7-2013

Nutrient intakes of healthy adults on a gluten-free diet

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Nutrient Intakes of Healthy Adults on a Gluten-Free Diet

by

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Thesis

Submitted to the School of Health Sciences

Dietetics and Human Nutrition Program

Eastern Michigan University

In partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Dietetics

Thesis Committee:

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July 2013

Ypsilanti, MI

ABSTRACT

Gluten-free diets have gained popularity in recent years among healthy individuals. However, gluten-free foods are typically lower in essential nutrients. It was hypothesized that the average intakes of fiber and the selected B vitamins and minerals would be lower in the gluten-free group than in the regular diet group. Fifty-eight adults, aged 25-35, participated in the study. Participants completed a five-day food record and questionnaire. FoodWorks® nutrient analysis software was used to evaluate intake of macronutrients and selected micronutrients. Gluten-free males consumed significantly lower amounts of carbohydrates, fiber, niacin, folate, and calcium but significantly higher amounts of fat and sodium than regular diet males. Gluten-free females consumed significantly lower amounts of carbohydrates, fiber, folate, iron, and calcium but significantly more fat, saturated fat, and cholesterol than regular diet females. Overall, gluten-free adults did not consume enough nutrient dense foods to meet all nutrient recommendations.

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Chapter 1: Introduction and Background

Introduction

Gluten is a term used to describe the two types of proteins found in wheat, known as prolamins and glutenins (Saturni, Ferretti, & Bacchetti, 2010). Many gluten containing grains such as wheat, rye, barley, triticale, kamut, and malt are staples in the American diet. Grains provide nutrients such as thiamin, riboflavin, niacin, folate, iron, magnesium, selenium, and dietary fiber (Raymond, Heap, & Case, 2006). Whole grains commonly consumed in the United States are wheat, rice, oats, barley, and maize (US Department of Agriculture [USDA], n.d.). Other foods may include gluten as part of a mixture, which may be less noticeable. Such foods include but are not limited to some dried fruits, marinades, seasoning, sauces, and salad dressings (O'Neil, Nicklas, Zamovec, & Cho, 2010).

There are a growing number of individuals who suffer from conditions that require elimination of gluten or wheat from the diet. One condition, celiac disease, affects more than two million Americans (The National Digestive Diseases Information Clearinghouse [NDDIC], 2012). Other conditions include wheat allergy, dermatitis herpetiformis, neurological complications, and non-celiac gluten-sensitivity (Pietzak, 2012; Sapone et al., 2012).

Wheat is one of the eight most common food allergens in the United States, affecting approximately 0.1% of the population (Pietzak, 2012). A wheat allergy is similar to celiac disease in that the immune system generates a reaction to the proteins in wheat. However, with this type of allergy the immune system is reacting to gliadins, and patients can usually ingest prolamin containing foods without an issue. For example, patients need to avoid all foods containing wheat but are typically able to eat foods containing rye, barley, and oats,

which do not contain gliadin. This type of allergy can affect the mouth, nose, eyes, throat, skin, and the gastrointestinal tract by causing swelling and irritation (Pietzak, 2012; Sapone et al., 2012). Rashes, hives, and swelling may appear on the skin, and the individual may also experience wheezing and have difficulty breathing (Pietzak, 2012). Gastrointestinal symptoms such as cramps, nausea and vomiting, flatulence, bloating, abdominal pain, and diarrhea are also common. Typically, a reaction occurs within minutes to hours after ingestion of foods containing wheat (Sapone et al., 2012). To avoid these symptoms and possibly death, individuals must avoid all foods containing wheat (Pietzak, 2012). If a wheat allergy is suspected, skin prick tests and *in vitro* Immunoglobulin E (IgE) assays are performed to confirm the diagnosis (Sapone et al., 2012). However, accuracy of these tests is somewhat limited due to low sensitivity of many skin prick reagents and cross-activity with grass pollens in adults. For some individuals, an oral food challenge is needed for diagnosis of this type of food allergy.

Celiac disease is an immune reaction to dietary proteins called prolamins found in certain grains that affects predisposed individuals (USDA, n.d.; Pietzak, 2012). More specifically, the prolamins in wheat, rye, and barley cause the immune reaction in the small intestine to occur (Pietzak, 2012). Ingestion of these foods causes adaptive and innate immune responses, which generate different reactions in the body (See & Murray, 2006). The innate response usually occurs within a few hours of gluten ingestion and causes lymphocyte activation on the surface of the epithelium. Changes in permeability occur as the epithelial cells mutate and produce cytokines. The adaptive immune response causes chronic inflammation in the small intestine and complete loss of gluten tolerance. Eventually, the chronic inflammation causes atrophy of the villi, which results in nutrient malabsorption

(USDA, n.d.; See, & Murray, 2006). In particular, the absorption of vitamin $B_{12,}$ iron, folic acid, and fat-soluble vitamins is reduced due to destruction in the upper portion of the small intestine (See, & Murray, 2006). In addition, elevated immunoglobulin A (IgA) tissue tranglutaminase (tTG) antibodies occur as a response to the condition (Presutti, Cangemi, Cassidy, & Hill, 2007). A strict gluten free diet is the only treatment for celiac disease, which allows reduction of inflammation and healing of the microvillus. Individuals adhering to this type of diet typically have decreased symptoms, improved digestion, and better absorption of nutrients within a few months. However, this is dependent on factors such as age, compliance, and severity of the disease. In addition, elevated antibody levels usually return to normal levels within three to 12 months (Presutti et al., 2007).

Diagnosing celiac disease can be difficult because there are more than 300 symptoms, which may vary among individuals, associated with the condition (National Foundation for Celiac Awareness [NFCA], 2012a). Gastrointestinal symptoms can include flatulence, bloating, diarrhea, and constipation. Others may experience rashes, mouth sores, joint pain, osteopenia or osteoporosis, infertility, weight loss, tingling or numbness anywhere in the body, irritability, headaches, depression, neurological disturbances, or discolored teeth (Sapone et al., 2012; Presutti et al., 2007; NFCA, 2012a). If celiac disease is suspected, the initial test performed is for the presence of serum tTG-IgA antibodies (Presutti et al., 2007). If antibodies are found, a small bowel biopsy is usually done to confirm the diagnosis. However, some individuals are deficient in these transglutaminase antibodies and will not have elevated levels (Sapone at al., 2012; Presutti et al., 2007). In such cases, a serum total IgA level is more accurate followed by a biopsy of the small bowel. Currently, it is estimated that one in every 133 people is affected by celiac disease in the United States, and

95% of those are believed to be suffering from additional undiagnosed or misdiagnosed conditions (NFCA, 2013a). Some reports have found that up to 36% of individuals with misdiagnosed celiac disease had been treated for irritable bowel syndrome (Presutti et al., 2007). Five to 22% of patients diagnosed with celiac disease have a first degree family member with the condition as well, and the diagnosis rate is anticipated to increase over time (NFCA, 2013a). The only treatment for celiac disease is a strict gluten-free diet, and some patients may find it helpful to consult with a registered dietitian.

Dermatitis herpetiformis is a skin rash that can appear almost anywhere on the body and is believed to be characteristic of celiac disease (Pietzak 2012; Sapone et al., 2012). The rash affects one in 10,000 people in the United States and is most commonly seen in individuals of European descent (Sapone et al., 2012). Although it can appear at any age, the rash typically occurs around 40 years of age and is more common in men than women. Similar to celiac disease, dermatitis herpetiformis occurs in approximately five percent of first degree relatives.

In almost all patients, the rash appears on the elbows and upper forearms but is also common on the face, neck, shoulders, torso, knees, and buttocks in a symmetrical pattern. While some individuals may be affected in multiple areas of the body, the rash can also be limited to one or two sites. Initially the rash appears as red, flat patches and then evolves to fluid-filled blisters causing severe itching (Pietzak 2012; Sapone et al., 2012). The rash is usually an ongoing problem for those affected, but in a small percentage of people it is sporadic (Sapone et al., 2012). Few individuals with this condition experience the same gastrointestinal symptoms associated with celiac disease, although the two conditions are related (Pietzak 2012; Sapone et al., 2012). However, 65-75% of patients with dermatitis

herpetiformis have villous atrophy in the upper portion of the small intestine. Also, antibodies present in patients with celiac disease are also found in those with dermatitis herpetiformis. These patients may also develop other conditions associated with celiac disease such as iron deficiency, anemia, and osteoporosis.

Diagnosis of this condition is confirmed by the presence of IgA antibodies in unaffected areas of the skin through a biopsy and through the diagnostic identification of celiac-type antibodies. Once a patient is diagnosed with dermatitis herpetiformis, it is likely that small bowel damage is present as well. Therefore, a biopsy of this area is unnecessary. However, if a biopsy is performed and the intestinal mucosa seems healthy, a gluten-free diet is still recommended because the rash is gluten-sensitive (Sapone et al., 2012). Dermatitis herpetiformis is treated with a combination of medication and a strict gluten-free diet to prevent flare-ups and further complications (Pietzak, 2012; Sapone et al., 2012).

Neurological complications associated with celiac disease include epilepsy, headache, depression, neuromuscular and movement disturbances, dementia, and autism (Rashtak et al., 2010). However, cerebellar ataxia, also referred to as gluten ataxia, is one of the more common conditions associated with celiac disease. Gluten ataxia is an autoimmune disease known to cause damage to the cerebellum, resulting in a lack of coordination of muscle movements (Pietzak, 2012; Sapone et al., 2012). Studies have indicated that patients with sporadic ataxia also tested positive for gluten sensitive antibodies (Sapone et al., 2012). Patients with this condition are typically affected in their early 50s, and the diagnosis rate between men and women is similar (Pietzak, 2012). In approximately 80% of cases, the eye muscles are affected and all patients experience gait ataxia (Sapone et al., 2012). A small percentage of patients have gastrointestinal symptoms, but roughly one third of them show

evidence of intestinal disease following a biopsy. In addition, magnetic resonance imaging (MRI) shows degeneration of the cerebellum in more than half of patients diagnosed with this condition.

Patients showing signs of advanced cerebellar ataxia should initially be tested for gluten sensitive antibodies (Sapone et al., 2012). If antibodies are present, a duodenal biopsy is performed for signs of intestinal disease. However, diagnosis of gluten ataxia can be complicated because not all patients with the condition test positive for these antibodies. A strict gluten free diet is recommended for patients positive for gluten sensitive antibodies even without signs of intestinal disease to improve symptoms associated with ataxia. If patients experience decreased symptoms of this condition by following a gluten free diet for at least one year, a diagnosis of gluten ataxia can be confirmed.

Non-celiac gluten sensitivity, more commonly referred to as just gluten sensitivity, is similar to celiac disease, yet the reaction is not immune mediated and individuals do not experience intestinal damage or malabsorption issues (Pietzak, 2012; Sapone et al., 2012). In recent years, there has been an increase in the number of individuals that appear to suffer from symptoms similar to celiac disease, which seem to lessen or disappear on a gluten-free diet. However, with this condition individuals usually have no evidence of intestinal disease or gluten sensitive antibodies. In addition, patients do not suffer from conditions associated with autoimmune disease (Sapone et al., 2012).

Similar gastrointestinal symptoms that appear with celiac disease and wheat allergy are also common with gluten sensitivity (Pietzak, 2012; Sapone et al., 2012). However, individuals more often experience changes in behavior, depression, bone or joint pain, muscle cramps, numbness in extremities, weight loss, chronic exhaustion, and "foggy mind."

Unlike celiac disease and wheat allergy, sensitivity to gluten cannot be determined diagnostically and is therefore difficult to determine. Initially, patients are tested for celiac disease and wheat allergy, and a biopsy of the small intestine may be performed (Sapone et al., 2012). If these tests are inconclusive, patients are placed on a strict gluten-free diet and regularly monitored for improvement of symptoms. If symptoms resolve, patients are thought to suffer from gluten sensitivity and must maintain this type of diet to prevent recurrence of symptoms. A gluten-free diet may also be used to control symptoms in conditions such as multiple sclerosis, irritable bowel syndrome, and autism (Pietzak, 2012; Sapone et al., 2012).

So, is a gluten-free diet healthier for everyone? Some celebrities, fitness, and nutrition professionals think so, and consumers have been quick to follow this growing trend. In recent years, the gluten-free diet has been perceived to be healthier by the general public, which has further increased the demand for high quality products with an appealing taste (Pietzak, 2012). Prior to recent awareness and consumer demand, gluten-free products could only be found in specialty stores and online. Recent popularity has allowed mainstream grocery stores to begin selling some of these products to meet consumer demands. Gluten-free foods such as bread, cereals, pizza, and baked goods, as well as many others, are starting to be more common in regular grocery stores, and their popularity continues to soar (Pietzak, 2012; Singh, & Whelan, 2011). Companies such as General Mills Inc. have also jumped on the band wagon and are now providing various gluten free foods, such as cake mix and cereals. The company has also developed a website dedicated to gluten-free living, which allows consumers to purchase products and provides hundreds of recipes. In 2008, sales of gluten-free products spiked by 16%, and there are now more than 3,000 products on the

market (Pietzak, 2012; NFCA, 2013b). In 2012, the sale of gluten-free products was estimated to be $12.4 billion, which increased 18% from sales in 2011(NFCA, 2012b).

Although the accessibility of gluten-free products has increased and Americans are spending more money on them, the price of these foods is significantly higher than their gluten containing counterparts (Singh & Whelan, 2011; Lee, Ng, Zivin, & Green, 2007). The cost of gluten-free foods such as cereals, breads, cookies, and crackers range from 76-518% more than their gluten-containing counterparts (Singh, & Whelan, 2011). In addition, the cost of gluten-free sauces are two to 124% greater than those containing gluten. In general, alternative grains are more costly than those containing wheat and may require improvements such as the addition of nutrients during processing, thus further increasing their cost. Regardless of the purchase venue, gluten-free products are more expensive than comparable foods containing gluten, even though their demand has increased.

Wheat-based foods are easily accessible, more affordable, and provide important nutrients in which some gluten-free foods are deficient. Whole grains are rich in nutrients such as starch, fiber, protein, and lipids (USDA, n.d.). They also provide the B vitamins thiamin, niacin, riboflavin, and vitamin E. In addition, whole grains provide minerals such as magnesium, calcium, potassium, phosphorus, iron, and sodium. These foods are also naturally rich in flavonoids and phytochemicals. Health benefits associated with the consumption of whole grains compared to refined grains are decreased risk of obesity, cardiovascular disease, stroke, type two diabetes, hypertension, metabolic syndrome, as well as some types of cancer (Slavin, 2004). The United States Department of Agriculture (USDA) recommends five to eight ounces of grains per day for most adults, and half of those should be whole grains (Raymond et al., 2006).

Many gluten-free substitute foods such as bread, cereals, and pasta have shown to lack certain nutrients as compared to similar wheat-based items (Saturni et al., 2010; Lee et al., 2007). These gluten-free foods are poor sources of vital nutrients such as fiber; the B vitamins folate, niacin, thiamin, riboflavin; and iron (Saturni et al., 2010). Dietary fibers found in foods such as fruits, vegetables, and grains are either soluble or insoluble based on their ability to dissolve in water (US National Library of Medicine [NLM], 2013a). Soluble fiber becomes gelatinous in water and is easily digested by bacteria in the intestines, while insoluble fiber does not dissolve in water and is less easily digested. Adequate dietary fiber intake improves blood glucose levels and decreases blood pressure and cholesterol levels, thus reducing the risk for heart disease, stroke, hypertension, obesity, and diabetes (Anderson et al., 2009). In addition, sufficient intake of fiber helps prevent constipation and promotes a healthy immune system and gastrointestinal tract. The Adequate Intake (AI) of dietary fiber for most adult women under 50 years of age is 25 grams (g) per day and 38 g per day for adult men under 50 years of age (USDA, 2012a). Folate, also referred to as folic acid, is a water-soluble vitamin used in Deoxyribonucleic acid (DNA) synthesis and therefore necessary for new cell formation (NLM, 2013b). Deficiency can lead to anemia, weakness and fatigue, gray hair, mouth ulcers, poor growth, and swollen tongue. The Recommended Dietary Allowance (RDA) for most healthy adults is 400 micrograms (mcg) per day (USDA, 2012a). Niacin or Vitamin B_3 takes part in multiple metabolic reactions such as energy metabolism and is found in a variety of foods, including enriched grains (NLM, 2013c). Deficiency of this vitamin can lead to a condition known as pellagra, which causes symptoms such as diarrhea, mental confusion, delusions, inflamed skin and mucous membranes, and skin sores. The recommended amount of niacin is 16 mg per day for adult men and 14 mg

per day for adult women (USDA, 2012a). Thiamin, or vitamin $B_{1,}$ plays an active role in energy metabolism and in nerve function throughout the body (NLM, 2013d). Inadequate amounts of thiamin over a long period of time can result in the deficiency disease known as beriberi. Beriberi can occur in two forms: "wet", causing swelling, or "dry", resulting in muscle wasting. Additionally, this disease can cause damage to the nervous system and heart as well as other muscles, affect short-term memory, and cause irritability, confusion, decreased appetite, and weight loss. The RDA of vitamin B_1 for men and women is 1.1-1.2 mg per day (USDA, 2012a). Riboflavin, or vitamin $B_{2,}$ is important for energy metabolism and red blood cell production and can easily be obtained from green leafy vegetables, meat, eggs, dairy products, and enriched grains (NLM, 2013e). Deficiency of this vitamin can lead to anemia, sores on the mouth and lips, skin disorders, sore throat, and inflammation of the mucous membranes. The RDA for adult men and women is 1.1-1.3 mg per day (USDA, 2012a). Iron, a mineral found in all cells of the body, is important for making oxygen-carrying proteins known as hemoglobin and myoglobin (NLM, 2013f). Iron-deficiency anemia, a severe depletion of iron stores, results in low hemoglobin levels, leaving patients feeling exhausted. Other symptoms include headaches, paleness, and sensitivity to cold temperatures. Additionally, these patients may experience a condition known as pica, which causes a desire to eat nonfood items such as ice, clay, and paste. The Dietary Reference Intake (DRI) for iron is eight milligrams per day and 18 mg per day for healthy adult men and women, respectively, under age 50 (USDA, 2012a).

Statement of the Problem

In the United States, enrichment of grains containing gluten is required, while enrichment of gluten-free foods is voluntary (Thompson, 1999). It is well known that grains

provide essential nutrients such as vitamins and minerals as well as flavonoids and phytochemicals reducing the risk for nutrient deficiencies (USDA, n.d.). Individuals adhering to a strict gluten free diet must avoid these foods, which may increase their risk for possible deficiencies. Gluten-free foods are often made from starchy plants processed into flours. Refined flours such as cornstarch, potato starch, tapioca starch, and white rice flour are all considered poor sources of these vitamins and minerals. Many individuals who follow a gluten-free diet may not obtain adequate amounts of essential nutrients, which might have a negative effect on their overall health.

Purpose of the Study

With the negative consequences associated with nutrient deficiencies, it is clear that a better understanding of gluten-free diets is needed. Conditions requiring a strict gluten-free diet can arise at any age, which places even a young child at a risk for nutrient deficiencies. In recent years, a gluten-free diet has been viewed as healthier than a regular diet, and this trend continues to grow. Many gluten-free products currently available meant to replace those containing gluten do not provide adequate amounts of iron, fiber, and B vitamins (Saturni et al., 2010). In addition, the typical gluten-free dietary pattern has been found to be higher in fat, sugar, and calories compared to a regular diet. Given the number of individuals following a gluten-free diet, it is important to identify common nutrient deficiencies associated with this dietary pattern.

Chapter 2: Review of Literature

Electronic databases were accessed for this literature review. Criteria for inclusion included a) publications between 1999-2013; b) publication in the English language; c) subjects over the age 18 years; and d) gluten-free diets. Eastern Michigan University's Library Database System was utilized to find all the studies discussed in this review. The following key words were used to conduct the search: gluten-free diets, celiac disease, iron, fiber, riboflavin, thiamin, niacin, folate, adults, nutritional quality, nutritional analysis, and gluten-free grains.

The literature review included the following areas: a) nutritional quality of gluten-free diets; b) nutritional quality of gluten-free products; c) impact of whole grain consumption on diet quality and nutrient intake; and d) the effect of alternative grains on the nutritional quality of the gluten-free diet.

Nutritional Quality of Gluten-Free Diets

Patients diagnosed with celiac disease must adhere to a strict gluten-free diet for life. The elimination of foods containing gluten for this length of time may lead to nutritional deficiencies, impacting the health of these patients. A study conducted in the United Kingdom (UK) by Wild et al. investigated the nutritional quality of celiac disease patients consuming a gluten-free diet (Wild, Robins, Burley, & Howdle, 2010). Those included in the analysis were 62 females with a mean age of 53 years and 31 males with a mean age of 56 years.

Participants were recruited through medical clinics and a teaching hospital over an 18- month period. Patients with a history of confirmed celiac disease following a strict gluten-free diet for a minimum of six months participated in this study. Both weight and

height were recorded for participants and used to calculate Body Mass Index (BMI) and energy needs.

The control group included 195 males and 256 females, non-celiac participants from the National Diet and Nutrition Survey conducted from June 2000-July 2001 in the UK. This survey provided a summary of the dietary intake for adults 19-64 years of age in the UK. In addition, 708 non-celiac, cancer-free participants from the UK Women's Cohort Study were also used in the control group of this study. The UK Women's Cohort Study, conducted in 1993, investigated the relationship between diet and cancer. The study group, recruited between January 2007 and May 2008, consisted of adults between the ages 18-79 years diagnosed with celiac disease.

During the initial visit with a registered dietitian, participants were instructed to record their food and beverage intake for three weekdays and two weekend days, totaling five days. Portion sizes were measured with household measuring devices or estimated by using photographs of portion sizes that were provided. All dietary supplements taken during this time period were also recorded.

Participants were also questioned regarding any conditions affecting their dietary intake. Reported conditions among participants were a diagnosis of type I or type II diabetes, pancreatic cancer, enteropathy-associated T-cell lymphoma, fibromyalgia, irritable bowel syndrome, Sjogrens syndrome, multiple sclerosis, Parkinson's disease, chronic constipation, thyroid disease, and ulcerative colitis. In addition, the desire to lose or gain weight affected the dietary intake of some participants. Furthermore, one participant reported being a vegetarian, while five others reported avoidance of lactose, soy, or eggs.

One week following the initial visit, participants were telephoned for any necessary clarification of the instructions. Completed diaries were returned to the investigator by mail. Estimated energy requirements for each participant were compared with reported energy intakes over the five-day period. This comparison was used to determine the amount of underreporting and overreporting of all the nutrients that were evaluated from the food diaries. An adjustment of $\pm$ 20% of each individual's estimated energy intake was made to counteract inaccurate reporting. Actual physical activity levels were not assessed; rather, it was assumed that all participants were sedentary. Intakes of protein, carbohydrates, fat, calcium, magnesium, iron, zinc, selenium, vitamin D, folate, manganese, added sugars, and dietary fiber were evaluated based on the dietary intake reported.

Analysis of the food diaries revealed that females on a gluten-free diet consumed four to 17% more calories and five to 19% more grams of protein and carbohydrates than both control groups combined. Females in the gluten-free group also consumed 0.4-17% more fat per day than the control groups. Starch intake was 31% less while added sugar intake was 41% more among females in the gluten-free group than those in the control group. Vitamin D intake was the same in both groups, while calcium intake was two to 13% more in the gluten-free group than both control groups. Also, magnesium intake in the gluten-free group was 11% more than the National Diet and Nutrition Study group, but 20% less than the UK Women's Cancer Study. Manganese and iron intake in the study group followed similar trends. Gluten-free females on average consumed eight percent more manganese and iron than the National Diet and Nutrition Survey group but 28% less manganese and 14% less iron than the UK Women's Cancer Study. In addition, the study group females consumed nine percent less folate, 32% less selenium, and nine percent less zinc than females in the UK

Women's Cancer Study. Compared to the National Diet and Nutrition Survey group, the study group consumed six percent more zinc and folate per day. The average daily nutrient intake for males in the gluten free group was compared to an age-and gender-matched population in the National Diet and Nutrition Survey group. The gluten-free group on average consumed 11% more calories, seven percent more protein, 14% more fat, 19% more carbohydrate, 23% more added sugar, 10% more calcium, and 10% more iron than the control group. In addition, the gluten-free group consumed the same amount of vitamin D but 12% less magnesium, 14% less manganese, and six percent less folate than the control group.

Of participants with celiac disease younger than 55 years, only 32% in the gluten-free group met the current recommendations of 1,000 mg per day of calcium. The calcium recommendation for postmenopausal women and men over 55 years is 1,200 mg per day. Of postmenopausal study participants, only 18% met the current recommendations. The average calcium intake for participants on a gluten-free diet was 888.3 $\pm$ 346.7 mg. The average calcium intake for participants in the control groups were 783.0 $\pm$ 285.8 mg (National Diet and Nutrition Survey of Adults) and 869.5 $\pm$ 286.5 mg (UK Women's Cohort Study). From the gluten-free group, 47% of women and 48% of men reported calcium and vitamin D supplementation.

This study revealed that female subjects on a gluten-free diet consumed more calories from protein, carbohydrates, and fat as well as more calcium than those in the control group. However, these females also had lower intakes of fiber, iron, zinc, manganese, selenium, and folate. The mean intake of magnesium among females in the gluten free group was greater than those in the National Diet and Nutrition Survey but less than those in the

UK Women's Cancer Study. Men in the gluten free group consumed considerably more calories from protein, carbohydrates, and fat. A comparison of carbohydrate intake among both men and women showed that 42% of participants consumed more than the recommended calories from carbohydrates per day.

In summary, this research showed that males and females consuming a gluten-free diet had adequate intake of calories from all macronutrients, but higher amounts of carbohydrates were consumed from simple sugars (Wild et al., 2010). Females in the gluten-free group had lower intakes of magnesium, iron, zinc, manganese, selenium, and folate than those consuming foods containing gluten. Few men in the gluten-free group met the recommendation for magnesium and selenium, and, on average, calcium intakes among both males and females were relatively low compared to current recommendations.

The authors recommended that patients on a gluten-free diet should not fully rely on gluten-free products but should increase intakes of naturally gluten-free nutrient dense foods such as legumes, starchy vegetables, or alternative grains.

A similar study to the one discussed above conducted by Thompson et al. included 47 adult (39 females, eight males) volunteers diagnosed with celiac disease, who followed a strict gluten-free diet (Thompson, Dennis, Higgins, Lee, & Sharret, 2005). This study evaluated nutrient intakes and dietary patterns of adults with celiac disease who followed a strict gluten-free diet.

The inclusion criteria for this study included residents of the United States, 20 years of age or older, not pregnant or breast-feeding, diagnosed with celiac disease through intestinal biopsy, and strictly adhered to a gluten-free diet. Participants recorded everything they ate and drank including brand names, food preparation methods, measurements or

quantity of foods, and recipes for three consecutive days. A description of restaurant meals and food labels for gluten-free products were requested as well. Participants also completed a questionnaire related to their diet history. Food records were manually analyzed using the USDA's Nutrient Database for Standard Reference and Bowes and Church's Food Values of Portions Commonly Used (17th edition). Manufacturer information included food labels and nutrition information from restaurants.

Body Mass Index was calculated for each participant; 30 of the 47 participants were considered healthy, 13 were in the overweight category, one participant was obese range, and three were in the underweight category. In addition, 16 participants reported being lactose intolerant at some point in their lives, while nine reported suffering from the condition at the time of the study.

Results indicated that 88% of gluten free males and 87% of gluten-free females had estimated carbohydrate intakes within the acceptable range of 45-65% of total calories. Eighty-eight percent of males and 46% of females had fiber intakes that met or exceeded the recommended daily amount of 20-35 g per day. The recommended daily amount of iron was consumed by all males but only 44% of females. Calcium intakes that met or exceeded the recommended amount were consumed by 63% of males and 31% of females. Last, only 63% of males and 21% of females consumed at least the minimum daily recommended servings of grains.

In the United States, grains containing gluten, like wheat, provide a significant amount of nutrients including fiber, thiamin, riboflavin, niacin, folate, and iron (Raymond et al., 2006). However, gluten-free flours, breads, pastas, and cold cereals are not typically enriched (USDA, n.d.; Thompson, 1999). The results of this study showed that gluten-free

females had the lowest percentage of intakes of fiber, iron, calcium, and grain servings. Although the majority of females met the daily recommended calories from carbohydrate intake, a small fraction of this intake was actually consumed from grains. In addition, the majority of males met the daily recommended calories from carbohydrates, amounts of fiber and calcium, and servings of grains. All males consumed 100% of the daily recommended iron intake. The researchers concluded that several limitations of this study may have affected the results. Those limitations include the use of a three-day food record for assessing nutrient intake, recording error by participants, and the small sample size of the study population.

Nutritional Quality of Gluten-Free Products

The aim of the next study conducted by Thompson was to determine whether gluten-free cereal products contained similar amounts of thiamin, riboflavin, and niacin as their enriched gluten-containing counterparts (Thompson, 1999). This study reviewed 368 gluten-free products including rice flours, breads, pastas, and cold cereals.

The enrichment status of all the products was evaluated, and the ingredient list of 268 of those products excluding those containing rice flour was reviewed for refined grains or starch. Sixty-four of the products were analyzed for thiamin, riboflavin, and niacin contents and compared to their gluten-containing counterparts. Nutrition information for these products was obtained from the USDA's Nutrient Database for Standard Reference (release 12, 1998), Bowes and Church's Food Values of Portions Commonly Used (17^{th} edition), manufacturers, distributors, and nutrition labels from the products. Nutrition information was available for most products except those containing bean flours.

Results of the study showed that of the 368 gluten-free foods assessed, only 35 foods were enriched. Of the flours evaluated, the only products that were enriched were corn flour and de-germed cornmeal. Fifteen varieties of white rice flour, the primary flour used in gluten-free products, were assessed and none were enriched. Only four of the 16 companies that supply gluten-free bread products carried enriched bread. Of the 95 gluten-free bread mixes, only five were enriched while none of the 80 types of gluten-free pasta were enriched. In addition, only 26 of the 157 gluten-free ready-made products were enriched. Of the gluten-free cereals evaluated, four of the 17 were enriched. From the 268 gluten-free foods evaluated for overall ingredients, the first ingredient in 196 of those foods was a refined grain or starch. Of those products the refined grain or starch was enriched in only 32 of the 196 products.

Of the 64 gluten-free products analyzed for thiamin, riboflavin, and niacin content, 39 products had lower amounts of all the vitamins; nine had lower amounts of thiamin and riboflavin; three had lower amounts of thiamin and niacin; two had lower amounts of riboflavin; and four had a lower amount of thiamin than their gluten-containing counterparts. In addition, four of these products contained higher amounts of thiamin, riboflavin, and niacin, and one product contained the same amount of all three vitamins.

Overall, several gluten-free products currently on the market are not enriched and therefore do not contain the same amounts of thiamin, riboflavin, and niacin as their gluten-containing counterparts. The researcher concluded that individuals who follow a gluten-free diet should be regularly evaluated for vitamin deficiencies, and more research on gluten-free dietary patterns is needed.

In a separate yet related study, the folate, iron, and dietary fiber content of gluten-free cereal products was assessed (Thompson, 2000). The nutrition information for 86 products consisting of 28 flours, 30 bread products, 14 pastas, and 14 cold cereals was analyzed.

The folate, iron, and dietary fiber information for gluten-free products was requested from 52 manufacturers and distributors. Of those, 16 companies supplied the necessary data. Other nutrient data was obtained from the internet using websites from Jower Foods and Dietary Specialties and from the USDA's Nutrient Database for Standard Reference (release 13, 1999). Once the nutrient content of these foods was assessed, they were compared to their gluten-containing counterparts.

Thirty of the 37 gluten-free cereal products analyzed contained lower amounts of folate, iron, and fiber when compared to products containing gluten. In addition, only three of the 58 breads, pastas, and cold cereals analyzed were enriched with folic acid. Eighty- three of 86 products assessed had available iron contents and of those 64 had lower iron contents than their gluten-containing counterparts. Fiber content was assessed for 85 of the 86 products which showed that 26 of these foods had lower amounts of fiber than those containing gluten. Overall, this study showed that gluten-free foods on the market are typically not enriched and do not contain the same amounts of folate, iron, and fiber as their gluten-containing counterparts.

Impact of Whole Grain Consumption on Diet Quality and Nutrient Intake

O'Neil et al. (2010) evaluated the relationship of whole grain intake to diet quality and overall nutrient intake in adults from a nationally represented population. Participants included 7,039 adults between the ages 19-50 years and 6,237 adults 51 years of age and older. Pregnant and breast feeding women were excluded. This study was a secondary

analysis of cross-sectional data from the National Health and Nutrition Examination Survey from 1999-2004.

Data from the National Health and Nutrition Examination Survey are grouped into two-year increments. Data from the 1999-2000 and 2001-2002 surveys collected 24-hour dietary recalls from participants. The 2003-2004 survey collected a two-day dietary intake from participants. Each participant was categorized based on the average number of whole grains consumed per day. Those categorizes were ≥ 0 to < 0.6 servings, ≥ 0.6 to <1.5 servings, ≥ 1.5 to <3.0 servings, and ≥ 3.0 servings.

Data collected in the 1999-2000 survey were analyzed with the USDA's 1994-1998 Survey Nutrient Database. Nutrients for the surveys conducted from 2001-2004 were analyzed with the USDA's Food and Nutrient Database for Dietary Studies, versions one and two.

Results of this study showed that adults 19-50 years of age consumed an average of 0.63 servings of whole grains per day and adults 51 years and older had an average daily whole grain intake of 0.77 servings. The average Healthy Eating Index scores were significantly higher among participants consuming the most whole grains. In addition, calories, carbohydrates, and fiber intakes were also considerably higher when a greater number of whole grain servings were consumed. Also, intakes of added sugars, saturated fat, monounsaturated fat, and cholesterol declined when whole grain consumption increased. Intakes of all micronutrients except vitamin B_{12} and sodium increased with greater whole grain consumption.

Overall, this study showed that whole grain consumption in the United States is low, but overall diet quality was improved with the inclusion of whole grains. In addition,

participants 19-50 years who consumed three or more servings of fiber per day nearly met the daily recommended amount based on gender. Participants 51 years of age and older met the daily fiber recommendation. Although grains were not enriched with vitamin E, five to 21% of this nutrient was retained in the processing of wheat. In this study, whole grain consumption did increase vitamin E intake, but on average none of the participants met the recommended daily amount. Participants on average consuming three or more servings of whole grains per day met the daily recommended amount of magnesium. The results of this study also showed that potassium intake was increased with whole grain consumption, but on average none of the participants met the daily requirement.

The Effect of Alternative Grains on the Nutritional Quality of the Gluten Free Diet

In another study by Lee et al. (2009), the nutritional quality of the standard gluten-free diet was improved with the inclusion of alternative grains like oats, high fiber gluten-free bread, and quinoa. Fifty randomly selected celiac disease patients were selected to participate.

Participants recorded their dietary intake for three days and returned the documents during a follow-up visit with a registered dietitian. Intakes of grains/starches were categorized by type, number of servings, and the time of day when they were consumed. Foods consumed between meals were recorded as snacks. The nutrient intake of all 50 participants was averaged to represent one standard gluten-free dietary pattern. The gluten-free dietary pattern with the inclusion of alternative grains was developed by substituting grains in the standard pattern with alternative grains. The grain portion of the alternative gluten free pattern consisted of oats for breakfast, high fiber brown rice bread for lunch, and quinoa for dinner. Nutrient analysis, which focused on the protein, fat, carbohydrate, fiber,

thiamin, riboflavin, niacin, folate, iron, and calcium content of the grain portion of each of the dietary patterns, was conducted using the USDA food composition databank, product websites, and nutrition labels.

Results of this study showed that 39% of the time, the grain portion of the meal was omitted by participants consuming the standard gluten-free diet. White rice was the most commonly consumed grain 38% of the time, while brown rice was consumed only six percent of the time. In addition, potatoes were used as the grain portion eight percent of the time, followed by oats five percent of the time, and corn four percent of the time. Of the meals consumed, 17% were consumed between meals and thus considered snacks. Over half of the total number of snacks consumed consisted of foods such as gluten-free chips, pretzels, cookies, donuts, and cakes. The standard gluten-free diet did not meet the six to 11 grain servings per day as recommended by the USDA. Also, this dietary pattern did not meet the recommended intake of fiber, thiamin, riboflavin, niacin, folate, iron, or calcium per day. The alternative dietary pattern increased the nutritional quality of the gluten-free diet by providing greater amounts of protein, iron, calcium, and fiber. On average, the standard dietary pattern provided 11 g of protein from grains per day, while the alternative dietary pattern provided 20.6 g per day. Iron content in the standard diet was 1.4 mg from grains per day, and the alternative diet provided 18.4 mg per day. The standard diet did not provide any calcium from grains, but the alternative diet provided 182 mg per day. Fiber was increased from five grams to 12.7 g with the inclusion of alternative grains. The amounts of thiamin, riboflavin, niacin, and folate were increased by substituting alternative grains, but there was not a significant difference between the two dietary patterns.

In summary, by substituting nutrient-dense alterative grains in place of grains commonly consumed in a gluten-free diet, nutritional quality of the diet was significantly increased. In addition, the alternative grains selected are readily available and affordable.

Overall, the research thus far showed that adults following a strict gluten-free diet typically consume more calories from sugar than adults consuming a diet including gluten-containing foods. In addition, females consuming a gluten-free diet typically had lower intakes of magnesium, iron, zinc, manganese, selenium, folate, fiber, calcium, and grain servings. Male participants consuming a gluten-free diet typically consumed higher amounts of nutrients than females but had lower intakes of magnesium, selenium, calcium, and grain servings than males on a regular diet. Furthermore, the majority of gluten-free products currently on the market are not enriched and therefore do not contain the same amounts of nutrients as products containing gluten (Thompson 1999, 2000). More specifically, the fiber, thiamin, riboflavin, niacin, folate, and iron contents of gluten-free products are typically lower than similar products containing gluten. Regardless of the type of diet consumed, research suggests that whole grain consumption among adults in the United States is low (O'Neil et al., 2010). However, even small amounts of whole grains consumed improved diet quality by providing nutrients such as fiber, magnesium, B vitamins, and potassium. Nutrient dense alternative grains have been shown to improve the nutritional quality of gluten-free diets by providing greater amounts of protein, iron, calcium, and fiber (Lee et al., 2009). Also, alternative grains provide slightly more thiamin, riboflavin, niacin, and folate than typical sources of grains in the standard gluten free diet.

Rationale for the Study

Previous research has developed a solid foundation for better understanding the

nutritional quality of the gluten-free diet. However, research has focused on patients with celiac disease and is lacking in how this type of diet affects healthy adults. In addition, previous studies have evaluated the nutritional quality of gluten-free foods, yet this body of research does not reflect the most recent gluten-free products on the market. In recent years, a gluten-free diet has been perceived as healthier than a regular diet, resulting in increased demand and sales of gluten-free products. The research available does not provide insight on whether a gluten-free diet provides adequate amounts of essential nutrients required by adults for optimum health. This study will evaluate the nutrient intake of healthy adults who predominantly consume a gluten-free diet. By determining the nutrient intakes of healthy adults on a gluten-free diet, the nutrient content of gluten-free foods currently on the market will also be better understood. This study will also identify potential nutrient deficiencies related to following a gluten-free diet that may have long-term health effects.

Chapter 3: Research Methodology

Hypothesis/Research Aims

The purpose of this study was to evaluate the nutrient intakes of healthy men and women between the ages 25-35 years, who consumed a gluten-free diet and compare their intakes with an age and gender matched control group, who consumed a regular diet that included foods containing gluten. The primary goal of this study was to determine the average intakes of fiber; the B vitamins thiamin, riboflavin, niacin, folate; and iron among adults, who consumed a gluten-free diet and compare those intakes to adults, who consumed gluten containing foods. The hypothesis investigated was that the average intakes of fiber; the B vitamins thiamin, riboflavin, niacin, folate; and iron would be lower in the gluten-free group than in the group consuming gluten-containing foods. The following specific aims were studied to evaluate the hypothesis: First, the average intakes of nutrients consumed were analyzed to determine if participants met the recommended daily amounts for adults. Secondly, the dietary sources of these nutrients in the gluten-free and regular diets groups were identified and compared.

The study was conducted on healthy men and women, 25 to 35 years of age, residing in the Hampton Roads area of Virginia. Test participants regularly ate a gluten-free diet, whereas control subjects ate a regular diet. Subjects selected for participation in the study group met the inclusion criteria, which consisted of (1) 25-35 years of age; (2) followed a strict gluten-free diet for at least one month; (3) were free of a diagnosis of celiac disease, irritable bowel syndrome, ulcerative colitis, Crohn's disease, diverticulitis, or wheat allergy; (4) not pregnant or breastfeeding; (5) had access to a computer and an email account.

Participants

A total of 70 study packets were distributed to adult men and women from the Hampton Roads area, who were recruited through notices placed in a fitness center and specialty health food stores. Signed Informed Consent documents (See Appendix G) were returned by 58 subjects who participated in the study. Twenty-five of those adults consumed a predominantly gluten-free diet, while 33 consumed a regular diet with gluten containing foods. Of the total participants, 24 were males and 34 were females.

Materials

The packets were organized prior to the start of the study and included five documents developed by the principal investigator. Those included instructions for participation (See Appendix A: Instructions for Participants in the Gluten-Free Study), a questionnaire (See Appendix B: Participant Questionnaire), blank food records (See Appendix C: Daily Food Record), and additional resources to assist participants in recording their dietary intake (Appendix D: Tools for Estimating Portion Sizes and Appendix E: Sample Nutrition Label). The questionnaire required the participants to provide their gender, age, weight, and height. Additionally, participants answered questions related to their past and present dietary habits.

The participant questionnaire (See Appendix B) was used to assess previous and current dietary habits as well as age, height, and weight for each participant. The first four questions were related to age, gender, height, and weight. The next four questions requested information regarding the participants' current and previous dietary habits, as well as supplement intake. The last three questions, completed only by participants on a gluten-free diet, pertained to the reasons for following this type of diet, benefits or drawbacks of eating

gluten-free, and the length of time the participants planned to follow the diet. The data from the questionnaires was organized into a Microsoft Excel document based on the participant's gender and whether they consumed a gluten-free or regular diet. In addition, each participant's BMI was calculated based on the weight and height reported.

To assess dietary intake, each participant recorded their food and beverage intake for five consecutive days, which consisted of four week days and one weekend day. Participants were provided with detailed instructions on how to properly complete the food logs and instructed to contact the investigator by email if they had additional questions. Completed food logs were manually entered into FoodWorks® nutrient analysis software and the total intake for all nutrients being evaluated was determined for each of the five days. All the data collected was then transferred to a Microsoft Excel document, which was programmed to calculate the average intake of each nutrient over the five day period for each participant. The average values for each nutrient was compared to the RDA established by the National Academy of Sciences, Institute of Medicine, and Food and Nutrition Board.

Procedure

The study protocol was approved by the College of Health and Human Services' Human Subjects Committee at Eastern Michigan University (See Appendix F). Individuals meeting the criteria initially picked up an informed consent form from the participating fitness center or it was mailed to an address provided to the investigator through email. Mailed consent forms included a return envelope addressed to the investigator with postage. The investigator informed potential subjects through an email message that they must read, sign, and return the form prior to participating in the study. Participants returned signed consent forms to the participating fitness center or by mail. A secure drop box

dedicated for the return of all study related materials was set up at the participating fitness center. The drop box was located behind the front desk of the facility and remained locked. Once documents were placed inside the box, only the investigator was able to retrieve them. Once a participant returned the signed consent form, they were sent an email from the investigator with instructions on how to proceed with the study. Participants were instructed to pick up an information packet from the participating fitness center or it was mailed to their address. Mailed packets included an envelope addressed to the investigator with postage for return of the completed documents. All participants were instructed to record everything they ate and drank for five consecutive days, including one weekend day, using the blank food records provided. All foods and beverages were to be measured using basic kitchen measuring devices or estimated using the tools provided (See Appendix D). In addition, participants were highly encouraged to record as much detail as possible including brand names and food preparation methods. Participants were also encouraged to include a copy of recipes for all homemade meals with all the other required documents. If the meal was consumed at a restaurant, participants were encouraged to record detailed description of the meal, including the name of the dish and the restaurant name. Although the information described above was preferred for accurate nutrient analysis, food logs were not excluded if these details were not provided. Participants returned the completed food records and related materials to the participating fitness center or by mail in the envelope provided. The control group consisted of men and women following a regular diet, while the study group included participants that consumed a gluten-free diet. Once each participant completed and returned the questionnaire and daily food records, the foods were entered in FoodWorks® and analyzed for intake of calories, carbohydrates, protein, fat, saturated fat, cholesterol, sugar,

fiber, thiamin, riboflavin, niacin, folate, iron, calcium, and sodium. Recipes provided were manually entered into the database for nutrient analysis. Manufacturer and restaurant websites were accessed to obtain nutrition information regarding foods not found in the database.

Data Analysis

Data was analyzed using SAS 9.3. Data is expressed as means $\pm$ standard deviation. T-tests were used to compare means between the control group and study group. A p-value of <0.05 was considered statistically significant.

Chapter 4: Results

The control group consisted of 15 adult males and 18 adult females who consumed a regular diet, while the study group consisted of nine adult males and 16 adult females who consumed a predominantly gluten-free diet. The average age of male participants was 32 years old, while the average age of female participants was 30 years old. Average BMIs, calculated from self reported height and weight, were 26.8 $\pm$ 2.5 kg/m^2 for all male participants and 23.3 $\pm$ 3.2 kg/m^2 for all female participants. Males consuming a regular diet had a BMI of 26.9 $\pm$ 2.9 kg/m^2, while males consuming a gluten-free diet had an average BMI of 26.6 $\pm$ 1.7 kg/m^2. Females consuming a regular diet had a BMI of 22.9 $\pm$ 2.4 kg/m^2 and gluten-free females had a BMI of 23.8 $\pm$ 3.8 kg/m^2. There were no significant differences in BMI between the control and gluten-free group in either males or females. According to the Centers for Disease Control and Prevention (2011), BMI between 18.5-24.9 kg/m^2 is considered normal while BMI between 25.0-29.9 kg/m^2 is considered overweight. In addition, a BMI below 18.5 kg/m^2 is considered underweight whereas a BMI greater than 30.0 kg/m^2 is considered obese. Females in both groups had a BMI in the normal category, whereas males in both groups were slightly overweight. Data regarding gender and average BMI values for all participants is shown in Table 1.

Table 1

Average Body Mass Index Data for All Participants

	Control Group (Regular Diet) (n= 15 males, 18 females)	**Study Group (Gluten-Free Diet) (n= 9 males, 16 females)**
Male	26.9 ± 2.9 kg/m^2	26.6 ± 1.7 kg/m^2
Female	22.9 ± 2.4 kg/m^2	23.8 ± 3.8 kg/m^2

Note. n= number of participants.BMI values calculated based on self-reported weight and height. Formula used: BMI (Body Mass Index) = (weight in pounds/ height in inches) x 703. A BMI 18.5-24.9 kg/m^2 is considered normal while a BMI 25.0-29.9 kg/m^2 is considered overweight (CDC, 2011).

Diet history for all participants was evaluated based on the answers provided on the questionnaire. The questions pertained to the type of diet each participant followed (regular or gluten-free), how long that type of diet was followed, and whether or not any other specific diets were followed in the past five years. Participants were also asked about supplement use and instructed to provide detailed information regarding supplements they took. All male and female participants that followed a regular diet reported following that particular type of diet longer than a year. Roughly 44% of gluten-free males reported following this type of diet less than six months, while the remaining gluten-free males reported following the diet for more than one year. Of the males that reported following a gluten-free diet for less than six months, half stated they had been on the diet for approximately two months. The other half stated they had been following a gluten-free diet for approximately five months. Of the gluten-free females, six percent reported following the diet for less than six months, 31% reported being gluten-free for six to 12 months, and 63%

indicated being on the diet for more than one year. Data for participants following a gluten-free or regular diet can be seen in Table 2. Next, participants were asked whether or not they had followed a diet within the past five years. If they answered yes, participants were instructed to list those specific diets as well as the length of time they followed them. In the control group, 36% of participants reported following at least one diet in the past five years. In the study group, 36% of participants stated that had followed a diet in the past five years. Of all the participants that reported dieting in the past five years, the most common diets listed were Weight Watchers®, a low carbohydrate diet, and a reduced calorie diet. The length of time participants followed a diet ranged from four months to two years. Of all the participants that reported following diets in the past, roughly half stated they had followed one diet in the past five years, while the other half indicated following two diets. None of the participants reported following more than two diets in the past five years. Data regarding dietary history can be seen in Table 2. In addition, all participants were asked to list supplements they were currently taking. Among all male participants 67% reported taking supplements, while 71% of all females reported supplement use. Eighty-four percent of all gluten free participants reported taking supplements, while only 58% of males and females on a regular diet reporting taking supplements. The most common supplements used among all participants were multivitamins, fish oil, whey protein, vitamin D_3, and calcium. Data related to the participants who took supplements is shown in Table 2.

Table 2

Diet History and Supplement Use for All Participants

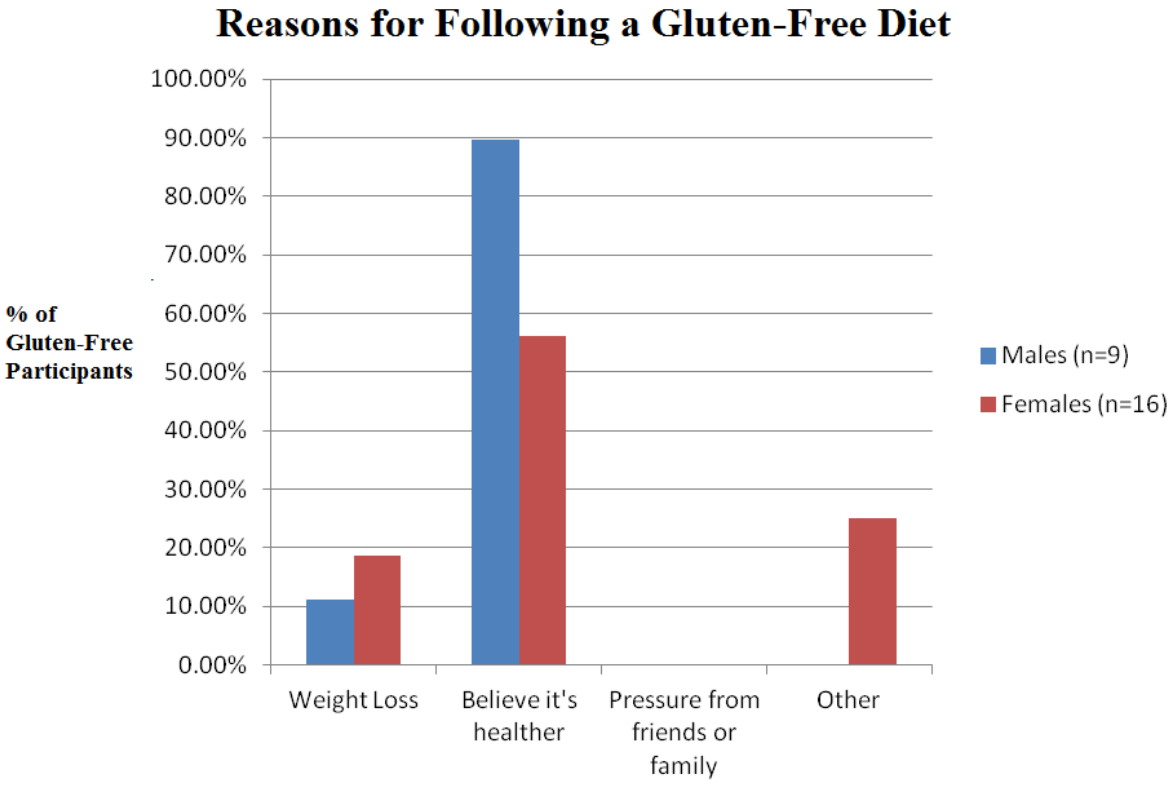
	Males (Regular Diet) (n= 15)	Males (Gluten-free Diet) (n= 9)	Females (Regular Diet) (n= 18)	Females (Gluten-Free Diet) (n= 16)
Length of Time Following Diet				
< 6 months		4		1
6-12 months				5
> 1 year	15	5	18	10
Followed a Diet in the Past 5 Years				
Yes	3	2	9	7
No	12	7	9	9
Take Dietary Supplements				
Yes	9	7	10	14
No	6	2	8	2

Note. n = number of participants. Data was compiled from the Participant Questionnaire found in Appendix B. All participants provided information on the type of diet they followed, length of time they followed this particular diet, length of time they followed diets in the past if applicable, and whether or not they consumed dietary supplements. Common supplements used were multivitamins, fish oil, whey protein, vitamin D_3, and calcium.

The last three items in the questionnaire pertained to gluten-free participants only. Those questions were related to the reasons participants chose to follow a gluten-free diet,

benefits or drawbacks of following the diet, and the length of time participants planned to follow a gluten-free diet. Participants following a gluten-free diet were instructed to choose one of the four reasons listed (weight loss, believe it is healthier, pressure from friends or family, or other) to indicate their reason for following this type of diet. A percentage of the actual number of each response was calculated for all participants in the study group and is shown in Figure 1. Over half of the females and nearly all males chose a gluten-free diet because they believed it was healthier. A small percentage of males and females selected weight loss as their reason for following the diet and a quarter of the females selected "other" as their reasoning for following a gluten-free diet. Those that selected "other" were instructed to list a reason in the space provided. The most common reasons listed were reduction in the number of headaches and perceived gluten intolerance. No participants indicated that they followed a gluten-free diet because of pressure from friends or family. Gluten-free participants were also instructed to list the benefits or drawbacks of following a gluten-free diet and indicate the length of time they planned to follow this type of diet. Listed benefits of following a gluten-free diet were reduction in the number of headaches and gastrointestinal issues such as diarrhea and stomachaches, as well as weight loss. No participant listed any drawbacks to being gluten-free and all participants indicated that they planned to follow a gluten-free diet more than one year.

Figure 1



Data was compiled from the Participant Questionaire from Appendix B. Participants were given a multiple choice question regarding their reason for consuming a gluten-free diet and instructed to select one answer. Those who selected "other" were instructed to provide a specific reason. The only responses provided were "Reduction in headaches" and "Gluten intolerance." A percentage of the actual number of each response was calculated for all particpants in the Study Group.

To analyze participants' dietary intake and determine their overall nutrient intake, their five-day food intake was collected and data manually entered into FoodWorks® nutrient analysis software. The data compared specific nutrient intakes in male and female subjects in the control group with the gluten-free study group. Nutrient intakes of participants on a gluten-free diet were compared to those on a regular diet with emphasis on fiber, B vitamins (thiamin, riboflavin, niacin, and folate), and iron. Subsequently, the dietary sources of the

nutrients analyzed were determined and emphasis was placed on possible nutrient deficiencies in the diet. The nutrient intakes of regular and gluten-free males were also compared followed by a comparison of the nutrient intakes of regular and gluten-free females. This was done by comparing the average intakes of each nutrient for each participant to the DRIs for adults (USDA, 2012a).

For moderately active males 19-50 years of age, the recommended amount of calories is 2,400- 2,800 kilocalories (kcal) per day (USDA, 2012b). To determine the amount of each nutrient consumed, the average intake was calculated from the five day food log obtained from each participant. On average, gluten-free males consumed fewer calories than males consuming a regular diet. Of the male participants consuming a regular diet, 47% consumed the recommended number of calories, while only 33% of males consuming a gluten-free diet met the calorie recommendation. The mean daily calorie intake for regular males was 2540.2 $\pm$ 438.2 kcal and 2304.3 $\pm$ 617.5 kcal for gluten-free males, which was not significantly different. The Acceptable Macronutrient Distribution Range (AMDR) for carbohydrate intake in adults is 45-65% of total calories consumed per day (USDA, 2012a). Based on the calorie range recommended for males that participated in this study, 270-455 g of carbohydrate per day was the acceptable range. Eighty-seven percent of males on a regular diet consumed carbohydrates within the acceptable range whereas only 44% of gluten-free males consumed carbohydrates within the recommended range. Males eating a regular diet on average consumed significantly ($p=<0.05$) more (313.5 $\pm$ 41.9 g of carbohydrate per day) compared with gluten-free males, who consumed 244.6 $\pm$ 81.3 g of carbohydrate on average per day. The AMDR for Protein in adults is 10-35% of total calories consumed per day and the RDA for protein intake is 0.8 g per kilogram (kg) of body weight per day (USDA,

2012a). Based on the calorie range recommended for males that participated in this study, 60-345 g of protein per day was the acceptable range. Based on the RDA, males on a standard diet are recommended to consume approximately 67 g of protein per day, whereas gluten-free males are recommended to consume approximately 65 g of protein per day. Males on a regular diet consumed 172.5 $\pm$ 52.1 g of protein on average per day, whereas gluten-free males consumed 194.7 $\pm$ 100.4 g of protein on average per day. All male participants consumed protein within the acceptable range, but their average intake was significantly ($p=<0.05$) greater than the RDA of 0.8 g of protein per kilogram of body weight per day. The majority of males (93%) on a regular diet consumed fat within the AMDR of 20-35% of calories per day (USDA, 2012a). Based on the recommended number of calories for male participants, 53-109 g was the acceptable range for fat intake for males per day. Gluten-free males consumed significantly ($p=<0.05$) more (125.6 $\pm$ 82.6 g) fat than males on a regular diet (70.0 $\pm$ 24.9 g). There was a significant difference ($p=< 0.05$) in fiber intake over the five day period between males consuming a regular diet and males consuming a gluten-free diet. The daily recommended amount of fiber for adult males is 38 g (NLM, 2013a). On average, males on a regular diet consumed 41.4 $\pm$ 9.2 g of fiber per day, while gluten-free males consumed only 31.8 $\pm$ 6.8 g of fiber per day. Of the males on a regular diet, 67% met or exceeded the recommendation for fiber intake. In the gluten-free group, all males consumed less fiber than recommended on average per day. The average daily fiber and macronutrient intake for male participants is shown in Table 3.

Table 3

Average Daily Macronutrient and Fiber Intake for Male Participants

	Calories (kcal)	Carbohydrates (g)	Protein (g)	Fat (g)	Fiber (g)
Control Group (Regular Diet)	2540.2 ± 438.2	313.5 ± 41.9	172.5 ± 52.1	70.0 ± 24.9	41.4 ± 9.2
Study Group (Gluten-Free Diet)	2304.3 ± 617.5	244.6 ± 81.3*	194.7 ± 100.4	125.6 ± 82.6*	31.8 ± 6.8*

Average nutrient intakes were calculated by analyzing 5-day food records using FoodWorks® nutrient analysis software (version 14). Values are means ± standard deviation. *Significantly different from controls (p=<0.05)

There was not a significant difference in the overall intake of cholesterol between males on a regular diet versus males on a gluten-free diet. Both regular and gluten-free males consumed more cholesterol than recommended (< 300 mg per day) (USDA, 2012a). On average, males on a regular diet consumed 340.3 ± 141.6 mg, while gluten-free males consumed 386.9 ± 116.3 mg of cholesterol per day. Of the males on a standard diet, 47% met the daily cholesterol recommendation whereas only 22% of gluten-free males met the daily cholesterol recommendation on average. Limiting saturated fat intake to no more than 10% of total calories per day is recommended to reduce the risk for cardiovascular disease (USDA, 2012b). Based on the calories recommended for male participants, less than 60-70 g of saturated fat per day was the acceptable range. All males in both groups on average met the daily recommendation for saturated fat intake. On average, males on a regular diet consumed 19.9 ± 8.4 g of saturated fat and gluten-free males consumed 22.9 ± 6.6 g of saturated fat on average per day. The recommendation for sugar intake is less than 25% of total calories per day (USDA, 2012a). Based on the calorie range recommended for male participants, 150-175 g of sugar per day was the acceptable range. All males on a regular and

gluten-free diet met the daily recommendation for sugar intake on average. Males on a regular diet consumed 76.7 ± 36.4 g of sugar while gluten-free males consumed 79.4 ± 23.0 g of sugar on average per day, which was not significantly significant. The average daily cholesterol, saturated fat, and sugar intake for male participants is shown in Table 4.

Table 4

Average Daily Cholesterol, Saturated Fat, and Sugar, Intake for Male Participants

	Cholesterol (mg)	**Saturated Fat (g)**	**Sugar (g)**
Control Group (Regular Diet)	340.3 ± 141.6	19.9 ± 8.4	76.7 ± 36.4
Study Group (Gluten-Free Diet)	386.9 ± 116.3	22.9 ± 6.6	79.4 ± 23.0

The average intake of nutrients was calculated by analyzing 5-day food records using FoodWorks® nutrient analysis software (version 14). Values are means ± standard deviation.

Although 100% of males consuming a regular diet met the daily recommendation for thiamin and riboflavin intake, males on gluten-free diet consumed less of these nutrients on average, but there was not a significant difference in the overall intake. The recommendation for thiamin intake for adult males is 1.2 mg per day and 1.3 mg per day for riboflavin. Males on a regular diet on average consumed 2.7 ± 1.1 mg of thiamin and 3.6 ± 1.6 mg of riboflavin per day, respectively. All males on a regular diet met or exceeded the daily thiamin and riboflavin recommendation on average per day. Gluten-free males on average consumed 2.5 ± 1.6 mg of thiamin and 2.4 ± 1.2 mg of riboflavin respectively per day. The majority (67%) of males on a gluten-free diet met or exceeded the daily thiamin recommendation. There was a significant ($p=<0.05$) difference in the niacin and folate intake between males consuming a regular diet and males consuming a gluten-free diet. The daily recommendation for adult males is 16 mg of niacin per day. The average niacin intake for males on a regular diet was

34.6 ± 9.4 mg, while gluten-free males consumed 23.2 ± 8.3 mg of the same nutrient on average per day. All males on a regular diet met or exceeded the daily niacin recommendation on average per day. Of the gluten-free males, 89% met or exceeded the daily niacin recommendation. The recommendation for folate is 400 mcg per day for adult males (USDA, 2012a). Males on a regular diet consumed significantly (p=<0.05) more folate (606.2 ± 180.3 mcg per day) than gluten-free males, who consumed 315.7 ± 169.1 mcg per day. The majority (87%) of males on a regular diet met or exceeded the daily folate recommendation on average, whereas only 22% of gluten-free males met or exceeded the daily folate recommendation. Both males on a regular diet (23.9 ± 5.9 mg per day) and gluten- free males (24.7 ± 8.9 mg per day) consumed adequate amounts of iron and met the recommendation of eight milligrams of iron per day (USDA, 2012a). Males on a regular diet consumed 2189.3 ± 352.1 mg of sodium per day, while gluten-free males consumed significantly (p=<0.05) more (2491.4 ± 479.0 mg) per day which is above the daily sodium recommendation (< 2,300 mg) (USDA, 2012a). Of the males on a regular diet, 53% met the daily sodium recommendation on average, whereas only 33% of gluten-free males met the daily sodium recommendation on average. There was a significant (p=<0.05) difference in the calcium intake between males consuming a regular diet and males consuming a gluten-free diet. The recommended amount of calcium for adult males is 1,000 mg per day (USDA, 2012a). Males on a regular diet on average consumed significantly (p=<0.05) more (1496.5 ± 402.5 mg) calcium than gluten-free males, who consumed only 926 ± 129.8 mg of calcium per day on average. Of the males on a standard diet, 93% met or exceeded the daily calcium recommendation, whereas only 22% of gluten-free males met or exceeded the daily calcium

recommendation on average. The average daily micronutrient intake for male participants is shown in Table 5.

Table 5

Average Daily Micronutrient Intake for Male Participants

	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Folate (mcg)	Iron (mg)	Sodium (mg)	Calcium (mg)
Control Group (Regular Diet)	2.7 ± 1.1	3.6 ± 1.6	34.6 ± 9.4	606.2 ± 180.3	23.9 ± 5.9	2189.3 ± 352.1	1496.5 ± 402.5
Study Group (Gluten-Free Diet)	2.5 ± 1.6	2.4 ± 1.2	23.2 ± 8.3*	315.7 ± 169.1*	24.7 ± 8.9	2491.4 ± 479.0	926.4 ± 129.8*

Average intake of micronutrients was calculated by analyzing 5-day food records of males 25-35 years old who consumed a regular diet (n=15) and gluten free diet (n=9) using FoodWorks® nutrient analysis software (version 14).Values are mean ± standard deviation.* Significantly different (p=<0.05) from controls

For moderately active females 19-50 years of age, the recommended amount of calories is 2,000-2,200 kcal per day (USDA, 2012b). Overall, calorie consumption of female participants was lower than the recommended amounts. Of the female participants consuming a regular diet, only six percent consumed the number of calories recommended, while none of the gluten-free females consumed calories within the recommended range. On average, females on a regular diet consumed 1723.8 ± 363.3 kcal per day and the gluten-free females consumed 1666.0 ± 379.4 kcal per day, which was not significantly different. The AMDR for carbohydrate intake in adults is 45-65% of the total calories consumed per day (USDA, 2012a). Based on the calorie range recommended for the female participants, 225-358 g of carbohydrate per day was the acceptable range. Seventeen percent of females on a

regular diet consumed carbohydrates within the acceptable range, while none of the gluten-free females consumed carbohydrates within the acceptable range. Females on a regular diet consumed 203.2 $\pm$ 38.7 g of carbohydrate per day, whereas gluten-free females consumed significantly ($p=<0.05$) fewer (133.3 $\pm$ 28.0 g per day) amounts of carbohydrate on average. The AMDR for protein intake in adults is 10-35% of total calories consumed per day and the RDA for protein intake is 0.8 g per kilogram of body weight per day (USDA, 2012a). The acceptable range for protein intake for female participants based on the calorie range recommended was 50-193 g of protein per day. Based on the RDA, females on a standard diet should consume roughly 49 g of protein per day, whereas gluten-free females should consume approximately 52 g of protein per day. Females on a regular diet on average consumed 104.7 $\pm$ 24.8 g of protein, whereas gluten-free females consumed 96.2 $\pm$ 25.9 g per day. Protein intakes for both females on a regular diet and gluten-free diet were within the acceptable range, but were significantly ($p=<0.05$) greater than the RDA. The AMDR for fat intake in adults is 20-35% of calories consumed per day (USDA, 2012a). Based on the calorie range recommended for female participants, 44-86 g of fat was the acceptable range. Regular females consumed 48.5 $\pm$ 16.6 g of fat per day, whereas gluten-free females consumed significantly ($p=<0.05$) more fat (77.8 $\pm$ 24.1 g) per day. There was a significant difference ($p=<0.05$) in fiber intake over the five day period between females on a regular diet and females on a gluten-free diet. The recommended daily fiber intake is 25 g per day for adult females (USDA, 2012a). On average, regular females consumed 26.7 $\pm$ 7.9 g of fiber per day, whereas gluten-free females consumed only 20.4 $\pm$ 2.9 g per day. For females consuming a regular diet, 67% met the daily fiber recommendation on average, whereas only six percent of gluten-free females consumed the recommended amount of fiber on average

per day. The average daily macronutrient and fiber intake for female participants is shown in Table 6.

Table 6

Average Daily Macronutrient and Fiber Intake for Female Participants

	Calories (kcal)	Carbohydrates (g)	Protein (g)	Fat (g)	Fiber (g)
Control Group (Regular Diet)	1723.8 ± 363.3	203.2 ± 38.7	104.7 ± 24.8	48.5 ± 16.6	26.7 ± 7.9
Study Group (Gluten-Free Diet)	1666.0 ± 379.4	133.3 ± 28.0*	96.2 ± 25.9	77.8 ± 24.1*	20.4 ± 2.9*

Average nutrient intakes were calculated by analyzing 5-day food records using FoodWorks® nutrient analysis software (version 14). Values are means ± standard deviation. *Significantly different from controls (p=<0.05)

There was a significant (p=<0.05) difference in the overall cholesterol intake between females on a regular diet versus females on a gluten-free diet. The average cholesterol intake of females on a regular diet was 238.3 ± 192.2 mg per day compared to 494.9 ± 236.2 mg per day on average consumed by gluten-free females. Thus, gluten-free females consumed more cholesterol than the recommended (< 300 mg per day) value (USDA, 2012a). Of the females on a standard diet, the majority (83%) met the daily cholesterol recommendation on average, whereas only 19% of gluten-free females consumed cholesterol within the recommended value. Limiting saturated fat intake to no more than 10% of total calories consumed per day is associated with reduced risk of cardiovascular disease and is therefore recommended for all adults (USDA, 2012b). Based on the calorie range recommended for female participants, less than 50-55 g of saturated fat per day meets this recommendation. All female participants in this study consumed less than the maximum recommendation of saturated fat. None the less, gluten-free females consumed significantly more saturated fat compared with females

on a regular diet. On average females on a regular diet consumed significantly (p=<0.05) less (13.0 ± 6.7 g) saturated fat per day compared with gluten-free females who consumed 21.8 ± 7.5 g per day. The recommendation for sugar intake is less than 25% of total calories consumed per day (USDA, 2012b). Based on the calories ingested for female participants, less than 125-138 g of sugar per day was recommended. Regular females consumed 67.4 ± 25.1 g of sugar on average per day compared with gluten-free females, who consumed 76.0 ± 5.5 g per day on average. The average daily saturated fat, sugar, and cholesterol intakes for female participants are shown in Table 7.

Table 7

Average Daily Cholesterol, Saturated Fat, and Sugar Intake for Female Participants

	Cholesterol (mg)	**Saturated Fat (g)**	**Sugar (g)**
Control Group (Regular Diet)	238 ± 192.2	13.0 ± 6.7	67.4 ± 25.1
Study Group (Gluten-Free Diet)	494.9 ± 236.2*	21.8 ± 7.5*	76.0 ± 5.5

The average intake of nutrients was calculated by analyzing 5-day food records using FoodWorks® nutrient analysis software (version 14). Values are means ± standard deviation.
*Significantly (p=<0.05) different from controls.

Although females following a regular diet consumed more thiamin, riboflavin, and niacin on average per day than females following a gluten-free diet, the difference was not significant. The recommended amounts thiamin, riboflavin, and niacin, for adult females are 1.1, 1.1. and 14 mg per day, respectively. Females on a regular diet on average consumed 2.0 ± 0.8 mg of thiamin per day, while gluten-free females consumed 1.5 ± 1.3 mg per day of the same nutrient. The majority (89%) of females on a regular diet met or exceeded the daily thiamin recommendation on average, whereas only 37% of gluten-free females met or

exceeded the daily thiamin recommendation on average. The average riboflavin intake for females on a regular diet was 2.7 ± 1.1 mg per day and 2.0 ± 1.2 mg for gluten-free females. Of the females on a regular diet, the majority (94%) met or exceeded the daily riboflavin recommendation on average. In the gluten-free group, 69% of females met or exceeded the daily riboflavin recommendation. Females on a regular diet consumed 23.3 ± 6.0 mg of niacin per day on average, whereas gluten-free females consumed 21.1 ± 11.6 mg per day. Almost all (94%) females on a regular diet met or exceeded the daily niacin recommendation. For gluten-free females, 62% met or exceeded the daily niacin recommendation on average. There was a significant ($p=<0.05$) difference in the folate and iron intake among females following a regular diet and those following a gluten-free diet. Females on a regular diet on average consumed 492.8 ±172.1 mcg of folate per day, while gluten-free females consumed 309.8 ± 175.2 mcg per day on average and did not meet the recommendation of 400 mcg per day (USDA, 2012a). The majority (72%) of females on a standard diet met or exceeded the daily folate recommendation, whereas only 19% of gluten-free females met or exceeded the daily folate recommendation on average. The average iron intake of 19.0 ± 5.6 mg per day for females on a standard diet was significantly ($p=<0.05$) different than the 13.4 ± 3.9 mg consumed by gluten-free females. For females consuming a regular diet, 67% met or exceeded the daily iron recommendation of 18 mg (USDA, 2012a). For gluten-free females, only 12% met or exceeded the recommendation for iron per day on average. Likewise, calcium intake for gluten-free females was significantly ($p=<0.05$) lower compared with women on a regular diet. The average calcium intake of regular females was 1008.4 ± 305.8 mg per day and 534.5 ± 177.3 mg per day on average for gluten-free females. For females on a standard diet, 67% met or exceeded the daily calcium recommendation per

day on average. All gluten-free females consumed less calcium than the daily recommendation of 1,000 mg (USDA, 2012). Sodium intake did not differ drastically between females consuming a regular diet and females consuming a gluten-free diet. The recommendation for sodium is less than 2,300 mg per day for adult females (USDA, 2012a). On average, females on a regular diet consumed 2240.7 ± 915.7 mg of sodium per day whereas gluten-free females consumed 2445.7 ± 288.9 mg per day on average. The majority (72%) of females on a standard diet met the daily recommendation for sodium, whereas only 44% of gluten-free females met the daily recommendation of less than 2,300 mg of sodium per day (USDA, 2012a). The average daily micronutrient intake for female participants is shown in Table 8.

Table 8

Average Daily Micronutrient Intake for Female Participants

	Thiamin (mg)	**Riboflavin (mg)**	**Niacin (mg)**	**Folate (mcg)**	**Iron (mg)**	**Sodium (mg)**	**Calcium (mg)**
Control Group (Regular Diet)	2.0 ± 0.8	2.7 ± 1.1	23.3 ± 6.0	492.8 ± 172.1	19.0 ± 5.6	2240.7 ± 915.7	1008.4 ± 305.8
Study Group (Gluten-Free Diet)	1.5 ± 1.3	2.0 ± 1.2	21.1 ± 11.6	309.8 ± 175.2*	13.4 ± 3.9*	2445.7 ± 288.9	534.5 ± 177.3*

Average intake of micronutrients was calculated by analyzing 5-day food records of females 25-35 years old who consumed a regular diet (n= 18) and gluten free diet (n=16) using FoodWorks® nutrient analysis software (version 14).Values are mean ± standard deviation.
* Significantly different (p=<0.05) from controls

The dietary sources of the nutrients discussed above were also determined according to the five-day food log collected from each participant. The main sources of carbohydrates for all participants were white and brown rice, as well as beans and lentils. In addition,

regular males and females obtained carbohydrates from whole wheat/enriched bread, pasta, hot and cold cereals, quinoa, cow's milk and yogurt, as well as snack foods such as pretzels, chips, crackers, cookies, and granola bars. Additional sources of carbohydrates for gluten free males and females were fresh/frozen fruits and vegetables, as well as gluten-free products such as bread, pasta, hot and cold cereals, and snack foods. The primary sources of fat and saturated fat for all participants were nuts/nut butters, eggs, cheese, salad dressings, snack foods, bacon, and beef. For gluten-free males and females other sources of fat and saturated fat were coconut oil, butter, and pork. Additional sources of fat for all participants were olive oil, salmon, and avocados. The main sources of protein for regular and gluten-free participants were beef, chicken breast, eggs, bacon, fish, a powdered protein supplement, cheese, beans and lentils, cow's milk, yogurt, and nuts/nut butters. Additional sources of protein for regular males and females were quinoa and oatmeal. Gluten-free males and females also obtained protein from canned or packaged tuna fish, pork, and luncheon meats. For regular and gluten-free females, egg whites were also a main source of protein. The main sources of cholesterol for all participants were beef, eggs, cheese, chicken breast, and salmon. Additionally, males and females eating a gluten-free diet obtained cholesterol from pork. Natural and added sugars for all participants came from fresh/frozen fruits, flavored yogurt, hot and cold cereals, snack foods, and condiments such as ketchup. The main sources of fiber for all participants were beans and lentils, brown rice, fresh/frozen fruits and vegetables, and nuts/nut butters. Regular males and females also obtained fiber from whole wheat/enriched bread, pasta, snack foods and similar foods, as well as quinoa and oatmeal. Gluten-free bread and similar products contributed to fiber intake for gluten-free participants. The primary sources of thiamin and riboflavin for all participants were enriched hot and cold

cereals, a powdered protein supplement, beans and lentils, and salmon. Participants eating a regular diet also obtained thiamin and riboflavin from whole wheat/enriched bread and pasta, enriched snack foods, and quinoa. Additionally, all participants obtained thiamin from enriched white and brown rice and other sources of riboflavin were cow's milk, yogurt, and eggs for all participants. For regular and gluten-free participants the main dietary sources of niacin were a powered protein supplement, enriched white and brown rice, nuts/nut butters, beans and lentils, and animal sources such as beef, chicken, fish, and bacon. For regular participants, enriched hot and cold cereals, whole wheat/enriched bread, enriched snack foods, and quinoa contributed to niacin intake. For gluten-free participants, additional sources of niacin were enriched gluten-free hot and cold cereals, avocados, and pork. The main sources of folate for all participants were a powdered protein supplement; beans and lentils; salmon; enriched white rice; and folate rich vegetables such as spinach, mixed greens, and asparagus. For regular participants other dietary sources of folate were quinoa, whole wheat/enriched bread and similar foods, as well as enriched hot and cold cereals. Additional sources of folate for gluten-free participants were enriched gluten-free hot and cold cereals and avocados. The primary dietary sources of iron for all participants were a powered protein supplement, beans and lentils, nuts/nut butter, beef, enriched white and brown rice, eggs, chicken, fish, and iron-rich vegetables such as spinach and asparagus. For regular participants iron was also obtained from quinoa; enriched hot and cold cereals; as well as whole wheat/enriched bread, pasta, and snack foods. For gluten-free participants, additional sources of iron were enriched gluten-free hot and cold cereals, pork, luncheon meats, and avocados. For all participants, the main sources of sodium were eggs, beef, chicken, cow's milk, yogurt, cheese, added table salt, bacon, salad dressings, nuts/nut butters, and

condiments such as ketchup. For regular males and females, foods such as whole wheat/enriched bread products, snack foods, as well as hot and cold cereals contributed to the overall sodium intake. Gluten-free males and females also obtained sodium from luncheon meats; canned or packaged tuna; butter; pork; and gluten-free bread, snack foods as well as hot and cold cereals. The main sources of calcium for regular and gluten-free participants were cow's milk, yogurt, a powdered protein supplement, cheese, beans and lentils, and eggs. In addition, fruits and vegetables rich in calcium as well as nuts/nut butters also contributed to the overall intake of this nutrient for all participants. Participants eating a regular diet also obtained calcium from whole wheat/enriched bread products, hot and cold cereals and snack foods. Gluten-free participants also obtained calcium from enriched gluten free hot and cold cereals.

Overall, participants from the regular diet and gluten-free diet groups obtained nutrients from similar foods. However, regular males and females that ate whole wheat/ enriched bread, pasta, hot and cold cereals, snack foods, and similar foods consumed a greater variety of nutrients from these sources than gluten-free participants who did not consume them. For participants on a regular diet, the main sources of carbohydrates, iron, fiber, folate, niacin, thiamin, riboflavin, and calcium were whole wheat/enriched bread, pasta, hot and cold cereals, snack foods, and similar foods. For gluten-free participants the main sources of these nutrients that were different from regular participants were fruits and vegetables, as well as gluten-free bread, pasta, hot and cold cereals, and similar foods, which were typically lower in these nutrients than whole wheat/enriched gluten-containing foods. The primary sources of nutrients for all male and female participants are shown in Tables 9 and 10.

Table 9

Dietary Sources of Nutrients for Male Participants

	Control Group Males	Study Group Males
Carbohydrates	White and Brown Rice; Beans and Lentils; Whole Wheat/Enriched Bread Products; Pasta, Hot and Cold Cereals, and Snack Foods; Quinoa; Cow's Milk; Yogurt	White and Brown Rice; Beans and Lentils; Fresh/Frozen Fruits and Vegetables; Gluten-Free Bread Products, Pasta, Hot and Cold Cereals, and Snack Foods
Fat	Nuts/Nut Butters; Eggs; Cheese; Salad Dressings; Snack Foods; Bacon; Beef; Olive Oil; Salmon; Avocados	Nuts/Nut Butters; Eggs; Cheese; Salad Dressings; Snack Foods; Bacon; Beef; Coconut Oil; Butter; Pork; Olive Oil; Salmon; Avocados
Fiber	Beans and Lentils; Brown Rice; Fresh/Frozen Fruits and Vegetables; Nuts/ Nut Butters; Whole Wheat/Enriched Bread Products, Pasta, and Snack Foods; Quinoa; Oatmeal	Beans and Lentils; Brown Rice; Fresh/Frozen Fruits and Vegetables; Nuts/ Nut Butters; Enriched Gluten-Free Bread Products
Niacin	Powdered Protein Supplement; Enriched White and Brown Rice; Nuts/Nut Butters; Beans and Lentils; Beef; Chicken; Fish; Bacon; Enriched Hot and Cold Cereals; Whole Wheat/Enriched Bread Products and Snack Foods; Quinoa	Powdered Protein Supplement; Enriched White and Brown Rice; Nuts/Nut Butters; Beans and Lentils; Beef; Chicken; Fish; Bacon; Enriched Gluten-Free Hot and Cold Cereals; Avocados; Pork
Folate	Powdered Protein Supplement; Beans and Lentils; Salmon; Enriched White Rice; Spinach; Mixed Greens; Asparagus; Quinoa; Whole Wheat/Enriched Bread Products; Enriched Hot and Cold Cereals	Powdered Protein Supplement; Beans and Lentils; Salmon; Enriched White Rice; Spinach; Mixed Greens; Asparagus; Enriched Gluten-Free Hot and Cold Cereals; Avocados
Calcium	Cow's Milk; Yogurt; Powdered Protein Supplement; Cheese; Beans and Lentils; Eggs; Whole Wheat/Enriched Bread Products, Hot and Cold Cereals, and Snack Foods	Cow's Milk; Yogurt; Powdered Protein Supplement; Cheese; Beans and Lentils; Eggs; Enriched Gluten-Free Hot and Cold Cereals
Sodium	Eggs, beef, chicken, cow's milk, yogurt, cheese, added table salt, bacon, salad dressings, nuts/nut butters, condiments, whole wheat/enriched bread products, snack foods, and enriched hot and cold cereals	Eggs, beef, chicken, cow's milk, yogurt, cheese, added table salt, bacon, salad dressings, nuts/nut butters, condiments, luncheon meats, canned/packaged tuna fish, butter, pork, gluten-free bread products, snack foods, and hot and cold cereals

Dietary sources of nutrients were determined for participants in FoodWorks® nutrient analysis software (version 14). There was a significant difference in intakes of control group males and study group males of nutrients shown.

Table 10

Dietary Sources of Nutrients for Female Participants

	Control Group Females	Study Group Females
Carbohydrates	White and Brown Rice; Beans and Lentils; Whole Wheat/Enriched Bread Products; Pasta, Hot and Cold Cereals, and Snack Foods; Quinoa; Cow's Milk; Yogurt	White and Brown Rice; Beans and Lentils; Fresh/Frozen Fruits and Vegetables; Gluten-Free Bread Products, Pasta, Hot and Cold Cereals, and Snack Foods
Fat	Nuts/Nut Butters; Eggs; Cheese; Salad Dressings; Snack Foods; Bacon; Beef; Olive Oil; Salmon; Avocados	Nuts/Nut Butters; Eggs; Cheese; Salad Dressings; Gluten-free Snack Foods; Bacon; Beef; Coconut Oil; Butter; Pork; Olive Oil; Salmon; Avocados
Fiber	Beans and Lentils; Brown Rice; Fresh/Frozen Fruits and Vegetables; Nuts and Nut Butters; Whole Wheat/Enriched Bread Products, Pasta, and Snack Foods; Quinoa; Oatmeal	Beans and Lentils; Brown Rice; Fresh/Frozen Fruits and Vegetables; Nuts and Nut Butters; Gluten-Free Bread Products
Saturated Fat	Nuts/Nut Butters; Eggs; Cheese; Salad Dressing; Snack Foods; Bacon; Beef;	Nuts/Nut Butters; Eggs; Cheese; Salad Dressing; Gluten-Free Snack Foods; Bacon; Beef; Coconut Oil; Butter; Pork
Cholesterol	Beef; Eggs; Cheese; Chicken Breast; Salmon;	Beef; Eggs; Cheese; Chicken Breast; Salmon; Pork
Folate	Powdered Protein Supplement; Beans and Lentils; Salmon; Enriched White Rice; Spinach; Mixed Greens; Asparagus; Quinoa; Whole Wheat/Enriched Bread Products and Hot and Cold Cereals	Powdered Protein Supplement; Beans and Lentils; Salmon; Enriched White Rice; Spinach; Mixed Greens; Asparagus; Enriched Gluten-Free Hot and Cold Cereals; Avocados
Iron	Powdered Protein Supplement; Beans and Lentils; Nuts/Nut Butters; Beef; Enriched White and Brown Rice; Eggs; Chicken; Fish; Spinach; Asparagus; Quinoa; Enriched Hot and Cold Cereals; Whole Wheat/Enriched Bread Products, Pasta, and Snack Foods	Powdered Protein Supplement; Beans and Lentils; Nuts/Nut Butters; Beef; Enriched White and Brown Rice; Eggs; Chicken; Fish; Spinach; Asparagus; Enriched Gluten-Free Hot and Cold Cereals; Pork; Luncheon Meats; Avocados
Calcium	Cow's Milk; Yogurt; Powdered Protein Supplement; Cheese; Beans and Lentils; Eggs; Whole Wheat/Enriched Bread Products, Hot and Cold Cereals, and Snack Foods	Cow's Milk; Yogurt; Powdered Protein Supplement; Cheese; Beans and Lentils; Eggs; Enriched Gluten-Free Hot and Cold Cereals

Dietary sources of nutrients were determined for participants in FoodWorks® nutrient analysis software (version 14). There was a significant difference in intakes of control group females and study group females of nutrients shown.

In summary, the hypothesis for this study was that the average intakes of fiber; the B vitamins thiamin, riboflavin, niacin, folate; and iron would be lower in the gluten-free group than in the regular diet group. The results of this study concluded the hypothesis to be partially true. Although males in the study group met the daily recommendations for protein, saturated fat, sugar, thiamin, riboflavin, niacin, and iron they were below the recommendations for calories, and significantly below or above the recommendations for carbohydrates, fat, fiber, folate, sodium, and calcium. In addition, gluten-free males consumed more cholesterol than recommended, but the difference was not significant. More specifically, males consuming a regular diet consumed calories and carbohydrates within the acceptable ranges, whereas gluten-free males consumed fewer calories and significantly fewer carbohydrates than control group males and their intakes were not within the acceptable ranges. The acceptable range of fat was consumed by males on a regular diet but gluten-free males did not consume fat within the acceptable range and their intake was significantly more than recommended. Males on a regular diet met the daily fiber recommendation on average, but gluten-free males did not meet the daily fiber recommendation and their intakes were significantly less than recommended. All males consumed more cholesterol than recommended per day, but their intakes were not significantly different than the cholesterol intake of males in the control group. All male participants met the daily recommendations of saturated fat, sugar niacin, thiamin, and riboflavin, but gluten-free males consumed significantly less niacin than males on a regular diet. In general, dietary sources of niacin were similar for all male participants, but males on a regular diet consumed whole wheat/enriched bread products whereas gluten-free males did not. Gluten-free males did obtain niacin from enriched gluten-free products, but these

products were not consumed regularly throughout the five-day period. In addition, the types of gluten-free products that were enriched were limited. On average, males on a regular diet met the daily folate recommendation, whereas gluten-free males consumed significantly less of this nutrient and their intake did not meet the daily folate recommendation. Dietary sources of folate were similar for all male participants, but males on a regular diet consumed whole wheat/enriched bread products, whereas gluten-free males did not. Gluten-free males did obtain some folate from enriched gluten-free products, but regular consumption was limited. Also, few gluten-free products consumed by participants were enriched. The recommended amount of iron was consumed by all male participants. The daily sodium recommendation was met by males on a regular diet, whereas gluten-free males consumed significantly more sodium than recommended. Gluten-free males regularly consumed foods high in sodium such as luncheon meats, canned or packaged tuna fish, butter, pork, and gluten-free bread products such as snack foods and cereals whereas males on a regular diet did not regularly consume these foods. Males on a regular diet met the daily calcium recommendation on average, while gluten-free participants did not meet the daily calcium recommendation and their intakes were significantly less than participants on a regular diet. Dietary sources of calcium were similar for all participants, but males on a regular diet consumed whole wheat/enriched bread products whereas gluten-free males did not consume these foods. Gluten-free males did consume enriched gluten-free hot and cold cereals but regular consumption was limited. In addition, not all gluten-free hot and cold cereals consumed by gluten-free participants were enriched.

Females in the study group met the daily recommendations for protein, fat, saturated fat, sugar, thiamin, riboflavin, and niacin. However, gluten-free females failed to meet the

daily recommendations for calories and consumed more sodium than recommended. Gluten-free females also consumed significantly below or above the daily recommendations for carbohydrates, fiber, cholesterol, folate, iron, and calcium. Females on a regular and gluten-free diet consumed less than the acceptable ranges of calories and carbohydrates, but the differences were not significant. All female participants consumed protein within the acceptable range but their intakes were significantly more than the RDA. All females consumed fat within the acceptable range, but gluten-free females consumed significantly more fat than females on a regular diet. Females on a regular diet met the daily fiber recommendation on average, but gluten-free females did not meet the daily fiber recommendation and their intakes were significantly less than recommended. Dietary sources of fiber were similar for all female participants, but females on a regular diet obtained fiber from whole wheat/enriched bread products, pasta, snack foods, and hot and cold cereals as well as quinoa whereas gluten-free females did not consume these foods. Gluten-free females consumed significantly more cholesterol than females on a regular diet and their intake exceeded the daily recommendation. All female participants met the daily recommendations of saturated fat, sugar, niacin, thiamin, and riboflavin, but gluten-free females consumed significantly more saturated fat than females on a regular diet. In addition, females on a regular diet met the daily reconditions for folate and iron whereas gluten-free females consumed significantly less folate and iron and their average intakes did not meet the daily recommendations. Female participants overall consumed similar dietary sources of folate and iron. However, females on a regular diet on a regular diet obtained folate and iron from regular consumption of foods not consumed by gluten-free females such as whole wheat/enriched bread products, pasta, snack foods, and hot and cold cereals as well as quinoa

Females on a regular diet consumed sodium within the recommended value. On the other hand, gluten-free females consumed significantly more sodium and their average daily intake exceeded the recommendation. Females on a regular diet also met the daily calcium recommendation on average, while gluten-free participants did not meet the daily calcium recommendation and their intakes were significantly less than participants on a regular diet. Dietary sources of calcium were similar for all female participants, but females consuming a regular diet obtained calcium from foods not consumed by gluten-free females such as whole wheat/enriched bread products, snack foods, and hot and cold cereals.

Overall, participants in the study groups consumed below the recommended daily values for several key nutrients including calories, carbohydrates, iron, fiber, folate, and calcium and above the recommended daily values for sodium and cholesterol.

Chapter 5: Discussion and Conclusion

Discussion

The consumption of gluten-free foods has become more popular in recent years among healthy people of all ages. However, the nutritional impact of following a gluten-free diet in healthy adults has not been fully evaluated. This study showed that healthy adults choose to follow a gluten-free diet because they believe it is healthier or that consuming a gluten-free diet will help them lose weight. The main aim of this study was to evaluate how eliminating gluten from the diet affected the nutrient intake in healthy adult males and females compared to adult males and females that consumed a diet containing gluten.

Although 15 different nutrients were analyzed for each participant, the primary focus was to evaluate nutrients such as fiber, thiamin, riboflavin, niacin, folate, and iron that are either limited or not present at all in gluten-free products. Therefore, the hypothesis for this study was that the average intakes of fiber; the B vitamins thiamin, riboflavin, niacin, folate; and iron would be lower in the gluten-free group than in the group consuming gluten containing foods. To fully evaluate the hypothesis the following specific aims were studied: First, nutrient intakes of subjects eating gluten-free and regular diets were measured with a focus on fiber; the B vitamins thiamin, riboflavin, niacin, folate; and iron and the nutrient intakes were compared to DRIs for adults. Then the dietary sources of these nutrients were identified and compared in both the regular diet group and gluten-free group.

The questionnaire completed by each participant provided information related to age, gender, height, weight, and diet history. The average BMIs in the control groups were 26.9 $\pm$ 2.9 kg/m^2 (males) and 22.9 $\pm$ 2.4 kg/m^2 (females). For the study group, the average BMIs were 26.6 $\pm$ 1.7 kg/m^2 (males) and 23.8$\pm$ 3.8 kg/m^2 (females). There were no significant

differences in BMI between the control and study group in males or females. The average age of male participants was 32 years of age and 30 years of age for female participants. All male and female participants in the control group reported that they had followed a regular diet for more than one year, while only five gluten-free males and 10 gluten-free females reported being gluten-free for more than one year. Of the remaining gluten-free participants, four males and one female reported being gluten-free less than six months. The other five gluten-free females reported they followed a gluten-free diet for six to 12 months. In addition, three males and nine females in the control group reported they had dieted in the past, while two males and seven females in the study group reported they had dieted in the past. In the control groups nine males and 10 females reported supplement use while in the study groups, seven males and 14 females reported use of supplements. Gluten-free participants were also instructed to indicate their reasoning for following a gluten-free diet. The majority of gluten-free participants indicated they believed that the diet was healthier. Gluten-free females indicated they followed this type of diet for weight loss and other reasons such as reduction in headaches and perceived gluten intolerance. Gluten-free males also indicated they followed a gluten-free diet for weight loss.

From the analysis described in the previous chapter, it was concluded that males following a regular diet on average consumed nutrients within the acceptable ranges or met the specific daily recommendation for 14 out of 15 nutrients that were evaluated. More specifically, males on a regular diet consumed the recommended amounts of calories, carbohydrates, protein, fat, saturated, fat, and sugar. In addition, males on a regular diet met or exceeded the daily recommendation for fiber, thiamin, riboflavin, niacin, folate, calcium, and iron. Regular diet males also consumed sodium within the recommendation on average

per day. Cholesterol intake (340.3 ± 141.6 milligrams) of men on a regular diet exceeded the recommendation of less than 300 milligrams of cholesterol per day (USDA, 2012a).

In contrast, men on a gluten-free diet met the daily recommendations of only seven out of the 15 nutrients that were evaluated. Gluten-free males did not consume calories, carbohydrates or fat within the acceptable ranges on average per day. In addition, gluten-free males did not meet the daily recommendations for fiber, cholesterol, folate, sodium, and calcium. Males consuming a gluten-free diet consumed fewer calories, significantly less carbohydrates and fiber and significantly more fat than recommended. Additionally, gluten-free males consumed more cholesterol than recommended. The average daily intakes of folate and calcium were significantly less than recommended for gluten-free males, whereas sodium intake was significantly more.

Women on a regular diet met specific daily recommendations for 13 of the 15 nutrients evaluated. Females following a regular diet on average consumed protein, fat, saturated fat, and sugar within acceptable ranges and met daily recommendations of fiber, thiamin, riboflavin, niacin, folate, calcium, sodium, and iron. On average, the daily number of calories and grams of carbohydrates consumed were less than the acceptable ranges for these women.

In contrast, women on a gluten-free diet met the recommendations for only seven out of 15 nutrients evaluated. Gluten-free females did not consume calories and carbohydrates within the acceptable ranges. Additionally, females consuming a gluten-free diet did not meet the daily recommendations for fiber, folate, iron, sodium, and calcium. Gluten-free females consumed fewer calories and significantly less carbohydrates than recommended per day. Also, females on a gluten-free diet consumed significantly less fiber, cholesterol, folate, iron,

and calcium than recommended per day. Gluten-free females also consumed significantly more sodium than recommended on average per day.

However, females consuming a gluten-free diet also consumed less fiber than recommended on average per day. Additionally, the daily cholesterol recommendation (<300 mg) was exceeded on average by gluten-free females (USDA, 2012a). Gluten-free females also consumed significantly less folate, iron, and calcium than recommended as well as significantly more sodium than recommended per day.

Results of the present study were similar to other studies previously discussed that evaluated the nutrient intakes of gluten-free adults. Like the current study, Wild et al., (2010) also evaluated the average intake of calories, fat, fiber, calcium, iron, and folate in gluten-free adults and compared those intakes to an age and gender matched control group consuming foods containing gluten as well as to daily recommended values. The results from the study conducted by Wild et al. were similar to the results in the present study, which found that gluten-free males and females consumed significantly more fat than participants in the control group. In addition, gluten-free females consumed significantly less fiber, iron, and folate than the control group females. Although the previous study conducted by Wild et al. and the current study had similar findings, there were also some notable differences. First, the study conducted by Wild et al. was composed of participants diagnosed with celiac disease, whereas the current study included individuals, who voluntarily followed a gluten-free diet for other reasons that were previously discussed. Also, in the Wild et al. study, gluten-free males and females consumed significantly more calories than control group participants, while gluten-free participants in the present study consumed fewer calories than the control group. Another notable difference between the study conducted by Wild et al.

and the current study was that gluten-free females in the Wild et al. study consumed significantly more calcium than females in the control group, whereas gluten-free females in the current study consumed significantly less calcium than females in the control group. In another similar study conducted by Thompson et al., (2005) gluten-free males consumed significantly less fiber than recommended per day and gluten-free females consumed significantly less iron and calcium than recommended per day, which reflected the results of the present study. However, the study conducted by Thompson et al. and the current study also had some prominent differences. First, the study conducted by Thompson et al. consisted of patients diagnosed with celiac disease, while the present study was composed of healthy adults who voluntarily followed gluten-free diet. In addition, the Thompson et al. study concluded that gluten-free males met the daily recommendation for carbohydrates, while the present study found that gluten-free males consumed significantly less carbohydrates than recommended per day. Also, gluten-free females in the study conducted by Thompson et al. study met the daily recommendation for fiber, whereas gluten-free females in the current study consumed significantly less fiber than recommended.

Consuming nutrients below or above the daily recommendations may have negative effects on overall health. Overall, gluten-free participants did not consume adequate amounts of calories, carbohydrates, fiber, folate, iron, and calcium to meet daily recommendations. In addition, gluten-free participants consumed more fat, cholesterol, and sodium than recommended per day. For the current study, daily calorie needs for participants were based on recommendations from Dietary Guidelines for Americans, 2010, which were estimated by age, gender, and physical activity level (USDA, 2012b). Although daily calorie requirements were estimated for participants, inadequate energy intake may contribute to insufficient

intake of other essential nutrients. Gluten-free males and females did not consume calories within the recommended range, but their intakes were not significantly lower than the calorie intake of males and females on a regular diet. Acceptable carbohydrate intakes were based on AMDR (USDA, 2012a). The acceptable ranges are related to a reduced risk of long-term diseases and if greater than the acceptable ranges are consumed, risk of long-term disease is increased (USDA, 2012a). Males and females on a gluten-free diet consumed significantly less carbohydrates than males and females on a standard diet and their intake was not within the acceptable ranges. Daily fiber recommendations are based on AI and vary based on gender (USDA, 2012a). Adequate fiber intake plays a role in maintaining healthy levels of blood glucose, cholesterol, and blood pressure and therefore reduces the risk for heart disease, stroke, hypertension, obesity, and diabetes (Anderson et al., 2009). In addition, adequate fiber intake reduces the risk for diverticular disease and colon cancer (University of California San Francisco [UCSF], 2013a). Fiber intake also helps to prevent constipation and contributes to a healthy immune system. Gluten-free males and females consumed significantly less fiber than recommended on average per day. Inadequate fiber intake may affect healthy levels of blood glucose, cholesterol, and blood pressure and therefore may increase the risk for chronic disease (NLM, 2013a; UCSF, 2013a). Insufficient intake of fiber may also contribute to constipation and increase the risk for diverticular disease and colon cancer (UCSF, 2013a). Daily folate, iron, and calcium recommendations are based on RDA, which provide daily recommendations that meet the nutrient needs of nearly all individuals in a group (USDA, 2012a). Folate is a water soluble vitamin that plays an important role in new cell formation (NLM, 2013b; National Institutes of Health [NIH], 2012). Folate also plays a significant role in normal fetal development and reduces the risk for birth defects and

therefore is necessary for all women of childbearing age (Lee & Haydu, 2005). Gluten-free males and females consumed significantly less folate on average per day than recommended. Deficiency of this vitamin can lead to megablastic anemia or abnormally large, underdeveloped red blood cells (NIH, 2012). This type of anemia may also cause fatigue, headaches, heart palpitations, an inability to focus, and difficulty breathing. A folate deficiency may also lead to swelling and ulcers of the mouth; increased homocysteine levels; and mutations in hair, skin, and nail color (NLM, 2013b; NIH, 2012). Iron, a mineral found in all cells of the body plays a vital role in the development of oxygen carrying proteins and proper cell growth (NLM, 2013f; NIH, 2007). Gluten-free females consumed significantly less iron than recommended on average per day. Deficiency of this mineral initially starts with low iron intake which depletes iron stores while normal hemoglobin levels are maintained (NIH, 2007). Iron deficiency anemia occurs when iron intake does not meet the body's needs and stored iron is depleted (NLM, 2013f; NIH, 2007). Patients with this condition may experience exhaustion, headaches, paleness and intolerance to cold temperatures. Calcium plays important roles in metabolic functions such as maintaining normal vascular, muscular, nervous system, and hormonal functions, as well as in maintaining healthy bones and teeth (NIH, 2013; UCSF, 2013b). Gluten-free males and females consumed significantly less calcium than recommended on average per day. Calcium deficiency may cause muscle cramps, fatigue, reduced appetite, lack of sensation and tingling in the extremities, irregular heart rhythms, and eventually may lead to death (NIH, 2013). However, blood calcium levels are well maintained regardless of dietary intake and initially signs of calcium deficiency are not present. Over a period of time, calcium deficiency can affect bone health resulting in osteopenia and eventually osteoporosis. Acceptable fat intakes

were based on AMDR which are acceptable ranges related to a reduced risk of long-term diseases and if consumed in excess the risk of long-term disease is increased (USDA, 2012a). Gluten-free males consumed significantly more fat than recommended on average, per day whereas both gluten-free males and females consumed significantly more sodium than recommended on average per day. Excess fat and sodium intake are associated with increased risk for cardiovascular disease (World Heart Federation [WHF], 2013). Excess sodium intake increases the risk for hypertension, which damages the vascular system and is associated with damage to the heart, arteries, and kidneys, and may contribute to erectile dysfunction and stroke (American Heart Association [AHA], 2013). Hypertension can also affect the health of blood vessels in the eyes and may lead to vision loss. Although individual nutrient requirements may vary, daily recommendations developed by organizations such as the Food and Nutrition Board, Institute of Medicine, and National Academies, as well the USDA, and the United States Department of Health and Human Services are generally adequate for healthy populations (USDA, 2012a, 2012b). In conclusion, adequate nutrient intakes are essential to maintain overall health and prevent adverse health effects associated with insufficient nutrient intakes.

Regular consumption of *nutrient dense* gluten free foods is necessary to reduce the risk of nutrient deficiencies. To ensure adequate nutrient intake, individuals following a gluten-free diet may benefit from nutrition counseling and education. Understanding the importance of proper meal planning and food selection may help gluten-free individuals choose more nutrient dense foods and avoid nutrient deficiencies. Enriched gluten-free products available seem to be somewhat limited, but gluten-free individuals would obtain more nutrients by selecting enriched gluten-free products over those that have not been

enriched. Manufacturers should consider enrichment of all gluten-free products they produce to help individuals following a gluten-free diet meet daily nutrient requirements.

Limitations

Although this study provided useful data overall, there were several limitations. First, the small sample size was not ideal for accurately evaluating the nutrient intakes of adults on a regular or gluten-free diet. The control group consisted of 33 (15 males, 18 females) participants and the study group consisted of 25 (nine males, 16 females) participants. The average intakes of nutrients may have been influenced by the intake of just one participant due to the small number of participants in each group.

Secondly, self-reported data such as height and weight as well as self-reported dietary intake possibly led to inaccurate calculations of BMI and nutrient intake. To provide an accurate dietary intake, it was recommended that participants provide as much detail as possible when recording their daily food intake. The instructions supplied to each participant recommended providing portion sizes, quantity, and brand names of foods consumed. Daily food records were vastly different in the amount of details recorded which did not provide an accurate description of the dietary intake of participants.

Next, the nutrient analysis software, FoodWorks® did not contain all the products consumed by participants. More specifically, the software did not contain products that are labeled gluten-free. Therefore, nutrition information for these foods was collected from manufacturer's websites. In addition, websites for restaurants were accessed to obtain nutrition information for other foods not found in the nutrient analysis software. Accessing nutrition information from restaurant and manufacturer's websites was time consuming and not all nutrition information was available for all foods consumed by participants.

In addition, it was difficult to know for sure whether some gluten-free products were enriched. The nutrient analysis software used (FoodWorks®) did not contain gluten-free products and therefore manufacturer websites were accessed to obtain nutrition information. However, the information found on these websites was limited overall. The amounts of some nutrients such as thiamin, riboflavin, niacin, and folate were not available for all gluten-free foods consumed by participants. Given that the nutrient information for some foods was not available, it was not included in the nutrient analysis.

Another limitation of this study is non-randomization. Participants were recruited from a fitness center and health food stores within a community, so they may have been more concerned about fitness and nutrition than if they were randomly selected participants. In addition, both the control and study participants were recruited from the same fitness center and health food stores within the community so it is possible that participants were influenced by one another.

Lastly, participants recorded their dietary intake for five consecutive days. This amount of time likely did not provide an accurate description of how participants eat on a regular basis and therefore the nutrient analysis may not have been fully accurate. Evaluating dietary intake over a longer period of time may have provided a better understanding of the nutrient intake of healthy adults on a gluten-free diet.

Conclusion

Gluten-free diets are popular in a variety of populations including healthy adults. In the past, few gluten-free products were not enriched unlike their gluten-containing counterparts. It does seem that some gluten-free products in general have evolved and include more nutrient dense ingredients than they once did. However, the nutrient content of

gluten-free foods still does not fully compare to the nutrient content of enriched gluten-containing foods. Therefore, individuals consuming these foods were at risk for nutrient deficiencies, particularly of fiber; B vitamins (thiamin, riboflavin, niacin, and folate); and iron. Healthy adults eating a gluten-free diet do not appear to fully rely on gluten-free substitute foods such as bread, pasta, cereal, and similar foods to replace comparable foods containing gluten. Rather gluten-free participants did include some naturally gluten-free foods that contributed to their overall nutrient intake. Although the naturally gluten-free foods consumed like fruits and vegetables; animal products such as meat, poultry, and eggs; and dairy products contributed to the overall nutrient intake of gluten-free participants, they did not provide adequate amounts of all the nutrients evaluated. Even though gluten-free participants consumed some foods rich in nutrients, they did not consume these foods regularly over the five-day period. For example, gluten-free males and females did consume dairy products like cow's milk, yogurt and cheese, but intake was not adequate to meet the daily calcium recommendation. In addition, participants consuming a regular diet obtained more nutrients overall with the addition of whole wheat/enriched bread products and similar foods that were not consumed by gluten-free participants. Also, gluten-free participants did not always include a grain portion in every meal rather gluten-free grains were consumed sporadically throughout the five-day period. In general, gluten-free participants did not consume sufficient amounts of nutrient dense foods to meet all nutrient recommendations.

Recommendations for Further Research

Further studies should try to recruit a larger sample to avoid data that is skewed by one or two participants. However, like the current study, it may be difficult to recruit a large number of adults that meet the inclusion criteria. To collect more data, several small cohorts

could participate in the study over a longer period time and the data from all participants could be combined. Also, randomization of participants in the control and study groups may prevent the results being impacted from participants discussing details of the study or dietary intake.

Adequate details related to brand names, the amount of food consumed, and preparation methods are essential to accurately analyze the nutrient intake of participants. Using a method for recording dietary intake in detail is ideal for this type of study. Also, a five-day record of dietary intake does not provide an accurate description of how participants eat on a regular basis. Having participants record their dietary intake for a longer period of time would allow investigators to see fluctuations, if they occur, in the amounts and types of nutrients consumed. In addition, a software program or other method to complete the nutrient analysis should have access to gluten free products currently on the market. This would eliminate the need to contact manufacturers for nutrition information.

In general, further research would benefit from a larger sample size, randomization of participants, detailed dietary intake, a longer data collection phase, and a nutrient analysis program including gluten-free products.

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APPENDICES

Appendix A: Instructions for Participants in the Gluten-Free Study

Thank you for participating in this study. Your time and information is greatly appreciated and will help the principal investigator better understand the nutrient intake of healthy adults like yourself.

Please read all of these instructions before starting the questionnaire or recording your dietary intake. Reference these instructions as you are completing the necessary documents. Do not discard these instructions until you have completed your portion of the study.

****Attention: Do not write your name on any document that will be returned to the investigator. All documents must be completed and returned by _____**

Appendix B: Participant Questionnaire

The questionnaire should be completed in blue or black ink. Please read each question carefully and select the answer that best describes your dietary habits. For questions requesting additional information, please write legibly in the space provided. Make sure to answer every question to the best of your ability and contact the investigator if you have any questions. To maintain your confidentially, do not write your name anywhere on this form. Do not return this form until you have recorded your dietary intake for five consecutive days. Complete instructions are described below regarding documents to be returned.

Appendix C: Daily Food Record

This packet includes five blank daily food record forms plus two additional forms if needed. Please record everything you eat and drink including all supplements every day for **five consecutive days including one weekend day.** Do not alter your eating habits from your normal intake during this study. A separate form should be used for each of the five days.

These forms should be completed in blue or black ink. First, circle the letter(s) that corresponds to the day of the week you are recording your dietary intake (SU= Sunday, M= Monday, T= Tuesday, W= Wednesday, TH= Thursday, F=Friday, S= Saturday), and write that day's date in the space provided. An accurate and detailed description of your dietary intake is very important, so it is suggested that you record each meal and snack immediately following consumption in the space provided. Please record individual measurements of every food and beverage you consume for each meal and snack. Basic kitchen measuring devices such as measuring cups or spoons, liquid measuring containers, and a food scale are all acceptable for measuring foods and beverages. If you are unable to measure foods and beverages consumed estimate your portion sizes using Appendix D: Tools for Estimating Portion Sizes. Each unit of measure such as cup, tablespoon, teaspoon, ounce, etc. should be recorded for each item consumed. Units of measure may be abbreviated and fractions as well as numbers to indicate your serving size do not need to be written out in their word form, but please write legibly. For packaged food items check the nutrition label printed on the packaging for the serving size. For assistance reading nutrition labels see Appendix E: Sample Nutrition Label. For mixed dishes such as soups, stews, and casseroles simply provide a measurement of how much you consumed; such as 1 cup and the name of the dish. Although not mandatory, you are highly encouraged to provide as much detail as possible including brand names for packaged foods, and food preparation methods such as baked, sautéed in oil, fried, grilled, etc. A copy of recipes for all homemade meals returned with the other required documents will be useful for proper nutrient analysis, but is not required. Recipes may be handwritten, typed or printed from a website. Also, make sure to indicate how many servings the recipe yields (see sample recipe below). If the meal was consumed at

a restaurant, recording a detailed description of the meal, including the name of the dish and the restaurant's name is also encouraged, yet not mandatory. If you need additional space use the reverse side of the sheet and specify which meal or snack you are recording. If you make a mistake; cross out the error with blue or black ink, use whiteout, or recopy your dietary intake on a new sheet. You may discard sheets with excessive errors if you recopied the information on the extra sheets provided. You do not need to return unused food record sheets.

Sample Daily Food Record:

Breakfast	2 eggs, ¼ cup egg whites, 1 cup chopped red bell pepper (pan fried in 1 Tablespoon Olive oil) 1 cup Kashi Go Lean cereal, ½ cup Diamond unsweetened almond milk 8 ounces coffee, 1 teaspoon International Delight Caramel Macchiato creamer

Sample Recipe:

Toasted Quinoa Salad with Scallops & Snow Peas
From EatingWell: March/April 2009

Yields 6 servings

Ingredients

- 12 ounces dry sea scallops, cut into 1/2-inch pieces, or dry bay scallops
- 4 teaspoons reduced-sodium tamari, or soy sauce, divided
- 4 tablespoons plus 2 teaspoons canola oil, divided
- 1 1/2 cups quinoa, rinsed well (see Tip)
- 2 teaspoons grated or minced garlic
- 3 cups water
- 1 teaspoon salt
- 1 cup trimmed and diagonally sliced snow peas, (1/2 inch thick)
- 1/3 cup rice vinegar
- 1 teaspoon toasted sesame oil
- 1 cup thinly sliced scallions

- 1/3 cup finely diced red bell pepper
- 1/4 cup finely chopped fresh cilantro, for garnish

Preparation

1. Toss scallops with 2 teaspoons tamari (or soy sauce) in a medium bowl. Set aside.
2. Place a large, high-sided skillet with a tight-fitting lid over medium heat. Add 1 tablespoon canola oil and quinoa. Cook, stirring constantly, until the quinoa begins to color, 6 to 8 minutes. Add garlic and cook, stirring, until fragrant, about 1 minute more. Add water and salt and bring to a boil. Stir once, cover and cook over medium heat until the water is absorbed, about 15 minutes. (Do not stir.) Remove from the heat and let stand, covered, for 5 minutes. Stir in snow peas, cover and let stand for 5 minutes more.
3. Meanwhile, whisk 3 tablespoons canola oil, the remaining 2 teaspoons tamari (or soy sauce), vinegar and sesame oil in a large bowl. Add the quinoa and snow peas, scallions and bell pepper; toss to combine.
4. Remove the scallops from the marinade and pat dry. Heat a large skillet over medium-high until hot enough to evaporate a drop of water upon contact. Add the remaining 2 teaspoons canola oil and cook the scallops, turning once, until golden and just firm, about 2 minutes total. Gently stir the scallops into the quinoa salad. Serve garnished with cilantro, if desired.

Documents To Be Returned

Please return the following items to the principal investigator (All other documents may be kept for your records or discarded):

1. Completed questionnaire

2. Completed Daily Food Records (5 consecutive days including one weekend day)

3. Recipes used for foods recorded on the Daily Food Records (optional)

The items listed above must be returned by _____ but you may return them before this date if they are completed.

* If you received your information packet from the participating fitness center, return the documents listed above to the fitness center. Place all the documents in the envelope provided and place it in the secure drop box set up at the fitness center.

* If you received your information packet by mail, return the necessary documents in the prepaid envelope provided. Leave the return address section on the envelope blank.

**** Do not write your name on the envelope or on any of the documents to be returned to the investigator. Make sure your envelope is securely sealed before returning it.**

Contacting the Investigator

If you have any questions or concerns throughout the study you may email the investigator, Julie Devlin at the following address:

glutenfreediet@hotmail.com

Appendix B: Participant Questionnaire

Please read all instructions on the cover sheet before completing this questionnaire.

Demographics/ Anthropometric Data

1. Age in years: _____

2. Gender (circle): M F

3. Weight in pounds: _____

4. Height: _____feet _____ inches

These questions pertain to your past and present dietary habits. If you DO NOT consume a gluten-free diet skip questions 5 through 7.

1. What type of diet do you consume on a regular basis?

(a). Gluten-free

(b). Regular (Includes foods containing gluten)

2. How long have you followed this type of diet?

(a). Less than 6 months; specify length of time: _____

(b). 6-12 months

(c). More than 1 year

3. Have you dieted in the past 5 years? If yes please list specific diets and how long you followed them. If additional space is needed, please attach additional sheets.

Example: Diet: Weight Watchers Length of time: 6 months

Diet: _____ Length of time: _____

Diet: _____ Length of time: _____

Diet: _____ Length of time: _____

Diet: _____ Length of time: _____

4. Do you take dietary supplements? If yes, list the brand name, type of supplement and amount taken per day. If extra space is needed, please attach additional sheets.

Example #1

Brand: Citracal **Type:** Calcium Citrate + D_3 Maximum

Amount per day: 630 mg calcium and 500 mg vitamin D

Example #2

Brand: Optimum Nutrition **Type:** Women's Multivitamin

Amount per day: 2 pills

Brand: _____ Type: _____

Amount per day: _____

Brand: _____ Type: _____

Amount per day: _____

Brand: _____ Type: _____

Amount per day: _____

Brand: _____ Type: _____

Amount per day: _____

5. If you consume a gluten-free diet, why do you choose to follow this type of diet?

(a). Weight loss

(b). I believe it is healthier

(c). Pressure from friends/family

(d). Other (please specify) _____

6. Have you experienced any benefits, drawbacks, or concerns by following a gluten-free diet? If yes, please specify. Otherwise leave blank.

_____

_____

_____

7. How much longer do you plan to follow a gluten-free diet?

(a). Less than 6 months

(b). 6-12 months

(c). More than 1 year

Appendix C: Daily Food Record

Day (circle) SU M T W TH F S Date _____

***Please read all instructions from Appendix A: Instructions for Participation before recording your daily food intake.**

Breakfast	_____ _____ _____
Snack	_____ _____
Lunch	_____ _____ _____
Snack	_____ _____
Dinner	_____ _____ _____
Snack	_____ _____

Appendix D: Estimating Portion Sizes

The most accurate way to determine serving sizes is to measure what you are eating. When that is not possible common household items can be used to estimate your serving size. Reference this form as often as needed to help you estimate your portion sizes.

1 cup = **Used for foods such as fruits, vegetables, pasta**	**Baseball** 
1/2 cup = **Used for foods such as ice cream**	**Racquet ball** 
1/4 cup = **Used for foods such as dried fruit**	**Golf ball** 
3 ounces = **Used for foods such as meats**	**Deck of Cards** 
1 ½ ounces = **Used for foods such as cheese**	**Six Stacked Dice** 
2 Tablespoons = **Used for foods such as peanut butter**	**Marshmallow** 
1 teaspoon = **Used for foods such as margarine**	**One Dice** 

Adapted From:
http://www.eatright.org/Public/content.aspx?id=4294967941&terms=portions#.UJc4YcXhqrg

Appendix E: Sample Nutrition Label

The most accurate way to determine how much you are eating is to measure food items. When measuring is not possible the nutrition label on packaged foods can help you estimate your portion size.

First check the serving size for this food item

Nutrition Facts

Serving Size 1 cup (35g)
Servings Per Container 10

Amount Per Serving	Cereal	Cereal with 1/2 cup Skim Milk
Calories	130	170
Calories from Fat	0	0
	% Daily Value*	
Total Fat 0g*	**0%**	**0%**
Saturated Fat 0g	**0%**	**0%**
Trans Fat 0g	**0%**	**0%**
Cholesterol 0mg	**0%**	**0%**
Sodium 200mg	**8%**	**11%**
Total Carbohydrate 30mg	**10%**	**12%**
Dietary Fiber 4g	**16%**	**16%**
Sugars 18g		
Protein 3g		
Vitamin A	25%	25%
Vitamin C	25%	25%
Calcium	0%	15%
Iron	10%	10%

*Amount in Cereal. One half cup skim milk contributes an additional 40 calories, 65 mg sodium, 6g total carbohydrates (6 g sugars), and 4g protein.

** Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Sat Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Then check the servings per container

How much of the pack did you consume?

Using this nutrition label above as an example; if you consumed half of the package, then you ate 5 cups. Record 5 cups as your serving size on your daily food record in the space provided.

Appendix F: Human Subject's Approval Letter

Dear Julie,

Congratulations! After careful review, your proposal "Nutrient intakes of healthy adults on a gluten free diet" has been accepted by the College of Health and Human Services Human Subjects Review committee.

The current version of your paper is available here:
http://commons.emich.edu/cgi/preview.cgi?article=1099&context=chhs_hs

The reviewers believe this will be a valuable study that will help to understand the effects of adults who only consume gluten free diets compared with those that do not. There is no risk involved to participants. No modification in diet is required.
The tool for reference of food portion is simple and easy to understand.

We stress that you do not stray from your proposed plan. Good luck with your research effort.

Sincerely,

Gretchen Dahl Reeves, PhD
Chair, CHHS_HSRC

Appendix G: Informed consent for participant in dietary analysis research

Purpose of study
The purpose of this research is to evaluate the typical daily nutrient intake of adults that predominantly consume a gluten free diet and compare their intakes on an age and gender matched control group who consume a regular diet that includes foods containing gluten.

Description of study procedures
Following the completion and return of this consent form, you will be contacted through email with instructions on how to proceed with the study. Once you are contacted you may pick up the information packet from the participating fitness center or it will be mailed to your address. Each packet will contain blank food records, a questionnaire, additional resources to help with recording your dietary intake, and instructions for completion.

As a participant, you will be asked to record everything you eat and drink for five consecutive days on the blank forms provided. Please do not alter your eating habits from your normal intake during the course of this study. In addition, you will be asked to complete a brief questionnaire regarding your demographic and anthropometric data as well as your dietary habits. Contact information for the principal investigator will be provided if you have any questions or concerns throughout the study. If you are unable to record your daily intake on the forms provided or fill out the necessary documents please inform the principal investigator prior to starting the study so other arrangements can be made. Once the food record and questionnaire are completed they must be returned to the participating fitness center or mailed by the specified date. At the fitness center a labeled drop box will be available behind the front desk.

Length of participation and withdrawal
Participation in this study will require approximately 15-20 minutes per day of your time over the course of five consecutive days. You may choose any five consecutive days over the course of the period of time specified. Participation is completely voluntary, and you may choose to withdrawal from the study at any time without penalty.

Confidentiality
Please be assured that your confidentiality will be protected throughout and following the study. All of your documents will be kept completely anonymous and no personal information will ever be used to identify you. The email account used for communication with participants and the investigator is solely dedicated for use during the study and the account will be deactivated following study completion. All your documents will be kept in a secure location during the study and destroyed once the study is completed.

Expected risks of participation
There are no expected risks associated with your participation in this study. As a participant you are not altering your eating habits during the course of this study. However, if you do experience any negative effects please contact the investigator.

Benefits of participation
Your participation in this study will help the investigator better understand types of foods consumed and the average amount of nutrients obtained by adults on a gluten-free diet.

Research results
Results of this study will be used in aggregate form and no identifying information will ever be released. The results may be presented at a professional meeting or published in a professional journal, and will be used as part of a Master's thesis.

Questions
If you have any questions regarding the project you may contact the principal investigator, Julie Devlin at glutenfreediet@hotmail.com. This research protocol and informed consent document has been reviewed and approved by the Eastern Michigan University, College of Health and Human Service, Human Subjects Review Committee for use for one year. If you have questions about the approval process, please contact Dr. Gretchen Dahl Reeves (734-487-3236; greeves@emich.edu) Chair CHHS Human Subjects Review Committee.

Consent to participate
I understand my rights as a research participant and voluntarily consent to participate in this study and follow its requirements. Additionally, I understand the purpose, intent, and necessity of the study. I will receive a signed copy of this consent form for future reference.

Signature _____ Date _____

Name (Printed)_____

Appendix H: Abbreviations List

AI- Adequate Intakes

BMI- Body Mass Index

CDC- Centers for Disease Control and Prevention

DNA- Deoxyribonucleic Acid

DRI- Dietary Reference Intakes

g- Grams

IgA- Immunoglobulin A

IgE- Immunoglobulin E

kcal- Kilocalories, Calories

kg- kilograms

kg/m^2- Kilograms per meters squared

mcg- Micrograms

mg- milligrams

MRI- Magnetic Resonance Imaging

RDA- Recommended Dietary Allowances

tTG- Tissue Tranglutaminase

USDA- United States Department of Agriculture