Exploring the Benefits of a Gluten Free Diet

by

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ABSTRACT

Background: Individuals in the general populations with a known gluten disorder is believed to be 6% and it is unclear why the gluten free diet (GFD) has risen sharply (28%) in recent years. However, science has revealed that gluten can cause colonic changes in those undiagnosed with a known gluten disorder. The ramifications of these changes are unknown. Three common ingredients found in gluten free products, such as pasta, are corn quinoa and rice. Evidence from the scientific literature has shown that corn and quinoa can produce more colonic hydrogen than refined wheat and rice, indicating that corn and quinoa have a reduced glycemic effect. Since rice and wheat have similar glycemic responses, corn and quinoa pastas would be expected to have a lower glycemic response than rice and wheat pasta.

Aim The aim of his study was to examine the glycemic response to three different types of pasta: wheat, rice and corn. Breath hydrogen, assessment of mood states, blood glucose and insulin were collected after ingestion of these pastas to determine the glycemic effects of these foods.

Methods: A double blinded crossover study design was utilized on a group of healthy individuals, and the test meals of wheat, rice and combinations of rice/corn, and corn/quinoa pastas were consumed one week apart in random order. Collections of fasting venous blood samples for insulin analysis, capillary blood from a finger stick for glucose analysis, breath hydrogen samples and satiety scales were used for glycemic response and mood states were collected prior to the meal (baseline) and then again after ingestion of the test meals. Attempts were made to explore the glycemic response of these test meals in relation to mood states.

Results: The glucose response showed no significant difference at baseline ($p = .683$) among all groups and no significant differences were seen post treatment at 30 minutes ($p = .875$). However, after 60 minutes all of the glucose concentrations began to decline except for the rice pasta which peaked at 90 minutes and the wheat pasta gave the most sustained decrease. The AUC glucose values showed no significant difference at both 120 ($p=0.196$) and 240 ($p=0.734$) minutes but with wheat pasta producing the lowest mean value. The POMS scores showed no

significant differences between groups over time (p=.239) but the wheat group produced the highest score (worsening moods states).

Conclusion: These results indicate that the formulation and processing of gluten free pastas may affect the rate and absorption and the subsequent glycemic response after the consumption of these foods. Whether or not wheat contains an ingredient that slows absorption and/or negatively affects mood remains undetermined warrants future research in this area.

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TABLE OF CONTENTS

CHAPTER PAGE

CHAPTER PAGE

APPENDIX

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

The use gluten of free products (GFP) has risen sharply in the last ten years. During the period of 2004 to 2011, the annual growth of these products rose 28%, and in 2008, sales surpassed that for both fat-free and low carbohydrate products. By 2010 sales reached 1.6 billion and then climbed to 4.2 billion in 2012. According to one recent consumer survey, 18% of adults are choosing gluten free products (4-8). Yet, the scientific evidence shows there are only three populations that should avoid gluten, those with Celiac disease, those who have an allergy to wheat, and those who are gluten sensitive. Individuals with these conditions react adversely to the ingestion of gluten. The prevalence of these conditions combined is only 5-6% of the U.S. population (5,6,14). Interestingly, recent research evidence suggests that gluten intake does alter colonic function in healthy adults as well as those with Celiac disease or gluten allergy (1,15,16). However, the ramifications of these changes in healthy adults are not known, and nutrition professionals generally do not recommend gluten-free diets for healthy individuals. Nevertheless, many individuals without known gluten disorders are switching to gluten free products and claim to feel better on this diet (8).

It is unclear why the gluten free diet has risen in healthy individuals but the adoption of this diet plan may force individuals to adjust what they eat. Reasons for this adjustment include low availability of product while dining out, lack of availability of product in grocery stores, and the economic burden of higher priced gluten free products. For these reasons, people may choose low-carbohydrate meal plans (e.g. substituting protein foods for carbohydrate foods) (9). In addition, many manufacturers of gluten free foods add corn to their products (9, 10). Both low-carbohydrate meals and high corn consumption may lower the overall glycemic response of the diet, a characteristic that may be associated with improved mood (11, 12). Hence, the reason why many healthy adults feel better on a gluten free diet may relate to the mealtime glycemic load. One study which tested the breath hydrogen of corn and other grains demonstrated that corn produced more colonic hydrogen than refined wheat, indicating that corn has a reduced glycemic effect (13, 14, 15). Three common ingredients substituted for wheat in gluten free foods, such as

pasta, are corn, quinoa or rice. Since rice and refined wheat have similar glycemic responses, the corn and quinoa pasta would be expected to consist of more slowly digestible starch (SD) and/or more resistant starch (RS) and therefore produce a lower glycemic response than both rice and wheat pasta (16, 17). Although many studies have examined the glycemic effects of specialty corn starch hybrids such as high amylase corn, waxy corn, and retrograded corn, no studies to date have tested the glycemic effects of normal (dent) corn pasta in comparison to refined rice or wheat pastas (12-16). Although some studies have tested the glycemic effect of whole quinoa, the scientific literature is devoid of any studies which have tested the glycemic effect of quinoa in the form of gluten free pasta (17, 18).

1.1 Purpose -

This study examined the glycemic response in healthy adults to four different types of commercial pasta: wheat, corn/rice, corn/quinoa and rice. The latter two pastas are marketed as 'gluten-free'. Mood states assessments, breath hydrogen, blood glucose and insulin were collected after ingestion of these pastas to determine the glycemic effects of these foods.

1.2 Hypotheses

H_1: The ingestion of gluten free corn-based pasta meal and gluten free corn-based pasta meal with the addition of quinoa will produce a lower degree of glycemia and more colonic hydrogen in healthy adults in comparison to the ingestion of either gluten free rice based or regular gluten containing wheat based pastas meals.

H_2: The glycemic response of the meal will be directly associated with mood states in healthy adults.

H_3: Satiety will be higher, and 24-h energy intakes lower, following corn-based pasta ingestion in comparison to the ingestion of either rice or wheat pastas in healthy adults.

1.3 Definition of Terms –

Gluten – A complex structural protein portion found in most grain cereals which consist of two portions, prolamins (proline) and glutelins (glutamine) and it is the glutens in wheat barley and rye species which have been found to be toxic to certain humans (19, 20).

Celiac Disease – A chronic autoimmune disorder (not an allergy) of the small intestines caused by the inability to metabolize gluten from wheat, barley and rye, When gluten is ingested in celiac patients, the body will 'attack' the lining of the intestine, flattening the villi, typically resulting in malnutrition. It has been estimated according to recent literature reviews that the prevalence of celiac disease in the general population is between .05% and 1% (21, 22).

Allergy – A hypersensitivity disorder of the immune system where the body defends itself against perceived invaders, 'attacking' the invader and releasing histamines.

Glycemic index – A system which ranks carbohydrate-rich foods on a scale of 1-100 by measuring how glucose levels rise after the ingestion of an amount of that food containing 50 grams of carbohydrate. Foods rank, high, medium and low (14, 23, 24).

Glycemic response – The glycemic response too food, which is described as "the change in blood glucose concentration induced by food ingestion" (14, 23, 24).

Breath hydrogen – hydrogen gas produced by the colonic fermentation of resistant starches and/or dietary fibers. Typically these gasses emanate from the colon, move to the blood then to the lungs (25).

Non-producers (of methane gas) – individuals who do not have a sufficient amount of methanogenic organisms in the colon to produce enough hydrogen or methane to be detected by the breath analyzer (25).

RDS – rapidly digested starch (26)

SDS – slowly digested starch (26)

RS – resistant starch (26)

1.4 Delimitations and limitations

- **Limitations**
    - This is an acute investigation; hence, long term benefits will not be assessed.
    - Study protocol compliance, including following specific meal plans and an overnight fast, may impact the results

- Whether or not the test subjects were ‘non-producers’ of colonic hydrogen gas which is contingent upon whether or not they have hydrogen producing intestinal micorbiota.
- Colonic gas can form several hours after (more than 4 hours) after the consumption of a meal consisting of either resistant starch or fiber.

- **Delimitation**
  - The results derived from the macaroni and cheese meal may not be generalizable to other gluten free products.
  - This test was performed on healthy college age students and therefore these results cannot be generalized to diabetics, elderly and those with certain digestive disorders.

CHAPTER 2

REVIEW OF THE LITERATURE

2.0 - Grains

Mankind began to plant and harvest grains nearly 10,000 years ago during the Neolithic Era. Wild wheat, barley and rice were first domesticated from the seeds of the grass species known as *Gramineae* (27). The harvesting of cereals enabled humans to have larger and more reliable food sources which helped populations transition from smaller tribes of hunters and gatherers to larger agrarian communities. These later societies farmed food and remained in place (28). Since then, grains have been regularly consumed by humans and today they are still a staple food source for most societies around the world. Wheat, rice, barley and maize make up nearly half of the energy consumed globally. Grain varieties can be found in all continents. In Africa, millets and sorghum are most common varieties whereas in Asia, rice is the staple grain. However in n Europe, wheat makes up the majority of the 131 kg per capita of grain consumed each year. In the U.S. and globally, wheat and rice are the most common food grains consumed (29, 30).

2.1 – Grain Structure

All grains are similar in structure and provide humans important nutrients. By weight, grains contain 10 – 20% protein, 2 – 5% fat, 50 – 60% starch and 10 – 15% dietary fiber (29). Grains also contain important micro nutrients like sterols, minerals, vitamins, phytic acid, and phenolic compounds. In the center of the grain is the germ. This is surrounded by a starchy inner core known as the endosperm. The bran and husks make up a protective outer layer. The germ (or embryo) makes up only a small portion of the grain but represents the main source of micronutrients in the grain (29, 31). By weight, the starchy endosperm makes up about 83% of the macronutrients found in grains and provides humans essential energy. While the outer layers of the grain kernel provide some minerals and vitamins, it consists mostly of dietary fiber for laxation, bulk, slower absorption of glycemic foods and the multiple systemic effects of colonic fermentation. However since the industrial revolution, grains have been commonly milled, particularly in the Western hemisphere. Milling strips the grains of the outer layers and inner

germ, taking out the essential dietary fiber, vitamins and minerals leaving mostly digestible starches in the endosperm also known as processed flour (31).

2.12 – Grain Starch, Molecular Structure

Chemically, grain starches are known as polysaccharides. The name “saccharide” means sugar or glucose and poly means many. Polysaccharides are literally many molecules of glucose held together with bonds. Dietary starches are bonded together with α 1-4 and α 1-6 linkages. The two main structures of starch are amylose and amylopectin. Amylose is a linear chain of glucose with α 1-4 bonds and typically makes up 15 – 20 % of the starch. The second component, amylopectin, is a branched chain of glucose with α 1-4 along its linear points and α 1-6 bonds at the branched points. These two structures make up and make the majority of most naturally occurring starches (32).

There are three crystalline structures of starch, A, B and C. These different starch types are classified partly by their density but also the make-up of the chain length of their amylopectin lattice structures. Type A starches contain amylopectin chain lengths of 23 – 29 glucose molecules. The hydrogen bonding of the hydroxyl groups in this structure creates a double helix structure, a pattern commonly found in cereals. Type B starches contain amylopectin chains of 30 – 44 glucose units and are associated with bananas and raw tubers. Both type A and type B starches are considered to be “true” crystalline structures but the third category, type C, is a mixed form. Type C starches are made up of 26 – 29 glucose units and consist of both type A and B types and are typically associated with legumes and peas (26, 32, 33).

2.13 – Grain Starch Digestion

With digestion, starches first need to be hydrolyzed (digested) into glucose before they are taken across the gut lumen. Generally, enzymes such as α-amylase, glucoamylase, and sucrase-isomaltase break the starch chain into free glucose units which are then free to be absorbed. The human enzyme α- amylase is secreted from the pancreas and it hydrolyzes the α-1-4 bonds in the starch granule. This enzyme works on the end points of the granule which explains why amylose is digested slower than amylopectin. Although amylopectin has the addition of α 1-6 bonds at its branch points, it has more of the end points containing the the α 1-4

bonds and will digest faster (34). After the action of enzyme α- amylase, the starch is then broken down into the disaccharides, maltose, maltotriose, and isomaltrose. The smaller units of glucose, galactose and fructose are then generated by the action of brush border enzymes for absorption into the gut lumen. Starch that escapes this process will then pass into the large intestine to be fermented by microbial colonies. Subsequent to this fermentation, the bi-products short chain fatty acids, and three gasses (methane, hydrogen and CO2) are then produced. (34).

2.14 – Resistant Starch

Resistant starches (RS) are starch molecules that escape enzymatic hydrolysis in the stomach and small intestines. These starches can end up in the colon where they can be used as a fuel for microbial fermentation, producing bi-products of hydrogen, methane, carbon dioxide and short chain fatty acids (32). In a starch molecule, higher amounts of amylose are associated with a slower rate of digestion and absorption. Raw starch is a crystallized form with amylose making up the amorphous region of the granule while the amylopectin makes up the crystallized area. Heating starch in water solutions to a sufficient temperature, past the gelatinization stage causes the starch crystals to melt or gelatinize into an amorphous structure. Gelatinized amorphous starch is easily digested and absorbed. However, these gels are unstable and when cooled can reform into more crystalline structures which are resistant to the hydrolysis action of the enzyme amylase, a process known as retrogradation. Type A starch form during slow cooling, while type B structures form during slow cooling in an aqueous solution. Generally, starches which are higher in amylose, are more resistant to digestion and have higher rates of retrogradation (26, 32).

2.15 – Resistant Starch, Classification

There are four general classifications of RS which have been put into a subcategory of RS1 – RS4. Briefly, RS1 is considered to be physically protected from digestion because of intact cell walls. These starches are found in whole or partly milled grains, legumes, seeds and pastas. Both milling and chewing can reduce the resistance qualities in these starches. RS 2 type starches are type B crystalline forms and are resistant due to the confirmation of its natural granule form. They are found in uncooked potatoes, green bananas, some legumes and high

amylose starches (26, 32). During the digestion process, these starches are typically, hydrolyzed slowly by α-amalase and during food processing, their resistant qualities are reduced by both cooking and milling. R3 type starch is a non-granule form that is typically re-formed (retrogradation) after cooking, during the cooling process. These starches can be cooked then cooled, as found in potato salad, bread, cornflakes, and other food products which have been put through repeated and/or prolonged moist heat preparations. The resistance quality in this type of starch is reduced during food processing conditions. The R4 type of starch is a modified version which has been cross bonded, esterified, or etherisised to reduce their digestibility. These starches can be called “high amylose” starch or flour and are typically found in certain breads, and cakes. The resistance in this type of starch is not easily reduced by processing or food preparation techniques (26, 32).

Caloric value of resistant starch – Experiments has calculated the energy from resistant starch to be approximately 2kcal/kg. This is considerably lower in comparison to normal digestible starch which has been calculated to be 4.2kalc/kg. However, food labels in both the U.S. and the U.K. have not added RS to their accounting of foodstuffs (32).

It has been said that it takes approximately 60 grams of non-digestible polysaccharides per day to sustain healthy colonies of microbiota found in the human colon. In the U.K, it is estimated that the average person obtains less than 20 grams of these non-digestible polysaccharides per day. Some have estimated the true number of RS in the average diet is sufficient to make the gap. However, other studies have said that worldwide, the average amount of RS in the daily diet varies considerably. In developed countries with high starch consumption, it the average daily amount of RS is estimated to be between 10 – 18g / day (32).

2.16 – Grain Fiber

The dietary fiber in grains is considered to be the non-digestible portion of the kernel. Typically, grain fiber is in the form of oligosaccharides and non-starch polysaccharides which then form complex non-digestible polysaccharides. Human beings lack the enzymes necessary to digest the dietary fiber and therefore, this substituent cannot be absorbed. They then pass freely into the colon where and can provide bulk or be fully or partially fermented (30,

39). Wheat fiber can be categorized as either soluble (dissolves in water) or insoluble (resists water) with each type having different health benefits. The water soluble fibers are non-starchy polysaccharides such as pentosans (arabinoxylan) and β-glucans which have viscous and fermentable qualities (31, 35). In humans, soluble dietary fibers have been shown to reduce serum cholesterol, postprandial glucose and insulin (31). Insoluble dietary fibers are cellulose, hemicellulose (water in-soluble) arabinoxylanand lignin mostly have bulking actions and are fermented in the colon only to a limited extent (31, 35, 36). β-glucans are found in the cell layers of the bran and are a major fraction of the fiber found in the grain. β-glucans are linear chains of fructose linked together by β 1-4 and β 1-3 bonds. Human digestive enzymes can only break α 1-4 bonds as seen in dietary starches and sugars. Therefore, dietary fibers with β bonds are resistant to digestion from human enzymes (31). Consumption of these soluble dietary fibers has been shown to reduce both cholesterol and glycemia and improve risks for cardio vascular disease. In addition, β-glucans can be used as a substrate for the colonic microbiota (36). Both oats and barley have higher amounts than wheat or rye. Another common dietary fiber in grains such as wheat, barley and rye is inulin. Inulin also has β bonds (β 2-1) bonds. It therefore passes into the colon for fermentation and is considered to be good for stimulating healthy growth of intestinal micro flora (probiotics) (31).

2.17 – Grain Lipids

The dietary fat portion of the grain is very small and is mostly found in the germ with lesser amounts in the bran. By weight, barley and wheat contain approximately 2-5% lipids while oats can contain up to 7% (29, 36). A small portion of grain lipids are phytosterols, a component that is structurally similar to cholesterol. However, plant sterols have more solubility qualities than cholesterol and therefore have the ability to inhibit cholesterol re-absorption from the intestines. The larger fraction of grains lipids (approximately 75%) is unsaturated fatty acids. This portion consists of equal amounts of both linoleic and oleic acids, both of which are said to lower cholesterol. Therefore both the fiber and the fat in whole grains are two components which are considered to be heart healthy nutrients (36).

2.18 – Grain Protein, Gluten

Gluten, a complex structural protein commonly found in cereals consists of two components, prolamins and glutelins. These two components of the grain have different soluble qualities. The glutelins are soluble in alkali and acid solutions while prolamins are soluble in aqueous alcohol solutions. Depending on the grain, the prolamins can take on different names. There are avenins in oats, hordeins in barley, secalins in rye and gliadins in wheat, all of which are considered to be prolamins (19). The amino acids glutamine and proline specifically give gluten elastic and viscous properties, making it capable of binding to other molecules. These binding properties make gluten a valuable substance in food manufacturing and gives products like bread and pasta vital structure (10, 20). Because of these qualities, gluten is commonly separated from the starch component and made into a substance called “vital dry gluten” which is widely used in many food and non-food products. While all grains have gluten, it is particularly the glutens found in wheat, barley and rye kernels that have become problematic to certain individuals. Interestingly, other recent studies have identified that some individuals with celiac disease (CD) can also be sensitive to the avenins found in oats (19). In this review however, when referring to “gluten disorders”, it is specifically covering the gluten found in wheat, barley and rye. The scientific literature has revealed three major digestive disorders in which people should avoid gluten from wheat, barley and rye - celiac disease (or coeliac disease), wheat allergy, non-celiac gluten sensitivity (20).

2.20 – Gluten disorders (Celiac Disease)

Celiac disease is chronic immune-mediated disorder. The ingestion of gluten in these genetically predisposed individuals causes a flattening and inflammation of the mucosa in the lining of the small bowel. Ultimately this results in atrophy of the villi and malabsorption of essential nutrients (21). Nutrient deficiencies in celiac disease patients can include calorie-protein deficiency, iron deficiency anemia, and numerous fat soluble and mineral deficiencies. Neurological symptoms can include depression, anxiety, schizophrenia, peripheral neuropathy, seizures and headaches (5).

As gliadin passes through the epithelial cells in the small intestine, an enzyme called tissue transglutaminase will deamidate the peptide, a process which increases its immunogenicicity. Once this occurs, antigen presenting macrophages which carry the HLA-DQ 2 and HLA-DQ8 receptors will present the gliadin peptides to a naïve (gluten specific) CD 4 T-cell which then causes the production of a particular cytokine called interleukin-15 (IL-15). Cytokine (IL-15) is a signaling protein which encourages the expression of a stress molecule MICA on the enterocytes of the intestinal lining and activates a specific white blood cell called intraepitheliallymphocites. The lymphocites then release a protein called NK-G2D, an immune cell which is believed to cause the flattening and atrophy of the intestinal lining. In addition, during this process, the immune system will create antibodies, IgG and IgA, which will then continue the cycle of inflammation (7, 22). When screening for celiac disease, antibody IgG and IgA tests in combination with endoscopy biopsies which detect intestinal flattening and atrophy are recommended (5).

Once the gluten is removed from the diet, this process will cease allowing the vili to fully recover in most individuals. Nearly all patients with celiac disease will carry either the HLA-DQ 2 or HLA-DQ2 gene in comparison to an estimated 1/3 of healthy Caucasian individuals. However, not all individuals with these HLA genes will have celiac disease. Possessing the HLA DQ-2 and HLA DQ-8 gene is necessary for having the condition but it is not a diagnosing factor (7, 21, 22). According to Sapone et al, a patient must have at least four of the five following criteria to meet the diagnosis for CD – 1. Typical Symptoms of CD, 2. Positivity of serum CD IgA class autoantibodies at higher titer, 3. HLA-DQ2 and/or HLA-DQ8 genotypes,
4. Celiac enteropathy found in the small bowel, 5. Positive response to a GFD (7). It has been estimated according to recent literature reviews that the prevalence of celiac disease in the general population is between .05% and 1% (7, 21, 22).

2.21 – Wheat Allergy

A wheat allergy is also an adaptive immune- mediated response to certain protein elements found in the wheat, in particular the water insoluble gliadins, but it differs from CD in that it that the immune cells involved are IgE rather than IgG and IgA. The immune response in

this allergy is targeting the invading element rather than the host and as such, wheat allergic patients do not experience the permanent gastrointestinal damage or other organ damage as seen in those with a celiac condition (5, 20).

The symptoms for a wheat allergy can be different from those with CD depending on the route of ingestion. Oral ingestion of wheat during a wheat allergy will manifest reactions in the throat, mouth, nose and eyes (itching, swelling and irritation), respiratory (difficulty breathing, wheezing and anaphylactic), and skin (hives rash and swelling). The gastrointestinal symptoms are similar to those found in CD patients (diarrhea, abdominal pain, gas, bloating and cramping). When wheat is ingested before exercise, a condition called “wheat-dependent exercise-induced anaphylaxis can occur and when wheat touches the skin, a reaction called “contact urticaria” can be produced. In addition, most wheat allergic patients typically do not need to restrict other prolamins from the diet, such as the ones found in rye, barley and oats (20).

Wheat allergies are most often found in infants and toddlers ages 3 to 5 and who also have other food allergies (5). Typically, the issue is resolved once the patient reaches adolescence or adulthood, but not always. Pursuant to recent literature reviews, the overall prevalence of wheat allergies in the U.S. is relatively low and estimated to be 0.1%, even lower than the rates of CD (5, 20). The treatment for a wheat allergy is removal of wheat from the diet. However, if wheat is encountered, the usage of medicines such as corticosteroids, and antihistamines can be helpful. In the case of a severe wheat allergy, sometimes epinephrine shots are necessary (20).

2.22 - Non-celiac Gluten Sensitivity

Non-celiac gluten sensitivity (NCGS) is a relatively new condition which can only occur in the absence of both celiac disease and a wheat allergy. To date, the precise definition and mechanism behind the disorders has not yet been determined. However, patients are currently being diagnosed with this condition mainly on the presence of a wide spectrum of both subjective and objective symptoms, some of which are similar to CD (table 1) (2, 4, 5, 20). Diagnosis is contingent upon the disappearance of these symptoms once gluten containing foods such as wheat, rye and barley have been removed from the diet (5). If the patient is already on a GFD

before seeing a doctor, the diagnosis can be difficult. Therefore, a gluten challenge is often recommended to determine the existence of NCGS and a randomly controlled environment using capsules containing wheat flour is the best way to perform this test (2). However, many have argued about the impracticalities and limitations of such procedures in a normal clinical setting. Generally, an open challenge using 4 slices of white bread is given. As a result, the concern for a placebo effect from such methods has been debated (2). Regardless of which gluten challenge method is used, the definitive diagnosis for NCGS comes after both celiac and a wheat allergy have been ruled out (by specific markers) and after a negative response to a gluten challenge presents the alleviation of the symptoms once gluten has been removed (2,5).

Table 1. Symptoms reported to be associated with CD, wheat allergy, & NCGS

	Gastrointestinal	Neurologic/psychiatric	Other
CD	Abdominal pain, Constipation & Diarrhea	Fatigue, Ataxia, Musculoskeletal pain, Brain fog, Headache, Tingling and/or numbness in hands/feet,	Weight loss, Dermatitis, herpetiformis,
Wheat Allergy	Vomiting, Abdominal pain, Diarrhea	Headache, Dizziness	Eczema, Asthma, Rhinititis, Nausea, Itchiness
NCGS	Nausea, Abdominal pain, Vomiting, Diarrhea, Constipation,	Fatigue, Headache, Musculoskeletal pain, Tingling and/or numbness in hands/feet, Brain fog, Other neurological & psychiatric conditions	Weight loss, Rash, Nausea,

Table 1 adapted from Lundin, Alaedini, et al) (2)

Due to the undetermined consensus among experts regarding the specific biological mechanism behind the disease, much of the evidence in the literature about NCGS lacks commonality in direction and is confusing (1, 2, 4, 5, 20). As a result, the prevalence of this condition is highly variable ranging from 0.63% - 6%, depending on the source (1). Health experts do not know precisely what the disease is, what causes it and who is affected by it. For instance, two studies, Biesiekierski et al and Saponi et al both found that NCGS patients are less likely to carry the HLA DQ2/8 gene and are less likely to produce gut permeability (20, 38). This information contrasts two other studies, Vazquez-Roque et al and Wahnschaffe et al who both

found evidence to the contrary, finding many NCGS patients who express the HLA DQ2/8 gene, evidence which suggests that NCGS actually fits into a spectrum of celiac disease (39, 40).

A few studies however have had common findings suggesting that NCGS belongs to a spectrum of functional bowl disorders such as irritable bowel syndrome (IBS). The same Vazqueze-Roque et al performed a 4 week randomized control trial on 45 gluten ingesting patients with irritable bowel syndrome. All participants were screened for celiac disease via upper-gastrointestinal endoscopy and found to have normal villi. Twenty two patients were placed into a group who tested positive for the gene HLA DQ2/8 and 23 patients were in the negative group. Both gluten containing foods and a GFD was given to the patients. Mucosal permeability, daily bowel function, small bowel and colonic transit time were tested. These tests concluded that gluten in the diet did create a higher amount of small bowel permeability and did produce an increase in stool frequency in those with IBS, particularly those in the positive HLA DQ2/8 suggesting not only that gluten changes small bowel function but that an adaptive immune response might explain the gluten barrier function (39).

Another study found a connection between non-celiac gluten sensitivity and abnormal bowel functions, in particular, gut permeability. Carroccio et al reviewed the medical files of patients with irritable bowel syndrome and have also been found to have a non-celiac wheat sensitivity (WS). All patients in this study had been screened for WS by using a double blinded placebo-controlled gluten challenge (DBPC). The investigators compared these patients to two groups, 100 celiac patients and 50 IBS patients. From these results, two groups were identified – those with strictly WS (group 1) and those with WS associated with multiple food hypersensitivity (group 2). As a whole, all WS patients when given gluten showed the histology of eosinophil infiltration of the duodenal and colonic mucosal, a condition commonly seen in patients with active IBS. Also, all WS groups showed higher frequency of self-reported wheat intolerance, weight loss, anemia, food allergy in infancy and current coexistence of atopy (hyperallergic) than the IBS control group. The patients in group 2 showed symptoms more similar to allergic patients and those in group 1 showed symptoms similar to celiac disease (41).

Most recently, the results of a later study performed by the same authors, Biesiekierski et al call into question the very existence of NCGS. These authors used a double blinded crossover design to test the effects of gluten on individuals who were self-diagnosed with NCGS and had symptoms of Irritable Bowel Disease (IBS) but had relief of symptoms when gluten was removed from the diet. Thirty seven subjects were shown to be free of celiac disease as evident by a normal duodenal biopsy and the absence of both HLA-DQ2 and HLA-DQ8 haplotype. All participants continued on their normal GFD for 1 week at baseline and then were first given a 2 week diet consisting of reduced FODMAPS (poorly absorbed carbohydrates). Next, each participant was randomly assigned to 1 of 3 arms of this study. The first arm was a 1 week diet consisting of either 2g gluten (low) or 16 g gluten (high) and lastly, 14 g of whey protein. These groups were compared to a control group given 16 g of whey protein. Each participant crossed over and all subjects received all 3 treatments. After a two week washout period, twenty two subjects were then crossed over again and were given test foods of high gluten, whey protein or the control (no protein) for three days. Serum and fecal markers for inflammation/immunity activation along with incidence of fatigue were assessed. In all subjects, gastrointestinal symptoms improved during the reduction of FODMAPS treatment but the investigators were unable to reproduce any kind of symptomatic or biological gluten specific effects. The results of this follow on study indicate that it was the FODMAPS instead of gluten which may be the reason why gluten free diets are relieving symptoms in those self-diagnosed with NCGS. (38).

2.23 – Gluten and Colonic Activity

As previously discussed, undigested gluten in the colon is a trigger for an inflammatory response that is associated with CD. Therefore, the presence of gluten in the colon may play a role in microbial activity. Some studies have seen differences in the metabolic function of gut microbes in both CD patients and healthy individuals. There is evidence of unbalanced microbial colonies in some CD patients when given both a gluten containing diet and gluten free diet (GFD) (4). To determine if these differences could be due to the diet, one study examined the colonic activity of healthy individuals after ingesting different amounts of gluten. It was hypothesized that

elements of dietary gluten can be malabsorbed in the small intestine, passing into the colon for fermentation (3).

In this study, the feces of 11 healthy individuals were screened for any intestinal disorders, in particular, CD and wheat allergy, were examined under 4 different diet conditions 1. A normal gluten diet, 2. A strict gluten free diet (GFD), 3. GFD with 9 grams of gluten supplemented, 4. A strict gluten free diet (GFD) with 30 grams of gluten supplemented / day. All fecal samples were analyzed for short chain fatty acids and fecal glutenasic activity. The results showed the presence of a newly discovered enzyme called glutenase. It was demonstrated that the more gluten that is introduced into the diet, the more gluten is found in the feces. The more gluten that was found in the feces, the more glutanase was seen as well. Interestingly, this extra protein in the colon did not produce a significant amount of colonic fermentation and production of SCFA in comparison to a strict gluten free diet. A significant amount of SFAS was only seen in the subjects who consumed more than 30 grams of gluten per day, whereas the average western diet contains between 13-15 grams per day (3).

It was pointed out however that gluten used in this study also had residues of a non-digestible olligosaccharide. It is believed that the diet and the presence non-digestible starches in the gluten capsules which most likely played a larger role in the colonic activity than the gluten itself. Furthermore, although gluten intake appears to slightly affect the metabolic activity of the microbiota colonies of the large intestines, the bigger discovery was the identification of the enzyme glutenase. This newly discovered glutanase activity indicates that microbrial protease activity is a factor in gluten digestion (3). More studies are needed to determine if CD patients have this enzyme higher up in the small intestine which would indicated the ability to more rapidly degrade gluten higher up, eliciting an inflammatory response not normally seen in healthy individuals.

Another literary review, Gaesser et al, concluded that wheat consumption can be beneficial to colonic health. The wheat kernel is naturally high in oligofructose and inulin, two resistant starches which are shown to have prebiotic effects and are highly beneficial in producing healthy colonies of gut bacteria (4).

2.24 – Gluten Free Diets and Colonic Health

Gluten free diets (GFD) can be either high or low in foods which promote colonic activity. This is generally contingent upon availability and price of gluten free (GF) products and/or whether the individual is using more fruits and vegetables in place of packaged gluten free products. In some cases, packaged commercially available gluten free products can be high in refined starches and energy. Most gluten free products in the market today are made from heavily processed starches obtained from alternative grains which do not contain the same proline and gliadin content as wheat, barley and rye. Interestingly, the alternative gains used for GFP are of the grass family but more closely related to corn (42). The typical non-gluten containing cereals which are the most common alternatives to wheat are rice, maize (corn), cassava, sorghum and also starchy potatoes. Next common are considered to be the minor cereals, millet, teff, fonio and Job's tears. Lastly are the pseudocereals, amaranth, quinoa and buckwheat (3, 10, 42). When these grains are used for baking, gluten substitutes like xanthum gum are added and often, these products are very processed and stripped of dietary fiber. As such, gluten free diets (GFD) in the general population can be either high or low in refined starches, whole grains and fiber (2, 9).

2.25 – Gluten Free Products, Availability and Costs

As previously mentioned, gluten free diets can be higher in fruits, vegetables and meats. However, the Lee et al, article did not look at this aspect of diet. Instead, it looked at availability of packaged gluten free foods and found that these products are limited in stores This can often lead to consumers to purchasing gluten free products from specialty stores and/or the internet. In addition, this study found that gluten free products are 2 – 3 times more expensive than regular gluten containing products. The availability of these products varied across regions but price did not. In addition, many of the gluten free products tested in this study contained fewer nutrients than gluten containing product. This may cause dietary conscience customers to buy supplements of vitamins and fiber, adding an additional burden to the adherence of this diet (9).

Lee concluded that in the U.S. gluten free foods are generally more expensive, can be less nutrient dense (lower in both vitamins and fiber), than their gluten containing counterparts and are often harder to find, making compliance to a gluten free diet hard to maintain (9).

2.30 – Glycemic Response

The glycemic response to food is described as "the change in blood glucose concentration induced by food ingestion" (24). The glycemic index (GI) and the glycemic load (GL) are elements of the glycemic response. All of these measures refer to incremental area under the blood glucose curve (IAUC) during a time period of 2 – 3 hours after the ingestion of a meal (postprandial). Specifically, the GI refers to the amount of available carbohydrate in a food which will raise the blood glucose in comparison to a reference, typically 50 grams of glucose (GI = IAUC food / IAUC glucose). The GI does not tell us how significant the rise in blood glucose is based on the amount one will typically eat (serving size). Whereas the GL refers to the typical amount of carbohydrate in a food consumed in a single intake and over a specific period of time (GL = GI / 100 x P x weight of food [where P = the proportion of available carbohydrate in the food]). Therefore, the GI is not necessarily a customary intake but the GL is more reflective of what an individual will likely consume in a single sitting (23).

2.31 – Glycemic Response Factors, Mechanism and Health Aspects

Glycemic carbohydrates, are those which can be broken down by the human digestive enzymes. After digestion of carbohydrates, individual glucose molecules are absorbed in the gut lumen through active transport via SGLT-1 then subsequently released in the portal vein by facilitated diffusion using GLUT 2, contributing to the rise of blood glucose. As glucose enters the bloodstream, a hormone called insulin is then released from the β–cells of the pancreas. Insulin takes glucose into the cells of the heart, muscle and adipose tissues via the insulin-sensitive GLUT 4 transporter. However, other organs such as the brain and the liver also uptake glucose but this occurs through facilitated diffusion that does not require the action of insulin (42).

Energy production occurs in the cell's mitochondria of the muscles and fat tissue where glucose turns into pyruvate then adenosine triphosphate (ATP). During muscle contractions ATP is hydrolyzed to form ADP (adenosine diphosphate) then if the demand for energy metabolism is

still active, ADP then gives a phosphate back to ATP in a cyclic process. When this happens, ADP turns into AMP (adenosine monophosphate). When the demand for ATP increases and the AMP to ATP ratio increases, AMPK (AMP-activated protein kinase) is released (44, 45,56). Circulating blood levels of AMPK have been shown to inhibit anabolic energy consuming pathways such as protein and fatty acid synthesis (weight gain) and stimulates energy production from catabolic systems such as glucose uptake and fatty acid oxidation which lessens blood triglycerides and cholesterol production (44,45, 46). Hence, if the body is consuming energy but not expending it and if energy stores are full, excessive insulin and the inhibition of circulating AMPK will also signal the body to synthesize tissue and gain weight.

In normal individuals, postprandial blood glucose will spike and then quickly regulate, returning to fasting levels within 2 – 4 hours (44). However, a condition known as insulin resistance occurs when the insulin receptors of the cells become impervious to the action of insulin (66). In many cases, this condition progresses into the development of type 2 diabetes mellitus. When the cell's insulin receptors are insensitive, the cell cannot efficiently uptake glucose for energy production. Individuals with insulin resistance and type 2 diabetes mellitus will have both elevated glucose and insulin levels in the blood for several hours during the postprandial period, in contrast to those who have type 1 diabetes. Type 1 diabetes mellitus is an autoimmune disorder that causes the destruction of the pancreatic β-cells and results in little to no insulin production. These individuals will have high blood glucose levels during the postprandial phase but they remain in a state of hypoinsulinemia (47). Those who have prolonged hyperinsulinemia from either overproduction or lack of clearance of insulin can have an allosteric inhibition of AMPK (66). In addition, those who have mitochondria dysfunction, a condition believed to be from over-activation of ATP and a subsequent inhibition of AMPK. Both hyperinsulinemia and mitochondria dysfunction, two conditions common among obese individuals, is believed to induce insulin resistance (48). In the general population, obesity is said to be one of the major risk factors for having insulin resistance (48). Interestingly, some studies have found that the attenuation of ATP production from weight loss, caloric restriction and exercise all lead to improved states insulin resistance. From this evidence, some researchers

believe that the surplus of ATP production is the most relevant risk factor for insulin resistance (48).

The glycemic response to a carbohydrate food is also contingent upon its bio-availability. The non-glycemic carbohydrates, resistant starches and dietary fiber, escape digestion and pass freely into the colon where they are utilized as substrates for colonic micorflora (Parada, Nugent). The byproducts of the fermentation are gasses such as methane, hydrogen and carbon dioxide and short chain fatty acids (SCFA), mainly acetate, propionate, and butyrate. Short chained fatty acids have multiple beneficial systemic effects, including cancer protection for the intestinal epithelial cells and in addition, they can be absorbed in small amounts through the portal vein and utilized for energy by the human host through a process called gluconeogenesis (14, 32). Generally, foods that have a moderate glycemic response are said to be of higher nutritional quality than in comparison to foods with a lowered glycemic response (14, 32).

2.32 – Glycemic Response and Mood

Although the evidence in the literature is lacking in the connection between low glycmic diets and mood, the overall consumption of carbohydrates in the diet has been connected to improved mood states (11, 12, 49-53). Authors Benton, Cheatham and Sathyanarayana et al, discusses the important relationship between insulin and brain functions - as the consumption of carbohydrate rich foods signals the release of insulin and the subsequent up-take of glucose into the cell for energy, insulin also acts by signaling the entrance of tryptophan into the brain. Neurotransmitters in the brain, like serotonin and its pre-curser tryptophan, are directly affected by carbohydrate ingestion and blood tryptophan levels. Since both of these neurotransmitters have been connected to the feeling of wellbeing, diets low in carbohydrates have been shown to bring on depression (11, 49, 50).

Decrastro et al used 38 undergraduate students and measured macro nutrient ingestion and mood by self-reports. Diet records were examined over a nine day period and significant correlations were found between the overall self-rated mood state over time and the percentage of each macronutrient in the diet. A positive correlation was found between diets high in carbohydrate consumption and improved moods and negatively with depression

(51). Conversely, a high protein and low-carbohydrate diet was positively correlated with depression and anger.

A similar effect was also seen in a study which tested a protein rich, carbohydrate poor (PR/CP at 40% carbohydrate) diet in comparison to a carbohydrate rich, protein poor diet (CR/PP at 60% carbohydrate). Although the macro nutrient content of the test meals were reported in carbohydrate, protein and fat, they did not report the amount of fiber nor the glycemic load of the test meals. These meals were tested on 24 individuals with high stress-proneness (HS) and individuals with low stress-proneness (LS) with the idea that those with HS had lower levels of brain tryptophan levels. Both mood states and serum tryptophan level were measured. A significant increase in serum tryptophan levels was seen in all of the CR/PP groups and not the PR/CP groups. And although they did not find improved moods in the CR/PP HS group, they did find a significant increase in depression in the PR/CP HS groups (52).

Keith et al tested experimental diets containing low (13% CHO), medium (54%) and high (72%) amounts of dietary carbohydrates on seven trained female cyclists. Each diet was tested for 7 days while normal training routines were prescribed. After each treatment, the subjects were asked to ride till fatigued then they were given POMS (profile of mood states) tests. The POMS test consists of 65 adjectives that are rated by participants on a 5-point scale. They concluded that the diets low in carbohydrate and high in protein and fat produced significant negative mood changes in the subjects that were detrimental to their training. When given more carbohydrates, their mood condition improved significantly. These authors have shown that diets rich in carbohydrates can improve moods (53).

Another study tested simple v. complex carbohydrates on blood parameters, satiety, hunger and mood. Twenty six healthy male subjects of normal weight were given breakfasts containing either a complex carbohydrate meal (71g CHO) or a simple carbohydrate meal (74g CHO) in a randomized crossover design. When given the simple carbohydrate meal, the subjects had higher postprandial glucose and insulin values and higher fatigue as expected. However, when given the complex carbohydrate meal, the subjects showed higher postprandial satiety scores and lower fatigue scores in comparison to the simple carbohydrate groups. Although the

POMS scores were improved in the complex carbohydrate group, the difference was not significant (12).

The results of these studies have shown a connection between carbohydrate rich diets and improved moods (11, 12, 49-53). In addition, complex carbohydrate foods that are lower on the glycemic load index, foods such as whole grains, fruits, vegetables and pastas will produce a more sustained release of insulin, better glycemic control, higher satiety and lower fatigue then in comparison to carbohydrate rich foods higher on the glycemic load index (52, 53)). Hence, longer term studies that test the relationship between complex carbohydrate diets and healthier moods may produce more significant results.

Cheatham et al, examined the long term effects of mood, weight loss and cognition in subjects who consumed high (60% carbohydrate) or low glycemic (40% carbohydrate) diets for six months. Thirty four healthy overweight volunteers were randomized and put into one of two diet groups consisting of foods either low glycemic (low GI) or high glycemic (high GI) foods. The test foods were provided by the researchers (3 meals/day, one snack and limited liquid calories) and all of the test foods in both groups were of equal energy (kcals) and fiber, however the groups differed by energy density, carbohydrate, and glycemic load. All participants in this study received 30% less of baseline energy requirements as determined by doubly labeled water procedure (50). Two things should be noted about this trial, although this study did lessen the overall amount of carbohydrates between groups, even the low carbohydrate group still had a significant amount of carbohydrate in the diet. In addition, when differentiating between lowered glycemic foods and higher glycemic foods, they are not only referring to a lower amount of carbohydrate in the diet, they are also referring to type of carbohydrate and ultimately the glycemic load of the food in the diet. Whereas only one of the previous studies mentioned, Passman, (12) used the glycemic load index. Secondly, this study tested the long term results of mood as compared to the shorter duration in the prior studies (11).

The results of this study found no significant change in cognition, however, a highly notable change in mood was seen when observing periods of the acute rather than the long term effects. A subclinical depression increased over the long term in the high GI group but was not

seen during the randomization of the low GI groups (11). Therefore, according to their findings, a low glycemic diet can improve moods in the long term. These results seem to contrast the carbohydrate depression hypothesis were the HC diets were seen to induce more tryptophan levels and improved moods. However, because the both groups of this study had significant amounts of CHO in the test meals, the improvement of moods may be directly related to the type of CHO and sustained release of glucose from the glycemic load of the test food.

2.33 – Glycemic Effect and Grains

Several studies have tested different carbohydrate foods to determine the glycemic response, in particular whole grains in comparison to refined wheat. Isaksson et al,tested the satiety effects after the consumption of breakfast meals made from either iso-caloric refined wheat bread or whole grain rye porridge. In this study, 24 healthy subjects were randomly assigned to daily consumption of either the porridge or the bread groups for two 3-week phases, separated by a washout of 3-4 weeks. Each phase had 3 visits on days 1, 8 and 22. On each day, appetite (hunger, satiety, and desire to eat) were registered under standardized conditions for 24 hours. In addition, orocecal transit time was measured and breath hydrogen to determine colonic fermentation on day 8 of each phase. Food diaries were also used to determine the effects of the test breakfasts on free living food intake (54).

During the four hour postprandial period, higher rate of satiety, lowered rates of hunger and lowered desire to eat was seen in the porridge groups. These results were sustained throughout the 3 week study phase. However, unlike prior studies with similar design, this study did not show these effects to last till the afternoon (4-8 hours). After consumption of both meals, the orocecal transit times were similar (5-6 hours) but the breath hydrogen results showed that colonic fermentation occurred after 4-8 hours and did not coincide with any change of appetite. However, the rye porridge did produce more colonic hydrogen in comparison to the refined wheat meal. Although a satiety effect was seen during the postprandial period, there was no change in energy intake during the hydrogen producing phase. These results did persist for 3 weeks, after daily repeated consumption, an indication of a protective role against weight gain.

This study showed that during the four hour postprandial period, a diet of whole grain rye porridge at breakfast time has the ability to increase satiety as compared to a breakfast meal of refined wheat and the consumption of whole grain porridge is sustaining during regular consumption. A diet rich in dietary fiber from whole grains leads to the passage of macro nutrients in the lowered intestines and may produce satiety signals in combination with slow passage time, hence a lowered glycemic effect (54).

Nilsson, Ostman et al, tested different breakfast foods (cereals) high in indigestible carbohydrates on the postprandial glycemic response. Twelve healthy subjects participated. The test foods were a reference product made of white wheat bread (WWB), a porridge made from barley kernels (and flour from the kernels), wheat kernels, oat kernels, rye kernels, and lastly white wheat bread enriched with barley (DF) (WWB + Barley DF), and assigned to the subjects in random order. In series one (1 or 2x/week w/ 3 d apart), the subjects were given the WWB and water the night before for dinner. The day of the test, the test meals were consumed at breakfast then after the breakfast. Postprandial glucose samples were collected at (before meal 0, 15, 45, 60, 90 & 120 mns) and incremental areas under the curve (IAUC) were plotted. Lastly, colonic fermentation was determined by using a breath hydrogen test upon fasting state and then ½ hour increments after the test meal (15).

In series two, the test meal was consumed in the evening and then the IAUC values were plotted after a standardized breakfast consisting of the WWB. Each participant was tested 1 or 2x/week on 3 separate occasions). Blood glucose and breath hydrogen were taken in the same manner after standardized breakfast (15).

In comparison to white wheat bread, the rye kernel breakfast produced lowered blood glucose at lunch and dinner but breath hydrogen was negatively correlated. But, the barley kernel breakfast produced the most colonic fermentation and significant lowered blood glucose in comparison to the white wheat bread. The consumption of high fiber whole grain cereals can produce a lowered glycemic effect, with a second meal and a mixture of carbohydrates having the greatest effect (15)

2.34 – Glycemic Effect and Resistant starch (corn starch)

Many studies have found that high fiber foods can produce colonic activity, a lowered glycemic effect and have greater satiety in comparison to refined grains. However, the scientific literature is limited in testing the health effects of resistant starches in the diet. Another study conducted by Nilsson, Ostman et al. tested the potential beneficial glycemic effects of different resistant starches (RS) in addition to dietary fiber (DF) in whole grain products. Glucose tolerance and the underlying mechanism for improved glucose regulation was measured after the consumption of these products after an evening meal and subsequent morning breakfast. The test products consisted of breads made with ordinary barley kernels (OB), OB that were cut 1 – 2 times (CutOB), barley kernels with elevated levels of amylose producing increased amounts of resistant starch (HAB), barley kernels with increased amounts of β-glucans (HBB), white wheat bread with added high RS cornstarch (WWB+RS), white wheat bread with added RS from cornstarch and dietary fiber from barley kernels (WWB+RS+DF), in comparison to the white wheat bread (WWB) (55).

Fifteen healthy subjects participated in this study and were asked to consume a diet low in DF the day before, refrain from smoking drinking, heavy exercise and could not have consumed antibiotics or probiotics for that last two weeks. Each subject was tested once a week on each test food. The night before the test, the subjects consumed the test product (in random order), then fasted throughout the night and then came into the lab, gave fasting samples, then ate the same test food again for breakfast, more tests resumed for several hours after (55).
For the glycemic index/load measure, blood was taken before and repeatedly after the standardized breakfast, finger prick for glucose, venous insulin, plasmaglucogon, plasma incretins [glucogon like peptides (GLP-1)] and gastric inhibitory peptides (GIP). Gastric emptying was measured using a breath hydrogen device andgastic emptying rate (GER) was measured using serum paracetmol as a marker. Lastly, satiety was measured using a bipolar rating scale of statements of feelings of satiety and hunger (55).

The different test foods did not produce significant differences in fasting blood glucose, serum insulin and/or serum glucagon by the subsequent morning. However, at breakfast, all of

the test meals produced lowered glucose responses in comparison to the control of WWB. In addition, all of the kernel meals (including the cut kernels) produced lowered blood glucose peak increments in comparison to the WWB control. The insulin response after an evening meal was lower in the OB group in comparison to the WWB. With satiety, all of the test foods had greater initial satiety results after breakfast, with OB being the highest in comparison to the WWB. With breath hydrogen, the HBB bread produced the highest results after an evening meal. The HAB bread produced the highest colonic activity after the breakfast meal. In the postprandial stage, the OB bread had the highest (55).

This study showed that whole grain based evening meal and a subsequent morning breakfast had the ability to produce a lowered postprandial glycemic effect and a higher amount of colonic hydrogen in comparison to refined wheat bread. In addition, the simulation of adding both resistant starch, from high amylose corn starch, and dietary fiber to refined wheat bread to equal the same macro nutrient content found in the whole grain barley bread was sufficient to elicit a significant lowered glycemic effect similar to the whole grain barley bread. These results show that it may be possible to tailor novel foods with added RS and dietary fiber which are capable of facilitating glycemic regulation.

Twenty two women were recruited for a crossover experiment and were asked to consume 5 different breakfast bars each one containing a different dietary fiber. The subjects were tested 5 times on each bar. Each bar had 10 grams of one of the following fibers; oligofuctose, inulin, soluble corn fiber or a resistant cross-linked starch made from wheat. These 4 bars were compared to a control consisting of no fiber. For the test, the subjects arrived in a fasted state, ate one of the bars with water, coffee or tea. Three hours later, they consumed a preselected meal of a Stouffers French Bread Pizza. A VAS questionnaire was collected at 0, 15, 30, 45, 60, 90, 120, and 180 minutes with lunch served at 180 minutes. Appetite sensations such as prospective meal satisfaction, satiety, and hunger were measured in addition to tolerance of the test product. Also, food intake diaries were recorded for 24 hours after each test. Lastly, colonic hydrogen was measured at baseline (0 minutes) and just before the pizza meal at 180 minutes. The results from these tests showed that all the test foods were well tolerated but there

were no differences in satiety or food intake at breakfast or at lunch. However, the colonic hydrogen tests showed that oligofructose produced the highest amount of colonic hydrogen with corn fiber and inulin producing the second highest. All three measures were much higher than both the RS wheat and the control (13).

In relation to hunger and fullness factor and after the adjusted mean and standard error, all of the test foods were similar to the control. However, in relation to the breath hydrogen tests, oligofructose had the highest, inulin and corn were the next highest. The wheat and the control had the lowest hydrogen. With methane, corn had the highest results with oligofructose and inulin showing the next highest. Both wheat and the control had similarly the lowest. Strangely, wheat had some of the highest bloating/flatulence factors, similar to oligofructose and inulin. Corn had some of the lowest bloating similar to the control. Stool frequency was similar in all categories with corn and the control being the lowest. The variations in the type of fiber and the way the fiber is processed all contribute to how the fiber is metabolized in the human body and which physiological effects are influenced (13).

Although these results show that the type of fiber did not affect satiety or food intake, it did affect the production of colonic hydrogen. Perhaps with a higher intake of both energy and fiber would produce these affects. One of the most surprising outcomes was that the corn produced a higher amount of colonic hydrogen than the wheat which was made of a cross-linked RS. From these results and from other studies which linked colonic hydrogen to a reduced glycemic response, it can be hypothesized that corn will produce a lowered glycemic response in comparison to wheat (13).

Another study wanted determine if the type of starch and/or fiber matters when measuring satiety another study, Willis et al tested the satiety effects of four muffins containing different types of dietary fiber, one having resistant starch. All of the high fiber muffins were compared to a low fiber muffin. All were similar in micro nutrient content except for the fiber and were similar in appearance. All muffins were made from the same recipe mix. The control was a low fiber muffin containing 1.6 grams of fiber, and the other four had approximately 10 grams one of the following – corn bran (CB), barley β-glucan + oat fiber (BG), resistant starch from high

amylose maize (RS) and polydextrose. Twenty healthy subjects participated and consumed one of the four muffins on five separate occasions. Each visit was in morning which allowed the subjects to arrive in the fasting sate. Satiety cannot be assessed by answering one question. Therefore, satiety was accessed through multiple questions at 15, 30, 45, 60, 120, and 180 minutes after baseline through a Visual Analogue Test (VAS) such as: hunger - how hungry do you feel? Not hungry at all (0mm) vs I have never been more hungry; fullness – how full do you feel? Not full at all (0mm) vs totally full (100mm); satisfaction – How satisfied do you feel? I am completely empty (0mm) vs I cannot eat another bit (100mm); prospective food intake - How much do you think you can eat? Nothing at all (0mm) vs a lot (100mm) (16).

With hunger and food intake, the AUC scores showed that the low fiber control had the highest effect with both polydextrose and BG having the second highest levels. The RS and the CB both produced the lowest hunger scores. Satisfaction and fullness were conversely the same. The low fiber and the polydextrose had the lowest values while both RS and CB produced the highest. The results from the VOS questionnaire indicated that the type of fiber does influence satiety with RS and CB consistently having the greatest effect in the short term, even more so than the barley β-glucan + oat fiber (BG). Conversely, the polydextrose and the low fiber muffin were similar and had the least effect on satiety overall (16). These findings warrant a closer look at the structure and health effects of resistant starches, especially those produced from corn.

Ward et al used differential scanning calorimetry to study the retrogradation properties of isolated amylopectin (AP) from both native wheat and corn starches. After an initial gelatinization and subsequent cooling, drying and storage, the corn AP produced significantly more crystalline structures (retrogradation) than did wheat AP. This was demonstrated by a higher enthalpy, the energy required to melt the crystalline material back to a gelatinous stage. Most of this crystallization occurred within one week of storage. In addition, the amount of enthalpy required to gelatinize the initial melting of the native starches was also higher in the corn than the wheat. It is theorized that the differences in retrogradation between the two starches may be explained by the differences in their glycolytic chain lengths. The native corn AP starch had shorter chains but more of them. The higher degree of chains in the corn may explain its higher

capacity to retrograde. From these results, it can be hypothesized that the drying and storage required to manufacture pasta can cause a corn pasta to develop more RS than a wheat pasta (37).

Berti et al tested both the in vitro starch digestibility of GF foods and the in vivo metabolic response to GF foods. The in vitro digestibility was performed by a multi-enzymatic process performed with pepsin and α-amylase to simulate digestion in test tubes. This was followed by an analysis of the reducing sugars. The in vivo test involved seven healthy women and six celiac women who were asked to arrive in the morning in a fasted state, consume the test meals and then an intravenous catheter was inserted into a vein for blood sampling. Both glucose and insulin values were recorded at 15,30,45,60, 90, 120, 150, 180 min after the start of the meal. The test on the healthy individuals compared the glycemic response of regular pasta, GF pasta (made from rice and potato starch), regular bread, GF bread and lastly whole grain quinoa. Satiety sensations from each test food were assessed using VAS tests. The test on the celiac subjects compared the glycemic response of GF bread and GF pasta, and this was compared to the glycemic response of the healthy individuals (17).

The in vitro results of starch digestibility showed that the AUC for GF bread was slightly higher than that of regular wheat bread but the AUC of GF pasta was the same and when comparing the AUC of the quinoa to the two pastas, there was no significant difference.
The in vivo test results showed that there were some glycemic response differences. Among the healthy and celiac individuals, the glycemic response of the GF bread and GF pasta was the highest in both groups but the celiac group was higher than the healthy individuals. With the insulin response, there were no significant differences between all products. With the glycemic index (GI), the results showed that the GF pasta was similar to the GI of the GF bread but the GI for the quinoa was lower than both the GF pasta and GF bread (17).

These test results show that that in vivo glycemic response to the GF bread did correlate to the in vitro digestibility results of the GF bread. However, no other in-vitro digestibility results correlated with the in-vitro glycemic responses. One of the most significant results from the in-vivo test was the lowered glycemic response to the quinoa in comparison to both the GF bread

the pasta and GF pasta. It can then be hypothesized that adding quinoa to GF pasta might have the ability to lower the glycemic response to GF pasta. In addition, it should by noted that this test used a GF pasta made from both rice and potato. It is therefore unknown what effect a GF pasta made from corn would have. Ultimately, it is difficult to interpret the meaning of these results because the amount of individuals tested were low and therefore significance was not obtained in other areas. These results warrant a closer look at the glycemic response of gluten free products (17).

Extrusion Processing - 2.35

Extrusion is a type of food processing used to make a variety of products. Typically food extrusion begins with a ground up mixture which is combined with other ingredients, then passed through a machine and condensed down to make a final product (56). First, raw ingredients, typically starch grains, are ground into flour then conditioning ingredients are added. Water, fat, protein or dyes bring the mixture together into dough and then the mixture is forced through, or injected into the extruder. Once the mixture is passed through, it can be used fresh or dried for longer shelf life. Extruded food products can be pastas, breakfast cereals, breads (breadsticks and croutons), cookie dough, ready to eat snacks, confectionary and even pet foods. This type of low cost food processing ensures speed and uniformity, three qualities highly valued by food manufacturers. (56).

Extruder machines are made up of rotating screws which pass the dough through a final perforated sheet called a die. Finally, a blade cuts the product as it passes through the die. Extruder machines pass food through at different speeds and at different temperatures (56). Foods are either extruded using a cold process or a cooking process. During the cooking process, steam is injected into the dough as it passes through the die (57). Both the steam and the friction of the extudate passing through the die cook and change the starch matrix. This step ultimately gelatinizes the product, the extent to which depends upon the amount of steam added and the speed of the extrusion (57). The volume of the final product typically expands due to the release of steam and reduction of forces, a process known as expansion ratio. Foods that are extruded using the cold method do not expand significantly (57).

As with many different food processing techniques, extrusion can influence the degree of digestibility of a food. Dahlin et al, tested the starch digestibility of extruded whole grain cereals (wheat, rye, corn, millet, and low and high tannin sorghum) and quinoa. This study found that four factors greatly favored the starch digestibility in the extruded products: temperature, moisture, screw speed and the type of grain being used (58). An extrusion temperature of 100/150 had the greatest effect. It also showed that an extrusion feed moisture of 25% was favorable and the screw speed of 100 rpm was the least favorable of the processes. These three factors showed to greatly increase the starch digestibility in all of the grains except for the quinoa (58). Therefore, in this study, the only factor which lessened the glycemic response proved to be the type of cereal being tested.

With fiber, author Eastman reports that the fiber content of pasta dough does not change after extrusion. However, the proportion of soluble to insoluble fiber does change making the soluble portion higher (59). Dietary fiber in extruded products, particularly insoluble fiber, can inhibit expansion and subsequent gelatinization during the cooking process. A lower expansion means lower moisture and a lowered gelatinization which can result in a lowered absorption rate. Thus, if the extrusion process of whole grain dough makes the soluble portion higher and the expansion higher, extruded whole grain products can produce a higher absorption rate than the un-extruded whole grain products. Other factors which can influence expansion and gelatinization of extruded products during the cooking process can be the protein content and the ratio of amylose to amylopectin. Products which have higher amounts of protein and higher amounts amylose can produce less expansion. Quinoa and wheat products have higher amounts of protein than the corn and rice. However, the proportion of amylose/amylopectin in rice, wheat corn and quinoa are similar (59).

Brennan et al tested effect of dietary fiber on starch digestibility in extruded cereal products by comparing a control of white wheat flour to various samples. Each sample had one of five different types of dietary fiber and one with hi amylose maize added into the wheat base. Each dietary fiber was added at different percentages, 5%, 10%, and 15%. During the test, digestibility was tested in vivo at 0, 20 min, 60 min and 120 min (60). It found that when comparing the starch

digestibility in the control from the raw sample to the extruded sample, the extrusion process significantly increased the availability of carbohydrate for absorption at all intervals. However, the different dietary fibers at different percentages did slow down the starch digestibility of both the raw and the extruded products depending on the type of the dietary fiber and the percentage added. Interestingly, the addition of the hi-maize to the extruded sample did not reduce the amount starch digestibility and produced similar and increased available when compared to the control (60).

Wheat pasta contains gluten, a structural protein which allows the product to maintain a degree of firmness after cooking. Because of this, wheat pastas are typically made using the conventional cold processing. However, gluten free pastas do not contain a structural protein and if made using the cold processing, these products will lack quality of texture and will result in a product that is too delicate (60). As a result, food manufactures have found other ways to ensure the desired firmness and integrity in their gluten free product. Some have resulted to adding structural ingredients such as modified starches, emulsifiers, gums, enzymes and other proteins. Yet other manufacturers have focused on the source of starch including corn, rice, legumes and pseudocereals. A third approach uses processing conditions. Recently, food manufacturers have discovered that by using parboiled grains in the cooked extrusion process, gluten free starches can undergo significant changes that can greatly improve the structural integrity of these pastas (60,61). This process can also affect the glycemic response. Researchers Marti et al performed two studies which tested the starch qualities of both pasta making processes (cold and cooked) on gluten free rice flour. These trials used parboiled rice flours from both milled and brown rice. Raw rice kernels were soaked, steamed then drying, a process that is known to cause retrogradation. With this method, the starch gelatinizes then as vitamins migrate toward the endosperm a lipid amylose complex is formed. This prevents further swelling and amylose leaching during cooling. The reorganization of the starch molecules help to increase hardness and reduce stickiness in the final product (61, 62).

Each mixture was extruded by the conventional cold method (50˚ max) and the cooked method (115˚ C). After drying, both pastas were cooked to determine different structural changes

in the product. The thermal properties of each pasta group were examined and it was determined that the parboiled pastas which were made using the cooked extrusion process required a higher melting point than the parboiled starches made using the cold extrusion process (61, 62). Furthermore, the pasta made from the heated method required a longer melting time, results that were confirmed again the second trial.

From these examples it can be determined that the level of starch digestibility in extruded gluten free food products is highly variable. Overall, extrusion does increase the glycemic index of food, in particular pastas. However, factors during processing such as temperature or the amount of liquid added or whether or not protein is present will also contribute to the efficiency of digestion. In addition to processing, the type of grain and dietary fiber will also lessen or increase the level of starch availability. Even more complex, recent manufacturing of gluten free pastas are using pseudocereals in combination with both parboiling of the raw product and cooking during extrusion, a process that greatly influences both the texture and the glycemic response of these products. From this, it can be concluded that because of the complexity of the processes and the different substrates used, the glycemic response of extruded gluten free products can be difficult to determine.

CHAPTER 3

METHODS

3.0 **Participants**

Seven individuals, aged 18 – 45 yrs, were recruited by way of college and departmental list serves at Arizona State University. Respondents were directed to a Survey Monkey for initial screening. Qualifying individuals were then contacted and invited to meet with researchers to sign a consent form and to complete more detailed screening for medications, food allergies, and disease states. Subjects who participated reported no food allergies, gastrointestinal disorders, or history of glucose intolerance, and had not been diagnosed with diabetes. All participants provided written informed consent and this trial was approved by the Arizona State University Institutional Review Board prior to the recruitment of participants (63, 64).

3.1 **Study Design**

Subjects participated in a trial which lasted for five weeks and consisted of four separate meal-based glucose tolerance tests. Each test lasted four hours and was spaced about 7 days apart. The randomized assignments of the different treatment groups were chosen from a block design using periods 1, 2, 3 and 4, provided by GraphPad.com. A double blind crossover study design was utilized, and the test meals (wheat, rice, rice and corn, and corn and quinoa pasta) will be consumed one week apart in random order (63). All participants followed their regular diets. For three days prior to each experimental day, the participants were instructed to consume at least one bagel daily (54 g carbohydrate/bagel). On the day prior to testing, participants will refrain from moderate to heavy activity. In addition, to control the amount of colonic hydrogen gas, participants were prescribed a standardized low residue dinner consisting of one 240 ml can of vanilla Ensure Plus drink (Abbott Laboratories Ross Products Division, Columbus OH) (24), which contained no fermentable carbohydrates and produces only negligible colonic fermentation. Additionally, participants were given one more bagel, enough to provide a third of each subjects daily caloric needs, which were calculated from the Harris-Bennedict equation multiplied by 1.3, a ‘very light’ activity factor (63). Participants fasted overnight (no foods or beverages with the exception of water) and arrive at the test facility the next morning at 7:00 and

8:00 in a fasting state (63). The study treatment meal consisted of 4 oz of cooked pasta cooked in salted water then combined with 2 oz of a pre-made processed cheese sauce.

3.2 Blood Glucose and Serum Insulin Analysis

Fasting venous blood samples were collected prior to the meal (baseline) for insulin analysis. Capillary blood from a finger stick was used to assess glucose. The test meal was consumed in 10 minutes and finger sticks were performed by using a portable glucose check monitor (Acu-Check Advantage Blood Glucose Monitoring System, Roche Diagnostics, Indianapolis, IN) at 0, 30, 60, 90, 120, 150, 180 and 240 minutes after the first bite of food (58). At thirty minutes after the ingestion of the test meal, a second venous blood sample (7-10 ml) was collected in serum separator tubes for insulin measurement. After centrifuging for 15 minutes, the samples were analyzed for insulin using a radioimmunoassay procedure at the nutrition laboratories at ASU.

3.3 Breath Hydrogen

Before the test meal was consumed and again four hours after ingestion of the meal, breath hydrogen was collected using a mouth piece and collection bag (QuinTronAlveoSampler bags model#QT00842-P, 122 QuinTron Instrument Company, Milwaukee, WI) (25). Breath samples for both the fasting state and the post meal were analyzed for hydrogen and methane by using a BreathTracker SC from QuinTron (64). All three values from the breath samples were corrected with a carbon dioxide measurement (64). Some individuals produce methane (CH_4) and some do not. If methane is present, it is made at the expense of hydrogen (25). Therefore total H_2 was calculated by accounting for the additional hydrogen molecules in the methane values (methane x 2 accounts for the two H2 that make up the methane).

3.4 Mood Sates

Mood states were evaluated by using a standard POMS (Profile of Mood States) questionnaire before the meal in a fasting state and again at two hours after the test meal. The POMS questionnaire has been commonly used in fields such as Nutrition, Dietetics, psychiatry, exercise physiology, and experimental psychology to test the effects of dietary components on

mood and behavior states. A response to questions about “how are you feeling” is graded on a 5 point scale of mood states: tension–anxiety, depression–dejection, anger–hostility, vigor–activity, fatigue–inertia and confusion–bewilderment (11, 53). The scores for tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia and confusion-bewilderment were first added up then the score for vigor-activity was subtracted. A higher the score, the more indicated a disturbed mood and a lower score indicated less of an effect.

3.5 Satiety

Satiety was measured by using a visual analogue questionnaire with 6 lines of 10 cm in length. These lines were anchored with words representing various degrees of hunger or satiety feelings with not particular feeling in the center. To the right of the center the lines represented feelings of feeling full or satisfied and to the left of the center the lines represented feelings of hunger by asking the following questions respectively: Extremely Hungry? Hungry?, Semi Hungry?, No particular Feeling (center)?, Semi Satisfied?, Satisfied?, and Extremely Full? Quantification of this data was performed by measuring the distance from the left end to the area marked by the respondent.

3.6 Statistical Analysis

Based on a previous trial, power analyses indicated that 7 subjects would be needed to produce 15% peak glucose concentrations with an 80% power (64). After the experiment, significant differences in outcome variances were determined by using a multivariate analysis which controlled for potential covariates. A Spearman’s correlation was performed on the data to determine any correlation between variables. These results are expressed as the mean ± SE. A repeated measure analysis was used on each variable in the randomized block design (65). Sample analysis were checked for normality using Shapiro-Wilk test. To improve any non-normality in variance, log data transformations were performed when necessary. If any log transformations failed to achieve normality, non-parametric tests were used. If P values of the results were <.05, the data was declared to be statistically significant (64). SPSS was used for all data analyses.

CHAPTER 4

RESULTS

4.0 - Subjects

Ten non-smoking, non-vegetarian, non-lactose intolerant, healthy individuals met the screening criteria and were enrolled in the study; two subjects never started the intervention and the second subject withdrew after 2 sessions due to their inability to tolerate the complexity of the study protocol. The data reported below represent the 7 subjects who completed the trial. Data were normally distributed unless noted. Participant characteristics and anthropometric measurements are displayed in Table 2. Three men and 4 females, 6 were Caucasian and 1 was Hispanic, had a mean age (± standard deviation) of 37.3± 13.8 years (range=19 to 53 years). The mean body weight was 156.5± 97.4 lbs. (range= 97.4 to 223.6lbs), with a mean body fat percentage of 24.3 ± 7.2 (range = 12.7 to 32.3) and a mean body mass index of 24.3±0.4 (range=17.8 to 30.5). Of the 7 subjects, 1 was overweight (BMI = 27.8) and 1 was obese (BMI = 30.5). The mean fasting glucose of the participants was normal (90.7 ± 6) but the mean fasting insulin showed mild insulin resistance (12.2 ± 5.1). At screening, all participants reported no history of gastrointestinal disorders, diabetes, glucose intolerance or infections. One participant reported partial removal of the sigmoid colon in years prior. None of the subjects reported any recent use of enemas or colon cleansers and not had been through recent corticosteroid therapy or was on antibiotic therapy. Upon the screening visit, all participants signed informed consents.

Table 2. Participant Characteristics n = 7		
	Mean & SD	**Range**
Males; Females	3;4	
Age (yrs)	37.3 ± 13.8	34
Weight (lbs.)	156.5 ± 45.6	127,2
Body Fat (%)	24.3 ± 7.2	19.6
BMI (kg/m^2)	24 ± 4.3	12,7
Fasting Glucose	90.7 ± 6 (normal = <110 mg/dl) [58]	13.25
Fasting Insulin ulU/mL	12.2 ± 5.1 (normal = 8 -11) [69]	7.41

4.1 - Blood Glucose and Insulin Concentrations

Mean glucose and insulin concentrations are displayed in Table 3. Mean glucose concentrations are graphed in Figure 1. Among all four groups, there were no significant differences in glucose concentrations at baseline (p = .908) nor were there any significant differences among the four groups from baseline to the completion of the test at 240 minutes (iAUC240; p=0.734).There were also no differences among the four groups from baseline to 120 min (iAUC120; p=0.196). At 30 min, the glucose spike was greater for corn/rice and next the corn/qinoa as compared to the rice and wheat pastas (p=0.052). After 60 minutes all of the glucose concentrations began to decline except for the rice pasta. The rice pasta's glucose spike continued after 30 minutes peaking at 90 minutes. All four of the pasta's glucose levels began to drop sharply after 60 minutes with the wheat pasta giving the most sustained decrease.
With the insulin concentrations, Table 3 and Figure 2, there were no significant differences at baseline (p = .683) among all groups and no significant differences were seen post treatment at 30 minutes (p = .875)

Table 3. Glucose/Insulin concentrations for the pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta)*

	Minute	Rice	Corn/Quinoa	Corn/Rice	Wheat
Glucose mg/dl	0	89.1 ± 6.0	91.3 ± 6.6	91.3 ± 9.7	91.1 ± 4.8
	30	111.0 ± 7.9	120.3 ± 12.1	122.7 ± 16.8	110.1 ± 12.8
	60	118.4 ± 20	118.1 ± 119.7	120.4 ± 24.3	108.9 ± 23.0
	90	106.7 ± 21	111.0 ± 21.2	111.9 ± 15.2	105.7 ± 19.9
	120	101.9 ± 14.3	94.9 ±7.6	102.6 ± 15.8	100.9 ± 12.7
	150	98.0 ± 13.7	90.9 ± 9.8	102.3 ± 19.6	97.0 ± 20.8
	180	93.1 ± 14.7	90.1 ± 11.4	95.6 ± 7.6	89.9 ± 7.9
	240	88.0 ± 10.5	89.4 ± 10.8	94.9 ± 10.6	94.0 ± 11.1
Insulin uIU/mL	0	12.3 ±5.1	12.9 ±4.7	11.4 ± 2.9	12.2 ± 3.6
	30	54.4 ± 20.7	52.6 ±28.2	57.6 ± 16.3	58.8 ± 14.8

*values are mean ± standard deviation; n = 7; p = 0.052 for incremental change in glucose at time 30 (repeated measures ANOVA)

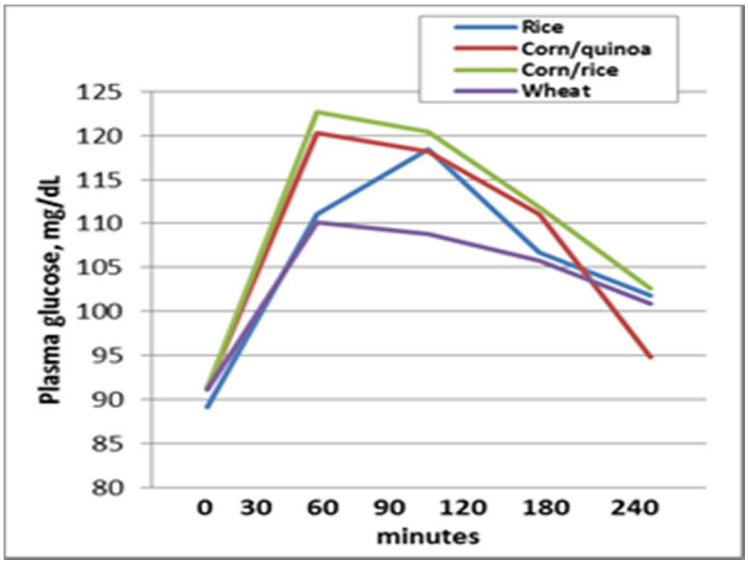

Figure 1. *Glucose concentrations at 30 min intervals for 240 min*

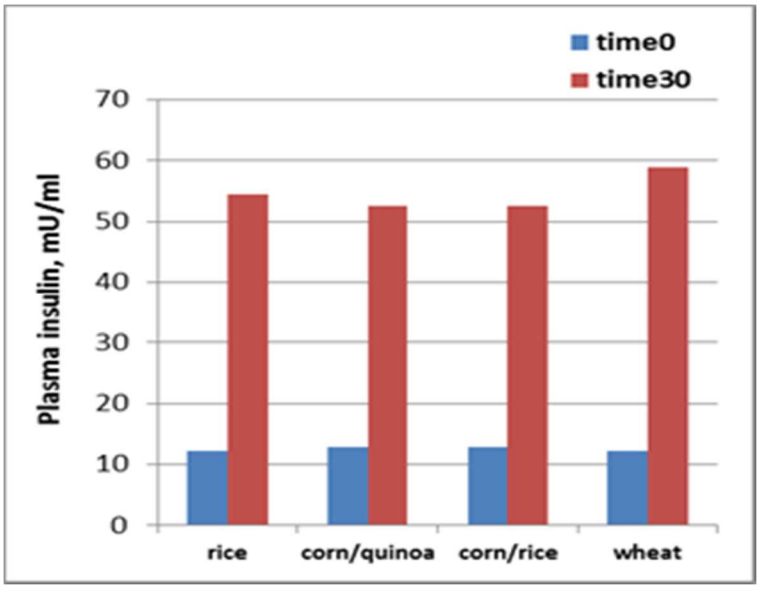

Figure 2. *Insulin concentrations at baseline (0 min and 30 min)*

4.2 – AUC for Blood Glucose

Table 4 reflects the area under the curve (AUC) values for 120 minutes. The AUC follows the trapizoidal rule where moving forward in time along the x axis, the entire AUC shows glucose

values from the beginning to the end of the test. The region is divided into sections or trapazoids. The region of each trapezoid is measured and represents the concentration of blood glucose values at a certain point in time.. Among all four groups, there were no significant differences in AUC values at 120 minutes (p=0.196). The highest mean was seen in the corn/rice pasta (86.8 ± 34.4), corn/quinoa pasta (77.4 ± 46.2) and lastly rice pasta at (77.4 ± 46.2) respectively. Although significance was not obtained, the wheat pasta had the lowest mean value (56.1 ± 47).

Table 4. Incremental AUC values ($mg \cdot dl^{-1} \cdot 120\ min^{-1}$) at 120 for the pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta)*	
Rice	75.1 ± 52.3
Corn/Quinoa	77.4 ± 46.2
Corn/Rice	86.8 ± 34.4
Wheat	56.1 ± 46.5

*values are means ± standard deviation; n = 7 ; p=.196 (repeated measures ANOVA)

Table 5 reflects the area under the curve (AUC) values for 240 minutes. Among all four groups, there were no significant differences in AUC values at 240 minutes (p=0.734). The highest mean was seen in the corn/rice pasta (109.5 ±67.2), rice pasta (93.7 ± 66.2) and lastly corn/quinoa pasta at (76.6 ± 49.9) respectively. Although significance was not obtained, the wheat pasta had the lowest mean value (67 ± 74.8).

Table 5. Incremental AUC values ($mg \cdot dl^{-1} \cdot 240\ min^{-1}$) at 240 min. for the pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta)*	
Rice	93.7 ± 66.2
Corn/Quinoa	76.6 ± 49.9
Corn/Rice	109.5 ±67.2
Wheat	67.0 ± 74.8

*values are means ± standard deviation; n = 7 ; p=.734 (repeated measures ANOVA)

4.3 – POMS Scores

Table 6 reflects the POMS values for all treatments. Among all four groups, there were no significant difference in total change POMS values between baseline (p value = 0.623), at 120 minutes (p=0.171) and for change over time (p=.239).There was no correlation between the

change in mood state and glycemic response after meal consumption but mood states improved during higher glucose responses. (Scatterplot r=-0.132, p=0.529), (Spearman correlation p = 0.042).

Table 6. POMS values for pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta)*

(0) Baseline	Rice	Corn/Quinoa	Corn/Rice	Wheat
Tension	5.3 ±2.7	3.6 ± 2.2	3.0 ± 2.8	5.0 ± 3.8
Depression	4.2 ±7.0	2.6 ± 4.3	1.7 ± 3.1	3.0 ± 6.9
Anger	3.8 ±4.6	0.9 ± 1.2	1.0 ± 1.3	0.5 ± 1.2
Vigor	8.1 ± 8.9	14.1 ± 8.4	17.0 ± 7.2	15.3 ± 6.4
Fatigue	3.2 ± 2.5	2.6 ± 3.3	2.2 ± 2.3	1.3 ± 2.4
Confusion	5.3 ± 2.8	3.7 ± 2.4	4.2 ± 2.1	3.7 ± 2
Total	12.3 ±21.7	-0.9 ±10.2	-5.0 ± 11.9	-1.8 ± 15.3
120				
Tension	5.9 ± 3.0	5.1 ± 2.3	4.0 ± 2.6	5.7 ± 3.5
Depression	5.7 ± 6.6	0.4 ± 0.5	1.7 ± 3.6	4.1 ± 6.4
Anger	3.6 ± 4.3	1.3 ± 1.1	0.7 ± 1.2	4.1 ± 7.0
Vigor	8.0 ± 7.7	11.7 ± 6.9	15.0 ± 5.7	10.7 ± 6.1
Fatigue	2.9 ± 3.8	4.0 ± 4.2	2.2 ± 2.2	2.9 ± 2.9
Confusion	4.9 ± 1.7	6.9 ± 3.8	6.7 ± 2.4	7.0 ± 4.2
Total	14.9 ±18.5	6.0 ± 13.3	0.2 ± 10.4	13.1 ± 23.4
Change	1.7 ± 13.9	6.9 ± 14.0	5.2 ± 7.0	12.2 ± 13.2

*values are means ± standard deviation. Abbreviations: T, tension, D, depression, A, anger, V, vigor, F, fatigue, C, confusion. Total POMS score is the sum of T,D,A,F, and C minus V. n = 6 for rice and corn/rice group; n= 7 for corn/quinoa and wheat group; p value = 0.623 and 0.171 at baseline and at 120. p=0.239 for change over time (repeated measures ANOVA)

Correlations

			AUCrice	chtotalPOMS1
Spearman's rho	AUCrice	Correlation Coefficient	1.000	-.409*
		Sig. (2-tailed)	.	.042
		N	28	25
	chtotalPOMS1	Correlation Coefficient	-.409*	1.000
		Sig. (2-tailed)	.042	.
		N	25	25

*. Correlation is significant at the 0.05 level (2-tailed).

Figure 3 – SPEARMAN Correlation between the glycemic responses of the pasta meal to the mood state

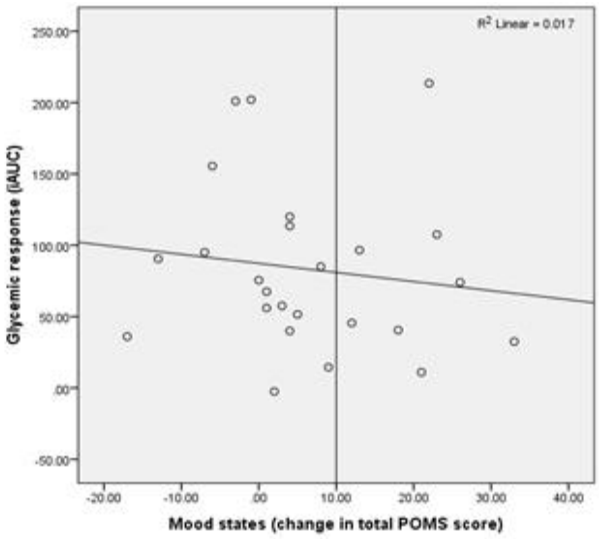

***Figure 4**. SCATTER PLOT Correlation between the glycemic responses of the pasta meal to the mood state*

4.4 – Breath Hydrogen Values

Table 7 reflects breath hydrogen (H_2) and total H_2 values (H_2 plus 2 methane). Breath H_2 and total H_2 decreased across all groups, p=0.396 and p=0.238 for change in H_2 and change in total H_2 respectively.

Table 7. Breath H_2 values for pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta)*

	Rice	Corn/Quinoa	Corn/Rice	Wheat
H_2 at 0 min (ppm)	9.3 ± 7.9	12.1 ± 12.4	10.7 ± 9.8	14.4 ± 13.5
H_2 at 240 min (ppm)	6.3 ± 7.1	6.0 ± 7.1	5.3 ± 5.6	7.9 ± 6.7
Change in H_2 (ppm)	-3.0± 9.7	-6.1 ± 7.2	-5.4 ± 7.2	-6.6 ± 14.0
Total H_2 at 0 min (ppm)	10.7 ± 8.7	15.6 ± 12.4	13.0± 9.7	15.6 ± 13.4
Total H_2 at 240 min (ppm)	7.1 ± 7.2	9.1 ± 8.6	7.3 ± 5.9	9.6± 6.5
Change in Total H_2 (ppm)	-3.6 ± 11.4	-6.4 ± 7.0	-5.7 ± 7.9	-6.0± 15.2

*Values are means ± standard deviation; n=7. Abbreviations; H_2 = hydrogen; total H_2 = H_2 plus 2 methane. P= 0.396 and p=0.238 for change in H_2 and change in total H_2 respectively.

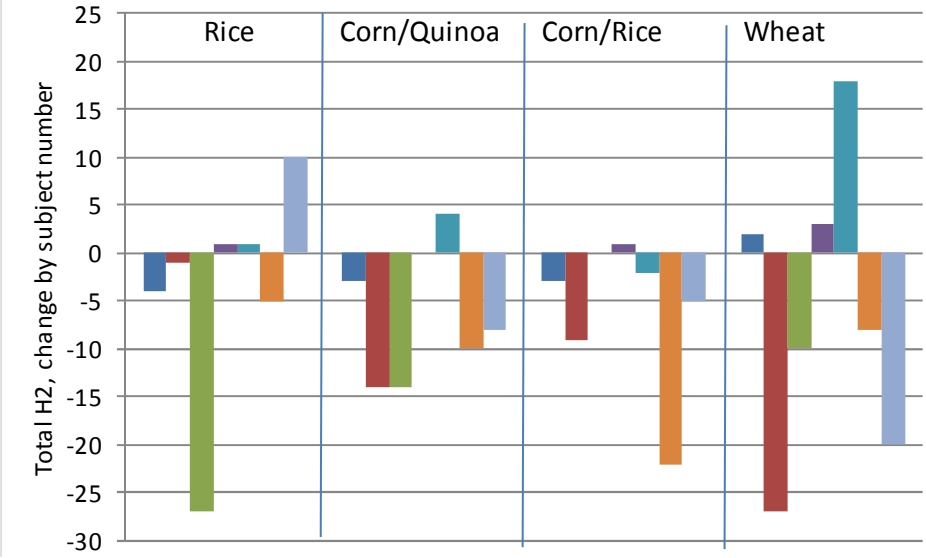

Figure 5 *– values represent the individual change in H2. Abbreviations; H_2 = hydrogen, n=7 for rice and wheat groups. N=6 for corn/quinoa and corn/rice groups.*

4.5 – Satiety Scores

Table 8 reflects the mean values for the satiety scores and the mean values for satiety area under the curve (AUC). Among all four groups, there was no significant difference in AUC satiety scores (p=0.194). With the AUC values, the corn/rice pasta had the highest satiety values (7.1 ± 6.8), followed by wheat (3.5 ± 8.3), rice (3.4 ± 7.6) and corn/quinoa (1.6 ± 6.7) respectively.

Table 8. Satiety Scores at four time points during the pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta)*

	Rice	Corn/Quinoa	Corn/Rice	Wheat
0 min	5.8 ± 4.6	5.0 ± 3.7	4.9 ± 4.5	5.5 ± 4
60 min	8.2 ± 4.9	8.0 ± 4.7	8.5 ± 5.1	7.5 ± 5.5
120 min	7.2 ± 3.7	4.4 ± 3	7.8 ± 4.6	7.3 ± 4.2
180 min	5.1 ± 2.3	3.4 ± 3.6	5.9 ± 4.5	4.8 ± 3.4
iAUC	3.4 ± 7.6	1.6 ± 6.7	7.1 ± 6.6	3.5 ± 8.3

*values are means ± standard deviation; n = 7; p for incremental AUC satiety across all groups =.194 (repeated measures ANOVA)

4.6 Total Energy Consumption Scores

Table 9 reflects the mean values for the total energy consumed for the remainder of the day (following the macaroni & cheese meal). Among all four groups, there were no significant differences in energy consumed (p=0.506). The highest amount of kcals consumed was seen in

the wheat group (2006± 1063), then corn/rice (1893± 741), corn/quinoa (1709 ± 703) and rice (1562 ± 578) respectively.

Table 9. Total energy consumption (kcals) of the pasta meals (three gluten free pastas [rice, corn/quinoa, corn/rice] or conventional wheat pasta for the remainder of the day *	
Rice	1561.6 ± 577.5
Corn/Quinoa	1708.9 ± 702.7
Corn/Rice	1893.3 ± 741.1
Wheat	2006.4 ± 1062.9

*values are means ± standard deviation; n = 6 for rice, corn/quinoa and wheat; n = 7 for corn/rice; p = .506 (repeated measures ANOVA)

CHAPTER 5

DISCUSSION AND CONCLUSION

The estimated number of individuals with known gluten disorders does not explain the recent and dramatic rise in gluten free products (GFP) sales. The cause for this market boom remains unexplained (1-7). However, many believe there are health benefits of consuming a gluten free diet (8). The scientific research on gluten free products, in particular, the glycemic response of GF pasta remains mostly unexplored. Due to the high levels of processing and the use of common gluten replacement ingredients such as potato and rice starch, several articles have claimed that gluten free products have a high glycemic index (10, 11, 18). More recently however, food producers are adding both corn and quinoa to their food formulas. To date, no studies have tested the glycemic index of these foods nor have they tested how these foods work in conjunction with mood states.

The International Table of Glycemic Index uses evidence from both published and unpublished verified sources and it lists the glycemic index for over 750 different types of foods (18). A few studies on this index did test the glycemic effect of both GF bread and pastas and demonstrated that the glycemic index (GI) of GF bread, biscuits, and pasta were comparable to the traditional foods. However, the test products used were made from either corn or rice but none of them used quinoa or mixtures of corn/quinoa, two combinations which are now common in the market.

In a small crossover trial, Berti et al, tested both the in vitro starch digestibility of GF foods and the in vivo metabolic response to GF foods (17). The in-vitro test compared the starch digestibility of whole grain quinoa in comparison to both regular wheat pasta, GF pasta and GF bread made from potato and rice flours. The in-vivo test involved both celiac (n = 6) and healthy individuals (n = 7). The test on the healthy individuals compared the glycemic response of regular pasta, GF pasta (made from rice and potato starch), regular bread, GF bread and lastly whole grain quinoa. The test on the celiac subjects compared the glycemic response of GF

bread and GF pasta, and this was compared to the glycemic response of the healthy individuals. (17).

The in vitro results of starch digestibility showed that the AUC for GF bread was slightly higher than that of regular wheat bread but the AUC of GF pasta was the same and when comparing the AUC of the quinoa to the two pastas, there was no significant difference. The in vivo test results showed that there were some glycemic response differences. Among the healthy and celiac individuals, the glycemic response of the GF bread and GF pasta was the highest in both groups but the celiac group was higher than the healthy individuals. With the insulin response, there were no significant differences between all products. With the glycemic index (GI), the results showed that the GF pasta was similar to the GI of the GF bread but the GI for the quinoa was lower than both the GF pasta and GF bread. The whole grain quinoa had a lower GI than GF pasta but, due to the small sample size, the results were not statistically significant. (17).

The reason for the higher AUC for some GF products could be due to the processing of the product (14, 17, 18, 26, 32, 37). With pastas, the products are made from refined flours and starches and the degree of processing of these ingredients can influence the glycemic response of the food (37). However, Ward et al tested the retrogradation properties of amylopectin (AP) made from both native wheat and corn. This study found that after an initial gelatinization and subsequent cooling, drying and storage, the corn AP produced significantly more crystalline structures (retrogradation) than did wheat AP. This was demonstrated by a higher enthalpy, the energy required to melt the crystalline material back to a gelatinous stage. Most of this crystallization occurred within one week of storage, a process comparable to the manufacturing of pasta products. It is theorized that the differences in retrogradation between the two starches may be explained by the differences in their glycolytic chain lengths. The native corn AP starch had shorter chains but more of them. The higher degree of chains in the corn may explain its higher capacity to retrograde (37). Nonetheless, this experiment produced a higher glycemic response in the corn pasta. It could be that the pasta making process did not produce the same or as much retrogradation as it did in the Ward experiment. Or, if the pastas used in this

experiment did have the development of retrogradation during the processing of the raw product, perhaps the longer cooking time and high temperatures in the cooking procedure caused re-gelatinization of the starch and a subsequent faster digestion and absorption. The present study found similar results to the Berti trial (17) where the highest AUC values were seen in the GF product in comparison to the wheat. Surprisingly though, the element which was expected to have a lowered glycemic response (quinoa/corn mixture) had the highest AUC values. A possible reason for the lowered glycemic response is the wheat pasta might be that during digestion, the gluten surrounds the starch granule and can slow down the hydrolysis by blocking the action of amylase. Therefore theoretically, the removal of gluten from a carbohydrate food might increase the glycemic response of a food.

The POMS test was utilized in this experiment to measure change in mood, a test which has been validated for both reliability and validity. The POMS questionnaire uses 65 words and/or statements which describe the subject's feelings. The test subjects rate their feelings in each question and their responses are categorized into anger, confusion, depression fatigue, tension and vigor. All the scores are added up then vigor is subtracted. The higher the participant's score on the POMS test, the more total disturbance in mood (TMD) (11, 53). Although significant differences were not obtained in this experiment. Scatter plot & Spearman's correlations shows sig ($p=0.042$), as glycemic response increases, POMS decreases (improved mood). Another unexpected result occurred when the mean values of the wheat group was found to have the highest POMS score, with corn/quinoa, and corn/rice having the next highest and lastly the rice pasta group having the lowest score. These results do not correlate with the lowered glycemic effect of the wheat group in comparison to the GF pastas. The scientific literature has shown that diets rich in carbohydrates positively correlate with improved moods and diets high in protein positively correlate with depression (11, 12, 49-53). This study produced POMS data which supports the findings in the literature, the more carbohydrate in the blood, the better the participants felt (figure 3). Passman et al and Cheatham et al tested the type of carbohydrate on their test subjects and found that a diet with complex carbohydrates produced higher satiety, lowered fatigue and improved mood in comparison to simple carbohydrates. Both

trials used a significant amount of carbohydrates in their test diets and therefore showed that the type of carbohydrate and glycemic load does have the ability to improve moods. All of the pasta meals in this study produced a high glycemic response. However, from the results in the Passman and Cheatham studies and from the fact that all of the test meals in the present study produced a high glycemic response, even in the meals that were considered lower, it would be expected that the lowered and more sustained glycemic effect from the wheat pasta would have produced an improved mood state in comparison to the groups which had the higher glycemic response. However, the opposite occurred. The wheat group produced a higher TMD than did the GF pastas which had a higher glycemic response. The reason for these results is unknown. However, it can be hypothesized from these results that an ingredient in the wheat, not the glycemic response, could be responsible for the disruption in mood.

The colonic hydrogen tests also produced unexpected results. The mean values for both the change in H_2 and total H_2 were higher in all groups at baseline than they were four hours (240 min) after the test meal. It has been documented in the scientific literature that some individuals can have residual colonic activity in the early morning hours after fasting (25, 63). To account for this, Heacock et al used the lowest value of 3 baseline breath samples during their trial, a procedure that was not performed in the current study (63). It is believed that these results could have been improved if the baseline data was collected in the same manner as the Heacock trial. However, other studies have followed similar procedures to this trial and have produced little to no colonic activity the morning of the test days at baseline. Instead, the opposite occurred during all treatments of this study.

In this trial, another reason for the high colonic activity after fasting could also be due to non-compliance of the protocol. When the test subjects arrived in the lab the next day, they were asked if they followed the diet protocol and if they brushed their teeth before arriving. All of the test subjects indicated that they were in compliance with the study protocol. As assurance, each subject was then asked to rinse their mouths with a mouthwash before baseline data was taken. Both baseline and post meal colonic tests were taken by the same investigator and in the same manner. Due to this, it is unlikely that all of the participants were non-compliant to any one

procedure but because this study consisted of only 7 subjects, it is more likely that the participants could have been non-compliant to one or any one of the procedures. It is believed that even being "slightly" non-compliant can produce undesired results. Ultimately though, the colonic hydrogen data from this trial was unexplainable and the statistical analysis produced insignificant results.

With satiety, it was originally hypothesized that both the corn and quinoa would produce higher satiety results than the wheat or the rice pastas. This was partially true in this study. The highest satiety was found in the corn/rice pasta but what's puzzling is why the corn/quinoa would produce the lowest. Both the wheat and the rice pasta produce similar satiety results and were under the corn/rice but above the corn/quinoa pastas. Therefore the anomaly in the satiety tests was the result of the corn/quinoa pastas.

It would be expected that the pasta group which produced the lowest glycemic response would, in this case the wheat, would also have the lowest amount of kcals consumed after the test meal. Typically, a lowered glycemic response of foods that are equal in macro nutrient composition would also produce more colonic hydrogen, more satiety and less kcals consumed in hours after the postprandial period. In this study, the wheat pasta group consumed the most kcals in the 24 hours after the test meal. The next highest was the corn/rice, the corn/quinoa and the rice respectively. Each treatment in this study was only tested once. It is likely then that the diet record after of only one test and only one day was not enough to fully produce or to fully capture the effect of these test meals. It is possible that a longer treatment would produce a desired result.

The limitations of this study include time constraints for the recruiting process, which resulted in a small sample size and subsequent results that were statistically insignificant. In addition to the limited time for recruiting, this was an acute investigation and long term benefits to this treatment are therefore unknown. With the colonic hydrogen tests, the limitations include whether or not the test subjects were "non-producers" of colonic hydrogen gas which is contingent upon if they had hydrogen producing intestinal microbiota and if they were producers of hydrogen, colonic gas can form more than 4 hours after the consumption of the test meal.

Lastly, the degree to which the test subjects were compliant with the study protocol is unknown and non-compliance can impact the results.

In conclusion these results indicate that the formulation and processing of gluten free pastas may affect the rate and absorption and the subsequent glycemic response after the consumption of these foods. Although no significant results were obtained, the gluten free pastas made from corn, quinoa and rice produced a higher postprandial glycemic response in comparison to the consumption of regular wheat based pasta. In addition, the total mood disturbance after the consumption was seen highest in the wheat pasta group and mood improvement was seen in the gluten free pastas. Whether or not wheat has an ingredient which negatively affects mood states remains undetermined and warrants future research in this area.

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APPENDIX A

DATA COLLECTED JANUARY- DECEMBER 2014

APPROVAL: EXPEDITED REVIEW

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

Dear Carol Johnston:

On 1/9/2014 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Diet strategies to improve the physiological and psychological responses to meal ingestion
Investigator:	Carol Johnston
IRB ID:	STUDY00000480
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:	Name: (Unspecified);
Grant Title:	
Grant ID:	
Documents Reviewed:	• Consent study #1, Category: Consent Form; • Consent study #2, Category: Consent Form; • Research Protocol, Category: IRB Protocol; • Health History Questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • POMS questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Satiety scale, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • calendar and instructions, Category: Participant materials (specific directions for them);

	• Verbal script, ad, email ad, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Funding information, Category: Sponsor Attachment;

The IRB approved the protocol from 1/9/2014 to 1/8/2015 inclusive. Three weeks before 1/8/2015 you are to submit a completed "FORM: Continuing Review (HRP-212)" and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 1/8/2015 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:

Christine Wilkins
Darren Snyder