An Analysis of the Dietary Iron

Intakes in Celiac Patients

by

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ABSTRACT

Recent research indicates that 1 out of 133 people living in the United States may have celiac disease (Fasano et al., 2003). As the only known treatment for celiac disease is the removal of gluten from the diet, the nutritional evaluation of gluten-free diets is an important component of the medical nutrition therapy for celiac disease. Three-day food journals were collected from volunteers (N = 13) recruited through celiac support groups in Wisconsin. The food journals were assessed for dietary iron intake as well as for grain servings. Each food journal was evaluated three times to assess the impact of gluten-free versus gluten-based foods as well as the impact of vitamin and mineral supplements on dietary iron intake.

Results indicated that the participants consumed a statistically significant portion of dietary iron from vitamin and mineral supplements. The substitution of gluten-containing foods also contributed a substantial amount of dietary iron for

males and females over 50 years old. The food records of females 19-50 which excluded the vitamin and mineral supplements resulted in a lower mean percent of the dietary reference intake for iron of 63.62% versus 101.43%. While this is not statistically significant, and is comparable with NHANES data, this is of concern. The number of grain servings consumed across all groups was also comparable with values from prior research, with females consuming below the recommended number of grain servings. A moderately-strong positive correlation exists between dietary iron intake and grain consumption, which suggests that an emphasis on the consumption of enriched and fortified gluten-free products or gluten-free whole grains may be beneficial in the nutrition education of celiac patients.

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Chapter I: Introduction

Celiac disease has been recognized by medical professionals since 1888, when Dr. Samuel Gee published a detailed clinical description of celiac disease (Gee, 1888). Gee determined that the physical symptoms he observed were linked to nutritional deficiencies caused by malabsorption in the small intestine. During World War II, the association of celiac disease with the consumption of bread products was established when improvement in the symptoms occurred after bread was eliminated from the diet, and the symptoms returned when bread was restored to the diet (cited in Connon, 2006). Over the past 60 years, further research studies have determined that gluten is the specific protein in bread which triggers the formation of antibodies which attack the villi of the small intestine. Removal of gluten from the diet, by eliminating wheat, rye, and barley products, results in the repair of the villi (Ciacci, Cirillo, Cavallaro, & Mazzacca, 2002). A gluten-free diet is the only known treatment for celiac disease (National Digestive Diseases Information Clearinghouse [NDDIC], 2007).

The villi are responsible for the majority of the absorption of the nutrients from food. Continued exposure to gluten, due either to a lack of diagnosis or a failure to comply with a gluten-free diet, will result in the deterioration and damage of the villi, which will then lead to the nutritional deficiencies first noted by Gee.

Nutrients which are typically absorbed by the small intestine and are impacted by celiac disease include iron, folate, calcium, carbohydrates, lipids, vitamins A, D, E, and K, and other micronutrients including thiamin, riboflavin, and niacin (Zarkadas & Case, 2005). If celiac disease is left undiagnosed or untreated, the deficiencies of these nutrients may lead to malnutrition, anemia, osteoporosis, infertility, congenital malformations, short stature, and intestinal lymphoma or adenocarcinoma (NDDIC, 2007). Prior to the development of these serious complications, some symptoms which may appear include gas, bloating, abdominal pain, chronic diarrhea or constipation, fatigue, anemia, bone or joint pain, delayed growth in children, a failure to thrive in infants, tooth discoloration or loss of tooth enamel, tingling or numbness in the legs, or an itchy, blistering rash called dermatitis herpetiformis (NDDIC, 2007).

Previously, celiac disease was thought to be rare in the United States, primarily affecting children and occurring in only 1:10,000 people (Connon, 2006). Recent improvements in diagnostic techniques and increased awareness of the disease have changed this thinking. Highly sensitive and specific serological tests can now accurately identify the antibodies associated with celiac disease in asymptomatic individuals (Fasano et al., 2003). The discovery of genetic markers indicating a predisposition for celiac disease has also been beneficial in identifying and diagnosing those people at risk for celiac disease (National Institutes of Health [NIH], 2004).

Using these new diagnostic techniques and genetic testing, a large multicenter study conducted over five years concluded that the prevalence of celiac disease in subjects previously considered not-at-risk is 1:133, the prevalence for symptomatic subjects is 1:56, the prevalence for subjects with second-degree relatives previously diagnosed is 1:39, and the prevalence for subjects with first-degree relatives previously diagnosed is 1:22 (Fasano et al., 2003). It is now estimated that 2 million people in the United States may have celiac disease (NDDIC, 2007). According to an expert in celiac diagnosis and prevalence (Fasano, 2006, p. xxiv), "celiac disease is now becoming a problem of the masses rather than the uncommon disease of a few."

As more people are diagnosed with celiac disease, more attention is being focused on the only treatment available: the adoption of a gluten-free diet or lifestyle. The relatively low cost of wheat and the historical importance of wheat in the American diet has led to the presence of this versatile grain in a wide range of foods and other products. Wheat is not only found in staple foods, such as breads and pasta, but it is also used as a thickener in sauces, and as a filler in medications and vitamins. Wheat can also be found in the glue of envelopes, and in cosmetics. The Food Allergen Labeling and Consumer Protection Act of 2004 mandates that any product containing wheat must be labeled as such as of January 1, 2006 (United States Food and Drug Administration, 2004a). While this will aid in identifying wheat-free products, it does not always ensure that the product is gluten-free as the Act does not require the identification of rye and barley as allergens (Korn, 2006). The Food and Drug Administration (FDA) is making headway in addressing this problem through the development of a voluntary labeling program of gluten-free products scheduled for implementation by August, 2008 (United States Food and Drug Administration, 2007).

Dietary counseling has traditionally focused on teaching patients to identify and avoid products containing gluten, and correcting any nutrient deficiencies identified at the time of diagnosis (Thompson, Dennis, Higgins, Lee, & Sharrett, 2005). Naturally gluten-free foods include fresh meats, fruits, vegetables, and most dairy products. These foods are considered to be healthy options which contain many necessary nutrients, but recent studies of gluten-free diets have indicated that this lifestyle may be contributing to nutritional deficiencies (See & Murray, 2006). Calcium and vitamin D deficiencies have been linked to lactose intolerance, which is often a result of an inability of the intestinal villi, damaged by celiac disease, to produce the enzyme necessary to digest dairy products. Traditional wheat breads and cereals are enriched and fortified to provide a significant source of vitamins and minerals in the average American diet (Thompson, 1999). However, many gluten-free products are lower in iron, folate, fiber, thiamin, riboflavin, and niacin when compared with similar products made with wheat (Thompson, 1999, 2000). With the increase in the diagnosis and prevalence of celiac disease, these dietary deficiencies will become increasingly important in providing dietary counseling for celiac patients living a gluten-free lifestyle.

Statement of the Problem

The purpose of this study was to determine whether adults who have been diagnosed with celiac disease, and who are living a gluten-free lifestyle, are deficient in the dietary intake of iron which would normally be ingested in gluten-based, fortified, and enriched cereals, breads, and pastas. During the Winter and Spring of 2008, three-day food journals were collected from volunteers within Wisconsin who participated in celiac support groups, attended a celiac educational event, or responded to a notice posted in the Menomonie Market Food Co-op.

Research Objectives

1. To analyze the dietary intakes of adults with celiac disease who have adopted a gluten-free lifestyle for a minimum of twelve months, to determine if adequate amounts of dietary iron are being consumed according to the Dietary Reference Intakes established by the Food and Nutrition Board.

2. To determine whether the consumption of gluten-free products by celiac patients results in decreased dietary iron intakes compared with consuming similar gluten-based products.
3. To compare the number of servings of grains consumed by adults with celiac disease with the number of servings of grains recommended by the United States Department of Agriculture Food Guide Pyramid.

Definition of Terms

The following words are defined in order to lend clarity to the content of this study:

Atypical presentation. Few or no gastrointestinal symptoms are exhibited in this form of celiac disease, but extraintestinal symptoms such as osteoporosis or iron-deficiency anemia may be presenting symptoms (NIH, 2004).

Autoimmune disorder. A condition or disease resulting from the body's defensive response to a substance (Korn, 2006).

Body mass index (BMI). "A mathematical formula that correlates with body fat and is expressed as weight in kilograms divided by height in meters squared" (Mahan & Escott-Stump, 2008, p. 532).

Celiac disease (CD). An inherited, multi-system autoimmune disorder triggered by the ingestion of gluten. It primarily affects the gastrointestinal tract by damaging the intestinal villi, which interferes with the digestion and absorption of nutrients from food (NDDIC, 2007).

Celiacs. People who have been medically diagnosed with celiac disease.

Classic presentation. Of the forms of celiac disease, this is the most commonly described form. Presenting symptoms occur as a result of fully-developed villous atrophy and include diarrhea and abdominal distention in adults, and diarrhea, vomiting, and failure-to-thrive in children (Green and Cellier, 2007).

Dietary Reference Intakes (DRI). A set of values established by the Food and Nutrition Board, in partnership with Health Canada, which identify acceptable levels of nutrient intakes (Institute of Medicine, 2005).

Enriched. The replacement of vitamins and minerals lost during the processing of food products (United States Food and Drug Administration, 2004b).

Fortified. The addition of vitamins, minerals, or amino acids to processed foods to increase the intake of nutrients that may be lacking in the diet (United States Food and Drug Administration, 2004b).

Gluten. Technically a protein found only in wheat, it gives wheat products an elastic quality. However, the use of the word *gluten* is universally accepted as a collective term to describe a group of storage proteins found in wheat, rye, barley, and their derivatives (Korn, 2006).

Gluten-free. Foods which do not contain any wheat, rye, barley, or related grain products.

Intestinal biopsy. The extraction of tissue samples of the villi from the small intestine. Samples are retrieved by threading a thin tube through the mouth, past the stomach, and into the small intestine. This test confirms a diagnosis of celiac disease following positive serological screening tests.

Iron-deficiency anemia. A hematologic condition diagnosed by the presence of low hemoglobin levels, low serum ferritin levels, and high total iron binding capacity (TIBC) levels (Cook, 2005).

Osteoporosis. "A condition in which the bones become weak, brittle, and [...] prone to breaking. Poor calcium absorption contributes to osteoporosis" (NDDIC, 2007, p. 7).

Serologic screening tests. Blood tests which are performed to identify specific antibodies produced by the body in response to the ingestion of gluten (Korn, 2006).

Serum ferritin. Measurement of the iron storage protein, ferritin, in the blood plasma which is used to estimate the amount of iron present in the bodies iron storage areas such as the liver, spleen, and marrow (Mahan & Escott-Stump, 2008).

Silent presentation. Celiac patients who have positive serologic tests and the presence of villous atrophy, but who have no presenting physical symptoms (NIH, 2004).

Villi. Finger-like projections in the small intestine which are responsible for the absorption of nutrients from foods (NDDIC, 2007).

Villous atrophy. The progressive deterioration or destruction of the villi of the small intestine caused by the antibodies produced in reaction to gluten (Zarkadas & Case, 2005).

Assumptions and Limitations

In the development of this study, two assumptions were recognized. First, it was assumed that members of celiac support groups within Wisconsin would be willing to volunteer as participants. This assumption was based on prior studies of gluten-free lifestyles which recruited members of celiac support groups in other areas of the United

States. The second assumption was that some of the participants would be more diligent in following the restrictions of their diet while participating in the study than they would be on a normal basis. Humans are inherently eager to please and willing to do what they perceive the researcher wishes.

Several limitations of this study were evident. In order to enroll participants with minimal food restrictions due to intestinal damage, only those adults who had been following a gluten-free diet for a minimum of twelve months were recruited for participation. Participants were also required to have had a diagnosis confirmed by intestinal biopsy as this provides an indisputable diagnosis of celiac disease. These requirements limited the pool of participants.

Although food journals are considered an appropriate instrument for the collection of dietary intake, participants may not have accurately or honestly recorded their intakes. In addition, many celiacs prepare their food from scratch in order to maintain a gluten-free diet. Any recipes the individuals used were provided to the researcher when the food journal was submitted so that the nutritional value of each dish could be calculated. Participants were also asked to include labels or photocopies of labels of manufactured gluten-free products as well as any vitamins which they consumed. These limitations may have impacted the accuracy of the nutrient analysis and may have deterred celiac patients from participating in the study.

Chapter II: Literature Review

Introduction

Included in this chapter are five main sections pertaining to the maintenance of a gluten-free diet in the management of celiac disease. First, the availability of gluten-free products is reviewed and is followed by an analysis of the development and acceptability of gluten-free products. Next, the nutritional quality of gluten-free manufactured products is examined. The fourth section focuses on nutritional deficiencies that are related to maintaining a gluten-free diet. The final section reviews the relationship of iron-deficiency and celiac disease.

Gluten-Free Product Availability

Historically, the availability of gluten-free breads, cereals, and baked goods has been limited (Zarkadas et al., 2006). In the past decade, however, the availability of gluten-free foods has increased dramatically (Korn, 2006). This follows reported increases in the development and availability of gluten-free products (Case, 2001). Food manufacturers have responded to an increased demand for gluten-free products brought about by the increased awareness and diagnosis of celiac disease (Zhang, 2005). Gluten-free foods, previously available only at specialty stores, by mail order, or through the internet, are now appearing on the shelves of mainstream supermarkets.

One company in the United States which produces gluten-free products in a dedicated facility is Whole Foods Market. With over 200 retail stores in the United States, they manufacture and market more than 25 gluten-free products (*Whole Foods Bakehouse*, n.d.). Within 10 months of opening their bakehouse in 2004, the demand for the products required them to increase their production staff by 400%.

Manufacturers such as Kinnikinnick Foods have expanded their facilities from 500 square feet in 1991 to 18,000 square feet in 2001 (*Company History*, n.d.). They have also increased their distribution from one retail store in Canada to 890 retail partners in the United States and 390 retail partners in Canada. Their production facilities are also entirely gluten-free to avoid cross-contamination.

Companies such as Kinnikinnick Foods, Bob's Red Mill, Glutino, Gluten-Free Pantry, Ener-G Foods, and Enjoy Life Natural Brands sell their products over the internet and also supply supermarkets. In June 2005, the market research firm SPINS Inc. reported that the sales of gluten-free products rose 18% over the prior 52 weeks (cited in Zhang, 2005). It also reported that approximately 2,000 gluten-free products were being marketed with annual sales exceeding $600 million.

In spite of the research and development and increase in production of gluten-free foods, a recent survey reports that 83% of the respondents had difficulty in finding gluten-free foods and that this has contributed to a reduction in their quality of life (Zarkadas et al., 2006). This may be due, in part, to the higher cost of gluten-free products in comparison to their gluten-containing counter parts (Zarkadas & Case, 2005). Although celiac patients may find cooking or baking from scratch a more economical alternative to purchasing prepared gluten-free products, they may find it more difficult to prepare a quality baked product.

Gluten-Free Product Development and Acceptability

In discussing the etiology of celiac disease, the word "gluten" refers to a group of ethanol-soluble prolamins which are found in wheat, rye, and barley. The specific proteins contained in these grains are gliadin, secalin, and hordein, respectively

(Thompson, 2006). Gluten is technically the combination of the various gliadin and glutenin protein fractions in wheat (Wieser, 2007). However, the term "gluten" has come to represent the group of proteins which trigger the gastrointestinal autoimmune response which characterizes celiac disease (Thompson, 2006).

Gluten forms a complex protein network which provides the structure in baked goods (Wieser, 2007). It determines the ability of the dough to absorb and retain water and provides the elastic, cohesive, and viscous properties of the dough. The lack of this unique protein network in gluten-free products prevents the carbon-dioxide released during yeast-leavened baking from being trapped, resulting in a shorter, more dense product (Gujral, Guardiola, Carbonell, & Rosell, 2003). The lack of elasticity and cohesiveness also leads to dry, crumbly products (Gallagher, Gormley, & Arendt, 2002). In a 2002 study published by Arendt, O'Brien, Schober, Gormley, and Gallagher, many commercially-available gluten-free products were found to have poor mouthfeel and flavor (cited in Gallagher, Gormley, & Arendt, 2004).

In a recent survey of celiac patients, 85% indicated they were unable to find gluten-free foods of acceptable quality (Zarakadas, et al., 2006). In an effort to improve the quality of life for celiac patients, recent research has focused on improving the acceptability of gluten-free cereals, breads, and baked goods. Many additives have been tested in attempts to replace the protein structure provided by gluten. Commercial products were originally unavailable and research published by Rotsch in 1954 noted that gluten-free dough had the consistency of batter, and produced breads with a crumbly texture and poor color (cited in Gallagher et al., 2004). Similar issues have arisen in the development of gluten-free pastas. The lack of the protein structure often causes gluten-free pasta to disintegrate during cooking (Gallagher et al., 2004). As cookies and biscuits are not as dependent on the strength of the gluten network, the development of gluten-free versions of these products has not been as problematic (Gallagher et al., 2004).

An early attempt to formulate a gluten-free loaf of bread used cellulose gum (now known as carboxymethylcellulose or CMC) to improve the properties of a potato bread (McGreer, 1967). Since then, a variety of additives have been tested in the development of gluten-free products made with rice flour, corn flour, potato flour, or a combination of these flours. Additives tested include starches, gums and hydrocolloids, prebiotics, and other non-gluten proteins. Each of the additives seems to improve some, but not all, of the sensory characteristics or properties of gluten-free products, depending on the type of product being tested. Various combinations of additives have also been used to maximize the quality of the products.

The most commonly tested groups of additives are starches and hydrocolloids. The addition of starches alone has not produced optimal results and requires the addition of hydrocolloids (Gallagher et al., 2004). Starches which are commonly used include rice, corn, potato, cassava, and tapioca. Hydrocolloids such as xanthan gum, pectin, hydroxypropylmethylcellulose (HPMC), carboxymethylcellulose (CMC), β-glucan, agar, agarose, carageenan, psyllium gum, and guar gum have all been tested to improve the quality of gluten-free baked goods. Their ability to mimic the hydrophilic nature of gluten and offset the hydrophobic nature of rice flour makes them ideal candidates for research (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007).

In a 2005 study which compared the staling characteristics of rice flour gluten-free bread to three different wheat breads, HPMC and xanthan gum were added to the gluten-free bread to improve the bread's properties (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005). While the gluten-free bread scored the lowest in yeast aroma and flavor, it was found to retain the highest level of moistness and had the lowest level of staling—one of the most common problems reported with gluten-free breads. The gluten-free bread was found to have a similar structure to the wheat bread control and also suffered the least amount of structural degradation after the storage period.

The rheological properties of gluten-free dough and the quality of the bread made from the dough were examined in a recent study (Lazaridou et al., 2007). The rice flour and corn starch base was augmented separately with two different levels of pectin, CMC, agarose, xanthan gum, and oat β-glucan. Examination of the dough showed that the sample prepared with xanthan gum had the highest viscosity and elasticity, followed by CMC, pectin, agarose, and oat β-glucan. This was inconsistent with the sensory evaluation of the breads by untrained panelists. This study concluded that the increased loaf volumes of CMC and pectin and their corresponding higher consumer acceptability made these hydrocolloids more desirable in the formulation of gluten-free breads.

The addition of oat β-glucan was in response to the identified inadequate fiber intake by celiac patients following a gluten-free diet (Thompson, 2000). In another study which addressed the low nutritional status of celiac patients, different prebiotics were added to a base of corn and potato starch (Korus, Grzelak, Achremowicz, & Sabat, 2006). Although inulin has been found to improve the loaf volume and crust quality of bread (Gallagher et al., 2004), the hydrocolloids pectin and guar gum were added to further improve the acceptability of the bread. The inulin-containing prebiotics tested were Frutafit inulin, bitter-free chicory flour, and fructooligosaccharide (FOS) syrup. Sensory evaluation by trained panelists found the chicory flour and inulin formulations to be preferable to the FOS formulation based on appearance, taste, aroma, crust, and crumb qualities. Chemical evaluation of the breads determined that the chicory flour formulation retained the highest level of inulin in the final bread products. In addition to increasing the fiber content in diets, Abrams and Courdray found that the inclusion of the prebiotic inulin into the diets of celiac patients is helpful in improving the absorption of various nutrients such as calcium (cited in Korus et al., 2006).

The ability of non-gluten proteins to improve the quality of gluten-free foods has also been investigated. Improvements in flavor, texture, and staling were found when dairy proteins were added to gluten-free bread formulas (Gallagher et al., 2004). However, since lactose intolerance is common in celiac patients (See & Murray, 2006), use of dairy proteins is not practical for use in gluten-free product formulations. The addition of surimi to gluten-free products has also produced improvements in volume, crust, and crumb properties but has not been accepted as a product additive (Gallagher et al., 2004). One other protein which has been studied is the addition of cyclodextrin glycosyl transferase (CGTase) to rice bread (Gujral et al., 2003). This enzyme catalyzes four reactions during bread production. By-products of these reactions include cyclodextrins which are able to reduce the hydrophobic nature of the rice flour and increase its solubility. The result is a more favorable dough and bread loaves with increased volume and soft crumb texture.

Nutritional Quality of Gluten-Free Manufactured Products

In the United States, enriched grain products such as cereals and breads constitute a significant source of iron, dietary fiber, and B vitamins (See & Murray, 2006). In the most recent analysis of the sources of nutrients, it was found that adults in the United States obtain over 43% of their dietary iron, over 41% of their thiamin, over 30% of their fiber, over 29% of their niacin, over 28% of their riboflavin, and over 25% of their folate from products which are typically wheat-based (Cotton, Subar, Friday, & Cook, 2004). Since adhering to a gluten-free diet requires the elimination of all wheat-containing products, celiac patients may be at-risk for deficiencies of these nutrients.

Although a gluten-free diet includes naturally gluten-free foods such as meats, fruits, vegetables, dairy products, corn, and rice, the typical gluten-free diet is lacking in grains (See & Murray, 2006). In one study, the thiamin, riboflavin, and niacin levels of gluten-free flours, breads, cereals and baking mixes were evaluated (Thompson, 1999). Of the 368 gluten-free products assessed for enrichment status, only 35 of these flours, breads, pastas, and cereals were determined to be enriched with niacin, riboflavin, and thiamin. In the United States, manufacturers are not currently required to provide the nutritional values of these three B vitamins unless the product has been enriched, which limited the scope of this study.

In a similar follow-up study, Thompson (2000) analyzed the iron, folate, and dietary fiber contents of gluten-free products. This research was more comprehensive due to the required inclusion of these three nutrients on all nutrition labels in the United States. The results were similar to the prior study, indicating that gluten-free cereals, breads, and pastas do not contain as much folate and iron as their refined, enriched, and fortified wheat-based counterparts. The study did find, however, that gluten-free products may contain more dietary fiber than the wheat-based products.

Many more companies are now producing more gluten-free products as the market for the products has increased with the rise in the number of diagnoses (Korn, 2006). It has been reported that some companies which produce gluten-free products are enriching and fortifying their products, although the nature of the gluten-free dough does not readily lend itself to the enrichment process, becoming even less elastic and cohesive, resulting in a more crumbly texture (Korus et al., 2006). Other companies are using naturally gluten-free grains which are already good sources of these B vitamins and iron (Zarkadas & Case, 2005). These grains include, but are not limited to, amaranth, quinoa, flax, buckwheat, millet, and wild rice. No additional published studies have reported an updated comparison of the new products.

Nutritional Deficiencies in Gluten-Free Celiacs

Traditionally, education regarding the gluten-free diet in the treatment of celiac disease has focused on identifying and avoiding foods which contain gluten and identifying foods which do not contain gluten and which can therefore be consumed (Thompson et al., 2005). Limited research has been conducted to evaluate the nutritional quality of the dietary intake of those celiacs following a gluten-free lifestyle. The results of studies which have been conducted indicate that dietary deficiencies do exist for this population.

In the earliest research reviewed, Sabry and Okada (1992) examined the nutrient intakes of 26 gluten-free celiacs from Canada. The intakes of the study population were compared with the average intakes of Ontario residents previously studied. Of the nutrients evaluated, the intakes of women following a gluten-free diet were found to be lower in calcium, iron, thiamin, and riboflavin than the intakes of women in the comparison group. The study concluded that the inadequate intake of calcium was a result of the high prevalence of lactose intolerance in this group. Due to the lack of calcium intake, concerns were raised regarding the possible inadequacy of vitamin D intake. In Canada and the United States, fortified milk is the primary source of vitamin D in the diet. Probability analysis performed on the data collected revealed that 27% of the participants "were at risk of dietary inadequacy of calcium" (Sabry & Okada, 1992).

Probability analysis also indicated that all participants had a 12% risk of dietary inadequacy of iron, a 13% risk of dietary inadequacy of thiamin, and a 9% risk of dietary inadequacy of riboflavin. The lack of dietary fiber, thiamin, and iron were attributed to two factors. First, gluten-free products were identified as having lower levels of these nutrients than grain products consumed by the general adult population. In addition, some of the female participants had low caloric intakes which would lead to lower intakes of these nutrients (Sabry & Okada, 1992).

A small, but comprehensive study performed in Sweden evaluated the vitamin nutrition status of 30 adults who had been following a gluten-free lifestyle for eight to twelve years (Hallert, et al., 2002). No other studies have examined such long-term effects of a gluten-free diet on nutritional status. As determined by intestinal biopsy and serum zinc levels, all of the subjects had healthy upper intestinal tracts. This eliminated intestinal malabsorption as an influence on serological vitamin status and indicated compliance with a gluten-free diet.

Dietary food records and biochemical data were obtained from the subjects (Hallert et al., 2002). Analysis of the dietary intakes of the subjects indicated that vitamin B_{12} and folate intakes were below the intakes of the control group. Serological tests revealed low plasma levels of folate and vitamin B_6. The lower levels of B_6, B_{12}, and folate all support the elevated plasma homocysteine levels which were also found in the serological tests. This study also raised a concern that the elevated homocysteine levels found may be indicative of increased risks for cardiovascular disease, hypercholesterolemia, and hypertension, which were found in a 1999 study conducted by Nygård, Vollset, Refsum, Brattström, & Ueland (cited in Hallert et al., 2002).

A long-term study conducted in Ireland assessed the dietary compliance, anthropometric measurements, hematological samples, bone mineral densities, and the quality of life of 50 adult celiacs who had been diagnosed as children approximately 28 years earlier (O'Leary et al., 2004). A clinical nutritionist determined that 50% of the subjects were fully compliant with a gluten-free diet, 18% were partially compliant, and 32% were non-compliant. All of the anthropometric values were within expected values based on the age and gender of the subjects. The only biochemical abnormality the researchers identified was low serum ferritin levels in 83% of the females and 21% of the males, indicating iron-deficiency. Thirty-two percent of the subjects were found to have sub-normal bone mineral densities. Of these, 28.9% were found to have osteopenia and 2.6% were found to have osteoporosis. The researchers concluded that non-compliance to a gluten-free diet was a major contributing factor for both the incidence of iron-deficiency and low bone mineral density.

Osteoporosis due to calcium malabsorption is a well-established consequence of undiagnosed and untreated celiac disease (Zarkadas & Case, 2005). Once a gluten-free diet is followed, the villi of the intestine heal and the ability of the body to absorb nutrients increases. This typically occurs between six months and two years after the elimination of gluten from the diet (NDDIC, 2007). In a recent study (Pazianas et al., 2005) the absorption of calcium and corresponding bone mineral density were studied in a group of adult celiac females who had been following a gluten-free diet for over four years. A control group of age- and sex-matched females was used for comparison.

Dietary intakes of calcium, serum calcium, serum vitamin D, and bone mineral density tests were used to evaluate and compare the two groups (Pazianas et al., 2005). The intakes of calcium were found to be similar. Similar levels of serum calcium and vitamin D indicated that the absorption of both groups was similar and that any prior malabsorption in the celiac subjects had been repaired. However, the lower bone mineral densities in celiac subjects for all but one of the four tests indicated that women with celiac disease have significant bone demineralization even after lengthy adherence to a gluten-free diet. Although the subjects were not deficient in vitamin D intake, the researchers concluded that vitamin D therapy may be beneficial for women with celiac disease to promote bone mineralization and prevent osteoporosis (Pazianas et al., 2005).

Only one published study has been conducted evaluating the nutritional adequacy of gluten-free diets in the United States (Thompson et al., 2005). This study was conducted in response to concerns which arose as a response to the lack of enriched and fortified gluten-free grain products. Self-reported food records were analyzed for the adequacy of calories, carbohydrates, dietary fiber, iron, calcium, and servings of grains.

Comparisons were made with the United States Department of Agriculture's Food Guide Pyramid and with data collected from the United States National Health and Nutrition Examination Survey (NHANES).

Nutrient intake was examined by gender (Thompson et al., 2005). Males had adequate intake in all of the nutrients except for dietary fiber and calcium. Females were found to have adequate intake in only carbohydrates and calories. Among the females, dietary fiber intake was below recommended levels in 54% of the subjects, iron intake was below recommended levels in 56% of the subjects, calcium intake was below recommended levels in 69% of the subjects, and the intake of grain foods was below recommended levels in 79% of the subjects.

The level of calcium intake was of particular concern to the researchers due to the incidence of osteoporosis among celiacs (Thompson et al., 2005). In a 2001 study of treated and untreated adult celiacs conducted by Meyer et al. (cited in Thompson et al., 2005), only 28% of the subjects had bone mineral densities which were considered normal. As a result of the dietary study in the United States, researchers concluded that comprehensive dietary education for celiacs must include an emphasis on the nutritional quality of the diet as opposed to focusing on the foods to avoid or include in the gluten-free diet (Thompson et al., 2005).

Iron Deficiency in Celiac Disease

Classic symptoms of celiac disease include gastrointestinal issues such as diarrhea, constipation, abdominal pain, abdominal bloating, nausea, vomiting, and lactose intolerance (Murray, Watson, Clearman, & Mitros, 2004). However, atypical, extraintestinal symptoms may now account for more diagnoses than classic symptoms

(NIH, 2004). Among adults, these atypical symptoms may include iron-deficiency anemia, infertility, recurrent fetal loss, osteoporosis, vitamin deficiencies, and fatigue. An Italian study which examined the clinical presentations of 1,026 subclinical (atypical) and silent celiacs found that 39% of the patients had iron-deficiency anemia as the symptom leading to diagnosis (Bottaro, Cataldo, Rotolo, Spina, & Corazza, 1999). Similar results were found in a more recent study conducted in the United States in which approximately 24% of the subjects were diagnosed as iron-deficient at the time of their diagnosis (Harper, Holleran, Ramakrishnan, Bhagat, & Green, 2007). The high prevalence of iron-deficiency and iron-deficiency anemia as presenting symptoms has resulted in several studies investigating the relationship between iron status and celiac disease.

Early research determined the effect of a gluten-free diet on the restoration of biochemical iron levels in the body (Souroujon, Ashkenazi, Lupo, Levin, & Hegesh, 1982). Conducted in Israel, this study examined 116 biopsy-proven pediatric celiac patients whose ages ranged from six months to 20 years. This study was unique as the subjects were placed on a gluten-free diet and then removed from the diet to determine the changes in biological iron status as measured by serum ferritin, hemoglobin, serum iron, total iron binding capacity, erythrocyte mean corpuscular volume, and transferrin saturation. Histological samples of the small intestine were also examined and correlated with the iron values. In reviewing the biochemical values, the researchers concluded that serum ferritin correlated most closely with intestinal recovery in response to a gluten-free diet and villous atrophy resulting from the reintroduction of a normal diet. Recovery of serum ferritin levels averaged 1 μg/L/month while the levels dropped approximately four times faster (4 μg/L/month) in response to a normal diet and returned to below-normal, indicating iron-deficiency. Once the subjects were returned to a gluten-free diet, serum ferritin values returned to a low-normal range for this pediatric population within six to 12 months.

A small study conducted in Italy examined the histological and biochemical changes in 26 adult celiac patients for an average of 55 months after instituting a gluten-free diet (Bardella et al., 1985). To determine their iron status, both hemoglobin and serum iron were evaluated. During the course of the study, five patients (19%) were intermittently diagnosed with iron-deficiency anemia as well as other abnormal biochemical values. These abnormal lab values correlated with some degree of villous atrophy. While all of the subjects reported compliance with a gluten-free diet, the researchers concluded that, due to these abnormal biochemical values and the minimal improvement in villous atrophy, the subjects' results were due to poor dietary compliance.

Further correlation between iron status and villous atrophy was found in a Finnish study which examined 40 newly diagnosed adult patients (Kemppainen et al., 1998). Histological, biochemical, and anthropometric values were collected and reviewed. After one year following a gluten-free diet, patients showed improvement in most of the initial abnormal lab values. Of the 29 patients who still had partial villous atrophy, three patients still had low erythrocyte folate levels, five patients had low serum vitamin A, one had low serum vitamin B_{12}, one had low serum protein, seven had low hemoglobin, five had low serum ferritin, 15 had low serum iron, and 10 had low serum zinc. Of the biochemical values examined, only the serum ferritin and erythrocyte folate levels correlated with the patients' degree of villous atrophy and recovery. This confirmed findings from the earlier study by Souroujon et al. (1982).

Annibale et al. (2001) specifically examined the relationship of a gluten-free diet on adult celiac patients' recovery from iron-deficiency anemia in a prospective study conducted in Italy. Twenty newly diagnosed celiac patients who presented with iron-deficiency anemia completed the study. Histological samples were collected at diagnosis and at six months to determine improvements in villous atrophy. Biochemical values were obtained at diagnosis and also at six, 12, and 24 months. Hemoglobin, mean corpuscular volume, serum iron, and serum ferritin were used to determine the iron status of each patient. No supplemental iron was given to these patients during the 24 month period.

Improvements in iron status were detected throughout the study, and at 24 months; only one patient had not recovered from iron-deficiency anemia, although 45% of the subjects were still considered iron-deficient as evidenced by low serum ferritin levels. While 77.8% of the patients recovered from anemia within six months due solely to the intestinal recovery which resulted from following a gluten-free diet, the authors concluded that iron supplementation may be necessary after six months to fully restore the iron status of celiac patients. Barisani, Ceroni, Del Bianco, Meneveri, and Bardella (2004) previously identified insufficient oral iron intake, decreased absorptive surface, and blood loss as factors which influence celiac patients' low iron status.

Recently, a cohort study was conducted which examined the serum ferritin levels of 405 celiac patients (Harper et al., 2007). Initial ferritin levels were collected within three months of diagnosis and again after following a gluten-free diet for one year.

The initial serum ferritin levels were compared to an age- and gender-matched cohort population obtained from NHANES III data. Approximately 70% of the celiac patients had ferritin levels below the control population, although only 24% were diagnosed with iron-deficiency anemia. Interestingly, 12-13% of the anemic subjects had serum ferritin levels which were higher than the control group. The authors indicated that as an acute-phase reactant, serum ferritin may rise as a reaction to chronic inflammation of the gastrointestinal tract. The serum ferritin levels of these subjects did decrease in response to a gluten-free diet, while the low ferritin levels increased in relation to intestinal recovery as shown in prior studies (Barisani et al., 2004; Kemppainen et al., 1998; Souroujon et al., 1982).

Chapter III: Methodology

The purpose of this study was to determine whether adults who have been diagnosed with celiac disease, and who are living a gluten-free lifestyle, are consuming adequate dietary intakes of iron which would normally be ingested in gluten-based, fortified, and enriched cereals, breads, and pastas. During the Winter and Spring of 2008, three-day food journals were collected from volunteers within Wisconsin who participated in celiac support groups, attended celiac educational events, or responded to a notice posted in the Menomonie Market Food Co-op. Included in this chapter is information detailing subject selection and description, instrumentation, data collection procedures and analysis, and limitations.

Subject Selection and Description

All participants in this study were 18 years of age or older. A self-reported biopsy-proven diagnosis of celiac disease was required for participation, as was adherence to a gluten-free lifestyle for a minimum of twelve months prior to the beginning of the study. Female participants could not be pregnant or lactating.

Participants were recruited within Wisconsin from celiac support groups, a celiac educational event, or in response to a notice posted in the Menomonie Market Food Co-op. Four celiac support groups were contacted, three of which allowed recruitment at their meetings. Meetings with the volunteers from the Madison support group were scheduled according to their availability. The support group at Luther Midelfort Chippewa Valley, in Bloomer, Wisconsin, required approval from the Luther Midelfort Institutional Review Board (IRB) prior to the support group meeting. Appropriate documentation was submitted and approval was obtained. Recruitment at the Eau Claire, Hudson, and Madison, Wisconsin; support groups did not require IRB approval other than the approval obtained from the UW-Stout IRB. Approval for recruitment at the Menomonie Market Food Co-op educational event and through posting of a notice at the facility was granted by the marketing manager.

Notification of recruitment at the support group meetings was given through the available support group newsletters. At the educational event, the marketing manager relayed the researcher's purpose and invited those attendees interested to contact the researcher following the event. Those interested in participating met with the researcher following the meetings, or the education session, and were provided with a consent form for their review (Appendix A). The form provided a description of the study, clarified the risks and benefits of participation, outlined the time commitment required, explained how confidentiality would be maintained, and stated the participant's right to withdraw from the study at any time.

Instrumentation

Subjects completed a brief questionnaire designed by the researcher (Appendix B). This questionnaire was used to assess eligibility and also to provide the researcher with data used to determine each participant's nutritional requirements. Included were questions regarding the participant's diagnosis of celiac disease, gender, pregnancy status, lactation status, and age group. Other nutritional complications were noted, as was the participant's consumption of a daily multivitamin.

The food journal forms were provided by the Mayo Clinic in Rochester, Minnesota (Appendices C and D), and were also reviewed with each participant. Labels with specific instructions pertaining to this study were applied to the front covers. A sample of a daily food record which was designed by the researcher to reflect a gluten-free diet was also provided to each participant (Appendix E). In addition, recipe forms designed by the researcher were supplied (Appendix F). These forms allowed for a more accurate nutritional assessment of foods prepared in the home.

Data Collection Procedures

In November, 2007, approval to begin this study was granted by the University of Wisconsin-Stout Institutional Review Board. During the late Winter and Spring of 2008, participants were recruited from the Bloomer celiac support group, the Eau Claire celiac support group, a gluten-free lunch-and-learn educational session sponsored by the Menomonie Food Market Co-op, and through a notice posted at the Menomonie Food Market Co-op (Appendix G).

Detailed instructions on the completion of the food journal were provided to, and reviewed with, each participant (Appendix H) following the completion of a consent form and questionnaire. Any questions or concerns raised by the participants were addressed by the researcher.

Participants were given a packet of information which included detailed instructions, a sample food record reflecting a gluten-free diet, a food record booklet, recipe forms, and a pre-addressed, postage-paid envelope for return of the materials. Participants completed a three-day food journal within the month following initial consent. The journal included three consecutive days, with at least one of those days being a weekend day. Nutrition labels or copies of the labels were also included by the participants for any gluten-free foods and multi-vitamins consumed. Inclusion of the labels facilitated the entry of manufactured gluten-free products into the nutrient database and ensured a higher level of accuracy. Height, weight, and age were self-reported and were collected following the return of the materials to the researcher.

Data Analysis

All three-day food records were analyzed for adequate intake according to the DRI values using the Food Processor SQL edition, version 9.9, software program for adequate intake according to the DRI values. Next, each food record was analyzed a second time. In this analysis, all vitamin and mineral supplements were removed. A third analysis was then performed in which any manufactured gluten-free foods were replaced with similar wheat-based products. These three analyses provided three food records for each participant which is referred to as "food record version" in the subsequent statistical analysis. The original food record has been designated the "actual" version, the second version (without vitamin or mineral supplements) has been designated the "no-vitamin" version, and the final version has been designated the "gluten-substitution" version.

Participants were grouped according to the size of the community in which they lived. Participants who resided in communities with populations under 100,000 were considered living in "small" locations, while participants who resided in communities with populations over 100,000 were considered to be living in "large" locations.

Due to varying DRI values based on age and gender, these two parameters were also used to classify the participants. The Institute of Medicine (2005) recommends that women between 19 and 50 years of age consume 18 milligrams of dietary iron per day. Therefore, all participants were classified as either "19-50" or "over-50".

The statistical analysis for this study was computed using SAS Release 9.1.3 (SAS Institute Inc., 2002-2003), Cary, North Carolina by Vickie King, PhD, Associate Director of VMRD-Biometrics at Pfizer, Incorporated. Differences of the DRI percentage of dietary iron intake and the number of grain servings by location were compared using a general linear mixed model with the fixed effect of location and the random effect of residual (Littell, Stroup, & Freund, 2002; Milliken & Johnson, 1984; Steel & Torrie, 1980). Least squares means and standard errors were calculated for each location.

Percentage of iron intake and the number of servings of grains were also analyzed using a repeated measures mixed model of variance (Littell, Stroup, & Freund, 2002; Milliken & Johnson, 1984; Steel & Torrie, 1980). The fixed effects in the model were gender, age group, food record version and gender by age group by food record version interaction. The random effects in the model were participant and residual. Contrasts were used to do comparisons of interest. Least squares means, standard errors, and 95% confidence intervals were calculated for each gender, age group, and food record version combination.

Pearson correlation coefficients were calculated between percentage iron intake and the number of servings of grains for each food record version (Steel & Torrie, 1980). The coefficients were used to determine the impact of grain servings on the dietary iron intake of the participants.

Limitations

Several limitations in the methodology have been recognized. The first limitation was the inability to verify by measurement the actual height and weight of each participant. Due to the nature of the collection venues, collecting this data was not possible and may have reduced participation, had it been possible. A second limitation in the collection of data was the selection of the wheat-based products for gluten-free manufactured products, although every effort was made to find comparable products. The third limitation recognized in the collection of data was the unwillingness of participants to record their food intake for three days. Upon inquiry by the support group leaders, many potential participants revealed concerns that their diets were inadequate and that they were reluctant to have them analyzed despite reassurances to the contrary. The final, but major, limitation of this study is the small sample size (N=13). Although least squares means and standard error were used to adjust for sample size variation, the statistical analysis should be viewed with caution.

Chapter IV: Results

Within this chapter, the anthropometric and demographic characteristics of the participants are reported. The grains, starches, and hydrocolloids which were consumed by the participants are described. Finally, the statistical analysis of the food records are reported as they pertain to the participants' dietary iron intakes as well as the number of grain servings consumed.

Fourteen of the 17 people who voluntarily signed consent forms returned the necessary materials. One of the 14 people failed to record three consecutive days in his/her food journal, and was therefore excluded. The exclusion of this participant's data resulted in a final population of N = 13. The anthropometric data which was collected to determine participants' dietary requirements is summarized in Table 1.

Table 1

Mean and Standard Error (SE) of Anthropometric Measurements of Participants

Measurement	Mean	SE
Height (inches)	65.00	0.79
Weight (pounds)	137.50	5.07
BMI	22.83	0.63

Of the 13 participants who were used in the final analysis, 15% were male ($n = 2$) and 85% were female ($n = 11$). The age of the participants primarily fell into the over-50 age group ($n = 9$, 69%) and 31% fell into the 19-50 age group ($n = 4$). The distribution of these demographics is displayed in Table 2. The mean age with standard error was 60.15

years ± 5.58. The youngest participant was 24 years old and the oldest was 82 years old. Sixty-nine percent ($n = 9$) of the participants resided in small locations, and 31% ($n = 4$) of the participants resided in large locations.

Table 2

Gender and Age Distribution of Participants

Gender	Age 19-50 Years	Age Over 50 Years
Male	0	2
Female	4	7

Note. Values are reported as *n*.

Composition of Gluten-Free Foods

The gluten-free food labels and recipes provided were analyzed to determine which grains, starches, and hydrocolloids were used to make gluten-free, carbohydrate-based foods. Brown rice, white rice, and corn were the most common grains found on nutrition labels. The most prominent starches used in recipes and listed on nutrition labels included brown-rice flour, white-rice flour, sweet-rice flour, rice bran, corn starch, potato starch, tapioca starch and flour, and corn meal. Starches and grains which were not prominent, but were of interest, include amaranth, quinoa, teff flour, quinoa flour, almond meal, flax meal, and flax seed. While the latter starches and grains are not common ingredients in gluten-containing diets, they are of superior nutritional value and are derived from grains recommended for celiac patients (Thompson, 2006).

The most prevalent hydrocolloids used in gluten-free foods were guar gum and xanthan gum. Guar gum was used most extensively in manufactured gluten-free products, while xanthan gum was most often found as an ingredient in foods which were prepared by the participants from recipes. Other hydrocolloids which were found in manufactured products include cellulose gum, carboxymethylcellulose (CMC), psyllium, pectin, and carageenan.

Dietary Iron Intake

The least squares means and standard error of the milligrams of dietary iron intake were calculated by age, gender, and food record version. Table 3 displays the values for the no-vitamin food records, as these records accurately reflect the participants' actual dietary intake of iron. As the raw intake of dietary iron, the no-vitamin food records are useful for further comparison with other studies, but are not useful in comparing among the groups of the present study due to the variation in the recommended intakes of iron based on age and gender.

Table 3

Least Squares Means and Standard Error (SE) of Dietary Iron Intake for the No-vitamin Food Records

Gender	Age Group	Least Squares Means	SE
Female	19-50	11.45 (18)	2.02
Female	Over-50	11.53 (8)	1.53
Male	Over-50	16.74 (8)	2.86

Note. Values are reported as milligrams of dietary iron, with recommended milligrams of dietary iron in parentheses.

Dietary iron intake was analyzed in detail as a percent of the amount of iron recommended according to the Dietary Reference Intakes as determined by the Institute of Medicine (2005). The percent dietary iron intake for each food record version for all participants (N = 39) was calculated and used as the basis in the analysis.

As shown in Figure 1, the mean percent of dietary iron intake varied between the three food record versions, and all means exceeded 100% of the DRI. The highest mean was that of the actual food record versions, at 244%. The lowest mean value of 129% belonged to the no-vitamin versions (which excluded any vitamin and mineral supplements but still contained gluten-free foods). When gluten-free foods were replaced with the gluten-containing foods, the mean percent of dietary iron intake in the gluten-substitution versions rose to 198%.

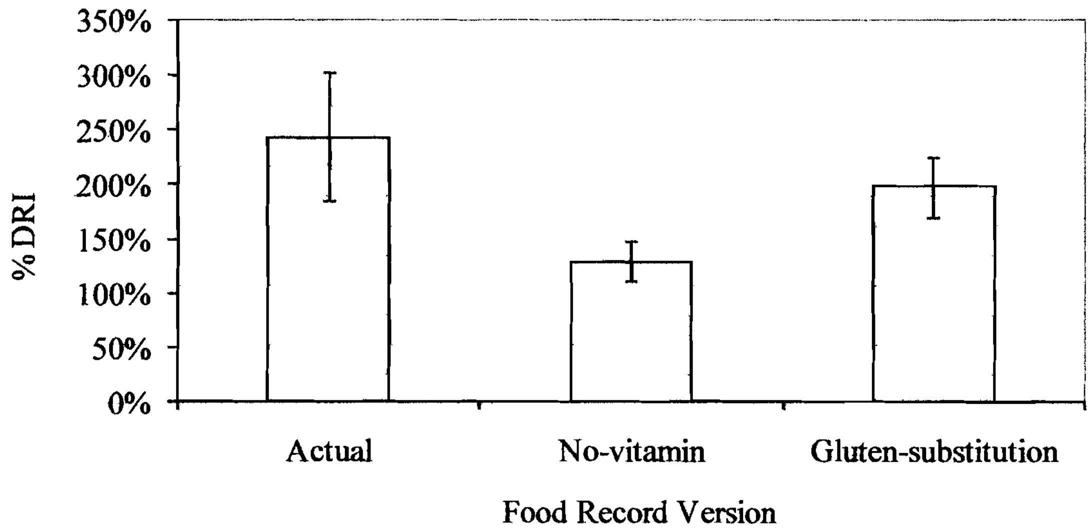

Figure 1. Percent DRI dietary iron intakes by food record version. Values are reported as means with standard error and $n = 13$ for all categories.

The fixed effect of location on the percent dietary iron intake was examined (Table 4). Using a general linear mixed model, no significant difference was found between large and small locations ($p > 0.05$).

Table 4

Least Squares Means and Standard Error (SE) of the Percent Dietary Iron Intake by Location

Location	Least Squares Mean	SE
Large	129.69	40.26
Small	217.50	26.84

The interactive effects of age group, gender, and food record version on the percent dietary iron intake were examined using a repeated measures mixed model of variance and are shown in Table 5. Statistically significant differences between means were found among the food record versions ($p \leq 0.05$) and among the age group effects. The statistically significant difference in food record versions was due not only to the effect of supplemental vitamins and minerals found in the actual food records as compared with the no-vitamin and gluten-substitution food records of several age and gender groups ($p \leq 0.05$), but was also found between the no-vitamin and gluten-substitution food records of both the over-50 males and the over-50 females ($p \leq 0.05$).

The difference in age groups can be attributed to several effects. The most significant contribution is among the gluten-substitution versions of the 19-50 females versus both the over-50 males and the over-50 females as well as the no-vitamin versions of the 19-50 females and the over-50 males ($p \leq 0.01$). The difference between the no-vitamin food record versions of the 19-50 females and over-50 females were also statistically significant ($p \leq 0.05$). An additional significant effect was found between the gluten-substitution versions of the over-50 males and over-50 females ($p \leq 0.05$) but it did not impact the overall gender effect ($p > 0.05$).

The least squares means of the no-vitamin food records for females 19-50 years old indicates that they receive only 63.62% of the DRI. Using these food records and substituting gluten-based foods resulted in an increase in the percent dietary iron intake to 94.87%. While this increase appeared to be of clinical interest, the difference of least squares means indicates that this is not a statistically significant increase ($p = 0.39$).

Table 5

Least Squares Means, Standard Error (SE), and Confidence Intervals (CI) of the Mixed Effects for Percent Dietary Iron Intake

						95% CI	
Food Record Version	Age Group	Gender	*n*	Mean	SE	Lower	Upper
Actual	19-50	Female	4	107.14[cd]	101.43	-118.85	333.13
Actual	Over-50	Female	7	320.97[a]	76.67	150.13	491.80
Actual	Over-50	Male	2	246.75[ab]	143.44	-72.85	566.35
No-vitamin	19-50	Female	4	63.62[d]	21.85	14.94	112.31
No-vitamin	Over-50	Female	7	144.18[c]	16.52	107.38	180.98
No-vitamin	Over-50	Male	2	209.25[bc]	30.90	140.40	278.10
Gluten-substitution	19-50	Female	4	94.87[d]	30.48	26.97	162.78
Gluten-substitution	Over-50	Female	7	219.06[b]	23.04	167.73	270.39
Gluten-substitution	Over-50	Male	2	332.38[a]	43.10	236.35	428.41

Note. All values are a percent of the dietary iron intake versus the DRI values recommended by the Institute of Medicine (2005).
[a b c d e] Means with different superscripts are significantly different at $p \leq 0.05$.

Grain Servings

The number of grain servings per participant was analyzed from several perspectives. Figure 2 shows the mean number of grain servings calculated from the food record versions, as well as the standard error values. As expected, the means for the actual and no-vitamin versions were identical, as the single variation between the versions was the inclusion or exclusion of the vitamin and mineral supplements (M = 4.35, SE = 0.85). The substitution of gluten-containing foods for gluten-free foods resulted in an increase in the number of grain servings (M = 6.35, SE = 0.71).

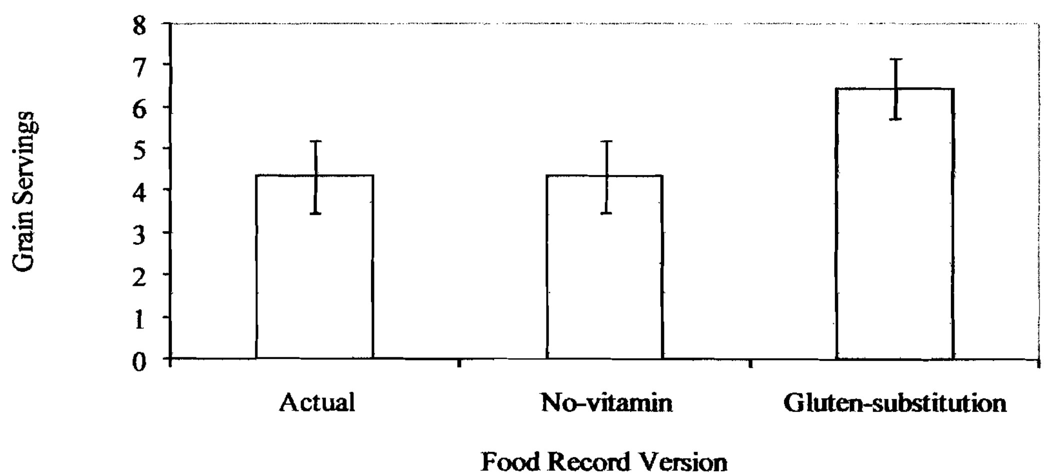

Figure 2. Number of grain servings consumed per food record version. Values are reported as means with standard error and n = 13 for all categories.

The effect of location on the number of grain servings was analyzed using a general linear mixed model and is displayed in Table 6. As with the percent dietary iron intake, no significant difference was found between large and small locations ($p > 0.05$).

Table 6

Least Squares Means and Standard Error (SE) of the Number of Grain Servings by Location

Location	Least Squares Means	SE
Large	3.88	0.84
Small	5.52	0.56

The number of grain servings for each food record version was also analyzed in comparison to the number of servings recommended by the Food Guide Pyramid (United States Department of Agriculture [USDA], n.d.). The percent of participants who met or exceeded the recommendation for their age and gender are shown in Figure 3. The actual and no-vitamin food record versions are identical with respect to grain content, and vary only in vitamin and mineral content. Only 30.8% of the participants met or exceeded the recommendation. The substitution of gluten-containing foods resulted in 53.8% of the participants meeting or exceeding the recommended number of grain servings.

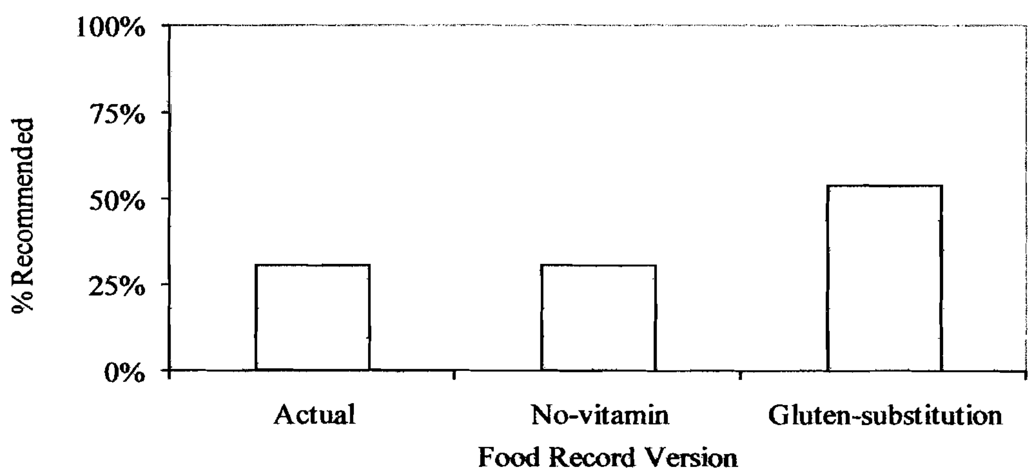

Figure 3. Percent of participants meeting or exceeding the number of recommended grain servings (USDA, n.d.) per food record version. For all categories, $n = 13$.

Table 7 shows the results of the analysis of the interactive effects of age group, gender, and food record version on the number of grain servings. Although the mixed model analysis showed statistically significant differences in the number of grain servings among the food record versions ($p \leq 0.05$), the differences of least squares means comparisons which contributed to this significance were found between the actual and no-vitamin food records of females 19-50 and the gluten-substitution food records of males over-50 ($p = 0.03$). For the purposes of this study, this comparison is not relevant. However, the differences of least squares means was nearly significant between the actual food record version of females 19-50 and the gluten-substitution food record for the same group ($p = 0.07$). While not actually statistically significant, this difference is of interest. The differences of least squares means of the actual or no-vitamin and the gluten-substitution food record versions of both the females and males over-50 were not statistically significant ($p = 0.10$ and $p = 0.41$, respectively).

Table 7

Least Squares Means, Standard Error (SE), and Confidence Intervals (CI) of the Mixed Effects for Number of Grain Servings

Food Record Version	Age Group	Gender	n	Mean	SE	95% CI Lower	95% CI Upper
Actual	19-50	Female	4	3.05[a]	1.41	0.09	6.01
Actual	Over-50	Female	7	4.26[ab]	1.07	2.02	6.50
Actual	Over-50	Male	2	7.25[ab]	2.00	3.06	11.44
No-vitamin	19-50	Female	4	3.05[a]	1.41	0.09	6.01
No-vitamin	Over-50	Female	7	4.26[ab]	1.07	2.02	6.50
No-vitamin	Over-50	Male	2	7.25[ab]	2.00	3.06	11.44
Gluten-substitution	19-50	Female	4	5.68[ab]	1.41	2.71	8.64
Gluten-substitution	Over-50	Female	7	6.03[ab]	1.07	3.79	8.27
Gluten-substitution	Over-50	Male	2	8.85[b]	2.00	4.66	13.04

[a b] Means with different superscripts are significantly different at $p \leq 0.05$.

Correlation of Dietary Iron Intake and Grain Servings

Pearson correlations were performed to determine whether a correlation existed between the number of grain servings and the percent dietary iron intake (Table 8). A moderately-strong positive correlation was found between the gluten-substitution food records and the percent of dietary iron intake ($r = 0.65, p \leq 0.05$).

Table 8

Correlation of Grain Servings and Percent Dietary Iron Intake

Food Record Version	*r*	*p*
Actual	0.08	0.81
No-vitamin	0.48	0.10
Gluten-substitution	0.65	0.02

Chapter V: Discussion

This study examined the dietary iron intakes and the number of grain servings consumed by adults who have been diagnosed with celiac disease, and who are living a gluten-free lifestyle. This chapter includes limitations of this study, discussion of the results of this study in comparison to other research, conclusions which may be drawn from this research, and finally, recommendations for further research.

Limitations

Several limitations existed within this study. Enrollment of participants proved to be extremely difficult due to several factors. Restricting the participation to celiac patients who had been following a gluten-free lifestyle for more than twelve months prevented several potential subjects from enrolling in the study. This was also true of the requirement that all participants have a biopsy-proven diagnosis of celiac disease. Many other celiac patients who wished to participate had only been diagnosed through antibody testing. Parents also wished to enroll their children in the study but were unable to do so due to the requirement that all participants be 18 years of age or older. Participation was also limited by the reluctance of support group members to record their food intake for three consecutive days. Other celiac support group members were hesitant to enroll out of fear that their dietary intakes would be criticized for its overall quality and content despite reassurances by both the researcher and support group leaders that this would not be the case.

Although food journals are considered an acceptable method of gathering and examining dietary intake, they are prone to inaccuracy. This may be due to the desire of the participants to follow a more nutritionally-adequate diet during the appointed time frame, or may be due to delayed or absent recording of foods or meals. Recording each food and requiring that recipes and nutrition labels be included in the information returned to the researcher may have caused participants to repeat their food choices over the course of the three days. Additionally, the gluten-free foods which were analyzed through the entry of recipes and nutrition label information may also limit the accuracy of the nutritional analysis.

The participants in the study were all members of celiac support groups, and as such, they may have been more knowledgeable regarding the management of their dietary intake than participants recruited from outside of support group settings. They were also aware of the focus of this study and may have altered their dietary intakes as a result.

Discussion

The participants in this study had a mean value for BMI of 22.83 kg/m^2 (SE = 0.63). This average indicates that, as a whole, the group was in the normal range and is neither underweight nor overweight. The mean age of the participants was 60.15 years old (SE = 5.58). These mean values, shown in Table 9, are comparable with the participants in the only published dietary study of United States celiac patients with 57 subjects from across the United States (Thompson et al., 2005). The gender distribution was also similar, with males comprising 17% of the population and females 83%. This similarity of the populations lends itself well to comparisons in discussion.

Table 9

Mean and Standard Deviation (SD) of Characteristics of Participants in Similar Study

Measurement	Mean	SD
Age	51	11
BMI	23.3	3.1

Note. Prior study conducted by Thompson et al., 2005.

Table 10 shows a comparison between the means of the dietary iron intakes of the no-vitamin food record versions with the values in the prior study (Thompson et al., 2005). Females were not sub-divided into groups based on dietary recommendations, so the same value from the previous study is used for both female groups of the current study. The males in the current study have a higher dietary intake compared with the prior study, but should be viewed with caution as the current study included only two male participants. The dietary iron intake of females in the current study is similar to the prior study.

Table 10

Comparison of the Mean Values of Dietary Iron Intake

Gender	Age Group	Current Study	Prior Study
Female	19-50	11.45	11.0
Female	Over-50	11.53	11.0
Male	Over-50	16.74	14.7

Note. Values shown represent milligrams of dietary iron. Prior study conducted by Thompson et al., 2005.

Another study conducted in Canada (Sabry & Okada, 1992) reported mean iron intakes of 17.1 milligrams for males and 10.8 milligrams for females, which are also comparable with the values found in this research. While neither of the two prior studies reported the total percent intake of dietary iron versus the DRI in the United States or Recommended Nutrient Intakes for Canadians (RNI), each reported females, 44% and 20%, respectively, as being below recommended values.

Data reported from NHANES 2003-2004 data (USDA, 2007) is divided into narrower age groups than this study. The mean dietary iron intake of females between 19 and 49 years old is between 13.1 and 14.0 milligrams, which are higher than the 11.45 milligrams reported for the females age 19-50 in this study, but does indicate that many females, celiacs and non-celiacs alike, are not meeting their recommended daily goal of 18 milligrams of dietary iron. This is of concern.

According to the NHANES data, females over 50 years old consume between 12.6 and 13.1 milligrams of iron. This is slightly higher than the 11.53 milligrams reported in the current research. As both males in the present study were over 70 years old, the mean value for the 70-and-over age category for males of 15.8 milligrams from the NHANES data was used as a comparison. This is slightly higher than the 16.74 reported for the two males in the present research. In all of these instances, all of the participants over the age of 50 years old have met or exceeded the recommended levels of dietary iron intake.

Although most of the participants consumed more than the DRI of dietary iron, the dietary iron status of these patients may still be of concern. As noted in a study which examined the efficacy of a gluten-free diet in resolving iron-deficiency anemia (Annibale et al., 2001), 45% of the patients were still categorized as iron-deficient (but not anemic) based on their serum ferritin levels after following a gluten-free diet for 24 months.

The difference in the percent of participants who met or exceeded the recommended grain servings for each of the food record versions was not found to be statistically significant. With 30.8% of the participants meeting the requirement for the actual and no-vitamin versions and 53.8% of the participants meeting the requirement for the gluten-substitution versions, this much higher percentage may be a result of bias of the research when the gluten-containing foods were substituted for the gluten-free foods. While all substitutions were consistent among the types of products, whole wheat products were usually substituted when available and provide a higher value of grain servings compared to the grains and starches commonly contained in gluten-free foods.

Based on NHANES 1999-2002 data, 34.6% of the participants aged 12 and over met or exceeded the recommended number of grain servings. Further divided by age, 39.1% of the participants 12-18 years old, 33.8% of those 19-50 years old, and 34.3% of those over 50 years old met or exceeded the recommendation. It is interesting to note that the actual and no-vitamin food record versions containing gluten-free foods fell below these levels, and that the gluten-substitution food records exceeded the NHANES levels.

The mean number of grain servings by age and gender can be compared to results from the prior study which was discussed (Thompson et al., 2005). While the age groups are smaller in the current study, comparisons between the number of grain servings found in the actual and no-vitamin versions with the prior study can be made as the overall demographics are similar (Table 11). As with the similar comparison of dietary iron, the males of the current study surpassed the males of the prior study in their number of grain servings per day. Again, this must be viewed with caution due to the limited number of male participants in this study ($n = 2$). A similar number of grain servings were consumed by the females over-50 years old as were consumed by all females in the prior study. Females 19-50 years old consumed fewer servings by comparison.

Table 11

Comparison of the Mean Values of the Number of Grain Servings

Gender	Age Group	Current Study	Prior Study
Female	19-50	3.05	4.6
Female	Over-50	4.26	4.6
Male	Over-50	7.25	6.6

Note. Prior study conducted by Thompson et al., 2005.

A moderately-strong positive correlation was identified between the number of grain servings and the percent of dietary iron intake within the gluten-substitution food record versions. In another recent study which used NHANES 1999-2000 data and included only females 19 years old and over, a similar relationship was found between whole grain consumption and dietary iron intake (Good, Holschuh, Albertson, & Eldridge, 2008). The women who consumed no whole grains were significantly more likely ($p \leq 0.05$) to consume less than the recommended amount of dietary iron. The consumption of any whole grains was found to be associated with significantly higher intakes of iron as well as other vitamins and minerals. These results are not surprising since 43.5% of the dietary iron intake in the United States comes from grain products (Cotton et al., 2004).

No statistical significance was found between the location of the participants and the percent dietary iron intake or the number of grain servings consumed by the participants. However, it was of interest to examine this as a possible contributing factor of gluten-free dietary quality since 83% of respondents in a recent survey reported being dissatisfied with the availability of gluten-free foods (Zarkadas et al., 2006). While the availability of gluten-free foods may still be higher in larger communities, the dietary quality of the products available appears to be similar.

Conclusions

While the results do not show statistical significance in the percent dietary intake among the food record versions of females 19-50 years old, the least squares means of 63.62% calculated for the no-vitamin food records does put them at-risk for iron deficiency. This, coupled with their lower intake of grains and the fact that whole grain consumption is positively associated with dietary iron intake, makes them a group which requires more attention regarding these two factors.

Celiac patients who participated in this study consumed a statistically significant amount of dietary iron from vitamin and mineral supplements. Females 19-50 years old did not show a significant difference between the actual and no-vitamin food record versions. This was most likely due to the fact that only one of the four participants (25%) in this group reported having consumed a multivitamin containing iron during the three-day periods. This is also of concern as this group has the highest dietary iron recommendation but appeared to have the lowest intake of supplemental iron.

The intakes of both dietary iron and grain servings are important in maintaining hematologic iron values. Among celiac patients, this may be of increased importance due to lower serum ferritin values (Annibale et al., 2001) and possible malabsorption due to non-compliance with a gluten-free diet (Bardella et al., 1985; Sourjouron et al., 1982).

Recommendations

Upon reviewing the methodology and results of this study, several recommendations for further research are evident. First, a larger sample size would provide stronger statistical evidence. Collecting data from a greater number of participants would require a longer enrollment period than the four months available for this study and would require the participation of a greater number of support groups.

The second recommendation would be to conduct a study which would correlate the serum ferritin values of participants with their dietary intake. Compliance with a gluten-free diet would also need to be assessed through antibody levels to determine whether hematologic values were impacted by dietary non-compliance.

Next, it would be of interest to assess the correlation of the consumption of whole grain servings and dietary iron in celiac patients. Another dimension of this research could include providing gluten-free whole grain bread to a randomly selected sub-group while providing gluten-free bread not containing whole grain to a control group. Serum ferritin levels could also be obtained to increase the strength of the research.

A fourth recommendation would be to assess the impact of increased nutrition education regarding dietary iron among females who are 19-50 years old. One focus of the educational materials and sessions could be on natural food sources of dietary iron, with an emphasis on whole grain foods. This would be of interest to both non-celiac and celiac females in this age group.

Another possible area for further research would be in product development efforts to create more fortified and enriched gluten-free products which are acceptable, in both taste and texture, to the consumer. Products must also be affordable and must be made available on a large scale.

The final recommendation for further research is to examine the purchasing patterns of gluten-free products by celiac patients, as prior research indicates that consumers have difficulty finding available manufactured gluten-free products. It would be interesting to determine the quantities of products purchased over the internet, at local stores, and at stores which are not local to the consumer. The frequency of purchases and the amount of money spent on the purchases could be additional factors to consider.

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Appendix A: Consent Form

This research has been approved by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46.

Consent to Participate In UW-Stout Approved Research

Title: Dietary Iron Intakes in Celiac Patients

Description:

This study will assess the amount of iron in the diet of celiac patients who are following a gluten-free diet. A brief questionnaire will be completed and will be used to determine eligibility and gather information which will be used to classify the data. The dietary intakes will be analyzed through a three-day food record which must be completed within two weeks after instructions are received.

In order to participate in this study, you must be 18 years or older, must have been diagnosed with celiac disease through intestinal biopsy, and must have been following a gluten-free diet for at least one year. You must also not be taking prescribed iron supplements, be pregnant, or be lactating.

Risks and Benefits:

Some people may become anxious when recording their diet. All food records will remain confidential and will not be identified by name, only by a code number.

Iron deficiency anemia is one of the most common presenting symptoms of celiac disease and the dietary intakes of iron in celiac patients following a gluten-free diet has not been studied in-depth. This research will be beneficial to health professionals who work with celiac patients and to those people with celiac disease to help ensure that your nutritional needs are met. This is of increasing importance due to the increased availability of processed gluten-free foods made with refined grains which are lacking in fortification or enrichment. Results of this study may also motivate gluten-free food manufacturers to fortify and enrich their products.

Time Commitment and Payment:

By participating in this study, you are agreeing to weigh, measure, and record all foods eaten during a three day period. This must include at least one weekend day. Instructions regarding completion of the food record will take less than 15 minutes.

Confidentiality:

Your name will not be included on any documents. A code number will be assigned to each participant and the list of code numbers and names will be held by a responsible individual not directly conducting the study. You cannot be identified from any of this information. Results will not be published by individual, but will be reported by groups. This informed consent will not be kept with any of the other documents completed with this project.

Right to Withdraw:
Your participation in this study is completely voluntary. You may choose not to participate without any penalty to you. Should you choose to participate and later wish to withdraw from the study, you may discontinue your participation at any time without any penalty.

IRB Approval:
This study has been reviewed and approved by The University of Wisconsin-Stout's Institutional Review Board (IRB). The IRB has determined that this study meets the ethical obligations required by federal law and University policies. If you have questions or concerns regarding this study please contact the Investigator or Advisor. If you have any questions, concerns, or reports regarding your rights as a research subject, please contact the IRB Administrator.

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Statement of Consent:
By signing this consent form you agree to participate in the project entitled, "Dietary Iron Intakes in Celiac Patients."

____

Signature Date

Appendix B: Questionnaire

This research has been approved by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46.

QUESTIONNAIRE

Dietary Iron Intakes in Celiac Patients

____________________Subject number (to be assigned by investigator)

1. Contact information*
   Name ____
   Address ____
   City, State, Zip ______________________________________
   If the investigator needs more information about your food record, how would you prefer to be contacted?
   ❒ Email ❒ US mail ❒ Phone ❒ Meeting ❒ I prefer not to be contacted
   Email address (optional) ______________________________________

   Phone number including area code (optional) ___________________________

   When is the best time to call you to discuss food record questions? ___________

2. How were you diagnosed with celiac disease?

   ❒ Intestinal biopsy

   ❒ other ____

3. I was diagnosed with celiac disease:

   ❒ Less than 1 year ago

   ❒ 1 – 2 years ago

   ❒ 2 – 5 years ago

   ❒ 5 – 10 years ago

   ❒ More than 10 years ago

4. I have been treated for iron-deficiency anemia:

   ❒ Yes, please specify date(s) ____________________

   ❒ No

5. I have been following a gluten-free diet for
   ❒ Less than 12 months
   ❒ One year or longer, please specify __________

6. Age and Gender:

I am ❐ Male ❐ Female: Are you pregnant? ❐ Or lactating? ❐

I am ❐ Younger than 18 ❐ 18 – 50 years old ❐ Over 50 years old

7. Other dietary restrictions:

❐ I have no other medical conditions that would require a very structured diet.

❐ I avoid dairy products due to lactose intolerance

❐ I follow a very structured diet to manage my diabetes

❐ I follow a vegetarian diet (I eat eggs and dairy products)

❐ I follow a vegan diet (no meat, fish, eggs, or dairy products)

❐ Other, please specify ______________________________

8. Dietary Supplements:

❐ I take a daily multi-vitamin

❐ I take iron supplements

❐ Other, please specify ______________________________

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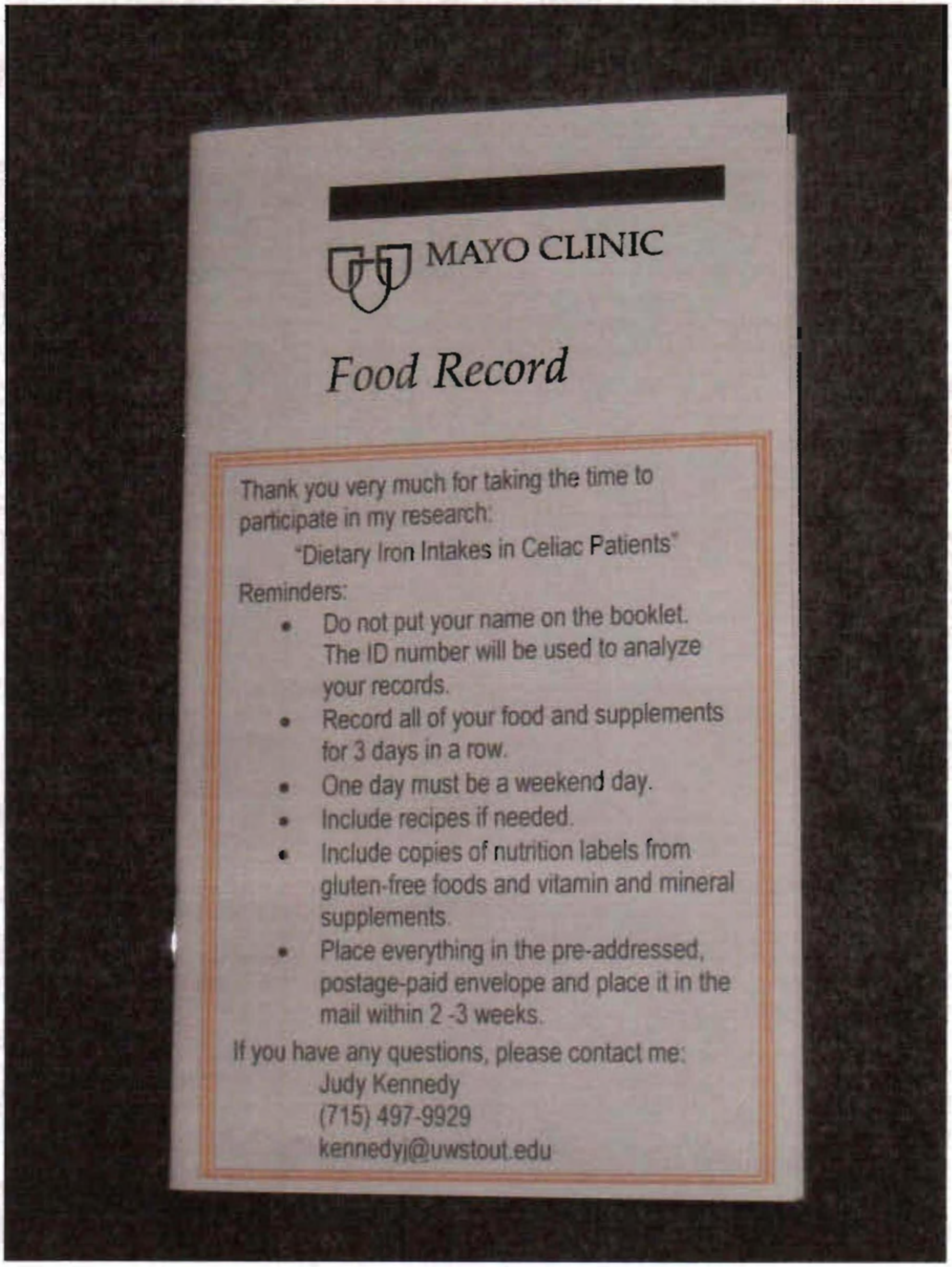
* All personal information will be secured and will remain confidential. It will not be shared with any other person or organization for any purpose.

Appendix C: Food Journal Record



Appendix D: Food Journal Form

Day_____________________________ Date_______________ Was this a usual day?____________________				
	Amount	Food, Beverage	Time	Location

Appendix E: Food Journal Sample

Day Thursday Date 1/3/2008

Was this a usual day? Yes

	Amount	Food, Beverage	Time	Location
B	4 oz 1 Short spray 12 oz ¼ cup ¾ cup	Orange Juice Egg, scrambled Pam, original Coffee, black 1% Milk Perky-Os, Apple Cinnamon	7:00 AM	Home
S	1 medium 8 oz	Banana Coffee, black	10:00 AM	Work
L	1 ½ packet 1 2 Tbsp 2 Tubs Value size	Side Salad Salad dressing, Creamy Ranch Baked Potato Shredded Cheese Butter Spread Diet Coke	12:00 PM	Wendy's
S	15 16 oz	Baby Carrots, Green Giant Water	2:30 PM	Work
D	2 slices of 12 total 1 cup 2 Tbsp ¼ cup ¼ cup 1 cup 16 oz	Meatloaf, recipe included Mashed potatoes, 3 cups total prepared with: butter milk Beef gravy, Maxwell's Kitchen Broccoli, carrot, cauliflower blend, Birds Eye Steam Fresh (dash salt and pepper) Water	6:00 PM	Home
S	1 mini bag	Popcorn, Act II, butter flavor	8:00 PM	Home

Appendix F: Recipe Form

Recipe: ____

Number of Servings in recipe: ______ **Number of Servings Eaten:** _______

Instructions:

- Please record each major ingredient and the amount used.
- Use standard measures such as teaspoons, tablespoons, cups, pounds, and ounces.
- Indicate whether ounces are dry or fluid ounces.
- Spices do not need to be included, but salt and any seasoning with salt should be listed if it calls for 1/8 teaspoon or more.
- In the Preparation Method, you must indicate if the food was fried, sautéed, broiled, baked, or boiled. If foods are fried, include the oil used in the Ingredient list. If foods are sautéed, include the butter or broth used.
- If you use a recipe from the internet or your computer, you can attach a printed copy, but be sure to note any changes or substitutions. For example, if a recipe calls for elbow macaroni, you may write down your substitution of American Harvest quinoa elbows.

Measurement	Ingredient

Preparation Method: ____

____

____

Appendix G: Notice of Study

This research has been approved by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46.

You're Invited

To participate in a Graduate Research Project evaluating Gluten-Free Diets

Who: Celiacs, 18 years or older
Biopsy-proven diagnosis
Following a gluten-free diet for at least 12 months
Not pregnant or lactating

What: Keep an anonymous & confidential 3-day food journal after signing a consent form and completing a short questionnaire

Why: To see how much iron is in the typical gluten-free diet

When: Enroll now through April 20, 2008

Contact: Judy Kennedy to participate in the study
"Dietary Iron Intakes in Celiac Patients"
kennedyj@uwstout.edu OR 715-497-9929

IRB Administrator: Sue Foxwell, Director, Research Services
UW-Stout, 152 Vocational Rehabilitation Building
Menomonie, WI 54751
foxwells@uwstout.edu 715-232-2477

Appendix H: Food Record Instructions

Food Record Instructions
Dietary Iron Intakes in Celiac Patients

1. Keep track of your food for 3 days in a row. You **must** include one weekend day.

2. Each time you eat, write down every food. This includes all meals and snacks.

3. Fill in the food record as soon as possible. This helps the record be as accurate as possible and makes the research more valuable.

4. In the left-hand column, indicate which meal is being eaten. You can use:
   B = Breakfast
   L = Lunch
   D = Dinner or Supper
   S = Snack
   V = Vitamin or supplement

5. Record each food in appropriate amounts such as teaspoons, tablespoons, cups, ounces (fluid or dry), and pounds. If you are not able to measure or weigh your food, record an approximate size or the number of items. Some quick and handy measurements are:

http://www.nestle.ca/NR/rdonlyres/FC3BB26D-FCEB-4D7F-9B22-B8CE914033C7/0/PortionControl_EN.jpg

6. Common abbreviations include:

Tablespoon	tbsp or T
Teaspoon	tsp or t
Ounce	oz
Fluid	fl
Pound	lb or #
Cup	C
Slice	sl
Gluten-free	GF

7. Include any and all details about your food.
   - Include brand names for any packaged foods.
   - Whenever possible, indicate how the food was cooked. This includes methods such as fried, sautéed, broiled, baked, or boiled.
   - For foods which come in a large variety of ways, be as specific as possible.
     - For example, milk can be skim, 1%, 2%, or whole.
     - Canned fruit can be in heavy syrup, lite syrup, or packed in juice.
     - Orange juice may be prepared many ways including, but not limited to, fresh-squeezed, from concentrate, or may include calcium.
   - List items you spread on, pour over or pour into your food. This includes all jams, jellies, ketchup, mustard, sugar, creamer, and butter or spreads.
   - Spices added to foods do not need to be recorded unless they include salt.

8. Foods that are prepared from recipes should be recorded on the recipe form. It includes more detailed instructions about recipes.

9. If a food, such as stir fry, is made from several ingredients, record each ingredient. For example: 3 ounces chicken breast, ¼ cup chopped celery, ¼ cup carrots, ½ cup broccoli, 1 cup white rice, ¼ cup gluten-free soy sauce.

10. If you eat at a restaurant, please indicate the restaurant's name. If you ask for substitutions or modifications to regular menu items, please note these requests. If you are not at a major chain restaurant, try to itemize the foods as well as you can. For example, if you order a hamburger patty, record the weight advertized on the menu and how it was cooked: 1/3 pound ground beef patty, grilled.

11. Record the time of day and location of your meal or snack.

12. Start a new page for a new day.

13. Your meal does not have to fit in one box. Use as much space as needed to record all of your food.

14. The nutrition information of packaged gluten-free foods may not be available for analysis, so PLEASE include the nutrition label (or a copy of the label) whenever possible.

15. Record each vitamin or supplement you take. Either include a copy of the label or record the amounts of each individual vitamin or mineral. For example, if you take a multi-vitamin which includes 20 vitamins and minerals, each one should be recorded with its correct amount. Only one detailed list is necessary, but be sure to record each day and time vitamins and supplements are taken.

Thank you for taking the time to participate in the "Dietary Iron Intakes in Celiac Patients" thesis research. If you have any questions or concerns, feel free to contact me and I'll gladly answer them!

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