# Glycemic Response to Gluten-Free Bread in Healthy Adults

by

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ABSTRACT

*Background*: Research has found that nearly a quarter of the American population follows a gluten-free diet in some capacity, while only about 1% of the population is diagnosed with celiac disease. Although the amount of research-based evidence supporting any health benefits of a gluten-free diet in an individual without a gluten-related disorder is limited, the number of people claiming to follow a gluten-free diet continues to rise. Also, despite an increasing belief that gluten is harmful for health, the potentially undesirable effects of gluten substitutions used in gluten-free foods are largely unknown. Due to the protein network encapsulating starch granules, gluten is thought to lengthen the amount of time needed during starch digestion, thereby reducing postprandial glycemia. Therefore, it is predicted that breads containing gluten will produce a lower glycemic response compared to gluten-free breads.

*Aim*: The aim of this study was to investigate the glycemic response of gluten-free bread made with different types of flour compared to bread made with gluten-containing wheat flour.

*Methods*: This study involved a 3-week, randomized, single blind crossover study in which 17 healthy individuals were asked to consume a different type of bread each week, 2 of which were gluten-free. Blood glucose was taken by finger prick at fasting as a baseline measurement, then for 2 hours after bread consumption in 30-minute increments.

*Results*: Across the three groups, there was no significant difference in iAUC values after 120 minutes ($p=0.192$ ). The greatest mean was seen in the gluten-containing bread ($145.3 \pm 82.6$), then the gluten-free bread made with rice flour ($125.5 \pm 62.8$), and lastly the gluten-free bread made with potato and fava bean flour ($112.4 \pm 64.5$).

*Conclusion*: The inconsistent results of this study compared to previous, similar studies suggests that the postprandial glycemic response of gluten-free products can not be generalized as a whole, but instead is dependent on the type of product and the ingredients used to replace the gluten. Although the results did not show a significant difference, it does argue against the belief that gluten-free products are invariably better for health in the general, non-gluten sensitive population.

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# CHAPTER 1

## INTRODUCTION

Recent research has found that nearly a quarter of the American population follow a gluten-free diet in some capacity, while only half of these individuals have a gluten-related disorder, such as celiac disease, gluten-sensitivity or wheat allergy (Newberry, McKnight, Sarav, & Pickett-Blakely, 2017). National Health and Nutrition Examination Surveys (NHANES) report that the prevalence of Celiac Disease has not changed from 2009 to 2014, with 1.74 million Americans diagnosed with Celiac Disease each year (Kim, Churrango, Patel, Kothari, & Ahlawat, 2016). The majority of non-celiac consumers who follow a gluten-free diet choose gluten-free products because they believe that the gluten-free version is healthier than the original product containing gluten (Gaesser & Angadi, 2012; D. Lis, Stellingwerff, Kitic, Ahuja, & Fell, 2015). While the amount of research-based evidence supporting any health benefits of a gluten-free diet in a healthy individual is limited, the number of healthy people claiming to follow a gluten-free diet in some capacity continues to rise (Kim et al., 2016; Newberry et al., 2017). This shift in consumption has given fuel to food companies and marketers to portray the more expensive, gluten-free options as being "better for your health," including foods that never even contained gluten. In addition to these tailored marketing strategies, celebrity endorsements frequently encourage a gluten-free diet for weight loss (Gaesser & Angadi, 2012). Although many in the lay public believe that gluten is harmful for health, the potentially undesirable effects of gluten substitutions used in gluten-free foods are largely unknown.

Substitute ingredients for the wheat in gluten-free breads include brown rice flour, corn flour and potato flour, as well as legume and nut flours. Additional ingredients are added to gluten-free products to produce a similar texture and taste, which may influence the way starch is digested (Berti, Riso, Monti, & Porrini, 2004). Gluten is a storage protein found in wheat, barley and rye (Aziz, Branchi, & Sanders, 2015; Lee, Ng, Dave, Ciaccio, & Green, 2009). Depending on the carbohydrate type and source, the rate of digestion and glycemia varies. The difference in glycemic index is determined by the rate that glucose is absorbed and removed from the bloodstream (Bacchetti, Saturni, Turco, & Ferretti, 2014). Certain complex carbohydrates have a lower glycemic index than simple carbohydrates (Bacchetti et al., 2014). Gluten has been found to help reduce the rate of amylolytic digestion because it encases the starch granules, which lengthens the amount of time needed for the breakdown of starch, thereby reducing postprandial glycemia (Smith et al., 2015). Thus, breads containing gluten are predicted to produce a lower glycemic response compared to gluten-free breads (Capriles & Aras, 2016).

Previous research has investigated the change in digestion due to processing and manufacturing of gluten-free foods (Berti et al., 2004; Capriles & Aras, 2016). One particular study found that the removal of gluten increased the rate in which starch was digested as compared to similar products containing gluten (Berti et al., 2004). This research concluded that the additional processing needed to remove gluten altered the product, resulting in expedited amylolytic digestion (Berti et al., 2004). Another study found that consumption of gluten-free pasta significantly increased postprandial glycemia when compared to similar pasta containing gluten (Johnston, Snyder, & Smith, 2017). To date the postprandial glycemic response of gluten-free bread made with different gluten

substitutes, compared to similar commercial bread containing gluten, is unknown.

In addition to changes in starch digestion and postprandial glycemia, other studies bring to light possible nutrient deficiencies when following a gluten-free diet (Vici, Belli, Biondi, & Polzonetti, 2016). This is necessary and important research as it will contribute empirical evidence regarding the impact of commercially available gluten-free breads on a biomarker linked to health outcomes in adults without gluten sensitivities.

## Purpose of Study

The intent of this research is to examine the postprandial glycemic response of gluten-containing bread compared to gluten-free breads composed of non-wheat ingredients in healthy adults. Blood glucose was recorded in 30-minute intervals after consumption of these different types of bread to observe the 2-hour postprandial glycemic response of these foods.

## Research Aim and Hypothesis

$H_1$ : The consumption of gluten-free bread will produce an increased degree of postprandial glycemia as compared to gluten-containing bread in healthy adults.

## Definition of Terms

- **Gluten –** A storage protein found in wheat, barley and rye that gives dough elasticity.
- **Celiac Disease –** A genetically predisposed autoimmune disease triggered by gluten, in which the gastrointestinal system is damaged during digestion (Amerine, 2006).

- **Glycemic Index –** A system that ranks food by the rate of which glucose is absorbed into circulation and removed from the bloodstream (Augustin et al., 2015).
- **Glycemic Response –** The change in blood glucose that a carbohydrate-containing food or meal evokes after consumption (Augustin et al., 2015).
- **Amylolytic digestion –** Digestion of starch into glucose.
- **Postprandial glycemia –** The term "prandial" refers to the time at which food is being consumed. Therefore, postprandial glycemia is the presence of sugar in the blood after consumption of food.

**Delimitations and Limitations**

Delimitations:

- Healthy adults between the ages of 18 – 60 were recruited for this study from the Phoenix, Arizona metropolitan area. Results of this study may not be generalized to other age groups or to adults with gluten sensitivities or other health conditions.
- Results observed from the selected gluten-free breads are not to be generalized conclusively to all gluten-free breads or products.

Limitations:

- The results of this study were observed over a short period of time; no long-term effects were assessed.
- Participants were asked to fast the night before blood glucose was assessed; adherence to this request is not guaranteed.

# CHAPTER 2

# REVIEW OF THE LITERATURE

## Grain Overview

In an effort to prevent associated health risks, the U.S. dietary guidelines recommend that at least half of the grains consumed in a person's diet should come from whole grains (Dietary Guidelines for Americans 2015-2020 eighth edition.2015). According to the 2015-2020 Dietary Guidelines for Americans, individuals usually meet their recommended total grain intake goals (Dietary Guidelines for Americans 2015-2020 eighth edition.2015). Although consumption of refined grains across sex and age transcend limitations, intake of whole grains falls short of the recommendations (Dietary Guidelines for Americans 2015-2020 eighth edition.2015). Research has found that less than 1% of children and 10% of adults are meeting their daily whole grain suggested consumption (Albertson, Reicks, Joshi, & Gugger, 2016). It seems there is still a strong need for education and awareness about what grains are and what differentiates a whole grain from a refined grain.

## Grain Structure

Grains are made up of a starchy endosperm, germ and outer bran. The endosperm and germ are rich in soluble fiber, while the bran is comprised of non-digestible and insoluble carbohydrates (Jonnalagadda et al., 2011). The fibrous bran acts as a shell designed to protect the germ and endosperm (Jonnalagadda et al., 2011). In addition to providing protection from natures elements, the bran also provides phenolic compounds, vitamins and minerals (Jonnalagadda et al., 2011). The endosperm is the largest portion of the grain and is the primary energy source for the plant, as it contains starch and

protein in addition to vitamins and minerals (Jonnalagadda et al., 2011). The germ represents the embryo that would develop into a new plant in the natural environment and is comprised of protein, fat, vitamins and minerals (Jonnalagadda et al., 2011). The germ contains the most amount of protein proportional to its size, but the size of the endosperm allows it to hold 75% of the grain protein (Brouns, Hemery, Price, & Anson, 2012). The processing of refined white flour involves the removal of the germ and bran, leaving the resulting product with fewer nutrients and fiber (Jonnalagadda et al., 2011). Unrefined whole grains maintain their fiber content, containing about 80% more fiber than grains and flours that have had the germ and bran removed (Jonnalagadda et al., 2011). In order to be classified as a whole grain, either the processed grain must contain the original endosperm, germ and bran, or the natural proportion of these components must be added back into the refined grain (Jonnalagadda et al., 2011; Lillioja, Neal, Tapsell, & Jacobs, 2013). Whole grains are classified as complex carbohydrates because the saccharide chains are longer, and more convoluted, increasing the amount of time needed for digestion and absorption compared to simple sugars (Harris & Kris-Etherton, 2010). A diet rich in whole grains has been found to be higher in fiber, vitamins and minerals, while lower in saturated fat, cholesterol, sodium and added sugars (Albertson et al., 2016). Evidence has shown that a diet high in whole grains is related to a reduced risk of obesity, cancer, heart disease and type 2 diabetes, among other chronic diseases (de Munter, Hu, Spiegelman, Franz, & van Dam, 2007; Lillioja et al., 2013).

**Whole Grains, Health and Nutritional Content**

Numerous epidemiological studies have found an inverse relationship between the consumption of whole grains and the risk of cardiovascular disease, ischemic heart

disease and coronary heart disease (Erkkilä, Herrington, Mozaffarian, & Lichtenstein, 2005; Mellen, Liese, Tooze, & Vitolins, 2007; Nettleton, Steffen, Loehr, Rosamond, & Folsom, 2008). There are a variety of explanations for this relationship, including the regulation of blood glucose and insulin response, which helps to modulate blood pressure (Jonnalagadda et al., 2011). This regulation in blood glucose is partially due to the fact that whole grains are high in resistant carbohydrates, meaning they slow the process of digestion, thus decreasing the amount of glucose let into the blood at one time (Harris & Kris-Etherton, 2010). Fiber also increases the density of gastrointestinal contents, which extends satiety and indirectly reduces additional food consumption. In this indirect way, whole grains in the form of fiber can also prevent hypertension and heart disease by helping to regulate and maintain a healthy weight. Research strongly suggests that whole grains should be used to replace refined grains in order to improve postprandial blood glucose levels and, ultimately, vascular health (Harris & Kris-Etherton, 2010). Heart disease is the number one cause of death in the United States while diabetes, currently listed as number seven, is not far behind (CDC National Health Report Highlights.).

Although epidemics generally seem to be out of an individual's control, type 2 diabetes is a result of not only genetics, but also dietary intake and lifestyle. Prevention and treatment for some individuals diagnosed with type 2 diabetes is possible through a nutritious diet, healthy lifestyle and weight control (de Munter et al., 2007). For the diabetic population, it is important to track and understand how each individual responds to different carbohydrates. When a grain is refined, the fibrous bran and germ are removed, resulting in an end product that has a much higher glycemic index than the original whole grain (Punder & Pruimboom, 2013). Many studies have found a positive

relationship between the consumption of whole grains, and a high fiber diet, with a reduced risk of type 2 diabetes (de Munter et al., 2007). A meta-analysis by Munter et al. found a 21% decreased risk of type 2 diabetes when intakes of whole grains were increased to two servings each day (de Munter et al., 2007).

**Types of Whole Grains**

Wheat is the most commonly consumed whole grain in the United States, with corn, oats, rice and barley close behind (Jonnalagadda et al., 2011). A comparison of the macronutrient composition of these common whole grains is shown in Table 1. Roughly 600 million tons of wheat is harvested each year around the world, making it one of the most popular cereal grains (Shewry, 2009). Wheat has been a dietary staple for about 10,000 years with over 25,000 genetically diverse cultivated forms (Shewry, 2009). Currently, about 95% of wheat in the world is a hybridized form called hexaploid wheat, which is also called “pasta wheat” or “bread wheat” (Shewry, 2009). The uses of wheat in a westernized diet are limitless, from breads and pastries to pastas and pizzas. Wheat provides strong, soft and elastic properties to dough, characteristics that make it a profoundly useful and unique from other cereal grains. These distinct properties are a result of the imbedded wheat proteins wound together to form gluten.

Corn, also known as maize, is one of the most substantial crops cultivated in the world and has been a dietary staple for over 9,000 years (Blake, 2015). As the cultivation of corn has evolved, it has become dependent on human farming to be planted and nurtured (Blake, 2015). In the United States, corn is currently planted on over 90 million acres, most of which is used as animal feed (USDA Economic Research Service: Crops. May 8, 2018). In the human diet, corn is consumed as a whole vegetable, but is also used

as an ingredient in other processed products. The processing of corn-based products is done either by “wet milling” or “dry milling”, depending on the desired end product (USDA Economic Research Service: Crops. May 8, 2018). High-fructose corn syrup, starch, corn oil and beverage alcohol are examples of products created through wet milling corn (USDA Economic Research Service: Crops. May 8, 2018). On the other hand, cereal flakes, corn flour, corn grits and corn meal are processed through dry milling (USDA Economic Research Service: Crops. May 8, 2018). Corn has historically been produced mainly for human consumption and animal feed, but its value has expanded over the years and is now used to produce ethanol in the form of fuel and alcohol (Blake, 2015).

Oats make up 1% of the total grain produced worldwide, and of that small percentage, 75% is used for animal feed (Tosh & Miller, 2016). The cultivation of oats is thought to have began 4,000-5,000 years ago and is still a prevalent crop in many countries (Tosh & Miller, 2016). Currently, the United States is the most substantial importer of oats worldwide (Tosh & Miller, 2016). Oats are used in the form of oatmeal and oat bran, which are often added to cereals while oat flour may appear in a variety of breads and baked products (Tosh & Miller, 2016). When processing oats, the high content of soluble fiber makes it difficult to get a clean severance between the bran and the endosperm, so oats are generally consumed in whole form (Jacobs & Gallaher, 2004). When broken down by dry weight, starch makes up 57% of oats, protein about 18%, insoluble fiber is 6% and soluble fiber a little more than 5% (Doehlert, 2013). Compared to wheat, barley and rye, oats are typically higher in some micronutrients such as

calcium, magnesium, zinc and folate, but often have lower niacin content (Tosh & Miller, 2016).

Cultivating the wild grass ancestors of rice for human consumption traces back more than 10,000 years (Kovach, Sweeney, & Mccouch, 2007). Different variations of rice are used as major meal components for more than half of the worldwide population (Batres-Marquez, Jensen, & Upton, 2009; Cheajesadagul, Shiowatana, Siripinyanond, & Szpunar, 2013; Setia, 1994; USDA Economic Research Service: Rice. September 20, 2018). Rice is consumed and available in the United States as a whole grain (brown rice) or a refined grain (white rice) (Batres-Marquez et al., 2009). The germ and bran portions of brown rice are removed to form white rice, utilizing only the endosperm of the original whole grain (Lamberts et al., 2007). Rice is often found in processed food products like breakfast cereals, crackers, soup, baby food and a variety of snack items (Setia, 1994). Rice products range from rice noodles and rice paper to rice vinegar and rice milk. Similar to corn, rice does not contain gluten as a protein, as do wheat and barley, so it is often used as gluten-free flour to substitute wheat flour in recipes for those following a gluten-free diet.

Barley has been grown and used as an ancient grain for human nourishment since 8,000 BC, but within the last couple centuries its use has shifted predominately to incorporation into animal feed (Aldughpassi, Wolever, & Abdel-Aal, 2016). Currently, 2-3% of the barley cultivated around the world is used for human intake, while 65% is incorporated into animal feed and the remainder is used as malting and brewing grain (Aldughpassi et al., 2016). “Pearling” is the most common technique used to process barley and involves the outer layers being removed (Aldughpassi et al., 2016). Barley can

be milled into flour or bran, and can be used to make products like muffins, pastas and noodles (Aldughpassi et al., 2016). Starch is the greatest component of barley, roughly 60-70% of dry matter (Aldughpassi et al., 2016). Fiber is the second largest component, 11-34%, followed by protein, which makes up 10-20% of dry matter (Aldughpassi et al., 2016). Like wheat, one of the grain proteins found in barley is gluten.

Table 1: Whole Grain Macronutrient Comparison (g/100 g)

| *Whole Grain Type* | Total Carbohydrates *(g/100g)* | Total Protein *(g/100g)* | Total Lipid Fat *(g/100g)* | Total Fiber *(g/100g)* |
|---|---|---|---|---|
| Wheat, Soft White | 75.4 | 10.7 | 2 | 12.7 |
| Corn, Yellow | 74.3 | 9.4 | 4.7 | 7.3 |
| Oats | 67.7 | 13.2 | 6.5 | 10.1 |
| Brown Rice, Long Grain | 76.3 | 7.5 | 3.2 | 3.6 |
| Barley, Hulled | 73.5 | 12.5 | 2.3 | 17.3 |

Source of nutritional information: USDA, Agriculture Research Service. USDA food composition database.

**Grain Protein, Gluten**

Gluten is a storage protein found in the endosperm of grain cells that is made up of gliadins and glutenins, and is naturally found in wheat, barley and rye (Aziz et al., 2015; Shewry, Halford, Belton, & Tatham, 2002). With the addition of water these two proteins, gliadins and glutenins, intertwine and create a strong yet flexible network. Gluten is made up of about 80% protein that twists and interlocks the remaining 20%, which is thought to be trapped starch (Shewry, 2009). The structure of gluten is quite

complex and involves an intricate mixture of over 50 individual protein ingredients (Shewry et al., 2002). Proline is an amino acid that is very prevalent in gluten and provides unique characteristics attributing to the elasticity and digestibility (Colgrave, Byrne, & Howitt, 2017). The structure of proline allows it to be resistant to digestion, where proteases are unable to reach and effectively breakdown the protein (Colgrave et al., 2017). Therefore, gluten travels into the small intestine before being digested, where it can cause problems for populations with gluten-related disorders. The biological function of gluten is to protect and carry carbon, nitrogen and sulfur in the endosperm of grain cells in order to sustain seed growth and development (Shewry et al., 2002). The most desirable characteristic of gluten seems to be its viscoelasticity, giving sticky and soft properties to dough and pasta when flour is mixed with water (Colgrave et al., 2017). The intricate network of proteins in gluten work to trap air and carbon dioxide, which cause the dough to rise and adds texture to various baked goods (Shewry, 2009).

Westernized diets are built around wheat as a staple, with many other processed foods using gluten as a thickener or binder to improve texture in food (Aziz et al., 2015; Singh & Whelan, 2011). Naturally found in plant seeds, gluten has been extracted and used as an additive to stabilize processed food and a supplement in low-protein food (Aziz et al., 2015). Research has found wheat to be one of the leading factors related to gastrointestinal symptoms, but there is much more to wheat than gluten alone (Biesiekierski, 2011). Grains are differentiated by their protein sequence, making certain grains safe for individuals with gluten-mediated immune responses, allergies and sensitivities (Lee et al., 2009). Grains often found as part of a gluten-free diet include rice, oats, corn, buckwheat, quinoa and millet (Lee et al., 2009). Populations that follow a

gluten-free diet as a form of treatment for disease or illness include those with celiac disease, a gluten sensitivity or a wheat allergy (Balakireva & Zamyatnin, 2016; Pietzak & Kerner, 2012).

**Gluten-Related Disorders, Celiac Disease**

Celiac disease is a genetic autoimmune disease of the gastrointestinal system that, without treatment, can lead to further disease and cancers of the gastrointestinal tract (Briani, Samaroo, & Alaedini, 2008). Celiac disease is multifactorial, meaning there is a genetic component and an environmental component that together cause symptoms. On the genetic side, individuals with celiac disease are predisposed to HLA-DQ2 and/or HLA DQ8 genotypes, which means they are more likely to develop celiac disease (Aziz et al., 2015; Balakireva & Zamyatnin, 2016). On the environmental side of this multifactorial disease is the consumption of gluten. Combining the genetic factor with the environmental trigger of gluten results in a T-cell reaction in which antibodies are produced followed by the release of damaging inflammatory cytokines (Abadie & Jabri, 2014). In response to gluten consumption, an individual with celiac disease will experience an intestinal inflammation and antibody production, resulting in the microvilli atrophy and altered intestinal absorption (Aziz et al., 2015; Lee et al., 2009). Inflammation is an immune response, in this case to gluten, which can become persistent, or chronic, after long-term repeated exposure (Punder & Pruimboom, 2013). Chronic inflammation has been found to be positively correlated with cardiovascular disease, cancer and other autoimmune diseases (Punder & Pruimboom, 2013).

Roughly 1% of the worldwide population is currently affected by celiac disease (Balakireva & Zamyatnin, 2016; Singh & Whelan, 2011). Celiac disease is typically

diagnosed by observing the antibodies within an individual's serum and other bodily fluids (Husby et al., 2012). Currently, because there is no cure for celiac disease, the only management and treatment is to follow a strict, lifelong gluten-free diet (Briani et al., 2008; Lee et al., 2009). Incompliance with a strict gluten-free diet causes damage to the intestinal mucosa, hindering adequate nutrient absorption (Singh & Whelan, 2011). Microvilli line the mucosal cells of the small intestine, acting like fingers picking up and absorbing nutrients. The delicate microvilli are damaged during inflammation, causing them to flatten against the intestinal wall. This flattening not only damages the microvilli, it hinders nutrient absorption resulting in gastrointestinal symptoms like persistent diarrhea and rapid weight loss. Early implications of weight loss and nutritional deficiencies due to dietary incompliance can evolve into long-term irreversible damage. Repeated damage has been linked to increased risk for osteoporosis, mucosal damage and cancer (Stuckey, Lowdon, & Howdle, 2009).

**Medical Condition Associated with Celiac Disease, Osteoporosis**

Research has found a positive correlation between celiac disease and low bone mineral density, osteopenia and osteoporosis (Choudhary, Gupta, & Beniwal, 2017). Intestinal damage and impaired absorption are consequences of celiac disease as mucosal cells are damaged, which results in reduced absorption of calcium and vitamin D (Larussa et al., 2012). For children who are not yet diagnosed with celiac disease, it can be implied that this population would possibly have reduced growth and bone strength. As those with celiac disease age, they are likely to experience bone fragility and fractures if they have been incompliant with the strict gluten-free diet or have permanent mucosal cell damage in the small intestine (Larussa et al., 2012). Research has found that bone

health can be maintained, and even improved, with the implementation of and compliance to a gluten-free diet in these individuals (Kalayci, Kansu, Girgin, Kucuk, & Aras, 2001; Mora et al., 1998). For most patients, bone mineral density can be rehabilitated within a year of adherence to a gluten-free diet (Choudhary et al., 2017). The earlier that celiac disease is diagnosed, and the level of compliance with a gluten-free diet, will determine the potential skeletal damage within this population. The impact on bone health is of concern for individuals with celiac disease as well as individuals with type 1 diabetes (Simmons et al., 2016; Szymczak, Bohdanowicz-Pawlak, Waszczuk, & Jakubowska, 2012).

**Medical Condition Associated with Celiac Disease, Type 1 Diabetes**

Research has shown that over 10% of individuals diagnosed with type 1 diabetes test positive for the serological marker used to diagnose celiac disease (Rewers, Liu, Simmons, Redondo, & Hoffenberg, 2004). Roughly 25% of individuals who have one autoimmune disorder, such as celiac disease, will develop a second autoimmune disease, such as type 1 diabetes (Mohan & Ramesh, 2003). When an individual is genetically susceptible to autoimmune conditions (roughly 18% of adults), various environmental factors can disturb immune regulation, resulting in additional immune mediated responses (Cojocaru, Cojocaru, & Silosi, 2010). For individuals experiencing two autoimmune disorders, such as celiac disease and type 1 diabetes, dietary restrictions and food processing methods must be considered. If a gluten-free diet is being followed, individuals with type 1 diabetes should be mindful of what ingredients are used to replace gluten in their diet. As mentioned above, the elimination of gluten has been found to reduce the digestion and absorption of starch, which may result in a spike in blood

glucose (Smith et al., 2015). Since this population does not produce insulin, the knowledge and understanding of ingredients in a gluten-free diet will be crucial to avoid hyperglycemia and possible nutrient deficiencies.

**Medical Condition Associated with Celiac Disease, Cancer**

For individuals with celiac disease who go undiagnosed for a portion of their life, or for those who do not properly follow a gluten-free diet, damage to intestinal mucosa may be significant. This long-term damage is thought to increase intestinal permeability, allowing ingested carcinogens the chance to proliferate and wreak havoc on the gastrointestinal system (Elfström, Granath, Ye, & Ludvigsson, 2012). This increased permeability might also be a result of chronic inflammation and antigen stimulation, paired with inconsistent immune responses and nutritional deficiencies (P. H. R. Green & Jabri, 2002). Some research has found that individuals with celiac disease in the United States have a heightened risk of certain types of cancer compared to the general population (P. H. Green et al., 2003). Other research concluded that a diagnosis of celiac disease comes with a predisposition for the development of gastrointestinal cancers, including lymphoma, esophageal carcinoma, oropharangeal carcinoma and small bowel adenocarcinoma (P. H. Green et al., 2003; Rostami Nejad et al., 2013). Additional research found parallels between celiac disease and an increased risk of melanoma, but these findings have not yet been investigated or explained (P. H. Green et al., 2003). In some cases, the cancer is diagnosed before celiac disease, and in others it comes after many years of being diagnosed with celiac disease, even in those who follow a strict gluten-free diet (P. H. Green et al., 2003). Either way, adherence to a gluten-free diet in

managing gastrointestinal health when diagnosed with celiac disease is imperative for preventing further life-altering complications, such as cancer and infertility.

### Medical Condition Associated with Celiac Disease, Infertility

After 12 consecutive months of attempting to conceive, failure to consummate clinical pregnancy is considered infertility (Balen & Rutherford, 2007). Research has found that women with undiagnosed and untreated celiac disease have a higher risk of infertility (Lasa, Zubiaurre, & Soifer, 2014). Celiac disease has also been associated with other pregnancy disorders, including low birth weights (Martinelli et al., 2000). Therefore, diminished nutrient absorption in women with celiac disease may result in deficient infant nutrition and growth. Celiac disease is related to many intestinal disorders, cancers and diseases but research has not yet concluded why this relationship exists between celiac disease and infertility (Lasa et al., 2014). Accepting and following a gluten-free diet may be a preventative and necessary measure for women with celiac disease to avoid the risk of infertility (Lasa et al., 2014).

### Gluten-Related Disorders, Gluten Sensitivity

Gluten sensitivity, also known as non-celiac gluten sensitivity, is thought to be a genetic immunologic reaction to gluten without the defining features of celiac disease (Hadjivassiliou, Grünewald, & Davies-Jones, 2002; Troncone & Jabri, 2011). A diagnosis of gluten sensitivity is more subjective than a diagnosis of celiac disease, contributing to the amount of individuals self-diagnosing and treating with a gluten-free diet (Gaesser & Angadi, 2012). Typically, a diagnosis of gluten-sensitivity follows the prescription of a gluten-free diet and improved symptoms related to this dietary change (Kerner & Pietzak, 2012). The lack in specificity for gluten sensitivity as a diagnosis

opens the door to self-diagnosis, self-prescription of a gluten-free diet and possible placebo effects (D. M. Lis, Stellingwerff, Shing, Ahuja, & Fell, 2015). Symptoms for gluten sensitivity include fatigue, headaches, gas, bloating and diarrhea (Gaesser & Angadi, 2012). These symptoms are similar to celiac disease, but are not likely to attribute to permanent gastrointestinal damage or nutritional deficiencies once gluten is removed from the diet (Kerner & Pietzak, 2012).

**Gluten-Related Disorders, Wheat Allergy**

A wheat allergy is similar to that of celiac disease in that both are immunological reactions. However, only a wheat allergy produces an IgE reaction to gliadins specifically, not to gluten as a whole (Inomata, 2009). Symptoms such as itching and irritation of the mouth, nose and eyes are common symptoms of a wheat allergy when ingested orally, and can advance to hives, anaphylaxis and severe abdominal discomfort (Kerner & Pietzak, 2012). Cramps, gas, bloating and diarrhea are all common gastrointestinal symptoms for individuals with celiac disease as well as those with a wheat allergy, making the initial diagnosis and differentiation difficult (Kerner & Pietzak, 2012). Unlike symptoms of celiac disease, an exposed wheat allergy coupled with physical activity can cause anaphylaxis, resulting in unconsciousness or death if left untreated (Inomata, 2009). Roughly 0.2-4.0% of the general population has been diagnosed with a wheat allergy (Bardella, Elli, & Ferretti, 2016). A gluten-free diet also seems to be a form of nutrition therapy for individuals with a wheat allergy, but in reality this population may not need to eliminate barley and rye, which are associated with a gluten-free diet (Kerner & Pietzak, 2012). A wheat allergy does not necessarily require complete elimination of gluten, but instead requires a wheat-free diet since the

immunological reaction is only a result of wheat-specific proteins (Pietzak & Kerner, 2012). Following a gluten-free diet might be more expensive and more restricting than following a wheat-free diet, so it is important that this is explained to and understood by this particular population.

For those diagnosed with celiac disease, a non-celiac gluten sensitivity or a wheat allergy, adherence to a gluten-free diet as an exclusive treatment can be very difficult and troublesome, particularly when common activities of daily living such as grocery shopping and dining at restaurants become a hassle. For individuals with celiac disease, research has observed that following a gluten-free diet can result in weight change, most often in the direction of weight gain due to the healing of the gastrointestinal tract which increases absorption of nutrients (Cheng, Brar, Lee, & Green, 2010). The heightened burdens of searching for gluten-free options at a grocery store or in restaurants, reading nutritional labels and the increased financial costs of following a gluten-free diet can be cause for incompliance by those diagnosed with celiac disease (Singh & Whelan, 2011). Recent research has found that substituting gluten-containing grains with alternative grains improves the nutritional profile of individuals following a gluten-free diet (Lee et al., 2009). Limited availability of gluten-free food paired with increased financial cost may make it difficult for this population to adhere strictly to a gluten-free diet (Singh & Whelan, 2011).

**Gluten-Free Diet, Composition**

The standard human diet is made up of a combination of carbohydrates, proteins and fats, which are broken down in different ways to provide energy to the body for function and development. Energy from carbohydrates is the preferred form of fuel for

the body and provides most of the energy in the human diet, about 50-65%, from consumption of starches, sugars and fiber (Babio et al., 2017; Newberry et al., 2017). On average, protein provides the second highest amount of energy in the diet, 20-30%, and fat provides around 15-20% of energy needs (Newberry et al., 2017). A gluten-free diet, on the other hand, manipulates this standard division of energy sources. Specifically, a gluten-free diet is often lower in carbohydrates altogether, and the carbohydrates that are consumed are often higher in sugar and lower in fiber (Alvarez-Jubete, Arendt, & Gallagher, 2009; Babio et al., 2017; Newberry et al., 2017). In addition to the obvious digestive regularity benefits of a high fiber diet, the fiber content in whole grain products extends satiety, by means of increased food density, while also producing a lower glycemic response (Jonnalagadda et al., 2011). The removal of gluten from the diet has been positively correlated with lower protein intake (Estevez, Ayala, Vespa, & Araya, 2016). This is of particular concern in vegetarian populations where protein intake typically comes from grains and plant-based sources. Fat intake has been found to increase when following a gluten-free diet because added fat is often part of the gluten-replacement to make up for the texture and palatability otherwise provided by gluten (Babio et al., 2017; Ferrara et al., 2009). On a micronutrient level, gluten-free food products lack sufficient amounts of thiamin, riboflavin, niacin, folate and iron since gluten-free products are often made with refined flours and are not fortified (Thompson, 1999; Thompson, 2000). Recent research has also seen a correlation between a gluten-free diet and inadequate calcium intake, resulting in deficient bone mineral content (Thompson, Dennis, Higgins, Lee, & Sharrett, 2005). Gluten-free products not only

change the nutritional content, but these replacement ingredients may alter the taste, texture and cost, compared to the gluten-containing counterpart.

**Gluten-Free Diet, Replacement Ingredients**

Some common replacement ingredients for wheat flour in gluten-free breads, pastas, pizza crusts and other processed products include different types of flour made from rice, almonds, oats, tapioca, sorghum, potatoes, corn, buckwheat, chickpeas, coconut and millet. Wheat starch is another possible substitute for gluten-containing products, but it is not often seen in commercial gluten-free products, possibly due to the risk of gluten-residues feared by consumers (FDA Guidance for Industry: Gluten-free labeling of foods; small entity compliance guide. September 16, 2018). The FDA considers a product with wheat starch to be "gluten-free" as long as the final product contains less than 20 parts per million gluten residues (FDA Guidance for Industry: Gluten-free labeling of foods; small entity compliance guide. September 16, 2018). It is also common to see amaranth, cornstarch, rice starch, potato starch, tapioca starch, flax seed, rice bran, quinoa, chia seeds, millet seeds, dates, date paste and eggs as additional ingredients in commercial gluten-free products.

Some of the most desirable characteristics of bread, pasta and other baked products are the structure, texture and taste, which are largely attributed to the gluten in the flour (Gallagher, Gormley, & Arendt, 2003). Gluten-free batter and dough is thinner and lacks the appealing elasticity and structure of products made with wheat flour (Foschia, Horstmann, Arendt, & Zannini, 2016; Gallagher, Gormley, & Arendt, 2004). Many of the products made without gluten often appear dull in color, more dense and tend to crumble apart more easily, which has led manufacturers to add different

ingredients to improve taste, texture and nutritional content in commercial gluten-free products (Foschia et al., 2016; Gallagher et al., 2004). Water retention, thickening and texture improvements can be made in gluten-free products by adding hydrocolloids, otherwise known as food gums, such as guar gum, xanthan gum and locust bean gum to help increase the volume of the product as well as crumb durability (Gallagher et al., 2004). A hydrocolloid is a long chain polymer that forms a gel when water is added, thereby increasing thickness, stability and gelling properties to the final product (Sciarini, Ribotta, León, & Pérez, 2010). The most common used hydrocolloid in food production is starch (Saha & Bhattacharya, 2010). The use of hydrocolloids, or gums, in gluten-free baking improves texture and dough viscosity (Saha & Bhattacharya, 2010).

Dairy proteins are similar to gluten in that they form strong, flexible matrices that help to maintain the structure in baked products (Hamaker Bruce, 2008). The addition of dairy in gluten-free products has been found to increase water absorption, which improves the shape and function of gluten-free dough (Gallagher et al., 2004). Protein fortification from dairy sources like sodium caseinate and milk protein isolate also help to give strength and structure to the dough of baked products, thereby improving the texture and color (Gallagher et al., 2004; Hamaker Bruce, 2008).

Historically, commercial gluten-free packaged products were primarily composed of carbohydrates, limiting the amount of protein intake when following a gluten-free diet (Matos Segura & Rosell, 2011). The addition of high-protein dairy powders has been found to drastically increase the protein content in gluten-free products (Gallagher et al., 2003; Gallagher et al., 2004). More recently, the introduction of legume flours (e.g. chickpea, lentil, lea and bean) have been used to increase the protein and fiber content of

gluten-free bakery items, while also increasing viscosity, volume and texture (Gularte, Gómez, & Rosell, 2012). Studies have also found that egg protein is a beneficial addition to gluten-free baked products, as it improves loaf mass and crumb structure while also increasing protein content (Crockett, Ie, & Vodovotz, 2011). The creation and evolution of gluten-free products with a balance of nutritional density and palatability is still being explored and researched.

**Gluten-Free Diet, Cost**

What the gluten-free diet often lacks in macro and micronutrients, it makes up for in cost. Previous research compared standard everyday food and found that some wheat-based gluten-free alternatives cost 76-518% more than the original product containing gluten (Singh & Whelan, 2011). A study by Lee et al. compared the cost of commercially available gluten-free products with similar gluten-containing products in the United States and found that the gluten-free items were all more expensive (Lee, Ng, Zivin, & Green, 2007). This increase in price is likely due to the need for alternative, more expensive, grains and ingredients, coupled with increased production costs since it takes more time and resources to engineer a gluten-free product that is similar to the original gluten-containing item.

The higher cost associated with gluten-free products may be especially impactful for low-income households or individuals who are prescribed a gluten-free diet as the only form of treatment for celiac disease. According to the Celiac Disease Foundation, if a family member is diagnosed with celiac disease the costs of a gluten-free diet may be claimed as a medical expense when itemizing deductions (Celiac Disease Foundation: Tax deductions for celiac disease.). This option may be beneficial for some but the

burdens of keeping all receipts for gluten-free items and applying for the deduction may be too cumbersome for others. For individuals who follow a gluten-free diet as a way of treating their gluten-related illness, the price and limited availability may significantly contribute to diet incompliance (Singh & Whelan, 2011).

### Gluten-Free Diet, Without a Gluten-Related Disorder

Previous market research has shown that the majority of individuals following a gluten-free diet are doing so by choice without being guided or instructed by their doctor (DiGiacomo, 2013). Many consumers who follow a gluten-free diet choose gluten-free products because they believe such products are healthier than the original product containing gluten (D. M. Lis et al., 2015; Marcason, 2011). Not only are gluten-free foods being marketed as being better for health, but celebrity endorsements are encouraging a gluten-free diet for weight loss (Gaesser & Angadi, 2012). Yet, no evidence-based research that has found that a gluten-free diet promotes weight loss in general populations (Gaesser & Angadi, 2012).

Contrary to popular belief, a gluten-free diet is not synonymous with low-energy foods, and many gluten-free products are lower in fiber and whole grains than their gluten-containing counterparts (Gaesser & Angadi, 2012). Other populations that have recently adopted gluten-free diets include athletes who believe eliminating gluten will result in better performance and decreased fatigue (D. M. Lis et al., 2015). Again, there is no evidence to support these beliefs (D. M. Lis et al., 2015). A gluten-free diet may in fact have adverse effects due to the diet restriction, including nutritional deficiencies and an inflation in food costs (Lee et al., 2009; D. M. Lis et al., 2015; Stevens & Rashid, 2008). Many gluten-free products are not fortified or naturally rich in certain nutrients,

contributing to potential dietary deficiencies in vitamin B12, iron, calcium, folate and fiber (Vici et al., 2016). The significant increase in interest and adherence to a gluten-free diet for the general population based solely on the belief that gluten is unhealthy, has never been published or proven through experimental research (Gaesser & Angadi, 2012). Rather, studies have found that gluten is beneficial and important in the diet for the general population, which discourages against avoiding gluten altogether (Gaesser & Angadi, 2012).

**Dietary Carbohydrates, Classification and Metabolism**

The avoidance of gluten often coincides with restricting other sources of carbohydrates in the diet, such as breads and pastas, which typically contain beneficial vitamins, minerals and useful energy for the body (Maughan, 2013). Carbohydrates provide four kilocalories per gram, and since carbohydrates account for about half of the total energy intake, it is important to understand the classification and use of carbohydrates in the diet (Macdonald, 1999; Maughan, 2013). Glucose, fructose and galactose are monosaccharide forms of carbohydrates, also known as "simple sugars" since they are the most basic and reduced form of carbohydrate and cannot be further digested (Maughan, 2013). Maltose, sucrose and lactose are disaccharide forms of carbohydrates because they have two monosaccharide units bonded together (Maughan, 2013). Olgiosaccharides are made up of three to ten saccharide units joined together by covalent bonds, while polysaccharides are made up of over ten sugar molecules in the form of a long chain (Chibbar, Jaiswal, Gangola, & Båga, 2016; Gropper, 2009; Maughan, 2013).

Carbohydrate digestion involves the breaking of these bonds between saccharide units by a water-based process called hydrolysis (Gropper, 2009). This process begins in the mouth with the use of salivary alpha-amylase, continues in the acidic environment of the stomach and is greatest in the upper part of the small intestine (Maughan, 2013). This is due to the release of brush border membrane enzymes, which are designed to break the bonds between saccharide units, allowing absorption of carbohydrates in the most basic form (Goodman, 2010; Maughan, 2013). The primary carbohydrate absorbed from the diet is in the form of glucose, by way of the SGLT1 (sodium-glucose co-transporter 1) membrane protein (Macdonald, 1999). Other forms of monosaccharides are absorbed through other glucose transporters but are often converted to glucose by hepatic or intestinal cells before absorption (Macdonald, 1999). Once absorbed, glucose is transported through the blood before being taken up into tissue cells by the release of insulin, a hormone produced by the pancreas. Glucose may then be transformed into adenosine triphosphate (ATP) and used as energy for all metabolic processes in the body or may be moved to the liver and skeletal muscle to be stored as glycogen (Maughan, 2013). These reserves are short-term storage, and can be tapped into when blood sugars are depleted to ensure the body has the energy it needs to perform functions.

Any intake of glucose greater than the endogenous storage capacity is used to make energy through the citric acid cycle (Maughan, 2013). If the carbohydrate intake is greater than the total energy requirement, saccharide units can be stored long-term as fatty acids in adipose tissue (Maughan, 2013). The body works to maintain blood glucose levels by absorbing exogenous glucose from carbohydrate sources in the digestive tract, and utilizing stored glycogen in liver to create endogenous glucose (Macdonald, 1999). In

the event of a fast, the body will utilize endogenous glucose production as a way of maintaining homeostasis and providing the body with enough energy to function normally (Macdonald, 1999). Exogenous sources of carbohydrates come in a variety of forms in the human diet, including fruits, vegetables, grains, dairy, seeds and legumes.

**Dietary Carbohydrates, Sources and Glycemic Response**

Dietary carbohydrates are primarily in the form of starch, a polysaccharide, and simple sugars, like sucrose (Gropper, 2009; Macdonald, 1999). Starches make up roughly half of the carbohydrates consumed in the human diet and are typically in the form of cereal grains and vegetables (Gropper, 2009). Simple carbohydrates include sucrose, fructose, maltose and lactose. Sucrose is a disaccharide composed of glucose and fructose that makes up about one-third of total carbohydrate intake. (Gropper, 2009). Simple carbohydrates, monosaccharides and disaccharides, enter the bloodstream quickly since they do not require digestion, which causes blood sugar to spike dramatically. These types of carbohydrates naturally give sweetness to foods, like fruit, but are also added to soda, candy and other processed foods (Paul, INSEL, & WALTON, 2001).

Complex carbohydrates, oligosaccharides and polysaccharides, are longer chains of sugar, like starches, and contain additional fiber, vitamins and minerals (Chibbar et al., 2016). Glycogen is an important polysaccharide found in certain animal tissues, while starch and cellulose are essential polysaccharides found in plants (Chibbar et al., 2016; Gropper, 2009). Since these types of carbohydrates have longer glucose chains, they provide more energy and slowly enter the bloodstream upon digestion. Starches and fibers are found in many different types of grains, legumes, tubers, vegetables and fruit (Paul et al., 2001). Complex carbohydrates can be classified as either refined or

unrefined. Refined carbohydrates are characterized by processing, while unrefined carbohydrates are whole grains (Paul et al., 2001). Refined grains have lower amounts of fiber, vitamins and minerals, compared to unrefined, whole grains (Paul et al., 2001). Whole grains typically take longer to chew and digest, and make consumers feel fuller, longer. This is largely due to the higher fiber content, compared to refined carbohydrates, which are digested and enter the bloodstream quickly, causing a greater spike in blood sugar.

**Glycemic Response, Glycemic Index**

Glycemic response is the change in the concentration of glucose in the blood following consumption of a carbohydrate source (Augustin et al., 2015). The postprandial glycemic reaction is displayed as an incremental area under the curve (iAUC) that begins with a fasting state and maps out blood glucose concentrations for a few hours after meal consumption. iAUC displays the concentration of glucose in the blood across time and shows how quickly glucose entered and left the bloodstream. iAUC allows conclusions to be made about different carbohydrate sources as it relates to postprandial glycemia.

In order to better understand and predict patterns in blood glucose levels following a meal, a glycemic index was designed to classify different carbohydrate-rich foods. The glycemic index is a scale that ranks food from 1 to 100 based on how much the blood glucose concentration rises after consumption of that food. Glycemic index is determined by measuring the glycemic response following the intake of 50 grams of carbohydrates within a specific food, typically 50 grams of glucose (Frost & Dornhorst, 2013). A high glycemic index food is digested and absorbed quickly, with a glycemic index score of 70 or greater on a glucose scale (Augustin et al., 2015). Foods with a high

glycemic index provide a rapid release of sugar into the blood, meaning there is minimal to no digestion needed. On the other hand, low glycemic index foods are digested and absorbed at a reduced rate, with a glycemic index score of 55 or less on a glucose scale (Augustin et al., 2015). The glycemic index provides an idea of how blood glucose will be affected following consumption of a specific food, but it does not take into consideration a realistic portion size.

Glycemic load differs from glycemic index because it represents the concentrated amount of carbohydrates in that particular food and the glycemic index of that food (GL = GI x available carbohydrate/given amount of food) (Augustin et al., 2015). In this sense, the glycemic load looks at both the quality and quantity of the carbohydrate-containing product that is consumed.

Interestingly, two unique foods can have the same number of carbohydrates but completely different glycemic responses. The glycemic index of individual foods or products are influenced and determined by a variety of factors, including the presence and quantity of fat, protein, fiber as well as the method in which the food was prepared (Macdonald, 1999). When carbohydrates are consumed with fat, protein and fiber, the meal sits in the stomach longer, reducing the rate of absorption and lowering the overall glycemic index (Macdonald, 1999). In regards to preparation, the method of cooking can alter the digestibility of starch and improve the ease of digestion compared to the original unaltered food (Macdonald, 1999). Research has shown that processing foods by way of extrusion cooking, explosion puffing and instantiation reduces the rate of starch digestion, therefore increasing glycemic index (Brand, 1985).

### Glycemic Response, Relation to Disease

Individuals with diabetes are encouraged to consume foods that have a low glycemic index to aid in the management of blood sugar levels (Macdonald, 1999). While the body maintains blood glucose levels by intestinal absorption and liver glycogen breakdown, insulin also plays a critical role in the delicate balance between hyperglycemia and hypoglycemia (Gropper, 2009). Insulin is a multi-purpose hormone secreted by the pancreas that inhibits gluconeogenesis by the liver, while also binding to specific cellular receptors that allow glucose to enter tissue cells throughout the body, reducing blood sugar levels (Gropper, 2009). Insulin also signals the conversion and storage of glycogen in the liver and muscle tissue (Macdonald, 1999). Insulin activity and sensitivity can vary between individuals for a variety of reasons, including overall health, weight, carbohydrate intake, frequency of physical activity and individual glycogen stores (Macdonald, 1999).

Glucagon is another hormone secreted by the pancreas, but its role is the opposite of insulin as it is released when blood sugar is too low, telling the body to eat something with sugar. Together these hormones work to naturally balance blood sugar levels throughout the day. Insulin resistance takes place when insulin receptors on cells no longer respond to the presence of insulin, which means glucose stays in the blood longer and can not be converted to energy in the cells. Insulin resistance and unmanaged postprandial glucose can eventually result in type 2 diabetes mellitus.

Cardiovascular risk factors have been positively linked to high GI foods (Frost & Dornhorst, 2013). When food is ingested by a healthy adult, there is an increase in cardiac output alongside the movement of blood from the limbs to the gastrointestinal

system (Macdonald, 1999). Together these systems work to maintain blood pressure throughout the body. Excess glucose in the blood is damaging to endothelial tissues and can increase risk of vascular damage, neuropathy and kidney problems (Gopi, Bir, & Kevil, 2012). Carbohydrates present in a meal increase cardiac output and can cause hypotensive problems in certain populations, like the elderly, when there is an abnormal or delayed autonomic nervous system response (Macdonald, 1999). The systems that work together to move blood throughout the body to maintain blood pressure before, during and after a meal, may be delayed in these populations, resulting in hypotension after a meal high in carbohydrates (Macdonald, 1999).

**Glycemic Response, Gluten-Free Products**

The glycemic response for many gluten-free products is yet to be determined. Theoretically, the removal of gluten would be associated with a higher glycemic response since the gluten network encapsulates starch granules acting as a strong obstacle to starch digestion (Smith et al., 2015). The ease with which starch is digested in different products determines the rise in blood glucose and, therefore, an increased glycemic index associated with that product. The processing of gluten-free products varies from standard products containing gluten and can influence the rate of starch digestion (Berti et al., 2004). Research has found that the additional processing required for gluten removal from products made with wheat flour increases the digestibility of starch in vitro (Berti et al., 2004). Although, the results between starch digestion in vitro does not always align with how starch digestion is impacted from the processing measures in gluten-free products (Berti et al., 2004).

# CHAPTER 3

# METHODS

## Participants

Upon approval from the Institutional Review Board (IRB), participants were recruited from departmental listservs from Arizona State University and the local community in Phoenix, Arizona by way of flyers, emails and word of mouth. Interested individuals were directed to SurveyMonkey to complete a questionnaire and determine initial eligibility. Power calculations based on three published crossover trials in healthy adults established that 19 participants were needed for this study to provide 80% power to detect a significant difference in AUC (Appendix B). Participants were between the ages of 18-45, free from any diagnosed gastrointestinal disorders, celiac disease, or diabetes, and had stable medication use. Individuals were excluded if they had any known food intolerances or allergies to any of the ingredients in the test breads. If initial eligibility was approved, an appointment with the researcher was made to further assess eligibility, sign a consent form and schedule subsequent appointments. At this time participants were provided with more detailed information about the study, including the length of the trial, data collection, protection procedures, potential risks and benefits, as well as additional contact information of the researchers. Qualifying individuals were required to be available for 2.5 hours every week, for three weeks, arriving between the hours of 7:00-9:00am at the Arizona Biomedical Collaborative (ABC) building (425 N. 5$^{th}$ Street, Phoenix, AZ 85004). At least one researcher was present at all appointments to answer additional questions. The Arizona State University Institutional Review Board approved this study, and written informed consent was obtained from each participant.

### Study Design and Procedure

This study was conducted as a 3-week, randomized, single blind crossover study (Appendix A). After meeting with the researcher and signing the consent form, each participant scheduled their 3 appointments approximately 7 days apart. Participants were randomly assigned to complete the 3 different treatment trials over the 3-week length of the study using a 3x3 block design. Two of the treatments were gluten-free breads, one made with potato and fava bean flour and the other with rice flour (see Table 2). The third treatment was traditional wheat bread containing gluten. This study protocol was mirrored after the work of Johnston, et al., in their research that studied the effects of gluten-free pasta and conventional wheat pasta on postprandial glycemia (Johnston, Snyder, & Smith, 2017). Participants were asked to consume one bagel every day for the two days leading up to each appointment, and on the night before their appointment they were to consume an additional bagel at the evening meal before fasting for 8-10 hours. Fasting was specified as a restriction of all food and beverages, except water. Participants were also asked to refrain from moderate to heavy activity the day before each appointment. Outside of bagel consumption, participants maintained their regular diets and physical activity. Eating bagels in this capacity, paired with limited activity the day before each appointment, was designed to saturate individual glycogen stores. In this way, glycogen stores were controlled between participants. This allowed any observed differences to be likely due to the treatment. Blood glucose levels were measured 5 times over the course of each appointment. To encourage retention, a $10 gift card was given to each participant at the second and third appointments, totaling $20 for participation.

Table 2: Comparison of Ingredients in Breads Used

| | Ingredients | Weight (grams) | Calories | Carbohydrates (grams) | Protein (grams) | Fat (grams) | Fiber (grams) | Sugar (grams) |
|---|---|---|---|---|---|---|---|---|
| **1. Oroweat Country White Bread** | **Unbleached Enriched Wheat Flour** [Flour, Malted Barley Flour, Reduced Iron, Niacin, Thiamin Mononitrate (Vitamin B1), Riboflavin (Vitamin B2), Folic Acid], Water, Sugar, Soybean Oil, Yeast, Salt, Preservatives (Calcium Propionate, Sorbic Acid), Monoglycerides, Cellulose Gum, Calcium Sulfate, Grain Vinegar, Datem, Soy Lecithin, Wheat Gluten, Soy, Whey (Milk). | 38 | 100 | 19 | 3 | 1.5 | 0.8 | 2 |
| | **3 Slices** | **114** | **300** | **57.0** | **9.0** | **4.5** | **2.4** | **6.0** |
| **2. Katz Gluten-Free Wholesome Bread** | **Gluten Free Flour (Potato, Fava Bean, Garbanzo Bean, Teff, Corn, Chia Seed, Flax Seeds**), Water, Canola Oil, Eggs, Honey, Salt, Xanthan Gum, Dry Yeast | 35 | 90 | 17 | 1 | 2.5 | 1 | 1 |
| | **3.4 Slices** | **119** | **306** | **57.8** | **3.4** | **8.5** | **3.4** | **3.4** |
| **3. Canyon Bakehouse Mountain White** | Water, **Brown Rice Flour, Tapioca Flour, Whole Grain Sorghum Flour**, Organic Agave Syrup, Extra Virgin Olive Oil, Xanthan Gum, Organic Cane Sugar, Eggs, Egg Whites, Yeast, Sea Salt, Cultured Brown Rice Flour, Organic Cane Sugar Vinegar, Enzymes. | 34 | 90 | 16 | 2 | 1.5 | 1 | 2 |
| | **3.6 Slices** | **121** | **321** | **57.1** | **7.1** | **5.4** | **3.6** | **7.1** |

*Manufacturer: 1= Bimbo Bakeries USA, Inc., 2 = Gluten Free Bake Shoppe Inc., 3= Canyon Bakehouse LLC. Price: 1=$2.98 /loaf, 2= $7.99/loaf, 3= $5.00/loaf*

**Surveys**

In order to better understand the general knowledge and beliefs pertaining to a gluten-free diet, a preliminary survey was sent out to the general public through social media (Facebook) and email addresses from Arizona State University listservs (Appendix D).

**Study Variables**

The independent variable in this study was the type of bread consumed at each appointment. The different types of breads were made with wheat flour (gluten-containing), rice flour or potato-fava bean flour, and all breads were commercially available (Table 2). The amount of bread and jelly fed to participants varied to ensure equal energy, carbohydrate and fat consumption at each treatment condition. The dependent variable in this study was blood glucose, or more specifically, postprandial glycemia. It was expected that postprandial glycemia would be higher after consumption of the gluten-free products.

**Laboratory Analysis**

Participants arrived on their scheduled days in a fasting state between 7:00 and 9:00 a.m. Blood glucose levels were taken using portable glucose check monitors (Accu-Check Advantage Blood Glucose Monitoring System, Roche Diagnostics, Indianapolis, IN). Each participant was assigned a glucometer to use throughout the entire study, and each glucometer was calibrated prior to the start of each feeding trial. Upon arrival, a finger prick of capillary blood was taken to assess a baseline, i.e., fasting, blood glucose concentration. Participants were given 10 minutes to finish their bread, then a finger prick of capillary blood was taken 30, 60, 90 and 120 minutes after the first bite of bread. This

process was repeated for each appointment, with the only difference being the treatment, or type of bread consumed.

**Statistical Analysis**

Data is reported as the mean ± standard deviation. The data from this study was analyzed using the Statistical Package for Social Science (SPSS) software, version 24 (SPSS Incorporated, Chicago, IL, USA). A p value of $<.05$ will be considered significant in this analysis. Shapiro-Wilk was used to determine the normal distribution of the data, and data was log-transformed if necessary to achieve normality. To assess the difference in means between the three treatment groups a repeated-measures ANOVA (analysis of variance) was used.

# CHAPTER 4

# RESULTS

Recruitment for this research took place in January 2018 by way of an initial online screening survey through SurveyMonkey (www.surveymonkey.com), which was distributed by email through Arizona State University departmental listservs. A total of 60 individuals completed the online survey and 40 people met the participant criteria. Eligible participants reported that their weight had been stable over the last 3 months, they had not been diagnosed with diabetes, pre-diabetes or gastrointestinal disorders, were not vegetarian or vegan and were free of any food allergies. Those who met the criteria were contacted by email to set up an initial appointment. Thirty-one individuals responded in some way to this initial email, and appointments were made with 21 respondents. During the initial appointments, the trial was explained in more detail and participant expectations were acknowledged. In addition, participant height, weight, BMI, body fat and waist circumference were recorded. Nineteen of those respondents who attended an initial appointment signed a consent form for participation in this study. Two participants dropped out of this study due to personal reasons not related to the research protocol. One had a last minute change of schedule, which would not allow her to participate, and the other found out she was pregnant prior to her last trial appointment. The study was then completed and the analysis was carried out on 17 participants: 3 men and 14 women.

Participants enrolled in this trial were between the ages of 19-36 years, with a mean age of 25.2±5.3 years. The height of the participants ranged between 144-181 cm., with an average height of 163.1±7.9 cm. Participant weight was between 47.4-83.6 kg,

with a mean weight of 61.7±10.3, and BMI ranged from 18.6-31.0, with an average of 23.2±2.9. Of the 17 participants who completed the trial, 2 were overweight (BMI=25.5 and 27.6) and 1 was obese (BMI=31.0). Participant waist circumference was between 26.0-40.5 in., with a mean of 29.8±3.5 in. These characteristics and anthropometric measures are displayed on Table 3. Data was normally distributed unless otherwise noted.

Table 3. Participant Characteristics

| Characteristics of Participants *(n=17)* | | | |
|---|---|---|---|
| | | Mean | SD |
| Males; Females | 3; 14 | | |
| Age (years) | | 25.2 | 5.3 |
| Weight (kg) | | 61.7 | 10.3 |
| Height (cm) | | 163.1 | 7.9 |
| Waist Circumference (in) | | 29.8 | 3.5 |
| BMI ($kg/m^2$) | | 23.2 | 2.9 |
| Average Fasting Glucose (mg/dL) | | 90.3 | 7.7 |

Mean glucose concentrations are displayed in Table 4 and are graphed in Figure 1. There was no significant difference seen between the three groups at baseline (p=0.920), with a mean fasting glucose of 90.3 ± 7.7 mg/dl. The gluten-containing bread (ORO) seemed to spike blood glucose concentrations faster and decline at a slower rate compared to the other breads. The gluten-free bread containing potato and fava bean flour seemed to have a less dramatic spike in blood glucose and declined at a greater rate towards baseline, compared to the other groups. However, there were no differences between groups for the glycemic response over the 2 hours post-meal (p= .358; Figure 1).

Table 4. Blood Sugar Response to Breads Used

| Blood Sugar Response to Breads Used | | | |
|---|---|---|---|
| | | Mean | SD |
| Oroweat Country White Bread | | | |
| | Fasting ORO (mg/dL) | 89.9 | 8.5 |
| | 30 min ORO (mg/dL) | 147.5 | 28.2 |
| | 60 min ORO (mg/dL) | 134.3 | 35 |
| | 90 min ORO (mg/dL) | 121 | 28.7 |
| | 120 min ORO (mg/dL) | 114.6 | 29.5 |
| | iAUC | 145.3 | 82.6 |
| Katz Wholesome GF Bread | | | |
| | Fasting KATZ (mg/dL) | 90.5 | 8.8 |
| | 30 min KATZ (mg/dL) | 138.2 | 33.2 |
| | 60 min KATZ (mg/dL) | 128.1 | 26.8 |
| | 90 min KATZ (mg/dL) | 110.7 | 17.8 |
| | 120 min KATZ (mg/dL) | 104.6 | 15.3 |
| | iAUC | 112.4 | 64.5 |
| Canyon Bakehouse GF Bread | | | |
| | Fasting CAN (mg/dL) | 90.3 | 8.5 |
| | 30 min CAN (mg/dL) | 138.2 | 22.7 |
| | 60 min CAN (mg/dL) | 133.2 | 29.7 |
| | 90 min CAN (mg/dL) | 118.2 | 19.5 |
| | 120 min CAN (mg/dL) | 103.9 | 15.9 |
| | iAUC | 125.5 | 62.8 |

Across the three groups, there was no significant difference in iAUC values after 120 minutes ($p=0.192$ ). The greatest mean was seen in the gluten-containing bread ($145.3 \pm 82.6$), then the gluten-free bread made with rice flour ($125.5 \pm 62.8$), and lastly the gluten-free bread made with potato and fava bean flour ($112.4 \pm 64.5$). (Figure 2)

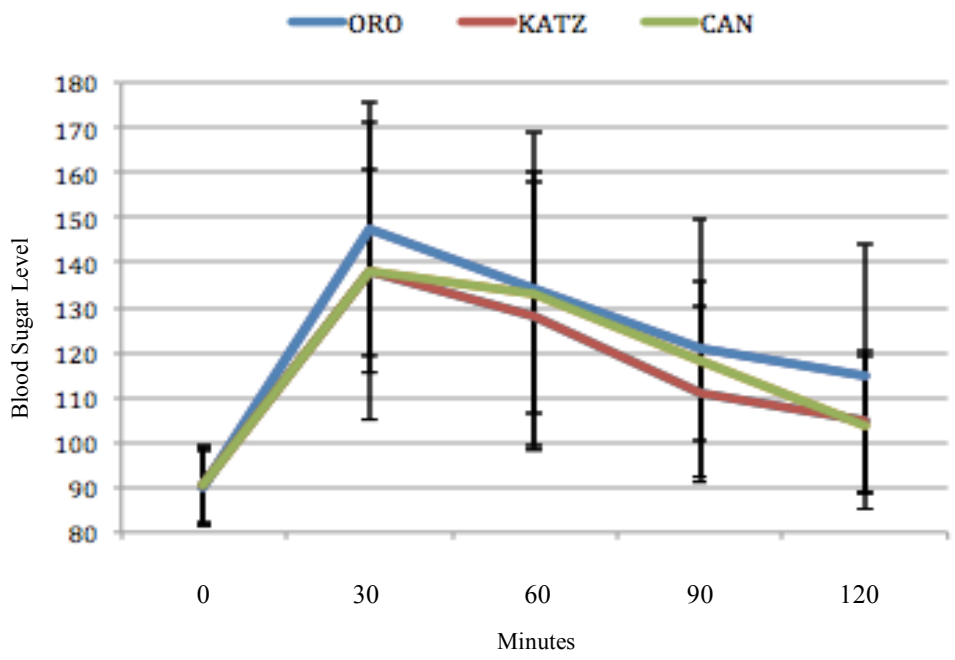


Figure 1. Comparison of blood glucose levels between and within groups at fasting, then 30, 60, 90 and 120 minutes after the first bite of bread was swallowed (p= 0.358). Data considered significant at p<0.05. Data represents participants who completed all three conditions (n=17).

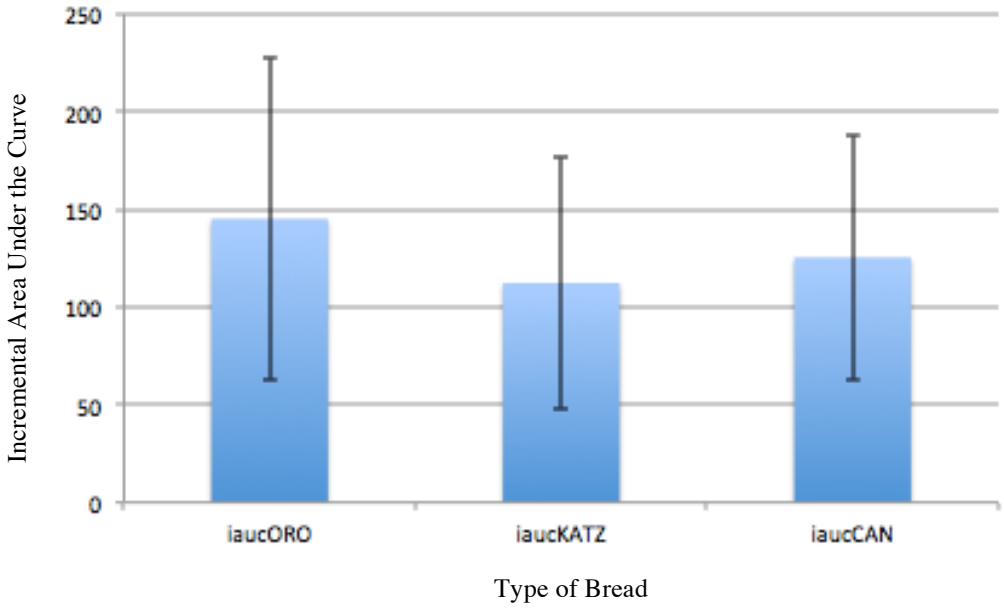


Figure 2. Comparison of incremental area under the curve between groups (p= 0.192). Data considered significant at p<0.05. Data represents participants who completed all three conditions (n=17).

# CHAPTER 5

# DISCUSSION AND CONCLUSION

Studies have found mixed results when observing the postprandial glycemic response of different gluten-free products compared to their gluten-containing counterparts. This research compared postprandial glycemia following the consumption of commercially produced gluten-free bread made with different types of flour and traditional gluten containing bread. The results of this study failed to show a significant difference in postprandial glycemia between gluten-free breads and similar bread containing gluten. Although these results do not show a significant difference, it does argue against the belief that gluten-free products are invariably better for health in the general, non-gluten sensitive population.

A similar study conducted in 2004 observed the postprandial response in 7 non-celiac women following the consumption of gluten-containing white bread and gluten-free white bread (Berti et al., 2004). The results of that study found a significantly greater AUC for the gluten-free white bread, but did not mention the ingredients in either of the breads used, and only controlled for carbohydrate content, leaving great variation in the amount of fat and fiber in each bread type (Berti et al., 2004).

Another study involving 8 healthy participants found that postprandial blood glucose was significantly higher following the consumption of white bread made from scratch with gluten-free flour, compared to bread made with gluten-containing flour (Jenkins et al., 1987). That study tightly controlled ingredients by making both types of bread from scratch, using wheat starch for the gluten-free bread rather than wheat flour.

The main ingredients used in the study presented in this paper followed the research by Johnston, et al., who found a significantly higher postprandial glycemic response following the consumption of gluten-free pasta made with rice and corn flour compared to that of gluten-containing wheat pasta (Johnston, Snyder, & Smith, 2017). These results are positively correlated with the other two studies mentioned, showing that some gluten-free products produce a significantly higher postprandial glycemic response compared to similar products made with gluten (Johnston, Snyder, & Smith, 2017). On the other hand, research by Johnston, et al. did not find a significant difference between pasta made with brown rice flour or pasta made with corn and quinoa flour, which parallels with the results from the study discussed in this paper (Johnston, Snyder, & Smith, 2017). These inconsistent results suggest that variation may be due to ingredients and the degree of product processing when it comes to the postprandial glycemic response of different gluten-free products.

The processing methods used to make pasta differ from those used to make bread, just as the glycemic index is inconsistent between the two products, even when the same type of flour is used (Fardet et al., 1998). Pasta goes through an extrusion process in which the semolina, or wheat flour, is combined with water and pushed through a die at high pressure (Fardet et al., 1998). This high pressure compacts the protein and starch granules together tightly, so the outside of the pasta is able to be hydrolyzed and digested before the inside, which reduces the rate in which pasta starch is broken down and absorbed (Zou, Sissons, Gidley, Gilbert, & Warren, 2015). The intricate gluten network slows the degradation of starch, and therefore the postprandial spike in blood glucose, by entrapping these granules. Consequently, the tight-packing extrusion process combined

with this gluten network is thought to be largely responsible for the reduced glycemic index in pasta, compared to bread made with the same type of flour (Zou et al., 2015). Comparing the variation of AUC in this study with the results from Johnston, et al. it appears that the AUC at 120 minutes is significantly higher in bread, regardless of ingredients or gluten content, compared to that of pasta (Johnston, Snyder, & Smith, 2017).

Based on the results from this study in light of previous research, it seems that when deciding between gluten-free and gluten-containing products for individuals who are not gluten sensitive, it may be more important to consider processing methods of the foods, rather than ingredients alone. If the primary health concern involves blood glucose management, as is the case for the diabetic population, these results indicate that pasta would be a better choice than bread. Specifically, traditional wheat-based pasta has been shown to produce a lower AUC, compared to corn, quinoa and rice-based pastas (Johnston, Snyder, & Smith, 2017). When deciding between commercially available gluten-free and gluten-containing bread, these results reveal that the postprandial response is indistinguishable between the breads selected for this research.

A possible limitation of this study is the potential for variability between participant compliance when fasting and consuming bagels prior to each appointment. The combination of maximizing glycogen stores with bagel consumption and fasting before each appointment was intended to keep glucose baselines as similar as possible between treatments. Incompliance in this sense could contribute to inconsistent results. Another limitation of this study is the variation of ingredients, other than flour, between the different breads. Commercial breads contain many different ingredients that could

influence blood glucose, or they could work together to produce different effects. Lastly, although the glucometers were calibrated before each appointment, there is always the possibility for variance that may have contributed to the findings. The outcome of this research was tailored to non-gluten sensitive individuals choosing to purchase and consume gluten-free bread that is commercially available at many grocery stores. Future research is suggested where more control over the ingredients can be exercised, possibly with homemade gluten-containing and gluten-free breads. Although it is not likely that many individuals will make gluten-free bread from scratch, this method of production would control for variation in processing and ingredients to draw conclusions on the effects of bread made with different types of gluten-free flour.

In conclusion, the hypothesis that the consumption of gluten-free bread will produce an increased degree of postprandial glycemia as compared to gluten-containing bread in healthy adults was not supported by the results of this study. The inconsistency between this study and previous studies suggests that it is not as simple as predicting the postprandial glycemic reaction to gluten-free products as a whole, but instead is dependent on the type of product and the ingredients used to replace the gluten. It is important to note that although the blood glucose response stayed nearly the same between all three breads used in the study, the gluten-free bread was at least twice the expense of the gluten-containing commercial bread. Thus, if the reason for purchasing gluten-cost bread is for perceived health benefits related to blood-glucose control, evidence from this study would indicate that choosing a gluten-free product produces the same results for a higher price. Although this research did not support the original hypothesis, the results indicated that there is no improvement in terms of postprandial

glycemia for healthy individuals consuming these commercial gluten-free breads. Depending on what is used to replace gluten in these products, and the method of processing, the change on blood glucose will vary.

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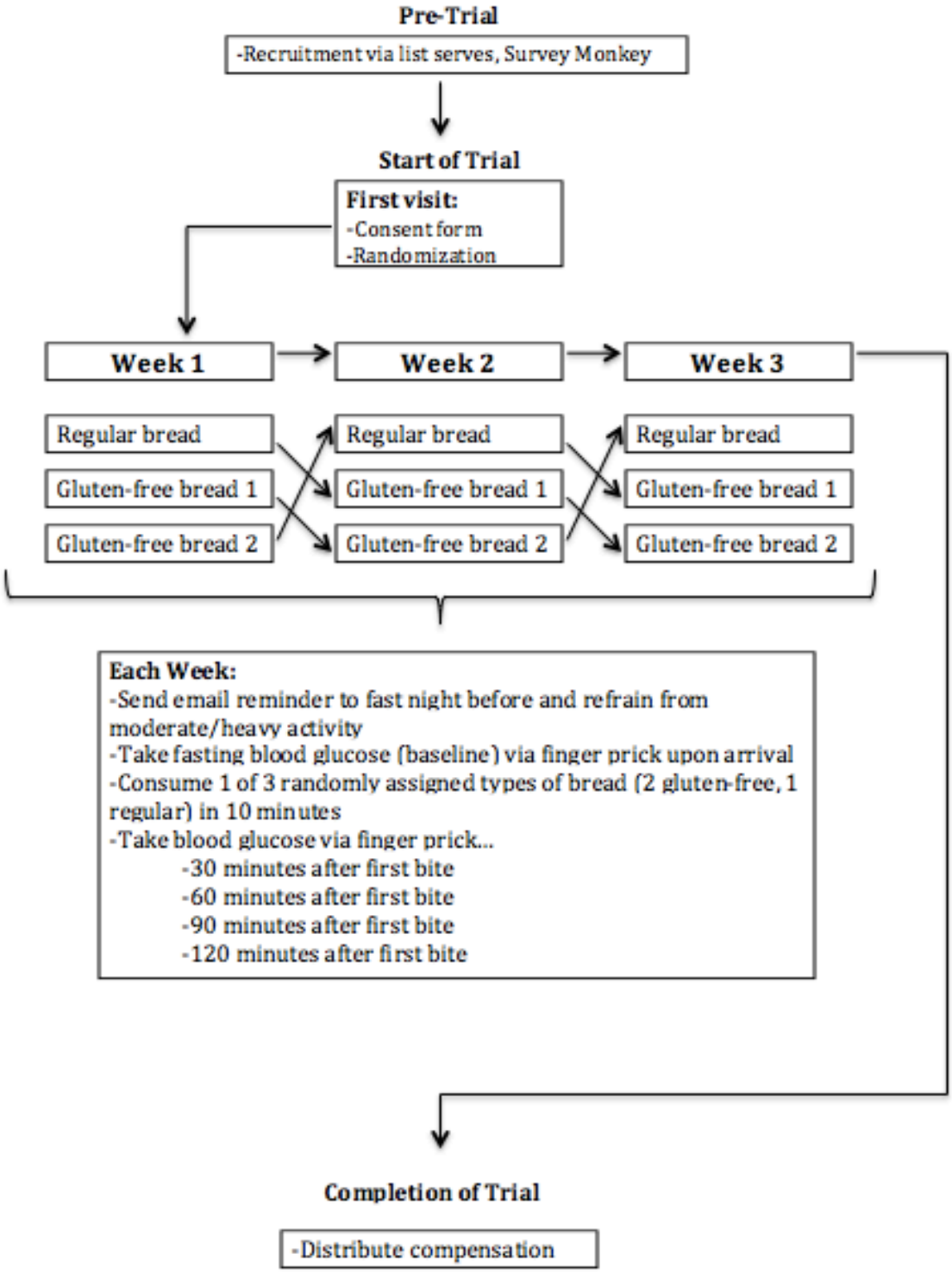
# APPENDIX A

## STUDY DESIGN FLOW CHART


Pre-Trial
-Recruitment via list serves, Survey Monkey
Start of Trial
First visit:
-Consent form
-Randomization
Week 1
Week 2
Week 3
Regular bread
Gluten-free bread 1
Gluten-free bread 2
Regular bread
Gluten-free bread 1
Gluten-free bread 2
Regular bread
Gluten-free bread 1
Gluten-free bread 2
Each Week:
-Send email reminder to fast night before and refrain from moderate/heavy activity
-Take fasting blood glucose (baseline) via finger prick upon arrival
-Consume 1 of 3 randomly assigned types of bread (2 gluten-free, 1 regular) in 10 minutes
-Take blood glucose via finger prick...
-30 minutes after first bite
-60 minutes after first bite
-90 minutes after first bite
-120 minutes after first bite
Completion of Trial
-Distribute compensation

# APPENDIX B

## SAMPLE SIZE CALCULATION

| Author | Year | Change in AUC | SD | n per group | Calculated n per group | Age range | Subject State | Test |
|---|---|---|---|---|---|---|---|---|
| Johnston, et al. | 2017 | 27 | 40 | 13 | 37 | 24-50 | Healthy, non-smoking adults | (Paired) |
| Jenkins, et al. | 1987 | 19 | 5.5 | 8 | 4 | 25-29 | Healthy adults | (Paired) |
| Berti, et al. | 2004 | 672 | 613 | 7 | 16 | 20-45 | Healthy women | (Paired) |
| **Average** | | | | **9** | **19** | | | |

# APPENDIX C

# IRB APPROVAL

APPROVAL: EXPEDITED REVIEW

Carol Johnston
SNHP: Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 1/8/2018 the ASU IRB reviewed the following protocol:

| | |
|---|---|
| Type of Review: | Initial Study |
| Title: | The effects of commercially available gluten-free breads on postprandial glycemia in comparison to conventional gluten-containing bread in healthy adults: a single blind randomized crossover trial |
| Investigator: | Carol Johnston |
| IRB ID: | STUDY00007539 |
| Category of review: | (2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (7)(b) Social science methods, (2)(b) Blood samples from others, (7)(a) Behavioral research |
| Funding: | None |
| Grant Title: | None |
| Grant ID: | None |
| Documents Reviewed: | • health history questionnaire, Category: Screening forms;<br>• calendar, Category: Participant materials (specific directions for them);<br>• protocol, Category: IRB Protocol;<br>• ad and script, Category: Recruitment Materials;<br>• consent, Category: Consent Form;<br>• online survey, Category: Recruitment Materials; |

The IRB approved the protocol from 1/8/2018 to 1/7/2019 inclusive. Three weeks before 1/7/2019 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 1/7/2019 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:
Lauren Waznik

# APPENDIX D

# GLUTEN KNOWLEDGE AND BELIEFS ONLINE SURVEY

Thank you for your interest in this research study conducted by Lauren LaRue, an Arizona State University (ASU) Master's student under the direction of Dr. Carol Johnston, ASU nutrition professor. This survey asks questions about your use, beliefs, and attitudes regarding gluten-free foods. You must be 18 years of age to participate in this survey. This survey will ask demographic questions such has age, education level, weight, and health status, and a series of questions regarding gluten-free products. You will NOT be asked to provide your name or other identifying information. You may quit the survey at any time if you do not want to continue answering questions. Survey participation will indicate consent. Participation is completely voluntary. The survey should take 10 minutes to complete. If you choose not to participate or to withdraw from the study at any time, there will be no penalty. There are no foreseeable risks or discomforts to your participation. The results of this study may be used in reports, presentations, or publications but your name will not be known. If you have any questions, please contact Dr. Johnston, ASU Nutrition Professor, at carol.johnston@asu.edu or 602-827-2265. Information collected from this survey may be used in research reports but your input is anonymous. If you have questions about your rights as a subject/participant in this research, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

Thank you for your interest in research conducted in the ASU School of Nutrition and Health Promotion.

## Survey/Questionnaire

1) What is your gender?
    a. Male
    b. Female

2) Please list your age (in years)

3) What is your weight? (in pounds)

4) What is your height? (in inches – note that 5 feet is 60 inches)

5) What is the highest level of formal education that you've completed?
    a. Attended High School
    b. Graduated High School
    c. Attended College
    d. Graduated College
    e. Post-Graduate Study without Degree
    f. Post-Graduate Degree

6) What is your marital status?
    a. Married
    b. Single, never married
    c. Separated of Divorced
    d. Widowed

7) What is your living situation?
    a. I live alone
    b. I live with family
    c. I live with roommates

8) What is your total annual income?
    a. Less than $30,000
    b. $30,000-$39,999
    c. $40,000-$49,999
    d. $50,000-$59,999
    e. $60,000-$69,999
    f. $70,000-$79,999
    g. $80,000-$89,999
    h. $90,000-$99,999
    i. More than $100,000

9) Has your doctor ever diagnosed you with diabetes or pre-diabetes?
   a. Yes
   b. No

10) Has your doctor ever diagnosed you with Celiac Disease or Irritable Bowel Syndrome (IBS)?
   a. Yes
   b. No

11) When given the choice between a product containing gluten and a similar product that is gluten-free…
   a. I always choose the gluten-free products
   b. I occasionally choose the gluten-free products.
   c. I never intentionally choose the gluten-free product.
   d. I have chosen gluten-free products in the past, but I no longer choose gluten-free products.

12) When out to eat at a restaurant, do you look or ask for gluten-free products?
   a. Always
   b. Never
   c. Sometimes

13) Aside from reactions due to Celiac Disease or closely related conditions, do you believe that there are health benefits of following a gluten-free diet?
   a. Yes
   b. No

14) Please check all that apply:
Gluten-free products:
   Taste better
   Have fewer allergens
   Have fewer hormones
   Are fresher
   Are more easily digested
   Are anti-inflammatory
   Are more nutritious
   Are heart-healthy
   Are better for the environment
   Have fewer pesticides
   Are less convenient to buy
   Are more costly
   Have fewer germs
   Lower blood sugar
   Promote health

15) How would you classify gluten?
   a. Fat
   b. Protein
   c. Carbohydrate
   d. Fiber
   e. Mineral
   f. Sugar
   g. Don't know

16) How do you classify your diet:
   a. Omnivore (eat meat daily)
   b. Plant-based (eat meat several times per week or less)
   c. Vegetarian (does not eat meat; will eat eggs and/or dairy products)
   d. Vegan (diet does not include any animal products)

# APPENDIX E

# ONLINE RECRUITMENT SURVEY

Thank you for your interest in research at ASU!

**I am a graduate student in the School of Nutrition and Health Promotion at Arizona State University, I am conducting a research study to examine the impact of gluten-free breads on blood glucose concentrations. I am inviting your participation in the screening process, which will consist of answering questions regarding health history, demographics, and scheduling availability. You have the right to not answer any question, and to stop participation at any time.**

**We are recruiting healthy adults between the ages of 18 and 45 y. Your participation in this survey is voluntary. If you choose not to participate or to withdraw from the survey at any time, there will be no penalty. Your responses to this survey will be confidential. If you meet the criteria for this study, you will be contacted to schedule an in-person appointment at the downtown campus of Arizona State University.**

**If you have any questions concerning the research study, please contact the research team at lauren.larue2@gmail.com. If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at (480) 965-6788.**

**By completing this survey, you are agreeing to be contacted by investigators (via e-mail) to schedule an appointment, should you qualify.**

* 1. Please provide your email address:

* 2. What is your age? (in years)

* 3. Has your body weight been stable (plus or minus 7 pounds) over the past 3 months?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 4. Are you healthy and free of chronic disease?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 5. Has your physician diagnosed you with either pre-diabetes or diabetes?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 6. Has your physician diagnosed you with any gastrointestinal-related conditions or malabsorption disorder? (such as Crohn's disease, Celiac sprue, polyps, irritable bowel syndrome, etc.)

- ◯ Yes
- ◯ No
- ◯ Unsure

If "Yes" please comment:

* 7. Do you have any food allergies?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 8. Do you follow any type of vegetarian or vegan diet?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 9. Would you be willing to visit the Nutrition Research labs on 4 occasions for up to 2.5 hours (located in the ABC1 building on the ASU downtown campus)?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 10. Would you be willing and able to tolerate five finger pricks on test days for this study?

- ◯ Yes
- ◯ No
- ◯ Unsure

* 11. Would you be willing to consume 2 bagels and restrict any moderate-to-vigorous physical activity on the days prior to testing?

- ◯ Yes
- ◯ No

* 12. Would you be able to fast overnight (no food or beverage with the exception of water) for 10 hours before each test period?

- ◯ Yes
- ◯ No

# APPENDIX F

# CONSENT FORM

**BLOOD GLUCOSE RESPONSE TO GLUTEN-FREE BREAD INGESTION**

**INTRODUCTON**

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

**RESEARCHERS**

Dr. Carol Johnston, a Nutrition professor at Arizona State University Downtown Campus, and Lauren Waznik, a nutrition graduate student, have requested your participation in a research study.

**STUDY PURPOSE**

The purpose of the research is to examine the effects of gluten-free bread ingestion on the blood glucose response.

**DESCRIPTION OF RESEARCH STUDY**

You have indicated to us that you are 18-45 years of age, a non-smoker and generally healthy. You have not been diagnosed with diabetes, you are not a vegetarian, and (if female) you have not recently been pregnant or lactating. Participants will be asked to maintain their usual diet and physical activity level throughout the trial with the exception of the days prior to testing. This study will initially involve the completion of a brief health history questionnaire to demonstrate the absence of medical conditions that may impact the study. Your weight, height, and girth will be measured at this time. This first meeting will take about 20 minutes. There are three additional visits (e.g., the test days) that will last about 2.5 hours each and scheduled a week or two apart. The procedures on all test days are identical. On the day prior to testing you are asked to avoid any moderate-to-vigorous exercise (normal activities such as walking to work or walking the dog is ok). You will be asked to eat a normal breakfast and to consume a bagel with a lunch of your choice. You will also be asked to consume a 2$^{nd}$ bagel at your dinner meal. The bagels will be given to you. Following dinner (at about 7 pm), you will fast overnight and not consume any food or beverage with the exception of water. On test days, you will travel to ASU (the Nutrition labs at the ABC1 Building on the ASU Downtown campus) early in the morning. Your finger will be pricked for a blood sample, and you will then consume a test meal (bread and butter). Your finger will be pricked 4 more times over the next 2 hours. Finger pricks will be conducted under sterile conditions using disposable, retractable lancets. Blood samples will be analyzed for glucose. You may drink water during this time but you are not to consume any food. Otherwise, you may read, study, or work on the computer at the test site. Once testing is complete, you may proceed with your normal activities. About 19 subjects will participate in this study.

**RISKS**

Bruising of the skin or a feeling of faintness is possible during the finger pricks. Sterile conditions will be used during the finger pricks.

**BENEFITS**

There is no direct benefit for participating in this trial. If you would like your personal data, you can sign a standard release form to receive your results.

**NEW INFORMATION**

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

**CONFIDENTIALITY**

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr.

Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators. No blood samples will be retained following the finger pricks.

**WITHDRAWAL PRIVILEGE**

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not incur negative treatment to you by the researchers.

**COSTS AND PAYMENTS**

The all test foods will be given to you during the study free of charge. You will receive a $10 Target card at test visits 2 and 3 ($20 total if the study is completed).

**COMPENSATION FOR ILLNESS AND INJURY**

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

**VOLUNTARY CONSENT**

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston; 500 N. 3rd Street Phoenix, AZ 85004; 602-827-2265.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

| _____ | _____ | _____ |
|---|---|---|
| Subject's Signature | Printed Name | Date |

| _____ | _____ |
|---|---|
| Contact phone number | Email |

**INVESTIGATOR'S STATEMENT**

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

# APPENDIX G

# HEALTH HISTORY QUESTIONNAIRE

MEDICAL HISTORY QUESTIONNAIRE ID#_____

Height _____ft. _____ in. Weight: _____lbs. Waist: _____ ins. To be completed by investigator

Age: _____

Gender: □ Male □ Female

Current smoker: □Yes □ No

1. Have you been diagnosed with pre-diabetes or diabetes? Y N

2. Have you been diagnosed with other chronic diseases (such as heart disease, neurological disease, autoimmune disease, or cancer)? Y N

3. Have you been diagnosed with celiac disease or gluten allergies? Y N

4. Do you take any medications regularly? Y N
If yes, please list what kind and how frequently:

| Medication | Dosage | Frequency |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

5. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Y N
If yes, what supplements and how often?

_____
_____
_____
_____
_____

6. Do you have medical conditions that you see a physician for on a regular basis? Y N
Please explain:

_____
_____
_____
_____
_____

7. Do you have any food allergies? Y N
If yes, please describe _____
_____

OVER ⟶

8. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.) Y N
If yes, please describe _____
_____
_____

9. Have you lost >10 lbs in the past 3 months? Y N

10. Are you OK with eating bagels and breads (gluten free and regular breads)? Y N

11. Will you have any problems fasting for 12 hours prior to testing sessions? Y N

12. Do you have any difficulty chewing or swallowing? Y N

13. Will you have a problem (such as fainting) providing blood samples via a finger prick? Y N

14. Typically, how many alcoholic beverages to you consume? (circle most appropriate answer)

0 1-2 weekly 3-4 weekly 5-6 weekly 1 daily 2 daily >2 daily

15. If you drink alcohol or caffeine, will you be able to abstain from these beverages for the 24-hour periods prior to test days? Y N

16. If you exercise regularly, will you be able to not exercise (other than basic walking and work activity ) for the 24-hour period prior to testing? Y N

Please describe any other conditions or medical reasons that may affect your participation below (i.e. recent pregnancy, infections, allergies, etc):