Folate Content of Gluten Free Food Purchases and Child Dietary Intake in Households with Children with Celiac Disease

by

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Abstract

Celiac Disease (CD) is gastrointestinal autoimmune disorder that can only be treated by the Gluten Free Diet (GFD). Children with CD on the GFD may face macro-and micronutrient imbalances (1-3) due to the high fat/sugar and low micronutrient content of many processed Gluten Free (GF) foods. (4, 5) Information is limited regarding the folate content of food purchases in households with children who have CD and follow the GFD. The study objective was to examine folate content and intake of GF-foods purchased by households with children with CD. The presented study is a secondary, cross-sectional analysis of households with children with CD (n=73) on the folate content of food purchases and the dietary folate intake of children with CD following the GFD (n=78). Median (IQR) age and family size was 10.5 years (8-14) and 4 people (4-5), respectively. Thirty six percent of families had a positive family history of CD. Naturally occurring and certified GF food made up 76% (n=12,460) of food purchases with the remaining 24% (n=4,010) of products containing gluten. Median folate content of purchased foods was 15µg DFE/100g weight of food (IQR: 5-36) with legumes being the most folate-rich food item. Twenty-two children (n=22, 29%) met the folate EAR with low folate intakes (198µg DFE [IQR: 138-259]). Higher folate intake/folate content of GF-food purchased was related to maternal education (university or above) and younger child age. Supplementation with 400µg DFE can provide enough to help all children and adolescents meet the EAR for folate for age and sex. Low folate intake/folate content of household GF-food purchases highlights the increased risk of folate deficiency. Consideration for supplementation or folate fortification policies is needed for the benefit of children with CD on the GFD.

Preface

This thesis is a secondary data analysis of original work done by Dr Mager's research group and Amanda Liu. The research project described in this thesis was part of a larger study approved by the University of Alberta Research Ethics Board under the name: "How do parents and child's perceptions of quality of life affect dietary adherence to the gluten free diet and nutritional intake in children with Celiac Disease?", No. Pro00033867, November 7, 2012.

No part of this thesis has been previous published. Amanda Liu *BASc* was responsible for the thesis write up. Dr. Diana R. Mager *PhD, RD* was responsible for and supervised all phases of the research, from study design to the thesis preparation and feedback for Amanda Liu *BASc*. Dr. Diana R. Mager *PhD, RD* and Dr. Sven Anders *PhD* co-developed the research question and study design. Dr. Diana R. Mager *PhD, RD* supervised data collection and analysis. Kristin Harms *BSc*, Samantha Cyrkot *RD*, Chelsea Kamprath *BSc*, Dr. Diana R. Mager *PhD, RD*, and Amanda Liu *BASc* implemented the study and recruited participants. Dr. Justine M. Turner *PhD, MD*, Dr. Margaret Marcon *MD*, Dr. Catherine Walsh *MD*, Esther Assor *RD*, Inez Martincevic *RD MSc*, Heather Mileski *RD*, and Dr. Herbert Brill *MD* assisted in participant recruitment at their respective clinics. Kristin Harms *BSc*, Samantha Cyrkot *RD*, Chelsea Kamprath *BSc*, Amanda Liu *BASc*, and various volunteers were responsible for data entry. Samantha Cyrkot *RD*, Chelsea Kamprath *BSc*, and Amanda Liu *BASc* were responsible for auditing the data. Samantha Cyrkot *RD* was responsible for the secondary audit. Dr. Diana Mager *PhD, RD* and Amanda Liu *BASc* were responsible for the statistical analysis. Dr. Diana R. Mager *PhD, RD*, Dr. Sven Anders *PhD*, Samantha Cyrkot *RD*, Chelsea Kamprath *BSc*, Kristin Harms *BSc*, Amanda Liu *BASc*, Heather Mileski *RD*, Dr. Jenna Dowhaniuk *MD*, Roseanne Nasser *RD MSc*, Dr. Margaret

Marcon *MD*, Dr. Herbert Brill *MD* and Dr. Justine M. Turner *PhD, MD* contributed to the interpretation of the results.

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List of Abbreviations

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ANGCY: Alberta Nutrition Guidelines for Children and Youth

ATTG: Anti-tissue Transglutaminase

CD: Celiac Disease

CNF: Canadian Nutrient File

DFE: Dietary Folate Equivalents

DRI: Canadian Dietary Reference Intakes

DV: Percent Daily Value

EAR: Estimated Average Requirements

GF: Gluten Free

GFD: Gluten Free Diet

IQR: Interquartile Range

RDA: Recommended Dietary Allowance

UL: Tolerable Upper Intake Level

USDA: United States Department of Agriculture

Presentation of Work within Thesis

Presentations

Diana M, Radmanovich K, Liu A*. Eating Healthy on the Gluten Free Diet. Nutrition for Optimum Health: Eating the Gluten Free Diet. Edmonton, AB, Canada May, 2017.

DRM presented 70% on Celiac Disease, FODMAPs, and the nutritional impacts of the Gluten Free Diet. KR presented 15% on the diet quality of the Gluten Free Diet. AL presented 15% on the Gluten Free Diet and the nutritional concerns.

Abstracts

Liu A, Marcon M, Turner JM, Harms K, Mager DR. Health professional and community perceptions regarding content development for a Gluten Free Diet Nutrition Guide. Women and Children's Health Research Institute Research Day 2018, Edmonton, Canada. October 2018. Poster Presentation.

University of Alberta Ethics ID: Pro00065489. DRM and JMT co-developed the research question and study design. DRM supervised the data collection and analysis. KH and AL implemented the study. DRM and AL were responsible for statistical analysis and coding of themes. DRM and AL drafted the abstract. MM, JMT, KH, DRM and AL contributed to the interpretation of results and approved the final abstract. AL created the poster. JMT, DRM and AL approved the final poster. (AL contributed to 25% of the final product.)

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University of Alberta Ethics ID: Pro00065489. DRM and JMT co-developed the research question and study design. DRM supervised the data collection and analysis. KH and AL implemented the study. DRM and AL were responsible for statistical analysis and coding of themes. DRM and AL drafted the abstract. MM, JMT, KH, DRM and AL contributed to the interpretation of results and approved the final abstract. AL created the poster. JMT, DRM and AL approved the final poster. (AL contributed to 25% of the final product.)

Liu A, Marcon M, Assor E, Mahmud F, Turner JM, Mager DR. Dietary intake and micronutrient supplementation in youth with Celiac Disease with and without Type 1 Diabetes. Alberta Diabetes Institute Research Day 2017, Edmonton, Canada. October 2017. Oral Presentation.

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Liu A, Marcon M, Assor E, Mahmud F, Turner J, Mager DR. Dietary intake and micronutrient supplementation in youth with Celiac Disease. Pediatric Research Day 2017, Edmonton, Canada. May 2017. Poster Presentation.

University of Alberta Ethics ID: Pro00048602. DRM, JMT, and MM developed the research question and study design. EA, DRM and AL implemented the study. DRM and MM supervised the data collection. DRM supervised the data analysis. DRM and AL were responsible for statistical analysis and coding of themes. DRM and AL drafted the

abstract. MM, EA, FM, JMT, DRM and AL contributed to the interpretation of results and approved the final abstract. AL created the poster. JMT, DRM, and AL approved the final poster. (AL contributed to 25% of the final product.)

Published Papers

Liu A, Marcon M, Assor E, Mahmud F, Turner J, Mager DR. (2018). Dietary Intake and Micronutrient Supplementation in Youth and Celiac Disease with and without Type 1 Diabetes. Can J Dietetic Practice and Research, 79(3):118-124.

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Mager DR, Liu A, Marcon M, Harms K, Brill H, Mileski H, Dowhaniuk J, Nasser R, Carroll MW, Persad R, Turner JM. (2019). Diet patterns in an ethnically diverse pediatric population with Celiac Disease and chronic gastrointestinal complaints. Clin Nutr ESPEN, 30:73-80.

University of Alberta Ethics ID: Pro00033867. DRM developed the research question and study design. KR, HM, RN, and AL implemented the study. DMR, MM, JMT, and HB supervised the data collection. DRM was responsible for the statistical analysis. DRM drafted the manuscript. DRM, MM, HB, AL, KR, HM, RN, AA, CMW, JY, RP and JMT contributed to the interpretation of results and approved the final manuscript. (AL contributed to 5% of the final product.)

Chapter 1: Literature Review

1.1 Introduction

Celiac Disease (CD) is a gastrointestinal autoimmune disease where the consumption of gluten causes damage to the small intestinal lining (**Figure 1.1**). (6) The treatment for CD is lifelong adherence to the Gluten Free Diet (GFD), which requires the elimination of gluten-containing grains such as wheat, barley and rye. The restrictive nature of the GFD may result in nutritional imbalances. High levels of saturated fat and sugar, and low levels of micronutrients, particularly iron, vitamin D, and folate, have been documented in children and adults following the GFD. (7) Reliance on processed Gluten Free (GF) food products help patients adhere to the GFD but may not have a balanced nutritional profile that can provide adequate nutrients. (5) This may also contribute to nutrient imbalances, particularly as these foods are often high in sugar and fat. (4, 8, 9)

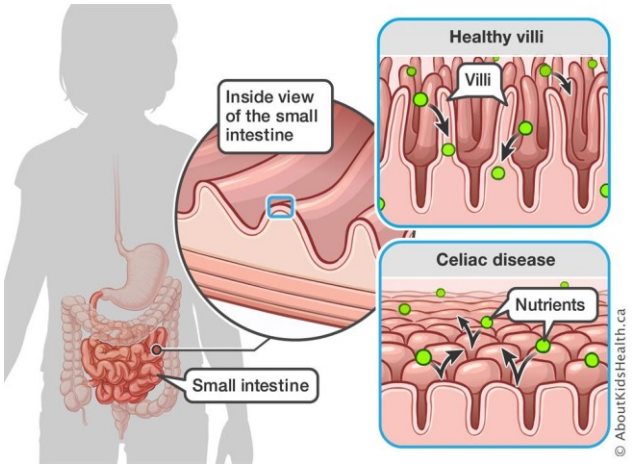

Figure 1.1: Presentation of the Small Intestinal Villi in Celiac Disease. The consumption of gluten causes an autoimmune reaction in Celiac Disease, which results in damage to the villi in the small intestine. In normal, healthy villi, nutrients can normally pass through the membrane and into the body. In Celiac Disease, the villi decrease the surface area available for nutrients to pass through making it difficult for the body to absorb enough nutrients influencing growth and development in children. (10, 11)
Reproduced from: Celiac Disease, Walsh et al. (10) with permission from AboutKidsHealth.

Folate and folic acid are B vitamins that play a role in metabolizing amino acids and DNA synthesis in cells. (12) Folic acid refers to the oxidized form of the vitamin, commonly found in supplements and fortified foods (**Figure 1.2**). (12) Folate is the reduced form of the vitamin and found in many naturally GF foods, such as spinach and eggs (**Figure 1.3**). Limited information is available on the folate content of processed GF foods due to the lack of folate information on nutritional labels in Canada. Commonly utilized processed GF grain flours (i.e. rice and corn) are low in folate making it difficult to determine the total folate provided in processed GF food items. (13) It is known that the processed GF food items available in Canadian retail stores are not nutritionally superior to their gluten containing counterparts. (4) Little is known regarding the micronutrient content of GF food items and naturally GF food items purchased by households with a child diagnosed with CD following the GFD. The background to this thesis work will focus on the nutritional limitations of the GFD in children and adults with CD in relation to dietary folate intake and the folate content of commonly purchased GF foods.

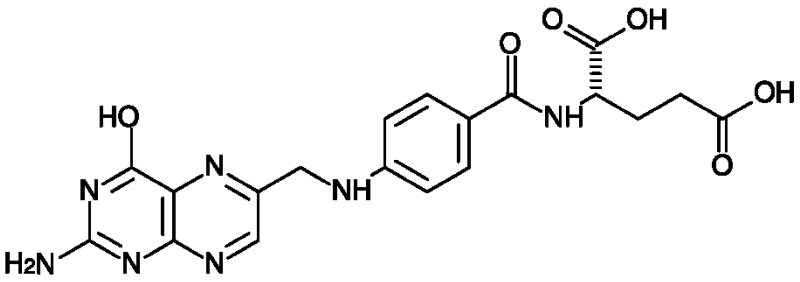

Figure 1.2: Folic Acid Chemical Structure. Folic acid is the oxidized form of folate, a water-soluble B vitamin. The basic chemical structure of folic acid includes one glutamate. The body's uptake of folic acid is easier than that of folate from natural food sources. (12, 14) About 70% of the folic acid consumed is utilized by the body. (15) Folic acid is most commonly used in supplements and for food fortification. (16)
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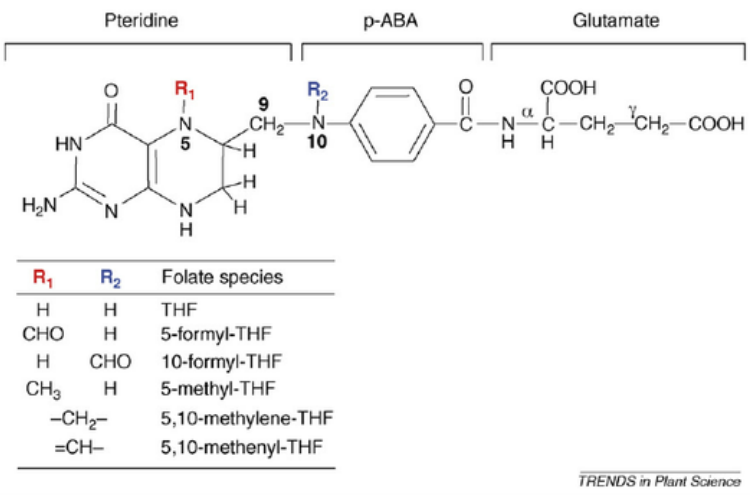

Figure 1.3: Common Structural Forms of Folate found in Food. Folate is the reduced form of folate, a water-soluble B vitamin. The chemical structure for folate can contain several functional groups and multiple glutamates. (17) Folate from food sources are not as bioavailable as folic acid, as is reported to be 80% that of folic acid. (18) Naturally occurring folate-rich food sources include green leafy vegetables, legumes and eggs. (12)
Reproduced with permission from: Bekaert S, Storozhenko S, Mehrshahi P et. al. Folate biofortification in food plants. Trends in Plant Science, 2008, 13, 28-35p. (17).

1.2 Celiac Disease and the Gluten Free Diet

1.2.1 Celiac Disease

CD is a gastrointestinal autoimmune disease where the consumption of gluten causes an immune reaction that damages the villi in the small intestine (**Figure 1.1**). (19) In western countries, CD has an estimated prevalence of 1% of the population. (6) Genetically susceptible individuals, individuals with a first-degree relative diagnosed with CD, or who have Type 1 Diabetes have an increased risk for CD. (6) Typical symptoms include abdominal pain, diarrhea, constipation, dermatitis herpetiformis and growth delay. (6) In CD, when gluten is consumed, it increases the permeability of the intestinal lining, allowing more gluten proteins to pass through. (20) Gluten is then broken down by tissue transglutaminase, this process of deamidation allows the gluten to bind to HLA-DQ2/DQ8, activating the cytotoxic CD4+ T cells, and attracting the

CD8+ lymphocytes to attack the intestinal lining. (20) Damage from the CD8+ lymphocytes causes the villi to flatten, reducing the surface area required for nutrient digestion and absorption. (6, 21) **Figure 1.1** shows a comparison of the villi in a healthy gut in contrast to CD. Damage to the villi causes malabsorption that may lead to nutritional deficiencies. Once on the GFD, the removal of gluten exposure removes the autoimmune reaction that causes damage to the small intestinal villi. (11) The GFD will allow the villi to return to health, restoring their normal length and absorption capacity, which will help correct any nutritional deficiencies. (22)

CD is diagnosed with an initial screening serological test, such as anti-tissue transglutaminase IgA (ATTG) or anti-endomysium IgA (EMA). (6) To confirm the diagnosis after positive serological test results a small intestinal biopsy is conducted and evaluated according to the Marsh criteria. (6) Total villous atrophy represented by a Marsh classification of 3 is the gold standard for the diagnosis of CD. (6) The treatment for CD is strict life-long adherence to the GFD, which will allow the intestine to heal. Dietary therapy is currently the only proven treatment for CD. (6)

1.2.2 The Gluten Free Diet

The GFD is a dietary restriction that excludes gluten containing food sources. Wheat, barley, rye and food items made from these components are removed from the diet and replaced with food items that do not contain gluten. **Table 1.1** includes examples of GF grains and ingredients that may be included in the GFD. **Table 1.2** includes a list of gluten containing grains that should not be included in the GFD. Many gluten containing food products, such as bread, pasta and baked goods, have GF counterparts that make it easier for patients to adhere to the GFD. (21) The GF grains and ingredients found in **Table 1.1** can be found in many processed

GF food products in various combinations. (13) Adherence to the GFD can affect disease activity as even low levels of gluten intake may still cause an autoimmune reaction. (6, 11) However, adherence to the diet can adversely impact nutritional adequacy. Greater adherence to the GFD seems to be associated with greater chances for nutritional deficiencies due to the unbalanced nutritional profile of the diet and deficient nutrient intakes currently seen in CD patients. (8, 23) This will be discussed further in the next section.

Table 1.1: Examples of Gluten Free Grains and Ingredients.

Gluten Free Grains	Gluten Free Ingredients
Amaranth	Arrowroot
Buckwheat	Flax
Corn/cornmeal/corn flour	Nut Flours (almond, walnut, cashew)
Millet	Soy Flour
Quinoa	Potato Flour
Rice	Bean Flours (chickpea, lentil, pea)
Sorghum	Cassava Flour/Tapioca
Oats*	Sago
Teff	Taro
*There is a potential for Oats to be cross-contaminated with wheat or other gluten containing grains in Canada, so only certified Gluten-Free oats can be considered gluten free.	

Adapted from Walsh et al., Celiac Disease, (11) and Gluten Free Eating, Canadian Celiac Association (24) and used with permission from AboutKidsHealth and the Canadian Celiac Association.

Table 1.2: Examples of Gluten Containing Grains.

Gluten Containing Grains not Allowed on the Gluten Free Diet		
Wheat (bran, flour, germ)	Durum flour	Graham flour
Bulgur	Einkorn	Emmer
Farro	Kamut	Spelt/Dinkel
Barley	Rye	Triticale

Adapted from Walsh et al., Celiac Disease, (11) and Gluten Free Eating, Canadian Celiac Association (24) and used with permission from AboutKidsHealth and the Canadian Celiac Association.

1.2.3 Nutritional Limitations of the Gluten Free Diet

Current research has highlighted multiple macro- and micro-nutrient imbalances in patients with CD following the GFD. (2, 21) The unbalanced nutritional profile of the GFD is of particular concern for CD children who must adhere to the diet for life, increasing the risk for nutrient deficiencies (6) or cardio-metabolic dysregulation. (25) Mager et al. found higher intakes of total energy, carbohydrates, fat, and total sugar in children following the GFD when compared to healthy controls. (8) Similar findings of higher macronutrient and sugar intake were seen in the adult CD population. (3, 26) There is conflicting evidence for low fiber intake for children and adults on the GFD. (2, 26, 27) Lower intakes of vitamin D, calcium, iron, B vitamins, and folate have been documented on the GFD. (8, 26, 28-31) High intakes of saturated fats and sodium in children have been documented by Penagini et al. and Mager et al. (8, 27), although not by all. (2) The nutritional deficiencies seen in pediatric patients are similarly seen in adult patients, which may indicate a continuation of the nutritional limitations of following the GFD for life. (31) Mager et al. has utilized cluster analysis to analyze the nutritional intake of children on the GFD, and found that children tend to consume processed meats and high fat dairy and limited amounts of fruits and green leafy vegetables. (8) Children on the GFD who had relatively healthier diets consumed more whole grains such as white rice and corn. (8) Family ethnicity also seems to be associated with adherence on the GFD, with better adherence seen in families of specific ethnic backgrounds, including Indian, Pakistani, and Sri Lankan. (1)

Recent research has focused on the nutritional profile of GF processed food products as a possible explanation for the nutritional imbalances found on the GFD. When compared to their gluten-containing counterparts, processed GF food products were found not to be nutritionally superior. (4) Mager et al. has documented low intakes of green leafy vegetables and legumes,

and reliance on processed GF food products in children following the GFD (8), thus, folate intake is at a particular disadvantage as the mandatory folate fortification in Canada excludes GF food products. (29) Processed GF food products are an important component of the GFD and the low folate content may put children and women of childbearing age with CD at risk for nutritional deficiencies.

1.3 Folate Requirements

1.3.1 Structural Forms and Uses of Folate

Folate is a water-soluble B vitamin that exists in multiple forms. When folate is consumed, it will be hydrolyzed in the gut before storage in the liver, bloodstream, and body tissues. (32) The term folate refers to the reduced from of the vitamin naturally found in green leafy vegetables, legumes and eggs. (12) The basic structure of folic acid and folate are shown in **Figure 1.2** and **Figure 1.3**. One particular form of folate is folic acid, which is the oxidized form of the vitamin that is added to fortified foods and used in vitamin supplements. (12) Folic acid is used in enriched wheat flours and breads in Canada. (33) The bioavailability of folic acid is much higher than folate due to the less complicated uptake of folic acid in comparison to folate in naturally occurring foods. (12, 14) The bioavailability of folate in foods is found to be 80% that of folic acid (34) and about 70% of the folic acid ingested becomes utilized by the body. (15) Folate plays a role in the metabolism of amino acids and acts as a precursor for nucleic acid metabolism. The vitamin's importance in nucleic acid metabolism makes it important in the prevention of neural tube defects in women of childbearing age. (32)

1.3.2 Folate Requirements for Children and Youth

Folate requirements for children and adolescents are presented in **Table 1.3**. Folate recommendation levels for children were extrapolated from adult recommendations made from studies in adult erythrocyte and plasma folate concentrations. (32) Higher recommendation levels for pregnant and lactating women were based on the prevention of neural tube defects, and derived from observational studies looking at neural tube defect risk at varying folate intake levels. (32) The Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) are measured in Dietary Folate Equivalents (DFE). (12, 32) Measuring folate intake in DFE takes into account the differing bioavailability of food folate and synthetic folic acid. (12) The folate DFE is measured as follows: 1 DFE=1µ food folate=0.6µg folic acid in fortified foods=0.5µg folic acid in supplements. (35) The Tolerable Upper Intake levels (UL) were set for synthetic folic acid, as the bioavailability is higher than that of food folate. (12) The folate UL for children was derived from adult UL determined using case reports of patients given oral folate supplements with vitamin B12 deficiency. (32) Folate toxicity has not been associated with food consumption nor has it been documented in children. (32) Nutrient deficiency may occur at time of CD diagnosis and while following the GFD, thus folate supplementation may be needed to compliment the low folate content of the GF food supply. (21, 36, 37) Once the small intestinal lining is healed on the GFD, absorption should normalize. (22, 38)

Table 1.3: Dietary Reference Intake Recommendations for Folate by Age for Males and Females (DFE μg/day)

Age (years)	Estimated Average Requirement (EAR)	Recommended Dietary Allowance (RDA)	Tolerable Upper Intake Level (UL)
4-8*	160	200	400
9-13*	250	300	600
14-18*	330	400	800
Pregnancy (≤18-50)	520	600	800-1000
Lactation (≤18-50)	450	500	800-1000

DFE = Dietary Folate Equivalents. *Includes males and females within the age range.

Adapted with permission from: Dietary Reference Intakes, Health Canada. (35)

Folate intake that consistently does not meet the recommended levels of intake will increase the risk for folate deficiency. (32) Folate deficiency is defined as a serum/plasma folate concentration lower than <10 nmol/L or an erythrocyte folate concentration lower than <340 nmol/L. (39) Since folate has an important role in amino acid and nucleic acid metabolism deficiency can lead to complications impacting the physical and mental development in children, further impacting future health. (40) Both vitamin B12 and folate are needed to form 5, 10-methylenetetrahydrofolate for thymidylate synthesis and deficiency of either vitamin can lead to megaloblastic anemia making it important to correctly identify the deficient nutrient. (32) The risk for intrauterine complications may be greater in pregnant women with CD possibly due to nutrient malabsorption from remnant villi damage (41), which is further complicated with the lack of folate intake seen on the GFD. (28) Undiagnosed CD can lead to spontaneous abortions and miscarriages (42), as seen in a 1971 case study where two women had 11 recurrent abortions total for six years each before being diagnosed with CD. (43) Folate deficiency and malabsorption due to the villi damage from CD was suspected to be the main culprit. (43) Folate deficiencies were also seen in a 2019 case report of a 26-year old pregnant woman with

preeclampsia and anemia who was subsequently diagnosed with folate deficiency and CD despite taking folate supplements. (44) For pregnant women folate deficiency places them at risk for infertility and reproductive issues (see **Table 1.4**). The risk for folate deficiency extends into childhood, as the GFD requires lifelong adherence (40) manifesting as developmental complications leading to increased medical burden on the child (see **Table 1.4**).

Table 1.4: Clinical Conditions Associated with Folate Deficiency in Women, Children and Adolescents with Celiac Disease

Clinical Conditions Associated with Folate Deficiency in Women with CD		
late menarche	premature menopause	secondary amenorrhea
recurrent miscarriages	unexplained infertility	
Clinical Conditions Associated with Folate Deficiency during Pregnancy with CD		
megaloblastic anemia	pre-term birth	stillbirth
intrauterine growth restriction	neural tube defects	miscarriage
low birth weight	small gestational size	
Clinical Conditions Associated with Folate Deficiency in Children and Adolescents*		
megaloblastic anemia	developmental delay	cognitive impairment
chronic diarrhea	failure to thrive	psychological impairment
mouth ulcers	seizures	
CD=Celiac Disease * The list of medical conditions may be seen in any child with folate deficiency and may apply to children and adolescents with CD who have chronically low folate intakes.		

Adapted with permission from: [Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline], Pitkin RM, Allen LH, Bailey LB, et al., National Academy Press, 2000, 196-305. (32), [Lanzkowsky's Manual of Pediatric Hematology and Oncology (6th Ed).], Lanzkowsky P, Academic Press, 2016, 84-101. (40), and Saccone G, Berghella V, Sarno L, et al. Celiac disease and obstetric complications: a systematic review and metaanalysis. Am. J. Obstet. Gynecol, 2016, 214(2), 225-234. (45)

1.3.3 Folate Intake and Status in Children and Adolescents with Celiac Disease following the Gluten Free Diet

Adherence to the GFD does not guarantee nutritional adequacy. (28) Folate intake is documented to be lower on the GFD than compared to the healthy population. (2, 8, 26, 28, 29, 46) Our research group has found low folate intakes in Canadian children and adolescents with

CD following the GFD when compared to healthy controls, where the majority could not meet the EAR for age and gender. (7, 8) The low dietary folate intake in children could be partially explained by the intake of low folate GF grains, such as rice flour, and limited intake of green leafy vegetables and legumes in the diet. (8) A table on folate intake in children and adults following the GFD is presented in **Table A.1**.

While folate intake on the GFD is well documented, knowledge on folate status in children and adolescents with CD is limited. (47) The research available is largely focused on the adult CD population. When measuring folate status the erythrocyte and plasma folate levels need to be measured over the course of one month to determine long-term status. (39, 48) No current studies exist looking at the long-term folate status of patients following the GFD long-term. **Table 1.5** presents a list of studies that have looked at folate status in children and adults with CD. These studies have only looked at folate concentrations at one point in time after following the GFD for a minimum of 3 months (49) and a maximum of 22 years. (50)

Folate intake during pregnancy with CD is impacted by the recovery of the villi and subsequent nutrient malabsorption. (51, 52) Untreated or undiagnosed CD can lead to folate malabsorption and a nine times increased risk for recurrent miscarriages when compared to treated CD patients. (42) A study of 13 women with CD showed that adhering to the GFD and taking folate supplements stops reoccurring miscarriages. (51) Six of the 13 women (46%) were able to have successful pregnancies after following the GFD for four years, indicating that once the small intestinal villi has healed folate absorption was able to normalize and pregnancy complications dissipated. (51) No research is available on the folate intake or status of pregnant and lactating women with CD; however, folate supplementation is highly recommended due to the micronutrient imbalances seen on the GFD. (41, 52) A Canadian study showed that healthy

pregnant women without CD have a mean plasma folate concentration of 36.4 nmol/L. (53) It is noted that the healthy mothers consumed folate-fortified gluten-containing food products and prenatal multivitamin supplements that contained folate. (53) It is possible that pregnant women with CD may have lower folate status due to the lack of folate fortification of GF food products in Canada (54) and limited folate intake on the GFD. (28) No information exists on the folate content of breast milk from lactating mothers with CD. Research in healthy lactating mothers without CD show that folic acid supplements increase total folate concentration in breast milk by 18% when compared to women who do not supplement. (55) Folate concentration of breast milk in healthy mothers was also found to be positively associated with folate intake in healthy infants at 6 months of age ($P<0.001$). (56) Indicating that folate supplementation may help mothers with CD provide more folate when breastfeeding by filling in the nutrient deficiencies in the GFD (28) and providing additional folate that can be utilized for breast milk. (55)

Knowing the folate status of this population will help health professionals identify patients with a greater risk of nutrient deficiencies earlier, so preventive measures can be taken. If the GFD leads to persistent nutritional deficiencies in the body this may influence childhood growth and development leading to further complications in adulthood. For women of child bearing age knowledge of folate status will help health professionals provide appropriate treatments and support.

Table 1.5: Studies on Folate Status in Adults and Children with Celiac Disease

Authors	Year	Country	Sample Size	Age Range	Serum, Plasma or Erythrocyte Folate	Folate Range	Results
Adult Studies							
Burger et al. (50)	2018	Netherlands	n=250	45.5±17.1y	Not Specified	9-36 mmol/L	There was a reoccurrence of folate deficiency after initial recovery on the GFD at a rate of 20% (n=50). (50)
Valente et al. (57)	2015	Brazil	n=20 (CD)	36.3±13.7y	Serum	17.5 ±8.0 nmol/L	6.6% of patients had lower folate concentrations compared to healthy controls. (57)
Dickey et al. (58)	2008	Ireland	n=100 (CD)	54.7±12.4y	Serum and Erythrocyte	25.7±31.5 nmol/L (serum) 1048±791 nmol/L (erythrocyte)	None (0%) of the patients was considered folate deficient. Folate status was lower in patients with continued villi atrophy compared to patients with healthy villi. (58)
Hallert et al. (30)	2002	Sweden	n=30	45-64y	Plasma	10.5 nmol/L (8.3-12.7; Males); 12.6 nmol/L (9.4-15.8; Females)	Low folate concentrations were seen in 20% (n=6) patients. Six additional patients (n=6) were on folate supplementation (prescribed) so folate status was normal. (30)
Kemppainen et al. (59)	1998	Finland	n=40	24-65y (M); 18-62y (F)	Erythrocyte	570±357nmol/L (partial atrophy); 407±156nmol/L (subtotal atrophy) 309±167nmol/L	1 of 5 patients with partial villous atrophy (20%), 4 of 17 patients with subtotal villous atrophy (24%) and 9 of 15 patients (60%) with total villous atrophy had abnormal folate concentrations. (59)

						(total atrophy)	
Children Studies							
Fernández et al. (60)	2019	Spain	n=70 (CD)	4-18y	Plasma	6.7ng/mL (4.3-9.1)	While dietary folate intake was at 67% of the folate recommendations for age and sex, plasma folate concentrations were within normal. (60) The number of children who had deficient folate concentrations was not specified.
Deora et al. (61)	2017	Canada	n=140	7.8±4.0y	Erythrocyte	Not Specified	7.5% (n=3) had sub-optimal folate concentrations after 6 months on the GFD. (61) One child (n=1, 1.5%) was still folate deficient after 18 months. (61)
Wessels et al. (62)	2016	Netherlands	n=182	6.3±4.3y	Not Specified	7.7±1.4 nmol/L	3% of children had deficient folate concentrations at follow-up. Two children (n=2) were folate deficient during their second year of follow-up and were provided with folate supplements. (62)
Nijhawan et al. (49)	2013	India	n=363	19±7y	Serum	Not Specified	22% (n=80) of children with CD had folate deficiency. (49)
Hjelt and Krasilnikoff (63)	1990	Denmark	n=20	1.2-16.6y	Plasma and Erythrocyte	27.5 nmol/L (5.8-54.5, Plasma); 1,467 nmol/L (817-2,260, Erythrocyte)	One patient (n=1, 7%) had abnormal erythrocyte folate after going on the GFD. (63)
GFD=Gluten Free Diet; CD=Celiac Disease; y=years; EAR=Estimated Average Requirement; RDA=Recommended Dietary							

Allowance; UL=Tolerable Upper Intake Level

The folate DRIs for children (male and female), 4-8y, is 160µg DFE (EAR), 200µg DFE (RDA), and 400µg DFE (UL). **(35)** The folate DRIs for children (male and female), 9-13y, is 250µg DFE (EAR), 300µg DFE (RDA), and 600µg DFE (UL). **(35)** The folate DRIs for adolescents (male and female), 14-18y, is 330µg DFE (EAR), 400µg DFE (RDA), and 800µg DFE (UL). **(35)**

Normal ranges for serum/plasma folate levels is 6-20ng/ml (13.5-45.3nmol/L) and for red blood cell folate is >100ng/ml (>226.5nmol/L). **(39)**

Adapted with permission from: Burger JPW, van der Laan JJH, Jansen TA et al. Low yield for routine laboratory checks in follow-up of coeliac disease. J Gastrointestin Liver Dis, 2018, 27, 233-239. (50); Valente FX, Campos Tdo N, Moraes LF et al. B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study. Nutr J, 2015, 14, 1-8. (57); Dickey W, Ward M, Whittle CR et al. Homocysteine and related B-vitamin status in coeliac disease: Effects of gluten exclusion and histological recovery. Scand. J. Gastroenterol, 2008, 43, 682-688. (58); Hallert C, Grant C, Grehn S et al. Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. Alimentary Pharmacology & Therapeutics, 2002, 16, 1333-1339. (30); Kemppainen TA, Kosma VM, Janatuinen EK et al. Nutritional status of newly diagnosed celiac disease patients before and after the institution of a celiac disease diet--association with the grade of mucosal villous atrophy. Am. J. Clin. Nutr., 1998, 67, 482-487. (59); Fernández BC, Varela-Moreiras G, Úbeda N et al. Nutritional Status in Spanish Children and Adolescents with Celiac Disease on a Gluten Free Diet Compared to Non-Celiac Disease Controls. Nutrients, 2019, 11(10), 1-22. (60); Deora V, Aylward N, Sokoro A et al. Serum Vitamins and Minerals at Diagnosis and Follow-up in Children With Celiac Disease. J Pediatr Gastroenterol Nutr, 2017, 65, 185-189. (61); Wessels MM, van V, II, Vriezinga SL et al. Complementary Serologic Investigations in Children with Celiac Disease Is Unnecessary during Follow-Up. J Pediatr, 2016, 169, 55-60. (62); Nijhawan S, Katiyar P, Nagaich N et al. Prevalence of associated disorders in Indian patients with celiac disease. Indian J Gastroenterol, 2013, 32, 330-334. (49); Hjelt K, Krasilnikoff PA. The Impact of Gluten on Haematological Status, Dietary Intakes of Haemopoietic Nutrients and Vitamin B12 and Folic Acid Absorption in Children with Coeliac Disease. Acta Paediatrica, 1990, 79, 911-919. (63)

1.3.4 Folate Content of Gluten Free Foods

Naturally occurring GF food items vary in folate content. (37) Several naturally occurring GF foods have low folate contents such as cucumbers (7µg DFE/100g), apples (3µg DFE/100g) and rice (boiled: 2µg DFE/100g). (37) With the exception of eggs (hard-boiled: 54µg DFE/100g), fresh meat products generally contain little to no folate. (37) The folate content of naturally GF grain varies; commonly used GF grains such as rice (white, cooked: 2µg DFE/100g) and corn (yellow, grits, cooked: 1µg DFE/100g) do not contain much folate when refined and processed. (13) Folate rich food items include green leafy vegetables, eggs, nuts, seeds and legumes, examples and their folate content are presented in **Table 1.6**.

Table 1.6: Examples of Folate Rich Foods and their Folate Content

Food Item	Folate Content (DFE µg/100g)
Asparagus, Boiled	149
Avocado	81
Spinach, Boiled	146
Black Beans, Boiled	149
Lentils, Boiled	181

Adapted and used with permission from: Nutrient Value of Some Common Foods, Health Canada. (37)

In Canada, no mandatory legislation exists requiring manufactures to folate fortify processed GF food products at the same levels in wheat flour. (54) The decision to fortify and at what levels are at the manufacturer's discretion. Recent explorations into the nutritional profiles of processed GF food products show that they are not nutritionally superior to their gluten-containing counterparts. (4) Canadian processed GF grain products (breads, flours and pasta) had documented higher levels of total sugars, saturated fats and lower levels of iron, vitamin B_{12} and folate. (4, 5, 64, 65) However not all nutritional labels on processed GF food products

reported folate, so some items may have unknown amounts of folate inside. (5, 65) These investigations on GF grain products examine a narrow selection of the GF foods children consume on the GFD, as the diet may include other sources of nutrients such as fruits, vegetables, meats and dairy products. (8, 66)

The low folate content seen in some of processed GF grain products may be due to the use of refined rice and corn flours, which are low in folate. (13) **Table 1.7** presents the folate content of commonly used GF flours. When the majority of ingredients consist of low folate flours this may result in a low folate food product that has an imbalanced nutritional profile. (13)

Table 1.7: Common Gluten Free Flours and their Folate Content

Food Item	Folate Content (DFE µg/100g)
White Rice Flour	4
Corn Flour	25
Tapioca Starch	4
Buckwheat flour	54

Adapted and used with permission from: Foschia M, Horstmann S, Arendt EK et al. Nutritional therapy – Facing the gap between coeliac disease and gluten-free food. Int. J. Food Microbiol., 2016, 239, 113-124. (13) and Nutrient Value of Some Common Foods Health Canada (37).

Kulai and Rashid analyzed the nutritional profile of 71 Canadian GF breads, cereals, pasta, baked goods, processed meats and frozen foods. (5) The investigation found that the folate content stated on the nutritional labels of GF food products were lower than their gluten containing counterparts. (5) GF breads had a difference of 11.1%DV when compared to their gluten containing counterparts (22.7%DV [GF] vs. 33.8%DV [gluten containing]). (5) GF pastas were found to have an 89.4% DV difference in folate when compared to their gluten containing counterparts (5.1%DV [GF] vs. 94.5%DV [gluten containing]). (5) A greater number of manufacturers were fortifying their products (six manufacturers fortified their GF cereals) in

Kulai and Rashid's sample, as opposed to Thompson's US sample of GF cereals, which only found three manufacturers who fortified. (5, 67) Jamieson, Weir, and Gougeon's sample of 398 Canadian processed GF food products (bread, cereal, pasta, processed meats, and frozen foods) had similar nutritional content findings to Kulai and Rashid. (65) The iron and folate content of the GF foods analyzed by Jamieson et al. show them to be 55% and 44% less than their gluten containing counterparts. (65) Limited research is available examining the nutritional profiles of Canadian GF food items outside of processed GF grain products such as breads and cereals. While GF grain products are the focus of the GFD, other food items such as fruits and vegetables also play an important role in providing nutrients in the diet and their nutritional quality will influence the nutrient intake on the GFD. (8, 66) Evidence is emerging on the impact from the use of low nutrient GF flours in GF food products and on the potential implications for the GFD. (13) The folate content of common processed GF grain products are presented in **Table 1.8**.

Table 1.8: The Folate Content of Common Processed Gluten Free Grain Products

Food Item	Folate Content (DFE µg/100g)
Corn Pasta, cooked	6
GF Bread, rice-based	36
Pancake, buckwheat (made with eggs, oil and milk)	23
GF Waffles	8
GF Crackers, multigrain/multi-seed	35
Granola, homemade	83

Adapted and used with permission from: Nutrient Value of Some Common Foods, Health Canada (37) and USDA National Nutrient Database for Standard Reference, USDA. (68)

When compared to enriched white bread in Canada (178µg DFE/100g) (37), processed GF grain products fall short in achieving a similar folate density. The lack of uniform fortification policies for GF food products in Canada places individuals with CD at risk for

suboptimal micronutrient intake, particularly for folate. The nutritional quality of the processed GF food products consumed directly influences the diet quality of the GFD (13), which can be further compromised when combined with other dietary restrictions such as vegetarian and lactose-free. (69)

1.4 Supplementation in Children

Supplementation includes the consumption of vitamins, minerals, protein and herbal preparations. (70) Supplement use in Canadian children and adolescents was the highest in children ages 1 to 8 years old at 45% of the population. (71) Older adolescents (14-18 years) are the least likely to use supplements. (71) According to the first cycle of the Canadian Health Measure Survey, a quarter of the Canadian population took folate supplements, with the lowest rates of usage in teenagers and young adults. (72) A study in the United States indicated that 23.6% of CD patients took a dietary supplement. (73) This is in contrast to Ball, Kertesz and Moyer-Mileur's findings of supplement use at 62% among children and adolescents with a chronic illness. (74) Based on a Canada wide internet survey, 77% of children with CD consumed a micronutrient supplement, the most popular being a multivitamin (33%). (75) In another study by Alzaben et al., 66% of children took a vitamin D supplement, providing an additional 379±85 IU/day of vitamin D to the diet. (7) The additional vitamin D supplementation helped increase dietary intake of vitamins D to meet the RDA in 41% of children with CD. (7) Calcium supplementation in the same cohort was able to provide an additional 20-100mg per day increasing dietary intake (1022mg/day [CD] vs. 763mg/day [control]). (7) Supplementation with folate can have the same impact as that seen with vitamin D and calcium. (36, 76) Bailey et al. examined the dietary intake of 7250 children, age 2-18y,

with and without supplementation and found that supplements can help children meet nutritional requirements (magnesium, vitamin C and E) that would otherwise be deficient on diet alone. (77)

Considerable research has been conducted into the motivations for non-prescribed supplementation in children and adolescents. The main reason cited for providing multivitamin supplementation was the belief that they help with chronic illness or athletic performance. (70, 78-80) Our research team has explored the barriers and facilitators for supplement use in children and adolescents with CD and Type 1 Diabetes. Health professional recommendation, parental influences and routine were identified as facilitators in taking supplements; while the lack of knowledge, forgetting, and priority on blood glucose control were the main barriers. (81) Belief in the performance enhancing abilities of supplements such as muscle maintenance and exercise recovery has also been documented in adolescent athletes. (82, 83) Parental belief in the positive use of supplements also greatly influences their use in children. (80, 81, 84) Multivitamin preparations are one of the most popular supplements taken by children and adolescents (70, 79, 84); however, caution is needed on their nutritional content as their levels vary by manufacturer. **Table 1.9** lists several brands of multivitamins that contain folic acid and their levels. As shown, the folic acid content varies greatly and the recommended dosage can further influence folate intake.

Table 1.9: Folate Contents of Popular Gluten Free Pediatric Multivitamins Sold in Canada

Multivitamin	Folate Content per Tablet (µg)	Recommended Dosage
Brand 1	100	4-12 years (1 tablet daily)
Brand 2	50	4-8 years (1 tablet daily) 9-13 years (2 tablets daily)
Brand 3	200	2+ years (1 tablet daily)
Brand 4	300	3-13 years (1 tablet daily) 14-18 years (2 tablets daily)
Brand 5	400	4+ years (1 tablet daily)
Brand 6	100	6+ years (4 tablets daily)

Created by author, Liu, 2019.

1.5 Conclusion

The consumption of gluten triggers an immune response in CD that damages the lining of the small intestine. The GFD is the only known treatment for CD, yet the diet has many nutritional deficiencies that can contribute to micronutrient imbalances, particularly with folate. Folate from food sources come in several forms while folate added to fortified foods and found in supplements is in the form of folic acid. The bioavailability of these folate forms varies, with folic acid being the most bioavailable. (12) Folate bioavailability may influence the ability for children and adolescents to meet folate requirements. Low bioavailability of folate may mean low folate uptake into the body and insufficient levels for cell metabolism leading to complications such as megaloblastic anemia and developmental delays in children. (32) Lower folate bioavailability in foods combined with the low folate content of GF foods on the GFD places pregnant women with CD and their child at greater risk for miscarriages and birth defects. This increased risk for folate deficiency resulting from lowered bioavailability and the low folate content found in GF foods follows into childhood and adulthood for a CD patient.

Naturally occurring GF food items such as spinach and legumes are rich in folate. While processed GF food products have unbalanced nutritional profiles and poor folate content. (4, 5)

Many GF grain products utilize low folate GF flours and ingredients, which leads to a low folate final product. (85) Coupled with the lack of mandatory folate fortification in GF food products seen in gluten containing flours, the available GF foods with high folate contents may be limited. To determine whether the current GFD and food supply is sufficient for adequate folate intake, current folate status in children and adolescents is needed. What is available at retail stores may not necessarily translate into purchases brought into the home. Limited information exists on the folate content of GF household food purchases made in households with a child with CD who is following the GFD. The role supplementation may play in optimizing the folate status of those following the GFD also needs to be explored. The purpose of this thesis was to examine the evidence that relates to folate intake and folate content in GF foods purchased in households with a child with CD following the GFD and what impact this may have on overall folate intake in the child with CD on the GFD.

Chapter 2: Research Plan

2.1 Study Rationale

CD is a gastrointestinal autoimmune condition where the consumption of gluten causes an autoimmune response that damages the small intestine. The only known treatment for CD is life-long adherence to the GFD. Many research groups have noted the GFD for being low in multiple micronutrients, including folate. (7, 26) Folate is a water-soluble vitamin and is essential for the development of red blood cells and the prevention of neural tube defects in newborns. (12, 86) Current evidence highlights the unbalanced nutritional profiles of processed GF food products (4, 64, 65); the resulting unbalanced micronutrient intake may place children with CD at risk for folate deficiency and its associated diseases. Supplementation can help to bridge the gap between intake on the GFD and the nutritional requirements for optimal growth and health; however, poor adherence has been documented. (81) Limited information is available on the impact folate supplementation has on dietary intake on the GFD.

Children who can consume gluten-containing products receive supplementary folate through the mandatory fortification of wheat flours. Folate fortified flours in Canada can provide children a minimum of 100- 200µg/day. (87, 88) The Canadian folate fortification regulations improve the folate content of gluten containing grains and their consumption can provide healthy children an additional 83-96µg of folate per day. (89, 90) However, the mandatory folate fortification regulations do not apply to GF food items and thus children with CD would not receive this boost in folate intake. Relying on the low folate content of commonly used GF ingredients such as rice and corn flour may present a barrier in meeting folate requirements on the GFD. The restrictive nature of the GFD limits folate-rich food sources to green leafy vegetables, legumes, nuts and seeds, which many children on the GFD inadequately consume.

(8) Selective food choices further restrict nutrient intake making it more difficult to meet nutritional adequacy. (2) More information is needed on the folate content of food items available in the home of a child diagnosed with CD following the GFD to explore the influences of the available food supply on the nutritional profile of the GFD. The study objective was to examine the dietary folate intake and the folate content of GF household food purchases in families with children with CD, following the GFD.

2.2 Hypothesis and Objectives

2.2.1 Objectives

a) To describe and quantify the dietary folate intake in children with CD.

b) To describe and quantify the folate content of GF household food purchases in families with children with CD following the GFD.

c) To describe the potential impact of folate supplementation on the dietary folate intake of children with CD (by simulating the inclusion of a multivitamin that contains folate to dietary intake and comparing against the EAR for folate).

2.2.2 Hypothesis

a) Dietary folate intake will be lower than the recommended folate EAR for age and gender in children diagnosed with CD, following the GFD.

b) The folate content of GF household food purchases of processed and unprocessed GF food products will predominately consist of folate-poor sources (≤20µg DFE/100g food).

c) The theoretical addition of a multivitamin supplement that contains folic acid will result in significantly higher dietary folate intakes in children with CD following the GFD

when compared to the dietary folate intake of children who do not take a folate supplement.

Chapter 3: Folate Content of Household Food Purchases, Dietary Folate Intake of Children and Adolescents following the GFD and the Impact of Folate Supplementation

3.1 Introduction

CD is an autoimmune gastrointestinal disease resulting in affected individuals' intolerant to gluten; a glycoprotein found in wheat, rye and barley. The only known treatment for CD is strict adherence to a GFD. (6) Current research shows that many commercially processed GF foods are in fact not nutritionally superior by having lower micronutrient contents and higher fat and sugar contents than their gluten containing counter parts. (4, 5, 91) One major concern associated with the GFD is dietary folate intake. Recent work by our group and others has shown that dietary folate intake in children and adolescents with CD on the GFD are substantially lower than recommended intakes (2, 7, 8, 26), placing the growing child and particularly females of childbearing potential with CD at increased risk of folate deficiency and adverse health and pregnancy outcomes. Common ingredients such as white rice or corn flour found in processed GF foods contain low levels of folate and few are consistently enriched with folate. (13) In contrast, Canada mandates folate enrichment of wheat-based flours resulting in children consuming a minimum of 100-200µg/day of folate above the amounts of naturally occurring folate in other foods. (87, 88) However, this mandatory regulation in Canada does not extend to GF foods, which are considered special dietary foods with folate fortification being voluntary. (54) Many other countries such as the USA and the UK also do not have mandatory fortification policies related to GF grains implemented. (92) The lack of routine folate fortification of processed GF foods brings into serious question if the folate content in the GFD and provided by commercially prepared GF foods purchased by CD households and children and adolescents with CD is adequate and how this may impact over-all folate intake. (3)

The low folate content of GF foods may pose a barrier in meeting folate requirements on the GFD; raising the question whether a mandated folate fortification policy for GF foods or manufacturing changes to the production of GF food products may be needed in Canada. The study objective was to examine the folate content of GF food purchases made by households with children with CD on the GFD, to describe folate intake, and to describe the impact of folate supplementation on the dietary intake of children with CD on the GFD. We hypothesized that folate content of food purchases and dietary intake will be low in households with children with CD. Finally, using our data we created a model simulation to assess the potential benefit of folate supplementation, which we hypothesize would increase dietary folate intake enough to meet folate requirements in children and adolescents with CD.

3.2 Methods

This study is a secondary analysis of data from a larger multi-site, cross-sectional cohort study (2014-2017) examining adherence to the GFD, dietary intake, food costs and health related quality of life in children with CD (n=243) following the GFD. (1) Families were recruited from pediatric celiac outpatient clinics at the Stollery Children's Hospital (Edmonton AB), the Hospital for Sick Children (Toronto, ON) and the McMaster Children's Hospital (Hamilton, ON). A total of 73 households (Edmonton=26, Toronto=23, Hamilton=25) representing 78 children with CD collected food receipts related to retail food purchases (e.g. grocery stores, fast food outlets) and restaurant food purchases for a 30 (IQR: 25-42) day period. (1) All children with CD were between 3 to 18 years old and were diagnosed with CD for more than 6 months via small intestinal biopsy. (93) Children who had food allergies or additional diagnosis known to influence dietary intake (i.e. type 1 diabetes, inflammatory bowel disease) were excluded. The

median number of children per household was 2 children (IQR: 2-3) with 1 child with CD (IQR: 1-1) following the GFD. Informed consent/assent was obtained prior to participant enrollment. This study was approved by the Human Research Ethics Board at the University of Alberta (Pro00033867), The Hospital for Sick Children's Research Ethics Board (#1000048112) and McMaster Children's Hospital Research Ethics Board, MREB (#1107).

3.2.1 Anthropometric and Demographic Data

Clinical data (child sex, child age, anthropometrics, serum ATTG, biopsy findings, age at diagnosis, medical history) were collected from medical health records. Adherence to the GFD was determined by gluten intake (> and < 10 mg/day). (1) Information on family history of CD was obtained from the parent and defined as the child having at least one parent, grandparent, or sibling with clinically diagnosed CD. Family ethnicity was defined according to Stats Canada 2006 census guidelines (94), but was classified as Caucasian vs. Other due to the smaller sample size. (1) Socio-demographic information (parental education, parental age, number of children in the household, number of family members consuming the GFD, household income) were collected at time of recruitment according to standard methodologies as described previously. (1)

3.2.2 Dietary Data (Objective 1)

Two non-consecutive 24-hour food recalls (one week-day/one weekend day) were collected with details on cooking methods. Portion sizes were estimated using measuring cups and spoons, additional information was obtained on the dimensions of the plates and bowls used to help aid estimation. Dietary data was assessed for folate intake using the Food Processor Nutrition Analysis Software (SQL 11.0.124, 2015, ESHA Research, Salem, OR, USA) and when available manufacturer food labels as described previously. (8) Folate intake was measured in

folate DFE (in µg) then compared to the folate EAR and UL of folate for age and sex. (35) Determination of gluten content in processed foods and overall dietary gluten intake was calculated from all grains and grain products using the Osborne method (1, 95), equation as follows:

$$\frac{20 * \text{Weight of Grain Portion of Food}}{1000} = \text{Gluten Content (mg)}$$

The weight of GF foods was multiplied with 20ppm (the maximum acceptable level of gluten for GF foods in Canada) to obtain the total gluten content for the grain product. (1, 95) For "mixed foods" (e.g. pizza, lasagna), the Canadian Diabetes Association exchange system was used to estimate the weight of the grain portion in the formula (1 exchange=30g). (95)

3.2.3 Determination of Folate Content of Household Food Purchases (Objective 2)

Parents were instructed to collect all household food purchase receipts for a 30-day period according to standardized procedures. (96, 97) Purchase data from supermarkets, farmers markets, speciality stores, fast food outlets, school cafeterias and restaurants were all included. (96, 97) Information on purchase date, food item(s), price (in $CAD) of each food item (gross and net) were retrieved from purchase receipts. Non-food expenditures were excluded in the analysis. Individual food items were grouped into 15 categories as described in the appendix (**Table A.2**). (8) Food categories were further separated into naturally occurring GF foods, certified GF foods, gluten containing foods and highly processed food products. Naturally occurring GF foods consisted of mainly unprocessed foods (i.e. vegetables, milk, fresh beef, and rice) and are known to be devoid of the gluten protein. (24) Certified GF foods consisted of products that carry the Canadian Celiac Association GF symbol or a GF statement on the

package. (98) Gluten containing foods consisted of any product made with wheat, barley, rye and their related ingredients as listed on the ingredient label. Highly processed foods consisted of processed and packaged food products made with multiple ingredients. (99) The highly processed category for household food purchase analysis included processed food products where GF status was not clearly identified on the package label. Individual food purchase entries from receipts were collectively tallied to identify the top-3 most frequently purchased food products within each food category.

The Canadian Nutrient File (CNF), food labels and manufacturer information were used to determine folate DFE content (**Table 3.1**). (100) For GF foods where the aforementioned sources did not provide folate content information, the United States Department of Agriculture's (USDA) nutrition database was used. (68) For processed food products, folate content was determined by the first three ingredients listed on the product ingredient label. All individual food purchases were coded for price, package weight, serving size in grams, the Daily Value (DV) or amount of folate per serving, and the total folate content of the product purchased. The Percent DV of 15% for folate (60µg DFE/serving) was used as the cut-off for the definition of a folate-rich food. (101, 102) A Percent DV <5% (20ug DFE/serving) was used to define a folate-poor food. (101, 102)

Table 3.1: Identification of Source Information for Folate Content of Household Food Purchases

Information Collected	Source of Information		
	Fresh & dried fruits, vegetables, meats, eggs, dairy products, and food products that are fairly consistent across brands (i.e. tomato sauce)	Packaged foods without folate on the nutritional label	Packaged foods fortified with folate or with folate on the nutritional label
Package Weight	Weight purchased stated on grocery receipt	Package Label	Package Label
Food Description	.	Package Label	Package Label
Calories	Canadian Nutrient File	Package Nutrition Label	Package Nutrition Label
Serving Size	100 g[1]	Package Nutrition Label	Package Nutrition Label
Folate Content	Canadian Nutrient File	Closest substitute available in the Canadian Nutrient File[2]	Package Nutrition Label

[1]100g was chosen as the standard weight of fresh meats, grains and produce to match standard nutrient analysis weights used in the Canadian Nutrient File.
[2]For example, the folate content for Oreo cookies would be obtained from "Cookie, chocolate sandwich, cream filling, regular; 3819" in the Canadian Nutrient File database.

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Household food purchases were ranked by folate content from highest to lowest within each food category and compared to the %DV to identify folate rich/poor food purchases. To quantify the percent shares of each food category in terms of total household food purchases, the number of purchases made within each food category was divided by the total number of food purchases. To quantify the share of folate-rich foods purchased in each food category, the total number of folate-rich foods in each food category was divided by the total quantity of purchases made within each food category.

3.2.4 Folate Supplementation Simulation (Objective 3)

An online search of the five most popular pediatric GF multivitamin supplements sold in Canada that contained folate was conducted on Google through major retailers (e.g. Walmart). The online Google search location was set to Edmonton, AB. Only Canadian websites of each retailer was used. The pediatric multivitamins were then sorted according to the retailer's best selling brands. The pediatric multivitamin brands were verified to be consistent with the brands taken by children with CD from our research group's publication on dietary adherence on the GFD. (1) Commercial multivitamin supplements that contain folic acid are considered to be 100% bioavailable. (12, 103) To account for the supplement's bioavailability, the stated folate content of the multivitamin supplement was assumed to be the amount absorbed. (104) Folate DFE from dietary intake was used to account for folate bioavailability found in naturally occurring foods. The folate content of one multivitamin tablet and the manufacturer's recommended dosage for the pediatric GF multivitamin supplements was used to calculate a daily folate supplement dosage (**Table 3.2**). The median supplement dosage was used as the standard folate supplementation level of a typical multivitamin (400µg DFE of folic Acid). The standard folate supplement dosage was then added to the folate DFE intake of each child to create a folate-supplemented intake. The new folate supplemented intake levels were compared to the EAR and UL for age and sex to assess for adequacy and excessive intake, respectively.

Table 3.2: The Five most Popular Gluten Free Pediatric Multivitamin Supplements sold in Canada

Gluten Free Pediatric Multivitamin Supplement	Folate content per tablet	Suggested Daily Dose	Total Daily Amount	Folate Type	Retail Locations
Brand 1	50 µg	4-8 years (1 tablet); 9-13 years (2 tablets)	75 µg	Folate	Nutritional Supplement stores
Brand 2	200 µg	2+ years (1 tablet)	200 µg	Folic Acid	Grocery Stores and Pharmacies
Brand 3	300 µg	3-13 years (1 tablet); 14-18 years (2 tablets)	450 µg	Folic Acid	Grocery Stores and Pharmacies
Brand 4	400 µg	2-3 years (0.5 tablet); 4+ years (1 tablet)	400 µg	Folic Acid	Grocery Stores and Pharmacies
Brand 5	100 µg	6+ years (2 tablets)	200 µg	Folic Acid	Grocery Stores

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3.2.5 Statistical Analysis

Statistical analysis was performed using SAS 9.4 Statistical Software (SAS V.9.04.01; SAS Institute Inc., Cary, NC, USA). Data was expressed as median (interquartile range, IQR: Q1-Q3) for variables demonstrating non-parametric distributions. A Shapiro-Wilk test was performed to assess the normality of distribution for each variable. The total number of members in the household (mother, father, all children) was used to represent family size. Household income, family size, child age, parental age, gluten intake (> and < 10mg/day(95)), serum ATTG (> and < 7 U/ml), parental education (University education/Other), and country of birth (Canada/Abroad) were divided into above and below the median as stated on **Table 3.3**. Chi-square analysis was performed on the categorized household socio-demographic factors to assess

associations of folate intake (met/did not meet folate EAR; **Objective 1**) and purchases of folate-rich foods (> and < 60µg; **Objective 2**). (102) Where necessary, data was adjusted for potential confounding variables such as positive family history of CD (+/-) and/or CD duration (> and < median). Statistical significance was based on a p-value <0.05.

To assess dietary folate intake with and without supplementation, a Mann-Whitney test was conducted to compare above and below the median folate supplemented intake (> and < 597.6µg), and folate intake with and without supplementation (+/-) (**Objective 3**). Chi-square analysis was used to assess the distribution of children above and below the EAR before and after folate supplementation. Fisher exact tests were used to assess the distribution of children above and below the UL before and after folate supplementation.

3.3 Results

3.3.1 Anthropometric and Socio-demographic Data

Data are described in **Table 3.3.** All children were growing within healthy reference ranges (**Figure A.3 and Figure A.4**). (105) All children (100%, n=77) were adherent to the GFD as defined by an estimated gluten content < 10mg from dietary intake. (95) Seventy-five percent (n=52) of children had ATTG levels < 7 U/mL. (106) Thirty-six percent of children (36%, n=28) had a parent, grandparent or sibling diagnosed with CD in their household. The typical family includes one mother, one father, and two children (totaling four members). Median age at diagnosis was 8 years (IQR: 6-11).

Table 3.3: Family Socio-Demographics and Child Anthropometrics

The Family[§]	**Ratio**	**Median**	**IQR**
Family Ethnicity (Caucasian : South Asian)[1]	70:2	.	.
Family History of CD (Y:N)[2]	24:42	.	.
Number of Family Members in a Household	.	4	4 - 5
Number of children with CD	.	1	1 - 1
Mother's Education (University : Other)[3]	46:28	.	.
Father's Education (University : Other)[4]	44:28	.	.
Household Income ($CAD)[5]	.	$99,979	$81,836 - $112,216
Mother's Age (years)[6]	.	42	37 - 46
Father's Age (years)[6]	.	43	40 - 47
The Child[*]	**Ratio**	**Median**	**IQR**
Child Sex (M:F)	25:53	.	.
Child's Age	.	10.5	8.4 - 14.2
Length of Diagnosis (years)[7]	.	1.8	0.8 - 3.3
BMI (kg/m^2)[8]	.	17.9	15.9 - 20.6
BMI-z score[8]	.	0.25	-0.30 - 0.90
Height (cm)[8]	.	142.8	125.2 - 163.3
Height-z score[8]	.	0.30	-0.30 - 1.00
Weight (kg)[8]	.	36.9	24.7 - 53.5
Weight-z score[8]	.	0.30	-0.35 - 0.90
ATTG (U/mL)[9]	.	2.1	2.0 - 6.8
Child Nutrient Intake[†]	**Ratio**	**Median**	**IQR**
Gluten (mg)[10]	.	3.4	2.3 - 4.2
Folate (µg)[11]	.	185	131 - 252
Folate DFE (µg)[11]	.	198	138 - 259

Analysis performed using SAS 9.4 Statistical Analysis program.
IQR=interquartile range; Y=yes; N=no; CD=Celiac Disease; GFD=Gluten Free Diet; M=male; F=female; BMI=body mass index. [§]n=73; *n=78; [†] n=77, one child (n=1) was removed due to inaccuracies associated with estimating food portion sizes.
[1]Ethnicity defined from Stats Canada. (94) South Asian includes Southern Asia, Pakistan and India. (n=72). [2](n=66). [3](n=74). [4](n=72). [5]Income was determined by household postal code and Superdemographics (https://www.superdemographics.com/) (n=77). [6] (n=75). [7](n=74). [8]Calculated using the World Health Organization anthropometric calculator (n=76). (105) [9]Anti-tissue transglutaminase (ATTG); reference cut-offs: <7 U/mL negative, 7-10 U/mL weakly positive, >10 U/mL positive (DynaLIFE, 2018); (n=69). n=52 (75%) children and adolescents fell in the negative range of ATTGs. [10]Calculated as described in Mager et al. from dietary intake. (1) A value <10mg is considered gluten free. (95) [11]Calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA).

3.3.2 Dietary Folate Intake in Children with CD consuming the GFD (Objective 1)

The median folate intake for children with CD was 198µg (IQR: 138-259). Folate intake divided by age groups 3-8y, 9-13y and 14-18y is presented in **Figure 3.1a-c**. Twenty-two

children (29%) met the folate EAR for age and sex. (35) Only 18% (n=14) met the RDA for age and sex. (35) Legumes, as the richest dietary source of folate for children (median folate content of legumes: 24µg, IQR: 12-45; 5% of average caloric intake) were consumed by 46% (n=35) of children with CD. Peanut butter was the most frequently consumed food item within the legume category (median folate content: 12µg, IQR: 12-24, 7% of total caloric intake) and found in the dietary intake of 30% (n=23) of children with CD. In contrast, forty-eight percent of children with CD consumed folate-fortified GF cereals and bread (median folate content: 32µg, IQR: 23-80; 7% of total caloric intake). Children who consumed folate-fortified GF cereals and breads had a higher median folate intake (215µg, IQR: 153-284; n=40) compared to children who did not consume these products (185µg, IQR: 121-239; n=37) (p=0.04). Of the children who consumed folate-fortified GF cereals and breads, 22% (n=17) met the EAR. In contrast, only 6.5% (n=6) of children who did not consume folate fortified GF cereals and breads met the EAR for folate (p=0.005).

Having being diagnosed with CD for < 1.8 years was associated with a higher percentage of children meeting the folate EAR for age and sex (22% met EAR, n=17) compared to children who had CD for > 1.8 years (6.5% met EAR, n=5) (p=0.008). Maternal university-level education was associated with more children meeting the EAR (23% met EAR, n=18) as compared to children whose mothers did not have university education (5% met EAR, n=4) (p=0.01). Younger children (<10.5 years) was associated with being more likely to meet the folate EAR (21% met EAR, n=16) in comparison to children who were above the median age (>10.5 years; 8% met the EAR, n=6) (p=0.009). Younger children who were <8 years at time of CD diagnosis were associated with meeting the folate EAR (21% met EAR, n=16) than children who were older (>8 years) at time of diagnosis (8% met EAR, n=6) (p=0.03). Younger maternal

age (<42 years) was associated with the child meeting the folate EAR (21% met EAR, n=16) as compared to older maternal age (> 42 years, 8% met EAR, n=6) (p=0.04). Serum ATTG concentrations, child sex, family history of CD, child gastrointestinal symptoms, birthplace (child/parental), family ethnicity (Caucasian vs. non-Caucasian), paternal age/education, household income (> and < median), family size (> and < median of 4) were not associated with folate intake levels.

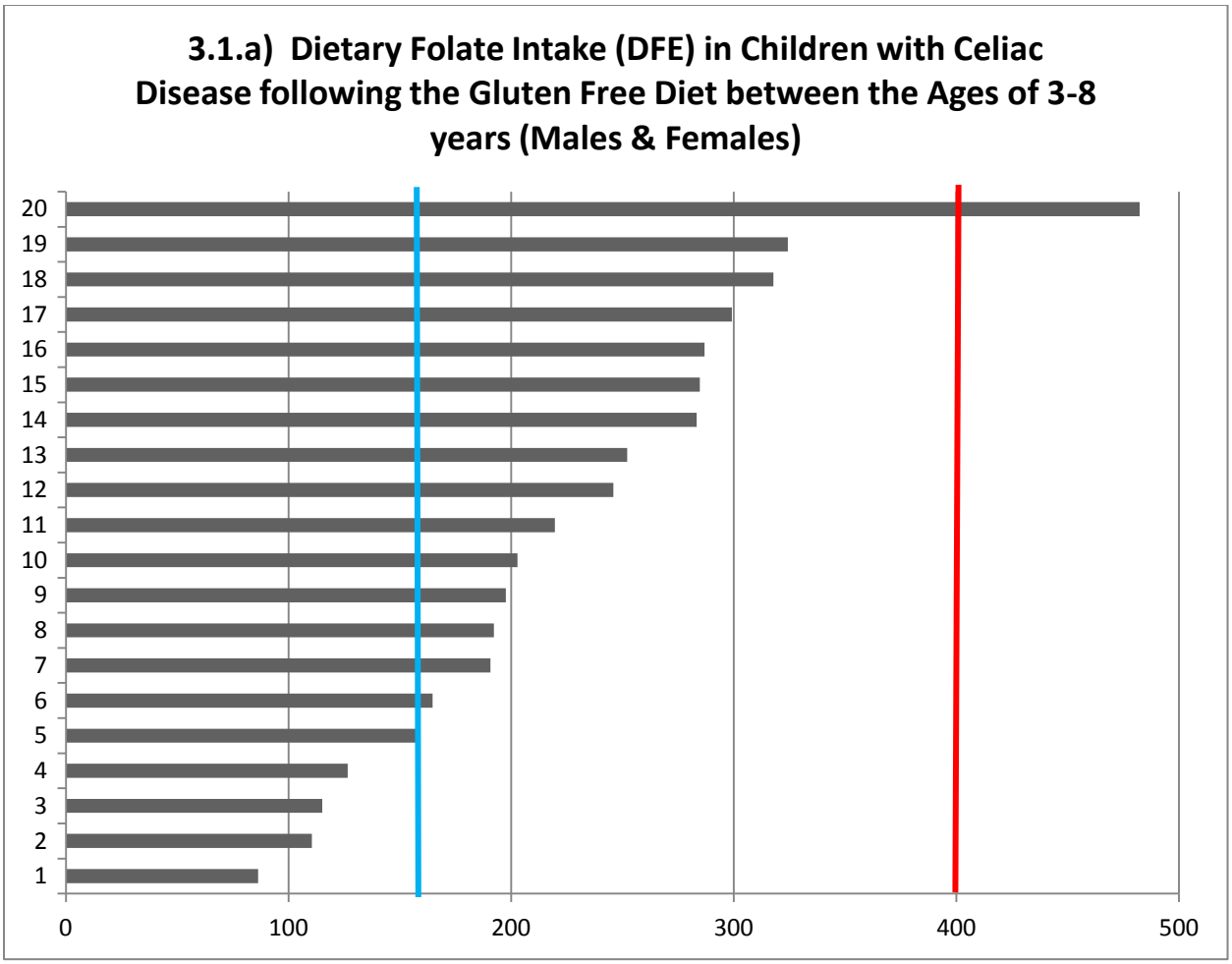


Figure 3.1.a: Mean Daily Intake of Folate in Children and Adolescents with Celiac Disease following the Gluten Free Diet between the ages of 3-8 years (Male & Female). Presented are the mean daily folate intakes (in DFE) of children and adolescents ages 3-8 years old with CD following the GFD (n=20). Both males and females are included in the dietary analysis. One child (n=1) was removed from the dietary analysis due to inaccuracies in portion size measurements. The folate EAR for this age and sex group (males and females, ages 3-8 years) is 160µg, represented by a blue line on the graph. The folate UL for this age and sex group (males and females, ages 3-8 years) is 400µg, represented by a red line on the graph. Each bar represents one child's dietary folate intake. Dietary intake of folate was assessed using two 24h food recalls, using one weekday and one weekend day. (8) Folate intake was calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA). Fifteen children (n=15, 75%) met the EAR. One child (n=1, 5%) exceeded the UL. CD=Celiac Disease; GFD=Gluten Free Diet; DFE=Dietary Folate Equivalents; EAR=Estimated Average Requirement; UL=Tolerable Upper Intake Level

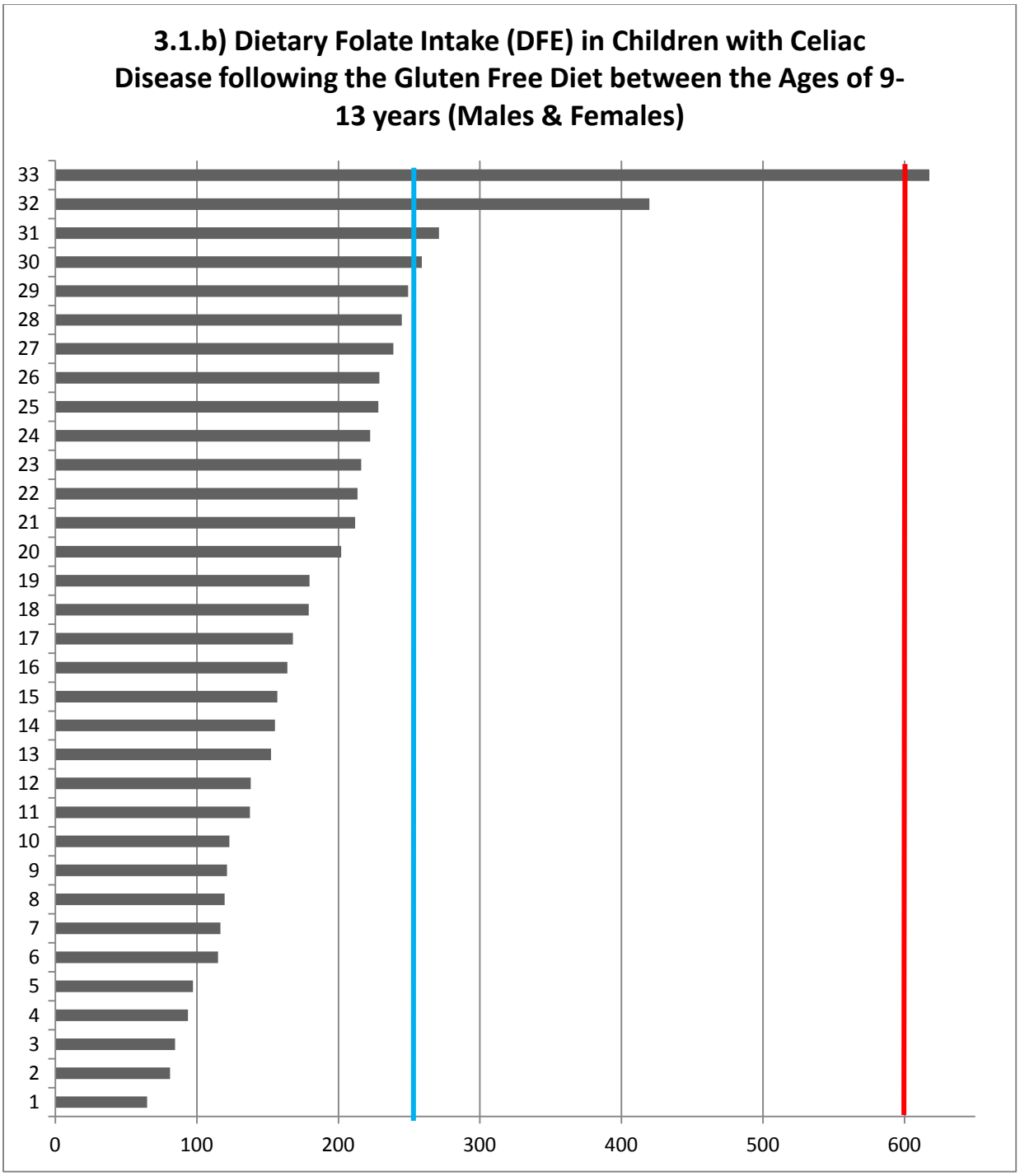


Figure 3.1.b: Mean Daily Intake of Folate in Children and Adolescents with Celiac Disease following the Gluten Free Diet between the ages of 9-13 years (Male & Female). Presented are the mean daily folate intakes (in DFE) of children and adolescents ages 9-13 years old with CD following the GFD (n=34). Both males and females are included in the dietary analysis. The folate EAR for this age and sex group (males and females, ages 9-13 years) is 250µg, represented by a blue line on the graph. The folate UL for this age and sex group (males and females, ages 9-13 years) is 600µg, represented by a red line on the graph. Each bar represents

one child's dietary folate intake. Dietary intake of folate was assessed using two 24h food recalls, using one weekday and one weekend day. (8) Folate intake was calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA). Four children (n=4, 12%) met the EAR. One child (n=1, 5%) exceeded the UL. CD=Celiac Disease; GFD=Gluten Free Diet; DFE=Dietary Folate Equivalents; EAR=Estimated Average Requirement; UL=Tolerable Upper Intake Level

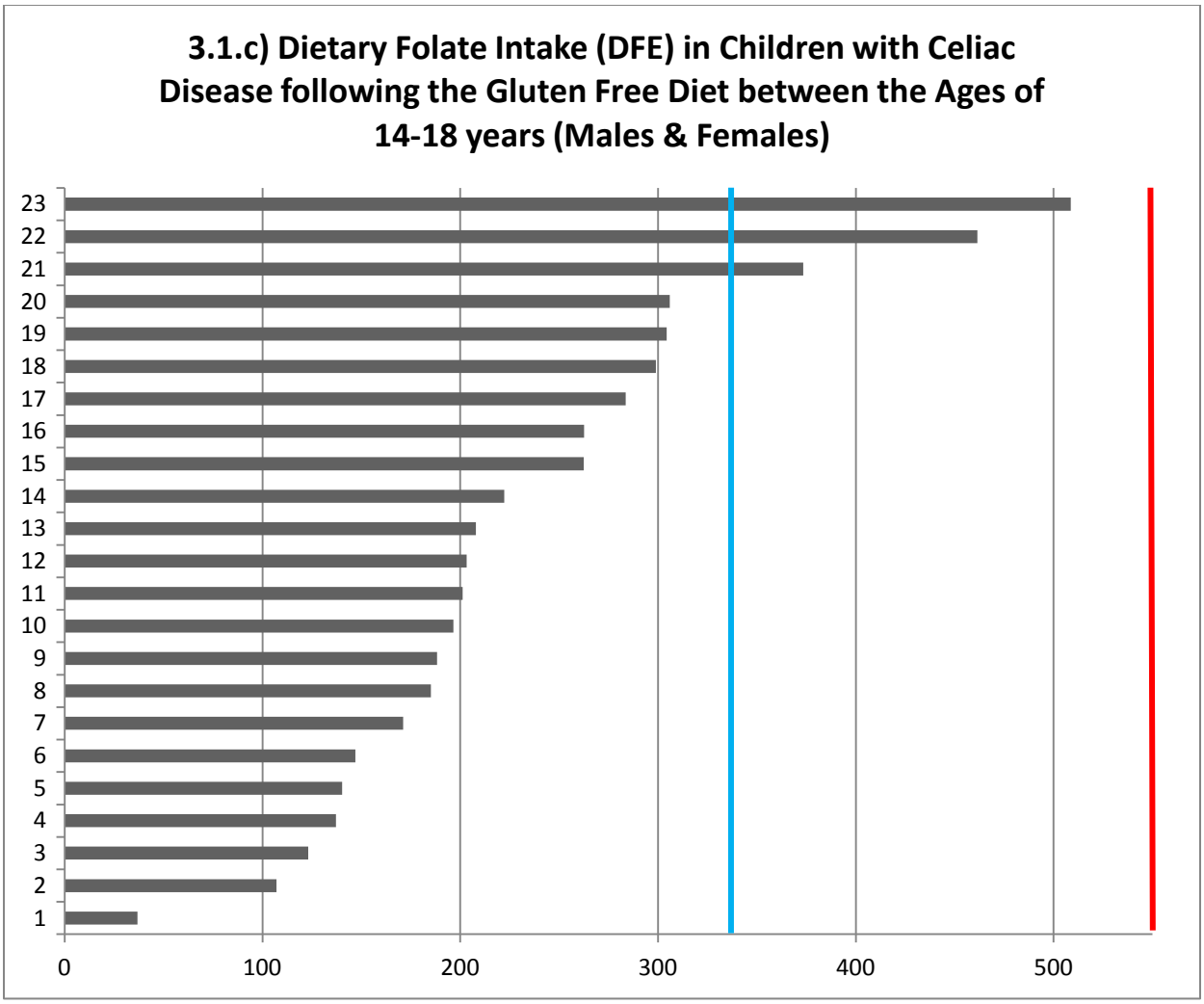


Figure 3.1.c: Mean Daily Intake of Folate in Children and Adolescents with Celiac Disease following the Gluten Free Diet between the ages of 14-18 years (Male & Female). Presented are the mean daily folate intakes (in DFE) of children and adolescents ages 14-18 years old with CD following the GFD (n=23). Both males and females are included in the dietary analysis. The folate EAR for this age and sex group (males and females, ages 14-18 years) is 330µg, represented by a blue line on the graph. The folate UL for this age and sex group (males and females, ages 14-18 years) is 800µg, represented by a red line on the graph. Each bar represents one child's dietary folate intake. Dietary intake of folate was assessed using two 24h food recalls, using one weekday and one weekend day. (8) Folate intake was calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA). Three children (n=3, 13%) met the EAR. No one exceeded the UL. CD=Celiac Disease; GFD=Gluten Free Diet; DFE=Dietary Folate Equivalents; EAR=Estimated Average Requirement; UL=Tolerable Upper Intake Level

3.3.3 Household Food Purchases

Household food purchases and folate intake are described in **Table 3.4** (**Objective 2**). The median duration for household food purchase receipt collection was 30 days (IQR: 25-42). A total of 20,323 items were purchased (food/ non-food items) and itemized. GF foods (naturally occurring, packaged products) made up 76% (n=12,460) of the total food purchases with 9% (n=1,529) containing gluten and 15% (n=2,481) were considered unsafe as the presence/absence of gluten could not be determined. The majority of food purchases came from chain supermarkets (90.0%, n=14,906) and speciality food shops (7.5%, n=1,248), with very few foods purchased at restaurants (0.6%, n=102), fast food (0.7%, n=110) and other locations (0.6%, n=104).

3.3.4 Folate Content of Household Food Purchases (Objective 2)

The median folate content of purchased food was 15µg DFE/100g weight of food (IQR: 5-36). Families purchased folate-rich foods such as eggs (1.4% of total food purchase entries, n=207), broccoli (0.8% of total food purchase entries, n=113), and avocados (0.6% of total food purchase entries, n=86). Folate-fortified GF breads and cereals were made up by a few brands (n=6) representing 15% (n=129) of GF bread and cereal purchases. The analysis of food cost per unit of folate (µg/$CAD) showed that legumes were the least expensive folate-rich food source (**Figure A.1**). Only 48% (n=35) of households purchased legumes and these represented 1.3% (n=216) of total food purchases.

Maternal university-level education was associated with increased purchases of folate-rich foods (35%, n=4,971) in comparison to non-university maternal education (26%, n=3,655) (p=0.0002). Households with children older than the median age (10.5 years) were associated

with a higher share of purchase of folate-rich foods (31%, n=4,437) compared to households with children younger than 10.5 years (29%, n=4,186) (p=0.0008). Paternal university-level education, CD duration (< and > 1.8 years), positive family history of CD (parents, grandparents, siblings), households with differences in family sizes (> and < 4) and household income (> and < median of $ 99,979 CAD) was not related to household food purchases of folate-rich GF foods (processed or naturally occurring) (p>0.05).

3.3.5 Household Food Purchases and Child Dietary Intake

Household receipt collection and dietary intake collection were completed at the same time (within 30-40 days). The proportions of household food purchases and child dietary intake were found to be similar for all food categories with the exception of dairy [12% of total household food purchases vs. 18% of total child dietary intake]. Purchases of vegetables made up 16% of total food household purchases and child dietary intake of vegetables made up 15% of total food items consumed. However, the median folate content of vegetable purchases was higher (26µg) than the median folate content of vegetables consumed by children on the GFD (8µg). Purchases of legumes were also at similar proportions found in child dietary intake (1.4% of total household food purchases vs. 1% of total child dietary intake). Nutritional supplements were found to be 0.6% of total household food purchases and 0.5% of total child dietary intake. Thirty percent of families in the cohort (n=21) were found to purchase nutritional supplements that contained folate.

Table 3.4: Breakdown of Folate Content found in Household Food Purchases and Child Dietary Intake

Food Category	Household Food Purchases (N=16,470 food items)				Child Dietary Intake (N=3,648 food items)	
	a) Percent of Total Food Purchases (%)	b) Food Items Purchased	c) Folate Content (µg/100g)[†]		d) Folate consumed (µg/serving)[†]	
			Median	IQR	Median	IQR
Naturally GF Food Items						
Fruits & Vegetables	31.0	5,104	19[a]	7 - 29[a]	6[aa]	2 - 17[aa]
Animal Protein[§]	10.7	1,769	6[b]	3 - 9[b]	4[bb]	1 - 10[bb]
Plant Protein[§]	2.4	391	61[c]	36 - 87[c]	12[cc]	6 - 32[cc]
Dairy Products[§]	11.3	1858	5[d]	5 - 12[d]	5[dd]	1 - 12[dd]
Certified GF Food Items						
GF Grain Products[§‖]	7.8	1284	22[e]	8 - 36[e]	13[ee]	3 - 32[ee]
GF Whole Grains[§]	0.6	105	9[f]	8 - 49[f]	4[ff]	2 - 7[ff]
GF Other Foods[§‖]	4.5	743	22[g]	8 - 23[g]	3[gg]	2 - 6[gg]
Gluten Containing Food Items						
Gluten Containing Grain Products[§]	7.5	1231	125[h]	32 - 171[h]	n/a	n/a
Gluten Containing Flours[§]	0.08	13	291[i]	25 - 335[i]	n/a	n/a
Highly Processed Food Items						
Other Foods[§]	24.1	3,972	10[j]	2 - 31[j]	0.1[jj]	0 - 2[jj]
Nutritional Supplements						
Nutritional Supplements	0.6	95	100[k]	76-200[k]	50[kk]	0-99[kk]

n/a=not applicable. GF=Gluten Free. All values are median (interquartile range, IQR: Q1-Q3).
[a](n=4,419); [b](n=1,589); [c](n=343); [d](n=1,659); [e](n=941); [f](n=91); [g](n=609); [h](n=1,052); [i](n=13); [j](n=3,406); [k](n=20); [aa](n=1,065); [bb](n=309); [cc](n=126); [dd](n=619); [ee](n=151); [ff](n=41); [gg](n=25), [jj](n=784); [kk](n=4).
[*]16% (n=2,635) of food purchases utilize GF ingredients but manufacturing process was not certified/labeled GF.
[†]Calculated from the Canadian Nutrient File. Food preparation methods have been taken into account when calculating folate intake on ESHA Food Processor Nutrition Analysis Software (SQL 11.0.124, 2015, ESHA Research, Salem, OR, USA).
[§]Animal Protein includes beef, chicken, pork, fish, eggs and other meats. Plant Protein includes legumes, nuts, seeds, tofu and processed meat substitutes. Dairy Products includes cow's milk, plant beverages, cheese and yogurt. GF Grain Products consist of GF grain products (bread, pasta and cereal). GF

Whole Grains include whole rice, corn, quinoa and buckwheat. GF Other Foods and Other foods include frozen entrees, condiments, and chips, GF Other Foods were certified GF. Gluten Containing Grain Products include bread, pasta, crackers, baked goods, and ect... that are made from gluten containing grains (i.e. wheat, barley and rye). Gluten Containing Flours include wheat, barley and rye flours. ‖Calculated from the United States Department of Agriculture (USDA) Food Composition Database, 2015. (68) Families N=73, totaling 20,323 items purchased. Children N=77, totaling 3,632 food items. Food items: 16,470 (81%), non-food items: 2978 (14.7%), nutritional supplements: 95 (0.5%), and unidentifiable items: 780 entries (3.8%).

3.3.6 Folate Supplementation (Objective 3)

The median folate supplement dosage based on folate content per tablet and manufacturer recommended dosage is 400µg DFE (IQR: 200-400). All GF pediatric multivitamin supplements contained folic acid except for one that stated folate (unspecified form). Dietary folate intakes in children and adolescents before and after simulated folate supplementation are depicted in **Figure 3.2a-c**. The median folate intake after simulated supplementation was 598µg (IQR: 540-659). Median dietary folate intake with supplementation was significantly higher in comparison to dietary intake alone (598µg [+supplementation] vs. 198 [-supplementation], p=<0.0001). All children (n=77, 100%) met the EAR and RDA for age and gender after supplementation. More children exceeded the UL with folic acid supplementation and dietary intake (n=34, 44%) as compared to dietary intake alone (n=2, 3%); however this was not statistically significant (p=0.21).

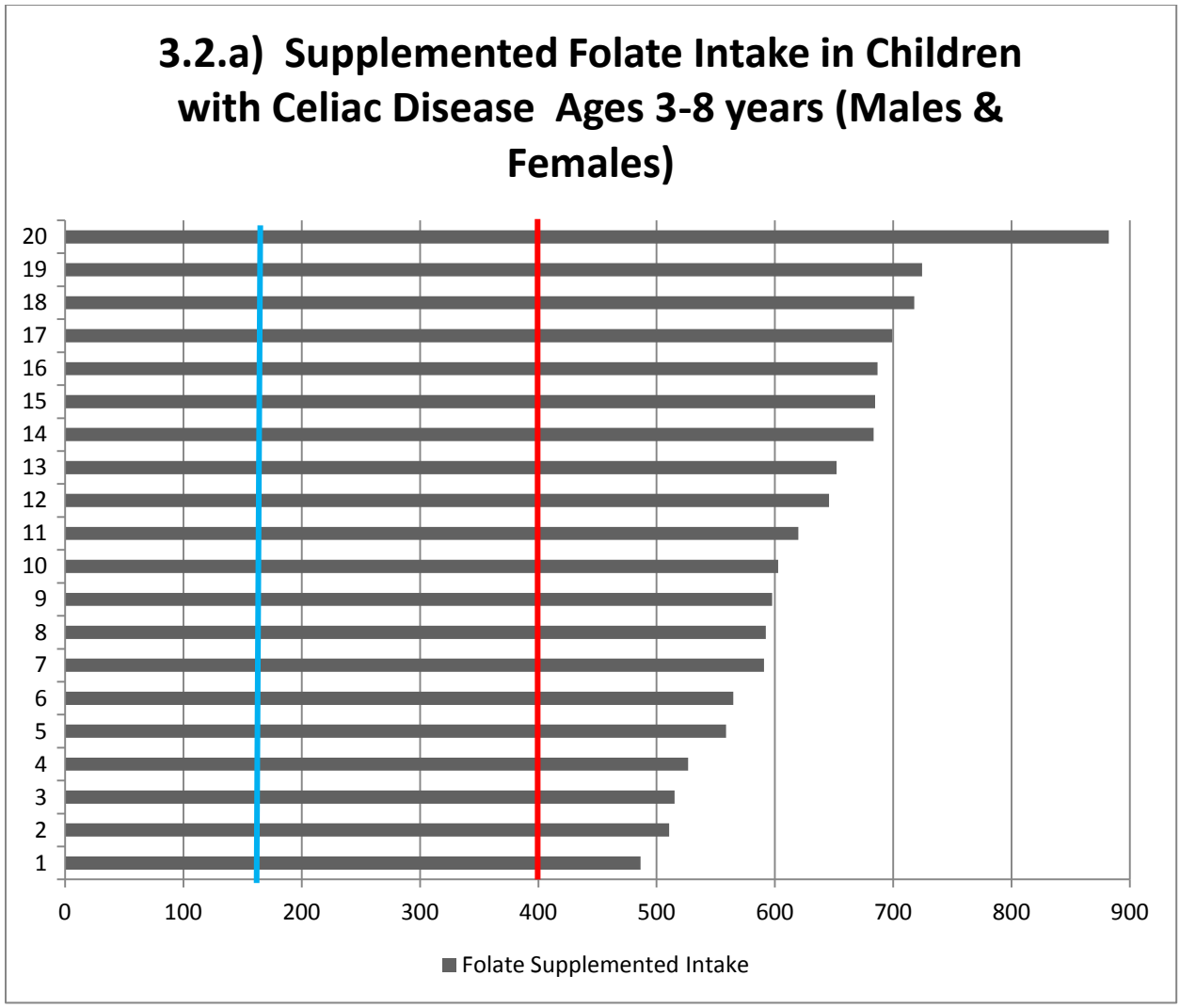


Figure 3.2.a: Folate Intake with Supplementation in Children and Adolescents with Celiac Disease following the Gluten Free Diet between the ages of 3-8 years (Male & Female). Presented is the mean dietary folate intake (in µg DFE) with supplementation for n=20 male and female children between the ages of 3-8 years with CD following the GFD. One child (n=1) was removed from the dietary analysis due to inaccuracies in portion size measurements. The EAR for folate in males and females ages 3-8 years is 160µg and is depicted with a blue line. The UL for folate in males and females, ages 3-8 years is 400µg and is depicted with a red line. The simulation provided each child one folic acid supplement containing 400µg DFE. The folic acid supplement was added to dietary folate intake to calculate the new folate supplemented intake values. Dietary intake of folate was assessed using two 24h food recalls, using one weekday and one weekend day. (8) Folate intake was calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA). The dark grey bars represent dietary folate intake after supplementation. Fifteen children (n=15, 75%) met the EAR before folate supplementation. All children (n=20, 100%) met the EAR after folate supplementation. All children (n=20, 100%) exceeded the UL after folate supplementation. CD=Celiac Disease; GFD=Gluten Free Diet; DFE=Dietary Folate Equivalents; EAR=Estimated Average Requirement; UL=Tolerable Upper Intake Level

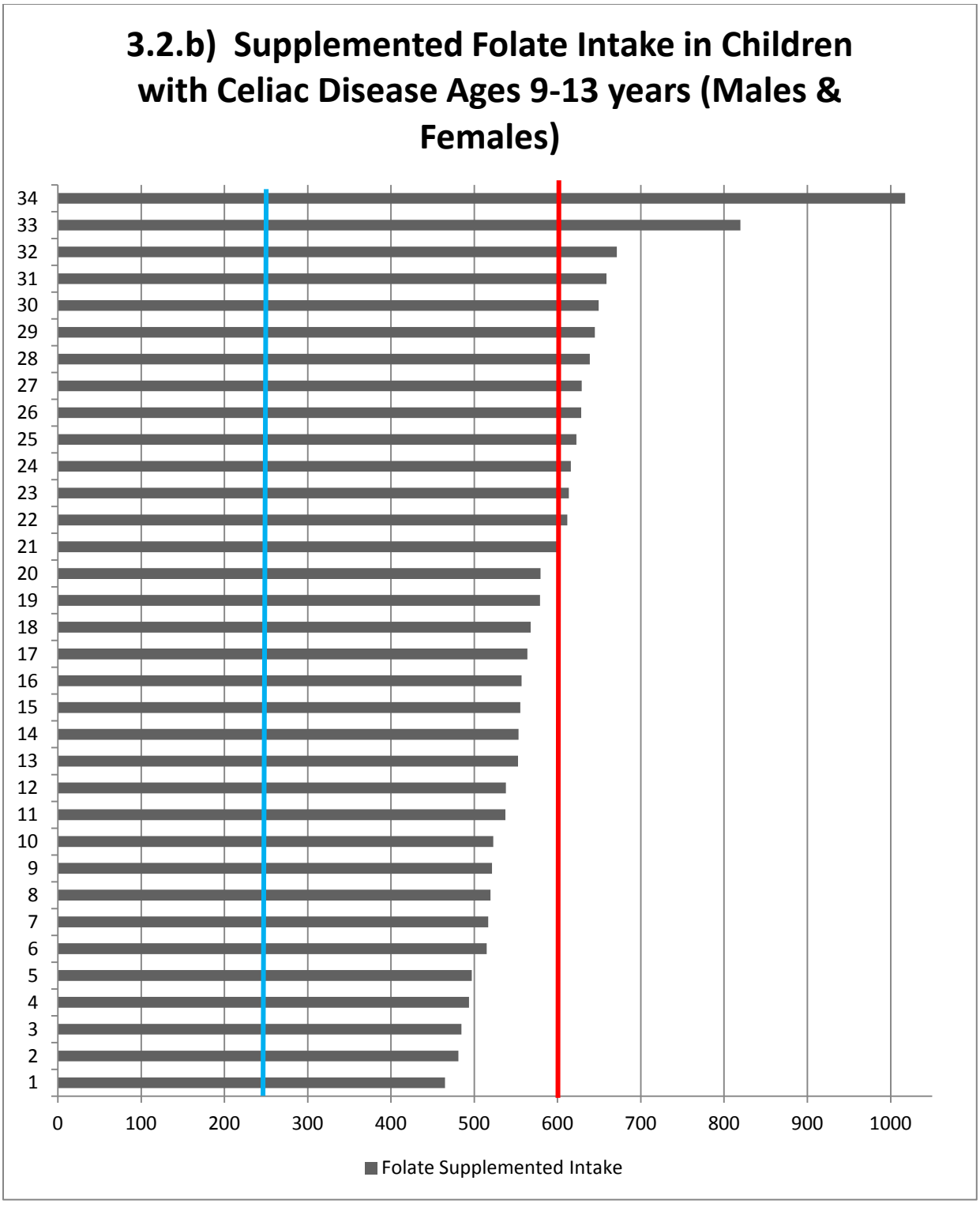


Figure 3.2.b: Folate Intake with Supplementation in Children and Adolescents with Celiac Disease following the Gluten Free Diet between the ages of 9-13 years (Male & Female). Presented is the mean dietary folate intake (in µg DFE) with supplementation for n=34 male and female children between the ages of 9-13 years with CD following the GFD. The EAR for folate

in males and females ages 9-13 years is 250µg and is depicted with a blue line. The UL for folate in males and females, ages 9-13 years is 600µg and is depicted with a red line. The simulation provided each child one folic acid supplement containing 400µg DFE. The folic acid supplement was added to dietary folate intake to calculate the new folate supplemented intake values. Dietary intake of folate was assessed using two 24h food recalls, using one weekday and one weekend day. (8) Folate intake was calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA). The dark grey bars represent dietary folate intake after supplementation. Four children (n=4, 12%) met the EAR before folate supplementation. All children (n=34, 100%) met the EAR after folate supplementation. Fourteen children (n=14, 41%) exceeded the UL after folate supplementation. CD=Celiac Disease; GFD=Gluten Free Diet; DFE=Dietary Folate Equivalents; EAR=Estimated Average Requirement; UL=Tolerable Upper Intake Level

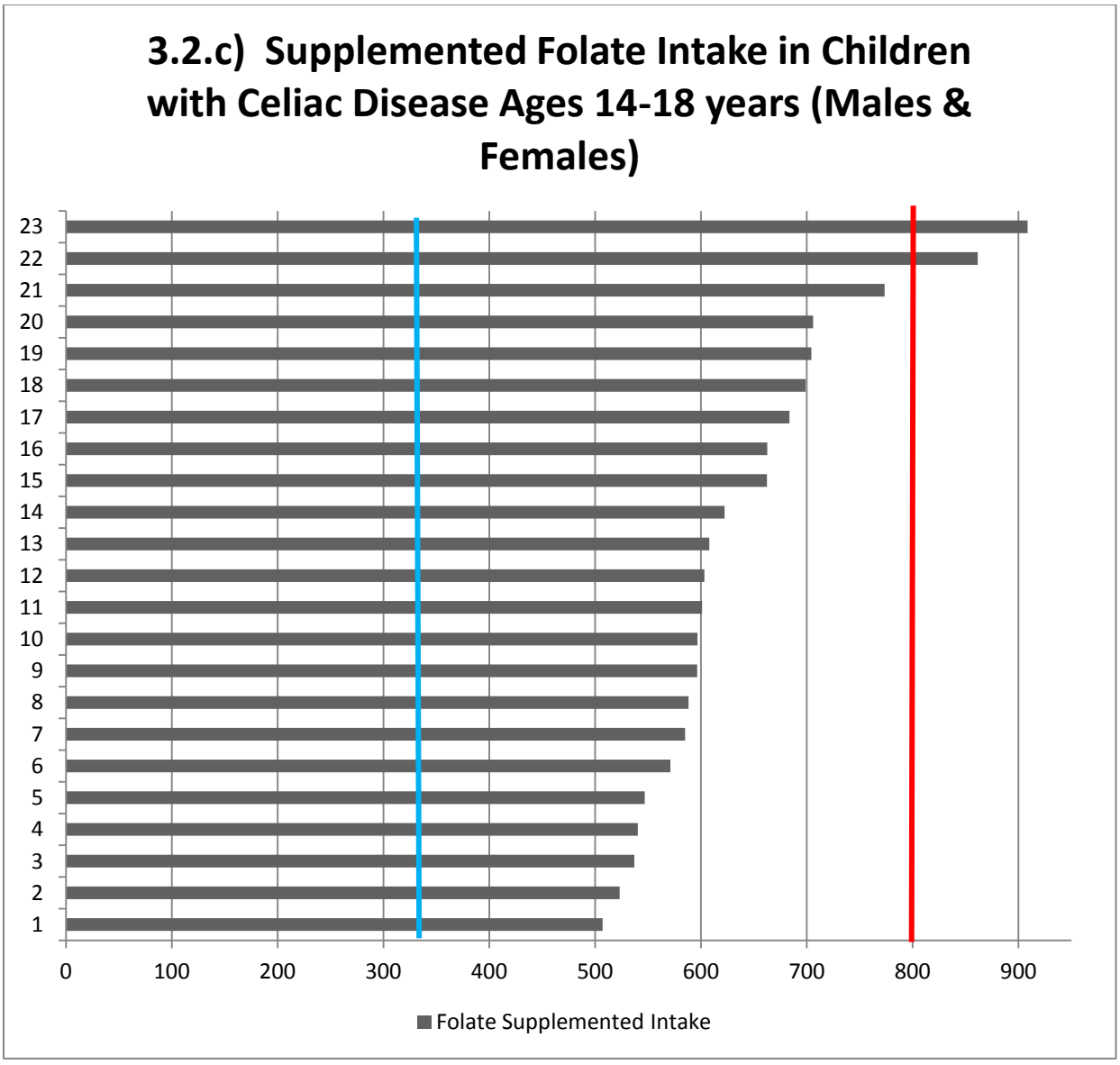


Figure 3.2.c: Folate Intake with Supplementation in Children and Adolescents with Celiac Disease following the Gluten Free Diet between the ages of 14-18 years (Male & Female). Presented is the mean dietary folate intake (in µg DFE) with supplementation for n=23 male and female children between the ages of 14-18 years with CD following the GFD. The EAR for folate in males and females ages 14-18 years is 330µg and is depicted with a blue line. The UL for folate in males and females, ages 14-18 years is 800µg and is depicted with a red line. The simulation provided each child one folic acid supplement containing 400µg DFE. The folic acid supplement was added to dietary folate intake to calculate the new folate supplemented intake values. Dietary intake of folate was assessed using two 24h food recalls, using one weekday and one weekend day. (8) Folate intake was calculated using ESHA Food Processor Nutrition Analysis Software (SQL11.0.124, ESHA Research, Salem OR, USA). The dark grey bars represent dietary folate intake after supplementation. Three children (n=3, 13%) met the EAR before folate supplementation. All children (n=23, 100%) met the EAR after folate supplementation. Two children (n=2, 9%) exceeded the UL after folate supplementation.

CD=Celiac Disease; GFD=Gluten Free Diet; DFE=Dietary Folate Equivalents; EAR=Estimated Average Requirement; UL=Tolerable Upper Intake Level

3.4 Discussion

The GFD is the only known treatment for CD and folate intake is known to be low on the diet. (3, 7, 8, 26) GF food products have documented poor nutritional profiles with low micronutrient content and higher levels of fat and sugar when compared to their gluten containing counter parts. (4, 5, 9) However, the folate content of food items (naturally occurring and GF food products) purchased in a household with a child with CD following the GFD is not well known. Folate is important for children and adolescents as it plays an important role in growth and development, as well as for females of childbearing potential in preventing adverse birth outcomes (**Table 1.4**). In this study, we examined the folate content of food purchases in households with children with CD who follow the GFD.

Recent literature has raised concerns over several macro and micronutrients related to the consumption of the GFD by children. (3, 8, 26) In particular, folate on the GFD has been shown to be a nutrient of concern as no mandatory fortification regulation exists in Canada. (54) Processed GF foods marketed to children (e.g. GF cereals) have been shown to be low in nutritional quality, containing high levels of sugar and added fat. (4) However, empirical evidence on household food purchase patterns in families with children with CD is limited. No data is currently available regarding the folate content of household food purchases in families with children with CD. The purpose of this study was to characterize household food purchases with an emphasis on folate content and to describe folate intake in the children with CD, in these households. We hypothesized that folate content in the diets of children with CD and in GF; foods purchased by CD households would be low. Furthermore, we simulated the impact of folate supplementation on the folate intake of children on the GFD. We found that folate content of food purchases/dietary intake was low and through dietary intake alone 29% of children met

folate requirements; this can be increased to 100% with the inclusion of a daily folate supplement (400µg DFE).

Analysis of household food purchase receipt data shows CD families trying to accommodate both gluten containing diets and the GFD. Households infrequently purchased folate-rich GF food products (legumes, folate fortified breads and cereals) even though these were the most cost effective GF sources of folate. Instead, households tended to purchase significant amounts of folate-poor GF foods (naturally occurring) such as cucumbers, carrots, bananas, and chicken (**Table A.3**). Such household food purchase patterns place the child with CD at risk of suboptimal folate status, as excessively large amounts of these foods would need to be consumed to ensure children were able to meet their folate EAR needs (i.e. 12.5 cup of sliced carrots provides 300µg folate DFE). In comparison, folate-rich foods, such as lentils, require relatively smaller amounts (i.e. 0.8 cup of boiled lentils is equivalent to the 300µg folate DFE). Even if families had purchased more folate-rich foods within the naturally occurring GF food categories such as legumes, broccoli, and spinach, these foods were not routinely consumed by the children and access to these foods were not necessarily the most cost-effective sources of folate. While GF folate-fortified breads and cereals were found to be a greater contributor of folate in the child's diet when compared to legumes, the challenges facing families with children with CD is the lack of uniform availability of GF processed grains that are fortified with folate. In this cohort, the number of food brands who fortified GF-processed grains was limited and the majority of GF-grains consumed were rice and corn, a common finding in the literature. (8, 13, 107) The folate content of GF food products found in the food purchases is similar to amounts stated by Kulai et al. and Jamieson et al., who also found low levels of folate in GF food products in Canada. (5, 65) Proportions of household food purchases and dietary intake were

found to be similar. It should be noted that the median folate content of purchased vegetable is higher than the vegetables consumed [26μg purchased by household vs. 8μg consumed by child] by the children with CD in the cohort, which implies that food selection can affect dietary folate intake. This may in part, explain why children and adolescents with CD consume diets low in folate.

While maternal education level, and age of the child was associated with overall dietary folate intake; household purchases of folate-rich foods, other socio-demographic factors did not. Multi-vitamin use was not documented in this study. Previous work in our clinics has shown that 73% of children are prescribed a multivitamin at time of CD diagnosis. (7, 76) While most of these multivitamins contained folate (75), many children with CD are not consistent in their supplement intake for a variety of reasons ranging from forgetfulness to lack of commitment. (81) Moreover, vitamin supplement purchases had limited representation in our data, at 0.6% of total purchases. This highlights the importance of examining the folate content of the GF food supply and the consideration of the need for the development of policies that mandate folate fortification of GF foods readily consumed by children with CD. This is particularly important for females with CD of childbearing potential due to the increased risk for adverse neonatal outcomes.

The addition of a standard folate supplement can increase the folate intake to ensure children and adolescents with CD meets the folate EAR recommendations for age and sex. To account for the difference in folate bioavailability in food sources and multivitamin supplements the folate DFEs from both sources were used. The bioavailability of folic acid in supplements is approximately 100% (12); however, bioavailability of folate from food sources can vary widely. (12) Winkels et al. has demonstrated that the bioavailability of food folate is approximately 80%

that of folic acid. (34) The lowered and varied bioavailability of food folate is mitigated in this simulation by utilizing the folate DFE of the food items.

Supplementation ensures that the basic folate requirements are more easily met especially when dietary restrictions (i.e. GFD) create imbalances due to restricted food selection. (3, 8) In younger children, it was easier to exceed the UL with folate supplementation than without (all children 4-8 years old exceeded the UL with supplementation). In contrast to older children (ages 14-18y), where only two children (n=2) exceeded the UL when a folate supplement was added. This would not be of major concern because the toxicity level has not been determined for folate in adults or children (32) and the folate bioavailability can be lowered when consumed with other food items. (12) Thus, the chances for adverse effects in younger children (≤8y) are low.

Study limitations included challenges associated with assessing folate content, lack of data on household socio-economic factors in families with children facing food insecurity, and the lack of information related to the actual folate status in the children with CD in the households studied. Folate content of many manufactured GF food products are not readily available and data related to the folate bioavailability in varying GF foods are scarce. (4) The use of ingredient lists, nutrition facts tables, and the use of reference values from CNF and the USDA for folate determination was used in some instances to determine folate content of foods purchased by households. While this may have led to underestimations of folate content in foods purchased (87), this appeared to be largely limited to gluten containing grain products (less than 8% of overall food purchases). Hence, it is unlikely that this had a major influence on the overall assessment of folate content of GF foods purchased. Labeling of GF products for folate may need to be considered so parents can make informed decisions in selecting more appropriate food products with better nutritional quality. In adults, up to 20% of individual's have folate

deficiency after following the GFD for over 7 years. (50) Hence, the extent to which persistent suboptimal folate intake may have on overall folate status in children and adolescents with CD is unknown. While adherence to the GFD may improve overall folate absorption in children and adolescents with CD, poor folate intake caused by low levels of folate within the GF food supply may be the most important driving force of folate status in children with CD. A current assessment of the blood folate status in children with CD is GFD is needed to fully understand the impact low dietary folate intake has in children with CD.

The differences in bioavailability of folate from food sources and the folic acid found in supplement sources may also influence the level of folate intake on the GFD. The bioavailability difference was mitigated by using the folate DFE as a standard measurement of folate in food when calculating dietary intake. However, the use of folate DFE is limited in that external factors such as variations in intestinal pH, folate digestion, and food composition can also influence absorption into the body. (12) To assess the bioavailability of folate on the GFD, more research is needed on folate bioavailability on GF food products and supplements.

While the large sample size related to food purchases examined (>20,000) was a conferred strength within this study design, the smaller number of families examined precluded the ability to examine GF purchases across a larger socio-economic spectrum. Low-income families and families of South Asian and Somali descent, where CD has been shown to be prevalent, are not largely represented in this cohort and may have different foods purchasing habits from families in this study. (108) While food cost are often cited as a major factor in food purchasing behaviors, the richest sources of dietary folate are also among the most cost effective (legumes, green leafy vegetables). This suggests that household incomes and food cost may not necessarily be driving forces in food purchase decisions by households with children with CD

with relatively high household incomes, hence may not be anticipated to be a major influence on overall folate intake. In contrast, income and food costs may be barriers to dietary adherence in families of lower socio-economic status. While GF grains only represented a small proportion of food products consumed, our data shows that the purchase of a few brand name GF folate-fortified breads and breakfast cereals contributed considerably to the improvement of folate intake in children and to meeting the EAR for folate. This may be particularly challenging in families living in rural centers where access to a wide variety of GF foods may not be possible and/or is expensive. As breakfast cereals have been shown to contribute to overall B vitamin intake in Canadian children (109), folate fortification of these GF food types (as with gluten containing grains) may be an important vehicle for consideration of folate fortification. Folate fortification of gluten-containing grains has resulted in significant improvements in folate status/folate intake in the general population in Canada and therefore is worthy of further discussion populations consuming the GFD. (87) Hennessy-Priest et al. found that Canada's current folate fortification levels set at 150µg/100g white flour, 60µg/100g breakfast cereals and 235µg/100g pasta (33), provided preschoolers (3-5 years) with 83µg/day of folate and contributing to 50% of dietary folate intake in the cohort. (89)

3.5 Conclusion

In conclusion, children following the GFD had a low intake of folate and have household food purchasing patterns reflective of folate-poor GF foods. This may lead to ongoing problems with folate insufficiency and contribute to adverse outcomes particularly for females with CD of childbearing potential and their children. These findings highlight the need for supplementation

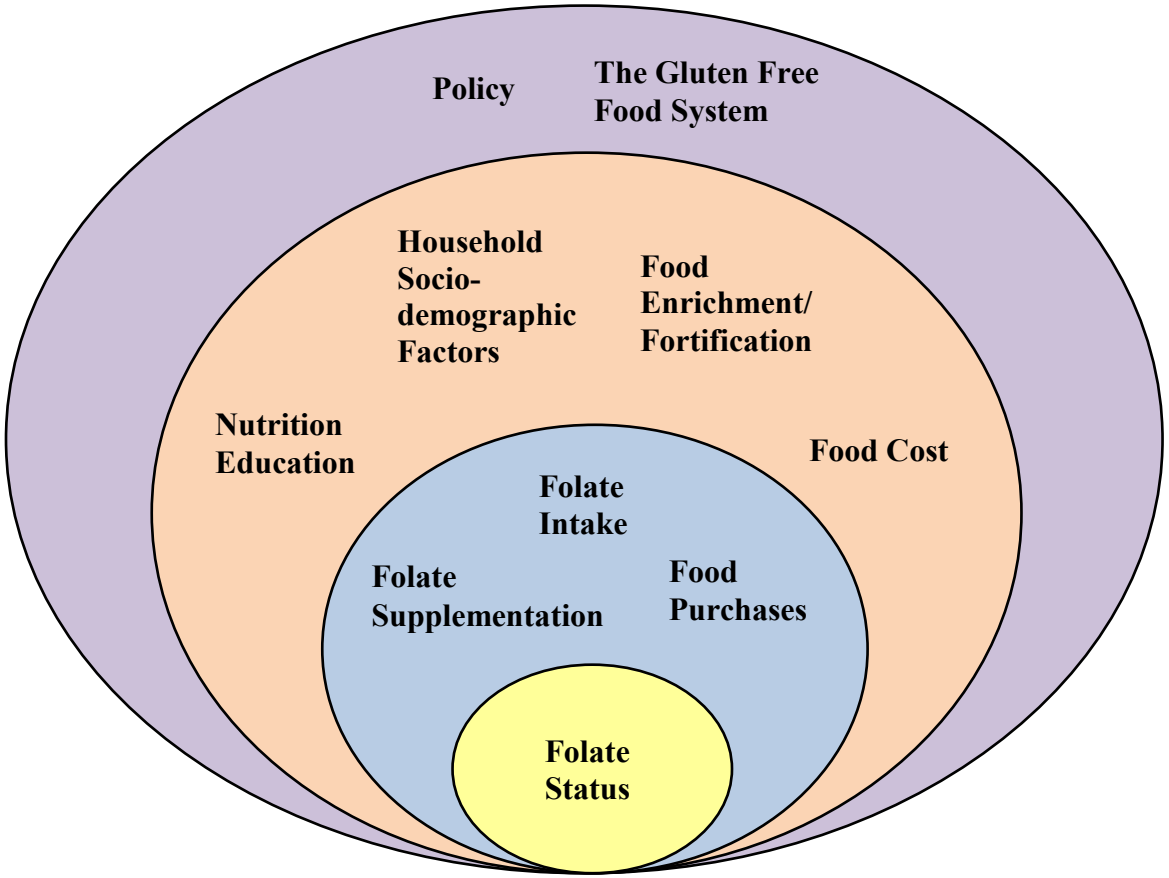
interventions or discussions on a folate fortification policy for children and adolescents with CD on the GFD.

Chapter 4: Integration Chapter

4.1 Summary of Research Findings and Implications

The only treatment for CD is the GFD. To help with adherence, many patients rely on processed GF food products. (110) GF processed foods (particularly those containing gluten grains) have been shown to have significant micronutrient imbalance including low folate levels. (5) Hence, consumption of these types of GF foods may influence overall folate intake in children and adolescents with CD, as these products are not typically fortified with folate because folate fortification is not mandatory for GF foods in Canada. (111) One important component of examining the factors influencing folate intake in children and adolescents with CD includes the types of foods present in the home and the expenditures made on food purchases outside of the home. Although, there is evidence that children and adolescents with CD have low folate intakes on the GFD, no information is available that links folate content of GF food purchases by families with children with CD with folate intake on the GFD. The purpose of this thesis was to examine the folate content of household food purchases (supermarkets, fast food outlets, retail stores etc) in Canadian families with children with CD and to describe folate intake in these households. **Figure 4.1** is a conceptual diagram of the variables that influence folate intake, folate supplementation, and purchase of folate-rich, GF food items.

Figure 4.1: Summary of the Connections between Child Dietary Folate Intake and Household Food Purchases



Created by author, Liu 2019

Presented is a conceptual diagram of the variables that connects dietary folate intake in children and adolescents, household food purchases and folate supplementation. The yellow circle represents folate status of the child. The blue circle lists the variables that can directly influence folate status (factors analyzed in the thesis). The orange circle lists the variables that can influence the purchase of folate-rich, Gluten Free food items. The purple circle lists the external influences on the food system, the food environment, food purchases and dietary intake.

4.1.1 Dietary Intake of Folate in Children and Adolescents

The major study findings illustrate that children and adolescents with CD consuming the GFD have low intakes of folate with the majority failing to consume levels above the EAR for age and sex (**Hypothesis 1**). Low folate intake seen in this cohort was consistent with previous research by Dickey et al. (2008), Wild, et al. (2010), Alzaben et al (2015), Valente et al (2015), Vici et al. (2016), Melini et al. (2019), Di Nardo et al. (2019), and Mager et al. (2019), where the folate intake of adults and children with CD was low. (2, 3, 7, 8, 26, 28, 57, 58) This may be due to the poor folate content of the ingredients used in many commercially available GF food items. (4, 13) Recent work by our group shows that low folate intake is consistent in children with CD across different diet patterns (Western diets vs. Prudent diets), with no major influences by ethnicity, CD diagnosis or duration. (8) Despite low folate intake, the consumption of fruits and vegetables in this cohort was similar to ANGCY food group serving recommendations (**Figure A.2**). This implies that the selection of folate-poor fruit and vegetables (e.g. carrots and cucumbers) is what drives the low levels of folate intake in children and adolescents.

Children do consume folate-rich GF foods, such as legumes, vegetables, juices, and GF breads and cereals (**Figure A.5)**; however, the intake of legumes (average serving: 52g) and vegetables (average serving: 57g) are low relative to other foods. Certain brands of GF breads and cereals are already folate fortified and would explain why the consumption of these food products made significant contributions to dietary folate intake. These findings were consistent with a study by Hennessy-Priest, Mustard, Keller, et al. who looked into the impact of folate fortification on the dietary folate intake of children. (89) The higher folate intake from folate fortified GF breads and cereals suggest that fortification can have positive impacts on the folate intake; consistent with findings in the healthy population with folate fortified gluten-containing

grains (see **Table A.5b** for an example of a GF menu with fortified grains). (33, 112) Food items consumed at restaurants and fast food outlets represented less than 1% of the total food items consumed by the cohort. This implies that food items from these locations would not be significant contributors to folate intake and it can be explained by the decreased frequency of eating at restaurants by many families that follow the GFD. (110) The selection of folate-rich food items was not influenced by income, as the selection of folate-rich foods was consistently low despite higher household income. This could be explained by the stronger influences on food choice by convenience, food availability, appeal, and parental influence as shown in a study by Neumark-Sztainer et al. (113)

4.1.2 Folate Content of Household Food Purchases and Influencing Factors

Folate-rich (≥60µg DFE/100g weight of food) household food purchases make up the minority of total food purchases (**Hypothesis 2**). These foods made up 18% of total household food purchases and of which 0.3% were related to items purchased at restaurants/fast-food outlets. Of the GF-foods purchased, the types of folate-rich food rich items purchased included legumes, eggs and broccoli; but these only represented 2.8% of total monthly food expenditures. This explains in large part why folate intake in children on the GFD remains quite low.

Six brands of folate fortified GF breads and cereals met the definition of folate-rich, representing 1.2% of total food purchases. These findings of folate fortification are similar to those found by Kulai and Rashid (5), indicating that a small number of companies continue to voluntary fortify their GF food products in Canada. In contrast, the gluten containing folate-rich foods that were purchased by the households were breads and cereals; these represented 3.3 % of total food purchases by the household. Two food guide servings of these food types for members

of the household without CD and not consuming the GFD would translate to 128µg and 82µg of folate (37), which highlights the importance of folate fortification in grains. The folate content of foods from restaurant and fast food outlets was low, but it represented less than 1% of total food items purchased of all households studied. Thus, purchases from these locations are unlikely to be a major contributor to folate intake in children or the purchase of folate-rich foods. In contrast, one potential source of higher folate, GF foods may be ethnic restaurant establishments, which sell ethnic cuisines that focus on folate-rich foods such as beans and legumes (e.g. Mexican, South East Asian establishments).

4.1.3 Effect of Micronutrient Supplementation on Folate Intake

The inclusion of a multivitamin supplement increased dietary folate intake, such that all children were able to meet the folate EAR for age and sex (**Hypothesis 3**). A supplement dosage of 400µg DFE/day does put folate intake over the UL of 400µg DFE/day (after taking into account dietary intake) for children ≤8 years. (32) A folate toxicity dose has not been determined for adults or children (32); thus, the chances for adverse effects with supplementation at 400µg DFE/day for young children ≤8 is low. The lowered concern is due to the lower bioavailability of folate in natural foods and fortified foods (12), and the fact that folic acid bioavailability can be impacted when consumed with other food items in the diet. (104) One study did show that a dosage between 5-10mg did not present with any toxicity symptoms in healthy patients. (114) Folate toxicity was seen in one study that provided 15mg folate/day to adults resulting in negative mental changes and gastrointestinal effects. (32) The typical micronutrient supplement provides 400µg folate per dosage, which is substantially lower than the UL of 600 µg DFE/day (for Females and Males, 9-13y) and 800 µg DFE/day (for Females

and Males, 14-18y). (115) This suggests that the risk for folate toxicity when providing an over-the-counter supplement with a dose of 400µg DFE/day to CD patients (≥8 years) is low. Observational studies suggested that high dose folic acid intake (ranged from 0.35 to 15 mg/day) may mask vitamin B12 deficiency; with more cases documented at the higher end of the range. (32) The risk for folate supplementation with over the counter supplements to mask vitamin B12 delicacy is low due to the lower dosage. Thus, routine use of over the counter folate supplements containing 200-400 µg (standard dosing in pediatric commercial vitamin supplements) should not pose a risk for younger children. Ideally, supplementation is routine so that daily variations in dietary intake will be covered. However, previous research has shown that supplementation in children with CD is not always consistent and it can be influenced by parental beliefs, a health professional recommendation and age of the child. (71, 75, 81) Adolescents in particular, may have a lower level of adherence and hence, consideration of other approaches (such as folate fortification of GF-foods) may help. (71, 116) Consideration for setting up a support system and routine establishment can help establish supplementation practices in children and adolescents with CD. (75, 81)

4.2 Relevance and Implications to Clinical Practice

Results from this study demonstrate that children and adolescents following the GFD have low folate intakes and household food purchases have low folate content. Health practitioners, especially Registered Dietitians, need to identify where dietary folate intake is derived from to give appropriate advice to help patients make achievable dietary changes. Education from a Registered Dietitian on how to change dietary practices can help to increase the intake of folate on the GFD. For example, Dietitians have the expertise to help educate

patients and their family on reading nutritional labels to identify folate-fortified GF food products. (117) Supplementation is another way to help children and adolescents following the GFD meet folate requirements (**Chapter 3**). Health professionals can support supplementation practices by encouraging facilitators such as routine development and providing a support system to ensure adherence. (75, 81)

4.2.1 Implications to Woman of Childbearing Potential and their Offspring

The low folate content of food purchases demonstrates that supplementation during pregnancy is still needed for females of childbearing potential with CD to meet folate requirements. (118) Continued consumption of GF food products with low folate contents may lead to folate deficiency during pregnancy, and pre-term birth and low birth weights in the offspring. (45) The symptoms of folate deficiency may not appear until it is too late and has already influenced the development of the fetus. (86) Thus, it is important to ensure that women with CD of child bearing age have sufficient folate intakes from dietary and supplementary sources before pregnancy occurs so that adverse outcomes can be prevented. This can mean in addition to recommending supplementation, education on food literacy directed towards increasing folate intake on the GFD (i.e. purchasing folate fortified breads and cereals) may be needed. Dietitian led supermarket tours are one way to provide nutrition education to new mothers to increase the selection of folate-rich vegetables for consumption and folate fortified GF food products. (119) This knowledge on food selection can also be applied towards future pregnancies and after pregnancy when selecting food items for children and adolescents.

4.2.2 Implications to Infants, Children and Adolescents

Consumption of folate fortified GF breads and cereals were associated with improved folate intake (**Chapter 3**). However, not all children consumed folate fortified food products so the benefits were not seen in all children. Encouragement for the selection of folate-rich foods and folate fortified food products needs to take into account the child's taste preferences, convenience of product and food availability. (113) Improving availability of nutrient dense food selection at schools and restaurants can also help improve dietary quality and micronutrient intake. (120) Interventions should target food accessible to CD children and adolescents to make an impact on dietary intake. One study showed that greater access to retailers that sold healthy food options was correlated with higher diet quality in children. (121)

Supplementation for children and adolescents will help to increase folate intake without major changes to dietary intake patterns. This is especially helpful when access to folate fortified GF food products is limited and incorporation of green leafy vegetables and legumes presents a challenge. Our research found that health professionals could help patients overcome the barriers of supplementation by providing information on the benefits of supplementation; how nutrition can influence CD, and where to access GF supplements. (75, 81) Known barriers to supplementation in this population, such as lack of knowledge and health beliefs (75, 81), can be addressed during consultations with a health professional by providing the information needed to make informed decisions.

An analysis on the weight and height scores of the cohort showed growth within healthy ranges (**Figure A.3 and Figure A.4**). These results match those found by Fernández et al., who also saw normal growth in a cohort of children, 4-18years old with CD. (60) Normal growth within this cohort implies that the small intestinal villi have healed after following the GFD. (6)

Furthermore, no folate related complications or symptoms of folate deficiency were documented in the cohort, despite low dietary folate intake. This may also be explained by the healing of the small intestine while on the GFD and the normalization of nutrient absorption. (6) However, folate status documentation in CD clinics may still be helpful to prevent any potential complications and monitor long-term folate intake.

4.2.3 Implications to Lactation and Breastfeeding

The nutritional status of the mother is important for the nutritional status of the breastfeeding infant (122), as the infant's nutritional intake derived from the mother's breast milk in the first 6 months. (123) When folate content of GF food items is poor and the diet is nutrient deficient, the micronutrient quality of the breast milk will be negatively affected. (123) Infants are especially vulnerable, as their bodies are smaller and thus cannot store as much as children or adults, hence any change in nutrient intake may affect growth and development. (124) Mothers may also be impacted by insufficient folate intake as the body may deplete folate stores to produce breast milk, placing the mother at risk for folate deficiency complications (**Table 1.4**). (122) While supplementation will help, adequate dietary intake of a variety of nutrients is still needed. Potential interventions may be implemented that can target the nutritional needs of pregnant and lactating women, such as the use of meal planners and education sessions with a Registered Dietitian. (125, 126) The use of meal planners have shown to be effective at increase the intake of green and orange vegetables in healthy lactating mothers (0.3 food guide serving, Control vs. 1.5 food guide servings, Intervention). (125)

4.3 Nutrition and Health Literacy

Current nutrition education for CD is focused on elimination of gluten on the GFD. This study has demonstrated that current food purchase choices made by CD households may not provide sufficient folate to meet the folate EAR for age and sex. More folate-rich food choices need to be made during food purchasing to improve folate intake. Nutrition education needs to include food selection at supermarkets and restaurants for improved folate intake.

4.3.1 Meeting Folate Needs through Diet Alone

It is possible to meet folate needs through dietary intake alone on the GFD, even without folate fortification of GF grains and food products, assuming a child or adolescent consumed ample amounts of folate-rich foods such as legumes, lentils, and green leafy vegetables (**Table A.5**, and **Figure A.6**). Indian-vegetarian and Mediterranean dietary patterns may have higher intakes of folate due to the ample inclusion of legumes and green leafy vegetables, thus making it possible to meet folate requirements on dietary intake alone. (127, 128) However, the greatest challenge is the need to consume significant servings of these food items to meet folate needs (i.e. 2.2 cups of cooked broccoli, 1.1 cups of cooked lentils; **Table A.4**). These levels of intake would translate to 300-400 µg/d of folate. The problem lies in the current eating patterns of Canadian children and adolescents with CD, consuming significantly less portions than cited here, which highlights the need for the development of GF nutrition guidelines. This has significant implications for nutrition policies and education by health care providers to members of the public with children with CD consuming the GFD. To increase the consumption of folate-rich food items, interventions that target nutritional and food knowledge can help patients make informed decisions at food retail outlets. A systematic review by Iacovou et al. found that

community kitchens have a positive impact on the participant by improving food preparation skills and increasing fruit and vegetable intake to 5+ servings in 47% of the cohort. (129)

4.3.2 Food Based Nutrition Guidelines

Nutrition literacy tools are one option to help newly diagnosed CD patients turn nutrition knowledge into action (i.e. making healthy food choices at the supermarket). A GF food guide can help direct families towards healthy food choices and purchases. Canada's current food guide does not address the nutritional imbalances seen in the dietary intake patterns of the GFD and is thus inadequate in assisting patients with CD. Thus, a GF food guide that can address the difficulties of following and adhering to the GFD is needed. Knowledge on the household food purchases and dietary intake is used to inform the development of optimal dietary intake on the GFD by providing representative examples of GF food items families will purchase. Nutritionally adequate menu simulations that are representative of the food choices children and adolescents make ensure that a GF nutritional resource will be accurate and suitable for families, making implementation easier.

Folate fortified GF food products and ethnic foods identified in this study help to improve dietary diversity and provide additional folate-rich food sources (i.e. mustard spinach) for menu simulations. Improved dietary diversity on the GFD may be a possible solution in improving nutritional adequacy in children with CD (8), thus, methods that will help introduce a wider selection of foods will help improve nutritional intake. To meet folate requirements, increasing the recommended intake of vegetables and legumes may be needed on the GFD since there are no mandatory folate fortification policies for GF food products in Canada. Nutrition education classes that target the consumption of green leafy vegetables may prove useful in increasing the

dietary diversity in children with CD. Arsenic consumption would also be a concern as rice is highly prevalent in many GF grain products [mean rice content of GF grain products is 81% (130)]. It is recommended for children and adolescents following the GFD to consume a variety of grain types (i.e. corn, quinoa, buckwheat). (130)

4.4 The Gluten Free Food Supply in Canada

The influence on food purchase choices from the dietary restrictions of the GFD can be seen despite the limited scope of exploration into the home food environment. Households with children who follow the GFD will try to accommodate both a regular gluten-containing diet and a GFD. Improvements can still be made to improve the nutritional quality of food purchases that can benefit the whole family (i.e. increased purchase of legumes). For example, intervention programs targeting attitudes and behaviours towards the consumption of fruits and vegetables can help increase their purchase. A Cochrane systematic review found that multi-component interventions targeting fruit and vegetable consumption in children were able to increase intake by 0.37 cups per day. (131)

Manufacturers can help improve the nutritional quality of GF food products through voluntary folate fortification and recipe formulation to improve nutrient content in the final product. (13) Retailers can increase availability of folate-fortified GF food products; provide shelf signage to help consumers identify folate fortified GF food products; and increase community/kitchen space for classes around nutrition. These measures can help direct families towards more nutrient dense and folate-rich food purchases.

4.5 Policy Implications

Legislation can be an effective tool to implement population wide change and can be targeted to specific sub-populations (i.e. CD). Multiple regulations that target transition and adherence to the GFD may provide better support for Canadian CD patients.

4.5.1 Meeting Folate Needs with Diet and Folate Fortification Policies.

Given the challenges of meeting folate requirements through natural food sources alone, fortification may help to enrich the current GF food supply with additional folate. Folate fortification of white flour has proven to be successful in Canada (88), with demonstrated benefits for the whole population, from women of child bearing age to preschoolers. (89, 90, 132) Shuaibi et al. found that Canadian folate fortification policies resulted in an increase in folate intake of 96µg/day in women of child bearing age (90), and Hennessy-Priest et al. found that Canadian pre-schoolers had an increase in intake of 83µg/day. (89) Similar benefits in dietary folate intake could be seen in the Canadian CD population if GF grains and food products were folate fortified.

4.5.2 Folate Fortification of GF Food/Grain Products in Canada

Currently no Canadian legislation addresses the nutritional quality of GF food products. (54) Thus any changes in the nutritional quality of GF food products is left in the hands of manufacturers, leaving a lack of consistency between food products in the GF market place. Nutrient fortification of GF food products is voluntary in Canada; thus, folate levels can vary between and within brands, making it difficult for CD patients to find folate fortified GF food products. Establishing minimum folate fortification levels in GF food products ensures consistency for folate content. The current Canadian folate fortification levels for white wheat

flour is 150 µg/100g (33), this can be used as a reference point for the fortification of GF flours and food products. The Canadian experience with this level of folate fortification shows that requirements are met for the majority of population (132) and women in particular can obtain a mean folate intake of 486.4µg per day. (90) There is no information regarding the bioavailability of folic acid in GF food products in combination with the malabsorption issues of CD. The lower folate bioavailability seen in folate fortified foods (12) and the malabsorption issues with CD (6) may mean that CD patients absorb a much lower amount of folic acid than what is stated on the nutrition label. **Table 4.1** lists the advantages and disadvantages of making folate fortification mandatory in GF grain products.

Table 4.1: The Advantages and Disadvantages of Introducing a Mandatory Folate Fortification Regulation for Gluten Free Food Products

Advantages	Disadvantages
Increases the folate content of GF food products (this can match the current Canadian folate fortification level of wheat flour)	Fortification levels may not be enough to overcome nutrient malabsorption in CD at initial diagnosis (supplementation needed)
CD patients will be able to receive the same benefits as the healthy population from folate fortification. (i.e. risk of neural tube defects during pregnancy are lowered)	Folate fortification in food is inadequate for pregnant women (additional supplementation needed)
The addition of folate to food products is relatively inexpensive for manufacturers	The bioavailability of folate in GF food products is unknown
Current practices are already established for folate fortification of wheat flours, no new methods are needed	Changes to the manufacturing process may need to be made to be able add folate to GF food products. This may involve upfront costs smaller manufacturers may not be able to pay.
GF=Gluten Free; CD=Celiac Disease	

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Concerns have arisen over the potential harms of excess folate intake from fortified food in the healthy population. The potential of folate fortification masking vitamin B12 deficiency was not found in studies examining folate intakes before and after fortification. (133) A 2010

meta-analysis on randomized control trials looking at the effects of lowered homocysteine with B vitamins and cancer found no increased risk of cancer incidence or mortality. (134) No direct link has been seen between the increased observations of colorectal cancer incidences and established mandatory folate fortification. (135) Consumption of folate fortified foods can increase the percentage of unmetabolized folic acid in the blood (9.4% before fortification vs. 19.1% after fortification) (136) due to due to saturation of the body's ability to reduce folic acid. (133) No evidence has been found pointing to adverse health effects from exposure to unmetabolized folic acid in the body. (133) Canada's experience with folate fortification has been positive and is a good example to follow for the fortification of GF food products. (89, 132)

White wheat flour, breads, cereals and pasta are fortified with folate in Canada. (133) This strategy is effective as flour is utilized in many commonly consumed grain products, and breads and cereals are popular grain products in Canada. (112) A study looking at the consumption patterns of grain products in Canada found that grain products (mainly whole grain and white bread) provided 45% of dietary folate intake. (112) Children and adolescents with a pasta based consumption pattern had the highest folate intakes in the cohort. (112) An Ontario study looking at the folate intake of healthy preschoolers found that 80% of children consumed folate fortified breakfast cereals. (89) The same types of grain products can be found in the dietary intakes of children in the study (**Chapter 3**). This would imply that if GF breads, cereals and pastas were fortified with folate, it should directly improve the folate intake with little need for change in dietary pattern. GF rice and maize flours must also be included in the folate fortification to ensure that homemade products will also receive the same nutritional benefit. Teixeira et al. demonstrated in a multi-ethnic population of pregnant women, that the folate fortification of rice was able to provide 38% of folate intake in Asian women through the

consumption of noodles, rice and pasta. (137) This has important implications for fortifying GF food products as rice and maize grains are prevalent in GF food products (13), fortification of these grains will ensure that children, adolescents and adults following the GFD will obtain the benefits.

4.6 Future Directions

Assessments outside of the home are also needed to assess the access and availability of folate-rich GF food items, as food intake can occur anywhere in and outside of the home. It is known that having increased access to healthy food retail establishments can help children improve diet quality. (121) Thus, interventions that increase the selection of folate-rich food items at schools, daycares and community centers can affect the availability and access and improve dietary intake.

Knowing the bioavailability of folate is important to determine the amount absorbed by the body. The bioavailability of folate-fortified foods is estimated to be 85%. (12) However, little is known about whether and how the folate bioavailability of folate fortified GF food products may affect the bioavailability of other nutrients and the effectiveness of fortification on micronutrient intake on the GFD. If folate fortification can influence the absorption of other nutrients, it may be beneficial to see if fortification with multiple vitamins and minerals including folate is better.

Other examples of future studies include the use of community kitchens and phone apps. Community kitchens can be effective in providing CD patients with targeted nutritional knowledge and cooking skills. There is evidence that family-based nutritional interventions are effective in implementing change for vegetable intake. (138) Phone apps can be used to change

consumer behaviours towards the purchase and incorporation of folate-rich vegetables and legumes. The phone application can be a teaching tool that can assist in continued nutritional education to improve folate intake on the GFD.

4.7 Final Conclusions

This thesis explores the gap between the nutritional quality of GF food products at supermarkets and the dietary intake of children with CD following the GFD. Results indicate that dietary folate intake is not sufficient for most children following the GFD to meet folate needs for age and sex. Household purchases of GF food items are folate poor and few GF food products are folate fortified. Supplementation can help increase folate intake to meet the EAR for age and sex; however, consideration on the barriers to adherence is needed. Health professionals should consider strategies that assist CD patients in selecting more folate-fortified, GF food products or folate-rich foods to help increase folate intake. The development of a nutritional literacy tool that targets adherence and nutritional adequacy on the GFD will help patients and their families make better decisions on food purchases. Fortification is one option to increase the folate content of GF food products without changing dietary habits. Future research may include the development of targeted interventions (i.e. community kitchens, phone apps); school, daycare and community centre assessment of access to GF food items; and the bioavailability of folic acid fortified GF food products and their interactions with other nutrients.

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Appendix

Table A.1: Studies on Folate Intake in Children and Adults on the Gluten Free Diet.

Author	Year	Country	Sample Size	Age Range	Folate Intake (µg DFE)	Foods Consumed
Adult Studies						
Burger et al. (50)	2018	Netherlands	n=250	45.5±17.1y	n/a	n/a
Valente et al. (57)	2015	Brazil	n=20 (CD)	36.3±13.7y	130.8±53.6	All CD patients (100%) had folate intakes below the EAR (320µg/day). 3-day food records were used to assess dietary intake.
Dickey et al. (58)	2008	Ireland	n=100 (CD)	54.7±12.4y	33.1µg/MJ	Dietary intake of folate did not differ from controls. A 4-day food diary was used to assess dietary intake.
Hallert et al. (30)	2002	Sweden	n=30	45-64y	184±54	CD patients had a lower folate intake when compared to controls ($p<0.05$) and was also below the Nordic Nutrition Recommendations (300µg). A 4-day food record was used to assess dietary intake.
Kemppainen et al. (59)	1998	Finland	n=40	24-65y (M); 18-62y (F)	n/a	A 4-day food record was used to assess dietary intake. Micronutrient intake was not assessed in this study.
Children Studies						
Fernández et al. (60)	2019	Spain	n=70 (CD)	4-18y	202.4 (69.9-571.5)	Folate intake was significantly lower in children with CD in comparison to controls ($p<0.05$). Children with CD were reported to have 8 (4-8) servings of vegetables, 2 (2-2) servings of legumes and 14 (11-20) servings of bread/pasta/rice per week. Three 24-h dietary records and a food frequency questionnaire were used to assess dietary intake.
Deora et al. (61)	2017	Canada	n=140	7.8±4.0y	n/a	n/a
Wessels et al. (62)	2016	Netherlands	n=182	6.3±4.3y	n/a	n/a
Nijhawan et al.	2013	India	n=363	19±7y	n/a	n/a

(49)						
Hjelt and Krasilnikoff (63)	1990	Denmark	n=20	1.2-16.6y	n/a	Average folate intakes were at 90% of the RDA when on the GFD; average intake in µg was not stated. A 7-day food record was used to assess dietary intake.
n/a: none available; DFE: Dietary Folate Equivalents; CD: Celiac Disease; F: female; M: male; EAR: Estimated Average Requirement; RDA: Recommended Dietary Allowance; GFD: Gluten Free Diet						

Adapted with permission from: Burger JPW, van der Laan JJH, Jansen TA et al. Low yield for routine laboratory checks in follow-up of coeliac disease. J Gastrointestin Liver Dis, 2018, 27, 233-239. (50); Valente FX, Campos Tdo N, Moraes LF et al. B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study. Nutr J, 2015, 14, 1-8. (57); Dickey W, Ward M, Whittle CR et al. Homocysteine and related B-vitamin status in coeliac disease: Effects of gluten exclusion and histological recovery. Scand. J. Gastroenterol, 2008, 43, 682-688. (58); Hallert C, Grant C, Grehn S et al. Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. Alimentary Pharmacology & Therapeutics, 2002, 16, 1333-1339. (30); Kemppainen TA, Kosma VM, Janatuinen EK et al. Nutritional status of newly diagnosed celiac disease patients before and after the institution of a celiac disease diet--association with the grade of mucosal villous atrophy. Am. J. Clin. Nutr., 1998, 67, 482-487. (59); Fernández BC, Varela-Moreiras G, Úbeda N et al. Nutritional Status in Spanish Children and Adolescents with Celiac Disease on a Gluten Free Diet Compared to Non-Celiac Disease Controls. Nutrients, 2019, 11(10), 1-22. (60); Deora V, Aylward N, Sokoro A et al. Serum Vitamins and Minerals at Diagnosis and Follow-up in Children With Celiac Disease. J Pediatr Gastroenterol Nutr, 2017, 65, 185-189. (61); Wessels MM, van V, II, Vriezinga SL et al. Complementary Serologic Investigations in Children with Celiac Disease Is Unnecessary during Follow-Up. J Pediatr, 2016, 169, 55-60. (62); Nijhawan S, Katiyar P, Nagaich N et al. Prevalence of associated disorders in Indian patients with celiac disease. Indian J Gastroenterol, 2013, 32, 330-334. (49); Hjelt K, Krasilnikoff PA. The Impact of Gluten on Haematological Status, Dietary Intakes of Haemopoietic Nutrients and Vitamin B12 and Folic Acid Absorption in Children with Coeliac Disease. Acta Paediatrica, 1990, 79, 911-919. (63)

Table A.2: Classification Categories on Receipt Purchases.

Food Category	Examples
Fruits & Vegetables	Fresh/Dried/Frozen fruits and vegetables, Juice
GF Gain Products	GF Breads, GF Pasta, GF Cereals, GF Bakery products
Gluten Containing Grain Products	Whole Wheat Bread, Wheat-based Pasta, Cereals made of wheat or barley, Baked Goods made from wheat flour, Pizza crust
GF Whole Grains	Rice, Corn, Oats, Quinoa, and Buckwheat
Gluten Containing Whole Grain Flours	Wheat, Barley, and Rye flours
GF "Other" foods products	GF versions of prepackaged meals, condiments, seasonings, snacks, and baked goods
Milk	Cow's Milk, Milk Alternatives (soy, almond), Cheese, Yogurt
Nuts	Almonds, Walnuts, Cashews, Seeds, Flax, Sunflower seeds
Meat Products	Beef, Pork, Chicken, Fish, Eggs, Deli meat, Canned Meats/Fish
Unprepared Meals	Canned Soups, Dry Soup mix, Boxed Pasta (Mac and Cheese)
Prepared Meals	Ready-to-eat Meals, Frozen Meals, Meal Replacements
Restaurant Meals	Fast Food, Family Restaurants, Fine Dining, Catering
Snacks	Cookies, Chips, Chocolate, Ice cream
Baking & Cooking Ingredients	Spices & Seasonings, Honey, Cooking oil, Salt, Sugar, Baker's Yeast
Condiments and Sauces	Butter, Peanut Butter, Salsa, Tomato sauce, Ketchup, Mayo
Beverages	Tea, Coffee, Soft Drinks, Alcohol, Bottled Water
Unidentifiable Food Items	General Grocery, General Produce, Open Food entries on receipts
Non food items	Kitchen Items, Books, Clothing, Coupons
GF=Gluten Free Number of families, N=73. Number of children, N=77. Categories adapted from Mager et al. (8)	

Table A.3: Examples of Common Food Items Purchased.

Food Category	Commonly Purchased Folate Poor Food Items*	Commonly Purchased Folate Rich Food Items*	Commonly Eaten Food Items in Children with CD[Δ]
Vegetables	Carrots, Cucumber, Tomatoes	Broccoli, Romaine lettuce, Kale, Spinach	Cucumber, Carrot, Tomatoes, Bell peppers
Fruit	Apple, Banana, Blueberry, Grape	Avocado	Banana, Apples, Grapes
Juice	Apple juice	n/a	Orange juice, Apple juice
Legume	Canned Baked beans	Black beans	Black beans, Kidney beans, Chickpeas
Eggs	n/a	Fresh eggs	Fresh eggs
Animal Protein	Ground Beef, Fresh Chicken	Pulled pork, hot dogs/sausages	Fresh chicken, Fresh beef, Deli meat
Plant Protein	Tofu	Peanuts, sunflower seeds, flax seeds	Almonds, Chia and Flax seeds
Dairy Products	1-2% Milk, Greek yogurt, Mozzarella	Brie cheese	1-2% milk, 2% yogurt, Cheddar cheese, Marble cheese
Beverages	Bottled water, Soda (incl. Diet), Orange flavoured juice drink	n/a	Water
GF Whole Grains	Rice noodles, White Rice	Enriched instant rice, Rolled Oats, Quinoa	White Rice, Rolled Oats, Rice flour
GF Grain Products	GF penne/fuisilli, GF flour blends	Folate fortified GF cereal, Folate fortified GF white bread/buns/bagels	GF Bread, Folate fortified GF Cereals
Gluten Free Processed Foods	GF cookies, crackers and muffins	GF potato chips, Bean flour based crackers	GF multigrain crackers/cookies/pretzels
Gluten Containing Grain Products	Corn Tortilla shells not stated to be GF, granola bars	Wheat-based cereals/pasta/crackers, White bread/hot dog/hamburger buns/bagels	n/a
Processed Meals	Canned soup, Frozen Hash browns	Frozen pizza (cheese/pepperoni), Boxed Marconi and Cheese	Frozen French Fries
Snacks & Sweets	Chocolate, Ice Cream, Gummy candies, Tortilla chips, Popcorn	Potato chips, Wheat based cookies	Chocolate, Ice Cream/Frozen Yogurt, Tortilla Chips, Popcorn, GF Rice Crackers
Condiments	Butter/Margarine, Salad Dressing, Pasta sauce	Peanut Butter, Hummus, Guacamole	Butter, Ketchup, Peanut Butter, Honey
Seasonings	Cooking oil, Sugar, Salt	Dry Yeast, Onion powder	Canola oil, Salt, Sugar

CD= Celiac Disease; GF=Gluten Free; n/a=Not Applicable. Number of families, N=73. Number of children, N=77.
*Folate-rich is defined as ≥ 60µg DFE folate/100g, equivalent to 15% of the Daily Value as defined by Health Canada. (101, 102) Folate-poor is ≤ 20µg DFE folate/100g, equivalent to 5% of the Daily Value as defined by Health Canada. (101, 102) Analysis is based on a sample of n=20,338 purchased items. [Δ]Analysis is based on a sample of n=3,648 food items consumed by children and adolescents with CD following the Gluten Free Diet, between the ages of 3-18 years.

Table A.4: Table of Food Guide Servings Needed to meet Recommended Dietary Intake Levels for Folate.

Food Item	Amount needed to provide 300µg/day	Amount needed to provide 400µg/day
Foods with 15µg DFE/100g, representing the median folate content of food purchases		
Fresh Tomatoes, diced	10.5 cups	14 cups
Greek Yogurt, 0% MF, plain	7.9 cups	10.6 cups
Butter Popcorn	241 cups	321 cups
Tofu, firm	7.6 cups	10.2 cups
Sushi Rolls (26g/roll)	77 rolls	103 rolls
Foods with 36µg DFE/100g, representing the average folate content of food purchases		
Generic Garden Salad	5.7 cups	7.6 cups
Celery, diced	6.6 cups	8.8 cups
Gluten Free Bread	24.5 slices	32.7 slices
Red Bell Pepper, diced	5.3 cups	7.1 cups
Green Leaf Lettuce	14.1 cups	18.8 cups
Folate-rich* food items (based on folate content), selected from commonly purchased folate-rich food items		
Broccoli, raw	5.1 cups	6.8 cups
Broccoli, cooked	1.7 cups	2.2 cups
Spinach, raw	4.9 cups	6.5 cups
Spinach boiled	1.1 cups	1.4 cups
Kale, raw	3 cups	4 cups
All food items were selected from commonly purchased food items in the household grocery receipts from 73 families in Edmonton, Toronto and Hamilton, Canada. *Folate-rich is defined as ≥ 60µg DFE folate/100g of food, equivalent to 15% of the Daily Value. Folate poor is ≤ 20µg DFE folate/100g of food, equivalent to 5% of the Daily Value. (101, 102)		

Table A.5a: Dietary Intake Simulation for a Child (Male or Female) following the Gluten Free Diet, 3-8 years old.

Day 1	Amount	Folate Content
Breakfast		
GF Honey Corn Flakes	0.75 Cup	n/a
Milk, skim	1 Cup	12.3
Orange, fresh	1 Medium	39.3
Snack #1		
Cashews, dry roasted	0.25 Cup	23.6
Pears, fresh	1 Medium	11.6
Lunch		
Chicken breast, roasting, roasted, skinless	75g	2.3
Cheese, cheddar, diced	28g	5.0
Spinach, fresh	1 Cup	58.2
Avocado, fresh, sliced	0.25 Cup	30.4
Carrots, fresh, sliced	0.5 Cup	12.2
Water, municipal	0.5 Cup	0
Snack #2		
Yogurt, fruit on the bottom, 2% to 4% milk fat	50g	5.9
Cracker, rice cake, mini	20g	4.2
Mandarin oranges, fresh	0.5 Cup	15.6
Water, municipal	0.5 Cup	0
Dinner		
White Rice, long grain, parboiled, cooked	0.5 Cup	2.6
Pork tenderloin, broiled, lean	50g	3
Cauliflower, boiled, drained	1 Cup	54.6
Peas, green, boiled, drained	0.5 Cup	50.4
Edamame, boiled, drained	0.5 Cup	86.0
Salt, table	0.25 Tsp	0
Milk, skim	1 Cup	12.3
Total Folate Intake		**429.5**

The menu simulation was designed to meet the target Estimated Average Requirement for folate, which is 160µg for both males and females, aged 3-8 years. (35) n/a: none available

Table A.5b: Dietary Intake Simulation for a Child (Male or Female) following the Gluten Free Diet, 9-13 years old.

Day 1	Amount	Folate Content
Breakfast		
Puffed Rice Cereal, Enriched	28g	28
Milk, 1%	0.5 Cup	6.1
Banana, fresh	1 Medium	23.6
Lunch		
Standard Gluten Free White Bread (1 slice=31.5g)	2 Slices	8.3
Eggs, boiled, hard cooked in shell	2 Each	54.2
Spinach, boiled, drained	0.5 Cup	131.43
Pears, fresh	1 Medium	11.62
Milk, 1% milk fat	1 Cup	12.2
Snack		
Blackberries, fresh	1 Cup	36.02
Walnuts, dried	0.25 Cup	28.69
Dinner		
Brown rice, long grain, cooked	0.5 Cup	3.9
Chicken breast, roasting, roasted, skinless	71g	2.13
Gravy, turkey, canned	0.5 Cup	2.38
Asparagus, boiled, drained	0.5 Cup	134.09
Peas, green, boiled, drained	0.5 Cup	50.4
Butter	1 Tsp	0.14
Milk, 1% milk fat	1 Cup	12.2
Total Folate Intake		**533.1**

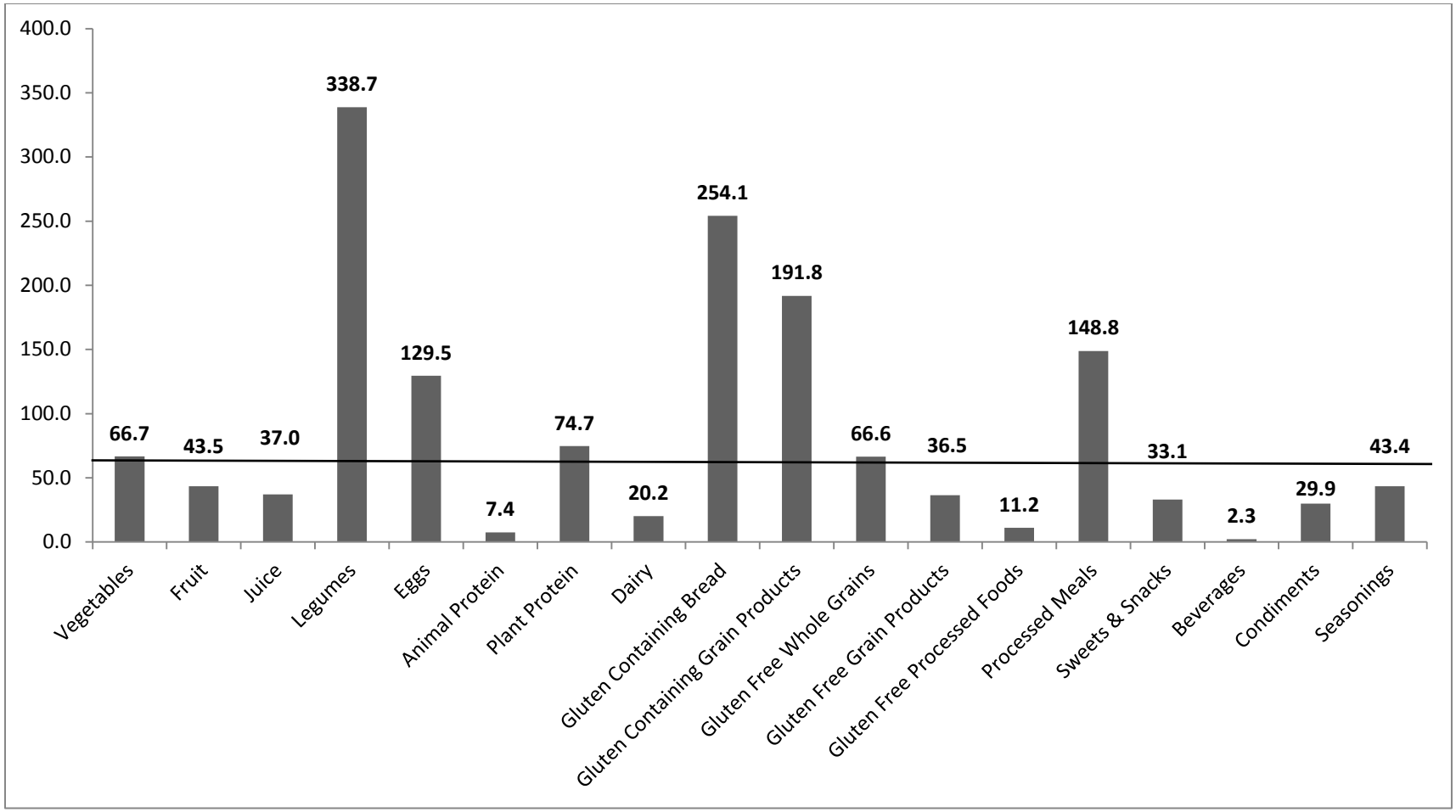
The menu simulation was designed to meet the target Estimated Average Requirement for folate, which is 250µg for both males and females, aged 9-13 years. (35) n/a: none available

Table A.5c: Dietary Intake Simulation for a Child (Male or Female) following the Gluten Free Diet, 14-18 years old.

Day 1	Amount	Folate Content
Breakfast		
Standard Gluten Free Breakfast Cereal	1 Cup	46.6
Banana, fresh	1 Medium	23.6
Strawberries, fresh, halves	0.5 Cup	18.24
Milk, skim	1.5 Cup	18.37
Lunch		
Standard Gluten Free White Bread	2 Slices	8.3
Margarine, canola & safflower, non-hydrogenated	1 Tsp	0.05
Turkey breast, broiler, roasted, skinless	50g	3
Cheese, cheddar, diced	25g	4.5
Cucumber, fresh, sliced	56g	3.92
Avocado, fresh, sliced	3 Tbsp	22.78
Lettuce, romaine, fresh	1.5 Cup	114.29
Salad dressing, ranch, food service	1 Tbsp	0.58
Snack		
Orange, fresh	1 Medium	39.3
Pudding, chocolate	92g	2.76
Dinner		
Rice, brown, cooked, medium grain	1 Cup	7.8
Chicken breast, roasting, roasted, skinless	4.5 Oz	3.83
Starch, corn	1 Tsp	0
Soy sauce, tamari	0.25 Tbsp	0.81
Pineapple, fresh	1 Cup	27.9
Bell pepper, green, fresh, chopped	0.25 Cup	3.72
Bell pepper, red, fresh, chopped	0.25 Cup	17.13
Black pepper	0.125 Tsp	0.03
Oil, olive	0.25 Tsp	0
Bok choy, cooked, drained	0.75 Cup	52.27
Carrots, boiled, drained	1 Tbsp	1.36
Onion, boiled, drained, chopped	1 Tbsp	1.97
Cashews, dry roasted	2 Tbsp	11.81
Milk, skim	1 Cup	12.25
Snack		
Walnuts, dried	0.25 Cup	24.52
Mango, fresh	1 Cup	23.09
Total Folate Intake		**494.9**

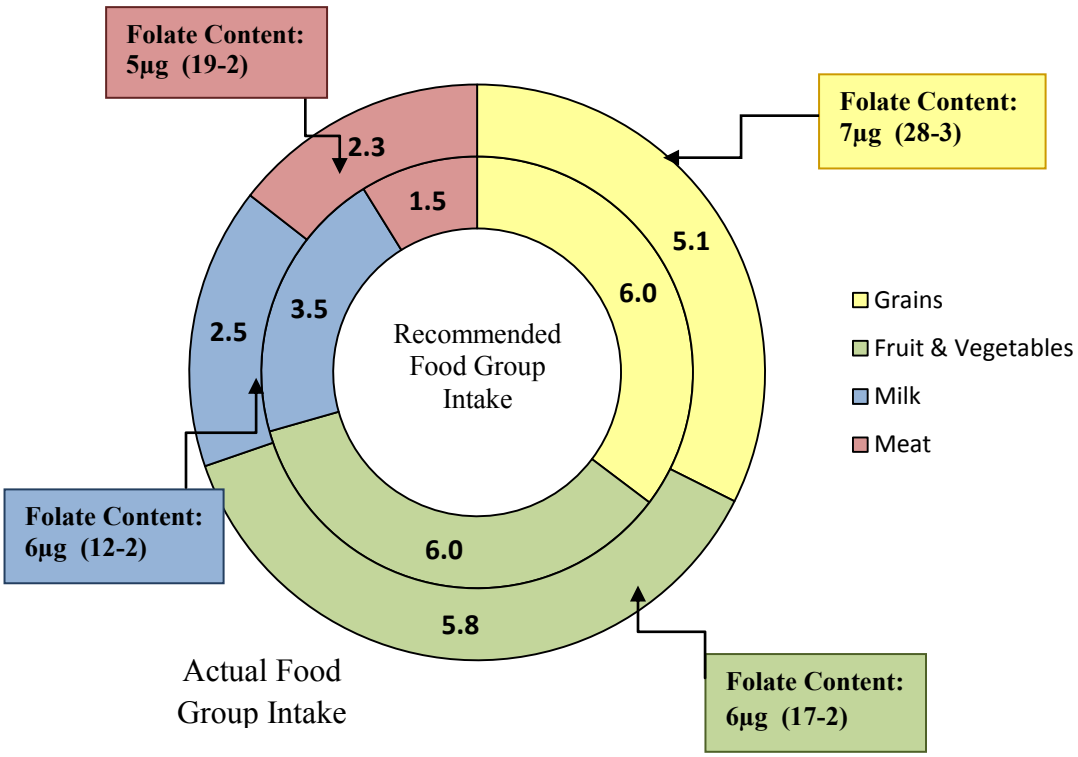
The menu simulation was designed to meet the target Estimated Average Requirement for folate, which is 330µg for both males and females, aged 14-18 years. (35) n/a: none available

Figure A.1: Folate Purchased (µg DFE/$CAD) within Individual Food Categories.



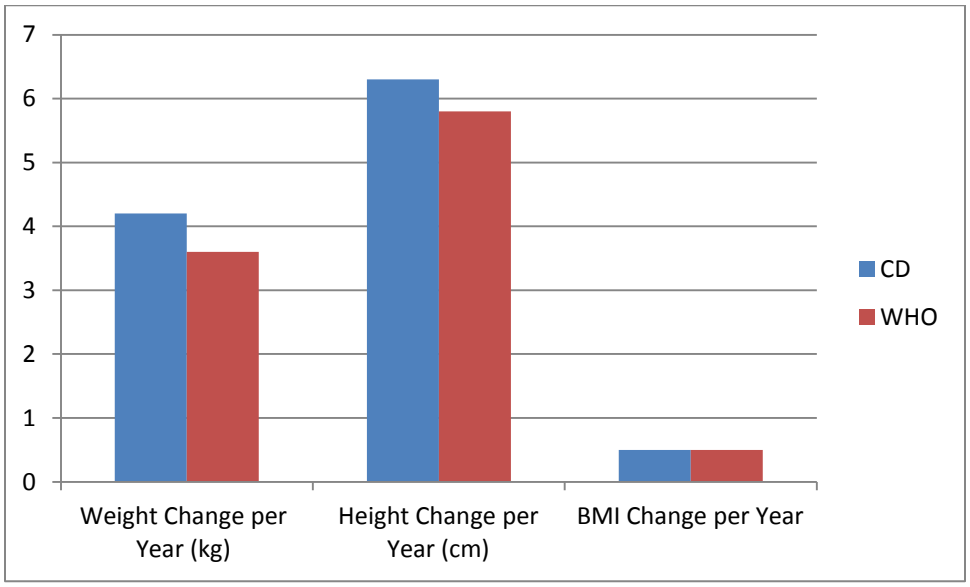
DFE = Dietary Folate Equivalents. This graph represents the amount of folate per dollar purchased divided by food category. The folate DFE (in µg) was used to calculate folate content. All prices are in Canadian Dollars. The line represents the cut-off definition for a folate-rich food source, set at 60µg DFE/100g food, as defined by Health Canada. (101, 102) Purchases made outside of Canada had the price converted to Canadian Dollars using the exchange rate on the date of purchase. All exchange rates were obtained from the Bank of Canada.

Figure A.2: A Comparison between the Recommended Food Group Intakes as stated by the Alberta Nutrition Guideline for Children & Youth and Actual Food Group Intakes of Children and Adolescents with CD following the GFD.



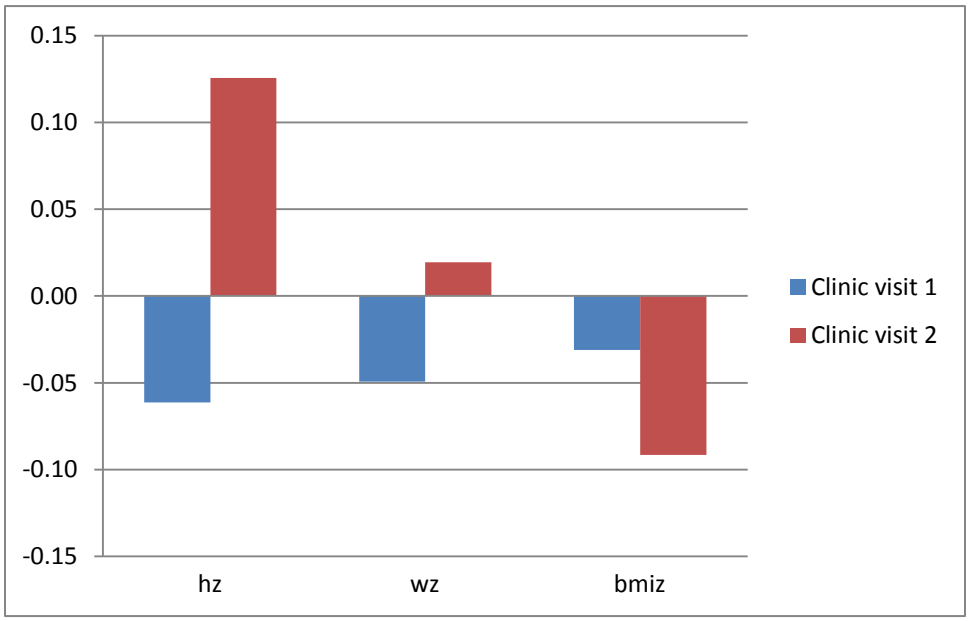
The values in the box represent the folate content (µg) of food consumed in each food group and presented as median (interquartile range Q3-Q1). Values in the circles represent the median number of food group servings consumed. The outer circle represents the food group servings children with Celiac Disease (CD) following the Gluten Free Diet (GFD) consumed and the inner circle represents the food group servings recommended by the Alberta Nutrition Guidelines for Children and Youth (ANGCY). Food group servings have been defined and calculated according to ANGCY. The dietary intake of n=77 children and adolescents between the ages of 3-18 years were included in the analysis. Dietary intake was collected through two 24-hour food recalls (one weekday and one weekend day). The "Gains" group includes naturally gluten free grains (rice, quinoa) and certified gluten free grain products (bread, cereal). The "Fruit & Vegetables" group includes fresh and processed fruits and vegetables. The "Milk" group includes cow's milk, cheese and yogurt. The "Meat" group includes animal (beef, chicken, pork) and plant sources (tofu, soy beverage, legumes, nuts, and seeds). Folate content was derived from the Canadian Nutrient File. Mixed foods were broken down into their components according to family recipes.

Figure A.3: Change in Weight, Height, and BMI per year in Children and Adolescents with Celiac Disease.



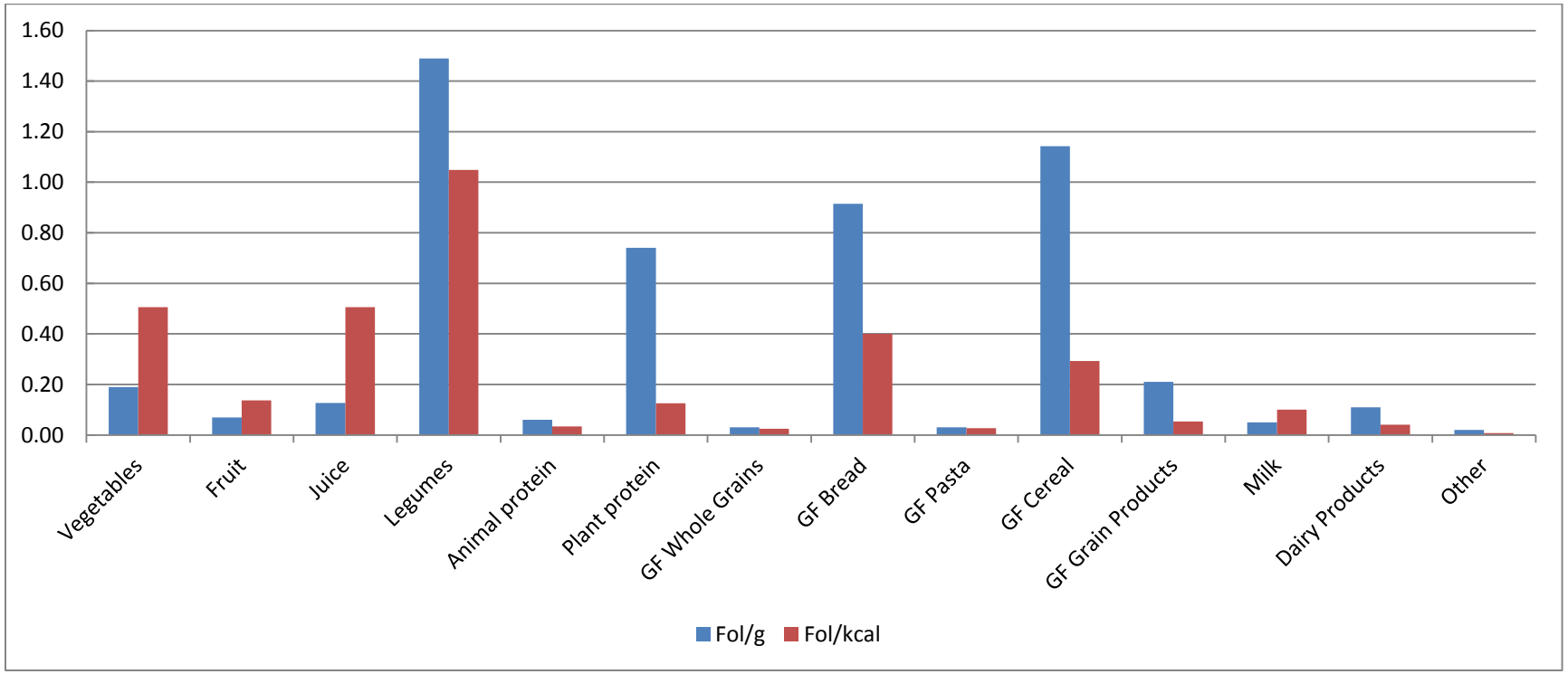
The changes in weight, height, and BMI were calculated on a yearly basis in children and adolescent with Celiac Disease. CD represents the weights, heights and BMI obtained from patients recruited from the Stollery Children's Hospital (Edmonton, AB). WHO represents the reference values obtained from the World Health Organization growth charts for male and female children between the ages of 9-11 years. A total of n=79 male and female children and adolescents between the ages of 3-18 years were included in the analysis. Fourteen (n=14, 18%) children from this analysis are also in the Receipt study cohort (Chapter 3). Sixty-five (n=65, 82%) children from this analysis are not in the Receipt study cohort. The change in weight, height and BMI was calculated as the difference between the first and second clinic visit and then divided by the time between clinic visits (in years). The mean duration between clinic visits is 1.3±0.6 years.

Figure A.4: Mean Height, Weight and BMI Z-score of Children and Adolescents with Celiac Disease at their First and Second Clinic Visit at the Celiac Clinic. (Stollery Children's, Edmonton, AB)



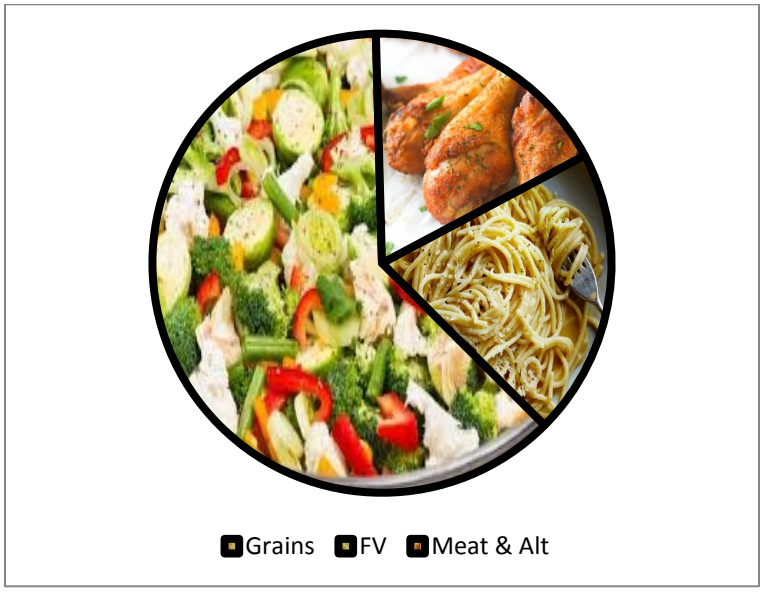
Presented are the mean z-scores of the height, weight and BMI that were documented on the first and second clinic visit at the celiac clinic at the Stollery Children's Hospital (Edmonton, AB). A total of n=79 male and female children and adolescents between the ages of 3-18 years were included in the analysis. Z-scores were calculated using WHO growth standards. (105) Values above the zero (0) line indicate a positive z-score and values under the zero line indicate a negative z-score. The mean duration between clinic visits is 1.3±0.6 years. The mean percentage change in weight z-score is 17.7±16.7 %. The mean percentage change in height z-score is 6.6±6.7 %. The mean percentage change in BMI z-score is 3.3±1.8 %. hz=height z -score, wz=weight z-score, bmiz=Body Mass Index z-score, BMI=Body Mass Index, WHO=World Health Organization

Figure A.5: Dietary Intake of Folate (DFE) in Children and Adolescents with Celiac Disease following the Gluten Free Diet broken down by Food Category.



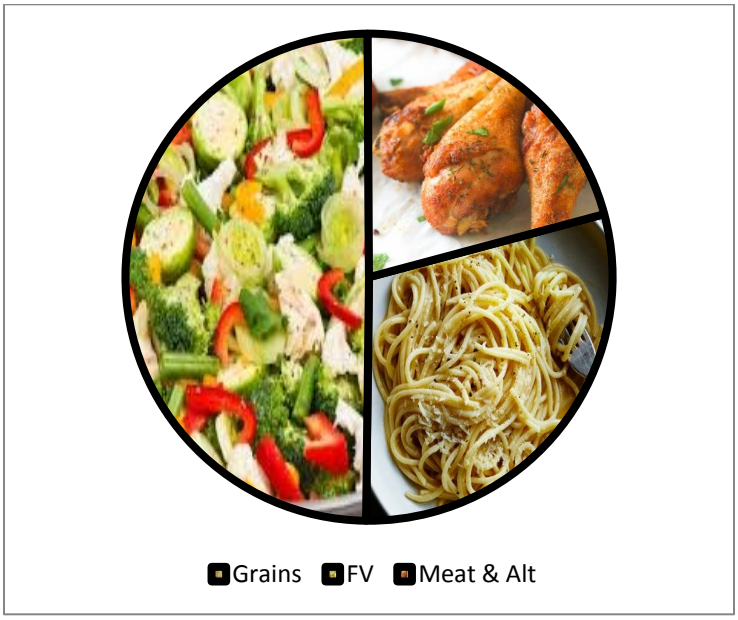
The dietary intake of folate (DFE) in children and adolescents with Celiac Disease following the Gluten Free Diet is presented in two formats. Folate calculated is in DFEs. Folate per gram (Fol/g) is the folate obtained per gram of food consumed. Folate per calorie (Fol/kcal) is the folate obtained per calorie consumed. The dietary intake of n=77 children and adolescents was obtained through two, non-consecutive 24-hour food recalls (one weekday and one weekend day). A total of 3648 food items were analyzed. Folate content was analyzed using ESHA Food Processor Nutrition Analysis Software (SQL 11.0.124, 2015, ESHA Research, Salem, OR, USA) and manufacturer food labels when available. Animal protein includes red meat, fish and their associated food products; Plant protein includes nuts, seeds and their associated food products; GF Whole Grains includes rice, oats, buckwheat and quinoa; GF Grain Products includes baking mixes, cereal bars, and GF crackers; Dairy Products includes yogurt, cheese and butter; and the Other category includes fries/chips, take out, condiments and seasonings. GF=Gluten Free, DFE=Dietary Folate Equivalents

Figure A.6a: Proportion of food group intake in menu simulation for children, 3-8 years old following the Gluten Free Diet.



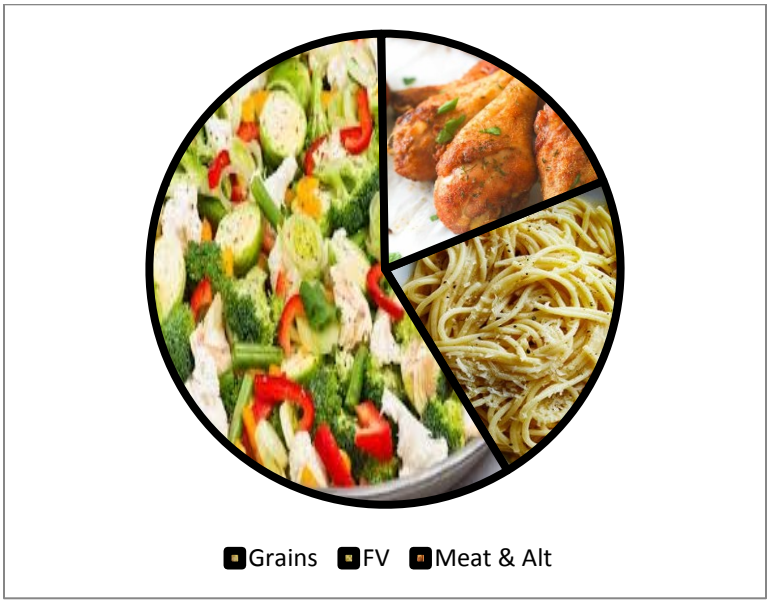
The pie graph represents the food group intake for Grains, Fruits & Vegetables (FV) and Meat & Alternatives (Meat & Alt) based on the menu simulation presented in **Table A.5a**. The total food group servings of grains consumed on the menu simulation were 3.1 and this group provided 1.6% of total folate intake (6.8µg DFE). The total food group servings of fruits & vegetables consumed on the menu simulation was 9.5 and this group provided 6.7% of total folate intake (28.9µg DFE). The total food group servings of meat & alternatives consumed on the menu simulation was 2.7 and this group provided 83.4% of total folate intake (358.2µg DFE). Food group servings were defined and calculated according to the Alberta Nutrition Guidelines for Children and Youth (ANGCY). Folate content was determined using the Canadian Nutrient File and manufacturer provided nutritional information. The menu was designed to meet the folate EAR of 160µg for males and females between the ages of 3-8 years. The "Gains" food group includes naturally gluten free grains (rice, quinoa) and certified gluten free grain products (bread, cereal). The "Fruit & Vegetables" food group includes fresh and processed fruits and vegetables. The "Meat" food group includes animal (beef, chicken, pork) and plant sources (tofu, soy beverage, legumes, nuts, and seeds).

Figure A.6b: Proportion of food group intake in menu simulation for children, 9-13 years old following the Gluten Free Diet.



The pie graph represents the food group intake for Grains, Fruits & Vegetables (FV) and Meat & Alternatives (Meat & Alt) based on the menu simulation presented in **Table A.5b**. The total food group servings of grains consumed on the menu simulation were 4.1 and this group provided 6.1% of total folate intake (31.9µg DFE). The total food group servings of fruits & vegetables consumed on the menu simulation was 7.0 and this group provided 16.7% of total folate intake (87.4µg DFE). The total food group servings of meat & alternatives consumed on the menu simulation was 2.9 and this group provided 73.8% of total folate intake (387.1µg DFE). Food group servings were defined and calculated according to the Alberta Nutrition Guidelines for Children and Youth (ANGCY). Folate content was determined using the Canadian Nutrient File and manufacturer provided nutritional information. The menu was designed to meet the folate EAR of 250µg for males and females between the ages of 9-13 years. The "Gains" food group includes naturally gluten free grains (rice, quinoa) and certified gluten free grain products (bread, cereal). The "Fruit & Vegetables" food group includes fresh and processed fruits and vegetables. The "Meat" food group includes animal (beef, chicken, pork) and plant sources (tofu, soy beverage, legumes, nuts, and seeds).

Figure A.6c: Proportion of food group intake in menu simulation for children, 14-18 years old following the Gluten Free Diet.



The pie graph represents the food group intake for Grains, Fruits & Vegetables (FV) and Meat & Alternatives (Meat & Alt) based on the menu simulation presented in **Table A.5c**. The total food group servings of grains consumed on the menu simulation were 4.5 and this group provided 11.2% of total folate intake (54.4µg DFE). The total food group servings of fruits & vegetables consumed on the menu simulation was 11.8 and this group provided 8.9% of total folate intake (43.1µg DFE). The total food group servings of meat & alternatives consumed on the menu simulation was 3.9 and this group provided 71.8% of total folate intake (349.6µg DFE). Food group servings were defined and calculated according to the Alberta Nutrition Guidelines for Children and Youth (ANGCY). Folate content was determined using the Canadian Nutrient File and manufacturer provided nutritional information. The menu was designed to meet the folate EAR of 330µg for males and females between the ages of 14-18 years. The "Gains" food group includes naturally gluten free grains (rice, quinoa) and certified gluten free grain products (bread, cereal). The "Fruit & Vegetables" food group includes fresh and processed fruits and vegetables. The "Meat" food group includes animal (beef, chicken, pork) and plant sources (tofu, soy beverage, legumes, nuts, and seeds).