempted to quantify food additive intake at the population level or in those with specific autoimmune conditions.

Epidemiological Evidence for Food Additive Contribution to CeD

Research has demonstrated regional variations in CeD prevalence and comorbidity rates with type 1 diabetes (T1D) that are not accounted for by differences in HLA genes or other genetic polymorphism associated with CeD, suggesting a role for environmental factors in disease development.[4] Based on studies of both mono- and dizygotic twins, it has been estimated that genetic factors account for approximately 1/3 of the risk for developing autoimmune disorders while environmental factors explain the remaining 2/3, with some variation, depending on the specific disease.[6,7] Considering this, it stands to reason that food additives in the diet may be a contributing factor in the development of CeD and other autoimmune diseases. This hypothesis is further supported by the increasing incidence of CeD over the past 10+ years and the global distribution of CeD incidence, which is higher in more industrialized nations where consumption of processed foods is also higher.[12,44,50] Studies conducted in the US,[51–53] United

Kingdom,[54,55] and Finland[56] have found a 2 to 6.4 time increase in CeD incidence over the past 9-50 years that cannot be solely explained by improvements in detection techniques.[56]

Preclinical Evidence for Nanoparticle Contribution to Mucosal Damage

Research suggests that adherence to a GF diet may not be sufficient to reverse existing mucosal damage, resulting in possible increased risk for the development of concurrent auto-immune conditions[57] and increased rates of all-cause mortality.[51] Animal models suggest that tight junction dysfunction likely precedes autoimmune disease development[10,58] and several studies have found that even in human subjects with strict, long-term adherence to a GF diet, abnormalities in the mucosal architecture,[9] decreased epithelial resistance, and reduced villous height and crypt depth remained.[13] While the positive impacts of a GF diet on mucosal healing and lab normalization depend on multiple factors including length of time in which the disease was untreated, degree of mucosal damage, severity of nutrient malabsorption,[59] and age at diagnosis,[60] they have also been associated with overall quality of the GF diet.[32] That elimination of gluten from the diet does not always result in full mucosal recovery highlights the possibility that the GF diet may contain additional components that can impact mucosal integrity. This suggests a need for CeD research to move beyond its focus on gluten content and begin to consider additional factors that have the potential to impact mucosal integrity including food additives and processing techniques, particularly as they related to GFPs.

To date, research specific to food additives and CeD has focused mainly on nanoparticles which fall into the FDA's GRAS category and have been part of the food supply for decades, despite a poor understanding of their long-term effects on the gut mucosa. The presence of nanoparticles such as microbial transglutaminase (mTG) and metallic nanoparticles (mNP) such as titanium dioxide (TiO2) and aluminosilicates (AlSi) (often *Sodium Silicoaluminate* on food labels) – whether intentionally or unintentionally added to foods and/or packaging – have raised concerns for those with CeD and other GI autoimmune diseases.[38]

Due to their minute size, resistance to GI degradation, and charged surfaces it has been suggested that metallic nanoparticles may pass more easily into the intestinal submucosa where they can both interact with local intestinal and immune cells and accumulate in intestinal lymphoid aggregates. *In vitro* studies have demonstrated increased inflammasome activity in cultured human intestinal epithelial cells exposed to TiO2[61] and altered responsiveness to lipopolysaccharide (LPS) in colonic biopsies and macrophages from IBD patients with exposure to both TiO2 and AlSi.[62,63] One *in vivo* study also found that oral ingestion of TiO2 exacerbated onset of colitis in DSS colitis model mice.[61,64] Such evidence suggests potential of mNPs to alter intestinal permeability and/or increase immunogenicity in individuals who are susceptible to GI-related autoimmune diseases including CeD.[38]

Perhaps more concerning, mTG is a molecular mimic of tissue transglutaminase (tTG) which is involved in the pathogenesis of CeD. Microbial transglutaminase is used mainly in meat processing to promote protein cross-linking[65] and is also capable of deamidation of both synthetic and natural gluten peptides. *In vitro* studies have found that deamidated gluten elicits a stronger immune response than native gluten particles.[38,66] This suggests that mTG may not only contribute to development of CeD in those who are genetically susceptible, but that its consumption in processed foods may also result in a more robust immune response in those with CeD. Microbial transglutaminase was not encountered on any food labels evaluated in this study.

Though nanoparticles are required to be listed on food labels if directly added to foods, they may also be present in the environment, such as in water and soil,[62] in food packaging, or indirectly added during processing[38] making it more challenging to quantify exposure. To date, no study that we are aware of has attempted to quantify nanoparticle exposure at the individual level.

Preclinical Evidence for Other Food Additive Contribution to Mucosal Damage

The development of CeD and subsequent ability of the intestinal mucosa to heal appears to vary, even among those with genetic predisposition to the disease, thus it is possible that different food additives, or classes of food additives, may be affecting different aspects of this process. The impact of more common food additives (polysorbate 80, carboxymethyl cellulose, xanthan gum, guar gum, soy lecithin, carrageenan, and maltodextrin) on those with CeD has yet to be elucidated. Many studies have been devoted to evaluating the impact of these food additives on mucosal integrity in animal models as it may relate to the development of other autoimmune conditions such as inflammatory bowel disease (IBD) in humans. Considering that IBD and CeD have similarities in underlying pathogenesis[67] and that individuals with CeD are at higher risk of developing additional autoimmune conditions,[2] it is possible that

children with genetic predisposition to any autoimmune disease impacting the GI tract may be similarly impacted by exposure to food additives.

Emulsifiers, due to their inherent surfactant characteristics, are thought to contribute to increased intestinal permeability by decreasing hydrophobicity of the mucosal lining of the intestine[7]. Polysorbate 80 (P80) has been approved for use by the Food and Drug Administration in some foods at concentrations up to 1% based on evidence from existing research.[68] Carboxymethylcellulose (CMC), also called *cellulose gum*, is GRAS according to the FDA[69] and can be found in concentrations of up to 2% in some foods. In mouse models, introduction of both CMC and P80 via drinking water were found to induce low-grade inflammation, obesity and metabolic syndrome in wild-type mice.[70] In genetically susceptible mice, CMC and P80 had even more deleterious effects including the development of robust colitis, increased colonic bacterial adherence, decreased microbial diversity,[70] increased leukocyte migration to the intestinal lumen, and development of wider spaces between the villi and the crypts of Lieberkühn.[71] Taken together, the microbial and morphological changes observed in the intestinal villi and increased leukocyte migration to the area were found to resemble findings in patients with Crohn's disease.

Carrageenan, a red seaweed extract, is another widely used food additive. While no adverse health effects have been found related to food-grade carrageenan, which has a high molecular weight and is extracted under alkaline conditions, degraded carrageenan (or poligeenan) has been associated with multiple negative health outcomes. Degraded carrageenan has a lower molecular weight and is produced by acid hydrolysis of food-grade carrageenan at high temperatures. Attempts have been made by the food industry to minimize risk to consumers by banning use of degraded carrageenan and altering the level of degradation and molecular weight of the food-grade carrageenan allowed in foods. Though at least one recent article has argued that this is not the case,[72] historically it has been believed that food-grade carrageenan may convert into degraded carrageenan during the digestive process. Additionally, there have been widespread claims of cross-contamination between food-grade and degraded carrageenan,[73] suggesting that there may be continued risk involved in the consumption of food-grade carrageenan.

Degraded carrageenan was designated as Group 2B in 1982 by the International Agency for Research on Cancer (IARC). This classification denotes sufficient evidence of carcinogenicity based on animal model studies which consistently observed exposure to degraded carrageenan to result in intestinal damage including colonic ulcerations and gastrointestinal neoplasms.[73,74] One study observed intestinal ulcerations, abnormal villous patterns, hyperplasia with microgranulomas, and severe inflammation in rat models exposed to oral carrageenan (1.5% concentration) for 30 days. Within those groups, half were pre-sensitized to carrageenan via parenteral administration of the same solution, an initial exposure that was found to exacerbated the effects of the oral carrageenan exposure.[75] Over the past four decades, a multitude of animal-model studies have observed similar negative outcomes related to exposure to degraded carrageenan, including increased intestinal permeability, inflammation and ulcerative lesions in guinea pigs, rats, mice and monkeys and colorectal tumors with long term exposure in mice and rabbits. A more recent study also found dose-dependent increases in inflammatory signaling molecules in rats.[72] Temporary guidelines for safe levels of carrageenan intake were proposed in 2018 by the European Food Safety Association while safety of consumption and risk of exposure to degraded carrageenan continue to be evaluated.[76]

Similarly, assessment of the impact of xanthan gum on intestinal health is ongoing. Though several short- and long-term studies have observed no adverse effects of xanthan gum ingestion in rats, other studies have observed the opposite. Increased cecal tissue weight and increased concentrations and volume of bile acids have been observed in rats and dogs. Low weight gain, possibly related to malabsorption, and minimal to moderate histological changes (goblet cell hypertrophy/hyperplasia) were observed in the large and small intestines of neonatal pigs at the highest level of exposure. Studies vary greatly in the length and amount of exposure and type of xanthan gum-containing product used. Overall, studies seem to suggest that higher levels of exposure may result in more deleterious effects in infants. Based on neonatal pig studies, clinical trials and post-marketing surveillance, a Committee of the World Health Organization (WHO) established an observed-adverse-effect level (NAOEL) of 220mg xanthan gum per kg body weight for infants and proposed a maximum use level of 1000mg/L in infant formulas. The same report also concluded that while there is evidence that xanthan gum exhibits biologic activity on exposed cells, the impact in humans remains unknown.[77]

Preclinical research has also found that consumption of food additives may lead to decreased ability to clear intestinal infections. One study found that consumption of maltodextrin (MXD) resulted in a decrease in *Salmonella* clearance in infected mice due to interruption of the mucosal layer of the intestine.[78]

Clinical Research on Food Additives and Health Implications

Clinical trials that assess the impact of food additive consumption on human health are understandably limited, with no known studies analyzing the link between CeD and food additive intake. A recent study published by the authors found that children with Crohn's disease frequently consume select food additives with exposures to xanthan gum, carrageenan, maltodextrin, and soy lecithin of greater than 0.1/day.[79] An observational study is also currently underway to assess the association between exposure to these and other specific food additives and the risk of relapse active inflammation in children with Crohn's disease.

One randomized control trial assigned participants with active corticosteroid-treated or ileo-colonic Crohn's disease to follow either a standard or a "low microparticle" diet for four months. The low microparticle diet excluded foods that contain titanium dioxide or aluminosilicates and involved washing and peeling fruits and vegetables to avoid environmental contamination from soil or water. The trial group experienced progressively improved Crohn's disease activity index (CDAI) throughout the 4-month trial period compared to the control group which returned to baseline levels but did not continue to improve. At the end of the study, 7 of 9 participants in the trial group were in remission compared to none in the control group.[80]

Another study – a review of 22 cases that was undertaken only after adverse events were reported to the FDA – found that consumption of infant formula which had been thickened with a xanthan gum-based thickener (SimplyThick) induced necrotizing enterocolitis (NEC) in premature infants.[81] NEC involves intestinal lesions, ulceration, hemorrhaging, and edema. Pathogenesis of this condition is not completely understood, but is thought to occur as a result of exposure of the immature intestinal mucosa to bacteria and other undigested food antigens leading to an inflammatory response.[82] Such a reaction is reminiscent of the development of CeD and also supports the hypothesis that exposure to certain substances, such as food additives, may induce inflammation and, particularly in those with existing mucosal damage and the genetic predisposition, autoimmune diseases. The FDA now recommends that SimplyThick formula not be given to preterm infants (born before 37 weeks gestation) or any infants under 12 months, and the SimplyThick brand also cautions against its use for any children under 12 years with a history of NEC.[83]

Taken together these studies suggest that nanoparticles and other food additives are not inert dietary components but that, similar to findings in animal models, they may play an active role in intestinal inflammation and autoimmune disease etiology in humans.

Study Aims

The frequency and impact of food additive intake in humans is yet unknown and requires further study. Characterization of the differences in diet composition based on processed foods and food additives consumed by children with and without celiac disease will provide insight and inform the direction of future research regarding the impact of specific types of products and/or food additives on the health of individuals with celiac disease.

The primary aim of the study is to quantify the frequency of exposure to specific food additives (polysorbate 80, methyl cellulose, xanthan gum, guar gum, soy lecithin, carrageenan, maltodextrin, aluminosilicates, and titanium dioxide) by children with and without CeD. The secondary aim is to examine and compare nutritional adequacy of these diets. The study also aims to evaluate the role of processed foods and GFPs in the diet and their contribution to food additive intake. We expect that, consistent with other research, fewer children in the celiac group will meet daily recommendations for fiber and micronutrient intake compared to their non-celiac counterparts, and that children in the celiac group will consume specific food additives more frequently than children in the control group.

Methods

Study Population & Design

Participants for this cross-sectional pilot study were recruited at Seattle Children's Hospital in Seattle, WA between June and December 2019. Children with CeD were recruited as cases from the Celiac Clinic and Celiac Support Group. A comparable group of non-celiac children were recruited from the Orthopedic Clinic as controls. A total of 51 participants (29 celiac and 22 controls) were enrolled in the study, of which 16 from the celiac group and 14 from control group returned 3-day food records. Of these 30 food records, data from 28 were included in the final analyses. One dairy from the celiac group was returned but was not included in the final analysis because it was missing

participant identifiers and therefore anthropometrics were not available to determine nutrition recommendations. Another diary, this one from the control group, was also removed from the study as it contained insufficient information to accurately input into the FoodPro database.

Eligible participants were identified via chart review. Inclusion criteria for cases included age between 6 and 18 years at time of consent/assent, diagnosed CeD (defined as biopsy proven, or positive celiac serologies with primary gastroenterologist confirming the diagnosis), duration of adherence to a GF diet for at least 6 months (as determined by chart notes and/or verbal confirmation at time of consent/assent), and English speaking/writing as translated study materials were not available. Exclusion criteria for cases included diagnosis of any comorbid GI diseases (including Crohn's, ulcerative colitis, irritable bowel syndrome, small intestine bacterial overgrowth), and adherence to any medically necessary diet outside of the GF diet. Controls were also reviewed for eligibility via medical records. Inclusion criteria for controls included age 6 to 18 years at time of consent/assent and exclusion criteria were diagnosis of CeD or any other GI disorder (including IBD, IBS, SIBO), and adherence to a GF or low-carbohydrate diet within the past 3 months. After initial screening, participants were introduced to the study by their primary treating provider. If interested, participants were then given more information and consented/assented in-person by the study researcher or coordinator. The study protocol was approved by the institutional review board at Seattle Children's Hospital. Informed consent was obtained from all young adults and parents/guardians.

Dietary Assessment Tools

A 3-day food record booklet was developed for this study by adapting the current Food Intake Record used by Seattle Children's Hospital and based on similar 3-day record booklets used by the Fred Hutchinson Cancer Research Center and the Clinical and Translational Research Center at the University of Colorado, Denver. Participants were asked to complete the 3-day food record according to the instructions printed in the food record booklet and provided orally by the researcher/coordinator. The provided 3-day food record booklets included an example food record for one day, detailed instructions and images to assist in reporting serving size, and 7 general questions on food-use patterns which were utilized by the researcher to assign default values in the case of incomplete food records. Participants were instructed to complete the food record over any three consecutive days and to provide detailed descriptions of all foods and beverages consumed including amount, brand name, method of preparation, and any diet claims on the label (e.g., low fat, sugar free, reduced sodium). Participants were also instructed to record meal type (breakfast, lunch, dinner, snack) and meal location (home, school, restaurant, other).

All 3-day records were entered into Food Processor (FoodPro) versions 11.3 and 11.6 (ESHA Research, Salem, OR) using United States Department of Agriculture (USDA) or Food and Nutrient Database for Dietary Studies (FNDDS) equivalent (standardized) products when possible to ensure inclusion of micronutrient information. If a standardized equivalent was not available, as in the case of many GFPs, a best-match was prioritized to ensure accuracy of nutrient and fiber content as these products often include unique varieties of grain that would not be reflected in standardized options. For all foods in which a product brand name, size, flavor or variety was not specified, a generic USDA or FNDDS item was used as the standard equivalent in all similar instances across all food diaries. Each participant's age, sex, height, and weight were entered into FoodPro, and an activity level of "lightly active" was used as the standard for all participants in order to generate individualized energy and nutrient intake recommendations for comparison to actual intake.

All food items recorded in the 3-day food records were also classified by whether they met the definition of *processed food* and *GFP*s. For the purpose of this study, we have defined processed foods as *any food classified as NOVA Group 3 or 4* and GFPs as *commercially prepared products specifically made to replace products that normally contain wheat, barley or rye and advertised as gluten-free on the label.* Note that nearly all, but not all GFPs are considered to be *processed* based on the NOVA definitions. All products defined as *processed* were entered into a separate spreadsheet without removing duplicate items in order to obtain a count of the number of times each item was consumed by each study participant. This list was cross-referenced to an existing database previously created by the authors which includes ingredient lists for all items developed through assessment of individual food labels made available online by food manufacturers and grocery establishments. Any products not already in the database were added. For all foods in which a product brand name was not specified, a brand was chosen based on its popularity in the online Google shopping search engine. In cases that a flavor was not specified, the *original* flavor was chosen. This brand and/or flavor was defined as the standard equivalent and the ingredient list was used in all similar instances across all food diaries to reduce introduction of bias.

The resulting dataset includes a list of ingredients for each item and was used to assess the presence/absence of any food additives of interest. The definition of food additives can vary widely and may be so broad as to include any substance added to food either directly or indirectly as a result of packaging, storage or handling.[84] For the purpose of this study, food additives were defined as *any non-nutritive substance that is intentionally added to food in order to enhance the product in some way such as improving color, texture, smell, flavor, or shelf-life*. This definition is consistent with food additives that may be found in NOVA Group 3 (preservatives, antioxidants, stabilizers) and/or Group 4 category foods (dyes, color stabilizers, flavors and flavor-enhancers, non-sugar sweeteners, and processing aids: carbonating, firming, bulking/anti-bulking, de-foaming, anti-caking and glazing agents, emulsifiers, sequestrants, and humectants).[44]

Specifically, we assessed each item for the presence of polysorbate 80, carboxymethyl cellulose, xanthan gum, guar gum, soy lecithin, carrageenan, maltodextrin, titanium dioxide, and aluminosilicates. To capture all instances of these additives, alternative names and spellings were also searched in the dataset including *carageenan*, *xantham gum*, *cellulose gum*, and *sodium silicoaluminate.* The number of exposures to each additive by each participant over the three-day study period was counted and averaged as was the number of ingredients listed on the label for each item.

This list was cross-referenced back to the FoodPro data where all processed foods were classified by presence/absence of at least one food additive. These classifications within the FoodPro data allowed for a novel analysis of nutrition information based on multiple sub-categories including: processed, GFP, number of ingredients, and food additive content.

Data Analysis

FoodPro automatically calculates a range for energy needs based on standard resting energy expenditure (REE) formulas for age, sex, and activity level,[85,86] and uses standard dietary reference intakes (DRI) and adequate intake (AI) values as defined by the USDA to determine recommended daily nutrient, vitamin, and mineral intake for age and gender. Reports were generated for each participant and included 3-day total intake for macro and micronutrients and the percentage of daily energy needs that were met as well as the percentage of DRI/AI recommendations met for macro and micronutrients. Meeting recommendations is defined as being within the range of calculated energy needs and meeting ≥100% DRI/AI for nutrients, vitamins, and minerals or ≤100% RDA for cholesterol, saturated fat, and sodium.[87] All nutrition data generated by FoodPro was analyzed using Stata software. Energy, macronutrient, and fiber intake were averaged and standardized to per 1000 calories for each group. Differences in mean values were assessed using Student's t test and the Wilcoxon rank-sum test. Group differences in categorical variables were assessed with the chi-square test. The percentage of participants meeting recommendations for energy, nutrients, vitamins, and minerals was also calculated and categorical variables were assessed with the Pearson's chi-squared test.

The number of exposures to each food additive in the 3-day period was counted for each participant and a mean 3-day exposure count was calculated for each group. The difference in mean 3-day exposure count between the two groups was also assessed using Student's t test and the Wilcoxon rank-sum test. The proportion of total products consumed that were considered processed foods, GFPs, or contained food additives was also calculated for each group and compared. Secondary analyses of the nutrition data were performed to compared differences in energy, macronutrient and fiber intake between participants in the celiac group for whom more or less than 15% of the products consumed were GFPs. Other analyses included the contribution of processed foods to the diet and energy intake in both groups, and the proportion of all food and processed foods containing at least one food additive in both groups. Finally, the proportion of GFPs and processed, non-GFPs containing food additives was compared.

Results

Study Population

Twenty-eight children (23 female, 5 male) with (n=15) and without (n=13) CeD were recruited as study participants from Seattle Children's Hospital. Participants had a mean age of 11.29 ± 3.2 years with 68% identifying as Caucasian, 7% as Asian and 25% as other/unspecified. There were no significant differences in mean age, race or z-scores for

height, weight and BMI between the celiac and control groups. All participants in the celiac group identified as female (100%), while 9 of 13 (69%) participants in the control group identified as female (p=0.02).

Table 1. Demographic and anthropometric data for participants in the celiac and control groups

	Celiac (n=15)	Control (n=13)	Difference (P-Value)
Gender – count (%)			
Male	0	4 (31%)	
Female	15 (100%)	9 (69%)	0.02*
Age – count (%)			
6-12	11 (73%)	7 (54%)	
>12-18	4 (27%)	6 (46%)	0.28
Race – count (%)			
White/Caucasian	11 (73%)	8 (62%)	
Asian	0	2 (15%)	0.29
Other/Unspecified	4 (27%)	3 (23%)	
Height-Z score	0.34 ± 1.05	0.82 ± 1.48	0.34
Weight-Z score	0.04 ± 0.93	0.26 ± 1.06	0.56
BMI-Z score	-0.07 ± 0.86	0.07 ± 0.89	0.69

*Statistically significant

Food Additive Exposure & Diet Composition

For the 9 food additives examined, participants in the celiac group were exposed to a mean of 19.6 ± 10.74 total additives while participants in the control group were exposed to a mean of 20.23 ± 10.17 total additives during a three-day period (P=0.88). The three-day average number of exposures to individual additives ranged from 0 to 5.27 ± 3.06. The food additive with the overall highest average of exposures was xanthan gum, followed by soy lecithin. The food additive with the overall lowest average number of exposures was aluminosilicates followed by polysorbate 80. Soy lecithin had the highest average exposures in the control group, however exposures in the celiac group were also high and the difference between groups did not reach significance (P=0.80). The celiac group had the highest average exposures to xanthan gum, a difference that was significant compared to the control group (P=0.009). No other differences in food additive exposure between the two groups reached significance.

Table 2. Three-day average number of exposures to specific food additives in celiac and control groups

	Total ± SD (n=28)	Celiac ± SD (n= 15)	Control ± SD (n=13)	Difference (P-Value)
Xanthan Gum	3.76 ± 3.15	5.27 ± 3.06	2.31 ± 2.43	0.009*
Soy Lecithin	3.69 ± 3.37	3.67 ± 3.92	4 ± 2.71	0.80
Maltodextrin	2.45 ± 1.94	2.33 ± 1.23	2.77 ± 2.52	0.56
Guar Gum	1.62 ± 1.29	1.8 ± 1.32	1.54 ± 1.27	0.60
Carrageenan	1.55 ± 1.82	1.47 ± 1.96	1.77 ± 1.74	0.67
Carboxymethyl Cellulose	1.31 ± 1.11	1.27 ± 0.88	1.46 ± 1.33	0.29
Titanium Dioxide	0.34 ± 0.77	0.33 ± 0.82	0.38 ± 0.77	0.87
Polysorbate 80	0.24 ± 0.51	0.13 ± 0.35	0.38 ± 0.65	0.21
Aluminosilicates	0.07 ± 0.26	0.00	0.15 ± 0.38	0.12

*Statistically significant

According to the analysis, 14.7% of all food items recorded contained at least one food additive, 13.9% of all products recorded by the celiac group contained at least one food additive compared to 15.9% in the control group (P=0.24). Processed foods accounted for 47% of total food items recorded by the celiac group and 48% of food items recorded by the control group (P=0.83). Overall, 33% of processed foods recorded contained food additives. In the celiac group, 32% of processed foods contained at least one food additive compared to 35% of processed foods in the control group (P=0.32).

Table 3. Characterization of the proportion of total foods and energy intake contributed by processed foods and the proportion and types of foods containing at least one food additive in celiac and control groups

	Celiac (n= 15)	Control (n=13)	Difference (P-Value)
% Diet from Processed Foods	47	48	0.83
% Energy from Processed Foods	67	65	0.72
% Total Foods Containing ≥1 Food Additive	13.9	15.9	0.24
% Processed Foods Containing ≥1 Food Additive	32	35	0.32

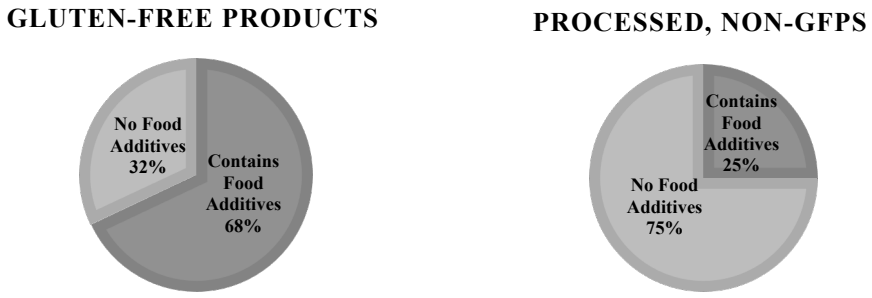
GFPs accounted for 15 ± 0.05% of total products and 34% of processed foods recorded by the celiac group compared to 1% and 2% respectively in the control group (P<0.0001). 68% of processed GFPs contained at least one food additive while only 25% of processed foods that were not considered to be GFPs contained at least one food additive (P<0.0001). Of those participants in the celiac group who reported GFPs accounting for >15% of total food items, 53% of total items consumed were processed foods and 19.5% contained food additives compared to only 41% processed foods (P=0.09) and 10.4% containing food additives (P<0.001) in the group that reported ≤15% of total items as GFPs.

Table 4. Characterization of the proportion of total foods and energy intake contributed by processed foods and the proportion of foods containing at least one food additive in celiac participants for whom either >15% or ≤15% of their total diet was comprised of gluten-free products (GFPs)

	Celiac >15% GFP (n=8)	Celiac ≤15% GFP (n=7)	Difference (P-Value)
% Diet from Processed Foods	53	41	P=0.09
% Energy from Processed Foods	72	62	P=0.29
% Total Foods Containing ≥1 Food Additive	19.5	10.4	P<0.001*

*Statistically significant

Figure 1. Proportion of gluten-free products (GFPs) versus processed, non-gluten-free products (non-GFPs) that contained at least one food additive



Diet Quality

In the celiac group 66.7 ± 16.1% of energy was contributed by processed foods, compared to 64.5 ± 16.6% in the control group (P=0.72). Within the celiac group, 29.3 + 13.3% of energy was contributed by GFPs. Those reporting >15% of total foods as GFPs consumed 71.7% ± 15% of energy from processed foods compared to 62.4 ± 16.8% of energy from processed foods in those reporting ≤15% of total foods as GFPs (P=0.29).

No significant differences in energy from fat, protein, carbohydrate or fiber intake per 1000 calories were found between the celiac and non-celiac groups. In the celiac group, significantly fewer participants met recommendations for thiamin, folate, magnesium, manganese and potassium compared to their non-celiac counterparts. Fewer than half of participants in the celiac group met recommendations for daily carbohydrate and pantothenic acid intake compared to more than half in the control group (P=0.14 and P=0.06). In the control group fewer than half of participants met recommendations for vitamin K intake, compared to more than half in the celiac group (P=0.43). In both groups, fewer than half of participants met recommendations for total dietary fiber, cholesterol, omega-3 and omega-6 fatty acids, biotin, vitamin E, calcium, iron, and molybdenum, however differences were not significant between the two groups.

Table 5. Energy from Fat, Protein, Carbohydrate and Fiber per 1000 calories

Calories from:	Celiac (n=15)	Control (n=13)	Difference (P-Value)
Fat (g/1000kcal)	44.7 ± 7.4	38.1 ± 5.8	0.015*
Protein (g/1000kcal)	39.1 ± 11.4	40.8 ± 6.1	0.64
Carbohydrates (g/1000kcal)	113.0 ± 23.2	125.9 ± 14.6	0.097
Fiber (g/1000kcal)	8.6 ± 3.4	8.5 ± 3.2	0.93

*Statistically significant

Table 6. Number and Percent of Participants Meeting Daily Energy and Nutrient Recommendations

	Celiac (n=15) (n, percent)	Control (n=13) (n, percent)	Difference (P-Value)
Energy (kcal)	9 (60)	8 (62)	0.93
Energy from Fat (kcal)^	12 (80)	11 (85)	0.75
Energy from SatFat (kcal)	14 (93)	12 (92)	0.92
Protein (g)	15 (100)	13 (100)	1
Carbohydrates (g)	5 (33)	8 (62)	0.14
Total Dietary Fiber (g)^	2 (13)	1 (8)	0.63
Cholesterol (mg)	5 (33)	4 (31)	0.89
Omega 3 Fatty Acid (g)	5 (33)	3 (23)	0.55
Omega 6 Fatty Acid (g)	4 (27)	2 (15)	0.47
Vitamin A - RAE (mcg)	7 (47)	9 (69)	0.23
Vitamin B1 - Thiamin (mg)	11 (73)	13 (100)	0.04*
Vitamin B2 - Riboflavin (mg)	14 (93)	13 (100)	0.34
Vitamin B3 - Niacin (mg)	13 (87)	13 (100)	0.17
Vitamin B3 - Niacin Equiv (mg)	14 (93)	13 (100)	0.34
Vitamin B6 (mg)	14 (93)	13 (100)	0.34
Vitamin B12 (mcg)	13 (87)	13 (100)	0.17
Biotin (mcg)^	6 (40)	3 (23)	0.34
Vitamin C (mg)	9 (60)	11 (85)	0.15
Vitamin D (mcg)^	15 (100)	13 (100)	1
Vitamin E - Alpha-Toco (mg)	3 (20)	4 (31)	0.51
Folate (mcg)	5 (33)	12 (92)	0.001*
Vitamin K (mcg)^	8 (53)	5 (38)	0.43
Pantothenic Acid (mg)^	4 (27)	8 (62)	0.06
Calcium (mg)^	5 (33)	5 (38)	0.78
Chromium (mcg)^	15 (100)	13 (100)	1
Copper (mg)	11 (73)	11 (85)	0.47
Iodine (mcg)	2 (13)	0 (0)	0.17
Iron (mg)	11 (73)	12 (92)	0.19
Magnesium (mg)	6 (40)	10 (77)	0.049*
Manganese (mg)^	9 (60)	13 (100)	0.01*
Molybdenum (mcg)	2 (13)	0 (0)	0.17
Phosphorus (mg)^	10 (67)	11 (85)	0.27
Potassium (mg)^	8 (53)	1 (8)	0.01*
Selenium (mcg)	12 (80)	13 (100)	0.08
Sodium (mg)^	14 (93)	11 (85)	0.46
Zinc (mg)	12 (80)	13 (100)	0.09

^ recommendations based on AI (all others based on DRI); *Statistically significant

Discussion

Food Additives Exposure

This study is one of the first to attempt to quantify exposure to food additives and is the first to quantify differences in food additive exposures in a GF diet. Additionally, the combination of two datasets has allowed for novel secondary analyses of the nutritional quality of a GF diet based on each food item's status as a processed item or GFP, and food additive content.

We found that overall, celiac and non-celiac participants had a similar number of total exposures to food additives during a three-day period and a comparable number of exposures to each individual additive assessed apart from xanthan gum, which was significantly higher in the celiac group. Similar to a previous study published by the authors which assessed food additive exposures in children with Crohn's disease, xanthan gum had the highest mean exposures.[79] Given the lack viscoelastic properties in GF grains, higher use of water-soluble gum or hydrocolloids such as CMC, guar gum, and xanthan gum in GFPs is expected. Hydrocolloids, due to their ability to interact with both aqueous and non-aqueous phases, are known to improve the rheology, or flow, of batters and doughs by acting as gelling agents and stabilizers, ultimately resulting in an improved structure and softer crumb in final products.[88,89] Hydrocolloids differ in their properties and thus their effects on baked goods will depend on the type of grain used as well as the pH and temperature. Xanthan gum is an effective stabilizer at a wide range of temperatures and pH and is able to maintain viscosity even at room temperature and in the presence of electrolytes. Other hydrocolloids are less versatile; for example, CMC does not maintain viscosity in the presence of electrolytes or at a low pH and guar gum similarly will degrade and lose viscosity at high temperatures and high and low pH.[90] In one study, addition of xanthan gum was also found to result in fewer larger cells per mm2 suggesting it results in a more open, less-dense structure compared to a control.[89] Both the versatility and effectiveness of xanthan gum in achieving desired texture and crumb structures in baked goods likely explains the observed high prevalence in processed foods in general and particularly in a wide variety of GFPs.

Given the ubiquity of xanthan gum in GFPs and evidence that it is potentially a biologically active molecule with an unknown impact on intestinal health, further research is warranted. As research to date has focused on healthy animal models and infants, it would be a prudent next step to consider the impact of xanthan gum on animal models with genetic susceptibility to CeD, similar to existing research designed to assess the impact of CMC and P80 in IBD-susceptible rats. Larger studies with more statistical power to assess intake of xanthan gum and other food additives in those with CeD would also help determine the necessity of future clinical studies. Clinical trials and/or retrospective studies to assess differences in microbiota and inflammatory response in those with CeD on high versus low GFP/xanthan gum diets may be warranted. A similar clinical trial is currently underway to assess the impact of a diet containing 15g/day CMC on microbiota composition and metabolic parameters in healthy human subjects.[91] However, caution should be used with consideration to exposing participants with CeD, who may already be experiencing higher levels of mucosal inflammation and damage, to additional sources of potential irritation. Pre-clinical studies to assess for dose-dependency and reversibility of intestinal inflammation or damage with elimination of a given food additive from the diet would also inform safety and design of future clinical trials with CeD participants.

Diet Composition: Processed Foods & Gluten-Free Products

We hypothesized that children on a GF diet would consume more food additives than their non-celiac counterparts due to the expected higher content of food additives in GFPs. However, we also thought that total processed food intake could be a confounding variable. Since participants with CeD were recruited from the celiac clinic and celiac support group, we assume that these families have received frequent nutrition education, which could result in increased propensity to make healthier dietary choices, such as choosing fewer processed food items. No studies that we are aware of have compared different types of GF diets to determine whether a GF diet that relies more heavily on processed products compared to one that is higher in naturally GF foods (such as rice and corn-based products) differs in nutritional quality or has differing impact on improvements in mucosal healing.

Only one study that we are aware of has attempted to compare the types and nutritional value of foods consumed by children with and without CeD. Zuccotti et al. found that children with CeD consumed, on average, more bread (7:4) and rice (2:1) than their peers and that they also more frequently consumed products with lower nutritional value (junk food 9:6.5, cookies 7:3, soft drinks 8.5:6.5) than their non-CeD counterparts.[16] In contrast, we did not find that children

with CeD had significantly different intake of processed foods compared to the control group. This may indicate that eliminating gluten from the diet did not have an impact on processed food intake, but rather that common processed foods are simply being swapped out for their GF equivalents by those consuming a GF diet. It is notable that despite no difference in processed food intake between groups, there was still a significant difference in xanthan gum intake. Considering that 62% of GFPs were found to contain food additives compared to only 25% of non-GF processed foods, this finding makes sense and is consistent with the hypothesis that GFPs require more food additives to be considered palatable. That the high GFP celiac subgroup had significantly higher exposures to food additives compared the low GFP subgroup suggests that food additive intake is associated with GFP intake regardless of processed food intake.

Zuccotti et al. also analyzed the extent to which GFPs play a role in the GF diet. The study found that GFPs accounted for 36.5% of total daily energy intake in children with CeD (18% of total energy from protein; 49.5% of total energy from carbs; 17.7% of total energy from fat).[16] We found that GFPs accounted for 29.3 ± 13.3% of total daily energy intake in the celiac group. This slightly lower percentage is likely related to differences in dietary patterns and the composition of available GFPs in Italy compared to the US. Similar to our study, this research also notes the difficulty of analyzing dietary contributions of GFPs in more detail due to a lack of available information on food labels.

Diet Quality: Energy & Nutrient Intake vs Recommended

A review of 21 studies, with subjects ranging from 4 to 80 years of age, found nutrient content of GF diets to be poor not only in fiber, but also in Vitamin D, Vitamin B12, folate, iron, zinc, magnesium and calcium[15] and one in ten people with CeD have a deficiency in magnesium.[31] Similar to these studies, our results show that significantly fewer participants in the celiac group met recommendations for folate and magnesium compared to their non-celiac counterparts, in addition to significantly fewer meeting recommendations for thiamin, manganese and potassium. In contrast to these studies, 100% of the celiac group met recommendations for Vitamin D, 87% met recommendations for Vitamin B12, 73% for iron, and 80% for zinc. While it should be noted that meeting dietary recommendations does not necessarily rule out deficiency, particularly in children with CeD who are at higher risk of experiencing malabsorption, it is possible that such results are an indication of improving nutritional quality of available GFPs and/or a sign of healthy diet choices related to the nutrition education received by study participants.

Historically, GF diets have been found to be lacking in many of these vitamins and minerals as a result of fundamental differences in nutrient content of cereals that contain or do not contain gluten in addition to inconsistent fortification and/or enrichment of GFPs. One study found that of 368 GF flours, breads, pastas, and cereals assessed, only 35 were enriched. More specifically, none of the GF rice flours or any pastas were enriched and only a small proportion of breads, mixes, cereals and ready-made products – all of which would be included in our definition of GFPs – were enriched. When 64 of these were assessed for thiamin, riboflavin and niacin content and compared to their wheat-containing counterparts, only 4 were found to contain higher amounts of all three and one contained the same amount of all three. In contrast, 39 contained lower amounts of all three, an additional 14 contained lower amounts of two of the vitamins, and 6 contained lowers amounts of one of the vitamins. [24] Another study by the same authors found that GFPs were generally lower in folate and iron than their gluten-containing counterparts.[92]

More recently, demand for healthier, tastier GFPs has been growing and studies suggest that the nutritional quality of GFPs is improving.[93,94] For example, some companies have begun to research options for the enrichment of GFPs using seeds to improve fiber content,[95] while others have experimented with increasing natural ingredients like egg albumin relative to other additives, such as methylcellulose and gum arabic, in order to improve GF bread texture.[37] Another study found that GFPs, are also 28% lower in ingredient diversity than their gluten-containing counterparts, and contained sodium and salt 60% less frequently than non-GF products,[36] which could indicate increasing demand for products with fewer ingredients by the consumers most likely to purchase these products.

We found that fiber and calcium intakes were minimal but similar in both groups, with only two participants in the celiac group and one participant in the control group meeting recommendations for fiber and only 5 in each group meeting recommendations for calcium. According to the Scientific Report of the 2020 Dietary Guidelines Advisory Committee, both of these are under consumed by the entire US population,[96] making it unsurprising that fiber and calcium intake would be low in both groups. Though the difference did not reach significance, that a slightly higher proportion of participants met the recommendation for fiber in the celiac group compared to the control group is in contrast with multiple studies that have observed lower fiber intakes associated with the GF diet.[19,97] Given that GF

cereals are often lower in fiber than grains such as whole wheat,[98] in future studies, a secondary analysis of specific foods or food groups that contributed to daily fiber intake in both groups would be informative. It is also possible that this slightly higher fiber intake also reflects the increasing diversity of GFPs in recent years with many products now using more fiber-rich and nutrient-dense grains including quinoa, amaranth and buckwheat and legumes such as lentils and chickpeas.

Fewer than half of participants in both groups met recommendations for cholesterol, omega-3 and omega-6 fatty acids, biotin, vitamin E, and molybdenum and only about 60% of participants met daily energy needs. Multiple studies have found that those on a GF diet have, on average, higher energy intake and consume more fat, protein and carbohydrates/complex carbohydrates, fewer nutrients, and less fiber than their non-GF diet counterparts,[16–21] though some studies have found that a GF diet may also result in reduced energy intake.[22,23] Consistent with this research, we found that children in the celiac group consumed a significantly higher proportion of fat per 1000 calories compared to the control group, however proportions of protein, carbohydrates, and dietary fiber were not significantly different. It is possible that the higher proportion of fat may be related to the use of higher fat ingredients such as nuts and seeds in GFPs[95] which can improve flavor and texture and/or substitution of higher carbohydrate foods for those with higher fat content such as dairy products. It is also notable that despite the higher proportion of fat intake in the celiac population, fewer than 50% of participants in both groups met daily recommended intake for omega-3 and omega-6 fatty acids. This is consistent with a recent study that analyzed diet quality of children ages 4-18 based on National Health and Nutrition Examination Survey (NHANES) data which found that at all ages less than half of children met recommendations for fatty acid intake per 1000 calories.[99]

We did not see a significant difference in energy intake between the two groups, though both groups reflected lower than recommended energy intake in about 40% of participants. While it is possible that our results are a true reflection of reduced energy intake in adolescents, similarity between the groups suggest that it is likely not related to adherence to a GF diet. These results may be a reflection of overall poor dietary habits which have been observed in older children and adolescents in the US[99,100] and is also likely influenced by underreporting of energy intake which is well-documented in self-reported dietary studies in both adults and children.[101]

Only 33% of those in the celiac group met recommendations for carbohydrate intake compared to 62% in the control group. In a similar study by Mariani et al., the nutritional habits of 47 adolescents with and without CeD in Italy were assessed using 3-day diet diaries. Intakes were compared to Italian and American RDAs and results analyzed with consideration to GF-diet adherence in the CeD group. Similar to our results, the study found that all three groups had lower than recommended intakes of carbohydrates, iron, calcium and fiber intakes suggesting that such dietary inadequacies are at least in part reflective of adolescent diets in industrialized nations. This observation is consistent with the Scientific Report of the 2020 Dietary Guidelines Advisory Committee which found that not only are calcium and fiber under consumed by all Americans, but iron is also under consumed by adolescent females (ages 12-19 years).[99] Interestingly, Mariani et al. concluded that CeD participants adhering to a strict GF-diet experienced the most unbalanced nutrition pattern with significantly lower carbohydrate and fiber intake but significantly higher intakes of iron and calcium compared to their CeD peers who did not adhere to a GF diet.[21] Similarly, our study reveals a somewhat more unbalanced nutrition pattern in the celiac group compared to the control group.

While no significance differences in carbohydrate intake was found between the two groups in our study, it is possible our study was statistically underpowered, and that significance may have been reached if the groups were larger. Lower carbohydrate intake in the celiac group is not surprising considering that the majority of foods containing gluten tend to come from common sources of carbohydrates, such as breads and pastas, as well as snack foods including crackers, cookies and pastries. Though our study suggests that GFPs contribute nearly 1/3 of energy in the diet, given the use of alternative flours and extra ingredients used to make them more palatable, it is likely that GFPs contain a higher ratio of fat to carbohydrates than their gluten-free counterparts. This difference in ingredients could influence overall macronutrient ratios and may also contribute to a more unbalance nutritional pattern.

Study Feasibility

Feasibility of the project was dependent on multiple factors. First, gaining access to an appropriate control group was a challenge. We initially applied to partner with the Odessa Brown Children's Clinic but were denied as they are a common control site for clinical research and were not able to accommodate our study at the time. We then applied to work with the Seattle Children's Hospital orthopedic clinic and were approved. This process delayed the recruitment

process by about two months and resulted in a discrepancy in timing between recruitment of the two participant groups. Recruitment from the orthopedic clinic also required a large amount of pre-planning and coordination with clinical managers, physicians and physician assistants at a site unfamiliar and geographically distant to the study researcher and coordinator. This also introduced more inconsistency to the recruitment process as the study was introduced by a number of different providers with varying levels familiarity with the project whereas eligible celiac patients were introduced to the project by a single provider who was personally involved in the project. Further complicating the recruitment process was timing of recruitment which took place during or after a clinic visit, resulting in some families feeling they did not have the time to complete the consent/assent process, despite their interest in participating in the study. In other cases, the consent/assent process was rushed to accommodate participant schedules. In future studies, ensuring sufficient time to complete the consent/assent process and thoroughly orient participants to the food record booklet should be prioritized to both improve participant retention and ensure inclusion of necessary details in completed food records. Future studies would also benefit from consideration of alternative methods for collecting food records by utilizing technology to ease the process for participants and ensure that all the required information is captured for each food item.

Participant retention is another consideration for similar studies in the future. While recruitment proved easier than anticipated with a high level of interest from eligible patients in both groups, the dropout rate was also higher than expected. We initially anticipated a dropout rate of approximately 20% and planned our recruitment dates and goals accordingly. Unfortunately, we had a dropout rate of 41%, requiring additional recruitment days and ultimately, we were unable to obtain our goal of 40 food records within the timeframe of the study. In future studies, a higher dropout rate should be anticipated with recruitment taking place over a longer period of time. In addition to increasing the number of participants, this would also reduce bias introduced by seasonality, particularly if recruitment were to be completed over the period of one full year.

Recruitment and retention were likely improved by the small amount of remuneration that was offered for participation in the study. Several participants expressed excitement about a way to earn some money during the summer. Unfortunately, gaining approval to send checks to individual participants proved challenging due to logistical issues with the Grants and Contract Administration office, resulting in a delay in payment – of several months in some cases – to participants. Ensuring that these processes are in place prior to starting recruitment would prevent this issue and foster better relationships with participants in future studies.

A final notable challenge was gaining access to comprehensive nutrition data for specialty GFPs. Unfortunately, this information was not available in the version of the FoodPro database that we had expected to utilize. A database upgrade was determined to be necessary partway through the project, but the process was delayed for approximately 8 months and ultimately necessitated re-entry of all of the celiac group diet diary data. Unfortunately, even with the upgrade, USDA/FNDDS equivalents for many of these products were not available, introducing some data inaccuracy. Ensuring that accurate nutrition data can be obtained for unique products prior to starting a project will be essential for the success of future studies.

Strengths & Limitations

This study is one of the first to attempt to quantify frequency of exposure to specific food additives and is the first to do so in a population with CeD and with a control population for comparison. While several studies in the EU have attempted to quantify food additive intake based on population-level survey data, this study is the first use individual self-reported 3-day food records. This study is also the first of its kind to use a combination of two datasets to characterize differences in diet quality and food additive intake based on the role of processed foods and GFPs in the diets of celiac and control populations. These data provide novel insights into the impact of a diet high GFPs on food additive exposure frequency in the CeD population and are the first to suggest an association between GFP intake and food additive exposure frequency independent of processed food intake. These results have the potential to inform the design and focus of future studies and have set the stage for additional research regarding the impact of specific food additives on the health of individuals with or susceptible to CeD.

Some limitations should be also noted. First, the sample of participants was fairly homogenous with 68% of participants identifying as Caucasian and 100% and 69% identifying as female in the celiac and control groups respectively. While we expected a higher proportion of female participants in the celiac group considering that approximately 60-70% (1.85-3.6:1) of those diagnosed with CeD are female,[102,103] that no males were included in this

group limits generalizability to the celiac population and comparability between the two groups. Additionally, because celiac participants were recruited from the celiac clinic and celiac support groups, it is possible that they represent a subset of those with CeD who are more closely monitored and thus more conscious of their lab values and nutritional choices compared to the other children with CeD.

Second, the accuracy of the results is limited by both the availability of data in the FoodPro and the quality of the food records obtained. Self-reported food intake always involves some bias due to a tendency to underreport intake as well as some degree of inaccuracy based on participants' ability to estimate quantity of foods eaten. In this study, we were faced with the additional challenge of missing or incomplete data. Nearly 27% of processed foods recorded did not specify a brand name. In such cases, it was not possible to obtain ingredient information from food labels. Rather than remove these items from the processed food database, the authors chose to use pre-determined, common brand equivalents for those items which may have resulted in a slight over- or underestimate of food additive content. Analysis of nutrition quality was similarly impacted as not all brands were available in the FoodPro database. In order to obtain micronutrient information, USDA or FNDDS equivalent items were prioritized where possible, adding a degree of inaccuracy to the nutritional analyses.

Third, the items reflected in the food records may have been influenced by availability of certain foods due to seasonality and/or geography, which may also limit generalizability of the results. Diet records were returned with dates between June 2019 and January 2020 and therefore may not reflect year-round average intake. It is also possible that some participants completed their diet records while on summer or holiday vacation while others may have been attending school, which could impact eating habits. Overall, a comparable number of participants from each group – 60% and 62% from the celiac and control groups respectively – were recorded during the summer months (June-August) and may therefore minimize some differences between the groups. Geography should also be considered as this research was conducted in Seattle, a metropolitan area where GFPs are readily available. The contribution of GFPs to the diet in other regions of the US or in other countries could be quite different.

Finally, this was a small study with data from only 28 participants which may have limited our ability to reach statistical significance. The cross-sectional design does not allow for comparison of diet composition before and after starting a GF diet, which would provide an improved understanding of whether diagnosis with CeD changes food choices in a way that impacts the proportion of processed foods and food additives consumed. This design also negates our ability to make any claims regarding causality. Finally, without serological data, we were only able to estimate potential for micronutrient deficiency based on short-term dietary intake rather than assess actual deficiency; nor did this study allow for assessment of mucosal health as it relates to dietary choices.

Conclusion

Through this study, we were able to gain insight into the nuances of the GF diet and the role that GFPs have come to play. Such an understanding has the potential to inform dietary recommendations for children with CeD to prioritize choosing naturally gluten-free, minimally processed products. This guidance is consistent with current US Dietary Guidelines which emphasize limiting saturated and trans fats, added sugars, and sodium. However, such recommendations should be approached with caution in the celiac population to avoid the burden of unnecessarily restricting foods or inciting an unfounded fear of food additives, especially considering that an association between food additive exposure and health outcomes has yet to be established. To better inform these recommendations and tie them to improved health outcomes, future research should focus on the impact of food additives most commonly ingested by those with CeD, such as xanthan gum and soy lecithin, in animal models with genetic predisposition to CeD. Studies should also compare mucosal healing in children following diets that are high and low in GFPs/food additives based on markers of intestinal inflammation and tight junction permeability. Finally, to better understand the level of exposure to food additives and possible health implications, more information is needed regarding food additive and micronutrient content, particularly in the case of specialty food items, such as GFPs. Development of a national food additive database containing food additive content by weight for all manufactured food items, and inclusion of more specialty products in existing databases should be prioritized. Evaluation of the quantity of food additives consumed rather than the number of exposures would allow for improved accuracy and evaluation of dose-dependence in future studies.

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